EVALUATION OF SEED HEALTH OF SOME RICE VARIETIES GROWN IN HIMACHAL PRADESH

THESIS

By

ANURADHA
(A-2008-30-19)

Submitted to

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M Sc

Tough were the days
Tougher were the nights
I recall your precious
love & care for my smiles

Affectionately Dedicated

To my

Respected 'Mummy ji'

And 'Papa ji'

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CERTIFICATE – I

This is to certify that the thesis entitled **"Evaluation of seed health of some rice varieties grown in Himachal Pradesh"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms Anuradha (Admission No. A-2008-30-19)** daughter of Shri Rajinder Sharma under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

(Dr. A.S. Kapoor)
Major Advisor

Place : Palampur
Dated : 17th August, 2010

CERTIFICATE- II

This is to certify that the thesis entitled **"Evaluation of seed health of some rice varieties grown in Himachal Pradesh"** submitted by **Ms Anuradha (Admission No. A-2008-30-19)** daughter of Shri Rajinder Sharma to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Needless to say, all omissions and errors are mine.

Anuradha.

Place : Palampur
Dated : 17th August, 2010

(Anuradha)

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LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviation	Meaning
1	*et al.*	et alii (and others)
2	*i.e.*	Id est (that is)
3	*viz.*	vi delicet (namely)
5	p.	Page
6	pp.	Pages
7	°C	Degree Celsius
8	g	Gram
9	kg	Kilogram
10	Mt.	Metric tonnes
11	/	Per
12	%	Per cent
13	Fig.	Figure
14	cm.	Centimeter
15	ml	Milliliter
16	mg	Milligram
17	mm	Millimeter
18	min.	Minute (s)

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ABSTRACT

Twelve seed samples of different rice varieties from two agroclimatic zones of Himachal Pradesh tested for associated microflora based on four categories, showed the presence of 15 fungi by blotter method and 17 fungi and 1 bacterium sp. by agar-plate method. Among the methods, agar plate method was more efficient in detection of microflora. *Curvularia lunata*, *Drechslera oryzae* and *Xanthomonas* sp. were the predominant pathogens with highest frequency whereas *Fusarium solani* was detected from all the varieties with 100 per cent sample mean in each seed category. Among the varieties maximum microflora was detected from variety Kasturi Basmati and minimum from variety Pusa-1121, Jhumka and HKR-126 in each seed category. Only one nematode species, *Aphelenchoides besseyi* was detected from varieties Pusa-1121 and Jattoo from original, partially discoloured and discoloured seed category. For both in-soil and in-between paper test, varieties Pusa-1121 and Parmal had highest normal seedlings and lowest abnormal seedlings and dead seeds and Kasturi Basmati, Jattoo and Nagardhan had lowest per cent of normal seedlings and higher number of abnormal seedlings and dead seeds. Variety Parmal had the highest vigour index in original, apparently healthy and discoloured seed categories and variety Jhumka in partially discoloured seed category. Variety Kasturi Basmati and Nagardhan had the lowest vigour index. Chemical treatment with Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed and bioagent treatment with *Trichoderma harzianum* talc based formulation @ 4 g/kg seed were found to influence the associated microflora to a significant level and increased seed germination, root length and shoot length and seedling vigour index of each rice variety in each seed category. The highest lethal seed infection was found to be caused by *Curvularia lunata* in original seed category and by *Fusarium solani* in apparently healthy, partially discoloured and discoloured seed categories which was significantly reduced by chemical and bioagent treatments. Maximum microflora and least germination and seedling vigour index was observed from discoloured seeds and minimum microflora and maximum germination and seedling vigour index from apparently healthy seeds. Many seedborne microflora were detected from parts of seeds, husk and kernel however, husk found to harbour more number and frequency of microflora with highest frequency of *Curvularia lunata* which was decreased gradually with increasing duration of surface sterilization with mercuric chloride (0.1%). The number of associated microflora and their frequency varied from location to location due to the variation in weather parameters.

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Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food grain for nearly half of the world's population. "Rice is life", the theme of International Year of Rice, 2004 reflects the importance of rice which holds the key to our country's ability to produce enough food for our people. Globally rice is cultivated now on 154 million hectares with annual production of around 600 million tonnes and average productivity of 3.9 tonnes/hectares. Various types of land management systems for rice cultivation exist in India because it is one of the most climatically adaptable cereals.In India, during the period 2008-09, rice was cultivated in an area of 44 million hectares with a production of around 99.15 million tonnes (Subba Rao *et al.* 2010). In Himachal Pradesh, area under rice during 2003-04 was 81.4 thousand hectares with an annual production of 120.03 thousand tonnes (Anonymous 2005-06).

One of the reasons for low yield of rice is, rice diseases. Humid environmental conditions during *kharif* season pre-dispose the crop to the attack of various fungal, bacterial and viral pathogens and enhances the chance of seed colonization by different micro-organisms during maturity, harvesting and storage (Christensen and Kaufmann 1955; Neergaard 1977). It suffers from a number of diseases which are mostly seedborne *viz.*, brown spot (*Bipolaris oryzae*), rice blast (*Pyricularia grisea*), stack head (*Alternaria padwickii*), rice bunt (*Tilletia barclayana*), bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), etc. They not only reduce the quality of seed but are also transmitted from one season to other. Seed is considered to be the basic critical and vital input for enhancing and stabilizing the productivity and harnessing higher monetary return per unit area, time and input. Seed helps in the perpetuation of many diseases as well as source of disease development, a medium for survival of pathogens, their transmission to distant places, distribution in field as well as infest the uninfested soils. Seed health analysis plays an important role in the management of

seedborne diseases. Many of the seed testing laboratories set up by the Government satisfy themselves by testing viability and germination percentage but due to ignorance of seed testing laboratories, the dangerous micro-organisms go from one place to another.

Seed health refers primarily to the presence or absence of disease causing organisms *viz.,* fungi, bacteria, viruses, nematodes and insects associated with seed. Farmers often use seeds that have impurities and contaminants and are infected with pathogen (Fujisaka *et al.* 1993).

The method of storing and storing conditions also affects the seed health in terms of biodegradation by microflora associated with seeds which results in poor seed germination, affecting seed quality, seed and seedling vigour (Vidhyasekaran *et al.* 1973; Krishnamurthy and Raveesha 2001).

The required seed replacement is 20 per cent in cereals for certified seeds which is rarely met by the farmers and this leads to poor health of cereals especially of rice saved by the farmers. Seed health testing is an essential management tool for control of seedborne and seed transmitted pathogens and continues to be an important activity for their regulation and control through phytosanitory certification and quarantine programmes. It is also critical for insuring the health of basic seed stocks used for seed production and for plant germplasm utilized in research and product development (Morrison 1999). As a consequence of increased product liability (Meijerink and Van Bruekelen 1995) as well as competitive pressures within the seed industry, seed health has also become an important quality trait in the market place.

The parameters of seed quality *viz.,* genetic and physical purity, high germination percentage and vigour and free from seedborne diseases and insects are needed for realizing the full potential of a variety (Seshu and Dadlani 1989).

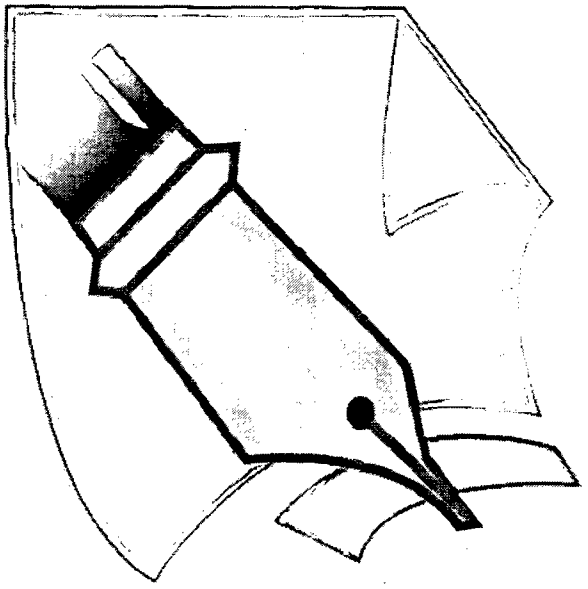
Seed vigour is recognized as an important seed quality parameter distinct from germinability (Seshu *et al.* 1988). One of the most important basic needs for the higher agricultural productivity is quality seeds, characterized by high viability and vigour (Yaklich *et al.* 1979).

In Himachal Pradesh, rice is mainly cultivated in Zone I and II. The seed health is influenced by climatic conditions of the area especially during harvesting time. Information on seed health status of rice varieties under different agroclimatic conditions is scanty under Himachal Pradesh conditions, especially as a management tool.

Therefore, the present research proposal was proposed with the following objectives:

1. To identify seedborne microflora of rice under different conditions
2. To establish the role of seedborne microflora in seedling abnormalities and
3. To determine the effect of seed treatments on germination and seed vigour.

Review of literature



2. REVIEW OF LITERATURE

Rice seeds harbour a wide range of microflora including the incidents of some serious rice diseases, which affect the seed health and quality of crop. Many different types of microflora associated with rice seeds which effects the germination, vigour of seeds and also leads to seedling abnormalities. The literature concerning the major aspects like distribution and identity of pathogens, methods used for seed health testing, role of associated microflora in causing seedling abnormalities by affecting germination and seedling vigour, management through seed treatment is briefly reviewed below under the following sub heads:

2.1 Distribution and identity of pathogens

2.2 Methods of seed health testing

2.3. Seeds as a carrier of various pathogens

2.4 Effect on germination and seedling vigour

2.5 Seed treatment with fungicides

2.6 Seed treatment with biocontrol agents

2.1 Distribution and identity of pathogens

The prevalence of organisms associated with rice seeds varies greatly. Due to associated microflora with seeds various harmful effects recorded in seeds are; loss of germination capacity, seed discolouration, decay, increase in fatty acids and utilization of carbohydrates for the synthesis of protein and toxin production (Christensen 1955; Christensen and Kaufmann 1969; Krogh *et al.* 1966).

Baldacci and Corbetta (1964) recorded 19 per cent *Alternaria* sp., 31.8 per cent bacteria, 92 per cent *Drechslera oryzae*, 30.2 per cent *Epicoccum* sp., 0.42 to 2.6 per cent *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. and *Pyricularia oryzae.*

Pavgi and Singh (1969) in Varanasi reported *Helminthosporium oryzae*, *H. sigmoideum* var. *irregulare*, *Leptosphaeria iwamotoi*, *Phaeosphaeria oryzae* and *Phyllosticta oryzina* as parasitic fungi on rice.

Ou (1972) gave a detail account of fungi, which were responsible for deterioration of rice grains both in the field and in storage. According to him, *Helminthosporium* sp., *Pyricularia oryzae, Alternaria (Trichoconis) padwickii*; *Gibberella fujikuroi, G. rosa, Fusarium cereali, Nigrospora* spp., *Epicoccum* sp., *Phyllosticta glumarum, Alternaria* spp. and *Helicoceras oryzae* are the common field fungi. The most common storage fungi were species of *Aspergillus* and *Penicillium, Absidia, Mucor, Rhizopus* spp. were also found and occasionally species of *Chaetomium, Dematium, Monilia, Oidium, Streptomyces, Syncephalastrum* and *Verticillium* have been reported from storage.

Babatola (1984) conducted a survey of plant parasitic nematodes associated with or affecting rice throughout Nigeria and identified some important nematode pests, especially the white tip disease nematode, *Aphelenchoides besseyi* and the rice root nematodes *Hirschmanniella spinicaudata* and *H. oryzae* from seed, soil and root samples from swamp rice fields respectively.

Dwivedi and Mehrotra (1984) in different parts of India reported that *Penicillium* spp. and Fungi Imperfecti were predominant in freshly harvested seed samples and *Aspergillus* in stored samples of rice. The percentage of stored fungi increased with the duration of storage. They reported the occurrence of 12 *Mucorales*, 27 *Aspergillus*, 29 *Penicillium* and 9 spp. of Fungi Imperfecti not reported earlier on rice seeds.

Incidence of *Drechslera oryzae*, *Alternaria padwickii, Fusarium* spp. *Curvularia* spp., *Aspergillus* spp. and *Penicillium* spp. in rice seed was also reported (Jha and Prasad 1984).

Basak and Mridha (1985) reported the isolation of *Alternaria longissima, Alternaria* spp., *Chaetomium* sp., *Curvularia* sp., *Drechslera oryzae*, *Fusarium moniliforme*, *Nigrospora oryzae, Penicillium* spp. and *Rhizopus* spp. from discoloured seeds of rice varieties. The prevalence of fungi varied with respect to variety and location.

Korolevo *et al.* (1985) isolated a bacterium *Xanthomonas campestris* pv. *oryzae* from rice seeds and proved its pathogenicity by inoculation. *Aphelenchoides besseyi* is widely distributed and occurs in most rice growing areas (Ou 1985).

Gajapathy and Kalyanasundarum (1986) described the distribution of seed borne mycoflora within grain with special reference to storage fungi present mainly in husk and outer layers of the kernel. The different fungi invading the peripheral layers were *Aspergillus flavus* and *A. nidulans* with *A. niger* to some extent and the fungi invading the interior of seed were *Aspergillus candidus, A. glaucus* and *A. versicolor*, *Penicillium* spp. but the grains having such pathogens were less common.

Mishra and Dharamvir (1988) detected the different fungi associated with discoloured seeds of rice cultivars. They detected the 28 fungi from 27 samples of discoloured rice grains from 4 agroclimatically different areas. Out of which 16 were associated with seeds from Bihar, 28 from Jammu, 21 from Andhra Pradesh and 23 from Orissa. *Drechslera oryzae* followed by *Curvularia lunata* predominant in Bihar; *Phoma* sp. followed by *Drechslera oryzae* and *Alternaria alternata* in Jammu; *Phoma* sp. , *Curvularia lunata* and *Nigrospora oryzae* in Andhra Pradesh; and *Curvularia lunata* dominated in Orissa. Among all *Curvularia lunata* was the most predominant fungus.

Riaz *et al.* (1995) studied the seedborne fungi of rice samples collected from Pakistan. *Alternaria* and *Helminthosporium* spp. occurred most frequently, followed by *Curvularia, Fusarium* and *Aspergillus* spp. in most of the accessions.

Ali and Deka (1996) recorded 10 fungal species of 11 genera (*Curvularia, Drechslera, Nigrospora, Trichothecium, Fusarium, Aspergillus and Penicillium*) that were associated with grain discolouration of 6 rice cultivars. The frequency of occurrence of these fungi varied considerably on different cultivars. The frequency of *F. moniliforme* was highest among the field fungi, while *Aspergillus* and *Penicillium* spp. were most frequent among the storage fungi after 8-10 months of storage.

Babu and Lokesh (1996) detected 24 fungal species associated with 51 paddy seed samples of different cultivars of paddy from different climatic regions of Karnataka. The highest incidence was of *Aspergillus* spp., *Drechslera* spp., *Chaetomium globosum*, *Fusarium moniliforme*, *Verticillium* spp., *Gerlachia oryzae* and *Rhizopus* spp.

Sharma and Chahal (1996) reported that two promising CMS rice lines PMS 3A and PMS 8A were highly susceptible to *Pyricularia oryzae* to varying degrees. Seed mycoflora *Alternaria alternata*, *Curvularia lunata*, *Drechslera oryzae*, *D. tetrame*ra and *Fusarium monilifor*me were predominantly associated with all types of rice genotype but the CMS lines were highly infested with *C. lunata*, *D. tetramera* and *F. moniliforme*.

Manandhar *et al.* (1998) studied the seedborne infection of rice by *Pyricularia oryzae* and its transmission to seedlings in Nepal. A linear relationship was found between panicle symptoms and seed infection *i.e.* more symptoms the higher seed infection.

Khan *et al.* (1999) detected *Fusarium moniliforme, F. semitectum, F. oxysporum, Alternaria alternata, A. padwickii, Curvularia oryzae, C. lunata, Drechslera oryzae, Pyricularia oryzae* and species of *Nigrospora, Phoma, Aspergillus* and *Penicillium* from different rice varieties.

Islam *et al.* (2000) conducted an experiment to evaluate seed health of some rice varieties and found that *Alternaria padwickii* was the dominant pathogen in best seed and farmers' original seed for all varieties except R30.

Pham Van Du *et al.* (2001) studied the effect of discoloured grains to rice grain quality in Mekong Delta. Total of 9 fungal species were reported from 60 samples of 12 cultivars. *Curvularia* spp. (13.4%) was the dominant pathogen through blotter method followed by *Alternaria padwickii* (12.0%), *Bipolaris oryzae* (4.9%), *Sarocladium oryzae* (1.9%), *Fusarium graminum* (1.5%), *Tilletia barclayana* (0.16%), *Phoma sorghina* (0.1%), *Cephalosporium oryzae* (0.34%), *Ustilaginoidea virens* (0.05%).

Cottyn (2002) detected *Xanthomonas* sp., *Pseudomonas* sp., *Bacillus* sp. and *Burkholderia* sp. from tropical fields of farmers in Philippine.

Mandhare *et al.* (2008) assessed the seed microflora in fourteen popularly grown paddy varieties by using standard blotter method and detected the 7 microflora from these varieties. *Fusarium moniliforme, Fusarium oxysporum, Curvularia lunata, Drechslera oryzae* and *Alternaria padwickii* were five pathogens pathogenic to paddy. The lowest microflora was reported in Palghar (4%) with 97 per cent germination and highest microflora in Indrayani (20%) with 80 per cent germination.

Bharathi and Raut (2009) studied the seed microflora of rice through blotter method and detected 14 fungi of 11 genera from the 40 rice seed samples stored for 8 months and 19 fungi of 11 genera from 30 samples immediately after harvest. *Curvularia lunata* and *Fusarium moniliforme* were the predominant fungi and few samples showed the presence of *Bipolaris oryzae*. Stored fungi, *Aspergilli* and *Penicillia* were predominant in stored samples than fresh samples.

Gopalkrishanan and Valluvaparidasan (2009) in Tamil Nadu studied the seedborne microflora of rice and detected total of 8 genera of fungi *viz., Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* comprising 12 spp. from seed samples. Among them most prominent was *Bipolaris oryzae* (58.89%) followed by *Alternaria padwickii* (52.96%), *Curvularia* sp. (44.60%), *Alternaria tenuis* (37.63%) and *Sarocladium oryzae* (26.83%).

2.2 Methods of seed health testing

Although different methods have been developed in different laboratories for testing the seed health but the information on seed health testing is still not adequate. Hiltner (1917) developed 'Hiltner's method' using sterile brick pieces to test the pathogenicity of seedborne fungi. Doyer (1938) tested the seed health and determined the seed infection by the direct inspection of dry seed samples, observation of seeds or seedlings in germination beds and inspection of seed washings. It was the first major breakthrough in seed health testing methods.

Muskett and Malone (1941) developed the 'Ulster method' for the examination of the presence of seed-borne parasites in flax seeds. This methods involves the planting of seeds on two per cent malt extract agar medium at 22^0C for five days, then the medium around each seeds is examined for presence of parasitic organisms. Hagborg *et al.* (1950) suggested the use of 2,4-D as an inhibitor of germination in routine examination of bean seeds for seed-borne infection.

Tempe (1957) suggested the routine investigation of seeds for their health conditions in Dutch Seed Testing Station at Wageningen. Neergaard and Saad (1962) recommended blotter test and potato dextrose agar plate test as standard procedures for laboratory seed health testing in routine studies.

Keeping in view the International uniformity of seed health testing, the International Seed Testing Association has standardized the following methods of seed health testing (Anonymous 1966).

a. Inspection of dry seeds

b. Examination after softening or soaking the seeds

c. Examination of material removed from seeds by washing

d. Examination after incubation:

   i. blotter method

   ii. agar plate method

e. Examination of growing plants

Mathur and Neergaard (1966) found agar plate method as a better method for isolation of *Trichoconis padwickii* and blotter method for isolation of *Heminthosporium oryzae* and *Pyricularia oryzae. H. oryzae* was isolated on agar only from one rice sample whereas from 8 out of 15 samples on blotter method and *Pyricularia oryzae* from one rice samples to the extent of 12 per cent through blotter method but was not observed on agar.

Puttoo (1969) reported washing test as a unsuitable method for identification of fungi. Among blotter and agar plate method, the later one is found to be more superior for isolating large number of fungi. Menten (1978) reported agar plate method could detect more pathogens than blotter method.

Shetty and Shetty (1985) in Mysore proposed new methods to detect seedborne *Alternaria padwickii*. They used standard blotter method, modified blotter method at pH 4, potato dextrose agar, deep freeze method, blotter immersed in paddy in paddy extract, guiacol agar method and paddy extract agar (40:20) method for detecting the fungus and reported that rice extract agar medium induced maximum expression of fungus and detect 48 per cent of seedborne *Alternaria padwickii* and gave better result than any other methods tested.

Khan *et al.* (1988) evaluated seed health of rice seeds for assessing seedborne mycoflora by using blotter, agar plate and deep freeze method and found the blotter method as suitable method for the detection of *Alternaria alternata, A. tenuissima, Aspergillus niger, A. flavus, A. terreus, Chaetomium globosum* and *Curvularia lunata. Drechslera* spp., *Curvularia* spp. were isolated more on agar plate and *Fusarium* spp., *Trichoconis padwickii, Myrothecium roridum* by deep freeze method

Farias *et al.* (2007) studied the occurrence of *Alternaria padwickii* in lots of rice seeds and its effect in plantlets and found blotter test method a best method in analyzing this pathogen. Bharathi and Raut (2009) studied the seedborne fungi of rice and effect of fungicides by using blotter method.

2.3 Seeds as a carrier of various pathogens

The presence of a pathogen with the seed favours the earliest possible infection of the seedling. Dorogin (1923) reported five different forms of contaminations with fungal parasites in seed samples which were as follows:

a. Admixture with seeds as sclerotia or as small agglutinated spore masses

b. Mummification of seeds by fungal stroma or presence of initial spores

c. Presence of mycelium, only in definite parts or organs within seeds

d. Fructifications on the surface of seeds

e. Spores

Orton (1926) found seed as carrier of pathogens and estimated the loss amounting to 2.3, 3.1, 3.2 and 6.1 per cent of wheat, barley, oats and bean crops, respectively.

Suzuki (1934) detected *Pyricularia oryzae* within the tissues of the embryo, endosperm, bran layers and glumes, and also between the glumes and the kernel.

The seedborne microflora of parasites and saprophytes, responsible for the reduction of quality, whether by discolouration or by more profound damage, in rice for milling or sowing, varies greatly from locality to locality, according to local conditions.

Several nematodes species are know to be associated with rice (Timm 1965; Sher 1968). Fazli and Schroeder (1966) studied the presence of mycelium of *Drechslera oryzae* in rice seeds.

In rice, 55 rice diseases are known to be caused by fungi out of which 43 are seedborne or seed transmittable (Ou 1985; Richardson 1979, 1981; Neergaard 1979). Goto *et al.* (1988) confirmed the seed transmission of *Xanthomonas oryzae* pv. *oryzicola*.

Sala *et al.* (1994) in Spain isolated several *Fusarium* spp. predominantly from section Liseola from cereals. Among all, *Fusarium moniliforme* was the predominant species (85.7%). *Fusarium* sp. has been well documented as seedborne pathogen on various crops including cereals (Bottalico 1997; Tankov 1998).

2.4 Effect on germination and seedling vigour

Majority of the fungi associated with stored seeds are chiefly responsible for seed deterioration and reduction in germination potential (Christensen and Kaufmann 1965). Apart from causing diseases, large number of fungi associated with seeds reduced germinability of seeds, caused seed and seedling rot (Baker and Smith 1966).

The rice seed associated fungi have been reported to cause discolouration to cause discolouration of seed, germination failure, root and stem rots and seedling blight of rice (Aguieroef *et al.* 1966; Ali and Deka 1996).

Chidambaram *et al.* (1973) and Neergaard (1977) reported the pathogenic behaviour of *Drechslera sorokiniana* in different cereals and vegetables.

Ahuja and Ahuja (1982) studied the germinabilty and seedling vigour of discoloured rice grains. Germination studies were carried out in two replicates of 30 spotted and normal grains in Petriplates incubated at 30^0C. The germination status was recorded after 96 hours of incubation. After 10 days incubation, 10 seedlings developed from each spotted and healthy seeds were taken randomly for shoot and root length measurements for studying seedling vigour. Among these, spotted seeds of all the genotype showed poor germination and seedling vigour.

Gora *et al.* (1987) conducted a test with 5 cultivars of rice grown in infested soil and reported that *Trichoconiella* (*Alternaria*) *padwickii* caused significant reduction in seed germination, root length and shoot length. Velazhahan *et al.* (1989) reported the reduction of seed germination, shoot and root length in rice seeds inoculated with *Acrocylindrium oryzae.*

Sharma *et al.* (1987) reported that discolouration reduced the germination of seeds and was proportional to severity of discolouration. Seventeen fungal species were isolated from different rice cultivars among which *Fusarium moniliforme*, *Alternaria alternata*, *Curvularia lunata* and *Trichoconis padwicki* were more common.

Fungi and bacteria associated with discoloured grains affect germinability (Misra *et al.* 1990; Ou 1985). Sharada *et al.* (1990) studied the seedborne bacteria of paddy and their effect on seed and seedling in 5 rice varieties *viz.*, Jaya, Gowrisanna, Rajamudi, Shakti, Rajakayame. Bacteria were isolated externally and internally from 5 cultivars and reported that most of the cultures of bacteria reduced vigour and were capable of producing symptoms to rice seedlings. Internal bacteria out numbered external bacteria. Most bacteria were gram negative and varied in size from cocco bacilli to long filamentous form. Only one gram positive strain was isolated from variety Shakti and it was internally seedborne.

Aspergillus and *Penicillium* are known to cause discolouration of grain, leading to low germination (Bokhary 1991). Sharada *et al.* (1992) studied the effect of different isolates of bacteria on rice seeds and reported that bacteria caused abnormalities like stunting growth, club root and 'comma'-shaped, wrinkled and damaged coleoptiles.

Sachan and Agarwal (1994) reported that fungi associated with all the eight type of seed discolourations in rice leads to loss of viability and seedling vigour. The maximum loss in seed viability, germination and seedling vigour was found in seeds having discolouration on both embryo plus endosperm.

Vaid *et al.* (1994) studied the effect of grain discolouration diseases on some important quality parameters of rice and reported decrease in hulling, milling and head rice yield.

Deka and Ali (1995) recorded fungi associated with freshly harvested discoloured rice grains as well as during storage. Among the fungi, *Curvularia lunata* [*Cochliobolus lunata*] was predominant occurring for approximately six months, *Aspergillus niger* was detected up to eight months in storage and may play a major role in seed deterioration particularly in combination with *C. lunata*. *Curvularia lunata* caused the maximum reduction in seed germination.

Mishra *et al.* (1995) detected 36 fungal species from rice varieties stored in warehouses for varying periods through standard blotter and agar plate methods. Significant correlation was found among the numbers of fungi, storage period and germinability.

Islam *et al.* (2000) conducted in-between and in-soil seed germination test and observed that the variety C-4/Malaqkit showed highest per cent of normal seedlings and R30 showed lowest per cent of normal seedlings in all treatments and varieties IR59 and R30 showed the highest per cent of abnormal seedlings in in-between paper test and dead seeds for in-soil test. IR59 had the highest and R30 had the lowest vigour in all the treatments. They also conducted the lethal seed infection experiment and found that it was caused by *Fusarium moniliforme*, *Alternaria padwickii* and *Curvularia* spp.

Pham Van Du *et al.* (2001) reported that seed germination was affected by discolouration. Khalid *et al.* (2001) studied the incidence of microflora in rice cultivars, their frequency and impact on seed germination. They reported five storage fungi *viz., Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Penicillium* spp., and *Rhizopus stolonifer* [*R. stolonifer* var. *stolonifer*] were associated with rice seeds. The highest fungal incidence (63.7%) was detected in Dilrosh-97 and lowest (29%) was observed in Pakhal than the other cultivars showing moderate incidence of microflora. The associated microflora reduced the germination of all the cultivars.

Mehrotra and Aggarwal (2003) reported that *Rhizopus nigricans*, *Mucor* spp. and *Fusarium oxysporum* fungi could seriously retard seed germination through softening and necrosis of tissues. Moss and Smith (2006) reported that pathogenic seed-borne fungi include *Rhizopus nigricans*, *Mucor* spp. and *Fusarium oxysporum.*

2.5 Seed treatment with fungicides

Seed treatment with Dithane M-45 (0.3% by seed weight) provides satisfactory control against *Alternaria padwickii* (Vir *et al.* 1971). Kauraw (1986) studied the effect of 6 fungicides namely, Bavistin, Captan, Dithane M-45, Foltaf, Thiride 75 and Vitavax @ 0.2 per cent on germination, root/shoot growth and incidence of seed borne pathogens in rice. Bavistin and Captan reported to increase germination by 3 and 4 per cent in pots than control (89% germination). Dithane M-45 and Bavistin increased germination percentage by 4 and 3 per cent

over control (92% germination) in blotter method whereas Dithane M-45 reported to reduce germination by 3 per cent in pots. Root length was increased by Thiride 75 (72.00 mm) in blotter and in pots (70.00 mm) and Vitavax (73.40 mm) in blotter and in pots (69.5 mm) as compared to control (66.5 mm) in blotter and in pots (60 mm) whereas, other fungicides reported to reduce the root length. Shoot length was reported to increase only by Vitavax in pots whereas other fungicides reduced in blotter test.

Rao and Ranganathaiah (1988) studied the effect of seed treatment with fungicides *viz.*, Captan, Dithane M-45, Thiram, Emisan-6 and Bavistin to control seed-borne *Drechslera oryzae* in paddy. The standard blotter method was used for studying the infection of *D. oryzae* on paddy seed after seed treatment with fungicides. Bavistin proved to be less effective than other fungicides.

Sharma *et al.*(1987) studied the effect of 12 fungicides *viz.,* Atapulgite dust, Bavistin, Bayleton, Baytan, Brassicol, Captan, Derosal, Emisan, Dithane M-45, Dithane Z-78, Thiram and Topsin M, to improve germinability of partially and fully discoloured seeds of rice. Bayleton was slightly reported as inhibitory to germination of seeds out of 12 fungicides evaluated. Derosal, Emisan and Thiram proved highly effective in improving the germinability of the seeds followed by Bavistin.

Dodan *et al.* (1994) tested 7 fungitoxicants in eradicating the seedborne infection and reported the effectiveness of carbendazim in providing maximum disease control (81.2%).

Sachan and Agarwal (1994) studied the effect of seed treatment with Captan, Ceresan, Dithane M-45, Thiram, Bavistin, Bavistin + Dithane M-45 (1:1) and Bavistin + Thiram (1:1) in reducing the inoculum of seedborne fungi causing seed discolouration. Seed treatment with Bavistin + Dithane M-45 and Bavistin + Thiram were reported to be superior among all other fungicides in increasing germination and seedling vigour and reducing the seedborne inoculum.

Sisterna and Ronco (1994) studied the efficacy of fungicides for controlling growth of five seedborne fungi associated with rice grain spotting. Vaid *et al.* (1994) reported that seed treatment with Carbendazim @ 2 g per kg of seed as a best treatment for improving germinability of discoloured rice.

Kabir *et al.* (2006) studied the effect of physical and chemical treatment on prevalence of seedborne fungi and seedling development of Boro rice in Bangladesh and found lowest prevalence of fungi in farmers' seeds treated with Vitavax 200, followed by the soaked, washed and cleaned seeds.

Ali and Deka (1996) found seed treatment of rice with Bavistin @ 1 g/kg seed as an effective treatment for maintaining seed germination (>70%) even after 8 months of storage. Deka *et al.* (1996) tested the effectiveness of seed treatment with 5 chemicals *viz.*, Bavistin (carbendazim), Hinosan (ediphenphos), Indofil M-45 (mancozeb), Tilt (propeconazole), Common Salt (NaCl) and reported mancozeb treatment as an effective treatment in controlling grain discolouration in rice.

The regular fungicides used for seed treatment are found to inhibit growth of the seedborne pathogens but their role in improvement of seed quality is poorly understood (Raju *et al.* 1999).

No pathogens were observed in chemically treated original seeds treated with Benlate @ 0.3 per cent and Dithane M-45 @ 0.3 per cent (Islam *et al.* 2000)

Bharath *et al.* (2005) tested the efficacy of fungicides in the management of fungal pathogens of watermelon. They found that *Fusarium* species were effectively controlled by Bavistin and Topsin showed the promising effect against all the fungal pathogens. Bavistin and Topsin also found to increase significantly the seed germination and vigour index.

Sagar and Hegde (2006) determined the effect of seed treatment using different systemic and non-systemic fungicides on seed mycoflora and seed vigour. Anjorin and Mohammed (2009) studied the effect of seed dressing fungicide on germination and seedling growth of watermelon.

Bharathi and Raut (2009) studied the effect of fungicides on seedborne fungi of rice through blotter method and found that Bavistin (0.1%) and a combination of Bavistin + Thiram (1:1) @ 0.3 per cent proved superior in controlling seedborne fungi and resulted into high germination of rice seed.

2.6 Seed treatment with biocontrol agents

Trichoderma species are capable of hyperparasitising the pathogenic fungi and found to involve in protecting number of crop plants (Durell 1968; Barnett and Binder 1973). Dennis and Webster (1971) found that many isolates of *Trichoderma* spp. produced volatile and non-volatile antibiotics active against wide range of fungi. Certain isolates of *Trichoderma* spp. are potential biocontrol agents and their activity in natural soils is well established (Wells *et al.* 1972).

Sumitha and Gaikwad (1995) reported that seeds coated with the antagonists *Trichoderma harzianum* and *Bacillus subtilis* showed higher and better germination and produced longer roots and shoots than untreated seeds. Mehta *et al.* (1997) reported that *Trichoderma* spp. *viz., T. harzianum* and *T. viride* promote plant growth by restricting pathogens as well as by its direct effect.

The efficacy of *Pseudomonas fluorescens* and *Trichoderma harzianum* against grain mold pathogen *Fusarium moniliforme* infecting sorghum seeds was reported by Raju *et al.* (1999).

Trichoderma harzianum population retained viability in talc, alginate pellets, vermiculite bran and gypsum formulations for longer periods when stored at $4^{o}C$ than at room temperature (Prasad *et al.* 2000). Hossain *et al.* (2002) studied the effect of *Trichoderma viride*, *T. harzianum*, *T. koningii*, *Gliocladium virens* and *Pseudomonas fluorescens* against *Pyricularia grisea* and found that all antagonists inhibited the growth of *P. grisea*.

Bharath *et al.* (2005) studied the effect of seed treatment with antagonists like *Trichoderma harzianum* and *T. viride* and found the improved seed germination, root/shoot length and seedling vigour by paper towel method and reduced the incidence of seedborne fungal pathogens. *Trichoderma harzianum* showed its efficacy against all *Fusarium* species.

Materials and Methods

3. MATERIALS AND METHODS

The present studies were conducted in the Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 2008-10. The material and methods followed during the course of study to fulfill the objectives are described under the following sub heads:

3.1 Collection of seed samples

3.2 Sterilization of glasswares/plastic wares

3.3 Soil preparation

3.4 Maintenance of biocontrol agent

3.5 Mass multiplication of *Trichoderma harzianum*

3.6 Studies of associated seed microflora

3.7 Isolation and identification of different pathogens

3.8 Seed health parameters as affected by microflora and effect of seed treatments

3.9 Lethal seed infection

3.10 Component analysis

3.11 Effect of chemical and bioagent treatments on seed microflora

3.12 Effect of weather parameters during harvesting period on seed microflora

3.13 Statistical methods

3.1 Collection of seed samples

3.1.1 Collection

Working seed samples were collected from different localities of Himachal Pradesh, where rice (*Oryza sativa* L.) is commercially cultivated. Samples of seed of three varieties of rice were collected from farmers of each agroclimatic zones *viz.*, I (Kasturi Basmati, Hybrid-6444 and Pusa-1121) and II (Jhumka, Parmal and Jattoo). Simultaneously samples of seed of two recommended

varieties were also be collected from HAREC, Dhaulakuan (PAU-201 and HKR-126), Bajaura (Nagardhan and Yunlen 18 (s)) and RWRS, Malan (HPR-2143 and HPR-1068) and samples so collected were designated as V_1 (Kasturi Basmati), V_2 (Hybrid-6444), V_3 (Pusa-1121), V_4 (Jhumka), V_5 (Parmal), V_6 (Jattoo), V_7 (PAU-201), V_8 (HKR-126), V_9 (Nagardhan), V_{10} (Yunlen 18 (s)), V_{11} (HPR-2143) and V_{12} (HPR-1068), respectively. The details of varieties along with their sources are presented in Table 1.

Table 1. Collection of rice seed samples from different locations of Himachal Pradesh

Location	Variety	Sample number
Farmer samples		
Zone I		
Una	Kasturi Basmati	V_1
Una	Hybrid-6444	V_2
Bharapur	Pusa-1121	V_3
Zone II		
Latwala	Jhumka	V_4
Kangra	Parmal	V_5
Kullu	Jattoo	V_6
Research station samples		
Hill Agriculture Research and Extension	PAU-201	V_7
Centre, Dhaulakuan (Zone I)	HKR-126	V_8
Hill Agriculture Research and Extension	Nagardhan	V_9
Centre, Bajaura (Zone II)	Yunlen 18 (s)	V_{10}
Rice Wheat Research Station, Malan (Zone II)	HPR-2143	V_{11}
	HPR-1068	V_{12}

3.1.2 Sampling of seeds

Sampling of seeds were made as per standard method (ISTA 1985):

i. **Primary sample** —small samples of equal size taken from one point in the lot.

ii. **Composite sample** —formed by combining and mixing all the primary samples taken from the seed lot.

iii. **Submitted sample** — all or part of the composite sample for testing.

iv. **Working sample** —all or part of the submitted sample on which the test is performed for testing.

3.1.3 Categorization of seeds

The collected seed samples of each rice varieties were critically examined and grouped into four categories on the basis of their morphological traits and external appearance by visual observations or with the help of hand lens (Plate 1):

(i) Original seed

(ii) Manually sorted best quality original seed (apparently healthy)

(iii) Partially discoloured seed and

(iv) Discoloured seed

The percentage of apparently healthy, partially discoloured and discoloured seeds in each collected variety was calculated by counting total number of seeds in 1200 seeds in 3 replications (400 seeds/replication) of each category.

3.2 Sterilization of glasswares/plastic wares

'Borosil' brand glasswares like test tubes, Petriplates, conical flasks, beakers and measuring cylinders etc. were dipped in chromic acid mixture (sodium dichromate 75 g, distilled water 500 ml and concentrated sulphuric acid

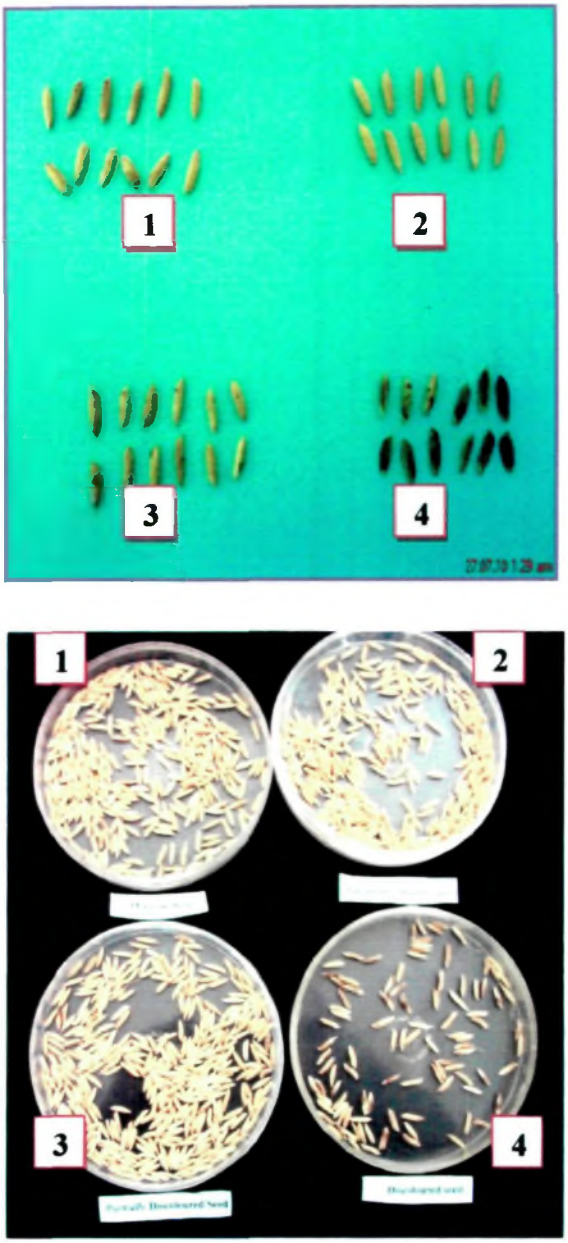


Plate 1. Four categories of rice seed varieties; original seed (1); apparently healthy seed (2); partially discoloured seed (3); and discoloured seed (4)

500 ml) overnight and washed in running tap water for 10 minutes and thrice in distilled water before use and sterilized in hot air oven at 160°C for 1 hour. Plastic wares were surface sterilized with 5 per cent alcohol solution or sodium hypochlorite (2%). Growth media were sterilized in autoclave at 1.05 kg/cm^2 pressure for 15 minutes.

3.3 Soil preparation

Soil was collected from the field area of Department of Plant Pathology, CSKHPKV, COA, Palampur and was mixed uniformly with FYM @ 200 g/kg of soil. Soil sterilization was done with formalin (40%) of 5 ml formalin diluted with 20 ml of water for 4 kg of soil. Treated soil was covered with polythene sheet and maintained as such for 7 days and then exposed for 7 days for aeration before filling the pot.

3.4 Maintenance of biocontrol agent

Culture of *Trichoderma harzianum* was procured from the Department of Plant Pathology. Stock culture was maintained by sub culturing on Potato Dextrose Agar (PDA) at 25 ± 1°C.

3.5 Mass multiplication of *Trichoderma harzianum*

For mass multiplication, potato broth was prepared by autoclaving at 1.05 kg/cm^2 pressure for 20 minutes. Culture of *T. harzianum* was prepared in potato broth in 250 ml conical flasks by inoculating 4 mm disc of 7 to 10 days old *T. harzianum* culture under aseptic conditions in laminar air flow chamber and incubated at 25 ± 1°C. The population of *T. harzianum* (2 x 10^9) were assessed in prepared broth and pH of talc powder was adjusted to 7 by adding 150 g calcium carbonate/kg talc powder. Talc powder sterilized in autoclave at 1.05 kg/cm^2 for 30 minutes for two consecutive days and 1 kg of sterilized talc powder and 5 kg of carboxyl methyl cellulose (CMC) transferred in a sterilized container under aseptic conditions. After that 400-500 g bioagent suspension added to it and mixed thoroughly. This produce then used for seed treatment with *T. harzianum.*

3.6 Studies of associated seed microflora

The different varieties based on different categories and treatments were tested for associated microflora based on standard procedures described by International Seed Testing Association (ISTA 1985) with slight modifications. Since the seeds are known to serve as a source of carrying wide variety of microflora, attempts were, therefore, made to isolate both ectophytic and endophytic microflora of the seeds with the help of following methods:

3.6.1 Blotter method

One hundred seeds of each category of different varieties untreated and chemical treated were placed in a plastic Petriplates (9 cm dia.) lined with two layers of blotting papers moistened with distilled water for studying the association of different microflora with rice seeds (Neergaard 1977). Twenty five seeds were placed in each Petriplates equidistantly. The petriplates were incubated at 25 $\pm$ 1^{O}C for seven days and the seeds were examined regularly for the presence of different fungi. There were four replications each having 25 seeds. Incubated seeds were examined visually and under stereobinocular microscope for the associated microflora. Associated fungi and bacteria, which could not be identified, were isolated in PDA and nutrient agar for further identification.

3.6.2 Nematode test

For the detection of associated nematodes, 2-3 longitudinal/cross cuts were given to 100 seeds of each category with the help of blade to almost separate the hull from seeds, then the entire mass of those seeds were soaked for overnight in sterilized water and suspension was examined for the presence of nematodes, if any. The nematodes were counted under a stereobinocular microscope.

3.6.3 Agar plate method

Agar plate method, as used by Neergaard (1977), was tried for the detection of seed microflora. Sterilized glass Petriplates (10 cm dia.) containing Potato Dextrose Agar (PDA) were used for the incubation of seeds.

The seeds were treated with 0.1 per cent mercuric chloride for three minutes, then washed three times in sterilized water, and then dried over sterilized blotting papers. Twenty seeds per Petriplates were placed equidistantly aseptically with the help of a sterilized pair of forceps. Total of 100 seeds of 4 category of each variety were tested. The plates were incubated under similar conditions as described under the blotter method. The seeds were examined on 3rd, 5th and 8th day of incubation under stereobinocular microscope for associated microflora.

3.7 Isolation and identification of different pathogens

3.7.1 Isolation

Mycoflora detected on rice seed by different methods tentatively were identified but for confirmation they were isolated on Potato Dextrose Agar (PDA) and bacteria detected transferred on Nutrient Agar medium. As soon as the colonies became visible, they were transferred to slants carefully with the help of sterilized inoculating needle. The inoculated tubes were incubated at 25 $\pm$ 1°C.

3.7.2 Identification

Isolated microflora were identified with the help of literature (Barnett 1962; Booth 1971; Mew and Misra 1994; Mew and Gonzales 2002).

Isolated mycoflora were identified on the basis of colony colour, number of colonies, sporulation, conidial characters and fruiting structures using stereobinocular and compound microscope. The microscopic examination of various fungi isolated in the present study was usually done by mounting a part of the colony in water on a clean slide.

Isolated nematodes were identified by using stereobinocular and compound microscope. As parasitic plant nematodes have stylets, which are not present in nonparasitic nematodes and these were viewed under a stereobinocular microscope with the light source coming from below and some characteristics, such as body morphology.

Isolated bacteria were identified on the basis of shape of the cell (rod shaped, spherical or spiral shaped bacteria), colony characters and pigmentation, gram staining (gram +ve or –ve reaction), presence or absence of extracellular polysaccharides (EPS) or slime layer or capsule.

i. Gram staining (Harley and Prescott 1993)

Reagent required: Crystal violet stain, alcohol, gram's iodine solution, safranin, cedar wood oil.

Procedure: Gram staining of bacteria, a technique used to distinguish whether the bacteria is gram positive or gram negative on the basis of difference in the structure of cell wall. For this, some drops of associated bacterial suspension were spread on a marked area of a clean grease free slide with the help of inoculating loop. The culture on the loop was dried and heat fixing of smear was done by passing the slide through flame called fixing of smear by which bacterium get killed and fixed on slide.

Crystal violet stain was spread on the marked area for about 1 to 2 minutes and washed down the excess stain in running tap water. Slide was put on the blotting sheet and flooded with gram's iodine solution and kept as such for 1 to 2 minutes and washed in running tap water for 1 to 2 minutes. After that few drops of absolute alcohol was spread to remove the stain and washed in the tap water. Slide was then stained with safranin for 1 to 2 minutes and again washed with running tap water for 5 seconds and dried with blotting sheet.

After staining it was observed under microscope at low power and then a few drops of cedar wood oil was put on it and moved the oil immersion lens on the slide so that it should touch the cedar wood oil.

If the bacterial cells remain coloured after staining procedure because of thick wall and appeared blue in colour then reaction was taken as gram positive. But if it did not retain dye in the staining procedure due to thin layered cell wall and appeared pink to red then reaction was taken as gram negative.

ii. Extracellular polysaccharides (EPS) or slime layer

Bacteria often produce the EPS outside the cell envelop. Small and dense layer with definite boundary is called capsule and water soluble, less adherent without definite boundary is refer to as slime layer. Capsule can be observed under the microscope after the capsule staining but the slime layer is difficult to recognize by staining. Bacterial species were identified on the basis of presence or absence of EPS or slime layer. The liquid culture of slime producing bacteria became viscous or glutinous and the colonies on agar media were dome shaped, umbellate and convex with the glistening or shining surface whereas which did not produced EPS were spreading type.

3.8 Seed health parameters as affected by microflora and effect of seed treatments

3.8.2 Germination percentage

Germination of rice seed was made with two methods, in-between paper method and in-soil method.

i. In-between paper method

Germination of 4 categories of each variety of rice used in different experiments was studied by employing rolled paper towel method as described by International Seed Testing Association (ISTA 1985). Three hundred seeds (100 seeds/replication) were used in each case. Two sheets of germination paper were wetted with distilled water and placed above butter paper used as a base for the paper, leaving adequate margin. Three hundred seeds in 3 replications (100 seeds/replication) both untreated and treated with chemicals, Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed and bioagent, *Trichoderma harzianum* talc based formulation @ 4 g/kg seed were sown on it evenly and rolled. Untreated seeds also served as a control for treated seeds. The rolls were placed in incubator at 28°C. Five and 9 days after sowing (DAS), all the normal seedlings were counted and removed from the paper. On the 14 DAS, normal, abnormal, diseased seedlings, and dead seeds were counted and placed separately in plastic Petriplates to examine the lethal seed infection in each case.

ii. In-soil method

From 4 categories of each rice variety 300 seeds both untreated and treated with chemicals, Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed and bioagent, *Trichoderma harzianum* talc based formulation @ 4 g/kg seed were sown in 3 replications in line in the sterilized soil (1-2 cm depth) @ 100 seeds/tray. Untreated seeds also served as a control for chemical and bioagent treated seeds. The trays were then kept in the glasshouse. Shoots rising more than 2 mm above soil surface were counted as germinated. Data were taken at 5 DAS and 9 DAS only for germination and final data were taken on 14 DAS (as final reading) in which normal, abnormal, diseased seedlings and dead seeds were counted in each untreated, chemical treated and bioagent treated seeds trays.

Though germination testing remains the principle and Internationally accepted, criterion for seed viability but for high germination seed lots, germination test result may not provide enough information as to potential seed lot performance. Therefore, vigour status of seed lot becomes important and vigour testing necessary.

3.8.3 Seedling vigour index

Seed vigour is defined as "sum total of those properties of the seed which determines the level of activity and performance of the seed or seed lot during germination and seedling emergence". During in-between paper germination experiments the observations were also recorded on root and shoot length of the 25 normally growing seedlings in all untreated, chemical (Bavistin + Dithane M-45) treated and bioagent (*Trichoderma harzianum*) treated seeds and their average were taken to calculate the seedling vigour index by using the formula given by Abdul Baki and Anderson (1973) as:

Seedling vigour index= (root length + shoot length) X % seed germination

3.9 Lethal seed infection

In the case of in-between paper germination experiments, the abnormal (physiological, diseased and decayed and dead) seeds of each untreated, chemical treated (Bavistin + Dithane M-45) and bioagent (*Trichoderma harzianum*) treated seeds were counted and placed in Petridishes separately for the blotter test and incubated for 4 days. After 4 days of incubation, seeds were examined under a stereobinocular microscope to confirm whether the abnormality or death of the seeds were due to pathogen or physiologically. If the adequate amount of pathogenic organisms was present near the embryo or in the middle portion of the seed or huge amount of necrotrophic organism was present all over the seed, a lethal seed infection was considered to have occurred. Data were taken as the number of seeds or seedlings that died due to lethal seed infection.

3.10 Component analysis

In order to detect whether the associated microflora was internally borne or externally seedborne, component plating technique was employed (Chisholm and Coates-Becford 1997). Analysis of associated microflora in husk and kernel of only discoloured seed category of variety Kasturi Basmati was made. One hundred seeds of discoloured category were separated into husk and kernel. The components were surface sterilized in 0.1 per cent mercuric chloride solution for half minute, one minute, one and a half minutes, two minutes, two and a half minutes and three minutes and control without sterilization, then washed three times with sterilized water and then dried over sterilized blotting sheets then these were transferred to PDA medium with the help of sterilized pair of forceps and incubated at 25 $\pm$ 1°C for 7 days. After incubation, each seed component was observed for the associated microflora visually as well as under stereobinocular microscope.

3.11 Effect of chemical and bioagent treatment on seed microflora

Each rice variety based on 4 categories was tested for effect of chemical and bioagent on seed microflora. Effect of chemical treatments on associated seedborne microflora was studied by blotter method and bioagent treatment effect was studied by agar plate method.

One hundred seeds of each variety having 4 categories after treatment with Bavistin + Dithane M-45 @ (1.25 + 1.25 g/kg seed) were placed in a plastic Petriplates (9 cm dia.) lined with two layers of blotting sheets moistened with distilled water and one layer of blotting sheet on the lid of Petriplates for studying the effect of treatment on associated microflora of seeds (Neergaard 1977). Twenty five seeds were placed in each Petriplates equidistantly and incubated at 25 $\pm$ 1^{O}C for seven days and examined regularly. Incubated seeds were examined visually as well as under stereobinocular microscope for the associated microflora after seed treatment with chemicals.

For studying the effect of bioagent treatment on associated seedborne microflora, one hundred seeds of each variety having 4 categories were treated with *Trichoderma harzianum* talc based formulation @ 4 g/kg of seed under aseptic condition and then placed in the Petriplates having PDA medium aseptically with the help of forcep. Twenty seeds were placed in each Petriplates equidistantly. Plates were then incubated under similar condition as described under chemical treatment method. The plates were examined regularly for studying the effect of bioagent treatment. The seeds were examined visually and under stereobinocular microscope for associated microflora after bioagent treatment.

3.12 Effect of weather parameters during harvesting period on seed microflora

Weather data of locality *viz.*, mean temperature (^{O}C), relative humidity (%) and rainfall (mm) during harvesting time were collected for ascertaining their role in seed associated microflora of different zones.

3.13 Statistical methods

The data of different experiments were subjected to appropriate statistical analysis wherever necessary after using square root and arc sine transformation. Transformed values of each treatment mean have been put in parenthesis. All the data were analyzed in the computer using CPCS-1 software. The significance of difference between the treatments was taken at 5 per cent level of significance.

Results & Discussion

4. RESULTS AND DISCUSSION

The present investigation entitled "Evaluation of seed health of some rice varieties grown in Himachal Pradesh" was undertaken with a view to identify the seedborne microflora of rice seed and their role in causing seedling abnormalities and effect of different treatments on germination and seed vigour.

The results obtained on various aspects are described under the following subheadings:

4.1 Collection and categorization of seed samples
4.2 Studies on seedborne microflora
4.3 Effect of chemical and bioagent treatments on seed microflora
4.4 Germination test (in-soil and in-between paper)
4.5 Effect of chemical treatment on germination
4.6 Effect of bioagent treatment on germination
4.7 Seedling vigour
4.8 Effect of chemical and bioagent treatment on seedling vigour
4.9 Lethal seed infection
4.10 Component analysis
4.11 Weather parameters

4.1 Collection and categorization of seed samples

Twelve rice seed samples comprising of recommended and local varieties were collected from two agroclimatic zones (I and II) of Himachal Pradesh. Cultivated samples of three varieties of rice were collected from farmers of each agroclimatic zones and two recommended varieties from each research station *viz.,* Hill Agriculture Research and Extension Centres, Dhaulakuan and Bajaura and Rice Wheat Research Station, Malan. The detail of which is presented in Table 1.

Seed samples collected were categorized on the basis of their morphological appearance into four categories *viz.,* original seed, apparently healthy, partially discoloured and discoloured seed. The proportion of discolouration of seeds in three categories *viz.,* apparently healthy, partially

discoloured and discoloured seed category is presented in Table 2. The percentage of categories of rice seeds based on discolouration among 12 varieties of rice ranged between 14.3 to 50, 30.3 to 54.3 and 17.3 to 62.5 per cent respectively in three categories.

Table 2. Extent of grain discolouration in different rice seed categories of twelve varieties

Variety	Discolouration (%)		
	Apparently healthy	Partially discoloured	Discoloured
Zone I			
Kasturi Basmati (V_1)	31.5	39.5	29.0
Hybrid-6444 (V_2)	25.8	54.3	20.0
Pusa-1121 (V_3)	30.8	33.5	35.8
Zone II			
Jhumka (V_4)	47.8	35.0	17.3
Parmal (V_5)	44.8	30.3	25.0
Jattoo (V_6)	23.3	54.3	22.5
HAREC, Dhaulakuan (Zone I)			
PAU-201 (V_7)	21.0	53.5	25.5
HKR-126 (V_8)	30.0	51.3	18.8
HAREC, Bajaura (Zone II)			
Nagardhan (V_9)	18.8	48.9	32.4
Yunlen 18 (s) (V_{10})	14.3	23.3	62.5
RWRS, Malan (Zone II)			
HPR-2143 (V_{11})	47.5	32.5	20.0
HPR-1068 (V_{12})	50.0	32.5	17.5

The extent of apparently healthy seeds was highest in RWRS, Malan (Zone II) collected samples (47.5 to 50%), followed by farmers' seed samples (23.3 to 47.8%) of same zone. Among varieties highest percentage of apparently healthy seed was in variety HPR-1068 (V_{12}) followed by variety Jhumka (V_4) and lowest percentage was observed in variety Yunlen 18 (s) (V_{10}) (14.3%). However, maximum number of partially discoloured seeds were noticed HAREC, Dhaulakuan (Zone 1) collected varieties (51.3 to 53.5%) followed by seed samples collected from farmers (33.5 to 54.3%) of same zone. Varieties Hybrid-6444 (V_2) and Jattoo (V_6) had highest number of partially discoloured seed (54.3%) and least percentage on variety Yunlen 18 (s) (V_{10}) (23.3%). Maximum percentage of discoloured seeds was observed in HAREC, Bajaura (Zone II) collected varieties (32.4 to 62.5%) with highest percentage on variety Yunlen 18 (s) (V_{10}) (62.5%) and least variety Jhumka (V_4) (17.3%).

Maximum number of partially discoloured seeds was observed in samples collected from farmers of Zone I and minimum from Zone II whereas, maximum percentage of discoloured seeds was observed in samples collected from HAREC, Bajaura (Zone II) (62.5%). Different climatic condition prevailing in these two zones and varietal differences might have accounted for difference in seed discolouration. The conditions like high rainfall along with comparatively higher temperature during cropping season also effect the activity of other microbes which might lead to discolouration. Consequently, these conditions affect the general plant growth and seed health during and after the harvesting of crop (Tarr 1955; Frederiksen 1974; Neergaard 1977).

4.2 Studies on seedborne microflora

The fungi, bacteria and nematodes associated with the seed samples of rice were detected by different methods:

4.2.1 Blotter method

The per cent incidence of microflora associated with 4 categories of each rice variety as detected by blotter method is given in Tables 3 to 6.

The data (Tables 3 to 6) indicated that in total 15 fungi *viz., Alternaria alternata* (3 to 49%), *A. padwickii* (7 to 13%), *Aspergillus* sp. (1 to 3%), *Chaetomium* sp. (5 to 15%), *Curvularia lunata* (2 to 54%), *Drechslera oryzae* (1 to 71%), *Epicoccum purpurascens* (2 to 28%), *Fusarium moniliforme* (1 to 23%), *Fusarium solani* (3 to 25%), *Fusarium* sp. (2 to 25%), *Mucor* sp. (1 to 2%), *Penicillium* sp. (1 to 2%), *Phoma sorghina* (1 to 71%), *Pyricularia oryzae* (5 to 15%), *Rhizopus stolonifer* (2 to 9%) and few non-sporulating fungus (1 to 3%) were found associated with 4 categories of each rice variety tested.

Significant variation with respect to associated microflora were observed in 4 seed categories and different varieties. Seed samples of Zone I showed the presence of 11 species of fungi on variety Kasturi Basmati (V_1) from original, 9 from apparently healthy and partially discoloured and 10 from discoloured seed category whereas 7 species were detected from variety Hybrid-6444 (V_2) in all categories except apparently healthy seed category which showed the presence of 6 fungal species and 3 species on variety Pusa-1121 (V_3) from original and partially discoloured, 2 from apparently healthy and 5 from discoloured seed category were recorded. In 3 rice varieties collected from this Zone; *Fusarium solani* and *Fusarium* sp. were recorded on 100 per cent samples in all four seed categories with a frequency ranging from 2 to 25 per cent with higher incidence in discoloured and partially discoloured seed category (25%). Frequency was lower (2 to 12%) in apparently healthy seed category. The frequency of *Alternaria alternata*, *Curvularia lunata* and *Epicoccum purpurascens* and *Fusarium moniliforme* were recorded on 66.7 per cent samples in 2 categories (original and partially discoloured), *Alternaria alternata, Curvularia lunata* and *Fusarium moniliforme* in apparently healthy and *Alternaria alternata*, *Epicoccum purpurascens* and *Fusarium moniliforme* in discoloured seed category. A higher frequency of *Curvularia lunata* in each category (ranging from 17 to 54 %) with a higher percentage in discoloured seed category (54%) and lower in apparently healthy seed category (17%) was observed. A*spergillus* sp., *Drechslera oryzae*, *Mucor* sp., *Penicillium* sp., *Phoma sorghina*, *Rhizopus stolonifer* and non-sporulating fungus colonizing 33.3 per cent samples with an incidence of 1 to 24

per cent was recorded in four seed categories. The maximum microflora was recorded from variety V_1 in all the categories with maximum frequency in discoloured seed category and low per cent frequency in apparently healthy seed category while minimum microflora was detected from variety V_3.

Seed samples collected from Zone II showed the presence of 5 fungal species on variety Jhumka (V_4) from all categories, 6 species on variety Parmal (V_5) from original and partially discoloured, 7 from apparently healthy and discoloured seed category and 7 species on Jattoo (V_6) from original and 6 from rest of the categories. *Drechslera oryzae* was predominant fungus recorded on 100 per cent samples collected from Zone II in 3 categories of seed (original, partially discoloured and discoloured) with an incidence of 1 to 71 per cent. Frequency of *D. oryzae* was higher in variety V_6 and lower in variety V_4 in all the categories followed by *Fusarium solani* with 100 per cent incidence in all seed categories and *Fusarium* sp. from 100 per cent samples in apparently healthy and discoloured seed categories and 66.7 per cent in original and partially discoloured seed categories. *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliforme* were also present on the seeds on V_5 and V_6, but were were not detected from V_4. *Alternaria padwickii, Chaetomium* sp., *Phoma sorghina* were present in 33.3 per cent samples with frequency of 3 to 71 per cent. *Phoma sorghina* was predominant fungal species in V_4 with higher frequency on original seed category (71%) and lower on healthy seed category (37%).

The seed samples collected from research station HAREC, Dhaulakuan (Zone 1) revealed the presence of total 5 fungal species in variety PAU-201 (V_7) in original and discoloured and 4 from apparently healthy and partially discoloured seed categories and 3 in variety HKR-126 (V_8) in each seed category. *Fusarium moniliforme* and *Fusarium solani* were detected from 100 per cent seed samples in each category followed by *Phoma sorghina* which was detected from 100 per cent seed samples in original and discoloured seed category. *Fusarium* sp. and *Epicoccum purpurascens* were detected from 50 per cent samples. The frequency of fungal microflora was minimum (1 to 7%) in variety V_8 in all the seed categories.

Table 3. Frequency (%) of rice seed microflora in original seed through blotter method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	10.0	0.0	2.0	0.0	53.0	0.0	26.0	14.0	10.0	10.0	2.0	2.0	0.0	0.0	9.0	1.0	11
V_2	11.0	0.0	0.0	0.0	24.0	14.0	2.0	0.0	15.0	12.0	0.0	0.0	5.0	0.0	0.0	0.0	7
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	9.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	3
% Sample mean	66.7	0.0	33.3	0.0	66.7	33.3	66.7	66.7	100.0	100.0	33.3	33.3	33.3	0.0	33.3	33.3	
Zone II																	
V_4	0.0	0.0	0.0	5.0	0.0	3.0	0.0	0.0	20.0	4.0	0.0	0.0	71.0	0.0	0.0	0.0	5
V_5	8.0	10.0	0.0	0.0	12.0	25.0	0.0	8.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
V_6	47.0	0.0	0.0	0.0	9.0	71.0	0.0	6.0	10.0	11.0	0.0	0.0	0.0	0.0	0.0	1.0	7
% Sample mean	66.7	33.3	0.0	33.3	66.7	100.0	0.0	66.7	100.0	66.7	0.0	0.0	33.3	0.0	0.0	33.3	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	10.0	15.0	10.0	7.0	0.0	0.0	1.0	0.0	0.0	0.0	5
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	5.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	3
% Sample mean	0.0	0.0	0.0	0.0	0.0	0.0	50.0	100.0	100.0	50.0	0.0	0.0	100.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	35.0	13.0	3.0	0.0	30.0	45.0	0.0	16.0	9.0	25.0	0.0	1.0	40.0	8.0	0.0	0.0	11
V_{10}	12.0	0.0	0.0	0.0	46.0	40.0	20.0	18.0	8.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	7
% Sample mean	100.0	50.0	50.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	50.0	50.0	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	20.0	0.0	0.0	0.0	29.0	26.0	0.0	19.0	10.0	8.0	0.0	0.0	22.0	15.0	0.0	1.0	9
V_{12}	12.0	0.0	0.0	0.0	42.0	22.0	0.0	23.0	9.0	20.0	0.0	0.0	5.0	0.0	0.0	0.0	7
% Sample mean	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	100.0	50.0	0.0	50.0	

***On the basis of 100 seeds tested of each cultivar**

Table 4. Frequency (%) of rice seed microflora in apparently healthy seed through blotter method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	4.0	0.0	1.0	0.0	41.0	0.0	9.0	5.0	12.0	8.0	1.0	0.0	0.0	0.0	2.0	0.0	9
V_2	8.0	0.0	0.0	0.0	17.0	10.0	0.0	0.0	6.0	5.0	0.0	0.0	3.0	0.0	0.0	0.0	6
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	10.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	2
% Sample mean	66.7	0.0	33.3	0.0	66.7	33.3	33.3	66.7	100.0	100.0	33.3	0.0	33.3	0.0	33.3	0.0	
Zone II																	
V_4	0.0	0.0	0.0	5.0	0.0	1.0	0.0	0.0	25.0	12.0	0.0	0.0	37.0	0.0	0.0	0.0	5
V_5	3.0	7.0	0.0	0.0	6.0	15.0	0.0	10.0	10.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	7
V_6	20.0	0.0	0.0	0.0	4.0	40.0	0.0	10.0	15.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	66.7	33.3	0.0	33.3	66.7	100.0	0.0	33.3	100.0	100.0	0.0	0.0	33.3	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	5.0	15.0	15.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	4
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	3
% Sample mean	0.0	0.0	0.0	0.0	0.0	0.0	50.0	100.0	100.0	50.0	0.0	0.0	50.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	20.0	10.0	0.0	0.0	18.0	30.0	0.0	9.0	11.0	20.0	0.0	1.0	28.0	5.0	0.0	0.0	9
V_{10}	7.0	0.0	0.0	0.0	30.0	25.0	10.0	15.0	14.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	7
% Sample mean	100.0	50.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	50.0	50.0	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	12.0	0.0	0.0	0.0	19.0	17.0	0.0	9.0	11.0	5.0	0.0	0.0	23.0	5.0	0.0	3.0	9
V_{12}	10.0	0.0	0.0	0.0	28.0	10.0	0.0	15.0	17.0	15.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	50.0	50.0	0.0	50.0	

*On the basis of 100 seeds tested of each cultivar

Table 5. Frequency (%) of rice seed microflora in partially discoloured seed through blotter method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	7.0	0.0	3.0	0.0	53.0	0.0	10.0	5.0	10.0	15.0	1.0	1.0	0.0	0.0	3.0	0.0	9
V_2	13.0	0.0	0.0	0.0	34.0	17.0	2.0	0.0	25.0	5.0	0.0	0.0	10.0	0.0	0.0	0.0	7
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	10.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	3
% Sample mean	66.7	0.0	33.3	0.0	66.7	33.3	66.7	66.7	100.0	100.0	33.3	33.3	33.3	33.3	33.3	0.0	
Zone II																	
V_4	0.0	0.0	0.0	8.0	0.0	1.0	0.0	0.0	25.0	5.0	0.0	0.0	65.0	0.0	0.0	0.0	5
V_5	4.0	8.0	0.0	0.0	14.0	30.0	0.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
V_6	41.0	0.0	0.0	0.0	10.0	60.0	0.0	9.0	10.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	66.7	33.3	0.0	33.3	66.7	100.0	0.0	66.7	100.0	66.7	0.0	0.0	33.3	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	5.0	12.0	15.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	4
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	3
% Sample mean	0.0	0.0	0.0	0.0	0.0	0.0	50.0	100.0	100.0	50.0	0.0	0.0	50.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	40.0	7.0	0.0	0.0	35.0	40.0	0.0	15.0	14.0	24.0	0.0	0.0	45.0	7.0	0.0	0.0	9
V_{10}	15.0	0.0	0.0	0.0	45.0	35.0	25.0	10.0	12.0	22.0	0.0	0.0	0.0	0.0	0.0	0.0	7
% Sample mean	100.0	50.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	0.0	50.0	50.0	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	26.0	0.0	0.0	0.0	32.0	25.0	0.0	6.0	10.0	7.0	0.0	0.0	25.0	13.0	0.0	2.0	9
V_{12}	15.0	0.0	0.0	0.0	45.0	18.0	0.0	18.0	9.0	20.0	0.0	0.0	5.0	0.0	0.0	0.0	7
% Sample mean	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	100.0	50.0	0.0	50.0	

*On the basis of 100 seeds tested of each cultivar

Table 6. Frequency (%) of rice seed microflora in discoloured seed through blotter method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	9.0	0.0	2.0	0.0	54.0	0.0	28.0	5.0	15.0	20.0	1.0	1.0	0.0	0.0	0.0	2.0	10
V_2	16.0	0.0	0.0	0.0	34.0	24.0	3.0	0.0	25.0	15.0	0.0	0.0	8.0	0.0	0.0	0.0	7
V_3	0.0	0.0	0.0	0.0	2.0	0.0	0.0	2.0	15.0	3.0	0.0	0.0	5.0	0.0	0.0	0.0	5
% Sample mean	66.7	0.0	33.3	0.0	100.0	33.3	66.7	66.7	100.0	100.0	33.3	33.3	66.7	0.0	0.0	33.3	
Zone II																	
V_4	0.0	0.0	0.0	15.0	0.0	3.0	0.0	0.0	25.0	10.0	0.0	0.0	69.0	0.0	0.0	0.0	5
V_5	10.0	11.0	0.0	0.0	15.0	35.0	0.0	3.0	18.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	7
V_6	49.0	0.0	0.0	0.0	14.0	71.0	0.0	6.0	11.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	66.7	33.3	0.0	33.3	66.7	100.0	0.0	66.7	100.0	100.0	0.0	0.0	33.3	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	14.0	12.0	15.0	5.0	0.0	0.0	1.0	0.0	0.0	0.0	5
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	3.0	0.0	0.0	0.0	7.0	0.0	0.0	0.0	3
% Sample mean	0.0	0.0	0.0	0.0	0.0	0.0	50.0	100.0	100.0	50.0	0.0	0.0	100.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	40.0	13.0	0.0	0.0	35.0	40.0	0.0	15.0	14.0	25.0	0.0	0.0	35.0	10.0	0.0	0.0	9
V_{10}	18.0	0.0	0.0	0.0	50.0	40.0	25.0	12.0	15.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	7
% Sample mean	100.0	50.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	0.0	50.0	50.0	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	33.0	0.0	0.0	0.0	40.0	31.0	0.0	16.0	15.0	10.0	0.0	0.0	33.0	13.0	0.0	3.0	9
V_{12}	15.0	0.0	0.0	0.0	50.0	23.0	0.0	13.0	12.0	10.0	0.0	0.0	5.0	0.0	0.0	0.0	7
% Sample mean	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	100.0	50.0	0.0	50.0	

***On the basis of 100 seeds tested of each cultivar**

However, seed samples collected from HAREC, Bajaura (Zone II) showed the presence of maximum 11 fungal species in variety Nagardhan (V_9) and 7 in variety Yunlen 18 (s) (V_{10}). *Alternaria alternata*, *Curvularia lunata, Drechslera oryzae*, *Fusarium moniliforme, Fusarium solani*, *Fusarium* sp. were detected in 100 per cent seed samples in all the category with a frequency ranging from 7 to 50 per cent with higher frequency on discoloured seed category (12 to 50%) and lower frequency on apparently healthy seeds (7 to 30%). *Alternaria padwickii, Epicoccum purpurascens, Penicillium sp., Phoma sorghina, Pyricularia oryzae* were also detected from 50 per cent seed samples of each category.

Alternaria alternata, *Curvularia lunata, Drechslera oryzae*, *Fusarium moniliforme, Fusarium solani*, *Fusarium* sp., *Phoma sorghina* were detected from 100 per cent seed samples collected from RWRS, Malan in 3 categories except from apparently healthy seed category all such other 6 fungal species were present on apparently healthy seed category except *Phoma sorghina* and it was not detected from variety HPR-1068 (V_{12}). *Pyricularia oryzae* was only detected from variety HPR-2143 (V_{11}) of all seed categories and frequency of detection ranged between 5 to 15 per cent.

The maximum mycoflora was detected from variety V_1 followed by V_9. *Curvularia lunata* was found to be predominant on variety V_1 with a frequency ranging from 41 to 54 per cent whereas *Drechslera oryzae* was predominant on seed samples of variety V_9 with a frequency ranging between 30 to 45 per cent. *Curvularia lunata* and *Drechslera oryzae* were found in higher frequency than the other fungi in each category and *Fusarium solani* was detected from all the varieties with 100 per cent sample mean (Figure 1 to 3). The frequency of *D. oryzae* was highest on variety V_6 ranging between 40 to 71 per cent. Least frequency of mycoflora were detected from variety V_8.

4.2.2 Agar plate method

The per cent incidence of microflora externally and internally associated with 4 categories of each rice variety on agar plate method is given in Tables 7 to 10.

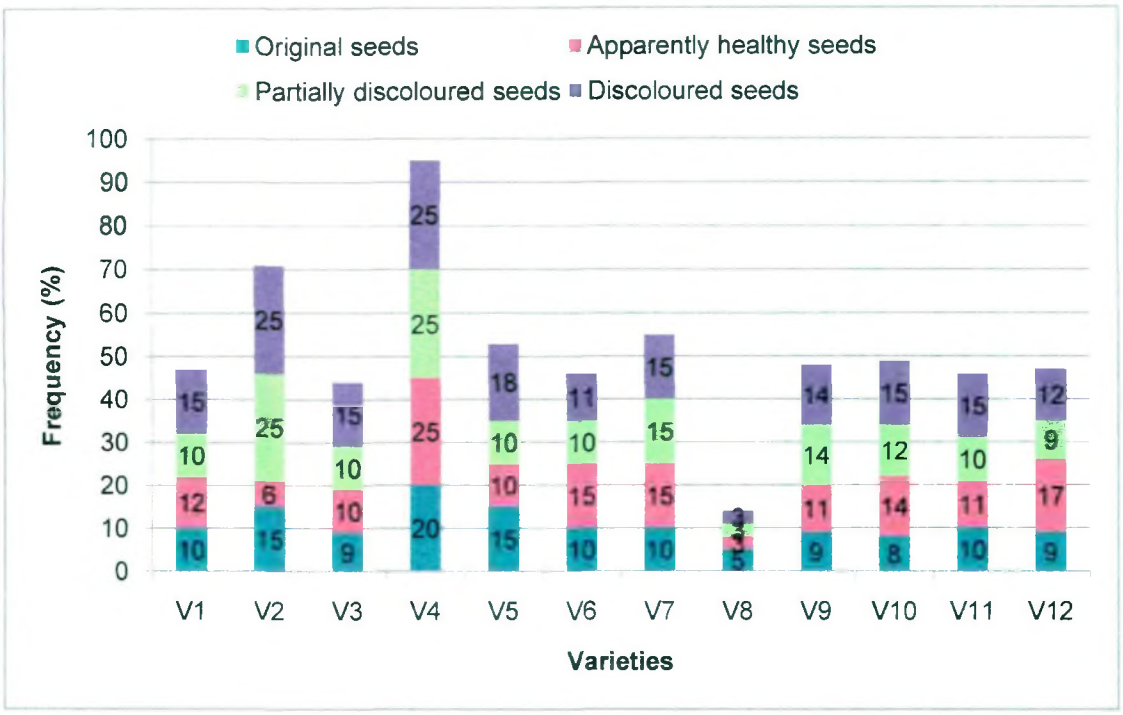


Figure 1. Frequency (%) of *Fusarium solani* in four categories of rice varieties through blotter method

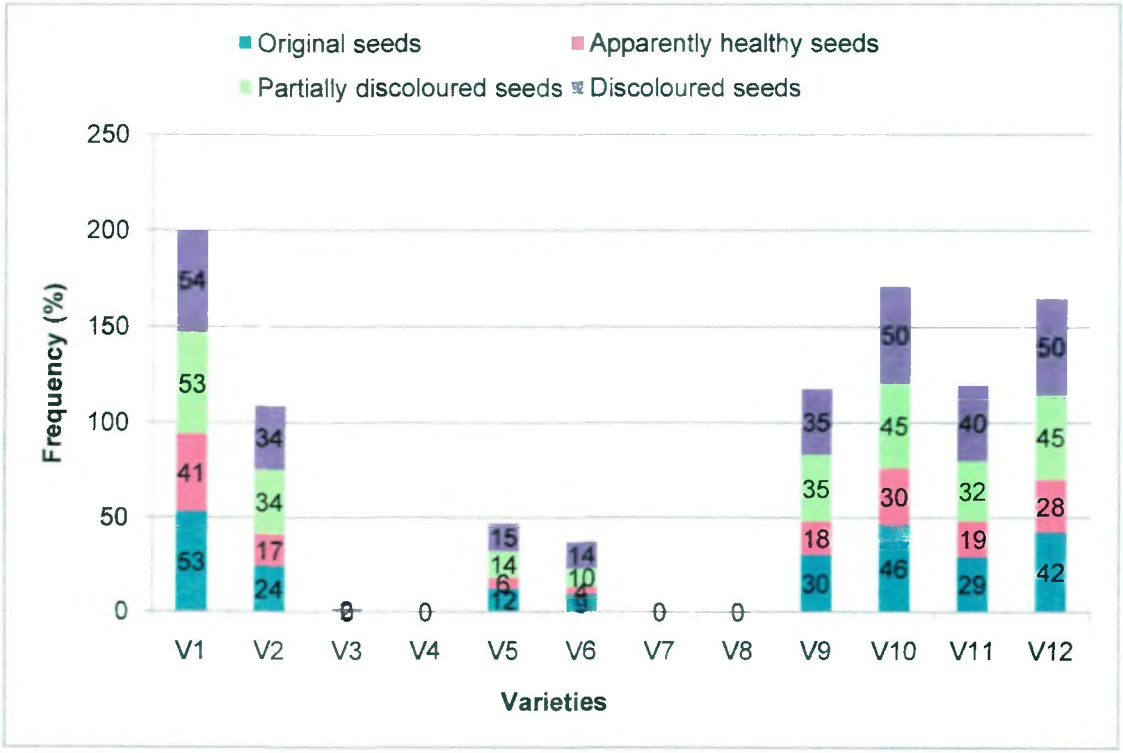


Figure 2. Frequency (%) of *Curvularia lunata* in four categories of rice varieties through blotter method

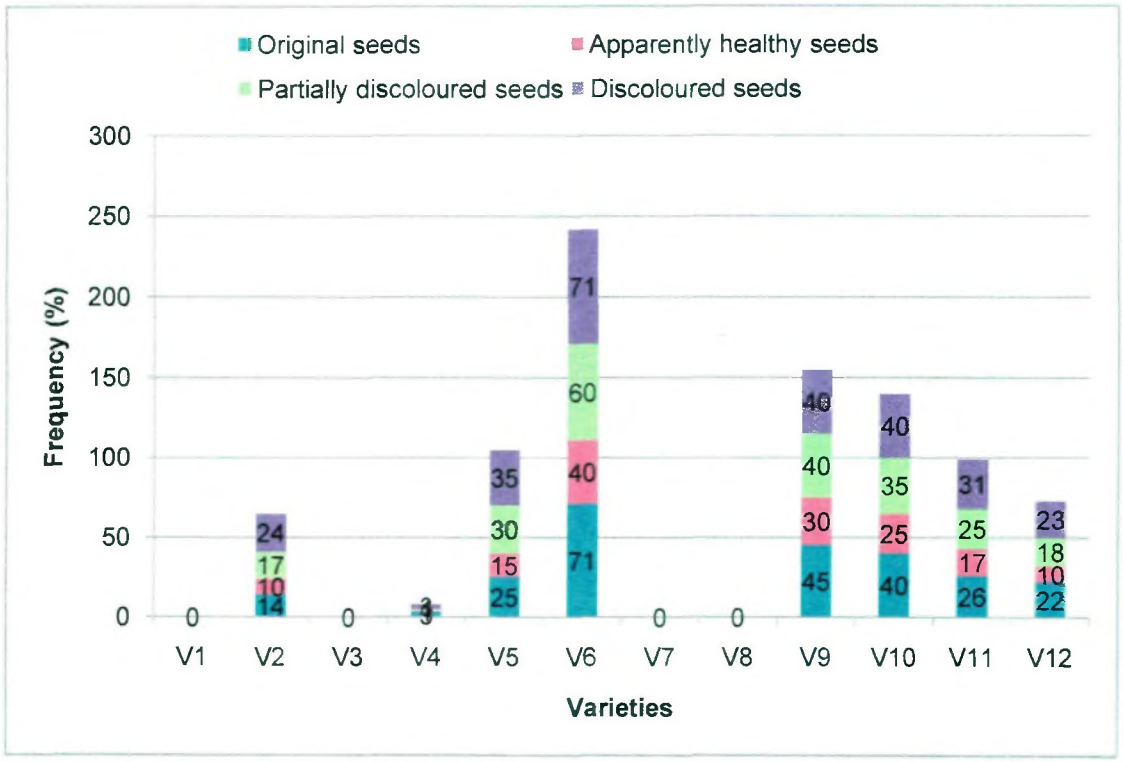


Figure3. Frequency (%) of *Drechslera oryzae* in four categories of rice varieties through blotter method

The data presented in Tables 7 to 10 showed that 17 fungi *viz., Alternaria alternata* (2 to 27%), *Alternaria padwickii* (1 to 11%), *Aspergillus* sp. (1 to 52%), *Chaetomium olivaceum* (16 to 29%), *Chaetomium* sp. (3 to 35%), *Curvularia lunata* (3 to 54%), *Drechslera oryzae* (2 to 60%), *Epicoccum purpurascens* (2 to 11%), *Fusarium moniliforme* (3 to 25%), *Fusarium solani* (5 to 30%), *Fusarium* sp. (2 to 12%), *Mucor* sp. (2 to 5%), *Penicillium* sp. (1 to 6%), *Phoma sorghina* (2 to 23%), *Rhizopus stolonifer* (15 to 37%), *Rhizoctonia* sp. (2 to 3%), and some non-sporulating fungi (2 to 5) and 1 bacterium *Xanthomonas* sp. (2 to 85%) were found associated with 4 seed categories of each rice variety.

Significant variations in microflora associated with seeds was observed in 4 seed categories and different varieties (Plate 2). In Zone I, 15 species were detected from variety V_1 on original and discoloured seed categories, 12 species from apparently healthy seed category and 13 species from partially discoloured seed category whereas, 8 species were detected from variety V_2 of original, partially discoloured and discoloured seed category and 6 species from apparently healthy seed category. Five species were recorded on variety V_3 from original, apparently healthy and partially discoloured seed categories and 7 species from discoloured seed category. *Fusarium* sp. and *Fusarium solani* were recorded on 100 per cent samples in all the categories with a frequency ranging between 2 to 30 per cent with maximum frequency in discoloured seed category (30%) and least in apparently healthy seed category (2%). *Aspergillus* sp. was recorded from 100 per cent samples of original, partially discoloured and discoloured seed category but with less frequency (2 to 6%). The frequency of *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliforme*, *Mucor* sp., *Penicillium* sp., and *Xanthomonas* sp. recorded on 66.7 per cent samples on each seed category (ranging from 1 to 70%). *Chaetomium olivaceum*, *Chaetomium* sp., *Drechslera oryzae*, *Epicoccum purpurascens*, *Rhizopus stolonifer*, *Rhizoctonia* sp. and some non-sporulating fungus were found to be present in only 33.3 per cent samples with a frequency of 2 to 35 per cent in all four seed categories. The maximum microflora were recorded from V_1 in all the

Table 7. Frequency (%) of rice seed microflora in original seed through agar plate method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium olivaceum*	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Rhizopus stolonifer*	*Rhizoctonia* sp.	Non-sporulating fungus	*Xanthomonas* sp.	Total microflor
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Zone I																			
V_1	5.0	0.0	2.0	25.0	28.0	54.0	0.0	5.0	5.0	9.0	3.0	2.0	1.0	0.0	15.0	3.0	2.0	5.0	15
V_2	10.0	0.0	6.0	0.0	0.0	48.0	16.0	0.0	0.0	10.0	5.0	2.0	3.0	0.0	0.0	0.0	0.0	0.0	8
V_3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	5.0	7.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	65.0	5
% Sample mean	66.7	0.0	100.0	33.3	33.3	66.7	33.3	33.3	66.7	100.0	100.0	66.7	66.7	0.0	33.3	33.3	0.0	66.7	
Zone II																			
V_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.0	2.0	0.0	0.0	20.0	0.0	0.0	0.0	5.0	4
V_5	10.0	11.0	0.0	0.0	5.0	15.0	25.0	0.0	3.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	8
V_6	0.0	0.0	0.0	0.0	0.0	6.0	52.0	0.0	4.0	10.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5
% Sample mean	33.3	33.3	0.0	0.0	33.3	66.7	66.7	0.0	66.7	100.0	66.7	0.0	0.0	33.3	0.0	0.0	0.0	66.7	
HAREC, Dhaulakuan	**(Zone I)**																		
V_7	6.0	0.0	0.0	0.0	0.0	18.0	7.0	2.0	12.0	9.0	5.0	0.0	3.0	0.0	25.0	0.0	3.0	0.0	10
V_8	8.0	0.0	0.0	0.0	0.0	20.0	12.0	0.0	5.0	30.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	100.0	0.0	0.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	0.0	50.0	0.0	50.0	0.0	
HAREC, Bajaura	**(Zone II)**																		
V_9	0.0	0.0	35.0	0.0	0.0	14.0	14.0	0.0	18.0	12.0	6.0	5.0	0.0	2.0	0.0	0.0	0.0	80.0	9
V_{10}	12.0	0.0	6.0	16.0	20.0	35.0	40.0	0.0	20.0	12.0	11.0	2.0	3.0	0.0	0.0	0.0	0.0	0.0	11
% Sample mean	50.0	0.0	100.0	50.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	50.0	50.0	0.0	0.0	0.0	50.0	
RWRS, Malan	**(Zone II)**																		
V_{11}	20.0	0.0	0.0	0.0	0.0	35.0	30.0	0.0	25.0	14.0	8.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	7
V_{12}	5.0	0.0	1.0	0.0	10.0	25.0	10.0	0.0	15.0	10.0	12.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
% Sample mean	100.0	0.0	50.0	0.0	50.0	100.0	100.0	0.0	100.0	100.0	50.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	

*On the basis of 100 seeds tested of each cultivar

Table 8. Frequency (%) of rice seed microflora in apparently healthy seed through agar plate method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium olivaceum*	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Rhizopus stolonifer*	*Rhizoctonia* sp.	Non-sporulating fungus	*Xanthomonas* sp.	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Zone I																			
V_1	2.0	0.0	0.0	16.0	23.0	37.0	0.0	5.0	5.0	9.0	3.0	4.0	2.0	0.0	0.0	3.0	0.0	3.0	12
V_2	11.0	0.0	0.0	0.0	0.0	25.0	14.0	0.0	0.0	18.0	5.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	6
V_3	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	8.0	9.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0	5
% Sample mean	66.7	0.0	33.3	33.3	33.3	66.7	33.3	33.3	66.7	100.0	100.0	33.3	66.7	0.0	0.0	33.3	0.0	66.7	
Zone II																			
V_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	15.0	0.0	0.0	0.0	2.0	4
V_5	2.0	1.0	0.0	0.0	0.0	3.0	15.0	0.0	9.0	10.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	7
V_6	0.0	0.0	0.0	0.0	0.0	3.0	29.0	0.0	7.0	12.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5
% Sample mean	33.3	33.3	0.0	0.0	0.0	66.7	66.7	0.0	66.7	100.0	100.0	0.0	0.0	33.3	0.0	0.0	0.0	66.7	
HAREC, Dhaulakuan	**(Zone I)**																		
V_7	3.0	0.0	0.0	0.0	0.0	10.0	3.0	3.0	10.0	15.0	5.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	8
V_8	5.0	0.0	0.0	0.0	0.0	15.0	10.0	0.0	8.0	20.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	100.0	0.0	0.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	
HAREC, Bajaura	**(Zone II)**																		
V_9	0.0	0.0	25.0	0.0	0.0	16.0	10.0	0.0	12.0	15.0	6.0	2.0	0.0	0.0	0.0	0.0	0.0	80.0	8
V_{10}	8.0	0.0	5.0	9.0	15.0	28.0	29.0	0.0	17.0	19.0	11.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	11
% Sample mean	50.0	0.0	100.0	50.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	50.0	0.0	0.0	0.0	0.0	50.0	1
RWRS, Malan	**(Zone II)**																		
V_{11}	15.0	0.0	0.0	0.0	0.0	20.0	25.0	0.0	5.0	16.0	10.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	7
V_{12}	3.0	0.0	3.0	0.0	3.0	15.0	2.0	0.0	6.0	19.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
% Sample mean	100.0	0.0	50.0	0.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	

*On the basis of 100 seeds tested of each cultivar

Table 9. Frequency (%) of rice seed microflora in partially discoloured seed through agar plate method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium olivaceum*	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Rhizopus stolonifer*	*Rhizoctonia* sp.	Non-sporulating fungus	*Xanthomonas* sp.	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Zone I																			
V_1	5.0	0.0	2.0	29.0	35.0	51.0	0.0	3.0	6.0	10.0	5.0	4.0	4.0	0.0	0.0	2.0	2.0	2.0	13
V_2	17.0	0.0	6.0	0.0	0.0	52.0	19.0	0.0	0.0	20.0	8.0	4.0	5.0	0.0	0.0	0.0	0.0	0.0	8
V_3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	3.0	10.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	70.0	5
% Sample mean	66.7	0.0	100.0	33.3	33.3	66.7	33.3	33.3	66.7	100.0	100.0	66.7	66.7	0.0	0.0	33.3	33.3	66.7	
Zone II																			
V_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0	6.0	0.0	0.0	23.0	0.0	0.0	0.0	3.0	4
V_5	5.0	3.0	0.0	0.0	6.0	14.0	17.0	0.0	9.0	12.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	15.0	8
V_6	0.0	0.0	0.0	0.0	0.0	5.0	60.0	0.0	2.0	13.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
% Sample mean	33.3	33.3	0.0	0.0	33.3	66.7	66.7	0.0	66.7	100.0	66.7	0.0	0.0	33.3	0.0	0.0	0.0	66.7	
HAREC, Dhaulakuan	**(Zone I)**																		
V_7	5.0	0.0	0.0	0.0	0.0	3.0	4.0	10.0	10.0	13.0	2.0	0.0	5.0	0.0	15.0	0.0	0.0	0.0	9
V_8	10.0	0.0	0.0	0.0	0.0	15.0	14.0	0.0	14.0	27.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	100.0	0.0	0.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0	
HAREC, Bajaura	**(Zone II)**																		
V_9	0.0	0.0	40.0	0.0	0.0	21.0	15.0	10.0	10.0	14.0	2.0	2.0	4.0	0.0	0.0	0.0	0.0	85.0	9
V_{10}	10.0	0.0	4.0	20.0	22.0	37.0	42.0	0.0	15.0	15.0	12.0	4.0	3.0	0.0	0.0	0.0	0.0	0.0	11
% Sample mean	50.0	0.0	100.0	50.0	50.0	100.0	100.0	50.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	50.0	
RWRS, Malan	**(Zone II)**																		
V_{11}	25.0	0.0	0.0	0.0	0.0	35.0	29.0	0.0	14.0	16.0	10.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	7
V_{12}	3.0	0.0	2.0	0.0	8.0	30.0	12.0	0.0	5.0	10.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
% Sample mean	100.0	0.0	50.0	0.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	

***On the basis of 100 seeds tested of each cultivar**

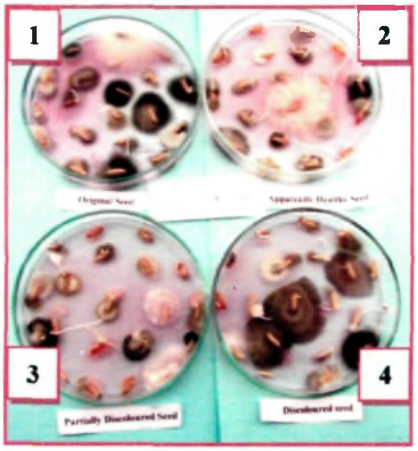
Table 10. Frequency (%) of rice seed microflora in discoloured seed through agar plate method*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* olivaceum	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Rhizopus stolonifer*	*Rhizoctonia* sp.	Non-sporulating fungus	*Xanthomonas* sp.	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Zone I																			
V_1	6.0	0.0	3.0	22.0	25.0	46.0	0.0	7.0	5.0	5.0	2.0	3.0	5.0	0.0	37.0	3.0	5.0	2.0	15
V_2	15.0	0.0	4.0	0.0	0.0	34.0	28.0	0.0	0.0	30.0	11.0	2.0	3.0	0.0	0.0	0.0	0.0	0.0	8
V_3	0.0	0.0	1.0	0.0	0.0	2.0	0.0	0.0	5.0	10.0	2.0	0.0	0.0	5.0	0.0	0.0	0.0	70.0	7
% Sample mean	66.7	0.0	100.0	33.3	33.3	100.0	33.3	33.3	66.7	100.0	100.0	66.7	66.7	33.3	33.3	33.3	33.3	66.7	
Zone II																			
V_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	22.0	0.0	0.0	0.0	2.0	4
V_5	7.0	3.0	0.0	0.0	8.0	10.0	20.0	0.0	10.0	12.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	15.0	8
V_6	2.0	0.0	0.0	0.0	0.0	7.0	43.0	0.0	5.0	10.0	10.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	7
% Sample mean	66.7	33.3	0.0	0.0	33.3	66.7	66.7	0.0	66.7	100.0	100.0	0.0	33.3	33.3	0.0	0.0	0.0	66.7	
HAREC, Dhaulakuan	**(Zone I)**																		
V_7	6.0	0.0	0.0	0.0	0.0	15.0	2.0	11.0	14.0	16.0	2.0	0.0	6.0	0.0	25.0	0.0	0.0	0.0	9
V_8	12.0	0.0	0.0	0.0	0.0	25.0	15.0	0.0	6.0	29.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
% Sample mean	100.0	0.0	0.0	0.0	0.0	100.0	100.0	50.0	100.0	100.0	100.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0	
HAREC, Bajaura	**(Zone II)**																		
V_9	0.0	0.0	52.0	0.0	0.0	20.0	17.0	0.0	16.0	15.0	5.0	2.0	3.0	0.0	0.0	0.0	0.0	85.0	9
V_{10}	12.0	0.0	6.0	20.0	22.0	39.0	42.0	0.0	18.0	20.0	10.0	4.0	6.0	0.0	0.0	0.0	0.0	0.0	11
% Sample mean	50.0	0.0	100.0	50.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	50.0	
RWRS, Malan	**(Zone II)**																		
V_{11}	27.0	0.0	0.0	0.0	0.0	30.0	35.0	0.0	10.0	20.0	10.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	7
V_{12}	2.0	0.0	2.0	0.0	15.0	20.0	15.0	0.0	12.0	16.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
% Sample mean	100.0	0.0	50.0	0.0	50.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	

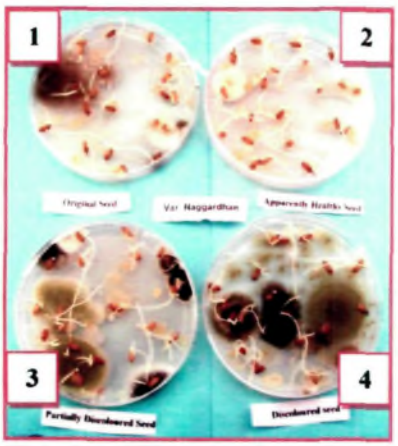
***On the basis of 100 seeds tested of each cultivar**

Pusa –1121 (Zone I)

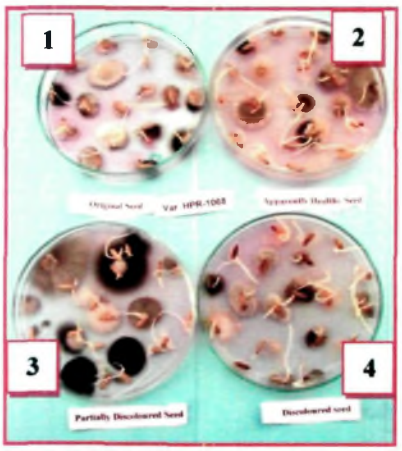
Parmal (Zone II)



HKR-126 (HAREC, Dhaulakuan Zone I)



Nagardhan (HAREC, Bajaura Zone II)



HPR-1068 (RWRS, Malan Zone II)

Plate 2. **Detection of seed microflora from different rice seed categories; original (1); apparently healthy (2); partially discoloured (3); and discoloured (4) of five varieties by agar plate method**

seed categories with highest frequency of *Curvularia lunata* (54%) in original seed category and lower in apparently healthy seed category (37%). *Xanthomonas* sp. was not detected from samples of variety V_2 in any of the seed categories from Zone I.

Seed samples of Zone II, showed the association of 4 microbial species on variety V_4 in all the seed categories, 8 species on original, partially discoloured and discoloured seed categories and 7 species on apparently healthy seed category of variety V_5 and 5 species were detected from original and apparently healthy, 4 species from partially discoloured and 7 species from discoloured seed categories on variety V_6. *Fusarium solani* from original seed and partially discoloured seed categories and *F. solani* and *Fusarium* sp. were detected from 100 per cent samples in apparently healthy and discoloured seed categories. *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme* and *Xanthomonas* sp. were detected from 66.7 per cent samples in each seed category with a frequency ranging from 3 to 60 per cent. *Alternaria alternata*, *A. padwickii*, *Chaetomium* sp. and *Phoma sorghina* frequency were recorded only from 33.3 per cent samples in all seed categories with higher frequency from partially discoloured seed (23%) and lower on apparently healthy seed category (1%). The maximum number of microflora were detected from V_5 having highest frequency of *Drechslera oryzae* in each category, with highest frequency on original seed category (25%) and lowest on apparently healthy seed category (15%). However, low percentage of microflora were detected from variety V_4 with higher frequency of *Fusarium solani* (20 to 25%) in all seed categories.

In the two seed samples collected from HAREC, Dhaulakuan, 10 species were detected from original seed category, 8 from apparently healthy seed and 9 from partially discoloured and discoloured seed categories on variety V_7 and 6 species were detected from each seed category in variety V_8. *Alternaria alternata*, *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforne*, *Fusarium solani* and *Fusarium* sp. were detected from 100 per cent seed samples in all seed categories with frequency ranging from 3 to 30 per cent. *Epicoccum*

purpurascens and *Penicillium* sp. were detected from 50 per cent samples with a frequency of 1 to 25 per cent in each category. *Rhizopus stolonifer* was not detected from apparently healthy seed category. *Fusarium solani* was predominant fungus (ranging from 9 to 30%) with higher frequency from variety V_8 and minimum frequency of *Penicillium* sp. was recorded from variety V_7 with higher frequency on discoloured seed category (6%) and lower on apparently healthy seed category (1%).

Seed samples collected from HAREC, Bajaura showed the presence of 9 species in original, partially discoloured and discoloured seed category and 8 species in apparently healthy seed category on variety V_9 and 11 species on variety V_{10} in each seed category. *Aspergillus* sp., *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme*, *F. solani*, *Fusarium* sp. and *Mucor* sp. were observed from 100 per cent samples with frequency ranging between 1 to 52 per cent with highest frequency of *Aspergillus* sp. on variety V_9 on discoloured seed category (52%) and lowest of *Mucor* sp. on variety V_{10} on apparently healthy seed category (1%). *Alternaria alternata*, *Chaetomium olivaceum*, *Chaetomium* sp., *Penicillium* sp., *Phoma sorghina* and *Xanthomonas* sp. were detected from 50 per cent samples on each seed category with higher frequency of *Xanthomonas* sp. on variety V_9 in partially and discoloured seed category (85%) and lower frequency (1%) of *Penicillium* sp. on variety V_{10} in apparently healthy seed category.

The seed samples collected from RWRS, Malan showed the presence of 7 species on variety V_{11} and 8 species on variety V_{12} in each seed category. *Alternaria alternata*, *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme*, *F. solani*, *Fusarium* sp. were detected from 100 per cent samples with a higher frequency of *Curvularia lunata* (20 to 35%) followed by *Drechslera oryzae* (2 to 30%) on the collected samples. *Aspergillus* sp. and some non-sporulating fungus were also recorded from 50 per cent samples.

Among the varieties the maximum microflora were detected from variety V_1 followed by V_{10} and here also *Curvularia lunata* was predominant fungal species detected from variety V_1 and *Drechslera oryzae* from variety V_{10} and minimum microflora were detected from variety V_4 in all the seed categories

showing highest infection on original seed category and lowest on apparently healthy seed category. *Xanthomonas* sp., *Curvularia lunata* and *Drechlera oryzae* were found in highest frequency than the other microflora in each category and *Fusarium solani* was detected from all the varieties with 100 per cent sample mean (Figure 4 to 7).

These results of the present study was in conformity with the results reported by Baldacci and Corbetta (1964), Ou (1972), Basak and Mridha (1985), Riaz *et al.* (1995), Sharma and Chahal (1996) and Khan *et al.* (1999) on associated microflora of rice seeds. Islam *et al.* (2000) they also conducted an experiment to evaluate seed health of some rice varieties through blotter method and detected 7 fungal species *viz., Alternaria padwickii*, *Curvularia* sp., *Fusarium moniliforme*, *Sarocladium oryzae*, *Pyricularia oryzae*, *Bipolaris oryzae*, *Microdochium oryzae*. Similarly, Mandhare *et al.* (2008) also detected 7 microflora from 14 popularly grown rice varieties by using blotter method *viz., Fusarium moniliforme, F. oxysporum, Curvularia lunata, Drechslera oryzae, Alternaria padwickii, Pseudomonas* spp. *and Aspergillus niger.* However, in the present study *Sarocladium oryzae, Microdochium oryzae* and *Pseudomonas* spp. were not detected from any rice seed samples collected from different zones.

Bharathi and Raut (2009) also detected 14 fungi of 11 genera from 8 months stored seed samples of rice by using blotter method and 19 fungi of 11 genera from seed samples tested immediately after harvest and found *Curvularia lunata* and *Fusarium moniliforme* as a predominant pathogen from all locations. Gopalkrishanan and Valluvaparidasan (2009) in Tamil Nadu also studied the seed microflora of rice and detected 8 genera of fungi comprising 12 species *viz., Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma.*

It is evident from the present studies that both the methods of seed health testing were found to be suitable for isolating a large number of microflora. Microscopic examination of associated microflora detected by two methods exhibited different microscopic structures (Plate 3). Khan *et al.* (1988) on rice; Tariq *et al.* (2005) on soybean and Dawar and Ghaffar (1991) on sunflower also found that blotter and agar plate methods were more suitable for the detection of

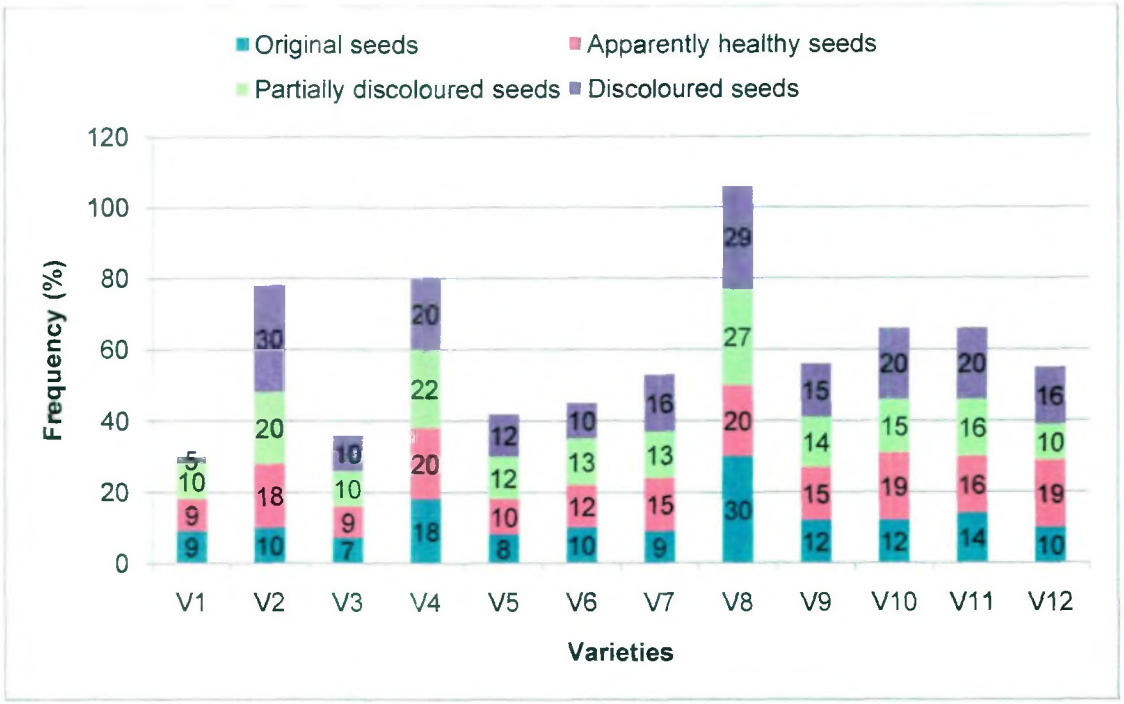


Figure 4. Frequency (%) of *Fusarium soiani* in four categories of rice varieties through agar plate method

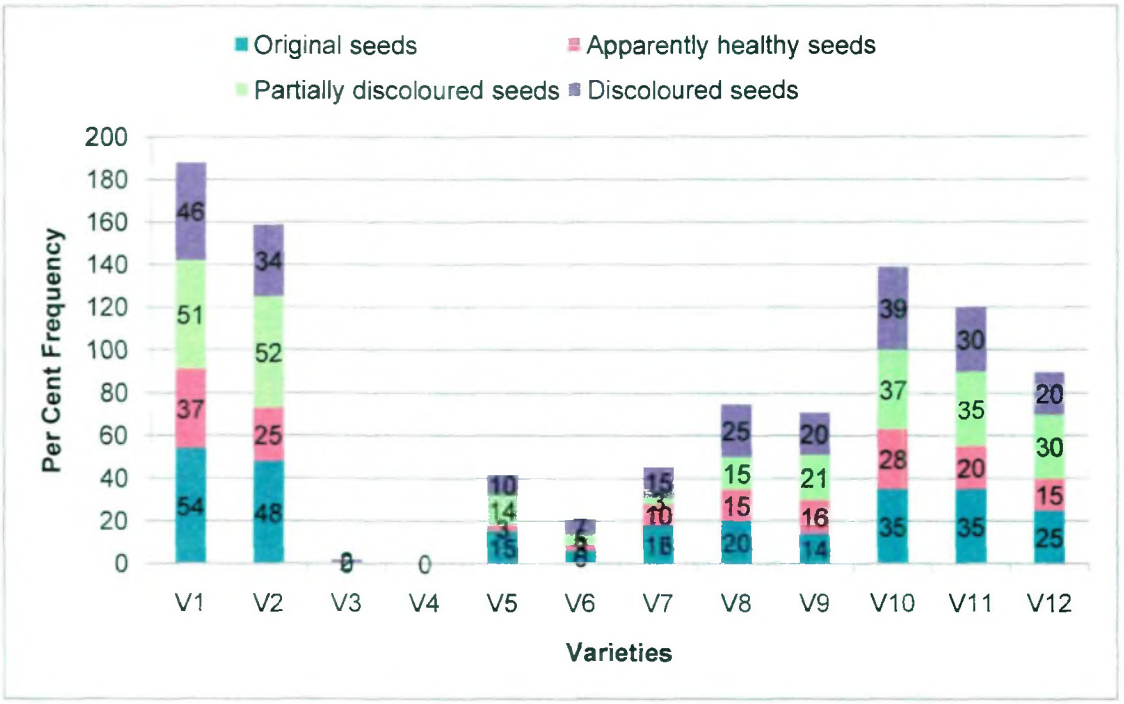


Figure 5. Frequency (%) of *Curvularia lunata* in four categories of rice varieties through agar plate method

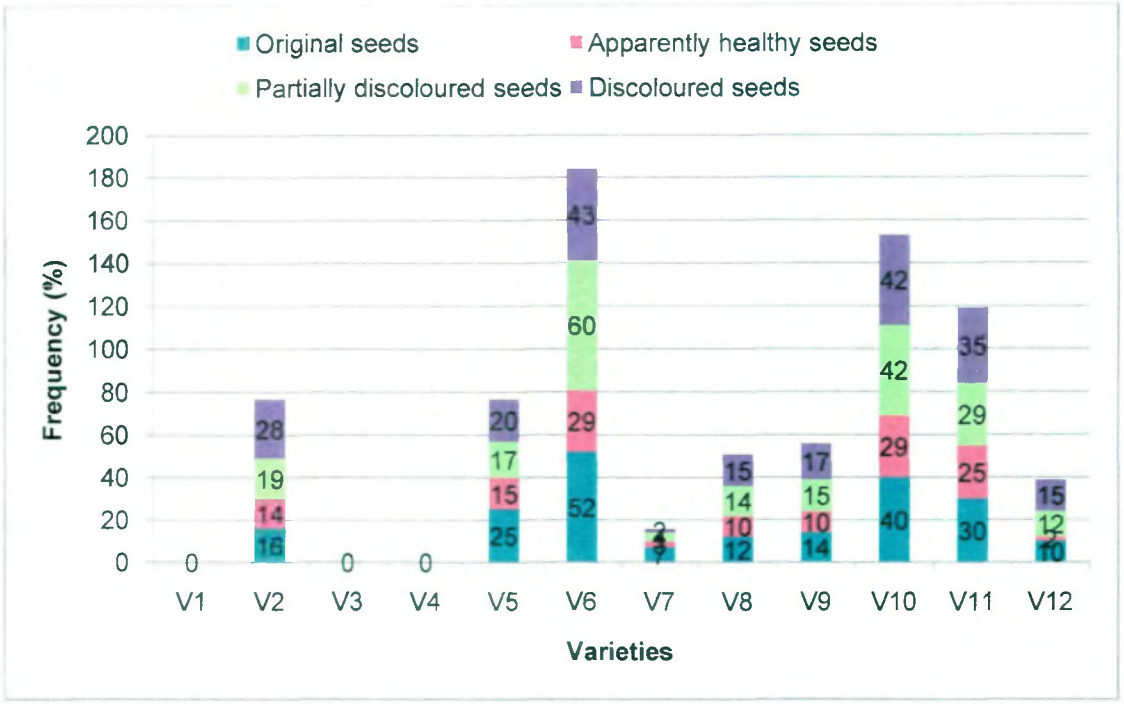


Figure 6. Frequency (%) of *Drechslera oryzae* in four categories of rice varieties through agar plate method

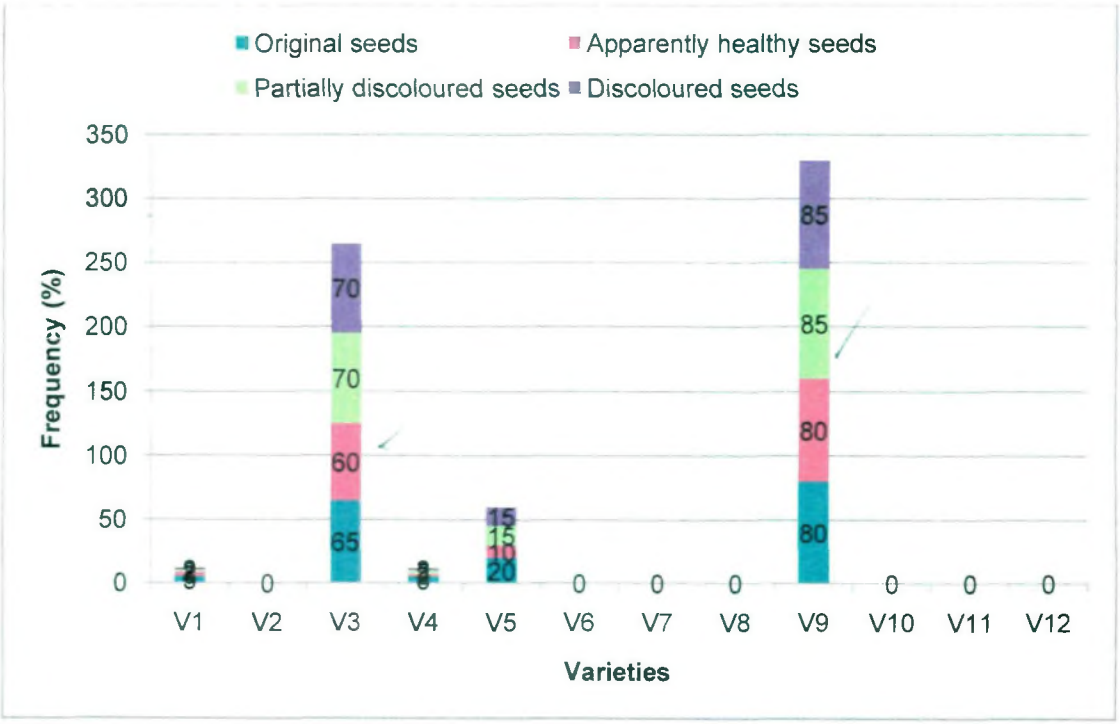
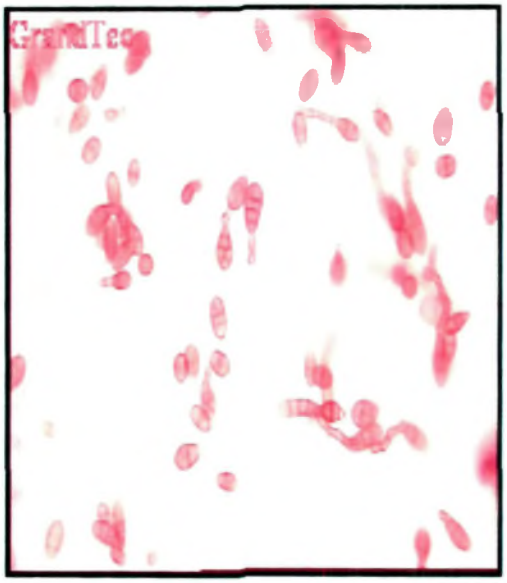
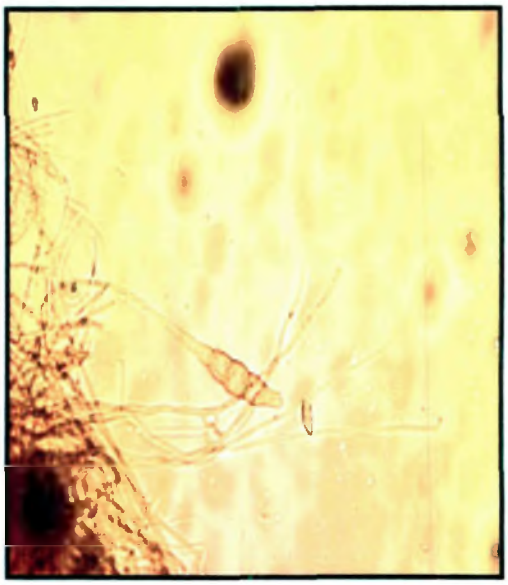


Figure 7. Frequency (%) of *Xanthomonas* sp. in four categories of rice varieties through agar plate method



Alternaria alternata

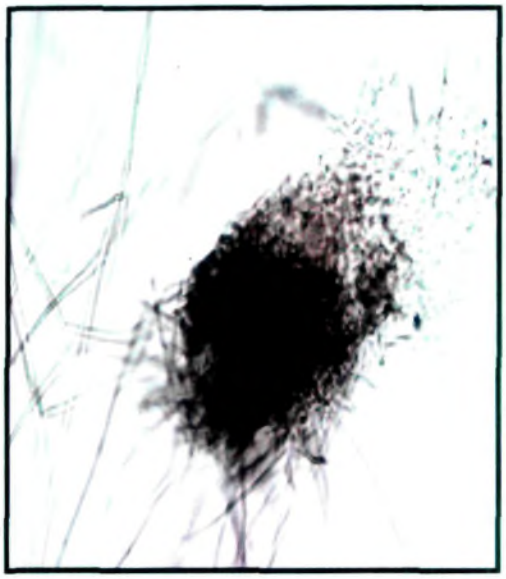


Alternaria padwickii

Aspergillus sp.

Chaetomium olivaceum

Plate 3: Microscopic observations of associated seedborne microflora of rice



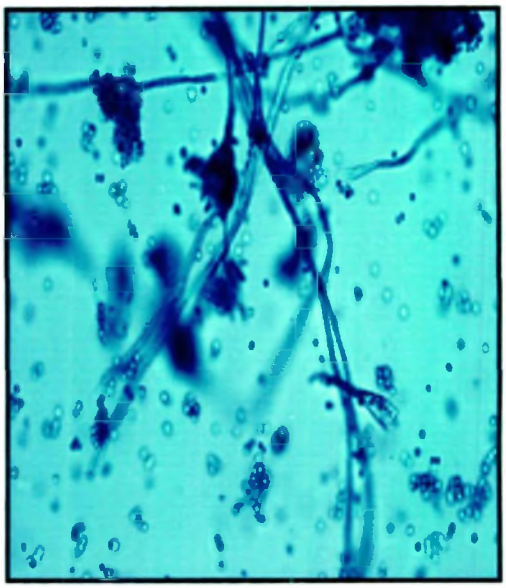
Chaetomium sp.

Curvularia lunata

Drechslera oryzae

Epicoccum purpurascens

Fusarium solani

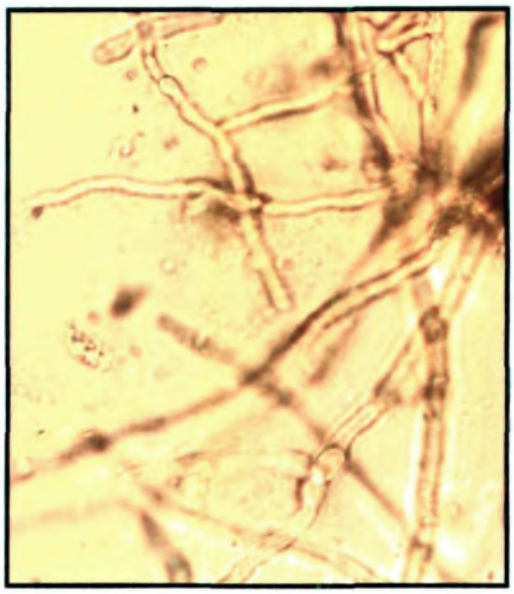


***Penicillium* sp.**

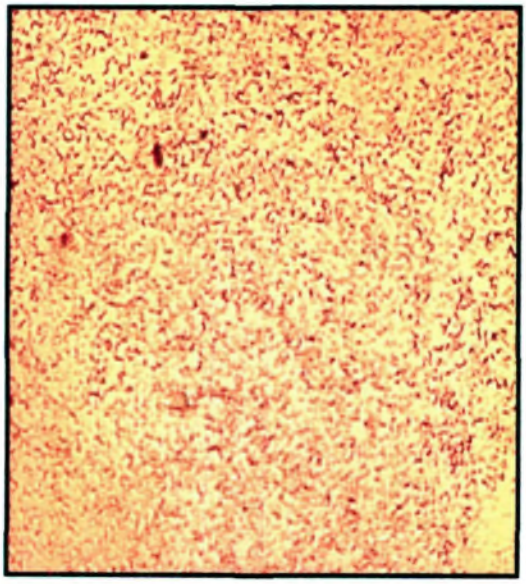
Phoma sorghina

Pyricularia oryzae

Rhizopus stolonifer



***Rhizoctonia* sp.**



***Xanthomonas* sp.**

seed-borne fungi. But, among the blotter and agar methods, the later had been found to be superior in isolating a large number of fungi. Total number of 15 fungal species were detected by blotter method whereas 17 fungal species and 1 bacterial sp. were isolated by agar plate in present study. The agar plate method was found to be superior in isolating *Curvularia* spp., *Drechslera* spp. and *Rhizopus stolonifer*. Khan *et al.* (1988) also found agar plate method as a more effective method for the isolation of *Curvularia* spp. and *Drechslera* spp. from disinfected seeds of rice. However, in present study frequency of all *Fusarium* spp. was found to be prominent on blotter method than the agar plate method. These findings were in accordance to the findings of Menten (1978) who also reported that agar plate method could detect more micro-organisms than blotter method and association of *Alternaria, Rhizoctonia* and *Macrophomina* was more on potato dextrose agar and *Fusarium* predominant on blotter method in beans. Apart from those, *Alternaria padwickii* was also predominant on blotter method than agar method. These observations corraborate with the findings of Agarwal *et al.* (1972) they found blotter technique better for the isolation of *Trichoconis padwickii* and *Drechslera oryzae* than agar plate method. Similarly, Khan *et al.* (1988) and Farias *et al.* (2007) also found *T. padwickii* as a predominant pathogen on blotter than agar method.

The results in the present studies showed that maximum microflora were detected from variety V_1 by both the methods with higher frequency of *Curvularia lunata* followed by varieties V_9 and V_{10} where, *Drechslera oryzae* was found a predominant pathogen. In support of present research Mishra and Dharamvir (1988) also detected *Drechslera oryzae* followed by *Curvularia lunata* as predominant pathogen in Bihar from discoloured seeds of rice cultivars. Pham Van Du *et al.* (2001) reported *Curvularia* spp. (13.4%) as a dominant pathogen through blotter method. In both the methods, maximum infection was recorded from discoloured seed category and minimum on apparently healthy seed category. This is because of the fact that fungal infections are mainly responsible for grain discolouration (Misra *et al.* 1994).

Baldacci and Corbetta (1964) reported that in addition to fungi, bacteria are also associated with grain discolouration. Only one bacterial genera *i.e. Xanthomonas* sp. was detected from all the varieties by using agar plate method..

Similarly, Goto *et al.* (1988) confirmed that *Xanthomonas oryzae* pv. *oryzicola* transmitted through seeds. Cottyn (2002) also detected *Xanthomonas* sp. from tropical rice fields of farmers in Philippines.

4.2.3 Nematode test

Data presented in Table 11 showed that nematode species were also found to be associated with rice seeds however; the frequency of detection was low. Only one nematode sp.; *Aphelenchoides besseyi* was detected from seed samples of variety V_3 and V_6. The per cent frequency of associated nematode on both varieties (V_3 and V_6) was lower in original seed category (2%), partially discoloured seed category (2%) and discoloured seed category (3%) whereas it was absent in apparently healthy seed category. Infested seed is a primary source of inoculum for white tip nematodes, (*Aphelenchoides besseyi*). Babatola (1984) detected and identified white tip disease causing nematode, *Aphelenchoides besseyi* from rice seeds in Nigeria.

Table 11. Frequency (%) of associated nematode (*Aphelenchoides besseyi*) with rice varieties in each category*

Variety	Original seed category	Apparently healthy seed category	Partially discoloured seed category	Discoloured seed category
V_1	0.0	0.0	0.0	0.0
V_2	0.0	0.0	0.0	0.0
V_3	2.0	0.0	2.0	3.0
V_4	0.0	0.0	0.0	0.0
V_5	0.0	0.0	0.0	0.0
V_6	2.0	0.0	2.0	3.0
V_7	0.0	0.0	0.0	0.0
V_8	0.0	0.0	0.0	0.0
V_9	0.0	0.0	0.0	0.0
V_{10}	0.0	0.0	0.0	0.0
V_{11}	0.0	0.0	0.0	0.0
V_{12}	0.0	0.0	0.0	0.0

*On the basis of 100 seeds tested of each cultivar

4.3 Effect of chemical and bioagent treatments on seed microflora

4.3.1 Blotter method:

Data presented in Tables 12 to 15 revealed that seed treatment with chemicals (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed) had significant effect against different seedborne fungi. In all 12 species were recorded on the chemical treated seeds though the number of fungi on individual seed lots ranged from 0 to 7 species in all categories. A maximum of 7 species were detected from varieties V_9 and V_{11} in original seed category, 6 from varieties V_2 and V_{12} from apparently healthy seed category, 7 from varieties V_2, V_{10} and V_{11} from partially discoloured seed category and 6 from varieties V_2, V_9, V_{10}, V_{11} and V_{12} whereas no microflora was detected from variety V_8 in all seed categories.Fungicidal treatment gave satisfactorily control of various fungi associated with rice seed (Plate 4).

The higher incidence of *Curvularia lunata* in variety V_1 and *Drechslera oryzae* in variety V_6 was reduced from the range 41 to 45 per cent and 40 to 71 per cent in untreated seeds (Table 3 to 6) to 5 to 18 per cent and 14 to 16 per cent (Table 12 to 15) respectively. Effectiveness of carbendazim (Bavistin) treatment against fungi of rice seed documented by earlier workers also (Lakshmanan and Mohan 1988; Dodan *et al.* 1994; Ali and Deka 1996). Seed treatment with mancozeb (Dithane M-45) has been suggested by several workers in controlling fungi of rice (Lakshmanan and Mohan 1988; Deka *et al.* 1996). However in the present study, both fungicides in combination gave better results. Sisterna and Ronco (1994) observed poor performance of Dithane M-45 against *Fusarium moniliforme* but carbendazim has been reported most effective against *F. moniliforme* (Lakshmanan and Mohan 1988; Ali and Deka 1996). Vir *et al.* (1971) reported that Dithane M-45 gives complete control of seedborne *Alternaria padwickii*. In present study also its infection was reduced to 0 to 4 per cent (Tables 12 to 15) from 7 to 13 (Tables 3 to 6).

Table 12. Effect of chemical treatment on associated microflora (%) of original seeds of rice*

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	2.0	0.0	1.0	0.0	15.0	0.0	0.0	7.0	3.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	6
V_2	6.0	0.0	0.0	0.0	12.0	8.0	0.0	0.0	4.0	10.0	0.0	0.0	1.0	0.0	0.0	0.0	6
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	2
Mean	2.7	0.0	0.3	0.0	9.0	2.7	0.0	2.3	3.7	5.3	0.0	0.0	0.3	0.0	0.0	0.0	
Zone II																	
V_4	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2
V_5	3.0	4.0	0.0	0.0	6.0	14.0	0.0	2.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6
V_6	10.0	0.0	0.0	0.0	2.0	16.0	0.0	0.0	5.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	5
Mean	4.3	1.3	0.0	0.7	2.7	10.0	0.0	0.7	3.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	5.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	3
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	5.0	0.0	0.0	0.0	7.0	9.0	1.0	0.0	6.0	0.0	0.0	0.0	3.0	1.0	0.0	0.0	7
V_{10}	3.0	0.0	0.0	0.0	10.0	15.0	5.0	0.0	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	4.0	0.0	0.0	0.0	8.5	12.0	3.0	0.0	7.0	4.5	0.0	0.0	1.5	0.5	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	5.0	0.0	0.0	0.0	9.0	12.0	0.0	10.0	6.0	1.0	0.0	0.0	10.0	0.0	0.0	1.0	7
V_{12}	3.0	0.0	0.0	0.0	15.0	7.0	0.0	11.0	4.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	4.0	0.0	0.0	0.0	12.0	9.5	0.0	10.5	5.0	5.5	0.0	0.0	5.0	0.0	0.0	0.5	

***On the basis of 100 seeds tested of each cultivar**

Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table 13. **Effect of chemical treatment on associated microflora (%) of of apparently healthy seeds of rice***

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	0.0	0.0	0.0	0.0	5.0	0.0	0.0	5.0	5.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	4
V_2	6.0	0.0	0.0	0.0	12.0	3.0	0.0	0.0	5.0	8.0	0.0	0.0	2.0	0.0	0.0	0.0	6
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	2
Mean	2.0	0.0	0.0	0.0	5.7	1.0	0.0	1.7	5.0	4.3	0.0	0.0	0.7	0.0	0.0	0.0	
Zone II																	
V_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1
V_5	0.0	1.0	0.0	0.0	5.0	12.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
V_6	8.0	0.0	0.0	0.0	4.0	15.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
Mean	2.7	0.3	0.0	0.0	3.0	9.0	0.0	0.0	3.7	0.0	0.0	0.0	0.3	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2
V_{10}	0.0	0.0	0.0	0.0	5.0	6.0	2.0	0.0	5.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	5
Mean	0.0	0.0	0.0	0.0	3.5	3.0	1.0	0.0	3.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	0.0	0.0	0.0	0.0	2.0	5.0	0.0	2.0	5.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	5
V_{12}	1.0	0.0	0.0	0.0	8.0	4.0	0.0	5.0	5.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	0.5	0.0	0.0	0.0	5.0	4.5	0.0	3.5	5.0	3.5	0.0	0.0	1.0	0.0	0.0	0.0	

***On the basis of 100 seeds tested of each cultivar**

Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table 14. **Effect of chemical treatment on associated microflora (%) of partially discoloured seeds of rice***

Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	1.0	0.0	0.0	0.0	8.0	0.0	0.0	5.0	6.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	5
V_2	8.0	0.0	0.0	0.0	18.0	3.0	1.0	0.0	8.0	8.0	0.0	0.0	2.0	0.0	0.0	0.0	7
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	2
Mean	3.0	0.0	0.0	0.0	8.7	1.0	0.3	1.7	7.3	6.0	0.0	0.0	0.7	0.0	0.0	0.0	
Zone II																	
V_4	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	3
V_5	2.0	3.0	0.0	0.0	7.0	15.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5
V_6	14.0	0.0	0.0	0.0	8.0	14.0	0.0	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
Mean	5.3	1.0	0.0	1.0	5.0	9.7	0.0	0.0	5.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	8.0	2.0	0.0	0.0	2.0	0.0	0.0	0.0	4
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	4.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	3.0	1.0	0.0	0.0	2
V_{10}	5.0	0.0	0.0	0.0	8.0	10.0	5.0	0.0	6.0	5.0	1.0	0.0	0.0	0.0	0.0	0.0	7
Mean	2.5	0.0	0.0	0.0	5.0	5.0	2.5	0.0	4.0	2.5	0.5	0.0	1.5	0.5	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	6.0	0.0	0.0	0.0	5.0	8.0	0.0	5.0	5.0	1.0	0.0	0.0	3.0	0.0	0.0	0.0	7
V_{12}	5.0	0.0	0.0	0.0	15.0	10.0	0.0	8.0	5.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	5.5	0.0	0.0	0.0	10.0	9.0	0.0	6.5	5.0	3.0	0.0	0.0	1.5	0.0	0.0	0.0	

*On the basis of 100 seeds tested of each cultivar

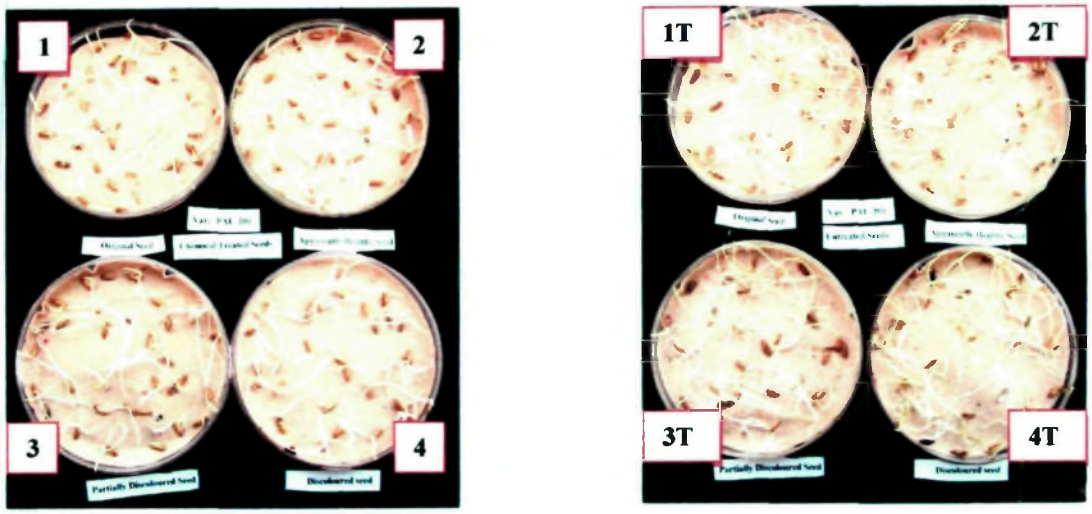
Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table 15. Effect of chemical treatment on associated microflora (%) of discoloured seeds of rice*

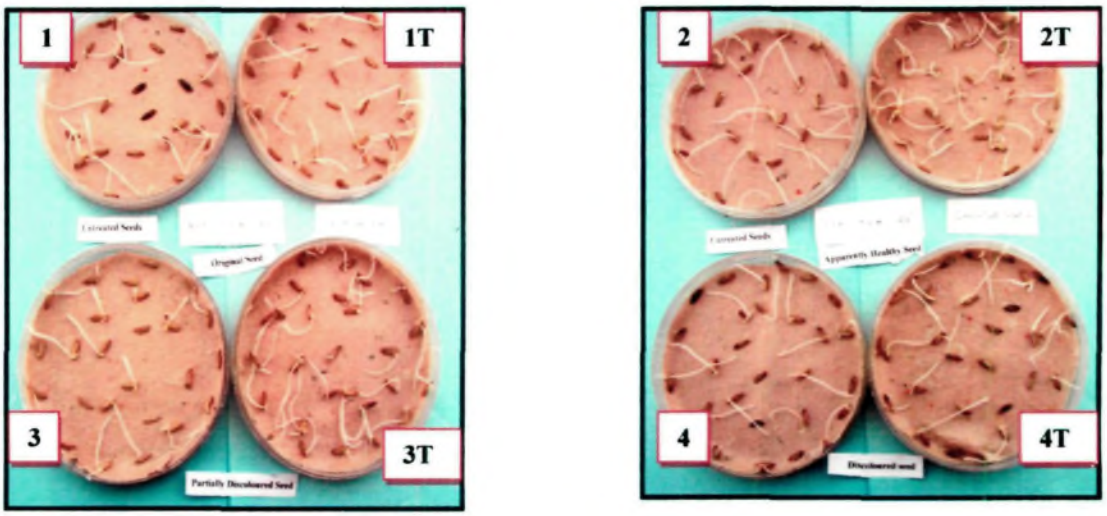
Variety	*Alternaria alternata*	Alternaria padwickii	*Aspergillus* sp.	*Chaetomium* sp.	*Curvularia lunata*	*Drechslera oryzae*	*Epicoccum purpurascens*	*Fusarium moniliforme*	*Fusarium solani*	*Fusarium* sp.	*Mucor* sp.	*Penicillium* sp.	*Phoma sorghina*	*Pyricularia oryzae*	*Rhizopus stolonifer*	Non-sporulating fungus	Total microflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Zone I																	
V_1	4.0	0.0	0.0	0.0	11.0	0.0	0.0	2.0	6.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	5
V_2	6.0	0.0	0.0	0.0	17.0	12.0	0.0	0.0	14.0	12.0	0.0	0.0	2.0	0.0	0.0	0.0	6
V_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	12.0	0.0	0.0	0.0	0.0	0.0	0.0	2
Mean	3.3	0.0	0.0	0.0	9.3	4.0	0.0	0.7	10.0	9.0	0.0	0.0	0.7	0.0	0.0	0.0	
Zone II																	
V_4	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	5.0	0.0	0.0	0.0	4
V_5	3.0	4.0	0.0	0.0	8.0	12.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5
V_6	12.0	0.0	0.0	0.0	10.0	15.0	0.0	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
Mean	5.0	1.3	0.0	0.3	6.0	9.0	0.0	0.0	4.3	0.3	0.0	0.0	1.7	0.0	0.0	0.0	
HAREC, Dhaulakuan (Zone I)																	
V_7	0.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0	7.0	3.0	0.0	0.0	3.0	0.0	0.0	0.0	4
V_8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	3.5	1.5	0.0	0.0	1.5	0.0	0.0	0.0	
HAREC, Bajaura (Zone II)																	
V_9	5.0	0.0	0.0	0.0	4.0	2.0	0.0	0.0	5.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	6
V_{10}	4.0	0.0	0.0	0.0	10.0	14.0	7.0	0.0	5.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	4.5	0.0	0.0	0.0	7.0	8.0	3.5	0.0	5.0	3.0	0.0	0.0	0.5	0.5	0.0	0.0	
RWRS, Malan (Zone II)																	
V_{11}	7.0	0.0	0.0	0.0	5.0	10.0	0.0	7.0	7.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	6
V_{12}	6.0	0.0	0.0	0.0	14.0	10.0	0.0	10.0	6.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	6
Mean	6.5	0.0	0.0	0.0	9.5	10.0	0.0	8.5	6.5	3.5	0.0	0.0	2.0	0.0	0.0	0.0	

*On the basis of 100 seeds tested of each cultivar

Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed



PAU-201 (HAREC, Dhaulakuan Zone I)



HKR-126 (HAREC, Dhaulakuan Zone I)

T = Treated

Plate 4. Effect of chemical treatment (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed) on associated microflora of different rice seed categories; original (1); apparently healthy (2); partially discoloured (3); and discoloured (4) by blotter method

4.3.2 Agar plate method

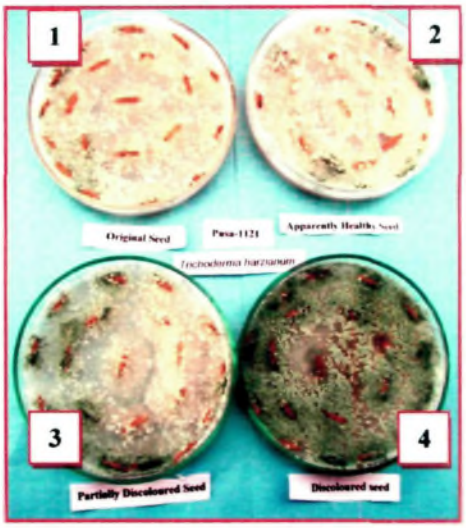
Seed treatment with bioagents restricted the growth of pathogens in all varieties of each category through agar plate method (Plate 5). Seed treatment with *Trichoderma harzianum* gave complete control of a number of fungi and bacteria associated with rice seeds. Mehta *et al.* (1997) also reported that *Trichoderma* spp. *viz.*, *T. harzianum* and *T. viride* promote the growth of plant pathogens by restricting pathogens as well as by its direct effect. *Trichoderma harzianum* has been proved to be more effective against *Fusarium* species (Bharath *et al.* 2005). However, in present study through agar plate method *Trichoderma harzianum* restricted the growth of all the associated microflora.

4.4 Germination test (in-soil and in-between paper)

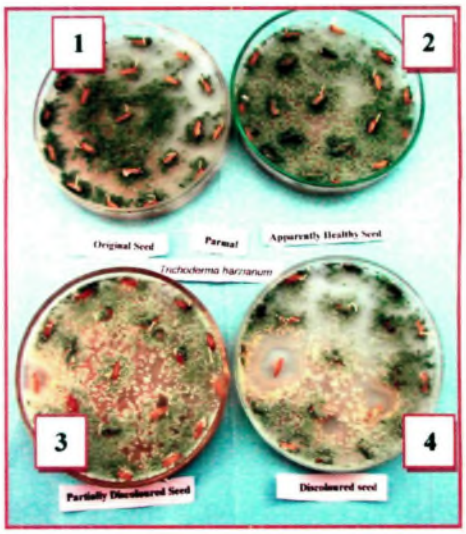
The germination status of different seed categories was studied by two methods; in-soil and in-between paper. Data pertaining to four seed categories are presented in Tables 16 to 19.

Germination in original seed category of 12 varieties varied from 83.67 per cent (V_6) to 96.33 per cent (V_3) in in-soil method and 82 per cent (V_{12}) to 95.33 per cent (V_3) in in-between paper method. The germination in both methods was higher than 80 per cent in all the varieties (Table 16). Percentage of abnormal seedlings varied from 0.67 per cent in variety V_3 to 4.33 per cent in variety V_{12} under in-soil method and zero per cent in variety V_3 to 7.33 per cent in variety V_9 under in-between paper method. Similarly, dead seeds varied from 3 per cent (V_3) to 11 per cent (V_1) under in-soil method and 4.67 per cent (V_3) to 13 per cent (V_{12}) under in-between paper method.

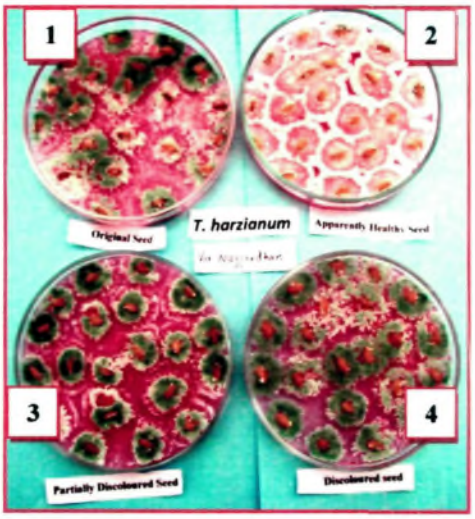
Variety V_3 and V_5 produced higher number of normal seedlings (98.67%) in apparently healthy seed category in in-soil method followed by variety V_7 (98%) and variety V_6 (90.33%) whereas variety V_{12} (90.33%) produced comparatively lower number of normal seedlings (Table 17). Variety V_5 (98.67%) also produced higher number of normal seedlings in in-between paper method followed by variety V_8 (96%), variety V_3 (95.67%) and variety V_4 (95.67%) which were statistically at par with each other whereas variety V_9 (85.67%) produced least number of normal seedlings.



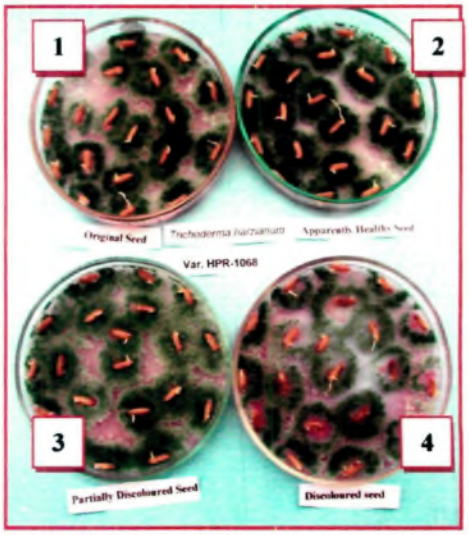
Pusa-1121 (Zone I)



Parmal (Zone II)



Nagardhan (HAREC, Bajaura Zone II)



HPR-1068 (RWRS, Malan Zone II)

Plate 5. Effect of treatment with talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed on associated microfiora of different rice seed categories; original (1); apparently healthy (2); partially discoloured (3); and discoloured (4) by agar plate method

Table 16. Germination status (%) of original seeds of rice*

Varieties	In-soil			In-between paper		
	N	Ab	D	N	Ab	D
V_1	87.00 (9.38)	2.00 (1.73)	11.00 (3.46)	83.00 (9.16)	6.67 (2.76)	10.33 (3.35)
V_2	93.33 (9.71)	2.00 (1.72)	4.67 (2.38)	92.00 (9.64)	1.33 (1.52)	6.67 (2.75)
V_3	96.33 (9.87)	0.67 (1.28)	3.00 (1.99)	95.33 (9.81)	0.00 (1.00)	4.67 (2.38)
V_4	92.67 (9.68)	1.33 (1.52)	6.00 (2.64)	89.67 (9.52)	2.33 (1.82)	8.00 (3.00)
V_5	93.67 (9.73)	1.67 (1.63)	4.67 (2.38)	92.67 (9.68)	2.67 (1.91)	4.67 (2.37)
V_6	83.67 (9.20)	3.33 (2.08)	13.00 (3.74)	82.33 (9.13)	5.00 (2.44)	12.67 (3.69)
V_7	94.00 (9.75)	1.67 (1.63)	4.33 (2.31)	87.67 (9.42)	3.67 (2.15)	8.67 (3.10)
V_8	89.33 (9.50)	2.00 (1.72)	8.67 (3.10)	86.33 (9.35)	2.67 (1.91)	11.00 (3.46)
V_9	87.67 (9.42)	2.67 (1.91)	9.67 (3.26)	82.33 (9.13)	7.33 (2.88)	10.33 (3.36)
V_{10}	86.00 (9.33)	3.00 (1.99)	11.00 (3.46)	84.67 (9.25)	3.33 (2.07)	12.00 (3.60)
V_{11}	92.33 (9.66)	2.33 (1.82)	5.33 (2.50)	85.00 (9.27)	5.33 (2.50)	9.67 (3.26)
V_{12}	87.33 (9.40)	4.33 (2.31)	8.33 (3.04)	82.00 (9.11)	5.00 (2.44)	13.00 (3.74)
Mean	90.28 (9.55)	2.25 (1.78)	7.47 (2.85)	86.92 (9.37)	3.78 (2.12)	9.31 (3.17)
CD(P= 0.05)	(0.13)	(0.34)	(0.39)	(0.19)	(0.37)	(0.45)
CV	(0.82)	(11.20)	(8.12)	(1.23)	(10.38)	(8.50)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds

Table 17. Germination status (%) of apparently healthy seeds of rice*

Varieties	In-soil			In-between paper		
	N	Ab	D	N	Ab	D
V_1	91.33 (9.61)	0.00 (1.00)	8.67 (3.10)	86.33 (9.34)	1.67 (1.63)	12.67 (3.69)
V_2	95.33 (9.81)	1.33 (1.52)	3.67 (2.16)	93.67 (9.73)	0.67 (1.28)	5.67 (2.56)
V_3	98.67 (9.98)	0.33 (1.14)	1.00 (1.41)	95.67 (9.83)	0.00 (1.00)	4.67 (2.38)
V_4	97.67 (9.93)	1.33 (1.14)	2.00 (1.72)	95.67 (9.83)	1.67 (1.63)	2.67 (1.91)
V_5	98.67 (9.98)	0.00 (1.00)	1.33 (1.52)	98.67 (9.98)	0.67 (1.28)	0.67 (1.28)
V_6	90.33 (9.56)	1.33 (1.52)	8.33 (3.05)	88.33 (9.45)	4.33 (2.31)	7.33 (2.88)
V_7	98.00 (9.95)	0.67 (1.28)	1.33 (1.52)	94.00 (9.75)	1.33 (1.52)	4.67 (2.35)
V_8	92.00 (9.64)	2.00 (1.73)	6.00 (2.64)	96.00 (9.85)	1.33 (1.52)	2.67 (1.91)
V_9	91.67 (9.63)	2.00 (1.72)	6.33 (2.70)	85.67 (9.31)	4.00 (2.23)	10.33 (3.36)
V_{10}	94.00 (9.75)	1.67 (1.61)	4.33 (2.31)	89.67 (9.52)	2.33 (1.80)	8.00 (3.00)
V_{11}	97.00 (9.90)	0.67 (1.28)	2.33 (1.82)	90.00 (9.54)	3.67 (2.13)	7.67 (2.92)
V_{12}	90.33 (9.56)	3.33 (2.08)	6.33 (2.71)	87.00 (9.38)	3.33 (2.08)	9.67 (3.26)
Mean	94.58 (9.78)	1.22 (1.42)	4.31 (2.22)	91.72 (9.63)	2.08 (1.70)	6.39 (2.62)
CD(P= 0.05)	(0.09)	(0.35)	(0.30)	(0.15)	(0.40)	(0.48)
CV	(0.52)	(14.62)	(7.98)	(0.94)	(13.89)	(10.95)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds

In in-soil method variety V_{12} (3.33%) had the higher number of abnormal seedlings followed by variety V_8 (2.00%). Variety V_1 (8.67%) produced highest percentage of dead seeds followed by variety V6 (8.33%). However, in in-between method, variety V_6 (4.33%) had the highest percentage of abnormal seedlings followed by varieties V_9 (4.00%), V_{11} (3.67%) and V_{12} (3.33%) which were statistically at par with each other. Variety V_1 (12.67%) produced highest percentage of dead seeds followed by variety V_{12} (9.67%).

In partially discoloured seed category variety V_5 (96.67%) produced the highest number of normal seedlings followed by variety V_7 (95%) which were statistically at par with each other whereas variety V_1 (85.67%) produced least number of seedlings in in-soil method (Table 18). Similarly, variety V_5 (97%) also produced higher number of seedlings in in-between paper method followed by variety V_8 (96%) which were statistically at par with each other. Variety V_9 (75.67%) produced the least number of normal seedlings.

In in-soil method, variety V_6 (10.33%) and variety V_{12} (10%) had the highest number of abnormal seedlings and V_1 (13%) had the higher number of dead seed whereas, in in-between method, variety V_9 (9.33%) had the higher number of abnormal seedlings and variety V_{12} (16.67%) and V_9 (15%) had the highest number of dead seeds.

In case of discoloured seed category variety V_5 produced the highest number of seedlings in both methods, 93 per cent and 89.67 per cent respectively followed by variety V_3 (86.33%) in in-between method and was statistically at par with variety V_5 (Table 19). Variety V_1 (81.00%) produced lowest number of seedlings in in-soil and variety V_{11} (65.67%) in in-between paper method.

Variety V_8 (5.67%) had the highest number of abnormal seedlings followed by variety V6 (5%) and variety V_{12} (5%) in in-soil method. Variety V_1 (16.67%) had the highest number of dead seeds followed by variety V_6 (14.33%) whereas, in in-between paper method variety V_9 (14.67%) had the highest number of abnormal seedlings and variety V_{11} (22%) had the highest number of dead seeds.

Table 18. Germination status (%) of partially discoloured seeds of rice*

Varieties	in-soil			In-between paper		
	N	Ab	D	N	Ab	D
V_1	85.67 (9.31)	1.33 (1.52)	13.00 (3.74)	80.33 (9.02)	6.33 (2.68)	13.33 (3.78)
V_2	93.67 (9.73)	1.67 (1.63)	4.67 (2.38)	92.33 (9.66)	0.33 (1.14)	7.33 (2.87)
V_3	94.33 (9.76)	1.67 (1.63)	4.00 (2.23)	92.33 (9.66)	0.00 (1.00)	7.67 (2.92)
V_4	90.33 (9.56)	2.33 (1.82)	7.33 (2.89)	91.67 (9.63)	3.67 (2.16)	4.67 (2.37)
V_5	96.67 (9.88)	0.67 (1.24)	2.67 (1.88)	97.00 (9.90)	1.33 (1.52)	1.67 (1.58)
V_6	86.33 (9.35)	10.33 (2.07)	10.33 (3.36)	85.33 (9.29)	5.33 (2.51)	9.67 (3.26)
V_7	95.00 (9.80)	3.67 (1.52)	3.67 (2.15)	87.33 (9.40)	3.33 (2.07)	9.67 (3.26)
V_8	89.67 (9.52)	8.67 (1.63)	8.67 (3.11)	96.00 (9.85)	1.33 (1.52)	2.67 (1.90)
V_9	89.67 (9.52)	8.00 (1.80)	8.00 (3.00)	75.67 (8.75)	9.33 (3.21)	15.00 (3.99)
V_{10}	89.00 (9.49)	9.33 (1.61)	9.33 (3.21)	82.33 (9.13)	4.00 (2.23)	13.67 (3.82)
V_{11}	90.33 (9.56)	7.00 (1.91)	7.33 (2.88)	81.33 (9.07)	6.67 (2.76)	14.33 (3.91)
V_{12}	87.33 (9.40)	10.00 (1.91)	10.00 (3.31)	79.00 (8.94)	4.33 (2.30)	16.67 (4.20)
Mean	90.67 (9.57)	5.39 (1.69)	7.42 (2.84)	86.72 (9.36)	3.83 (2.09)	9.69 (3.15)
CD(P= 0.05)	(0.10)	(0.42)	(0.36)	(0.18)	(0.42)	(0.55)
CV	(0.63)	(14.61)	(7.56)	(1.15)	(11.79)	(10.27)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds

Table 19. Germination status (%) of discoloured seeds of rice*

Varieties	In-soil			In-between paper		
	N	Ab	D	N**	Ab	D
V_1	81.00 (9.06)	2.33 (1.82)	16.67 (4.20)	76.67 (61.12)	5.67 (2.58)	17.67 (4.31)
V_2	88.67 (9.47)	2.33 (1.82)	9.00 (3.16)	84.00 (66.48)	2.67 (1.91)	13.67 (3.82)
V_3	89.67 (9.52)	1.33 (1.52)	9.00 (3.16)	86.33 (68.32)	0.00 (1.00)	13.67 (3.82)
V_4	88.67 (9.47)	2.33 (1.82)	9.00 (3.16)	84.67 (66.94)	4.00 (2.23)	11.33 (3.50)
V_5	93.00 (9.70)	1.67 (1.63)	5.33 (2.49)	89.67 (71.22)	3.67 (2.16)	6.67 (2.77)
V_6	80.67 (9.04)	5.00 (2.44)	14.33 (3.92)	78.67 (62.47)	6.00 (2.63)	15.33 (4.04)
V_7	89.67 (9.52)	3.67 (2.16)	6.67 (2.77)	79.67 (63.17)	3.33 (2.02)	17.00 (4.24)
V_8	86.33 (9.35)	5.67 (2.58)	8.00 (3.00)	80.00 (63.41)	5.00 (2.44)	15.00 (4.00)
V_9	85.67 (9.31)	3.00 (1.99)	11.33 (3.51)	70.00 (56.77)	14.67 (3.95)	15.00 (4.00)
V_{10}	83.67 (9.20)	4.33 (2.29)	12.00 (3.60)	76.33 (60.87)	4.67 (2.38)	19.00 (4.47)
V_{11}	84.00 (9.22)	3.67 (2.16)	13.00 (3.74)	65.67 (54.14)	12.33 (3.64)	22.00 (4.79)
V_{12}	83.33 (9.18)	5.00 (2.44)	12.00 (3.60)	77.00 (61.33)	3.67 (2.16)	19.33 (4.51)
Mean	86.19 (9.34)	3.36 (2.06)	10.53 (3.36)	79.06 (63.02)	5.47 (2.42)	15.47 (4.02)
CD(P= 0.05)	(0.13)	(0.32)	(0.33)	(2.69)	(0.45)	(0.42)
CV	(0.83)	(9.35)	(5.85)	(2.53)	(10.99)	(6.23)

Figures within parentheses are square root transformed values
** Figures within parentheses are arc sine transformed values**
* Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds

Among the two methods, in-between paper method exhibited higher number of dead seeds, which might have been due to dormancy problem in rice seeds and in-soil method due to natural scarification, breakdown of this dormancy lead to higher germination.

Among varieties, variety V_5 produced highest number of seedlings and among seed categories, apparently healthy produced highest number of seedlings in all varieties whereas discoloured seeds produced lowest number of normal seedlings. Discolouration of seeds lowered their germinability in all the cultivars and lead to seedling abnormalities (Plate 6 and 7).

Sharma *et al.* (1987) observed that the loss in germinability was directly proportional to discolouration. The microbial agents inducing discolouration can also inhibit the germination of the seeds (Misra *et al.* 1990; Ou 1985).

The reason behind the higher number of abnormal seedlings and dead seeds and least percentage of germination in V_1, V_6 and V_9 might be due to heavy colonization of the seeds by *Curvularia lunata*, *Drechslera oryzae*, *Fusarium moniliforme*, *Fusarium solani*, *Rhizopus* sp., *Xanthomonas* sp. Reddy and Khare (1977) and Imolehin (1983) reported that grain discolouration and poor germination of rice seed are due to *Curvularia* spp. and *Alternaria padwickii*. Sachan and Agarwal (1994) also studied that fungi associated with seed discolouration in rice resulted in loss in seed germination. Babu and Lokesh (1996) observed the least percentage of germination in IR-64 due to heavy colonization of the seeds by *Chaetomium globosum*, *Gerlachia oryzae*, *Fusarium moniliforme*, *Drechslera* spp., *Verticillium* sp. and *Rhizopus* spp. These fungi colonise the seeds right from the post flowering stages upto storage. Studies by Zope and Trimurty (2004) reported that *Fusarium moniliforme* and *Curvularia* spp. were most frequently occurred thereby reducing seed germination of paddy cultivars.

4.5 Effect of chemical treatment on germination

Data pertaining to effect of chemical treatment on seed germination are given in Tables 20 to 23. All the varieties produced a statistically higher number of normal seedling in each category compared to control (untreated seeds). Among the varieties, variety V_3 had the highest number of normal seedlings in

Original seed

Apparently healthy seed

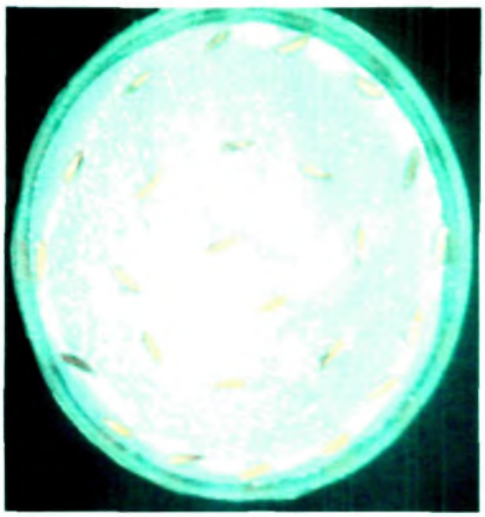
Partially discoloured seed

Discoloured seed

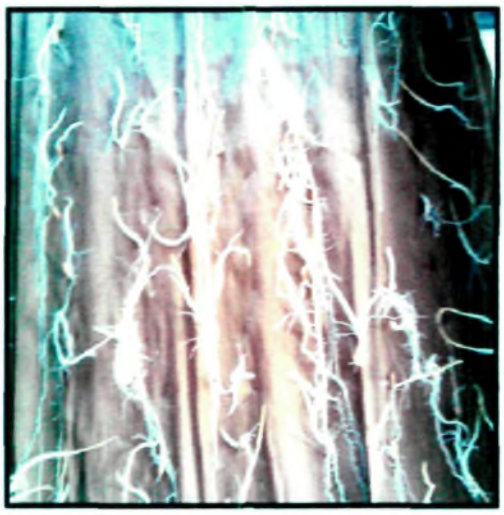
Plate 6. In-soil germination of rice seeds of variety Parmal

Normal seedlings

Abnormal seedlings



Dead seeds



In between paper germination

Plate 7. Abnormalities caused by associated rice seedborne microflora

both in-soil and in-between paper methods in original seed category (99.67% and 98.0%) (Table 20). Similarly, in apparently healthy seed category produced 99.67 per cent and 99.33 per cent and variety V_5 also produced 99.67 per cent and 99.33 per cent seedlings respectively (Table 21). Partially discoloured seed produced 99 per cent and 98 per cent (Table 22) whereas discoloured seed category produced 94.67 per cent and 91.67 per cent (Table 23) normal seedlings in variety V_5. Chemical treated seeds produced minimum per cent of abnormal seedlings as compared to control. Among the varieties, V_1 and V_{12} produced the highest number of abnormal seedlings in all the seed categories. All the varieties had lower number of dead seeds in chemical treated seeds as compared to control in both methods in each category. The reason behind enhanced germination due to promising chemicals may be confined to suppression of pathogenic fungi.

Chemical treatment with a mixture of fungicides Bavistin + Dithane M-45 (1.25 + 1.25 g/kg seed) was found to be effective in increasing the germination percentage of all varieties in each category as compared to untreated control. Germination is increased by 10.33 per cent (in-soil) and 12.33 per cent (in-between paper). In support of finding Sachan and Aggarwal (1994) also reported the beneficial effect of seed treatment with Bavistin + Dithane M-45 (1:1) and Bavistin + Thiram (1:1) in increasing germination and seedling vigour and reducing the seedborne inocula.

Similarly, Vaid *et al.* (1994) observed improving germinability of discoloured rice by seed treatment with Carbendazim @ 2g/kg seed. Bharath *et al.* (2005) also found that seeds treated with Topsin and Bavistin showed high percentage of germination of watermelon than any other treatment where, compared to control germination increased by 12 per cent and 13 per cent. Khalid *et al.* (2001) suggested the use of chemical treatment for raising healthy seedlings of rice.

Bharathi and Raut (2009) also observed that Bavistin (0.1%) and a combination of Bavistin + Thiram (1:1) @ 0.3 per cent proved superior in controlling seedborne fungi over untreated control and also resulted in high germination of rice seed.

Table 20. Effect of chemical treatment on germination (%) of original seeds of rice*

Varieties	Untreated control						Chemical treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
V_1	87.00	2.00	11.00	83.00	6.67	10.33	90.33	2.00	8.00	85.67	5.00	9.33
	(9.38)	(1.73)	(3.46)	(9.16)	(2.76)	(3.35)	(9.56)	(1.72)	(3.00)	(9.31)	(2.44)	(3.21)
V_2	93.33	2.00	4.67	92.00	1.33	6.67	95.67	0.67	3.67	94.67	0.00	5.33
	(9.71)	(1.72)	(2.38)	(9.64)	(1.52)	(2.75)	(9.83)	(1.28)	(2.16)	(9.78)	(1.00)	(2.51)
V_3	96.33	0.67	3.00	95.33	0.00	4.67	99.67	0.00	0.67	98.00	0.00	2.00
	(9.87)	(1.28)	(1.99)	(9.81)	(1.00)	(2.38)	(10.03)	(1.00)	(1.28)	(9.95)	(1.00)	(1.72)
V_4	92.67	1.33	6.00	89.67	2.33	8.00	99.00	0.00	1.00	94.33	2.33	3.33
	(9.68)	(1.52)	(2.64)	(9.52)	(1.82)	(3.00)	(10.00)	(1.00)	(1.38)	(9.76)	(1.80)	(2.07)
V_5	93.67	1.67	4.67	92.67	2.67	4.67	97.00	0.67	2.33	93.33	2.67	4.67
	(9.73)	(1.63)	(2.38)	(9.68)	(1.91)	(2.37)	(9.90)	(1.28)	(1.80)	(9.71)	(1.91)	(2.37)
V_6	83.67	3.33	13.00	82.33	5.00	12.67	94.00	1.33	4.67	88.33	4.33	7.33
	(9.20)	(2.08)	(3.74)	(9.13)	(2.44)	(3.69)	(9.75)	(1.52)	(2.37)	(9.45)	(2.31)	(2.88)
V_7	94.00	1.67	4.33	87.67	3.67	8.67	96.67	0.67	2.67	88.67	4.33	7.00
	(9.75)	(1.63)	(2.31)	(9.42)	(2.15)	(3.10)	(9.88)	(1.28)	(1.91)	(9.47)	(2.31)	(2.81)
V_8	89.33	2.00	8.67	86.33	2.67	11.00	96.67	0.67	2.67	97.67	0.67	1.67
	(9.50)	(1.72)	(3.10)	(9.35)	(1.91)	(3.46)	(9.88)	(1.28)	(1.91)	(9.93)	(1.28)	(1.58)
V_9	87.67	2.67	9.67	82.33	7.33	10.33	94.67	1.67	3.67	90.33	1.33	8.33
	(9.42)	(1.91)	(3.26)	(9.13)	(2.88)	(3.36)	(9.78)	(1.63)	(2.16)	(9.56)	(1.52)	(3.05)
V_{10}	86.00	3.00	11.00	84.67	3.33	12.00	93.00	1.33	5.67	88.00	3.33	8.67
	(9.33)	(1.99)	(3.46)	(9.25)	(2.07)	(3.60)	(9.69)	(1.52)	(2.56)	(9.43)	(2.08)	(3.10)
V_{11}	92.33	2.33	5.33	85.00	5.33	9.67	94.33	1.00	4.67	90.33	1.67	8.00
	(9.66)	(1.82)	(2.50)	(9.27)	(2.50)	(3.26)	(9.76)	(1.41)	(2.37)	(9.56)	(1.61)	(3.00)
V_{12}	87.33	4.33	8.33	82.00	5.00	13.00	91.67	1.67	6.67	85.67	4.67	9.67
	(9.40)	(2.31)	(3.04)	(9.11)	(2.44)	(3.74)	(9.63)	(1.63)	(2.77)	(9.31)	(2.38)	(3.27)
Mean	90.28	2.25	7.47	86.92	3.78	9.31	95.22	0.97	3.86	91.25	2.53	6.28
	(9.55)	(1.78)	(2.85)	(9.37)	(2.12)	(3.17)	(9.81)	(1.38)	(2.14)	(9.60)	(1.80)	(2.63)
CD(P= 0.05)	(0.13)	(0.34)	(0.39)	(0.19)	(0.37)	(0.45)	(0.11)	(0.33)	(0.41)	(0.13)	(0.33)	(0.48)
CV	(0.82)	(11.20)	(8.12)	(1.23)	(10.38)	(8.50)	(0.65)	(14.05)	(11.27)	(0.79)	(10.74)	(10.82)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table21. Effect of chemical treatment on germination (%) of apparently healthy seeds of rice*

Varieties	Untreated control						Chemical treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
V_1	91.33	0.00	8.67	86.33	1.67	12.67	91.67	1.33	7.00	88.67	4.00	7.33
	(9.61)	(1.00)	(3.10)	(9.34)	(1.63)	(3.69)	(9.63)	(1.52)	(2.82)	(9.47)	(2.24)	(2.88)
V_2	95.33	1.33	3.67	93.67	0.67	5.67	96.00	0.67	3.33	95.33	0.00	4.67
	(9.81)	(1.52)	(2.16)	(9.73)	(1.28)	(2.56)	(9.85)	(1.28)	(2.08)	(9.81)	(1.00)	(2.37)
V_3	98.67	0.33	1.00	95.67	0.00	4.67	99.67	0.00	0.33	99.33	0.00	1.00
	(9.98)	(1.14)	(1.41)	(9.83)	(1.00)	(2.38)	(10.03)	(1.00)	(1.14)	(10.02)	(1.00)	(1.38)
V_4	97.67	1.33	2.00	95.67	1.67	2.67	99.33	0.00	0.67	97.00	0.67	2.00
	(9.93)	(1.14)	(1.72)	(9.83)	(1.63)	(1.91)	(10.02)	(1.00)	(1.24)	(9.90)	(1.28)	(1.72)
V_5	98.67	0.00	1.33	98.67	0.67	0.67	99.67	0.00	0.33	99.33	0.33	0.33
	(9.98)	(1.00)	(1.52)	(9.98)	(1.28)	(1.28)	(10.03)	(1.00)	(1.14)	(10.02)	(1.14)	(1.14)
V_6	90.33	1.33	8.33	88.33	4.33	7.33	95.33	1.00	3.67	97.33	1.00	1.67
	(9.56)	(1.52)	(3.05)	(9.45)	(2.31)	(2.88)	(9.81)	(1.41)	(2.16)	(9.92)	(1.38)	(1.63)
V_7	98.00	0.67	1.33	94.00	1.33	4.67	97.33	0.33	2.33	96.00	1.00	3.00
	(9.95)	(1.28)	(1.52)	(9.75)	(1.52)	(2.35)	(9.92)	(1.14)	(1.82)	(9.85)	(1.38)	(1.99)
V_8	92.00	2.00	6.00	96.00	1.33	2.67	97.00	0.67	2.33	99.33	0.00	0.67
	(9.64)	(1.73)	(2.64)	(9.85)	(1.52)	(1.91)	(9.90)	(1.28)	(1.80)	(10.02)	(1.00)	(1.28)
V_9	91.67	2.00	6.33	85.67	4.00	10.33	95.33	1.33	3.67	93.33	1.67	5.00
	(9.63)	(1.72)	(2.70)	(9.31)	(2.23)	(3.36)	(9.81)	(1.52)	(2.16)	(9.71)	(1.63)	(2.44)
V_{10}	94.00	1.67	4.33	89.67	2.33	8.00	95.00	1.33	3.67	92.00	3.67	4.33
	(9.75)	(1.61)	(2.31)	(9.52)	(1.80)	(3.00)	(9.80)	(1.52)	(2.16)	(9.64)	(2.16)	(2.29)
V_{11}	97.00	0.67	2.33	90.00	3.67	7.67	96.33	0.33	3.33	93.00	2.00	5.00
	(9.90)	(1.28)	(1.82)	(9.54)	(2.13)	(2.92)	(9.87)	(1.14)	(2.08)	(9.69)	(1.72)	(2.43)
V_{12}	90.33	3.33	6.33	87.00	3.33	9.67	93.67	1.67	4.67	89.67	4.00	6.33
	(9.56)	(2.08)	(2.71)	(9.38)	(2.08)	(3.26)	(9.73)	(1.61)	(2.38)	(9.52)	(2.23)	(2.71)
Mean	94.58	1.22	4.31	91.72	2.08	6.39	96.36	0.72	2.94	95.03	1.53	3.44
	(9.78)	(1.42)	(2.22)	(9.63)	(1.70)	(2.62)	(9.87)	(1.28)	(1.91)	(9.80)	(1.51)	(2.02)
CD(P= 0.05)	(0.09)	(0.35)	(0.30)	(0.15)	(0.40)	(0.48)	(0.08)	(0.32)	(0.37)	(0.12)	(0.37)	(0.47)
CV	(0.52)	(14.62)	(7.98)	(0.94)	(13.89)	(10.95)	(0.47)	(14.98)	(11.39)	(0.75)	(14.45)	(13.87)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table 22. Effect of chemical treatment on germination (%) of partially discoloured seeds of rice*

Varieties	Untreated control						Chemical treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
V_1	85.67	1.33	13.00	80.33	6.33	13.33	93.67	1.33	5.00	84.33	6.33	9.33
	(9.31)	(1.52)	(3.74)	(9.02)	(2.68)	(3.78)	(9.73)	(1.52)	(2.44)	(9.24)	(2.70)	(3.21)
V_2	93.67	1.67	4.67	92.33	0.33	7.33	96.33	0.33	3.33	92.00	0.00	8.00
	(9.73)	(1.63)	(2.38)	(9.66)	(1.14)	(2.87)	(9.87)	(1.14)	(2.08)	(9.64)	(1.00)	(2.99)
V_3	94.33	1.67	4.00	92.33	0.00	7.67	98.33	0.00	1.67	94.33	0.00	5.67
	(9.76)	(1.63)	(2.23)	(9.66)	(1.00)	(2.92)	(9.97)	(1.00)	(1.61)	(9.76)	(1.00)	(2.58)
V_4	90.33	2.33	7.33	91.67	3.67	4.67	97.00	0.67	2.33	91.67	3.67	4.67
	(9.56)	(1.82)	(2.89)	(9.63)	(2.16)	(2.37)	(9.90)	(1.28)	(1.82)	(9.63)	(2.16)	(2.37)
V_5	96.67	0.67	2.67	97.00	1.33	1.67	99.00	0.00	1.00	98.00	0.67	1.33
	(9.88)	(1.24)	(1.88)	(9.90)	(1.52)	(1.58)	(10.00)	(1.00)	(1.41)	(9.95)	(1.24)	(1.49)
V_6	86.33	10.33	10.33	85.33	5.33	9.67	96.33	1.00	2.67	91.00	1.67	7.33
	(9.35)	(2.07)	(3.36)	(9.29)	(2.51)	(3.26)	(9.87)	(1.41)	(1.91)	(9.59)	(1.63)	(2.88)
V_7	95.00	3.67	3.67	87.33	3.33	9.67	96.67	0.33	3.00	92.00	1.67	6.33
	(9.80)	(1.52)	(2.15)	(9.40)	(2.07)	(3.26)	(9.88)	(1.14)	(2.00)	(9.64)	(1.63)	(2.69)
V_8	89.67	8.67	8.67	96.00	1.33	2.67	96.67	0.67	2.67	99.00	0.00	1.00
	(9.52)	(1.63)	(3.11)	(9.85)	(1.52)	(1.90)	(9.88)	(1.28)	(1.91)	(10.00)	(1.00)	(1.38)
V_9	89.67	8.00	8.00	75.67	9.33	15.00	96.33	1.00	2.67	82.67	5.00	12.33
	(9.52)	(1.80)	(3.00)	(8.75)	(3.21)	(3.99)	(9.87)	(1.41)	(1.91)	(9.15)	(2.45)	(3.64)
V_{10}	89.00	9.33	9.33	82.33	4.00	13.67	93.00	1.33	5.67	85.67	4.67	9.33
	(9.49)	(1.61)	(3.21)	(9.13)	(2.23)	(3.82)	(9.70)	(1.52)	(2.58)	(9.31)	(2.37)	(3.21)
V_{11}	90.33	7.00	7.33	81.33	6.67	14.33	95.33	1.33	3.00	90.00	1.33	8.67
	(9.56)	(1.91)	(2.88)	(9.07)	(2.76)	(3.91)	(9.83)	(1.52)	(1.99)	(9.54)	(1.49)	(3.09)
V_{12}	87.33	10.00	10.00	79.00	4.33	16.67	94.00	1.67	4.33	86.67	4.00	9.33
	(9.40)	(1.91)	(3.31)	(8.94)	(2.30)	(4.20)	(9.75)	(1.63)	(2.30)	(9.36)	(2.24)	(3.21)
Mean	90.67	5.39	7.42	86.72	3.83	9.69	96.06	0.81	3.11	90.61	2.42	6.94
	(9.57)	(1.69)	(2.84)	(9.36)	(2.09)	(3.15)	(9.85)	(1.32)	(2.00)	(9.57)	(1.74)	(2.73)
CD(P= 0.05)	(0.10)	(0.42)	(0.36)	(0.18)	(0.42)	(0.55)	(0.07)	(0.29)	(0.31)	(0.15)	(0.37)	(0.50)
CV	(0.63)	(14.61)	(7.56)	(1.15)	(11.79)	(10.27)	(0.44)	(13.18)	(9.30)	(0.91)	(12.59)	(10.95)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

Table 23. Effect of chemical treatment on germination (%) of discoloured seeds of rice*

Varieties	Untreated control						Chemical treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N**	Ab	D	N	Ab	D	N	Ab	D
V_1	81.00	2.33	16.67	76.67	5.67	17.67	86.33	2.33	11.33	77.33	4.00	18.67
	(9.06)	(1.82)	(4.20)	(61.12)	(2.58)	(4.31)	(9.35)	(1.82)	(3.51)	(8.85)	(2.23)	(4.42)
V_2	88.67	2.33	9.00	84.00	2.67	13.67	88.33	2.33	9.33	86.33	1.00	13.33
	(9.47)	(1.82)	(3.16)	(66.48)	(1.91)	(3.82)	(9.45)	(1.82)	(3.21)	(9.35)	(1.38)	(3.78)
V_3	89.67	1.33	9.00	86.33	0.00	13.67	90.67	2.33	7.00	89.33	0.00	10.67
	(9.52)	(1.52)	(3.16)	(68.32)	(1.00)	(3.82)	(9.57)	(1.82)	(2.82)	(9.50)	(1.00)	(3.41)
V_4	88.67	2.33	9.00	84.67	4.00	11.33	92.33	2.67	5.00	89.00	2.67	8.33
	(9.47)	(1.82)	(3.16)	(66.94)	(2.23)	(3.50)	(9.66)	(1.91)	(2.44)	(9.49)	(1.90)	(3.05)
V_5	93.00	1.67	5.33	89.67	3.67	6.67	94.67	1.67	3.67	91.67	2.00	6.33
	(9.70)	(1.63)	(2.49)	(71.22)	(2.16)	(2.77)	(9.78)	(1.63)	(2.16)	(9.63)	(1.72)	(2.69)
V_6	80.67	5.00	14.33	78.67	6.00	15.33	86.00	2.33	11.67	85.67	3.33	11.00
	(9.04)	(2.44)	(3.92)	(62.47)	(2.63)	(4.04)	(9.33)	(1.82)	(3.56)	(9.31)	(2.08)	(3.46)
V_7	89.67	3.67	6.67	79.67	3.33	17.00	91.33	1.67	7.00	82.33	5.67	12.00
	(9.52)	(2.16)	(2.77)	(63.17)	(2.02)	(4.24)	(9.61)	(1.63)	(2.82)	(9.13)	(2.58)	(3.60)
V_8	86.33	5.67	8.00	80.00	5.00	15.00	87.67	1.67	10.67	92.33	2.67	5.00
	(9.35)	(2.58)	(3.00)	(63.41)	(2.44)	(4.00)	(9.42)	(1.63)	(3.41)	(9.66)	(1.90)	(2.44)
V_9	85.67	3.00	11.33	70.00	14.67	15.00	87.67	2.00	10.33	80.67	8.33	11.33
	(9.31)	(1.99)	(3.51)	(56.77)	(3.95)	(4.00)	(9.42)	(1.73)	(3.36)	(9.04)	(3.04)	(3.51)
V_{10}	83.67	4.33	12.00	76.33	4.67	19.00	88.67	2.00	9.33	80.67	6.33	13.00
	(9.20)	(2.29)	(3.60)	(60.87)	(2.38)	(4.47)	(9.47)	(1.73)	(3.21)	(9.04)	(2.71)	(3.74)
V_{11}	84.00	3.67	13.00	65.67	12.33	22.00	88.00	1.33	10.67	73.33	8.00	18.67
	(9.22)	(2.16)	(3.74)	(54.14)	(3.64)	(4.79)	(9.43)	(1.52)	(3.41)	(8.62)	(3.00)	(4.43)
V_{12}	83.33	5.00	12.00	77.00	3.67	19.33	91.67	1.33	7.00	80.67	6.67	12.67
	(9.18)	(2.44)	(3.60)	(61.33)	(2.16)	(4.51)	(9.63)	(1.52)	(2.80)	(9.04)	(2.77)	(3.69)
Mean	86.19	3.36	10.53	79.06	5.47	15.47	89.44	1.97	8.58	84.11	4.22	11.75
	(9.34)	(2.06)	(3.36)	(63.02)	(2.42)	(4.02)	(9.51)	(1.71)	(3.06)	(9.22)	(2.19)	(3.52)
CD(P= 0.05)	(0.13)	(0.32)	(0.33)	(2.69)	(0.45)	(0.42)	(0.12)	(NS)	(0.36)	(0.17)	(0.39)	(0.42)
CV	(0.83)	(9.35)	(5.85)	(2.53)	(10.99)	(6.23)	(0.77)	(9.03)	(6.99)	(1.08)	(10.58)	(7.13)

Figures within parentheses are square root transformed values
** Figures within parentheses are arc sine transformed values**
* Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Chemical: Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed

4.6 Effect of bioagent treatment on germination

Data pertaining to the effect of bioagent treatment on seed germination are given in Tables 24 to 27. All the varieties after treatment with talc based formulation of *Trichoderma harzianum* produced statistically higher number of normal seedlings in each category compared to control (untreated seeds). Among the varieties, variety V_3 had the highest number of normal seedlings in both in-soil and in-between paper methods in original seed category (99.33% and 97.33%) (Table 24). Similarly, apparently healthy seed category produced 99.33 per cent normal seedlings in both the methods in variety V_3 and 99.67 per cent and 99 per cent normal seedlings in variety V_5 respectively (Table 25). Partially discoloured seed produced 98.67 per cent and 98 per cent (Table 26) whereas discoloured seed category produced 94.33 per cent and 90.33 per cent (Table 27) normal seedlings in variety V_5. Bioagent treated seeds also produced minimum per cent of abnormal seedlings as compared to control. All the varieties had lower number of dead seeds in bioagent treated seeds as compared to control in both methods in each category.

Treatment with biocontrol agent (*T. harzianum* talc based formulation @ 4 g/kg seed) was also found to be highly effective in increasing germination of rice seeds in both in-soil and in-between paper method as compared to untreated control. Compared to control germination was increased by 10 per cent and 13 per cent.

Trichoderma species are capable of hyperparasitising the pathogenic fungi and found to involve in protecting number of crop plants (Durell 1968; Barnett and Binder 1973).

Similar findings were reported by Sumitha and Gaikwad (1995). They observed higher and better germination in seeds coated with *Trichoderma harzianum* and *Bacillus subtilis* than untreated seeds. Bharath *et al.* (2005) also reported that seed treatment with biological control agents like *T. harzianum* and *T. viride* showed their efficacy by improving germination, in paper towel method.

Table 24. Effect of bioagent treatment on germination (%) of original seeds of rice*

Varieties	Untreated control						Bioagent treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N**	Ab	D	N	Ab	D	N	Ab	D
V_1	87.00	2.00	11.00	83.00	6.67	10.33	90.00	1.67	8.33	85.67	4.33	10.00
	(9.38)	(1.73)	(3.46)	(9.16)	(2.76)	(3.35)	(9.54)	(1.63)	(3.05)	(9.31)	(2.31)	(3.31)
V_2	93.33	2.00	4.67	92.00	1.33	6.67	95.33	1.33	3.33	94.67	1.33	4.00
	(9.71)	(1.72)	(2.38)	(9.64)	(1.52)	(2.75)	(9.81)	(1.52)	(2.08)	(9.78)	(1.52)	(2.23)
V_3	96.33	0.67	3.00	95.33	0.00	4.67	99.33	0.33	0.67	97.33	0.67	2.00
	(9.87)	(1.28)	(1.99)	(9.81)	(1.00)	(2.38)	(10.02)	(1.14)	(1.28)	(9.92)	(1.28)	(1.72)
V_4	92.67	1.33	6.00	89.67	2.33	8.00	98.67	0.00	1.33	94.33	1.00	4.67
	(9.68)	(1.52)	(2.64)	(9.52)	(1.82)	(3.00)	(9.98)	(1.00)	(1.49)	(9.76)	(1.38)	(2.38)
V_5	93.67	1.67	4.67	92.67	2.67	4.67	96.67	1.00	2.33	93.00	1.33	5.67
	(9.73)	(1.63)	(2.38)	(9.68)	(1.91)	(2.37)	(9.88)	(1.41)	(1.82)	(9.70)	(1.52)	(2.57)
V_6	83.67	3.33	13.00	82.33	5.00	12.67	93.33	1.33	5.33	87.00	3.33	9.67
	(9.20)	(2.08)	(3.74)	(9.13)	(2.44)	(3.69)	(9.71)	(1.52)	(2.50)	(9.38)	(2.07)	(3.26)
V_7	94.00	1.67	4.33	87.67	3.67	8.67	96.00	0.67	3.33	88.33	3.00	8.67
	(9.75)	(1.63)	(2.31)	(9.42)	(2.15)	(3.10)	(9.85)	(1.28)	(2.08)	(9.45)	(1.99)	(3.11)
V_8	89.33	2.00	8.67	86.33	2.67	11.00	96.67	0.67	2.67	98.33	0.00	1.67
	(9.50)	(1.72)	(3.10)	(9.35)	(1.91)	(3.46)	(9.88)	(1.28)	(1.90)	(9.97)	(1.00)	(1.58)
V_9	87.67	2.67	9.67	82.33	7.33	10.33	94.33	1.33	4.33	90.00	2.67	7.33
	(9.42)	(1.91)	(3.26)	(9.13)	(2.88)	(3.36)	(9.76)	(1.52)	(2.31)	(9.54)	(1.91)	(2.89)
V_{10}	86.00	3.00	11.00	84.67	3.33	12.00	93.00	1.67	5.33	87.00	4.00	9.00
	(9.33)	(1.99)	(3.46)	(9.25)	(2.07)	(3.60)	(9.70)	(1.63)	(2.50)	(9.38)	(2.23)	(3.15)
V_{11}	92.33	2.33	5.33	85.00	5.33	9.67	95.00	1.00	4.00	89.33	3.67	7.00
	(9.66)	(1.82)	(2.50)	(9.27)	(2.50)	(3.26)	(9.80)	(1.41)	(2.23)	(9.50)	(2.16)	(2.82)
V_{12}	87.33	4.33	8.33	82.00	5.00	13.00	91.33	1.33	7.33	86.00	4.00	10.00
	(9.40)	(2.31)	(3.04)	(9.11)	(2.44)	(3.74)	(9.61)	(1.52)	(2.89)	(9.33)	(2.23)	(3.30)
Mean	90.28	2.25	7.47	86.92	3.78	9.31	94.97	1.03	4.03	90.92	2.44	6.64
	(9.55)	(1.78)	(2.85)	(9.37)	(2.12)	(3.17)	(9.80)	(1.40)	(2.18)	(9.58)	(1.80)	(2.69)
CD(P= 0.05)	(0.13)	(0.34)	(0.39)	(0.19)	(0.37)	(0.45)	(0.10)	(0.30)	(0.39)	(0.14)	(0.37)	(0.47)
CV	(0.82)	(11.20)	(8.12)	(1.23)	(10.38)	(8.50)	(0.60)	(12.57)	(10.66)	(0.86)	(12.08)	(10.42)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Bioagent: Talc based formulation of *Trichoderma harzianum* @ 4g/kg seed

Table 25. Effect of bioagent treatment on germination (%) of apparently healthy seeds of rice*

Varieties	Untreated control						Bioagent treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N**	Ab	D	N	Ab	D	N	Ab	D
V_1	91.33	0.00	8.67	86.33	1.67	12.67	91.00	1.67	7.33	88.67	3.33	8.00
	(9.61)	(1.00)	(3.10)	(9.34)	(1.63)	(3.69)	(9.59)	(1.63)	(2.88)	(9.47)	(2.08)	(2.99)
V_2	95.33	1.33	3.67	93.67	0.67	5.67	95.67	0.67	3.67	95.00	1.67	3.33
	(9.81)	(1.52)	(2.16)	(9.73)	(1.28)	(2.56)	(9.83)	(1.28)	(2.16)	(9.80)	(1.63)	(2.06)
V_3	98.67	0.33	1.00	95.67	0.00	4.67	99.33	0.00	0.67	99.33	0.00	0.67
	(9.98)	(1.14)	(1.41)	(9.83)	(1.00)	(2.38)	(10.02)	(1.00)	(1.28)	(10.02)	(1.00)	(1.28)
V_4	97.67	1.33	2.00	95.67	1.67	2.67	99.00	0.00	1.00	98.00	0.67	1.33
	(9.93)	(1.14)	(1.72)	(9.83)	(1.63)	(1.91)	(10.00)	(1.00)	(1.38)	(9.95)	(1.28)	(1.47)
V_5	98.67	0.00	1.33	98.67	0.67	0.67	99.67	0.00	0.33	99.00	0.00	1.00
	(9.98)	(1.00)	(1.52)	(9.98)	(1.28)	(1.28)	(10.03)	(1.00)	(1.14)	(10.00)	(1.00)	(1.38)
V_6	90.33	1.33	8.33	88.33	4.33	7.33	95.67	1.00	3.33	96.67	0.67	2.67
	(9.56)	(1.52)	(3.05)	(9.45)	(2.31)	(2.88)	(9.83)	(1.38)	(2.08)	(9.88)	(1.28)	(1.91)
V_7	98.00	0.67	1.33	94.00	1.33	4.67	97.33	0.33	2.33	95.00	1.67	3.33
	(9.95)	(1.28)	(1.52)	(9.75)	(1.52)	(2.35)	(9.92)	(1.14)	(1.79)	(9.80)	(1.63)	(2.08)
V_8	92.00	2.00	6.00	96.00	1.33	2.67	97.67	0.33	2.00	99.33	0.00	0.67
	(9.64)	(1.73)	(2.64)	(9.85)	(1.52)	(1.91)	(9.93)	(1.14)	(1.72)	(10.02)	(1.00)	(1.28)
V_9	91.67	2.00	6.33	85.67	4.00	10.33	94.67	1.00	4.33	93.33	2.33	4.33
	(9.63)	(1.72)	(2.70)	(9.31)	(2.23)	(3.36)	(9.78)	(1.41)	(2.30)	(9.71)	(1.82)	(2.31)
V_{10}	94.00	1.67	4.33	89.67	2.33	8.00	94.67	1.33	4.00	91.33	3.67	5.00
	(9.75)	(1.61)	(2.31)	(9.52)	(1.80)	(3.00)	(9.78)	(1.52)	(2.23)	(9.61)	(2.16)	(2.44)
V_{11}	97.00	0.67	2.33	90.00	3.67	7.67	96.33	0.67	3.00	92.33	2.67	5.00
	(9.90)	(1.28)	(1.82)	(9.54)	(2.13)	(2.92)	(9.87)	(1.28)	(1.99)	(9.66)	(1.91)	(2.44)
V_{12}	90.33	3.33	6.33	87.00	3.33	9.67	93.67	2.00	4.33	90.00	2.67	7.33
	(9.56)	(2.08)	(2.71)	(9.38)	(2.08)	(3.26)	(9.73)	(1.73)	(2.30)	(9.54)	(1.90)	(2.88)
Mean	94.58	1.22	4.31	91.72	2.08	6.39	96.22	0.75	3.03	94.83	1.61	3.56
	(9.78)	(1.42)	(2.22)	(9.63)	(1.70)	(2.62)	(9.86)	(1.29)	(1.94)	(9.79)	(1.56)	(2.04)
CD(P= 0.05)	(0.09)	(0.35)	(0.30)	(0.15)	(0.40)	(0.48)	(0.11)	(0.32)	(0.44)	(0.12)	(0.29)	(0.47)
CV	(0.52)	(14.62)	(7.98)	(0.94)	(13.89)	(10.95)	(0.67)	(14.67)	(13.62)	(0.72)	(11.00)	(13.62)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Bioagent: Talc based formulation of *Trichoderma harzianum* @ 4g/kg seed

Table 26. Effect of bioagent treatment on germination (%) of partially discoloured seeds of rice*

Varieties	Untreated control						Bioagent treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N**	Ab	D	N	Ab	D	N	Ab	D
V_1	85.67	1.33	13.00	80.33	6.33	13.33	91.00	2.00	7.00	83.67	4.67	11.67
	(9.31)	(1.52)	(3.74)	(9.02)	(2.68)	(3.78)	(9.59)	(1.73)	(2.82)	(9.20)	(2.38)	(3.55)
V_2	93.67	1.67	4.67	92.33	0.33	7.33	95.67	0.67	3.67	91.00	3.00	6.00
	(9.73)	(1.63)	(2.38)	(9.66)	(1.14)	(2.87)	(9.83)	(1.28)	(2.16)	(9.59)	(1.99)	(2.64)
V_3	94.33	1.67	4.00	92.33	0.00	7.67	98.67	0.00	1.33	94.00	1.33	3.67
	(9.76)	(1.63)	(2.23)	(9.66)	(1.00)	(2.92)	(9.98)	(1.00)	(1.52)	(9.75)	(1.52)	(2.16)
V_4	90.33	2.33	7.33	91.67	3.67	4.67	97.00	0.33	2.67	91.33	3.33	5.33
	(9.56)	(1.82)	(2.89)	(9.63)	(2.16)	(2.37)	(9.90)	(1.14)	(1.88)	(9.61)	(2.08)	(2.49)
V_5	96.67	0.67	2.67	97.00	1.33	1.67	98.67	0.00	1.33	98.00	0.33	1.33
	(9.88)	(1.24)	(1.88)	(9.90)	(1.52)	(1.58)	(9.98)	(1.00)	(1.52)	(9.95)	(1.14)	(1.49)
V_6	86.33	10.33	10.33	85.33	5.33	9.67	94.33	2.00	3.33	90.33	3.33	6.33
	(9.35)	(2.07)	(3.36)	(9.29)	(2.51)	(3.26)	(9.76)	(1.72)	(2.07)	(9.56)	(2.08)	(2.71)
V_7	95.00	3.67	3.67	87.33	3.33	9.67	95.67	1.33	3.00	91.33	3.00	5.67
	(9.80)	(1.52)	(2.15)	(9.40)	(2.07)	(3.26)	(9.83)	(1.52)	(1.99)	(9.61)	(1.99)	(2.58)
V_8	89.67	8.67	8.67	96.00	1.33	2.67	95.67	1.00	3.33	98.67	0.00	1.33
	(9.52)	(1.63)	(3.11)	(9.85)	(1.52)	(1.90)	(9.83)	(1.41)	(2.08)	(9.98)	(1.00)	(1.49)
V_9	89.67	8.00	8.00	75.67	9.33	15.00	95.33	1.33	3.33	83.33	5.67	11.00
	(9.52)	(1.80)	(3.00)	(8.75)	(3.21)	(3.99)	(9.81)	(1.52)	(2.08)	(9.18)	(2.58)	(3.46)
V_{10}	89.00	9.33	9.33	82.33	4.00	13.67	91.67	1.67	6.67	83.67	3.67	12.67
	(9.49)	(1.61)	(3.21)	(9.13)	(2.23)	(3.82)	(9.63)	(1.63)	(2.77)	(9.20)	(2.15)	(3.69)
V_{11}	90.33	7.00	7.33	81.33	6.67	14.33	94.33	2.00	3.67	88.67	3.67	7.67
	(9.56)	(1.91)	(2.88)	(9.07)	(2.76)	(3.91)	(9.76)	(1.72)	(2.16)	(9.47)	(2.16)	(2.94)
V_{12}	87.33	10.00	10.00	79.00	4.33	16.67	92.33	1.33	6.33	85.67	4.00	10.33
	(9.40)	(1.91)	(3.31)	(8.94)	(2.30)	(4.20)	(9.66)	(1.52)	(2.70)	(9.31)	(2.23)	(3.36)
Mean	90.67	5.39	7.42	86.72	3.83	9.69	95.03	1.14	3.81	89.97	3.00	6.92
	(9.57)	(1.69)	(2.84)	(9.36)	(2.09)	(3.15)	(9.80)	(1.43)	(2.15)	(9.53)	(1.94)	(2.71)
CD(P= 0.05)	(0.10)	(0.42)	(0.36)	(0.18)	(0.42)	(0.55)	(0.09)	(0.32)	(0.36)	(0.13)	(0.32)	(0.46)
CV	(0.63)	(14.61)	(7.56)	(1.15)	(11.79)	(10.27)	(0.53)	(13.09)	(10.06)	(0.79)	(9.64)	(9.95)

Figures within parentheses are square root transformed values
*** Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Bioagent: Talc based formulation of *Trichoderma harzianum* @ 4g/kg seed

Table 27. Effect of bioagent treatment on germination (%) of discoloured seeds of rice*

Varieties	Untreated control						Bioagent treated					
	In-soil			In-between paper			In-soil			In-between paper		
	N	Ab	D	N**	Ab	D	N	Ab	D	N	Ab	D
V_1	81.00	2.33	16.67	76.67	5.67	17.67	85.33	1.67	13.00	77.67	6.33	16.00
	(9.06)	(1.82)	(4.20)	(61.12)	(2.58)	(4.31)	(9.29)	(1.63)	(3.74)	(8.87)	(2.71)	(4.12)
V_2	88.67	2.33	9.00	84.00	2.67	13.67	88.00	1.67	10.33	86.33	3.00	10.67
	(9.47)	(1.82)	(3.16)	(66.48)	(1.91)	(3.82)	(9.43)	(1.63)	(3.37)	(9.35)	(2.00)	(3.41)
V_3	89.67	1.33	9.00	86.33	0.00	13.67	90.67	2.00	7.33	88.67	2.67	8.67
	(9.52)	(1.52)	(3.16)	(68.32)	(1.00)	(3.82)	(9.57)	(1.72)	(2.89)	(9.47)	(1.91)	(3.11)
V_4	88.67	2.33	9.00	84.67	4.00	11.33	90.33	2.33	7.33	87.67	3.00	9.33
	(9.47)	(1.82)	(3.16)	(66.94)	(2.23)	(3.50)	(9.56)	(1.82)	(2.89)	(9.42)	(1.99)	(3.21)
V_5	93.00	1.67	5.33	89.67	3.67	6.67	94.33	1.67	4.00	90.33	1.67	8.00
	(9.70)	(1.63)	(2.49)	(71.22)	(2.16)	(2.77)	(9.76)	(1.63)	(2.24)	(9.56)	(1.63)	(3.00)
V_6	80.67	5.00	14.33	78.67	6.00	15.33	85.67	3.00	11.33	85.67	4.00	10.33
	(9.04)	(2.44)	(3.92)	(62.47)	(2.63)	(4.04)	(9.31)	(1.99)	(3.51)	(9.31)	(2.24)	(3.37)
V_7	89.67	3.67	6.67	79.67	3.33	17.00	91.00	2.33	6.67	83.00	5.33	11.67
	(9.52)	(2.16)	(2.77)	(63.17)	(2.02)	(4.24)	(9.59)	(1.82)	(2.76)	(9.16)	(2.51)	(3.55)
V_8	86.33	5.67	8.00	80.00	5.00	15.00	86.00	3.00	11.00	93.00	1.67	5.33
	(9.35)	(2.58)	(3.00)	(63.41)	(2.44)	(4.00)	(9.33)	(1.99)	(3.46)	(9.69)	(1.63)	(2.50)
V_9	85.67	3.00	11.33	70.00	14.67	15.00	85.67	2.33	12.00	75.67	9.67	14.33
	(9.31)	(1.99)	(3.51)	(56.77)	(3.95)	(4.00)	(9.31)	(1.82)	(3.60)	(8.76)	(3.26)	(3.91)
V_{10}	83.67	4.33	12.00	76.33	4.67	19.00	88.33	4.00	9.33	79.00	5.67	15.33
	(9.20)	(2.29)	(3.60)	(60.87)	(2.38)	(4.47)	(9.45)	(2.19)	(3.21)	(8.94)	(2.58)	(4.04)
V_{11}	84.00	3.67	13.00	65.67	12.33	22.00	86.00	2.33	11.67	74.33	6.33	19.33
	(9.22)	(2.16)	(3.74)	(54.14)	(3.64)	(4.79)	(9.33)	(1.82)	(3.56)	(8.68)	(2.70)	(4.51)
V_{12}	83.33	5.00	12.00	77.00	3.67	19.33	89.67	1.67	8.67	80.00	6.67	13.33
	(9.18)	(2.44)	(3.60)	(61.33)	(2.16)	(4.51)	(9.52)	(1.63)	(3.11)	(9.00)	(2.76)	(3.78)
Mean	86.19	3.36	10.53	79.06	5.47	15.47	88.42	2.33	9.39	83.44	4.67	11.86
	(9.34)	(2.06)	(3.36)	(63.02)	(2.42)	(4.02)	(9.45)	(1.81)	(3.19)	(9.18)	(2.33)	(3.54)
CD(P= 0.05)	(0.13)	(0.32)	(0.33)	(2.69)	(0.45)	(0.42)	(0.08)	NS	(0.25)	(0.12)	(0.30)	(0.35)
CV	(0.83)	(9.35)	(5.85)	(2.53)	(10.99)	(6.23)	(0.52)	(14.03)	(4.62)	(0.80)	(7.60)	(5.81)

Figures within parentheses are square root transformed values
**** Figures within parentheses are arc sine transformed values**
* **Average of three replications**
N= Normal seedlings; Ab= Abnormal seedlings; D= Dead seeds
Bioagent: Talc based formulation of *Trichoderma harzianum* @ 4g/kg seed

4.7 Seedling vigour

The results of effect of associated microflora on seedling vigour of different varieties on each category are presented in Tables 28 to 31.

Among varieties of untreated original seeds category, variety V_5 showed highest seedling vigour index (2073) which is statistically significantly different from other varieties (Table 28). Second highest seedling vigour was recorded in variety V_2 (1733.50) followed by varieties V_3 (1658.47) and V_{12} (1588.43). Seedling vigour was significantly lowest in varieties V_1 (558.93), V_9 (573.90) and V_{11} (596.60), whereas it was in medium range in varieties V_4, V_6 and V_7.

Almost similar trend was observed in two other seed categories *viz.*, apparently healthy and discoloured seed. Highest seedling index (2190.67) was found in variety V_5 of apparently healthy seed category (Table 29) whereas in partially discoloured seed category, it was maximum in variety V_4 followed by varieties V_2, V_3, V_5 and V_{12} which was in decreasing order of 1881.87, 1760.17, 1747.87, 1700.53 and 1651.60 respectively. Least seedling vigour was found in varieties V_9 (466.43), V_{11} (514.33) and V_{10} (675.27).

Among seed categories, the seedling vigour was comparatively low in discoloured seed category, lowest having 403.63 (V_9) and highest 1805.13 (V_5).

The 12 varieties in each seed categories can be groups into three subcategories based on seedling vigour *viz.*, more than 1500 (V_2 to V_6 and V_{12}), between 900 to 1500 (V_7 and V_8) and less than 900 (V_1, V_9, V_{10} and V_{11}).

Gora *et al.* (1987) reported that *Alternaria padwickii* caused significant reduction in seed germination, root and shoot length in rice seeds grown in infested soil. Studies by Sachan and Agarwal (1994) also reported that fungi associated with seed discolouration in rice leads to loss of seedling vigour. The lower seedling vigour in discoloured seed category is probably due to high infection of seedborne fungi.

4.8 Effect of chemical and bioagent treatment on seedling vigour

Data presented in Tables 28 to 31 indicated the effect of different fungicides and bioagent on root length, shoot length and seedling vigour index of rice varieties in different categories. Both the treatments (chemical and bioagent)

Table 28. Effect of associated microflora on seedling vigour of original seeds of rice*

Varieties	Germination (%)			Root Length (cm)			Shoot Length (cm)			Seedling Vigour Index		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	83.00	85.67	85.67	1.20	1.87	1.70	5.53	6.17	6.10	558.93	688.23	668.20
	(9.16)	(9.31)	(9.31)	(1.48)	(1.69)	(1.64)	(2.56)	(2.68)	(2.66)			
V_2	92.00	94.67	94.67	12.23	13.47	13.03	6.60	7.43	7.30	1733.50	1978.33	1924.80
	(9.64)	(9.78)	(9.78)	(3.64)	(3.80)	(3.75)	(2.76)	(2.90)	(2.88)			
V_3	95.33	98.00	97.33	10.53	12.73	12.73	6.87	7.47	7.20	1658.47	1979.93	1940.20
	(9.81)	(9.95)	(9.92)	(3.40)	(3.71)	(3.71)	(2.80)	(2.91)	(2.86)			
V_4	89.67	94.33	94.33	10.20	13.20	12.10	7.03	8.77	8.47	1545.23	2073.10	1940.40
	(9.52)	(9.76)	(9.76)	(3.35)	(3.77)	(3.62)	(2.83)	(3.12)	(3.08)			
V_5	92.67	93.33	93.00	14.57	14.10	14.03	8.37	8.87	8.30	2073.00	2143.20	2076.77
	(9.68)	(9.71)	(9.70)	(3.95)	(3.88)	(3.88)	(3.06)	(3.14)	(3.05)			
V_6	82.33	88.33	87.00	12.30	13.57	13.20	6.20	7.47	7.33	1523.00	1857.97	1785.87
	(9.13)	(9.45)	(9.38)	(3.65)	(3.82)	(3.77)	(2.68)	(2.91)	(2.89)			
V_7	87.67	88.67	88.33	8.17	8.50	8.40	3.97	4.13	3.97	1063.37	1120.17	1066.00
	(9.42)	(9.47)	(9.45)	(3.03)	(3.08)	(3.02)	(2.23)	(2.27)	(2.23)			
V_8	86.33	97.67	98.33	10.13	11.33	10.73	2.43	3.03	2.80	1084.97	1403.33	1330.87
	(9.35)	(9.93)	(9.97)	(3.34)	(3.51)	(3.43)	(1.85)	(2.01)	(1.95)			
V_9	82.33	90.33	90.00	1.73	2.03	1.90	5.23	5.47	5.43	573.90	677.43	660.03
	(9.13)	(9.56)	(9.54)	(1.65)	(1.74)	(1.70)	(2.50)	(2.54)	(2.54)			
V_{10}	84.67	88.00	87.00	2.43	3.23	3.10	6.03	6.27	5.67	716.37	835.90	762.53
	(9.25)	(9.43)	(9.38)	(1.85)	(2.06)	(2.02)	(2.65)	(2.70)	(2.58)			
V_{11}	85.00	90.33	89.33	1.23	2.00	1.83	5.77	6.23	5.57	596.60	744.13	660.97
	(9.27)	(9.56)	(9.50)	(1.49)	(1.73)	(1.68)	(2.60)	(2.69)	(2.56)			
V_{12}	82.00	85.67	86.00	12.70	13.37	13.20	6.67	7.70	7.53	1588.43	1804.73	1783.60
	(9.11)	(9.31)	(9.33)	(3.70)	(3.79)	(3.77)	(2.77)	(2.95)	(2.92)			
Mean	86.92	91.25	90.92	8.12	9.12	8.83	5.89	6.58	6.31	1226.31	1442.21	1383.35
	(9.37)	(9.60)	(9.58)	(2.88)	(3.05)	(3.00)	(2.61)	(2.73)	(2.68)			
CD(P= 0.05)	(0.19)	(0.13)	(0.14)	(0.10)	(0.10)	(0.07)	(0.09)	(0.10)	(0.07)	109.60	91.77	69.64
CV	(1.23)	(0.79)	(0.86)	(2.16)	(1.88)	(1.35)	(2.14)	(2.17)	(1.56)	5.30	3.78	2.99

Figures within parentheses are square root transformed values

*** Average of four replications**

T_1= Untreated control

T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)

T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 29. Effect of associated microflora on seedling vigour of apparently healthy seeds of rice*

Varieties	Germination (%)			Root Length (cm)			Shoot Length (cm)			Seedling Vigour Index		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	86.33	88.67	88.67	1.63	1.90	1.73	5.83	6.47	6.37	644.80	741.90	718.53
	(9.34)	(9.47)	(9.47)	(1.62)	(1.70)	(1.65)	(2.61)	(2.73)	(2.71)			
V_2	93.67	95.33	95.00	12.87	13.60	13.43	7.17	7.73	7.43	1876.17	2033.77	1982.40
	(9.73)	(9.81)	(9.80)	(3.72)	(3.82)	(3.80)	(2.86)	(2.96)	(2.90)			
V_3	95.67	99.33	99.33	11.53	12.63	12.43	7.07	7.87	7.43	1780.00	2036.53	1973.47
	(9.83)	(10.02)	(10.02)	(3.54)	(3.69)	(3.67)	(2.84)	(2.98)	(2.90)			
V_4	95.67	97.00	98.00	12.93	13.80	13.90	8.10	8.63	8.37	2012.10	2176.43	2181.93
	(9.83)	(9.90)	(9.95)	(3.73)	(3.85)	(3.86)	(3.02)	(3.10)	(3.06)			
V_5	98.67	99.33	99.00	13.90	14.50	14.13	8.30	8.77	8.80	2190.67	2311.03	2270.77
	(9.98)	(10.02)	(10.00)	(3.86)	(3.94)	(3.89)	(3.05)	(3.12)	(3.13)			
V_6	88.33	97.33	96.67	12.83	14.27	14.17	6.90	7.90	7.73	1743.27	2158.20	2116.93
	(9.45)	(9.92)	(9.88)	(3.72)	(3.91)	(3.89)	(2.81)	(2.98)	(2.96)			
V_7	94.00	96.00	95.00	8.43	8.67	8.50	4.13	4.37	4.20	1181.63	1250.80	1206.50
	(9.75)	(9.85)	(9.80)	(3.07)	(3.11)	(3.08)	(2.27)	(2.32)	(2.28)			
V_8	96.00	99.33	99.33	10.20	11.50	10.80	2.57	3.20	3.00	1225.57	1460.10	1370.90
	(9.85)	(10.02)	(10.02)	(3.35)	(3.54)	(3.44)	(1.89)	(2.05)	(2.00)			
V_9	85.67	93.33	93.33	1.90	2.10	1.90	5.60	6.00	5.23	643.03	756.13	665.87
	(9.31)	(9.71)	(9.71)	(1.70)	(1.76)	(1.70)	(2.57)	(2.65)	(2.50)			
V_{10}	89.67	92.00	91.33	2.40	3.00	3.23	6.20	6.27	5.77	771.00	852.80	822.13
	(9.52)	(9.64)	(9.61)	(1.84)	(2.00)	(2.06)	(2.68)	(2.70)	(2.60)			
V_{11}	90.00	93.00	92.33	1.30	2.00	1.83	6.13	6.23	5.57	669.80	736.33	683.30
	(9.54)	(9.69)	(9.66)	(1.51)	(1.73)	(1.68)	(2.67)	(2.69)	(2.56)			
V_{12}	87.00	89.67	90.00	12.73	14.60	13.37	7.20	8.53	7.80	1734.80	2074.20	1905.00
	(9.38)	(9.52)	(9.54)	(3.71)	(3.95)	(3.79)	(2.86)	(3.09)	(2.97)			
Mean	91.72	95.03	94.83	8.56	9.38	9.12	6.27	6.83	6.47	1372.74	1549.02	1491.48
	(9.63)	(9.80)	(9.79)	(2.95)	(3.08)	(3.04)	(2.68)	(2.78)	(2.71)			
CD(P= 0.05)	(0.15)	(0.12)	(0.12)	(0.09)	(0.06)	(0.06)	(0.07)	(0.08)	(0.05)	89.71	82.11	69.95
CV	(0.94)	(0.75)	(0.72)	(1.89)	(1.17)	(1.10)	(1.60)	(1.80)	(1.06)	3.88	3.15	2.78

Figures within parentheses are square root transformed values

*** Average of four replications**

T_1= Untreated control

T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)

T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 30. Effect of associated microflora on seedling vigour of partially discoloured seeds of rice*

Varieties	Germination (%)			Root Length (cm)			Shoot Length (cm)			Seedling Vigour Index		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	80.33	84.33	83.67	1.53	1.90	1.60	5.63	6.27	5.97	575.77	688.70	633.30
	(9.02)	(9.24)	(9.20)	(1.59)	(1.70)	(1.61)	(2.58)	(2.70)	(2.64)			
V_2	92.33	92.00	91.00	12.33	13.30	13.00	6.73	7.27	7.10	1760.17	1892.27	1829.10
	(9.66)	(9.64)	(9.59)	(3.65)	(3.78)	(3.74)	(2.78)	(2.88)	(2.85)			
V_3	92.33	94.33	94.00	11.80	12.63	12.27	7.13	7.67	7.53	1747.87	1914.77	1861.20
	(9.66)	(9.76)	(9.75)	(3.58)	(3.69)	(3.64)	(2.85)	(2.94)	(2.92)			
V_4	91.67	91.67	91.33	12.77	13.43	13.60	7.77	8.13	8.00	1881.87	1977.60	1972.53
	(9.63)	(9.63)	(9.61)	(3.71)	(3.80)	(3.82)	(2.96)	(3.02)	(3.00)			
V_5	97.00	98.00	98.00	10.60	11.43	11.57	6.93	8.23	8.20	1700.53	1927.67	1936.67
	(9.90)	(9.95)	(9.95)	(3.41)	(3.53)	(3.54)	(2.82)	(3.04)	(3.03)			
V_6	85.33	91.00	90.33	12.20	13.43	13.07	6.93	7.23	7.13	1632.70	1880.17	1824.70
	(9.29)	(9.59)	(9.56)	(3.63)	(3.80)	(3.75)	(2.82)	(2.87)	(2.85)			
V_7	87.33	92.00	91.33	8.50	8.53	8.30	4.00	4.33	4.10	1091.67	1183.33	1132.73
	(9.40)	(9.64)	(9.61)	(3.08)	(3.09)	(3.05)	(2.24)	(2.31)	(2.26)			
V_8	96.00	99.00	98.67	9.90	11.10	10.90	2.30	2.97	3.03	1171.00	1392.23	1374.67
	(9.85)	(10.00)	(9.98)	(3.30)	(3.48)	(3.45)	(1.82)	(1.99)	(2.01)			
V_9	75.67	82.67	83.33	1.27	2.00	1.87	4.90	5.17	5.00	466.43	591.73	572.27
	(8.75)	(9.15)	(9.18)	(1.51)	(1.73)	(1.69)	(2.43)	(2.48)	(2.45)			
V_{10}	82.33	85.67	83.67	2.23	3.07	2.80	5.97	6.00	5.67	675.27	785.37	708.30
	(9.13)	(9.31)	(9.20)	(1.80)	(2.02)	(1.95)	(2.64)	(2.66)	(2.58)			
V_{11}	81.33	90.00	88.67	0.87	2.00	1.80	5.47	5.77	5.60	514.33	699.33	656.17
	(9.07)	(9.54)	(9.47)	(1.36)	(1.73)	(1.67)	(2.54)	(2.60)	(2.57)			
V_{12}	79.00	86.67	85.67	13.23	13.47	13.27	7.67	7.80	7.40	1651.60	1842.97	1770.57
	(8.94)	(9.36)	(9.31)	(3.77)	(3.80)	(3.78)	(2.94)	(2.97)	(2.90)			
Mean	86.72	90.61	89.97	8.10	8.86	8.67	5.95	6.40	6.23	1239.10	1398.01	1356.02
	(9.36)	(9.57)	(9.53)	(2.87)	(3.01)	(2.98)	(2.62)	(2.70)	(2.67)			
CD(P= 0.05)	(0.18)	(0.15)	(0.13)	(0.09)	(0.09)	(0.06)	(0.09)	(0.07)	(0.04)	70.35	86.73	46.46
CV	(1.15)	(0.91)	(0.79)	(1.89)	(1.74)	(1.26)	(2.11)	(1.62)	(0.92)	3.37	3.68	2.03

Figures within parentheses are square root transformed values

*** Average of three replications**

T_1= Untreated control

T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)

T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 31. Effect of associated microflora on seedling vigour of discoloured seeds of rice*

Varieties	Germination (%)			Root Length (cm)			Shoot Length (cm)			Seedling Vigour Index		
	T_1**	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	76.67	77.33	77.67	1.50	1.90	1.77	5.67	6.33	6.10	549.00	636.60	611.07
	(61.12)	(8.85)	(8.87)	(1.58)	(1.70)	(1.66)	(2.58)	(2.71)	(2.66)			
V_2	84.00	86.33	86.33	12.53	13.17	13.17	6.83	7.33	7.33	1626.40	1769.70	1769.80
	(66.48)	(9.35)	(9.35)	(3.68)	(3.76)	(3.76)	(2.80)	(2.89)	(2.89)			
V_3	86.33	89.33	88.67	11.70	12.93	13.03	7.33	7.70	7.53	1643.30	1842.87	1823.53
	(68.32)	(9.50)	(9.47)	(3.56)	(3.73)	(3.75)	(2.89)	(2.95)	(2.92)			
V_4	84.67	89.00	87.67	12.00	13.67	13.67	7.50	8.57	8.00	1650.60	1979.07	1899.43
	(66.94)	(9.49)	(9.42)	(3.60)	(3.83)	(3.83)	(2.91)	(3.09)	(3.00)			
V_5	89.67	91.67	90.33	11.97	12.97	11.20	8.17	8.43	8.03	1805.13	1960.97	1737.47
	(71.22)	(9.63)	(9.56)	(3.60)	(3.73)	(3.49)	(3.03)	(3.07)	(3.01)			
V_6	78.67	85.67	85.67	11.73	13.93	12.73	6.10	7.27	7.00	1402.97	1816.13	1690.57
	(62.47)	(9.31)	(9.31)	(3.57)	(3.86)	(3.71)	(2.66)	(2.88)	(2.83)			
V_7	79.67	82.33	83.00	8.07	8.33	8.20	3.83	4.03	4.00	948.00	1018.13	1012.60
	(63.17)	(9.13)	(9.16)	(3.01)	(3.05)	(3.03)	(2.20)	(2.24)	(2.24)			
V_8	80.00	92.33	93.00	9.83	10.60	10.90	2.37	2.73	2.83	975.97	1249.07	1255.50
	(63.41)	(9.66)	(9.69)	(3.29)	(3.44)	(3.42)	(1.83)	(1.93)	(1.96)			
V_9	70.00	80.67	75.67	1.50	1.70	1.90	4.27	4.83	4.67	403.63	526.83	497.10
	(56.77)	(9.04)	(8.76)	(1.58)	(1.64)	(1.70)	(2.29)	(2.41)	(2.38)			
V_{10}	76.33	80.67	79.00	2.13	3.00	2.67	5.80	5.90	5.63	605.80	718.00	655.60
	(60.87)	(9.04)	(8.94)	(1.77)	(2.00)	(1.91)	(2.61)	(2.63)	(2.58)			
V_{11}	65.67	73.33	74.33	1.00	1.50	1.70	5.23	5.87	5.70	411.57	540.50	550.07
	(54.14)	(8.62)	(8.68)	(1.41)	(1.58)	(1.64)	(2.49)	(2.62)	(2.59)			
V_{12}	77.00	80.67	80.00	12.70	14.60	13.40	7.17	8.67	7.60	1529.13	1876.73	1680.00
	(61.33)	(9.04)	(9.00)	(3.70)	(3.95)	(3.79)	(2.86)	(3.11)	(2.93)			
Mean	79.06	84.11	83.44	8.06	9.04	8.69	5.86	6.47	6.20	1129.29	1327.88	1265.23
	(63.02)	(9.22)	(9.18)	(2.86)	(3.02)	(2.98)	(2.60)	(2.71)	(2.66)			
CD(P= 0.05)	(3.43)	(0.17)	(0.12)	(0.11)	(0.13)	(0.06)	(0.10)	(0.08)	(0.05)	79.50	94.45	40.61
CV	(3.26)	(1.08)	(0.81)	(2.30)	(2.49)	(1.24)	(2.34)	(1.65)	(1.10)	4.2	4.24	1.91

Figures within parentheses are square root transformed values
** Figures within parentheses are arc sine transformed values**
* Average of three replications**
T_1= Untreated control**
T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)**
T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)**

invariably improved the seedling vigour appreciably in comparison to untreated control in each variety and seed category. However, chemical treatment showed better results than bioagent treatment.

The chemical and bioagent treatment improved the seedling vigour index of variety V_9 from 573.90 to 677.43 and 660.03 in original seed category, from 643. to 756.13 and 665.87 in apparently healthy seed category, from 466.43 to 591.73 and 572.27 in partially discoloured seed category and from 403.63 to 526.83 and 497.1 in discoloured seed category respectively. Similar trend was observed in all varieties and seed categories. Sumitha and Gaikwad (1995) reported that seeds coated with the antagonists *Trichoderma harzianum* and *Bacillus subtilis* produced longer roots and shoots than untreated seeds. Enhanced root and shoot length may be due to effect of suppression of pathogenic microflora by treatment to seeds with bioagent. Seed treatment, pathogens, seed quality and genetic constitution are some of the factors influencing vigour, distinct from germinability (Seshu *et al.*, 1988). Bharath *et al.* (2005) also reported increased root and shoot length and vigour index in watermelon by treatment with chemicals and bioagents and dominance of chemical fungicides over bioagents. Sagar and Hegde (2006) also studied the effect of seed treatment with fungicides on seed vigour.

4.9 Lethal seed infection

The data in lethal infection of different categories of rice seeds and varieties are given in Tables 32 to 35.

The highest lethal seed infection was found to be caused by *Curvularia lunata* in original seed category of 12 varieties having highest infection on variety V_1 (3.33% dead seeds & 2.33% abnormal seedlings) (Table 32). Second highest lethal infection was recorded to be caused by *Fusarium solani* and highest infection was recorded on variety V_8 (3.33% dead seeds & 1.67% abnormal seedlings) followed by *Fusarium moniliforme* with highest infection on variety V_{10} (3% dead seeds & 1.67% abnormal seedlings) and V_{11} (2.67% dead seeds & 2.33% abnormal seedlings).

Table 32. Lethal seed infection level (%) of original seeds of rice under different treatment levels*

Variety	Condition	Physiological			*Fusarium moniliforme*			*Fusarium solani*			*Curvularia lunata*			*Alternaria alternata*			*Alternaria padwickii*			*Drechslera oryzae*			*Phoma sorghina*		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	Ab	2.67	2.67	2.00	1.00	0.00	1.00	0.33	0.67	0.00	2.33	1.67	1.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.67	4.33	4.33	1.00	1.33	2.00	1.67	1.00	1.00	3.33	2.00	1.67	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_2	Ab	0.00	0.00	1.33	0.00	0.00	0.00	1.00	0.00	0.00	2.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
	D	1.67	2.67	2.00	0.00	0.00	0.00	1.67	1.00	1.33	1.67	1.00	0.33	1.00	0.67	0.33	0.00	0.00	0.00	0.67	0.67	0.00	0.00	0.00	0.00
V_3	Ab	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	2.00	1.00	1.00	0.67	0.00	0.67	2.00	0.67	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_4	Ab	0.67	0.33	0.78	0.22	0.00	0.22	1.00	0.22	0.22	0.67	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
	D	0.56	0.89	0.78	0.00	0.00	0.11	0.56	0.33	0.44	0.56	0.33	0.11	0.33	0.22	0.11	0.00	0.00	0.00	0.22	0.22	0.00	0.00	0.00	0.00
V_5	Ab	1.00	1.67	1.00	0.67	0.67	0.33	0.67	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
	D	1.33	1.67	2.00	1.00	1.33	1.33	0.67	0.67	1.33	0.33	0.33	0.67	0.00	0.00	0.00	1.33	0.67	0.67	0.33	0.00	0.00	0.00	0.00	0.00
V_6	Ab	1.33	2.00	1.33	1.00	1.67	1.33	1.00	0.33	0.67	0.00	0.67	0.33	0.67	0.00	0.00	0.00	0.00	0.67	1.00	0.33	0.00	0.00	0.00	0.00
	D	3.00	3.33	3.67	2.00	2.00	2.00	0.67	0.67	1.33	1.00	1.00	1.67	0.67	0.33	0.67	0.00	0.00	0.33	5.33	0.00	0.00	0.00	0.00	0.00
V_7	Ab	1.00	2.00	1.67	1.67	1.67	1.33	0.67	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
	D	3.00	2.67	3.00	2.33	2.00	2.67	1.67	1.33	1.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	1.00	1.00
V_8	Ab	1.00	0.67	0.00	0.00	0.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	4.33	1.00	0.67	0.00	0.00	0.00	3.33	0.67	1.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	1.00	0.00
V_9	Ab	3.00	1.00	1.33	2.00	0.33	1.33	0.33	0.00	0.00	1.67	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.33	3.67	2.67	2.00	2.33	2.33	0.67	1.00	0.00	2.33	1.33	1.67	2.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_{10}	Ab	1.33	1.67	1.67	1.67	1.67	1.67	0.00	0.00	0.33	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00
	D	2.33	2.67	3.67	3.00	2.00	2.00	1.00	1.00	0.67	2.67	1.67	1.67	2.00	1.00	1.00	0.00	0.00	0.00	1.00	0.33	0.00	0.00	0.00	0.00
V_{11}	Ab	1.33	1.33	1.67	2.33	0.33	1.33	0.00	0.00	0.33	0.67	0.00	0.00	1.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	2.33	3.67	2.67	2.67	2.00	2.67	1.00	0.00	0.33	2.00	1.33	0.67	1.33	0.67	0.67	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
V_{12}	Ab	1.67	2.33	1.67	2.00	2.00	2.33	0.00	0.00	0.00	0.67	0.33	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	4.67	3.33	4.67	2.67	2.33	2.67	0.33	0.67	0.67	2.00	1.00	1.00	1.67	1.00	1.00	0.00	0.00	0.00	1.67	1.33	0.00	0.00	0.00	0.00

*** Average of three replications**
Ab= Abnormal seedlings
D= Dead seeds
T_1= Untreated seeds
T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)
T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 33. Lethal seed infection level (%) of apparently healthy seeds of rice under different treatment levels*

Variety	Condition	Physiological			*Fusarium moniliforme*			*Fusarium solani*			*Curvularia lunata*			*Alternaria alternata*			*Alternaria padwickii*			*Drechslera oryzae*			*Phoma sorghina*		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	Ab	1.67	2.00	2.00	0.33	1.33	1.00	0.67	0.00	0.00	0.67	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.67	4.67	3.00	1.33	1.00	2.00	1.67	0.00	1.33	2.67	1.00	1.33	1.67	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_2	Ab	0.00	0.00	1.33	0.00	0.00	0.00	0.67	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	2.00	2.33	1.33	0.00	0.00	0.00	1.00	1.00	1.33	1.67	1.00	0.67	0.67	0.00	0.67	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
V_3	Ab	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.67	0.67	0.33	0.00	0.00	0.00	3.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_4	Ab	1.33	1.00	0.67	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.67	1.00	0.67	0.00	0.00	0.00	1.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_5	Ab	0.33	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	0.67	0.00	0.67	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_6	Ab	1.67	0.67	0.67	0.33	0.00	0.00	1.67	0.33	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.00	1.00	1.00	0.33	0.00	0.00	1.67	0.67	1.67	1.33	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_7	Ab	0.67	0.33	1.00	0.67	0.67	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.67	1.33	1.33	1.67	1.00	1.33	1.00	0.67	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
V_8	Ab	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.33	0.33	0.67	1.00	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_9	Ab	2.00	1.33	1.33	1.00	0.00	1.00	0.67	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.67	2.00	1.67	2.00	1.67	1.33	1.33	0.67	0.33	2.00	0.67	0.67	1.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_{10}	Ab	1.33	1.67	1.67	1.00	1.00	1.67	0.00	1.00	0.00	0.00	0.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	2.67	1.33	1.33	1.67	1.33	1.33	0.33	0.67	0.67	1.33	0.67	0.33	1.33	0.33	0.33	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
V_{11}	Ab	1.33	1.33	1.67	0.33	0.33	0.33	1.33	0.33	0.67	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.00	1.67	2.00	1.00	0.67	0.67	0.67	1.33	1.00	1.67	0.67	1.00	1.00	0.33	0.33	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.00
V_{12}	Ab	2.00	1.67	1.67	0.33	0.67	0.33	1.00	1.33	0.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	4.00	2.00	2.33	0.67	1.67	0.67	1.33	0.33	2.00	1.33	1.00	1.00	1.67	1.00	1.00	0.00	0.00	0.00	0.67	0.33	0.33	0.00	0.00	0.00

*** Average of three replications**
Ab= Abnormal seedlings
D= Dead seeds
T_1= Untreated seeds
T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)
T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 34. Lethal seed infection level (%) of partially discoloured seeds of rice under different treatment levels*

Variety	Condition	Physiological			*Fusarium moniliforme*			*Fusarium solani*			*Curvularia lunata*			*Alternaria alternata*			*Alternaria padwickii*			*Drechslera oryzae*			*Phoma sorghina*		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	Ab	3.33	3.00	2.67	0.33	0.67	1.00	1.00	0.67	1.00	2.00	2.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	4.67	4.67	4.67	1.33	0.67	1.67	1.33	1.33	1.33	5.67	2.33	2.67	0.67	0.33	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_2	Ab	0.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.33	3.33	2.67	0.00	0.00	0.00	1.00	1.33	2.00	1.00	1.67	0.67	1.00	0.67	0.33	0.33	0.00	0.00	1.00	0.67	0.00	0.00	0.00	0.00
V_3	Ab	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.33	3.00	2.00	1.00	0.33	0.33	3.33	2.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_4	Ab	2.00	1.67	1.67	0.00	0.00	0.00	1.67	1.67	1.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.67	1.67	2.67	0.00	0.00	0.00	2.00	2.67	2.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.33	0.33	0.00	0.00	0.00
V_5	Ab	1.00	0.67	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	0.67	0.67	1.33	0.00	0.00	0.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_6	Ab	2.00	1.00	1.67	1.00	0.67	0.33	1.00	0.33	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00
	D	4.33	2.33	2.33	0.67	1.00	0.67	1.00	1.67	2.00	1.33	1.00	1.00	0.67	0.67	0.33	0.00	0.00	0.00	1.67	0.67	0.00	0.00	0.00	0.00
V_7	Ab	2.00	1.33	2.00	1.00	0.00	0.33	0.33	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	3.00	1.67	2.33	1.33	1.00	0.67	2.00	2.00	2.00	0.67	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	1.00	0.00
V_8	Ab	1.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	1.67	0.67	1.33	0.00	0.00	0.00	1.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_9	Ab	2.67	2.33	2.33	1.67	1.67	0.67	1.33	1.00	1.33	1.67	0.67	0.67	1.33	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	6.33	4.67	4.67	1.67	1.67	1.00	2.33	2.00	2.00	2.33	2.00	1.67	2.33	1.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_{10}	Ab	1.67	2.00	2.33	1.33	1.00	1.00	0.67	1.00	0.33	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.33	4.00	6.00	1.00	1.33	1.33	2.00	2.00	3.00	1.67	1.00	1.00	1.67	1.00	1.00	0.00	0.00	0.00	1.67	0.00	0.67	0.00	0.00	0.00
V_{11}	Ab	2.67	1.00	2.00	1.33	0.33	1.33	0.67	0.00	0.33	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.00	3.67	4.00	1.00	1.33	1.00	2.00	1.67	1.33	2.00	0.67	1.00	1.67	0.67	0.33	0.00	0.00	0.00	2.00	0.00	0.00	0.33	0.00	0.00
V_{12}	Ab	1.67	2.00	2.33	1.00	1.00	1.00	1.00	1.00	1.00	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	6.00	2.67	4.67	1.67	1.00	1.67	2.00	2.00	1.33	2.00	1.33	1.67	2.67	0.67	1.00	0.00	0.00	0.00	1.33	1.00	0.33	0.00	0.33	0.00

*** Average of three replications**
Ab= Abnormal seedlings
D= Dead seeds
T_1= Untreated seeds
T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)
T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

Table 35. Lethal seed infection level (%) of discoloured seeds of rice under different treatment levels*

Variety	Condition	Physiological			*Fusarium moniliforme*			*Fusarium solani*			*Curvularia lunata*			*Alternaria alternata*			*Alternaria padwickii*			*Drechslera oryzae*			*Phoma sorghina*		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
V_1	Ab	1.33	2.00	2.67	1.33	0.33	1.00	1.67	1.33	1.00	1.33	0.33	1.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.00	8.33	5.67	3.67	3.33	4.00	4.33	2.33	2.00	4.00	3.67	4.00	0.67	0.33	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_2	Ab	0.00	0.00	2.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00	0.67	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.00
	D	6.00	6.33.	4.67	0.00	0.00	0.00	2.33	2.67	3.33	2.67	2.67	1.67	1.67	1.00	1.00	0.00	0.00	0.00	2.00	1.00	0.00	0.00	0.00	0.00
V_3	Ab	0.00	0.00	2.00	0.00	0.33	0.33	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	6.00	4.00	3.33	3.00	2.67	2.00	4.00	3.00	3.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V_4	Ab	1.67	1.67	2.00	0.00	0.00	0.00	2.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00
	D	4.33	3.00	3.00	0.00	0.00	0.00	4.33	3.33	3.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	2.00	2.67	0.00	0.00	0.00
V_5	Ab	1.67	1.00	1.33	1.00	0.67	0.33	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	2.00	3.00	4.00	1.33	2.33	1.67	2.00	0.67	1.67	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.33	0.67	0.00	0.00	0.00	0.00	0.00	0.00
V_6	Ab	2.67	1.67	1.67	1.00	1.33	1.33	1.00	1.00	1.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
	D	5.67	3.67	3.67	2.00	3.00	2.00	1.67	1.00	1.67	2.67	2.00	1.67	1.33	1.33	1.33	0.00	0.00	0.00	2.33	0.00	0.00	0.00	0.00	0.00
V_7	Ab	1.33	2.33	2.67	1.00	1.33	1.33	0.67	0.67	1.33	0.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	6.00	4.67	4.00	1.67	2.00	2.33	2.33	2.33	1.67	3.33	1.67	1.67	2.33	1.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.33	0.67
V_8	Ab	1.67	1.67	0.67	1.67	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00
	D	4.33	2.00	2.33	3.00	1.33	1.67	2.67	1.67	1.33	0.67	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.67	0.00	0.00
V_9	Ab	5.67	2.33	3.33	3.00	1.67	3.67	2.00	1.67	0.00	2.00	1.67	1.67	1.33	1.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.33	0.00	0.00
	D	5.00	6.00	5.33	2.33	2.33	2.33	2.33	2.67	0.00	2.33	3.00	1.33	2.00	2.33	2.33	0.00	0.00	0.00	1.00	2.33	1.67	0.67	1.00	0.67
V_{10}	Ab	1.67	2.67	2.33	2.00	2.00	1.67	0.33	1.00	1.33	0.67	0.33	0.67	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.33	4.00	5.67	3.67	3.00	3.33	2.33	1.67	2.33	3.67	2.00	2.00	2.67	1.67	1.67	0.67	0.00	0.00	0.67	0.67	0.33	0.00	0.00	0.00
V_{11}	Ab	4.33	2.67	2.67	2.33	2.33	2.33	1.67	0.67	1.67	2.33	1.33	0.33	1.67	0.67	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
	D	6.33	6.67	7.00	3.67	3.67	4.67	3.00	2.33	2.33	3.67	2.67	2.67	3.00	1.67	2.00	0.00	0.00	0.00	2.33	2.00	0.67	0.00	0.00	0.00
V_{12}	Ab	1.67	2.67	2.33	1.67	2.00	2.33	0.33	1.00	1.00	0.00	0.67	0.67	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	D	5.67	4.00	4.33	3.67	2.67	3.00	2.33	2.00	2.00	2.67	1.67	1.67	3.33	1.33	1.33	0.00	0.00	0.00	1.67	1.00	1.00	0.00	0.00	0.00

*** Average of three replications**
Ab= Abnormal seedlings
D= Dead seeds
T_1= Untreated seeds
T_2= Chemical treated seeds (Bavistin + Dithane M-45 @ 1.25 + 1.25 g/kg seed)
T_3= Bioagent treated seeds (Talc based formulation of *Trichoderma harzianum* @ 4 g/kg seed)

The highest lethal seed infection in apparently healthy seeds category of 12 varieties was found to be caused by *Fusarium solani* with highest infection on variety V_3 dead seeds category (3%) and on variety V_6 (1.67% dead seeds & 1.67% abnormal seedlings) (Table 33). Second highest lethal seed infection was caused by *Curvularia lunata* with highest infection on variety V_1 (2.67% dead seeds & 0.67% abnormal seedlings) followed by *Fusarium moniliforme* on variety V_9 (2% dead seeds & 1.00% abnormal seedlings).

In partially discoloured seed category the highest lethal seed infection in 12 rice varieties were found to be caused by *Fusarium solani* with highest infection on variety V_3, dead seeds (3.33%), V_4 (2% dead seeds & 1.67% abnormal seedlings) and on variety V_9 (2.33% dead seeds & 1.33 abnormal seedlings) (Table 34). Among varieties the highest infection was found to be caused by *Curvularia lunata* on variety V_1 (5.67% dead seeds & 2% abnormal seedlings).

The highest lethal seed infection in discoloured seed category of 12 varieties was also found to be caused by *Fusarium solani* with highest infection on variety V_4 (4.33% dead seeds & 2% abnormal seedlings) followed by on variety V_1 (4.33% dead seeds & 1.67% abnormal seedlings) (Table 35). Second highest lethal seed infection was caused by *Curvularia lunata* with highest infection on variety V_1 (4% dead seeds & 1.33% abnormal seedlings) followed by on variety V_{11} (3.67% dead seeds & 2.33% abnormal seedlings).

Alternaria alternata, *Drechslera oryzae*, *Phoma sorghina* and *Alternaria padwickii* also caused lethal seed infection in each category of 12 rice varieties.

The highest lethal infection of *Curvularia lunata* was observed in partially discoloured seed category on variety V_1 (5.67% dead seeds & 2% abnormal seedlings) followed by discoloured seed category for V_1 variety (4% dead seeds & 1.33% abnormal seedlings) (Tables 32 to 35). The highest infection of *Fusarium solani* was observed in discoloured seed category on V_4 variety (4.33% dead seeds & 2% abnormal seedlings) followed by V_1 (4.33% dead seeds & 1.67% abnormal seedlings) in same category. Among the categories, discoloured

seed category had the highest level of infection and apparently healthy seed category had the lowest infection level. Among the varieties V_6 had the highest infection level in original seed category, V_1 in apparently healthy seed category, V_9 in partially discoloured seed category and V_{11} in discoloured seed category and the lowest infection level was observed from V_3 on original, partially discoloured and discoloured seed category and from V_8 in apparently healthy seed category. Ou (1985) reported death of the seedlings due to *Alternaria padwickii*. Fluffy mycelia and microconidia of *Fusarium moniliforme* cover the entire seed, and infected seedlings with necrotic lesions on roots die before or after transplanting (Misra *et al.* 1994b). The highest lethal seed infection by *Fusarium moniliforme* (7.33% dead seed & 1.42% abnormal seedlings) was observed in farmers' original seed for R30 variety (Islam *et al.* 2000).

Among the treated seeds, the highest lethal seed infection of *Curvularia lunata* in original seed category on variety V_1 reduced from 3.33 to 2 per cent in dead seeds and 2.33 to 1.67 per cent in abnormal seedlings by chemical treatment whereas by bioagent treatment it reduced from 3.33 to 1.67 per cent in dead seeds and 2.33 to 1.33 per cent in abnormal seedlings (Table 32). The highest lethal infection of *Fusarium solani* on variety V_8 reduced from 3.33 to 0.67 and 1.00 per cent in dead seeds and 1.67 per cent to complete control in abnormal seedlings by chemical and bioagent treatment respectively. The lethal infection of *Fusarium moniliforme* on variety V_{10} reduced from 3 to 2 per cent in dead seeds and remained same in abnormal seedlings in both chemical and bioagent treatment and on variety V_{11} reduced from 2.67 to 2 per cent in dead seeds and 2.33 to 0.33 per cent in abnormal seedlings by chemical treatment whereas by bioagent treatment remained same in case of dead seed category and reduced to 1.33 per cent in abnormal seed category.

The highest lethal seed infection of *Fusarium solani* in apparently healthy seeds category on variety V_3 reduced from 3 to 0.33 per cent in dead seeds category by both chemical and bioagent treatment and on variety V_6 reduced from 1.67 to 0.67 per cent in dead seeds by chemical treatment whereas

remained same by bioagent treatment and 1.67 to 0.33 per cent in abnormal seedlings by chemical treatment whereas complete control by bioagent treatment (Table 33). The highest lethal seed infection of *Curvularia lunata* on variety V_1 reduced from 2.67 to 1.00 per cent in dead seeds whereas remained same by chemical treatment and 1.33 per cent in abnormal seedlings and complete control in abnormal seedlings by bioagent treatment. The infection of *Fusarium moniliforme* on variety V_9 reduced from 2 to 1.67 per cent in dead seeds whereas complete control in abnormal seedlings by chemical treatment and 1.33 per cent in dead seeds and 1 per cent in abnormal seedlings by bioagent treatment.

In partially discoloured seed category the highest lethal seed of *Fusarium solani* on variety V_3 reduced from 3.33 to 2.33 and 1.33 per cent in dead seeds by chemical and bioagent treatment but almost remained same on variety V_4 and on variety V_9 from 2.33 to 2 per cent in dead seeds in both treatments and 1.33 to 1 per cent in abnormal seedlings by chemical treatment and remained same by bioagent treatment (Table 34). The highest infection of *Curvularia lunata* on variety V_1 reduced from 5.67 to 2.33 per cent in dead seeds but remained same in abnormal seedlings by chemical treatment whereas by bioagent treatment reduced from 5.67 to 2.67 per cent in dead seeds and complete control in abnormal seedlings.

The highest lethal seed infection in discoloured seed category by *Fusarium solani* on variety V_4 reduced from 4.33 to 3.33 per cent in dead seeds and 2 to 1 per cent in abnormal seedlings by chemical treatment and from 4.33 to 3.67 per cent in dead seeds and 2 to 1 per cent in abnormal seedlings by bioagent treatment whereas on variety V_1 from 4.33 to 2.33 per cent in dead seeds and 1.67 to 1.33 per cent in abnormal seedlings by chemical treatment whereas by bioagent treatment it reduced 4.33 to 2 per cent in dead seeds and 1.67 to 1 per cent in abnormal seedlings (Table 35). The highest lethal seed infection of *Curvularia lunata* on variety V_1 was reduced from 4 to 3.67 per cent in dead seeds and 1.33 to 0.33 per cent in abnormal seedlings by chemical treatment whereas by bioagent treatment remained same in dead seed category and reduced to 1 per cent in abnormal seedlings and on variety V_{11} from 3.67 to

2.67 per cent in dead seeds on both treatments and 2.33 to 1.33 and 0.33 per cent in abnormal seedlings. Similarly, infection by *Fusarium moniliforme*, *Alternaria alternata*, *Drechslera oryzae*, *Phoma sorghina* and *Alternaria padwickii* also reduced by chemical and bioagent treatment especially in partially discoloured and discoloured seed category. Literature pertaining to improvement of lethal seed infection by seed treatment with chemical/bioagent treatment is not available. Hence, it is the first report in this aspect.

4.10 Component analysis

Data given in Table 36 indicated the frequency of associated microflora on seed components (kernel and husk) of discoloured seed category of variety Kasturi Basmati (V_1) after surface sterilization for different time period with mercuric chloride (0.1%) (Plate 8).

4.10.1 Kernel:

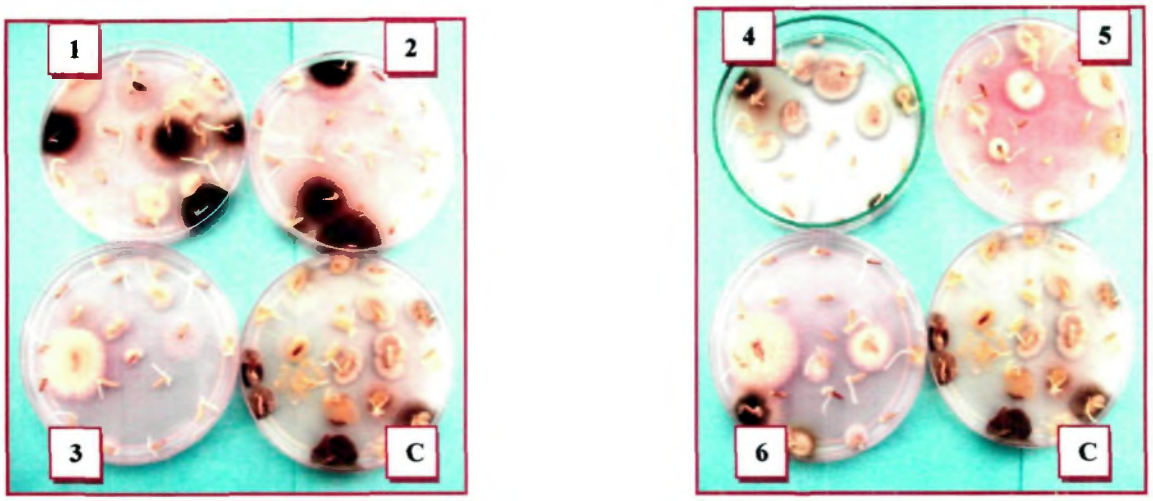
Maximum number of microflora (11) was detected from kernel surface sterilized for two minutes followed by 9 in half and one minute sterilization as compared to control (14) (Table 36). Among the microflora frequency of *Curvularia lunata* was highest (28%) after half minute sterilization which was almost similar to control (30%). Frequency of detection was 25 and 24 per cent in one and a half minutes and one minute sterilization and 18 per cent recorded from two and a half minutes sterilization. It is evident that with the increasing duration of sterilization detection of *C. lunata* also decreased gradually.

Detection of mycoflora *Chaetomium olivaceum, Chaetomium* sp. was also high in kernel ranging from 8 to 16 and 15 to 22 per cent as compared to 20 and 25 per cent respectively in control. *Alternaria alternata, Fusarium solani, Fusarium* sp. and *Aspergillus* sp. were other microflora detected from kernel. *Epicoccum purpurascens, Fusarium moniliforme, Mucor* sp., *Penicillium* sp., *Rhizopus stolonifer* and non-sporulating fungus were almost negligible, which otherwise except *Mucor* sp. were detected from control seeds. Maximum frequency (10%) of *Xanthomonas* sp. was also detected at half minute sterilization and appreciably decreased with the increasing period of surface sterilization.

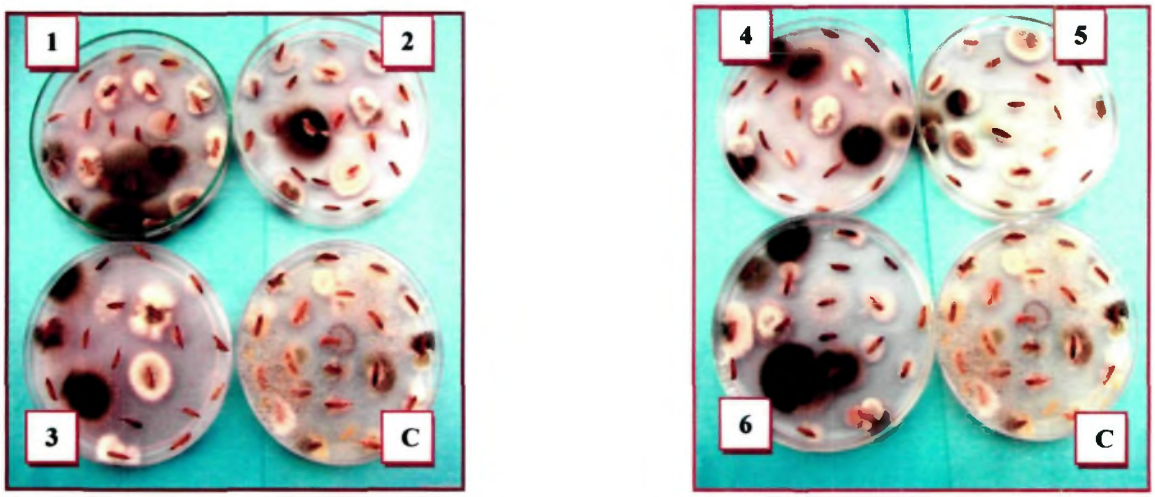
Table 36. Frequency (%) of associated microflora in kernel and husk of rice by agar plate technique*

Pathogen	Kernel							Husk						
Variety	½ min.	1 min.	1 ½ min.	2 min.	2 ½ min.	3 min.	Control	½ min.	1 min	1 ½ min.	2 min.	2 ½ min.	3 min.	Control
Kasturi Basmati														
Alternaria alternata	7	5	4	5	3	1	8	5	3	1	1	1	1	10
Aspergillus sp.	3	3	2	3	0	3	5	4	5	10	21	1	2	1
Chaetomium olivaceum	15	16	13	10	8	10	20	15	18	20	20	15	18	10
Chaetomium sp.	20	22	20	18	15	15	25	30	20	25	20	25	20	10
Curvularia lunata	28	24	25	20	18	20	30	30	32	28	10	25	20	10
Epicoccum purpurascens	0	0	0	1	0	0	2	0	0	1	1	0	1	2
Fusarium moniliforme	0	1	0	0	1	0	2	0	1	0	0	0	0	1
Fusarium solani	4	6	10	8	10	10	3	5	10	12	10	15	10	12
Fusarium sp.	3	5	3	2	1	0	8	0	3	0	0	0	0	1
Mucor sp.	0	0	0	0	0	0	0	3	4	2	16	1	0	4
Penicillium sp.	0	0	0	1	0	0	8	3	3	2	4	2	2	3
Rhizopus stolonifer	0	0	0	0	0	0	15	0	0	0	35	0	0	50
Rhizoctonia sp.	2	0	0	1	0	0	3	1	0	1	3	2	0	2
Non-sporulating fungus	0	0	0	0	0	0	1	0	0	0	2	0	0	1
Xanthomonas sp.	10	5	3	2	1	1	15	15	5	6	5	2	5	32
Total microflora	9	9	8	11	8	7	14	10	11	11	13	10	9	15

***On the basis of 100 seeds tested of each seed component**



Kernel



Husk

Plate 8. Detection of seedborne microflora in kernel and husk of rice variety Kasturi Basmati after surface sterilization with mercuric chloride (0.1%) at different timings; ½ minute (1); 1 minute (2); 1½ minutes (3); 2 minutes (4); 2½ minutes (5); 3 minutes (6); control (C)

4.10.2 Husk:

Microflora detected on husk were comparable with those detected from the variety by employing agar-plate method with a variable frequency. *Curvularia lunata* was also found predominant (10 to 32%) in all the treatments except at two minutes sterilization where *Rhizopus stolonifer* was predominant (35%) as compared to control where it was maximum (50%) followed by *Xanthomonas* sp. (32%). However, with surface sterilization *Xanthomonas* sp. was decreased (2 to 15%) significantly.

Component analysis of rice seeds showed that higher frequency of microflora were detected from seed husk as compared to kernel and the most of microflora were found to be located on seed husk. Maximay (1984) and Prasanna (1985) reported 92 per cent seed coat infection of cowpea seeds. Gajapathy and Kalyanasundarum (1986) also studied the distribution of seedborne mycoflora within rice grain mainly in husk and outer layers of the kernel. Sud (2002) conducted the seed component plating anyalysis of *Phaseolus vulgaris* L. and also found that seed coats harboured maximum mycoflora. Dawar *et al.* (2007) also conducted component analysis of chickpea and found that most of the fungi were located on the seed coat and observed higher incidence from seed coat.

4.11 Weather parameters

Data given in Table 37 showed the role of weather conditions on associated microflora of different locations. One of the reasons of variation in number of microflora and their frequency of detection among different samples could be the variation in the weather condition of agroclimatic zones, especially during harvesting period (Oct-Nov.). Weather parameters *viz.*, mean temperature (^{O}C), relative humidity (RH) and rainfall (RF) were different in different locations and these factors may be responsible for differences in discolouration and per cent frequency of microflora in different locations. This collaborates the earlier reports of Roy (1983), Ou (1985) and Sunder *et al.* (1989) who reported that the extent of discolouration varied with season, locality and variety.

Temperature has potential impact on plant disease through both the host crop plant and the pathogen. Generally, fungi that cause plant disease grow best in moderate temperature ranges. Temperate climate zones that include seasons with cold average temperatures are likely to experience longer periods of temperatures suitable for pathogen growth and reproduction if climates warm. Similarly, relative humidity and rainfall also has great impact on plant diseases.

High mean temperature (23.7^{O}C) and rainfall (59.2 mm) was recorded from Una during the month of October and in total 15 number of microflora were found associated with seed sample collected from Una with higher frequency of *Curvularia lunata* (53%). Similarly, high mean temperature and RH were recorded from Dhaulakuan were 22.5^{O}C and 91 per cent during month of October but comparatively less number (10) of microflora were found to be associated with the seed samples collected from Dhaulakuan with highest frequency of *C. lunata* (20%). This could be due to varietal differences.

At Bajaura location during harvesting period RH was 66 per cent and 64 per cent and RF 5.08 mm and 2.90 mm during October and November respectively which may have contributed for higher (14) microflora detection with highest frequency of *C. lunata* (46%) whereas a mean temperature of 20.9^{O}C, RH of 50 per cent and rainfall 49.2 mm were recorded from Malan during month of October but comparatively less (9) microflora were detected from seed samples collected from Malan but highest frequency was again of *C. lunata* (42%). The low RH and no rainfall during November might have contributed for detection of less number of microflora at Malan as compared to other locations.

Among all regions the maximum number of microflora and maximum frequency of *C. lunata* was observed in Una samples which could be due to congenial temperature (23.7^{O}C) and rainfall (59.2 mm), during October when harvesting is done in this location.

The effect of weather factors during harvesting period, though indicated their effect in the variation of number of microflora associated with seed samples and frequency of *C. lunata*, but it could not be ascertaining definitely the role of

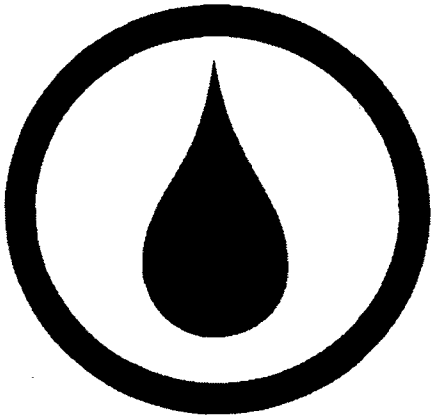
Table 37. Effect of weather factors during rice harvesting on total number of associated microflora and frequency of predominant microorganism

Location	No. of associated microflora	Frequency of *Curvularia lunata*	Weather factor during harvesting					
			October			November		
			Mean Temp. (^{O}C)	RH (%)	RF (mm)	Mean Temp. (^{O}C)	RH (%)	RF (mm)
Una	15	53.00	23.7	-	59.20	19.0	-	0.00
Dhaulakuan	10	20.00	22.5	91.0	11.60	17.4	90.0	2.60
Bajaura	14	46.00	18.0	66.0	5.08	12.8	64.0	2.90
Malan	9	42.00	20.9	50.0	49.20	16.5	33.4	0.00

- = not recorded
RH = Relative Humidity
RF = Rainfall

these factors due to variation in varieties in different locations and want of weekly weather factors. So more research in this aspect is needed. The effect of weather factors especially temperature and RH on rice blast has been documented by Kapoor (1975) under Himachal Pradesh conditions.

Summary and Conclusions



5. SUMMARY AND CONCLUSIONS

The present studies on seedborne microflora of rice were carried out with the objectives to identify the seedborne microflora associated with rice seeds under different agroclimatic conditions of Himachal Pradesh, effect of chemical (Bavistin + Dithane M-45) and bioagent treatment (*Trichoderma harzianum*) on associated microflora, role of associated microflora in causing seedlings abnormalities by studying effect on germination, seedling vigour, causing lethal seed infection, effect of treatments on germination and seedling vigour, to detect the location of associated microflora (husk and kernel) and role of weather parameters on associated microflora of rice seeds.

Twelve rice seed varieties were collected from different locations and research stations of Himachal Pradesh and varieties were categorized into 4 categories, that is, original, apparently healthy, partially discoloured and discoloured seed category on the basis of morphological appearance. The study on associated microflora of each rice varieties based on such categories were carried out by blotter and agar-plate method. The associated microflora of rice varieties of 4 categories as detected by blotter method indicated the presence of 15 fungi out of which the frequency of *Curvularia lunata* and *Drechlera oryzae* were higher than the other fungi in all seed categories. The maximum microflora were detected from variety Kasturi Basmati (9 to 11) followed by variety Nagardhan (9 to 11) whereas minimum from the variety HKR-126 (3). The microflora detected by agar-plate method indicated the presence of 17 fungi and one bacterium sp., out of which, *Xanthomonas* sp., *Curvularia lunata* and *Drechslera oryzae* were predominant pathogens with highest frequency than the other microflora in each seed category. *Fusarium solani* was detected from all the varieties with 100 per cent sample mean in each category from both the methods. Among the varieties, maximum microflora were also detected from variety Kasturi Basmati (13 to 15) followed by Yunlen 18 (s) (11) and mimimum from variety Jhumka (4) in all the seed categories. Among both methods agar-plate method was more efficient in detection of associated microflora.

Nematode test were also carried out and only one nematode species, *Aphelenchoides besseyi* was detected from variety Pusa-1121 and Jattoo with low frequency from original, partially discoloured and discoloured seed categories whereas, it was not be detected from apparently healthy seed category.

The seed health status of all the collected varieties based on 4 categories was assessed and it was found that seed health parameters like seed germination and seedling vigour varied with locations, varieties and seed categories. Varieties Kasturi Basmati, Jattoo and Nagardhan showed higher number of abnormal seedlings and dead seeds and least percentage of germination due to heavy colonization of the seeds by associated microflora and varieties Pusa-1121 and Parmal produced higher number of normal seedlings and lower abnormal seedlings and dead seeds. In majority, the germination was above 80 per cent in both the methods in all the seed categories but in some varieties of partially discoloured and discoloured seed categories it was also below 70 per cent.

Seedling vigour also varied with locations, varieties and seed categories. The variety, Parmal exhibited higher vigour index in original (2073), apparently healthy (2190.67) and discoloured seed (1805.13) categories, Jhumka in partially discoloured seed category (1881.87) and the variety, Nagardhan showed lower vigour index in all seed categories.

Chemical treatment (Bavistin + Dithane M-45) was found to influence the associated microflora of rice seeds in all the varieties and seed category to a significant level. After chemical treatment, total of 12 fungal species were recorded from all seed categories. Seed treatment with bioagent (*Trichoderma harzianum*) restricted the growth of all associated microflora. Chemical and bioagent treated seeds produced higher number of normal seedlings and produced minimum percentage of abnormal seedlings and dead seeds than untreated seeds. Bavistin + Dithane M-45 and *Trichoderma harzianum* treatments also increased the seedling vigour as majority of the varieties in each seed categories registered higher seed vigour index after treatments.

The highest lethal seed infection was found to be caused by *Curvularia lunata* in original seed category on variety Kasturi Basmati (3.33% dead seeds & 2.33% abnormal seedlings) and by *Fusarium solani* in apparently healthy, partially discoloured and discoloured seed categories. The highest infection by *Curvularia lunata* was observed in partially discoloured seed category on variety Kasturi Basmati (5.67% dead seeds & 2% abnormal seedlings) and that of *Fusarium solani* in discoloured seed category on variety Jhumka (4.33% dead seeds & 2% abnormal seedlings). The lethal seed infection was reduced to a significant level by chemicals and bioagent treatments in all the seed categories especially in partially discoloured and discoloured seed categories.

Various seedborne fungi were found located on different parts of the seed, husk and kernel. Husk harboured more number and frequency of microflora as compared to kernel. The frequency of *Curvularia lunata* was highest in both the seed components after half minute sterilization with mercuric chloride (0.1%) and decreased gradually after increasing the duration of sterilization.

The number of associated microflora and their frequency varied with the locations, the reason for which could be due to the variation in weather parameters *viz.,* temperature (^{O}C), relative humidity (%) and rainfall (mm) of agroclimatic zones especially during harvesting period (Oct-Nov.).

It can be concluded from the present investigation that occurrence of seed microflora and its role in causing seedling abnormalities by affecting seed germination, seedling vigour and causing lethal seed infection varied with location and seed categories. Discolouration in seeds caused by associated microbial agents which lowered its germination, root and shoot length, and seedling vigour whereas, apparently healthy seeds showed higher percentage of germination, root and shoot length and seedling vigour than discoloured seed. Environmental conditions prevailing in a particular agroclimatic situation had great impact on microflora development. Associated microflora leads to failure in

seed germination and caused lethal seed infection. These were managed to significant level by chemical and bioagent treatments. Thus, management of microflora is of utmost importance and seed dressing fungicides and bioagent like *Trichoderma harzianum* treatment can effectively ensure high germination and seed vigour, which ultimately increase the productivity of crop.

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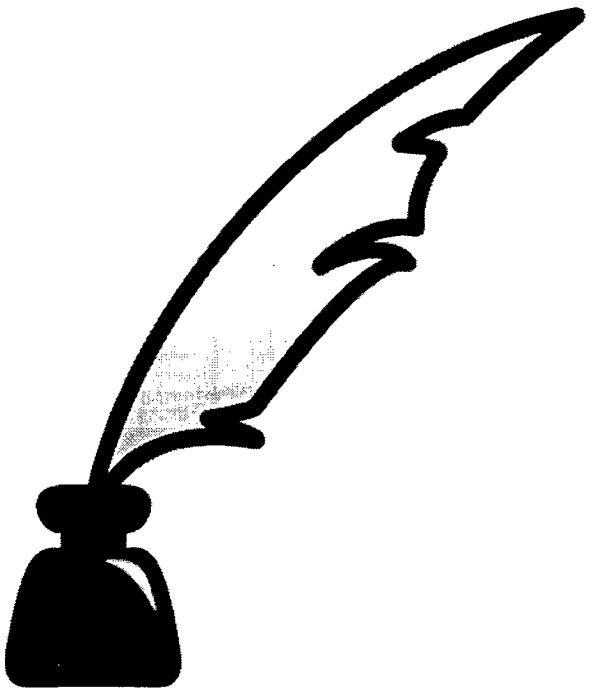

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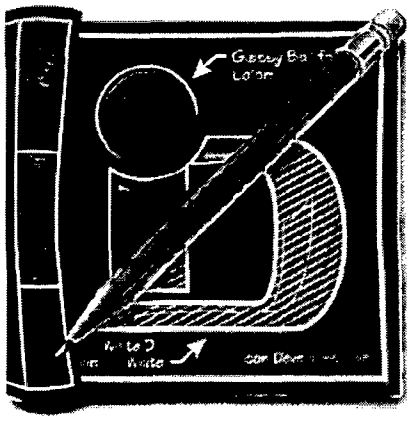
Appendix



Appendix-1

Composition of media used

Potato Dextrose Agar (PDA)

Peeled Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	1000 ml

Nutient Agar

Peptone	5 g
Beef extract	3 g
Agar	20 g
Distilled water	1000 ml

Nutrient Broth

Peptone	5 g
Beef extract	3 g
Distilled water	1000 ml

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10+2	March	2004	HPBSE, Dharamsala	73.00	1st
B.Sc. Agriculture	June	2008	CSK HPKV, Palampur	77.90	1st