Detection and Identification of Major SeedMycoflora Associated with Rice (*Oryza sativa* L.) Cultivars grown in Kashmir

Abstract

Worldwide, rice is a significant crop for food production. The main requirement for increased yield and production is high-quality seed. While being exported, seeds and grains have the risk of being associated with pathogenic as well as non-pathogenic microflora. As a result, they are frequently inspected in accordance with phytosanitary laws to stop diseases from spreading into new areas and also to have an impact on the quality of the seeds. Fungal infections that infect rice seeds are regulated by phytosanitary laws and can be identified using several techniques for checking seed health. Therefore, in accordance with the recommendations of International Seed Testing Association (ISTA), stored seeds of seeds of four rice varieties (SR-2, SR-3, SR-4, and SR-5) were studied to investigate the occurrence of seed-associated fungal mycoflora using the blotter paper and agar plate method. In both blotter and agar tests, four fungal species namely as *Fusarium oxysporium*, *Rhizopus stolonifer*, *Alternaria alternata*, and*Tricodermaharzianum*were isolated from different test varieties of rice. There was 5.667%, 8.333%, 4.333% ,9.667% by blotter test and 7.667%, 11.667%, 6.333%, 12.667% by agar test mycoflora associated with the seeds of SR-2, SR-3, SR-4 and SR-5.

Keywords: Rice seeds, blotter paper, agar plate, seed-associated mycoflora, pathogen frequency

Introduction

One of the world's staple food crops is rice (*Oryza sativa* L.). China and India are the top two rice-growing nations in terms of production. 782 million tons of rice are produced annually from 167 million hectares of rice plantings worldwide (FAO 2022). With 112.91 million tonnes produced, China is the top

rice producer, with India coming in second (Anonymous, 2022). Higher grain yield and improved crop growth are dependent on both seed quality and seed health (Haque *et al*., 2012). The availability of high-yielding, disease-free, and farmer-preferred seeds of high-yielding varieties is the most crucial requirement for successful crop production. A major economic impact on agricultural output is caused by fungi and other pathogens that attack plants, as they can cause diseases in farmed crops that lead to significant yield losses (Paplomatas, 2006). Plant diseases that are specifically spread by seeds are referred to as seed-borne diseases. In contemporary agricultural research, a healthy seed bank is crucial for a desired plant population and a successful harvest (Rahman *et al*., 2008). Seedborne pathogens are a persistent issue that may even be to blame for the introduction of new illnesses into previously uninfected areas as well as the resurgence of old ones (Walcott *et al*., 2007). Seed associated infections pose a significant risk to the establishment of seedlings (Walcott *et al*., 2003).Crops' capacity to establish themselves and reach their maximum yield and value potential is significantly impacted by the quality of the seeds that are sown (McGee, 1995). One of the most significant biotic barriers to seed production globally is the presence of seed-borne and seed associated fungi. They cause diversity in plant shape, pre- and post-emergence death of grains, and vigor of seedlings, which in turn reduces germination to some extent (Van Du *et al*., 2001; Rajput *et al*., 2005; Niaz and Dawar, 2009).

Materials and Methods

i). Planting material: The planting material viz. SR-2, SR-3, SR-4 and SR-5 for the study was obtained from Mountain Research Centre for Field Crops, Khudwani, SKUAST-Kashmir

ii). Seed Health Testing: Following seed health testing methods were used for the detection of seed associated mycoflora

a). Blotter test

In accordance with the International Seed Testing Association [ISTA, 1996], the four different rice types seeds were examined using the blotter method

in Petri plates to determine the presence or absence of seed associated mycoflora. ThePetri plates were incubated at 25±1°C with a 12/12 hour light and dark cycle. The developing fungal colonies were examined under stereomicroscope or transferred to temporary mounts to be examined under compound microscope for identification of fungi.

b). Agar Plate Method

Seeds were scrutinized using Agar Plating Technique for the examination of seed associated mycoflora. The plated seeds were cultured for five to seven days at 22 to 25°C with 12-hourly light and dark cycles. Fungi developing from the seeds on the agar medium were inspected and identified at the conclusion of the incubation time. Under a compound microscope, identification was performed using the morphology of the sporulation structures and colony characteristics.

iii). Identification of fungi

The various colonies were then identified visually, first under a stereomicroscope, and then under a compound microscope, which allowed for a closer look at the fruiting structures (Mathur and Kongsdal, 2003). All the fungi were identified on the basis of standard manuals and descriptions (Malone and Muskette,1964; Misra *et al.,* 1994;Mathur and Kongsdal, 2003), Ellis, 1971; Chidambaram and Mathur, 1975; Neergaard and Saad, 1962; Booth, 1971 and Agarwal et al., 1990)

iv). Analysis of data: All the findings were displayed as a percentage of each pathogen's frequency. All the data was subject to standard analysis for accuracy and clarity.

Results and Discussion

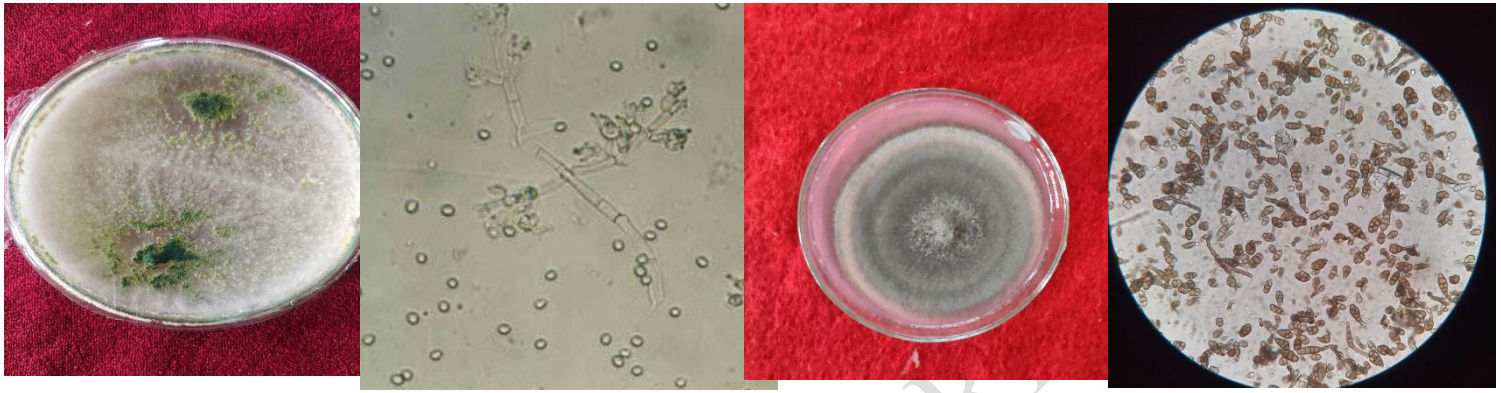
In both the blotter and the agar tests, variety SR-4 showed the least amount of seed-borne infection out of the four types examined. In comparison to other types, there was also reduced SR-4 infection from all fungal pathogens.

Based on colony morphology, four members of the fungal flora—*Tricodermaharzianum*, *Alternaria alternata*, *Rhizopus stolonifer*, and *Fusarium oxysporum*—were identified using spore/conidial morphological analyses.

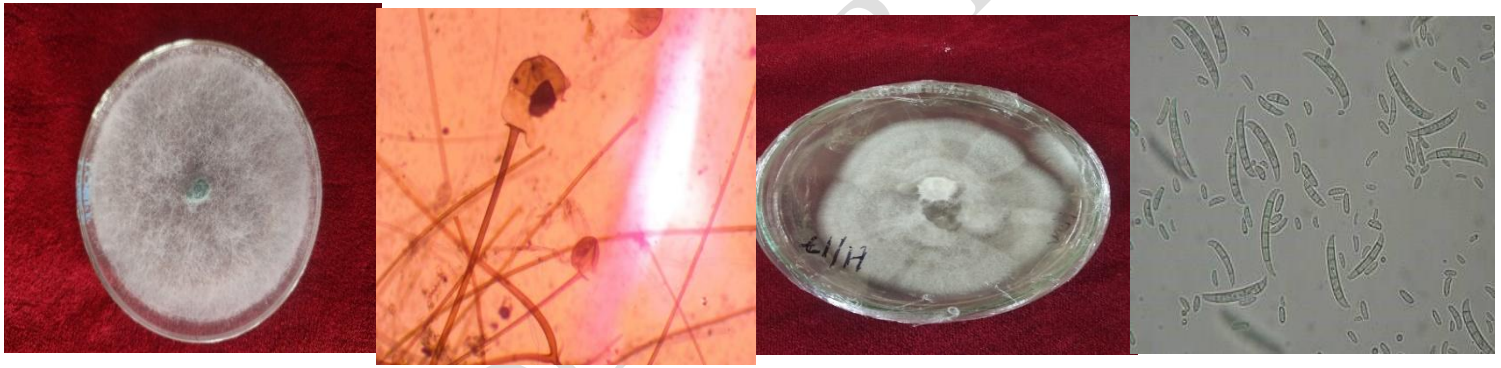
By blotter paper method highest per cent frequency of *Tricodermaharzianum*was noticed in SR-3 variety (6.667 %) where as lowest infection in SR-4 (2.667 %). Highest infection of *Alternaria alternata*was noticed on SR-5 (18.667 %) and lowest infection on SR-4 (9.33 %). *Rhizopus stolonifer*showed highest infection on SR-3 (10.667 %) and lowest on SR-4 (2.667 %). *Fusarium oxysporum*highest infection detected on SR-5 (5.333 %) and lowest infection on SR-2 (1.333 %) (Table 1).

Table 1: Per cent frequency of rice seed mycoflora associated with different varieties byblotter paper and agar plate method.

Fungi	Frequency of seed mycoflora (%) associated with different rice varieties									
	Blotter paper method					Agar plate method				
	SR-2	SR-3	SR-4	SR-5	Mean	SR-2	SR-3	SR-4	SR-5	Mean
Tricoderma*harzianum*	2.667	6.667	2.667	5.333	4.333	5.333	9.333	5.333	6.667	6.667
Alternaria *alternata*	13.333	12.000	9.333	18.667	13.333	13.333	21.333	9.333	22.667	16.667
Rhizopus *stolonifer*	5.333	10.667	2.667	9.333	7.000	6.667	10.667	6.667	14.667	9.667
Fusarium *oxysporum*	1.333	4.000	2.667	5.333	3.333	5.333	5.333	4.000	6.667	5.333
Mean	5.667	8.333	4.333	9.667		7.667	11.667	6.333	12.667	
CD(0.05)	Variety =1.9801 Fungi =1.9203 Variety* Fungi =3.958					Variety =1.8600 Fungi =1.7600 Variety* Fungi =3.720				



Trichoderma harzianum ***Alternaria alternata***



Rhizopus stolonifer ***Fusarium oxysporum***

Plate 1: Culture plate of different isolated fungi and their microscopic characters

Four seed-borne diseases were found and identified using the Agar plate method. They are *Fusarium oxysporum*, *Rhizopus stolonifer,Alternaria alternata*, and *Tricodermaharzianum*. *Tricodermaharzianum* exhibited lowest infection on SR-4 and SR-2 (both 5.333%) and maximum infection on SR-3 variety (9.33%). Infection rates for *Alternaria alternative* ranged from lowest on SR-4 (9.333%) to highest on SR-5 (22.667%). *Rhizopus stolonifera* observed that SR-5 had the highest infection rate (14.667%), while SR-4 and SR-2 had the lowest infection rates (6.667%). *Fusarium oxysporium* found that infection levels were lowest on SR-4 (4.000%) and highest on SR-5 (6.667%)(Table1). Plate 1 shows colony growth and spore/conidial formations observed under a compound microscope.

The information about the seedborne nature of pathogensprovided by Kumar and Bijendar (2022), Karan *et al*., (2021), Umbrey*et al*., (2021), Indira (2022), Khan (2006), Islam (2005) and Fakir (2001) was consistent with the current findings of the fungal organisms that are spread through seeds. The current study's findings indicate that the fungal flora that is carried by seeds includes *Trichoderma harzianum*, *Alternaria alternata*, *Rhizopus stolonifer*, and *Fusarium oxysporum*. Compared to the other studied rice seed varieties, the SR-4 variety showed less infection. The most important input in agriculture is pathogen-free seed. The majority of grain and seed varieties that are exported from India undergo testing in accordance with phytosanitary laws. The blotter and visual tests are often conducted as part of the phytosanitary rules on a regular basis. The results of the current investigation showed that certain pathogens were only detected in the blotter and agar tests for seed microflora. Since pathogen-free seed is a crucial component of commerce, this study will assist in performing both the blotter test and the agar test.

Conclusion

This study shows that the dominant rice cultivars in the Kashmir valley have a varied mycoflora of pathogenicand non-pathogenic fungus. Better seed health management is essential for effective rice farming because rice is a staple

meal. Understanding seed health is crucial to managing crops and pests in developing nations where farmers must conserve their own seeds for sowing. Testing for seed health is another way for farmers to optimize seed stocks for crop yield through quality management. It helps with seed certification for farmers from public seed providers and seed growers. Additionally, the results point to the necessity of properly storing rice seed in order to reduce fungal infestation in the near future.

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