# Screening of mycota associated with *Aijung* rice seed and their effects on seed germination and seedling vigour

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Experiments were conducted to evaluate the effect of some dominant seed borne fungi of *Aijung* rice variety on seed germination and seedling vigour. Twenty dominant fungi were found associated with *Aijung* rice seeds. Analysis of seed borne fungi by blotter method and agar plate method showed that species of *Aspergillus, Fusarium, Alternaria* and *Curvularia* are the dominant genera. Seed germination and seedling vigour tests were conducted using seed inoculation, soil inoculation and seed submergence method. Maximum reduction in seed germination and seedling vigour was caused by species of *Fusarium* in seed inoculation method, by species of *Rhizopus* and *Fusarium* in soil inoculation method and by species of *Aspergillus* in seed submergence method. In another experiment healthy rice seeds were soaked in 25, 50, 75 and 100% concentration of 7-, 14- and 21-day-old culture filtrates of the isolated seed borne fungi. Maximum reduction in seed germination was recorded from 21-day-old culture filtrates. The inhibitory effect on seed germination was found to decrease with increase in dilution of the filtrates.

**Key words –** germination – incubation – seed inoculation – vigour index

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## Introduction

Every seed is a potential harbour of a wide variety of microfungi containing both pathogenic and saprophytic microorganisms, both externally and internally (Utobo et al. 2011). These microfungi may reduce seed quality and impair seed germination resulting in the production of abnormal seedlings (Paul 1989, Vijayan & Rehill 1990, Bateman & Kwasna 1999, Khanzada et al. 2002). In case of severe infection the seed completely deteriorates and the grain may become unsuitable even for animal consumption due to production of mycotoxic substances by seed fungi.

Rice (*Oryza sativa* L.) is the most widely grown cereal crop in the world. Rice seeds are infected by large number of fungi that are reported to perpetuate from one season to another through infected seeds (Zope & Thrimurty 2004). Moreover, high rainfall and humidity during *Kharif* season expose paddy seeds to fungal invasions. Several fungal pathogens have been isolated from rice grains and have been reported to be responsible for a number of diseases from the nursery to the field (Ibiam et al. 2006). These fungi have been shown to play a role in reducing germination and seedling vigour (Subramanyam 1991, Gupta & Chouhan 1970, Dharamvir 1973).

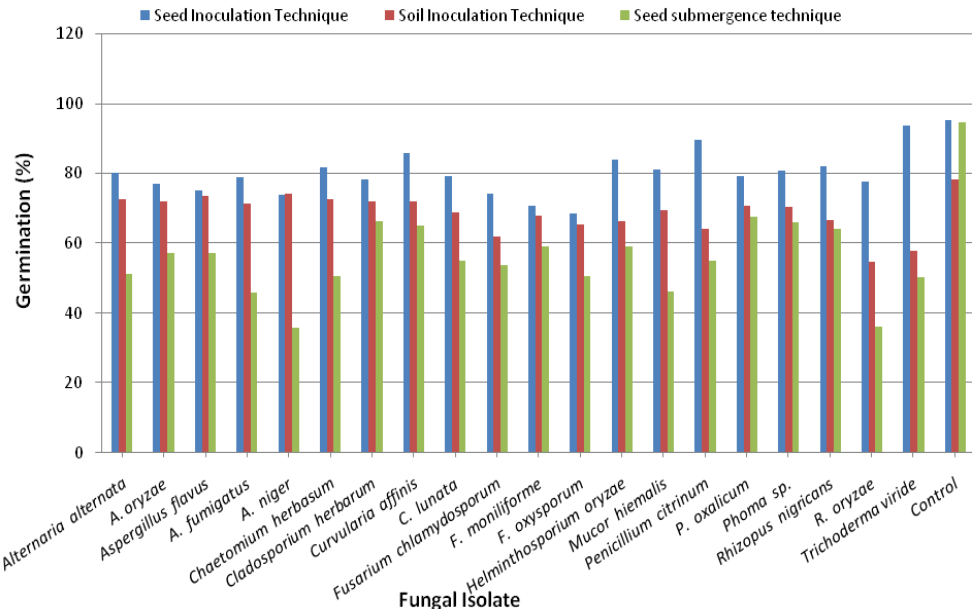


**Fig. 1 –** Effect of seed borne fungi (seed inoculation, soil inoculation and seed submergence method) on rice seed germination

The present investigation deals with isolation of fungi associated with *Aijung* variety of rice seed and their effects on seed germination and seedling vigour.

## Materials and methods

### *Site description*

The study area is situated in south of the river Brahmaputra, Assam, North-East India (26°12′N, 91°50′E) with an average altitude of 54 m (a.s.l). Healthy rice seeds of *Aijung* variety were collected from a local farmer in the study area. The variety is most widely cultivated in this region due to its high yield and adaptability to the prevailing climatic condition. The climate of the area is monsoonal, characterised by a long rainy season (May-September) and dry and cold winter (November-February). The average annual rainfall was about 1782 mm during the study period, with highest rainfall (315.5mm) in September. The hottest month of the year was August with a mean maximum temperature of 33.0°C and the coldest month was January with a mean minimum temperature of 9.9°C. Maximum relative humidity recorded during the study period was 85% in the month of July.

### *Microbial assay of rice seeds*

Microbiological assay of the mycota associated with rice seeds were carried out using standard blotter method (ISTA 1976) and nutrient agar plate method (Muskett 1948).

*Standard Blotter Method*

Unsterilized as well as sterilised rice seeds were placed on three sets of moist blotter paper at the rate of 10 seeds/ Petri plate. The experiment was laid with 20 replications each for sterilised and unsterilised seeds. For analysis of internal seed borne fungi seed were surface sterilised with 2.5% sodium hypochlorite solution for one minutes followed by 3-4 washings with sterile distilled water before plating, while unsterilised seeds were plated without treatment. The plated seeds were incubated at 25 ± 2°C in an incubator for 7 days. Sterile distilled water was sprayed aseptically on the Petri plates every third day in order to keep the blotters sufficiently moist. After incubation fungi were isolated by pure culture method. Further slides were prepared and examined under a compound microscope for identification of the isolated fungi.

*Agar Plate Method*

The other set of experiment was carried out on potato dextrose agar (PDA) medium. Similar to blotter method, 20 replicates were taken each for unsterilised and sterilised seeds at a rate of 10 seeds/Petri

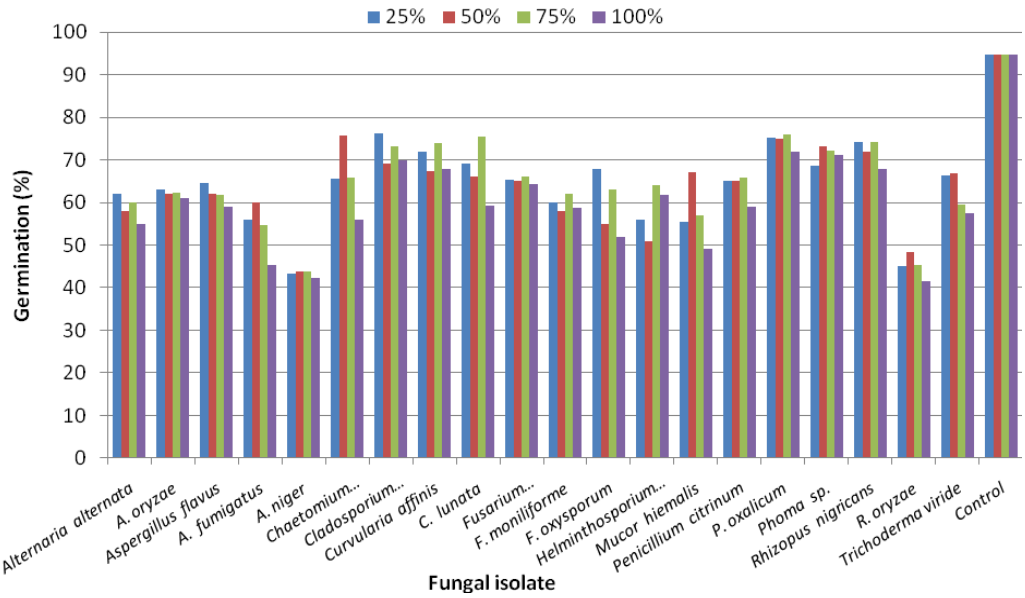


**Fig. 2 –** Effect of 7-day-old culture filtrate at different concentrations on rice seed germination

plate. The seeds were placed on sterilised glass Petri plates containing 20 mL potato dextrose agar medium. The plates were incubated at 25 ± 2°C for 7 days. Through constant observation during the incubation period, growth characteristics of fungal colonies were recorded. At the end of the incubation period, pure cultures of fungi growing out of seeds were prepared on suitable agar slants for further examination.

***Effect of seed borne fungi on germination and seedling vigour***

The effect of seed borne on germination and seedling vigour was analysed as per method of Singh & Swami (2004).

*Seed inoculation method*

Rice seeds were surface sterilised with 2.5% sodium hypochlorite solution for one minute followed by several washings with sterile distilled water before plating. Sterilised seeds were inoculated by rolling over 10-days-old sporulating culture of each fungus grown on PDA. The inoculated seeds were air dried for 72 h at room temperature. The inoculated seeds were plated over three layers of moist blotter paper. Three replicate of 10 seeds each were taken for each treatment. Equal number of healthy sterilised seeds without inoculation plated over moist blotter paper served as control. Treated and control plates were incubated at 25 ± 2°C for 14 days. After incubation, observation on percent germination, radical and plumule length and seedling vigour were recorded.

Germination percent was recorded as:
Germination (%) = Number of seeds germinated/ Total number of seeds used × 100

Seedling vigour was determined following the formula of Baki & Anderson (1972):
Vigour index = (Mean of root length + Mean of shoot length) × Percentage of seed germination

*Soil inoculation method*

The isolated fungi were grown separately on autoclaved rice medium (10:5 rice/water) in 100 mL conical flasks. The flasks were incubated at 25 ± 2°C for 10 days. Sterilised earthen pots (30 cm) were filled with pre-sterilised soil. For inoculation, the upper 5 cm layer of soil was thoroughly mixed with rice medium for supporting the fungal growth. The pots were covered with polythene bags and left for 24 h in a green house. After 24 h healthy sterilised rice seeds were sown in each pot. Three replicates of 10 seeds each were taken for each treatment. Equal number of healthy sterilised seeds sown in uninoculated pot served as control. After 14 days, observation on percent germination and seedling vigour were recorded.

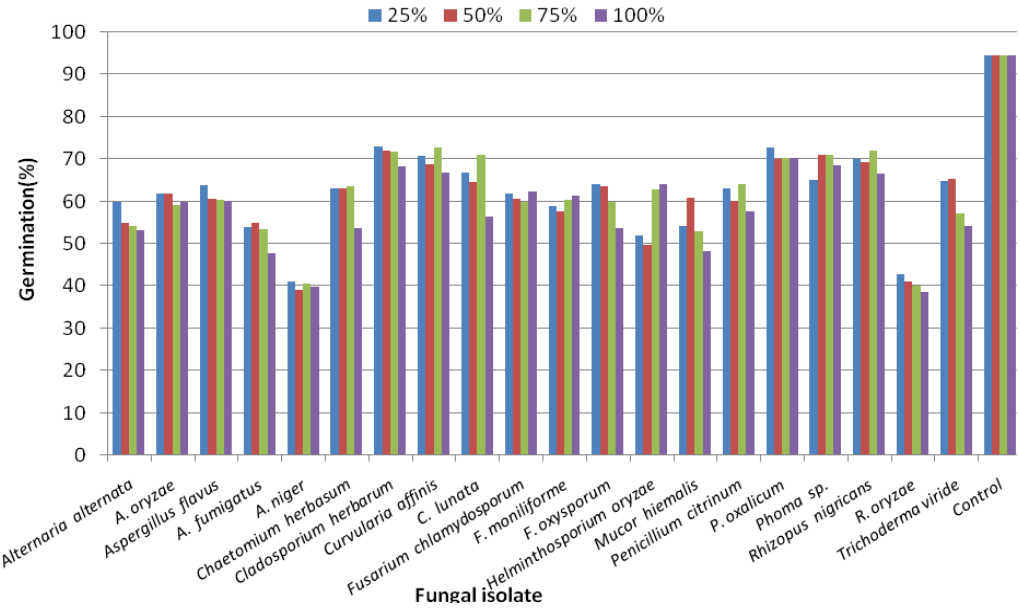


**Fig. 3 –** Effect of 14-day-old culture filtrate at different concentrations on rice seed germination

*Seed submergence method*

The isolated fungi were grown separately in Richard's broth medium and incubated for 21 days at temperature of 25 ± 2°C. Culture filtrates were obtained by filtering the contents through Whatman filter paper no. 44. Healthy surface sterilised rice seeds were separately soaked in these culture filtrates for 24 h and allowed to germinate in sterilised plates containing three layers of moist sterile blotter paper. Seeds soaked in uninoculated sterilised medium and sterile water served as control. Petri plates were incubated at 25 ± 2°C for 14 days. After incubation, percent seed germination and seedling vigour were recorded.

**Effect of different concentration of culture filtrates on rice seed germination**

Surface sterilised seeds were soaked for 24 h in different concentration (25, 50, 75, and 100) of 7-, 14- and 21-day-old culture filtrate of each fungus. Dilution was made with sterile distilled water. Three replicates of 10 seeds were taken for each concentration, days and fungus. Equal number of seeds soaked in sterile distilled water served as control. The soaked seeds were plated on three layers of moist blotter paper and incubated at 25 ± 2°C for 14 days. After incubation, percent germination was recorded.

## Results

**Microbial assay of seed mycota**

Twenty fungal species belonging to 12 genera were isolated from *Aijung* rice seeds (Table 1). More fungi were recorded in agar plate method than in the blotter method. In blotter method the highest percent incidence was recorded by *Alternaria alternata* (4.7%), followed by *Aspergillus fumigatus* (4.5%) and *A. niger* (3.7%) in case of surface sterilised seeds. The lowest incidence was detected for *Penicillium citrinum* (1.2%). In case of unsterilised seeds, the highest percent incidence was noted with *Curvularia affinis* (5.2%) followed by *Penicillium oxalicum* (4.9%) and *P. citrinum* (4.8%) and the lowest by *Fusarium chlamydosporium* and *Rhizopus nigricans* (2.6% each). Similarly, in agar plate method, the highest percentage of occurrence was recorded with *Alternaria alternata* (5.5%), followed by *Aspergillus fumigatus* (4.7%) and *A. niger* (4.5%) in case of surface sterilised seeds. The lowest incidence was detected in *Cladosporium herbarum*, *Fusarium moniliforme* and *Rhizopus nigricans* (2.1% each). In case of unsterilised seeds the highest percent of incidence was shown by *Penicillium oxalicum* (5.2%), followed by *Aspergillus fumigatus* and *Fusarium moniliforme* (5.1% each) and the lowest by *Fusarium chlamydosporium* (2.8%).

**Table 1** Incidence of fungi associated with rice seed evaluated by standard blotter method and agar plate method

| Fungi | % incidence | | | |
|---|---|---|---|---|
| | Standard blotter method | | Agar plate method | |
| | SS | US | SS | US |
| *Alternaria alternata* (Fr.) Keissl. | 4.7 | 4.0 | 5.5 | 4.3 |
| *A. oryzae* Hara | 2.5 | 3.5 | 3.2 | 4.2 |
| *Aspergillus flavus* Link. | 2.1 | 3.5 | 3.5 | 4.3 |
| *A. fumigatus* Fresen. | 4.5 | 4.2 | 4.7 | 5.1 |
| *A. niger* van Tiegh. | 3.7 | 4.2 | 4.5 | 4.8 |
| *Chaetomium* sp. | 2.2 | 4.0 | 3.5 | 4.1 |
| *Cladosporium herbarum* (Pers.)Link | 2.0 | 4.2 | 2.1 | 4.6 |
| *Curvularia affinis* Boedijn | 3.0 | 5.2 | 0.0 | 6.0 |
| *C. lunata* (Wakker) Boedijn | 2.4 | 3.9 | 3.3 | 4.4 |
| *Fusarium chlamydosporum* Wollenw. & Reinking | 0.0 | 2.6 | 2.2 | 2.8 |
| *F. moniliformae* J. Sheld. | 1.7 | 4.2 | 2.1 | 5.1 |
| *F. oxysporum* Schltdl. | 2.2 | 3.9 | 4.2 | 4.9 |
| *Helminthosporium oryzae* Breda de Haan | 2.0 | 4.2 | 4.2 | 4.4 |
| *Mucor hiemalis* Wehmer | 2.4 | 3.9 | 2.5 | 4.0 |
| *Penicillium citrinum* Thom | 1.2 | 4.8 | 2.3 | 5.0 |
| *P. oxalicum* Currie & Thom | 2.7 | 4.9 | 0.0 | 5.2 |
| *Phoma* sp. | 1.7 | 3.9 | 0.0 | 4.0 |
| *Rhizopus nigricans* Ehrenb. | 0.0 | 2.6 | 2.1 | 3.2 |
| *R. oryzae* Went & Prins. | 2.0 | 3.9 | 2.3 | 4.0 |
| *Trichoderma* sp. | 2.5 | 3.1 | 2.5 | 3.5 |
| Seeds without mycota | 54.5 | 21.3 | 45.3 | 12.1 |
| No. of genera | 12 | 12 | 11 | 12 |
| No. of species | 18 | 20 | 17 | 20 |

US- unsterilized seeds; SS-sterilized seeds;

Some fungi viz., *Fusarium chlamydosporium* and *Rhizopus nigricans* were absent in surface sterilised seeds but present in non-surface sterilised seeds in blotter method. In agar plate method *Curvularia affinis*, *Penicillium oxalicum* and *Phoma* sp. were absent from surface sterilised seeds.

**Effect of seed borne fungi on seed germination and seedling vigour (seed inoculation method)**

Results on the effect of seed borne fungi on germination and seedling vigour obtained by seed inoculation method are presented in the Table 2. The percent germination of seeds was markedly suppressed by all fungi. Maximum percent inhibition in germination over control was observed in the seeds inoculated with *Fusarium oxysporum* (28%) followed by *Fusarium moniliforme* (25.8%), *Aspergillus niger* (22.4%) and *Fusarium chlamydosporum* (22.3%). The lowest inhibition of germination was shown by seeds treated with inoculum of *Trichoderma viride* (1.8%) and *Penicillium citrinum* (6.1%). Germination percentages of treated seeds were statistically significant at .05 level. Maximum decrease in shoot length (7.3 cm) was observed in seeds inoculated with *Fusarium moniliforme* and root length (3.4 cm) in *Chaetomium herbarum* inoculated seeds. The mean differences were statistically significant. The highest vigour index (1394.8) of seedling was obtained from seeds inoculated with *Phoma* sp. and the lowest (817.6) was recorded in *Fusarium moniliforme* inoculated seeds.

**Effect of seed borne fungi on seed germination and seedling vigour (soil inoculation method)**

Table 3 reveals the effect of seed borne fungi on germination and seedling vigour by soil inoculation method. Highest percent inhibition in germination (30.1%) over control was recorded in the seeds sown in *Rhizopus oryzae* inoculated soils followed by

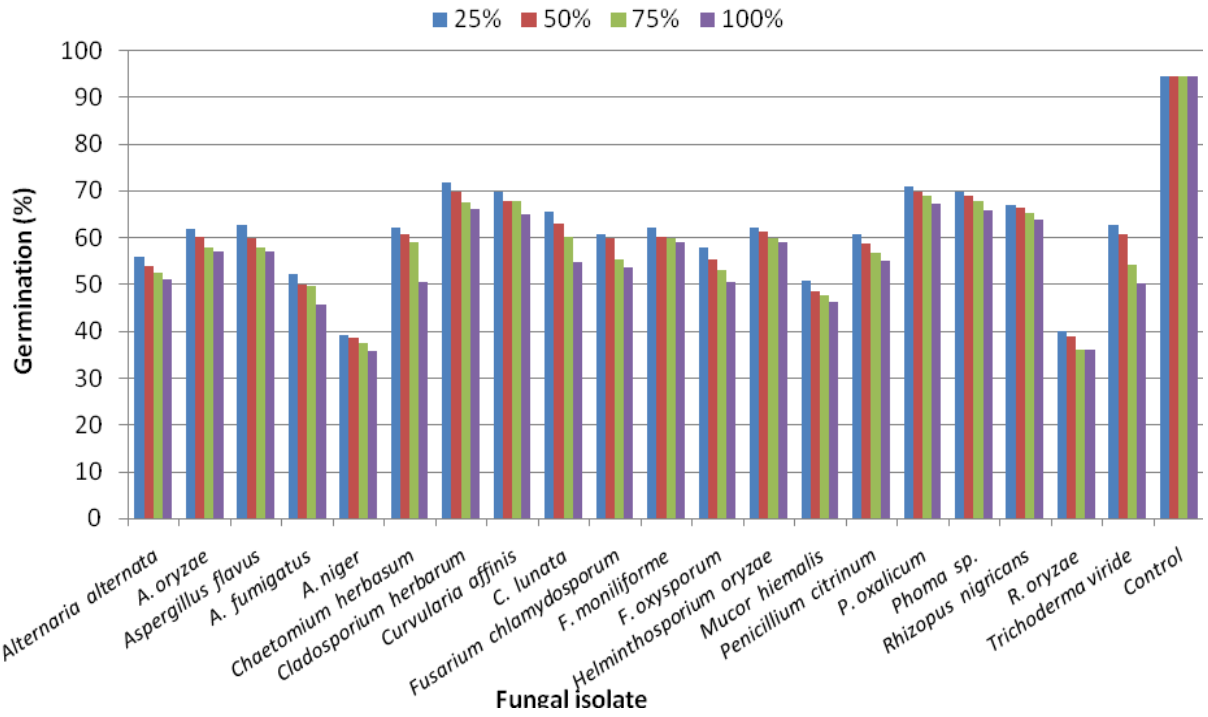


**Fig. 4 –** Effect of 21-day-old culture filtrate at different concentrations on rice seed germination

*Trichoderma viride* (26.2%), *Fusarium chlamydosporium* (20.6%) and *Penicillium citrinum* (18.2%). Lowest inhibition was observed in soil treated with inoculum of *Aspergillus niger* (5.3%). Germination percentages were found to be statistically significant at .05 level. Maximum reduction in shoot length (6 cm) was observed in *Rhizopus nigricans* inoculated soils and root length (3.3 cm) in *Chaetomium herbasum* inoculated soils. The highest vigour index (1038.2) was in seedlings obtained from *Aspergillus flavus* inoculated soils and the lowest (603.5) was recorded from *Fusarium chlamydosporum* inoculated soils.

**Effect of seed borne fungi on seed germination and seedling vigour (seed submergence method)**

Rice seeds soaked in culture filtrate of individual fungi showed reduction in percent germination and seedling vigour over control (Table 4). Maximum inhibition in germination (62%) over control and reduction in seedling vigour (322.7) was recorded from seeds soaked in culture filtrate of *Aspergillus niger* (62.05%) followed by filtrate of *Rhizopus oryzae* (61.7%), *Aspergillus fumigatus* (51.5%) and *Trichoderma viride* (46.9%). Minimum inhibition in germination was recorded with culture filtrate of *Penicillium oxalicum* (28.69%). Maximum reduction in shoot length was recorded in culture filtrate of *Penicillium oxalicum* (5.5 cm) and root length in culture filtrate of *Fusarium moniliforme* (3 cm). The highest vigour index (861.9) of seedlings was observed in culture filtrate of *Curvularia affinis* and the lowest (322.7) was recorded in filtrate of *Aspergillus niger.*

Fig.1 shows a comparison between seed germination as affected by seed borne fungi recorded by the three methods. Germination percentage ranged from 36% to 94 %. Maximum inhibition in seed germination over control were due to the effect of culture filtrates of the isolated fungi compared to inhibition shown by fungi inoculated through seed and soil. Maximum inhibition was shown by *Aspergillus niger* (62%) as estimated by seed submergence method and minimum by *Trichoderma viride* (2%) estimated through seed inoculation method.

**Effect of culture filtrates at different concentration on rice seed germination**

Figs 2, 3 and 4 reveals efficacy of 7, 14, and 21 day old culture filtrates at four different concentrations on seed germination. Data

**Table 2** Effect of seed borne fungi on seed germination and seedling vigour (seed inoculation method)

| Fungi | % Germination | % inhibition of germination over control | Shoot length (cm) | Root length (cm) | Vigour index |
|---|---|---|---|---|---|
| *Alternaria alternata* | 80.10±0.66 | 15.97 | 9.15±1.27 | 4.58±0.93 | 1099.73 |
| *A. oryzae* | 77.03±3.42 | 19.19 | 8.78±0.75 | 4.93±0.11 | 1056.08 |
| *Aspergillus flavus* | 75.00±2.08 | 21.32 | 9.95±1.07 | 5.53±0.32 | 1161.00 |
| *A. fumigatus* | 79.00±1.77 | 17.12 | 8.84±0.58 | 3.83±0.63 | 1000.93 |
| *A. niger* | 73.93±2.46 | 22.44 | 7.59±0.20 | 4.30±0.30 | 879.02 |
| *Chaetomium herbasum* | 81.83±5.92 | 14.16 | 10.73±1.19 | 3.36±0.27 | 1152.98 |
| *Cladosporium herbarum* | 78.23±6.41 | 17.93 | 8.63±0.37 | 4.73±0.37 | 1045.15 |
| *Curvularia affinis* | 85.80±0.75 | 9.99 | 8.56±0.75 | 6.56±0.24 | 1297.29 |
| *C. lunata* | 79.20±3.08 | 16.92 | 10.86±0.24 | 4.33±0.88 | 1203.04 |
| *Fusarium chlamydosporum* | 74.00±1.92 | 22.37 | 13.06±1.27 | 4.03±0.38 | 1264.66 |
| *F. moniliforme* | 70.73±2.94 | 25.80 | 7.33±0.93 | 4.23±0.13 | 817.63 |
| *F. oxysporum* | 68.63±1.04 | 28.00 | 8.99±0.61 | 3.56±0.28 | 861.30 |
| *Helminthosporium oryzae* | 84.00±2.28 | 11.88 | 9.58±1.32 | 4.90±0.58 | 1216.32 |
| *Mucor hiemalis* | 80.99±3.60 | 15.04 | 8.92±0.60 | 3.93±0.57 | 1040.72 |
| *Penicillium citrinum* | 89.50±1.15 | 6.11 | 8.45±0.27 | 4.30±0.41 | 1141.12 |
| *P. oxalicum* | 79.30±7.40 | 16.81 | 9.21±0.71 | 5.33±0.33 | 1153.02 |
| *Phoma* sp. | 80.86±3.55 | 15.17 | 10.29±1.29 | 6.96±0.57 | 1394.83 |
| *Rhizopus nigricans* | 82.00±2.51 | 13.98 | 9.62±0.31 | 6.66±0.61 | 1334.96 |
| *R. oryzae* | 77.60±6.20 | 18.59 | 9.22±0.37 | 5.53±0.63 | 1144.60 |
| *Trichoderma viride* | 93.57±1.96 | 1.84 | 9.66±0.71 | 4.33±0.66 | 1309.04 |
| Control | 95.33±0.89 | | 13.89±0.37 | 6.95±0.71 | |
| SEm | 1.00 | | 0.22 | 0.15 | |
| LSD(.05) | .004 | | .016 | .007 | |

Mean±SEm, n = 3

revealed that there was marked inhibition in the rate of seed germination from 7-day to 21-day-old filtrate. Filtrates of 100% concentration were found to be more effective in inhibiting seed germination than that of other concentrations. Seven-day old filtrate produced 41 to 76% seed germination, whereas 14 and 21 day old filtrates produced 39 to 73% and 36 to 71% seed germination as compared to control (95%). The results indicate that rice seeds were sensitive to filtrates of all the isolated fungi. The inhibitory effect of the filtrates on seed germination decreased with increase in dilution but in no case was seed germination observed to be more than that of the control.

## Discussion

The study revealed a wide range of fungi associated with rice seeds. Twenty different fungi were isolated from the rice seeds. Some of these fungi may be associated with the whole seed, some with the tissues of seed coat, embryo and cotyledon (Popoola & Akueshi 1986).Percent incidence of seed mycota was higher in agar plates as compared to blotter method. This may be attributed to the ability of agar plates to support growth of various fungal species, and due to the higher osmotic potential of PDA medium that showed its substrate efficiency for promoting the colonising rates of both external and internal seed fungal types. The results reveal both qualitative and quantitative variations of the prevalence of the fungal population in terms of percent incidence in two methods of isolation. In the present investigation some of the species or genera are found more frequently, some are noticed occasionally and some of were entirely absent. Seed treatment with sodium hypochlorite is observed to minimise or eliminate some of the externally seed borne (Jaffe 1968). Fungi isolated from unsterilised rice seed are externally seed borne and can probably be considered as either epiphytic or as mere chance contaminants (Suhag & Suryanarayan 1975, Vishnuvat & Shukla 1979, Ram et al. 1997, Prasad & Choudhary 1978, Khare 1996). These externally associated fungi are also known to cause deterioration

**Table 3** Effect of seed borne fungi on seed germination and seedling vigour (soil inoculation method)

| Fungi | % Germination | % inhibition of germination over control | Shoot length (cm) | Root length (cm) | Vigour index |
|---|---|---|---|---|---|
| *Alternaria alternata* | 72.66±2.09 | 7.04 | 7.77±0.54 | 4.83±0.60 | 915.51 |
| *A. oryzae* | 71.96±2.58 | 7.94 | 8.26±0.89 | 4.43±0.29 | 913.17 |
| *Aspergillus flavus* | 73.53±2.58 | 5.93 | 9.12±0.94 | 5.00±0.36 | 1038.24 |
| *A. fumigatus* | 71.26±0.37 | 8.88 | 7.14±0.19 | 4.33±0.20 | 817.35 |
| *A. niger* | 74.00±2.67 | 5.33 | 6.44±0.28 | 3.76±0.14 | 754.80 |
| *Chaetomium herbasum* | 72.46±1.07 | 7.30 | 9.23±0.84 | 3.26±0.17 | 905.02 |
| *Cladosporium herbarum* | 71.96±1.51 | 7.94 | 7.26±0.26 | 3.71±0.22 | 789.40 |
| *Curvularia affinis* | 72.05±1.01 | 7.82 | 7.81±0.68 | 5.72±0.14 | 974.83 |
| *C. lunata* | 68.76±0.62 | 12.03 | 9.18±0.76 | 4.26±0.89 | 924.13 |
| *Fusarium chlamydosporum* | 62.03±1.73 | 20.64 | 6.33±0.88 | 3.40±0.17 | 603.55 |
| *F. moniliforme* | 67.83±1.08 | 13.22 | 6.56±0.29 | 3.68±0.11 | 694.57 |
| *F. oxysporum* | 65.33±0.88 | 16.42 | 8.93±0.54 | 3.65±0.28 | 821.85 |
| *Helminthosporium oryzae* | 66.40±0.80 | 15.05 | 8.55±0.73 | 4.32±0.61 | 854.56 |
| *Mucor hiemalis* | 69.30±0.98 | 11.34 | 7.66±0.13 | 4.55±0.17 | 846.15 |
| *Penicillium citrinum* | 63.93±3.08 | 18.21 | 7.03±0.69 | 3.79±0.20 | 691.72 |
| *P. oxalicum* | 70.83±0.42 | 9.38 | 7.43±0.93 | 4.80±0.46 | 866.25 |
| *Phoma* sp. | 70.33±3.08 | 10.02 | 6.16±0.83 | 6.19±0.44 | 868.57 |
| *Rhizopus nigricans* | 66.60±3.57 | 14.80 | 6.00±0.55 | 7.22±0.33 | 880.45 |
| *R. oryzae* | 54.63±2.68 | 30.11 | 6.33±0.20 | 5.64±0.35 | 653.92 |
| *Trichoderma viride* | 57.66±4.97 | 26.23 | 8.18±0.29 | 4.32±0.66 | 720.75 |
| Control | 78.17±0.88 | | 9.57±0.23 | 6.55±0.20 | |
| SEm | 0.78 | | 0.18 | 0.14 | |
| LSD(.05) | .002 | | .003 | .001 | |

Mean±SEm, n = 3

of seeds by reducing their viability or germinability (Jayaweera et al. 1988). Such a type of deterioration could be due to seed borne fungi which are not only associated externally but also harboured internally in tissues of seed coat, embryo, and cotyledon or through cracks or pits.

The effect of all the isolated fungi as estimated by seed inoculation, soil inoculation and seed submergence method showed significant reduction in the seed germination and seedling vigour. It is likely that fungi which reduced seed germination are pathogenic to host seedlings at the pre-emergence stage (Jayaweera et al. 1988). *Fusarium* inoculated seeds showed maximum inhibition of germination in both the seed inoculation and soil inoculation method. The role of *Fusarium*in inhibiting germination has been reported earlier by Utobo et al. (2011). *Fusarium* is known to invade the seed coat, endosperm and embryo resulting in failure in germination. Further, species of *Fusarium* are known to produce phytotoxins which probably interfere with germination (Ellis 1971, Neergard 1977, Suryanarayana 1978, Kanapathipillai & Hashim 1982). Species of *Aspergillus* and *Rhizopus* were the next important inhibitor of germination encountered in the present study. In seed submergence method *Aspergillus* was found to be most potent inhibitor of germination followed by *Fusarium*. The adverse effect of *Aspergillus* on the germination of cereals has been reported in the recent years (Kanujia & Singh 1975). Species of *Aspergillus* although regarded as surface contaminant were also responsible for production of aflatoxins and also been known to deteriorate rice grains (Imolehin 1987).

Among the isolated fungi *Fusarium moniliforme*, *Rhizopus nigricans* and *Penicillium oxalicum* caused marked reduction in shoot length, whereas *Chaetomium herbasum* and *Fusarium moniliforme* caused marked reduction in root length. *Fusarium moniliforme*, *F. chlamydosporum* and *Aspergillus niger* caused reduction in vigour index.

**Table 4** Effect of seed borne fungi on seed germination and seedling vigour (seed submergence method)

| Fungi | % Germination | % inhibition of germination over control | Shoot length (cm) | Root length (cm) | Vigour index |
|---|---|---|---|---|---|
| *Alternaria alternata* | 51.23±0.53 | 45.85 | 7.77±0.16 | 5.49±0.28 | 679.30 |
| *A. oryzae* | 57.13±0.52 | 39.61 | 6.55±0.29 | 4.44±0.29 | 627.85 |
| *Aspergillus flavus* | 57.23±0.39 | 39.50 | 6.86±0.44 | 3.44±0.28 | 589.46 |
| *A. fumigatus* | 45.83±1.09 | 51.55 | 6.40±0.17 | 3.70±0.19 | 462.88 |
| *A. niger* | 35.90±1.05 | 62.05 | 5.81±0.15 | 3.18±0.15 | 322.74 |
| *Chaetomium herbasum* | 50.62±0.26 | 46.49 | 6.84±0.10 | 3.27±0.14 | 511.76 |
| *Cladosporium herbarum* | 66.30±1.16 | 29.92 | 6.90±0.11 | 3.33±0.19 | 678.24 |
| *Curvularia affinis* | 65.00±0.57 | 31.29 | 7.75±0.14 | 5.51±0.26 | 861.90 |
| *C. lunata* | 54.93±0.88 | 41.94 | 6.79±0.15 | 4.54±0.25 | 622.35 |
| *Fusarium chlamydosporum* | 53.63±1.31 | 43.31 | 6.44±0.25 | 3.50±0.21 | 533.08 |
| *F. moniliforme* | 59.00±0.57 | 37.63 | 6.49±0.29 | 3.00±0.14 | 559.91 |
| *F. oxysporum* | 50.66±1.20 | 46.45 | 8.33±0.22 | 3.36±0.20 | 592.21 |
| *Helminthosporium oryzae* | 58.96±0.98 | 37.68 | 7.41±0.29 | 3.65±0.24 | 652.09 |
| *Mucor hiemalis* | 46.20±1.77 | 51.16 | 6.76±0.11 | 4.13±0.14 | 503.11 |
| *Penicillium citrinum* | 55.13±1.12 | 41.72 | 7.41±0.22 | 3.48±0.27 | 600.36 |
| *P. oxalicum* | 67.46±0.86 | 28.69 | 5.49±0.26 | 4.54±0.26 | 676.62 |
| *Phoma* sp. | 66.02±0.75 | 30.21 | 6.18±0.15 | 5.60±0.22 | 777.71 |
| *Rhizopus nigricans* | 64.02±0.84 | 32.33 | 5.78±0.14 | 6.14±0.59 | 763.11 |
| *R. oryzae* | 36.20±0.65 | 61.73 | 5.80±0.13 | 5.25±0.39 | 400.01 |
| *Trichoderma viride* | 50.27±0.52 | 46.86 | 7.21±0.16 | 3.58±0.30 | 542.41 |
| Control | 94.61±0.46 | | 8.89±0.19 | 6.65±0.17 | |
| SEm | 1.17 | | 0.10 | 0.13 | |
| LSD(.05) | .011 | | .005 | .000 | |

Mean±SEm, n = 3

All the fungi produced toxic metabolites and especially *Aspergillus niger* and *Rhizopus oryzae* produced large quantities as indicated by the reduction of percentage germination of seeds. The result corroborate with the findings of Vidhyasekharan et. al. (1970). High concentrations of filtrate (100 and 75%) were found to have more inhibitory effect on seed germination than lower concentrations (50 and 25%). Reduction in seed germination was found to be directly proportional to the concentration of culture filtrate. These effects on seed germination may be due to inhibitory factor present in the fungal culture filtrate (Tiwari 1993). Reduction in seed germination percentage when seeds were soaked in the filtrates of *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Fusarium oxysporum* and *Penicillium* sp. has been reported earlier (Ibraheem et al. 1987). The role of toxic metabolites of *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. in reducing germination and seedling development has been reported by pervious workers (Umecharuba & Nwachukwu 1997, Nema 1992, Vazquez et al. 1993, Madhosing 1995).

It is evident that the seeds of *Aijung* rice variety carry a heavy load of seed borne fungi which are responsible for loss in seed germination and seedling vigour. It may be stated that it needs a long-term detailed study involving various aspects of seed mycota of *Aijung* rice in order to exploit the potentiality for improvement of quality of seed in Assam.

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