The Pharma Innovation Journal 2024; 13(5): 35-43

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2024; 13(5): 35-43

www.thepharmajournal.com
Received: 21-03-2024
Accepted: 24-04-2024

Tumula Snehankita
M.Sc, Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. MV Totawar
Associate Professor, Plant Pathology Section, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Sarika W More
Assistant Professor, Plant Pathology Section, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Dr. AV Zope
Assistant Professor, Plant Pathology Section, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Corresponding Author:
Tumula Snehankita
M.Sc, Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Studies on seedborne mycoflora of rice and their management

Tumula Snehankita, Dr. MV Totawar, Sarika W More, Dr. AV Zope

Abstract
Six varieties of rice (*Oryza sativa* L.). viz. MTU 1121, MTU 1061, MTU 1064, MTU 1224, Sona masuri and Swarna collected from Andhra Pradesh State Seed Development Corporation Ltd (APSSDC. Ltd.) Tanuku, West Godavari District, Andhra Pradesh were tested for the detection and identification of seed borne mycoflora and to ascertain their management by various methods viz. Standard blotter paper, Agar plate, 2,4-D and Rolled paper towel methods. Agar plate method recorded the highest seed infection (68.13%) among all the methods tested.

A total of 7 seed borne mycoflora including saprophytic and pathogenic were found associated with all the 6 varieties of rice viz. *Fusarium moniliforme*, *Curvularia lunata*, *Trichoconiella padwickii*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp. and *Rhizoctonia* sp.

Different fungicides used to investigate their effect on seed born mycoflora of rice. Seed treatment with fungicides indicated that Carboxin (37.5%) + Thiram (37.5%) DS @ 2 g/kg seed was most effective in reducing the frequency of seed borne mycoflora (96.43 to 100%) and also highest germination % was observed in the seed treatment with Carboxin (37.5%) + Thiram (37.5%) DS (84.33%).

Keywords: Rice varieties, seed borne mycoflora, detection methods

Introduction
Rice is an annual grass that belongs to the family Poaceae and genus *Oryza*. Among the 23 species only 2 species *Oryza sativa* (Asian Rice) and *Oryza glaberimma* (African Rice) have been known for their commercial value and cultivation. Of the two species *Oryza sativa* is the most widely grown species throughout the world. In Asia, based on geographical conditions *Oryza sativa* is divided into 3 sub species *viz*. indica, japonica, and javanica. Rice cultivation extends from 8° to 35° N latitude and from sea level to as high as 3000 m. The suitable conditions for rice growth are high humidity, prolonged sunshine, and assured supply of water. The average temperature required for the crop ranges from 21-37 °C while maximum temperature which the crop can tolerate is 40°-42 °C.

Major seed borne diseases of rice are Blast (*Pyricularia oryzae*), Brown spot (*Bipolaris oryzae*), Foot rot (*Fusarium moniliforme*), Stackburn (*Trichoconiella padwickii*), Sheath rot (*Sarocladium oryzae*), Sheath blight (*Rhizoctonia solani*), Stem rot (*Sclerotium oryzae*), Brown leaf spot (*Drechslera oryzae*), Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), Bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) (Khan *et al.*, 1990; Wahid *et al.*, 2001; Gill *et al.*, 1999) [11, 26, 6].

Rice crop is known to be infected by various disease causing organisms among which many are identified as plant pathogens. It is known to be infected by 56 fungal pathogens, (Ou, 1985) [18] of which 41 are reported to be seed borne (Richardson, 1979). The fungi are one of the major causes of seed quality deterioration as well as decreased crop yields. They are responsible for both pre and post-germination death of grains, affect seedling viability, vigour and thus cause some reduction in germination capability and also variation in plant morphology (Rajput *et al.* 2005) [20]. They cause serious damage to rice production with yield losses ranging from 3 to 100%. In India, yield losses due to brown spot caused by *Bipolaris oryzae* ranged from 50 to 90% (Ghose *et al.*1960) [4] and yield loss due to sheath blight (*Sarocladium oryzae*) were considered higher than 50% in India. (Mohan and Subramanian, 1979) [14]. Hence the experiment was conducted on "Studies on seed borne mycoflora of rice and their management with fungicides".

Materials and Methods

The experiment was conducted at Department of Plant Pathology, PGI, Dr. PDKV Akola, Maharashtra during 2020-2021. Seeds were taken from Andhra Pradesh State Seed Development Corporation Ltd (APSSDC Ltd.) Tanuku, West Godavari District, Andhra Pradesh. The seeds of each variety were collected in envelops, labelled and stored at room temperature for further use.

The detection of fungi from seeds was done by standard technique i.e. Agar plate method, Blotter method, Rolled paper towel and 2-4 D method.

Standard blotter paper method

The collected seed samples of rice were analyzed for the presence of seed borne fungal pathogens by Standard blotter paper method developed by (ISTA1996). As per ISTA rules, four hundred seeds were tested for each variety. In each 90 mm plastic petri plate ten seeds were placed on three layers of blotter paper (Whatman No 1) moistened with sterile distilled water in such a manner that eight seeds are at the outer ring and two seeds at the centre of the plate. The petri plates were incubated at 27±2 °C under 12 h alternating cycles of light and darkness for 7-10 days. Each seed was observed under Stereo microscope in order to record the presence of fungal colonies 7-10 days after incubation based on growth habit. Slides were prepared from the fungal colony and observed under research microscope to identify the fungi based on morphological characters. Most of the associated pathogens were identified by their growth characters following the keys outlined by Mathur and Kongsdal, 2003 [15]. The results were presented as frequency of occurrence for individual pathogen.

Agar plate method

The agar plate method is the most common method for detecting the fungal pathogens (Kameswara Rao &Paula Bramel, 2000) [10] as the PDA medium provides adequate conditions for the development of mycelial growth and sporulation. As per ISTA rules, four hundred seeds were tested for each variety. Approximately 15-20 ml of sterilized PDA medium was poured into each petri plate. After solidification of the medium, 10 seeds (8 seeds at the outer ring and 2 seeds at the centre of the plate) were placed in each petri plate (Ainsworth, 1961) [1]. Then the petri plates were incubated at 27±2 °C under 12 h alternating cycles of light and darkness for 7 days. Slides were made and observed under research microscope to identify the fungi based on colony characters and morphology of sporulation structures within 4-7 days after incubation.

2,4-D method

This method is the modification of the Standard blotter paper method (Limonard, 1966) [13]. Blotter papers were soaked in 25 ppm (2.5 g in 100 l of distilled water) of 2,4-D solution. 2,4-D inhibits the germination of the seeds which makes the detection of pathogens easy because seeds are not displaced and remain on the place where they are plated, unlike the germinating seeds which lie in many planes. Four hundred seeds were tested for each variety. In each 90 mm plastic petri plate ten seeds were placed on three layers of blotter paper (Whatman No 1) moistened with 2,4-D solution in such a manner that eight seeds are at the outer ring and two seeds at the centre of the plate. The petri plates were incubated at 27±2 °C under 12 h alternating cycles of light and darkness for 7-10 days. The seeds were observed for the detection of seed borne fungi after 7-10 days of incubation on the basis of cultural and morphological characters of fungi.

Rolled Paper Towel Method

Germination paper of standard size was used in this method. As per ISTA rules, four hundred seeds were taken from each variety and 50 seeds were placed between a pair of germination paper moistened with sterile distilled water (Warham, 1990) [27]. The germination paper was rolled and the ends were closed by threads. After 7-10 days of incubation period, seeds were observed to record the fungal incidence. Also germination % was recorded.

The frequency of occurrence of seed borne pathogens on the seeds in all the above mentioned methods was calculated by the following formula (Butt *et al.*, 2011) [3].

$$\text{Frequency of occurrence (\%)} = \frac{\text{No. of seeds on which a species occurs}}{\text{Total no. of seeds evaluated (400)}}$$

Effect of fungicides on seed borne mycoflora

Efficacy of different fungicides in eliminating the seed borne mycoflora of rice was tested *in vitro*. The seeds were treated with desired quantity of fungicides and the treated seeds were tested against seed borne fungi by Standard blotter paper method, 2,4-D blotter paper method, Agar plate method and Rolled paper towel method. To assess the effect of fungicides on germination, Rolled paper towel method was used. The procedure and the observations with respect to frequency of occurrence and germination percentage were recorded as per the methodology of each detection method.

The fungicides used in the experiment are given below

Carboxin (37.5%) + Thiram (37.5%) DS Vitavax powder-2 g/kg
Carbendazim 50% WP Bavistin-1 g/kg seed
Thiophanate methyl 70% WP Hexastop-1 g/kg seed
Metalaxyl 35% WS, Targon-2 g/kg seed

Results and Discussion

In the present investigation entitled “studies on seed borne mycoflora of rice and their management” seed borne mycoflora were associated with different varieties of rice and their management by seed treatment with fungicides were studied and the results of the study are presented and discussed as under.

Detection of seed borne mycoflora of rice by various seed health testing methods

Seeds of 6 varieties of rice *viz*. MTU 1121, MTU 1061, MTU 1064, MTU 1224, Sona masuri, Swarna were obtained from Andhra Pradesh State Seed Development Corporation Ltd. (APSSDC Ltd.) Tanuku, West Godavari District, Andhra Pradesh. The seeds were tested for the association of seed borne mycoflora by Standard blotter paper, Agar plate, 2,4-D and Rolled paper towel methods.

Detection of seed borne mycoflora associated with rice by Standard blotter paper method

The data pertaining to estimation of seed mycoflora of rice analysed through standard blotter method is presented in Table 1. A total of 7 seed borne mycoflora belonging to 6

genera including saprophytic and pathogenic were identified. The pathogenic mycoflora recorded were *Fusarium moniliforme*, (11.25 to 16.25%), *Curvularia lunata* (11.00 to 16.00%), *Trichoconiella padwickii* (2.25 to 5.00%), *Rhizoctonia* sp. (0.00 to 1.00%) and the saprophytic mycoflora *Aspergillus flavus* (10.00 to 12.50%), *Aspergillus Niger* (6.25-8.75%), *Rhizopus* sp. (1.50 to 2.25%) were recorded. Among all the varieties tested by Standard blotter paper method, maximum mean frequency of occurrence was recorded by *Fusarium moniliforme* (14.25%) and *Curvulari alunata* (14.25%) followed by *Aspergillus flavus* (11.08%), *Aspergillus Niger* (7.50%), *Trichoconiella padwickii* (3.29%), *Rhizopus* sp. (1.88%), and the minimum was observed with *Rhizoctonia* sp. (0.46%).

The highest frequency of occurrence of *Fusarium moniliforme* (16.25%), *Trichoconiella padwickii* (5.00%), *Aspergillus Niger* (8.75%) and *Rhizoctonia* sp. (1.00%) were recorded in the variety Swarna whereas the highest frequency of occurrence of *Curvularia lunata* (16.00%) were recorded with the variety MTU 1224. Similarly the highest frequency of occurrence of *Aspergillus flavus* (12.50%) was recorded with MTU 1061 and the highest frequency of occurrence of *Rhizopus* sp. (2.25%) was recorded with the variety MTU 1121.

The maximum association of seed borne mycoflora were recorded in the variety Swarna (58.75%) followed by MTU 1064 (54.75%), MTU 1224 (53.00%), Sona masuri (51.50%), MTU 1121 (50.75%) and the lowest association of seed bornemycoflora was recorded in MTU 1061 (47.50%).

The associations of seed borne mycoflora detected in the present study were in agreement with the findings of Ravindra Kumar *et al*, (2014) [21] who examined a total of 30 fungal species belonging to different groups recorded on farmers saved seed of different paddy varieties in Haryana. Out of which *Curvularia lunata* (67%), *Trichoconiella padwickii* (10.51%), *Rhizopus stonlonifer* (8.96%), *Aspergillus flavus* (8.26%) and *Fusarium moniliforme* (7.15%) were recorded as major mycoflora associated with the seeds. Similar results were also found by Kumar *et al.* (2014) [21], Gopala Krishna *et al.* (2010) [5].

Detection of seed borne mycoflora associated with rice by Agar plate method

The data in respect of detected seed borne mycoflora of 6 varieties of rice by Agar plate method was recorded and presented in Table 2 2. A total of 7 seed borne mycoflora belonging to 6 genera including saprophytic and pathogenic were identified. The pathogenic mycoflora recorded were *Fusarium moniliforme*, (15.00 to 20.00%), *Curvularia lunata* (14.50 to 19.00%), *Trichoconiella padwickii* (2.50 to 7.50%), *Rhizoctonia* sp. (0.00 to 0.50%) and the saprophytic mycoflora recorded were *Aspergillus flavus* (14.00 to 18.00%), *Aspergillus niger* (7.50 to 12.00%), *Rhizopus* sp. (2.50 to 4.00%).

Out of 6 varieties of rice tested by Agar plate method, maximum mean frequency of occurrence was recorded by *Fusarium moniliforme* (17.71%) followed by *Curvularia lunata* (17.17%), *Aspergillus flavus* (16.08%) and *Aspergillus Niger* (9.17%) whereas it was recorded minimum with *Rhizoctonia* sp. (0.42%) followed by *Rhizopus* sp. (3.00%) and *Trichoconiella padwickii* (4.58%).

The maximum frequency of occurrence of seed borne mycoflora on seeds of rice was recorded by *Fusarium moniliforme* (20.00%), *Curvularia lunata* (19.00%), *Trichoconiella* padwickii (7.50%), *Aspergillus flavus* (18.00%) in variety Swarna whereas the highest frequency of occurrence of *Aspergillus Niger* (12.00%) was recorded in MTU 1224 variety. Similarly the highest frequency of occurrence of *Rhizopus* sp. (4.00%) was recorded in MTU 1064 variety and the highest frequency of occurrence of *Rhizoctonia* sp. (0.50%) was recorded in MTU 1121 and Swarna variety of the total 6 varieties of rice, maximum total frequency of mycoflora 77.50% was recorded in the variety Swarna followed by MTU 1224 (69.00%),MTU 1064 (67.50%), MTU 1121 (67.50%) Sona masuri (64.75%), whereas the lowest of total mycoflora was recorded with the variety MTU 1061 (60.75%).

The results of present investigation are in agreement with the findings of Habib *et al.* (2012) [7] who tested fifteen varieties of rice for the detection of seed borne mycoflora by agar plate method. The mycoflora recorded were *Trichoconiella padwickii* (16.66-20.83%), *Aspergillus Niger* (16.65-20.85%), *Rhizopus* sp *(*13.83-16.65%), *Fusarium moniliforme* (12.5-18.05%), *Aspergillus flavus* (9.73-19.43%), *Curvularia* sp (6.95-11.11%). Higher infestation was recorded in agar plate method as compared to blotter paper method. Similar results were recorded by Naveen Kumar *et al.* (2016) [16] who tested health status of 7 cultivars of rice by blotter paper and agar plate method. A total of 9 pathogens were identified. Among them the most predominant one was *Helminthosporium oryzae* which was associated with 62.36 per cent seed samples, followed by *Alternaria padwickii* (36.63%), *Sarocladium oryzae* (30.63%), *Fusarium moniliforme* (28.63%) and *Curvularia lunata* (26.00%).

Detection of seed borne mycoflora associated with rice by 2,4-D method

The data presented in Table 3 showed the association of seed borne mycoflora of different varieties of rice. A total of 7 seed borne mycoflora belonging to 6 genera including saprophytic and pathogenic were identified (Plate 3). The pathogenic mycoflora recorded were *Fusarium moniliforme*, (9.00 to 13.00%), *Curvularia lunata* (8.00 to 12.75%), *Trichoconiella padwickii* (0.00 to 3.50%), *Rhizoctonia* sp. (0.00 to 0.75%) and the saprophytic mycoflora recorded were *Aspergillus flavus* (8.75 to 10.50%), *Aspergillus Niger* (3.00 to 6.25%), *Rhizopus* sp. (0.00 to 2.00%). Among all the varieties tested by 2,4-D method maximum mean frequency of occurrence was recorded by *Fusarium moniliforme* (11.25%) followed by *Curvularia lunata* (11.08%), *Aspergillus flavus* (9.46%), *Aspergillus Niger* (5.25%), *Trichoconiella padwickii* (1.67%), *Rhizopus* sp. (1.17%), and the minimum was observed with *Rhizoctonia* sp. (0.17%).

The maximum frequency of occurrence of *Fusarium moniliforme* (13.00%), *Trichoconiella padwickii* (3.50%) and *Rhizoctonia* sp. (0.75%) were recorded in Swarna variety whereas the highest frequency of occurrence of *Curvularia lunata* (12.75%) was recorded in MTU 1064 variety. Similarly the maximum frequency of occurrence of *Aspergillus flavus* (10.50%) was recorded in Sona masuri variety whereas the highest frequency of occurrence of *Aspergillus Niger* (6.25%) was observed in MTU 1061 and MTU 1064 varieties and the highest frequency of occurrence of *Rhizopus* sp. (2.00%) was recorded with variety MTU 1121 Among the 6 varieties of rice taken the highest association of total seed borne mycoflora (44.00%) was recorded in the

variety Swarna followed by MTU 1064 (43.00%), MTU 1224 (41.75%), Sona masuri (40.50%), MTU 1121 (37.00%) and the lowest association of total mycoflora was recorded in MTU 1061 (34.00%). The results obtained are in agreement with the findings of Kawrau LP who studied different detection methods *viz*. blotter, agar plate, soaking of blotter in 0.1% solution of 2,4-D, etc. and reported that soaking in 2,4-D reduce infection percentages

Detection of seed borne mycoflora associated with rice by Rolled paper towel method

The results in respect of frequency of occurrence of seed borne mycoflora of different varieties of rice by Rolled paper towel method were obtained and presented in Table 4. A total of 7 seed borne mycoflora belonging to 6 genera including saprophytic and pathogenic were identified. The pathogenic mycoflora recorded were *Fusarium moniliforme*, (6.50 to 8.00%), *Curvularia lunata* (0.00 to 7.00%), *Rhizoctonia* sp. (0.00 to 0.25%), *Trichoconiella padwickii* was recorded to be absent and the saprophytic mycoflora recorded were *Aspergillus flavus* (5.00 to 7.50%), *Aspergillus Niger* (0.00 to 4.00%), *Rhizopus* sp. (0.00 to 2.00%).

Out of all the varieties tested by Rolled paper towel method, maximum mean frequency of occurrence was recorded by *Fusarium moniliforme* (7.00%) followed by *Aspergillus flavus* (6.08%), *Curvularia lunata* (3.58%), *Aspergillus Niger* (1.83%), *Rhizopus* sp. (1.17%), and the minimum was observed with *Rhizoctonia* sp. (0.04%). *Trichoconiella-padwickii* was found to be absent in all the varieties.

The highest association of seed borne mycoflora on the seeds of rice was recorded by *Fusarium moniliforme* (8.00%), *Curvularia lunata* (7.00%) in the variety Swarna whereas highest frequency of occurrence of *Aspergillus flavus* (7.50%) was recorded in MTU 1064 variety. Similarly the highest frequency of occurrence of *Aspergillus Niger* (4.00%) and *Rhizopus* sp. (2.00%) was recorded in the variety MTU 1121 whereas the highest frequency of occurrence of *Rhizoctonia* sp. (0.25%) was recorded in the variety Sona masuri. *Trichoconiella padwickii* was recorded to be absent

The highest total mycoflora27.00% was recorded in the variety Swarna followed by MTU 1224 (22.00%), MTU 1121 (18.00%), MTU 1064 (17.50%), Sona masuri (17.25%) and the lowest frequency of occurrence of total mycoflora was recorded in MTU 1061 (16.50%).

Highest germination percentage was recorded in the variety MTU 1061 (82.00%) followed by MTU 1064 (80.00%), Sonamasuri (80.00%), MTU 1121 (78.00%), MTU 1224 (76.00%) and the lowest was recorded in the variety Swarna (75.00%). This clearly indicated that the varieties showing maximum association of seed borne mycoflora recorded lowest seed germination percentage such as Swarna (75.00%) and MTU 1224 (76.00%) whereas, the varieties showing minimum association of seed borne mycoflora recorded maximum seed germination percentage such as MTU 1061 (82.00%) and Sona masuri (80.00%). This implies that the seed borne mycoflora associated internally and externally with the seed effect the germination of seed. The results obtained are in agreement with the findings of Ora *et al.* (2011) [17] who assessed the seeds of cultivated hybrid rice varieties for seed borne pathogens and identified a total of 12 pathogens by blotter paper, agar plate and paper towel methods. In case of rolled paper towel method, the highest seed germination (96.38%) was observed on Hira-1. The lowest pathogenic incidence recording varieties showed lowest rotten seed, dead seed and highest seed germination. Similar findings were also recorded by Ravindra kumar *et al.* (2014) [21], Pandey S (2015) [19].

Evaluation of different seed health testing methods in detecting the seed borne mycoflora of rice

In the present investigation, frequency of occurrence of seed borne mycoflora was detected from 6 varieties of rice by Standard blotter paper, Agar plate, 2,4-D and Rolled paper towel methods (Plate 4). From the data presented in Table 5 and Fig.5, it was found that the maximum frequency of seed borne mycoflora was detected by Agar plate method (68.13%) followed by Standard blotter paper method (52.71%) whereas the lowest frequency of seed borne mycoflora was detected by Rolled paper towel method (19.71%) followed by 2,4-D method (40.04%).

Agar plate method was found to be superior over the other methods in the detection of seed borne mycoflora. The results are in correlation with the findings of Habib *et al.* (2012) [7] who worked on 15 rice varieties for the detection of fungi by agar plate and blotter paper methods and found out that higher infestation was recorded in agar plate method as compared to Standard blotter paper method. The mycoflora recorded in blotter paper method were *Helminthosporium spp.*(13.88-20.85%), *Alternaria alternate* (12.50-19.45%), *Aspergillus Niger*(12.50-16.66%), *Fusarium moniliforme* (9.73-16.66%), *Rhizopus spp.*(8.33-18.35%), *Aspergillus flavus* (4.15-12.50%), *Curvularia spp.*(0.00-8.35%) and the fungi identified in agar plate method were *Helminthosporium spp.*(2.23-31.95%) *Alternaria alternate* (16.66-20.83%), *Aspergillus Niger* (16.65-20.85%), *Rhizopus spp (*13.83-16.65%), *Fusarium moniliforme* (12.5-18.05%), *Aspergillus flavus* (9.73-19.43%), *Curvularia spp* (6.95-11.11%). Similar results were also recorded by Tanmay Gosh *et al.* (2018) [23].

From the data in Table 5 it was also found that the maximum frequency of occurrence of Fusarium moniliforme (17.71%), Curvularia lunata (17.17%), *Trichoconiella padwickii* (4.58%), *Aspergillus flavus* (16.08%), *Aspergillus Niger* (9.17%), *Rhizopus* sp. (3%) were recorded by Agar plate method whereas maximum frequency of occurrence of *Rhizoctonia* sp. (0.69%) was recorded in Standard blotter paper method.

Management of seed borne mycoflora of rice

Effect of fungicide seed treatment Carboxin (37.5%) + Thiram (37.5%) DS, Carbendazim 50% WP, Thiophanate methyl 70% WP and Metalaxyl 35% WS) on seed borne mycoflora of rice was tested by Standard blotter paper, Agar plate, 2,4-D and Rolled paper towel methods. Effect of fungicide seed treatment on germination percentage of rice was tested by Rolled paper towel method.

Evaluation of fungicides by Blotter paper method

Efficacy of different fungicides was tested against seed borne mycoflora of rice by Standard blotter paper method and the results were given in Table 6 and shown in Plate 8.All the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control.

Maximum reduction in seed borne mycoflora was observed in the seed treatment with Carboxin + Thiram (96.43%) followed by Carbendazim (93.76%), Thiophanate methyl (88.36%) whereas the lowest reduction of seed borne

mycoflora was noticed in the seed treatment with Metalaxyl (84.62%) in all varieties tested.

These results are in accordance with the findings of Naher *et al.* (2016) [28] who conducted experiment on three rice varieties using dry seed inspection and blotter methods. Three seed treating fungicides *viz*. vitavax 200 (0.25%), thieved (0.25%) and cupravit (0.25%) were used. Vitavax 200 was found the most effective against the seed borne pathogens of rice among all the fungicides tested. Similar observations were also recorded in present investigation that Carboxin 37.5% + Thiram 37.5% DS was found effective in the management of seed borne mycoflora of rice.

Bhuiyan *et al.* (2013) [2] worked on forty rice seed samples. Seed treating fungicides *viz*. Vitavax-200, Bavistin 50WP and Captan were tested to control seed borne mycoflora. Seed treatment with Vitavax-200 @ 0.3% of seed weight eliminated all seed borne mycoflora and increased germination by 25.70% over control.

Evaluation of fungicides by Agar plate method

The influence of 4 different fungicides were screened against seed borne mycoflora of rice by Agar plate method and the data was presented in Table 7 and shown in Plate 9. All the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control. Maximum inhibition of seed borne mycoflora was observed in the seed treatment with Carboxin + Thiram (98.77%) followed by Carbendazim (97.58%), Thiophanate methyl (95.35%) whereas the lowest inhibition of seed borne mycoflora was noticed in the seed treatment with Metalaxyl (94.58%). Similar results were also obtained by Waqar Islam and Manzoor Ahmed (2017) [29].

Evaluation of fungicides by 2,4-D method

The data in respect to the efficacy of different fungicides tested against the seed borne mycoflora of rice by 2,4-D method was presented in Table 8. All the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control. The maximum reduction of seed borne mycoflora was observed in the seed treatment with Carboxin + Thiram (98.81%) followed by Carbendazim (96.74%), Thiophanate methyl (95.28%) whereas the lowest was noticed in the seed treatment with Metalaxyl (93.07%). While literature hunted no evidence has been found related with evaluation of seed dressing fungicides by 2,4-D method.

Evaluation of fungicides by Rolled paper towel method

The influence of fungicide seed treatment on the seed borne mycoflora was recorded by Rolled paper towel method and the data was presented in Table 9. All the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control. The highest per cent reduction of seed borne mycoflora was observed in the seed treatment with Carboxin + Thiram (100%) followed by Carbendazim (99.31%), Thiophanate methyl (97.59%) whereas the lowest per cent reduction of seed borne mycoflora was noticed in the seed treatment with Metalaxyl (95.86%).

Effect of fungicide seed treatment on germination of rice was recorded by Rolled paper towel method and the results were given in Table 10. Highest germination percentage was observed in the seed treatment with Carboxin + Thiram (84.33%) followed by Carbendazim (83.63%), Thiophanate methyl (83.33%) and the lowest germination per cent was recorded in Metalaxyl (83.25%). The results obtained are in correlation with the findings of Huynh Van Nghiep and Ashok Gaur (2005) who studied 8 seed lots to evaluate the efficacy of chemical seed treatment against seed borne fungi. The germ inability of seeds were determined by using rolled paper towel method and the results showed that seeds treated with Vitavax, Thiram and Mancozeb maintained germination above (≥ 80%) after 6 months of storage.

From the data it was found out that among all the fungicides tested Carboxin 37.5% + Thiram 37.5% DS was found to be effective in the reduction of seed borne mycoflora (96.43 to 100%) followed by Carbendazim50% WP (93.76 to 99.31%) and the least reduction of seed borne fungi was recorded in the seed treatment with Metalaxyl35% WS (84.62 to 95.86%) followed by Thiophanate methyl 70% WP (88.36 to 97.59%).Carboxin + Thiram was more effective due to the synergistic effect of systemic fungicide (Carboxin) and contact fungicide (Thiram). However, all the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control.

Table 1: Frequency of occurrence of seed borne mycoflora associated with rice by Standard blotter paper method

Seed borne mycoflora	Frequency of occurrence of seed borne mycoflora (%) on different varieties of rice						Mean
	MTU 1121	MTU 1061	MTU 1064	MTU 1224	Sona masuri	Swarna	
Fusarium moniliforme	11.25	12.75	15.50	16.00	13.75	16.25	14.25
Curvularia lunata	14.00	11.00	13.75	16.00	15.00	15.75	14.25
Trichoconiella padwickii	3.75	3.00	3.25	2.25	2.50	5.00	3.29
Aspergillus flavus	11.25	12.50	11.75	10.00	11.00	10.00	11.08
Aspergillus Niger	7.50	6.75	8.25	6.25	7.50	8.75	7.50
Rhizopus sp.	2.25	1.50	2.00	1.75	1.75	2.00	1.88
Rhizoctonia sp.	0.75	0.00	0.25	0.75	0.00	1.00	0.46
Frequency of mycoflora	50.75	47.50	54.75	53.00	51.50	58.75	52.71

Table 2: Frequency of occurrence of seed borne mycoflora associated with rice by Agar plate method

Seed borne mycoflora	Frequency of occurrence of seed borne mycoflora (%) on different varieties of rice						Mean
	MTU 1121	MTU 1061	MTU 1064	MTU 1224	Sona masuri	Swarna	
Fusarium moniliforme	16.25	15.00	18.75	17.50	18.75	20.00	17.71
Curvularia lunata	17.50	14.50	16.25	18.75	17.00	19.00	17.17
Trichoconiella padwickii	3.75	6.25	5.00	2.50	2.50	7.50	4.58
Aspergillus flavus	17.50	14.00	16.25	15.75	15.00	18.00	16.08
Aspergillus Niger	8.75	8.00	7.50	12.00	8.75	10.00	9.17
Rhizopus sp.	3.50	2.75	4.00	2.50	2.75	2.50	3.00
Rhizoctonia sp.	0.50	0.25	0.00	0.00	0.00	0.50	0.42
Frequency of mycoflora	67.75	60.75	67.75	69.00	64.75	77.50	67.92

Table 3: Frequency of occurrence of seed borne mycoflora associated with rice by 2,4-D method

Seed borne mycoflora	Frequency of occurrence of seed borne mycoflora (%) on different varieties of rice						Mean
	MTU 1121	MTU 1061	MTU 1064	MTU 1224	Sona masuri	Swarna	
Fusarium moniliforme	9.00	10.00	11.75	12.50	11.25	13.00	11.25
Curvularia lunata	10.00	8.00	12.75	11.25	12.50	12.00	11.08
Trichoconiella padwickii	0.00	0.00	2.25	2.25	2.00	3.50	1.67
Aspergillus flavus	10.00	8.75	10.00	8.75	10.50	8.75	9.46
Aspergillus niger	6.00	6.25	6.25	5.00	3.00	5.00	5.25
Rhizopus sp.	2.00	1.00	0.00	1.75	1.25	1.00	1.17
Rhizoctonia sp.	0.00	0.00	0.00	0.25	0.00	0.75	0.17
Frequency of mycoflora	37.00	34.00	43.00	41.75	40.50	44.00	40.04

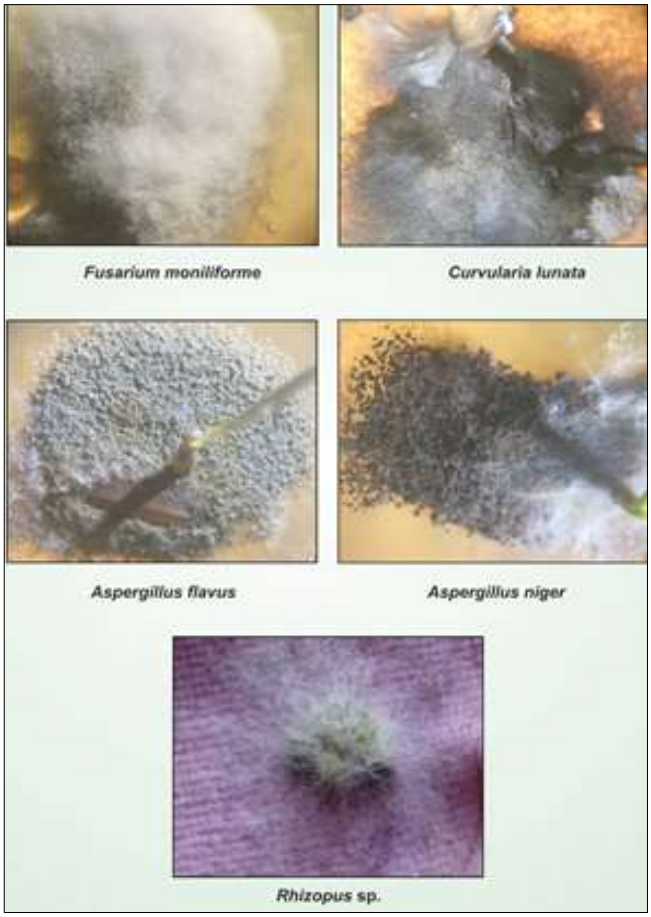
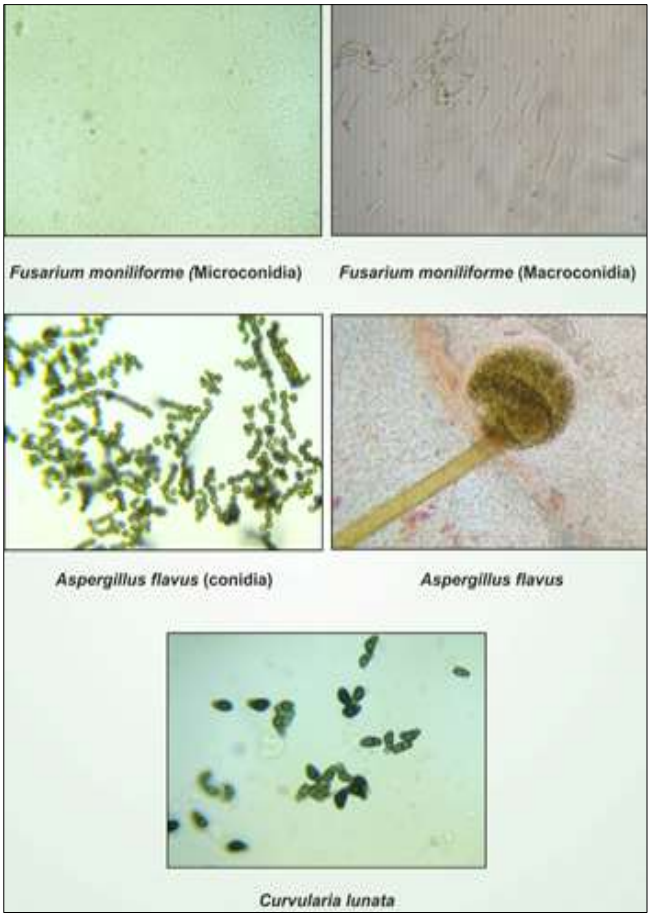


Plate 5: Stereoscopic photographs of different seed borne mycoflora of rice



Plato 6: Microphotographs of different seed borne mycoflora of rice

Table 4: Frequency of occurrence of seed borne mycoflora associated with rice by Rolled paper towel method

Seed borne mycoflora	Frequency of occurrence of seed borne mycoflora (%) on different varieties of rice						Mean
	MTU 1121	MTU 1061	MTU 1064	MTU 1224	Sona masuri	Swarna	
Fusarium moniforme	7.00	6.50	6.50	7.00	7.00	8.00	7.00
Curvularia lunata	0.00	4.00	0.00	5.50	5.00	7.00	3.58
Trichoconiella padwickii	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus flavus	5.00	6.00	7.50	6.00	5.00	7.00	6.08
Aspergillus Niger	4.00	0.00	2.00	2.00	0.00	3.00	1.83
Rhizopus sp.	2.00	0.00	1.50	1.50	0.00	2.00	1.17
Rhizoctonia sp.	0.00	0.00	0.00	0.00	0.25	0.00	0.04
Frequency of mycoflora	18.00	16.50	17.50	22.00	17.25	27.00	19.71
Germination %	78.00	82.00	80.00	76.00	80.00	75.00	78.50

Table 5: Evaluation of different seed health testing methods in detecting the seed borne mycoflora of rice

Seed borne mycoflora	Frequency of seed borne mycoflora (%)			
	Standard blotter paper method	Agar plate method	2,4-D method	Rolled paper towel method
Fusarium moniliforme	14.25	17.71	11.25	7.00
Curvularia lunata	14.25	17.17	11.08	3.58
Trichoconiella padwickii	3.29	4.58	1.67	0.00
Aspergillus flavus	11.08	16.08	9.46	6.08
Aspergillus Niger	7.50	9.17	5.25	1.83
Rhizopus sp.	1.88	3.00	1.17	1.17
Rhizoctonia sp.	0.46	0.42	0.17	0.04
Frequency of mycoflora	52.71	68.13	40.04	19.71

Table 6: Evaluation of different fungicides against seed borne mycoflora of rice by Standard blotter paper method

Treatments	Frequency of occurrence of mycoflora on seed and their per cent inhibition by different fungicides (Mean value of six varieties of rice)														Frequency of mycoflora (Mean)	Reduction of mycoflora over control (%)
	Fusarium moniliforme		*Curvularia lunata*		*Trichoconiella padwickii*		*Aspergillus flavus*		*Aspergillus Niger*		*Rhizopus* sp.		*Rhizoctonia* sp.			
	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I		
Carboxin + Thiram	0.10	99.26	0.43	96.93	0.33	90.79	0.39	96.18	0.38	94.58	0.18	87.98	0.00	100.00	0.26	96.43
Carbendazim	0.73	94.62	0.63	95.48	0.05	98.60	0.79	92.27	0.86	87.83	0.10	93.44	0.02	98.20	0.45	93.76
Thiophanate methyl	1.21	91.03	1.06	92.35	0.87	75.72	1.33	86.98	1.30	81.60	0.05	96.72	0.10	90.98	0.85	88.36
Metalaxyl	1.85	86.27	1.83	86.82	0.63	82.42	1.77	82.75	1.68	76.23	0.02	98.69	0.05	95.49	1.12	84.62
Control	13.48		13.84		3.58		10.24		7.07		1.53		1.11		7.26	

*F = Frequency of occurrence
P.I = Per cent inhibition over control

Table 7: Evaluation of different fungicides against seed borne mycoflora of rice by Agar plate method

Treatments	Frequency of occurrence of mycoflora on seed and their per cent inhibition by different fungicides (Mean value of six varieties of rice)														Frequency of mycoflora (Mean)	Reduction of mycoflora over control (%)
	Fusarium moniliforme		*Curvularia lunata*		*Trichoconiella padwickii*		*Aspergillus flavus*		*Aspergillus Niger*		*Rhizopus* sp.		*Rhizoctonia* sp.			
	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I		
Carboxin + Thiram	0.05	99.71	0.16	99.05	0.15	96.76	0.12	99.25	0.11	98.80	0.09	96.95	0.15	86.05	0.12	98.77
Carbendazim	0.25	98.56	0.34	97.95	0.18	96.22	0.24	98.45	0.23	97.50	0.22	92.80	0.18	83.72	0.23	97.58
Thiophanate methyl	0.48	97.21	0.42	97.49	0.43	90.65	0.75	95.17	0.40	95.56	0.43	85.87	0.22	79.84	0.45	95.35
Metalaxyl	0.72	95.82	0.70	95.79	0.43	90.83	0.64	95.87	0.42	95.37	0.48	84.21	0.22	79.84	0.52	94.58
Control	17.32		16.63		4.63		15.54		9.01		3.01		1.08		9.60	

*F = Frequency of occurrence
P.I = Per cent inhibition over control

Table 8: Evaluation of different fungicides against seed borne mycoflora of rice by 2,4-D method

Treatments	Frequency of occurrence of mycoflora on seed and their per cent inhibition by different fungicides (Mean value of six varieties of rice)														Frequency of mycoflora (Mean)	Reduction of mycofloraover control (%)
	Fusarium moniliforme		*Curvularia lunata*		*Trichoconiella padwickii*		*Aspergillus flavus*		*Aspergillus Niger*		*Rhizopus* sp.		*Rhizoctonia* sp.			
	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I		
Carboxin + Thiram	0.03	99.76	0.12	98.91	0.05	97.74	0.08	99.07	0.03	99.32	0.12	93.17	0.08	88.10	0.07	98.81
Carbendazim	0.28	98.00	0.11	98.99	0.18	91.70	0.16	98.24	0.33	93.23	0.18	89.76	0.18	75.00	0.20	96.74
Thiophanate methyl	0.40	97.18	0.33	96.88	0.30	86.42	0.40	95.56	0.23	95.43	0.22	87.32	0.18	75.00	0.29	95.28
Metalaxyl	0.42	97.06	0.46	95.72	0.52	76.60	0.61	93.25	0.62	87.48	0.28	83.90	0.13	81.43	0.43	93.07
Control	14.18		10.70		2.21		9.01		4.93		1.71		0.70		6.20	

*F = Frequency of occurrence
P.I = Per cent inhibition over control

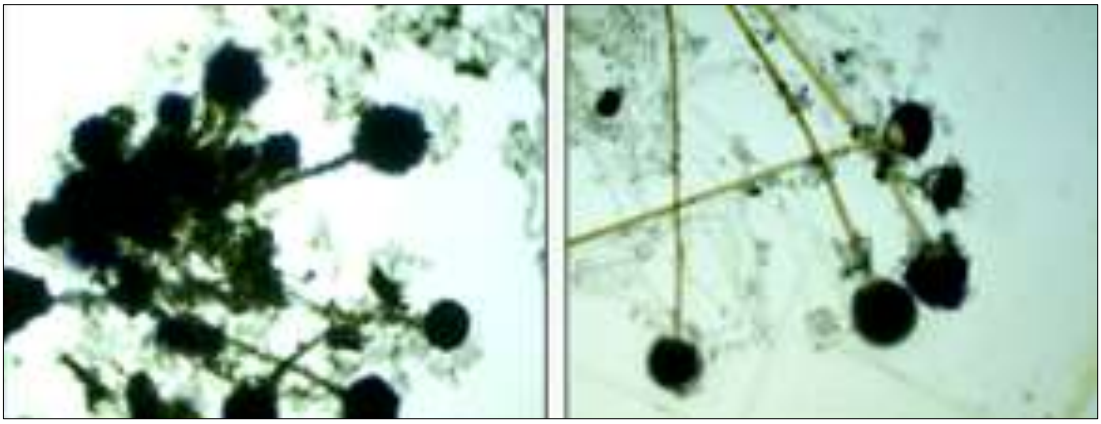
Table 9: Evaluation of different fungicides against seed borne mycoflora of rice by Rolled paper towel method

Treatments	Frequency of occurrence of mycoflora on seed and their percent inhibition by different fungicides (Mean value of six varieties of rice)														Frequency of mycoflora (Mean)	Reduction of mycoflora over control (%)
	Fusarium moniliforme		*Curvularia lunata*		*Trichoconiella padwickii*		*Aspergillus flavus*		*Aspergillus Niger*		*Rhizopus* sp.		*Rhizoctonia* sp.			
	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I	F	P.I		
Carboxin + thiram	0	100.00	0.01	99.83	0	100.00	0.02	99.72	0	100.00	0	100.00	0	100.00	0.00	100.00
Carbendazim	0.04	99.33	0.04	99.16	0.00	100.00	0.02	99.72	0.02	99.17	0.03	97.39	0.02	92.86	0.02	99.31
Thiophanate methyl	0.12	97.88	0.13	97.47	0.06	80.00	0.08	98.62	0.05	97.41	0.07	94.77	0.02	92.86	0.07	97.59
Metalaxyl	0.18	96.67	0.13	97.47	0.12	60.00	0.18	96.96	0.10	95.02	0.10	92.16	0.04	82.14	0.12	95.86
Control	5.51		4.95		0.29		6.03		2.01		1.28		0.23		2.90	

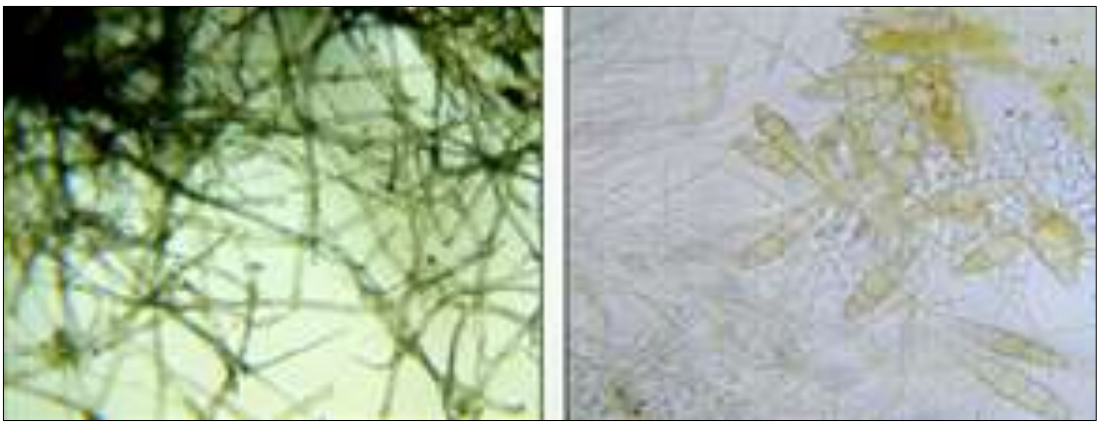
*F = Frequency of occurrence
P.I = Per cent inhibition over control

Table 10: Effect of different fungicides on germination of rice

Treatments	Germination (%)						Mean
	MTU1121	MTU1061	MTU1064	MTU1224	Sonamasuri	Swarna	
Carboxin + Thiram	85.00	84.00	85.00	84.00	83.00	84.00	84.33
Carbendazim	83.75	85.00	84.00	83.50	83.50	82.00	83.63
Thiophanate methyl	85.00	83.00	84.50	81.50	83.00	83.00	83.33
Metalaxyl	82.50	80.00	84.50	85.00	83.50	84.00	83.25
Control	79.00	81.00	80.00	78.00	80.00	75.00	78.83



Aspergillus Niger *Rhizopus* sp



Rhizoctonia sp *Trichoconiella padwickii*

Plate 7: Microphotographs of different seed borne mucoflora of rice

Conclusions

All the six varieties of rice were found to be associated with seed borne mycoflora. Seven seed borne mycoflora belonging to six genera were identified including pathogenic and saprophytic *viz*. *Fusarium moniliforme*, *Curvularia lunata*, *Trichoconiella padwickii*, *Aspergillus flavus*, *Aspergillus Niger*, *Rhizopus* sp. *Rhizoctonia* sp. Seed borne mycoflora *viz*. *Fusarium moniliforme*, *Aspergillus flavus*, *Curvularia lunata* and *Aspergillus Niger* exhibited highest infection frequency on rice seeds in all varieties tested. Agar plate method was found to be effective in the detection of seed borne mycoflora of rice whereas rolled paper towel method was least effective in detecting the seed borne mycoflora associated with rice. All the treatments were found effective to inhibit the seed borne mycoflora of all the varieties over control. In the seed treatment with fungicides Carboxin 37.5% + Thiram 37.5%

DS proved effective in reducing the seed borne infection and increasing the germination percentage of rice.

References

1. Ainsworth GC. Dictionary of fungi. Common Wealth Mycological Institute, Kew Burrey, England; c1961. p. 547.
2. Bhuiyan MR, Khan MAI, Hoque M, Nessa B, Rafii MY, Latif MA. Eco-friendly management of Seed Borne Fungi for Sustainable Crop Production. Life Sci J. 2013;10(4):1640-1650.
3. Butt AR, Yaseen SI, Javaid A. Seed Borne Mycoflora of Stored Rice Grains and its Chemical Control. J Anim Pl Sci. 2011;21(2):193-196.
4. Ghose RLM, Ghatge MB, Subramanian V. Rice in India (revised edn). New Delhi: ICAR; c1960. p. 474.
5. Gopalakrishnan C, Kamalakannan, Valluvaparidasan V. Survey of Seed Borne Fungi Associated with Rice Seeds in Tamil Nadu, India. Libyan Agriculture Research CenterJournal International. 2010;1(5):307-309.
6. Gill MA, Wahid A, Javed MS, Khan TZ. Major diseases of rice crop in the Punjab and their management strategies. In: Proc. 2nd Natl. Conf. Pl. Pathol., Univ. Agri. Faisalabad; c1999. p. 27-29.
7. Habib A, Javed N, Sahi ST, Waheed M. Detection of Seed Borne Mycoflora of Different Course and Fine Rice Varieties and Their Management Through Seed Treatments. Pak J Phytopathol. 2012;24(2):133-136.
8. Huynh Van Nghiep, Ashok Gaur. Efficacy of Seed Treatment in Improving Seed Quality in Rice (*Oryza sativa* L.). Omonrice. 2005;13:42-51.
9. ISTA. International Rules for Seed Testing. Seed Sci Technol. 1996;24:39-42.
10. Kameswara Rao N, Paula Bramel J (Eds.). Manual of Genebank Operations and Procedures. Technical Manual no.6. Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics; c2000.
11. Khan SA, Anwar SA, Bhutta AB. Studies on seed borne fungi, bacteria, and nematodes of rice in the Punjab. Pak J Sci Ind Res. 1990;33(11):489-492.
12. Kumar R, Gupta A, Mahechwari VK, Atwal SS. Health status of farmers' saved seed of various paddy varieties in Haryana, India. Pl Patho J. 2014;13(3):186-192.
13. Limonard T. A modified blotter test for seed health. Government seed testing station, Wageningen; 1966.
14. Mohan R, Subramanian CL. Yield losses due to sheath rot disease caused by Acrocylindrium oryzae Sawada. Madras Agricultural Journal. 1979;66:195.
15. Mathur SB, Kongsdal O. Common Seed Health Testing Method for Detecting Fungi. First edition. International Seed Testing Association, Bassersdorf, Switzerland; c2003. p. 425.
16. Naveenkumar R, Muthkumar A, Mohanapriya R. Survey of Seed borne fungi associated with seeds of rice in Tamil Nadu. Oryza. 2016;53(1):106-110.
17. Ora N, Faruq AN, Islam MJ, Akhtar, Rahman MM. Detection and Identification of Seed Borne Pathogens from Some Cultivated Hybrid Rice Varieties in Bangladesh. Middle East J Sci Res. 2011;10(4):482-488.
18. Ou SH. Rice diseases. CAB International Mycological Institute, Kew, Surrey, U.K.; c1985. p. 380.
19. Pandey S. Seed associated mycoflora of rice from Kymore region, Central India. Indian J Trop Biodiv. 2015;23(2):167-173.
20. Rajput MA, Pathan MA, Lodhi AM, Shah GS, Khanzada KA. Studies on seed-borne fungi of wheat in Sindh Province and their effect on seed germination. Pak J Bot. 2005;37(1):181-185.
21. Ravindra Kumar, Anuja Gupta, Maheswari VK, Atwal SS. Health Status of Farmers' Saved Seed of Various Paddy Varieties in Haryana, India. Pl Patho J. 2014;13(3):186-192.
22. Richardson MJ. An annotated list of seed-borne diseases. 3rd Ed. Commonwealth Agricultural Bureaux, U.K.; c1979.
23. Tanmay Ghosh, Biswas MK, Chiranjb Guin, Pradipta Roy, Kaustav Aikat. A Review on Seed Borne Mycoflora Associated with Different Cereal Crop Seeds and their Management. Plant Cell Biotechnology and Molecular Biology. 2018;19(3&4):107-117.
24. Thobunluepop P. Characterization of a botanical fungicide from Thai origin and its efficiency in Rice production. Cuvillier Verlag, Gottingen, Thailand; 2008.
25. Uma V, Wesely EG. Seed borne fungi of rice from South Tamil Nadu. J Acad Indus Res. 2013;1(10).
26. Wahid A, Javed MS, Indrees M, Gill MA. Association of F. solani to rice seeds in the Punjab, Pakistan. Abstracts Published in 3rd Natl Conf Pl Path. 2001;1-3 at NARC, Islamabad. p. 41.
27. Warham EJ. Effect of *Tilletia indica* infection on viability, germination and vigour of wheat seed. Pl Dis. 1990;74:130-135.
28. Naher UA, Panhwar QA, Othman R, Ismail MR, Berahim Z. Biofertilizer as a supplement of chemical fertilizer for yield maximization of rice. Journal of Agriculture Food and Development. 2016;2(0):16-22.
29. Islam W, Ahmed M. Identification of different fungi associated with grain discoloration complex disease of rice and management of infested grains through fungicides. Int. J. Sci. Res. Agric. Sci. 2017;4(2):30-35.