ECO-PATHOLOGICAL STUDY OF SEED MYCOFLORA AND ITS IMPACT ON BIODETERIORATION OF *Phaseolus vulgaris* L. IN HIMACHAL PRADESH

THESIS

By

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Submitted to

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IN

Partial fulfillment of the requirements for the degree

OF

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(PLANT PATHOLOGY)

(2002)

Dedicated

to my

Adorable
Daughter

Phalguni (Charu)

ACKNOWLEDGEMENT

In this highly complex society no work can be accomplished by a single individual but it needs inspiration and sincere guidance of intellectuals as well as the grace of that *Almighty God* for so many things. Inspite of all this, I can never forget my affectionate parents and mother-in-law who encouraged me to undergo higher studies. Their selfless persuation and sacrifices, heartfelt blessings and firm faith has made this manuscript a remuneration to translate their dreams into reality.

It is my privilege to express deep sense of gratitude and profound personal regards to my esteemed teacher and advisor **Dr O.P. Sharma,** Sr. Scientist (Plant Pathology) **and Head of the Department** for his constant encouragement, keen interest, constructive criticism and invaluable guidance, without which this study could not have been completed successfully. I shall always remain indebted to him for his benevolence bestowed upon me.

I owe my sincere thanks to Dr P.N. Sharma, Associate Professor (Plant Pathology) and member of my Advisory Committee, for his judicious, impeccable benovoden guidance and for critically going through the manuscript.

Grateful thanks are also due to other worthy members of my Advisory Committee namely Dr B.R. Sood, Professor (Agronomy), Dr B.C. Sood, Professor (Plant Breeding) and Dr K.P. Singh, Plant Physiologist for their valuable guidance and ready help.

My thanks are due to Dr V.K. Gupta, Dean Post Graduate Studies; Head, Department of Plant Pathology and Dr B.S. Katoch, Dean (Retd.), College of Veterinary and Animal Sciences for providing necessary facilities for conducting research work.

I offer my profound thanks to all the teachers of Department of Plant Pathology for their time to time guidance and help.

Language seems to be inadequate media to express my heartful gratitude for the eterual affection, lively sentiments and constant encouragement from my brother-Sachin; Sisters'-in-law Neelam, Neeraj and Sucheta ji; brothers' in law - Sanjeev, Yogesh and Rakesh ji and kids Garima, Mayank, Heena, Vidhu, Shreya and Shery. Besides, I am highly thankful to all the relatives for their affection.

It would be crucial if I do not acknowledge the aid and stimuli of my cateri comprising of all my friends, particularly Dubeys', Sharmas', Uppals', Dr Surender Sharma, Preeti, Menakshi, Anju, Meena, Shivani Mandeep and Aditya,

Help rendered by Laboratory Assistant Sh. Himat Ram is gratefully acknowledged.

I am very much grateful to Sh. Dina Nath Sharma for typing this manuscript against an impossible deadline.

Perhaps, above all, much more than the usual depth of gratitude must be expressed to my husband, Dr. Ajay Sood and daughter, Phalguni. The most revered personalities in my life, who after facing so many hardships spared no pains in looking after the comforts and ponder me the time, support, inspiration and love needed to complete these studies.

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CERTIFICATE - I

This is to certify that the thesis entitled `**Eco-pathological study of seed mycoflora and its impact on biodeterioration of *Phaseolus vulgaris* L. in Himachal Pradesh**` submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy (Agriculture)** in the subject of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mrs. Deepika Sud** daughter of **Sh. S.P. Sud** 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 fully acknowledged.

Place : Palampur
Dated: the 5th August, 2002

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Chairman
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CERTIFICATE - II

This is to certify that the thesis entitled `**Eco-pathological study of seed mycoflora and its impact on biodeterioration of *Phaseolus vulgaris* L. in Himachal Pradesh**` submitted by **Mrs. Deepika Sud** daughter of **Sh. S.P. Sud** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Agriculture) in the subject 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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INTRODUCTION

INTRODUCTION

Kidney bean (*Phaseolus vulgaris* L.), is a prime pulse crop among various grain legumes and is an excellent source of relatively cheap dietary protein. With continued human population growth and the reduction in meat consumption by health conscious consumers, legumes are expected to play an increasingly important role in meeting nutritional needs. Hence, they form an important part of current research (Chisholm and Coates-Becford, 1997). Common bean occupies prime place among grain legumes not only in the world but also in India (Sharma and Joshi, 1993). Kidney bean is extensively cultivated the world over in an area of about 25,160 thousand hectares with an annual production of 17,353 thousand metric tones, whereas in India the crop is grown in 9,500 thousand hectares with an annual production of 3,600 thousand metric tones (Anon., 1999).

One hundred gram of green pods contain protein (1.7 g), fat (0.1 g), carbohydrate (4.5 g), fiber (0.8 g) and minerals (0.5 g) (Aykroyd, 1983). Whereas, dry beans contain protein (22.9 g), fat (1.3 g), carbohydrate (60.6 g) and minerals (3.2 g) (Gopalan *et al.*, 1982).

The green pods of french bean contain traces of glycosides and certain other substances that reduce blood sugar level besides high protein content (15-31%) that makes it an excellent substitute for animal protein in the diet of predominantly vegetarian Indian population. The plant parts like straw/ discoloured pods are fed to the livestock.

In Himachal Pradesh, kidney bean locally called as 'rajmash' occupies a pivotal position amongst the legumes grown in mid hills (900-1300 m asl) and high hills (1800-3000 m asl). It is grown under varied agro-

ecological situations ranging from sub-tropical to dry temperate regions and is grown in an area of about 2,037 hectares with an annual production of 19,206 tones (Anon., 1995). Being a high quality legume crop, it fetches remunerative price (Rs 2500-3500/quintal). The pulse type of bean crop is cultivated in mid and high hills of Chamba, Kangra, Kinnaur, Kullu, Mandi, Shimla and Sirmour districts of Himachal Pradesh (Kingra and Singh, 1986) whereas, snap beans (vegetable type) are cultivated in low lying areas of the state.

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 maturation, harvesting and storage (Christensen and Kaufmann, 1955; Neergaard, 1977). Majority of these micro-organisms cause biodeterioration of seed whereas, some of them like *Colletotrichum lindemuthianum* (Sacc. and Magn.), *Sclerotinia sclerotiorum* (Lib) de Bary, *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn. (Dhingra, 1978; Dhyani, 1989) are serious pathogens of beans. The method of storing and storage environment also affects the seed health in terms of biodeterioration by seed mycoflora and this results in poor seed germination, seed and seedling vigour, viability and also seed quality (Vidhyasekaran *et al.*, 1973; Bilgrami *et al.*, 1976; Krishnamurthy and Raveesha, 2001).

One of the most important basic needs of higher agricultural productivity is quality seed, characterized by high viability and vigour (Yaklich *et al.*, 1979). These two characters can not be maintained in storage especially in crops like rajmash and soybean that rapidly deteriorate under ambient storage in tropical and sub tropical environments, with high temperature and humidity (Arulnandhy *et al.*, 1984). Viability and vigour tend to decline with the age of seed and in turn determine the crop stand and finally production (Roberts, 1972; Singh and Gupta, 1982).

Therefore, maintenance of seed viability and vigour from harvest till next growing season is of utmost importance. It has been reported that storage conditions and type of containers used for storage influence the germinability of soybean seeds (Arulnandhy and Senanayake, 1991). The significance of environment in seed health has been well advocated by McGee (1981) who described three environments viz. the seed production field, harvesting, processing and storage which affects the seed quality. McGee (1995) further stated that the quality of planted seed has a critical influence on the ability of crops to become established.

To realize the full potential of bean seed, the study of seed mycoflora and its impact on biodeterioration of rajmash/kidney bean is an essential aspect of seed health testing. Therefore, the present studies were undertaken with the following objectives:

1. To find out the spatial and varietal distribution of the mycoflora of french bean;
2. To determine the pathological significance of the mycoflora and its impact on seed quality, seedling health; and
3. To manage the fungi during storage through seed treatment.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Bean seeds harbour a wide range of mycoflora including the incitants of some serious bean diseases, which affect the seed health and quality of crop. Though literature pertaining to biodeterioration of kidney bean seed is scanty, yet the available literature relating to present studies is reviewed in this chapter under the following heads:

2.1 Spatial and varietal distribution of seed mycoflora

2.2 Factors affecting seed mycoflora

2.3 Pathological significance of seed mycoflora

2.4 Seed quality parameters as affected by mycoflora

2.5 Management of seed mycoflora

2.1 Spatial and Varietal Distribution of Seed Mycoflora

Kidney bean is known to be attacked by many devastating seed borne diseases (Neergaard, 1977) and the role of climatic conditions in affecting the seed infections has been well documented (Tarr, 1955; Frederiksen, 1974). 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).

Suryanarayana and Bhombe (1961) isolated *Alternaria, Fusarium and Colletotrichum* spp. from the kidney bean seeds by using agar plate method, while Watanabe (1972) reported the presence of *Macrophomina phaseolina* on bean seeds and found that the occurrence of the fungus was affected greatly by temperature. Christensen (1974) established the

significance of *Penicillium* and *Aspergillus* spp. as important storage fungi on bean, cowpea and groundnut samples from Jamaica. Osipyan and Batikyan (1975) isolated *Botrytis lanea* from green bean pods in Armenia. Fulco *et al.* (1977) recorded the presence of *Fusarium, Rhizoctonia, Colletotrichum* and *Diaporthae* spp. on bean seeds through blotter method. Lasca (1978) while studying the bean mycoflora in Brazil through blotter, agar plate and growth tests observed the symptoms of *Elsinoe phaseoli* on bean seedlings through grow on tests and established the seed transmission of the fungus. Dhingra (1978) observed the presence of *Fusarium semitectum, Rhizoctonia solani* and *Macrophomina phaseolina* on bean seeds using blotter and agar plate methods. Abdel Hafez (1984) while studying the mycoflora of bean seeds observed the association of 60 species of fungi belonging to 24 genera with the seeds of kidney bean, faba bean, lentil, lupin and pea seeds in Saudi Arabia using seed plating method on glucose czepeck's agar. The most frequent genera encountered were *Aspergillus, Penicillium, Rhizopus* and yeasts, followed by *Drechslera, Fusarium* and *Mucor.* Rao *et al.* (1985) studied seed borne nature of *Aspergillus* spp. in french bean seed samples collected from different ecological situations. They recorded heavy incidence of *Aspergillus* spp. along with *Macrophomina phaseolina*, *Phoma* and *Fusarium* spp. by blotter method. *Aspergillus flavus, A. niger, A. versicolour* occurred in high percentage. Component plating technique revealed the internally seed borne nature of *A. flavus, A. niger* and *A. versicolour. A. flavus* was found in all the components of the seed viz. seed coat, cotyledon and embryonic axis. Lokhande *et al.* (1986) assessed seed mycoflora of french bean on commonly grown cultivars in Sitara distt. of Maharashtra. They graded various seed lots into five categories on the basis of external appearance of the seeds and found seeds of grade-2 showing seed discolouration , having black grey and yellow spots, which harboured maximum mycoflora. However, apparently healthy seeds showed rare occurrence of seed borne fungi like *A. niger, A. flavus* and *Penicillium citrinum.* The graded seeds

were tested by the blotter test and agar plate method and in all 14 species of fungi belonging to 10 genera were recorded. Out of these 5 fungi viz. *Alternaria, Colletotrichum, Drechslera, Fusarium* and *Macrophomina* were pathogenic. Dhyani *et al.* (1989) observed *Phoma medicaginis* on bean seeds from different agro-climatic regions of Kumaun in north-western Himalayas, while studying the mycoflora by blotter and agar plate method. Similarly, Weidenborner and Hinderof (1989) isolated four species of *Aspergillus* viz. *A. glaucus, A. flavus, A. ochraceous* and *A. niger* from seeds of soybean, kidney bean, pigeon pea, cotton and pods of groundnut by employing malt extract agar plate method. The frequency of field fungi observed was less as compared to that of storage moulds in different samples, as only *Cladosporium* was noticed on both seeds and pods of bean and soybean.

Gupta *et al.* (1992) reported the occurrence of ten new fungi not reported earlier on french bean seeds from India viz. *A. ochraceous, A. tamari, Chaetomium globosum*, *Drechslera oryzae*, *Fusarium acuminatuji kuroi* var. *subglutinans*, *F. semitectum*, *Penicillium griseo-fulvum* and *Phomopsis* spp. Sati *et al.* (1993) found moderate to heavy incidence of *Drechslera sorokiniana* in rotted seeds and seedlings of bean (*Phaseolus vulgaris)* collected from Kumaun hills. Seed component plating revealed the presence of fungus in seed coat, cotyledon and embryo, however, incidence was highest on seed coat and least on embryos. Tseng *et al.* (1995) developed a profile of bean mycoflora and determined the frequency of various fungi isolated from the bean seeds in Taiwan and Ontario. They found that the average percentage of fungi in seed lots from Ontario and Taiwan was 54.8 and 58.5 per cent, respectively. The most commonly occurring fungi were *Alternaria, Fusarium, Rhizoctonia, Penicillium, Rhizopus, Sclerotinia, Gliocladium* and *Mucor* in different seed lots. However, *Fusarium* and *Aspergillus* were the most probable mycotoxin producing fungi associated with bean seeds. Tseng *et al.* (1996) while comparing profiles of seed borne fungi of bean and soybean found that

beans were more susceptible to fungal infections than soybean when grown under same environmental and cultural conditions. *Aspergillus, Penicillium, Rhizopus, Eurotium* and *Curvularia* were common on bean and soybean seed. Similarly Ruiz *et al.* (1996) studied the mycoflora of green beans grown in different locations and height under green house conditions in Spain and recorded *Aspergillus, Penicillium, Aureobasidium* and *Alternaria* without any significant difference in the occurrence of mycoflora on the basis of height and location of their cultivation.

Chisholm and Coates-Beckford (1997) assessed the seed health of bean, cowpea and groundnut collected from different areas of Jamaica after harvest and storage of seeds in different environments. Thirty nine species of fungi representing twenty genera were detected and beans harboured all the twenty genera and thirty nine species. Out of the nineteen fungi observed through component plating technique in beans, 94.7, 68.4 and 31.6per cent were observed on seed coat, cotyledons and embryonic axis, respectively. Ushamalini *et al.* (1998) detected *Macrophomina phaseolina, Fusarium oxysporum, Alternaria alternata, Aspergillus* spp., *Penicillium* spp. and *Rhizopus stolonifer* collected from different districts of Tamilnadu. Sinha *et al.* (1999) detected externally and internally seed borne mycoflora of french bean using agar plate and blotter methods on two varieties viz. Pusa Parvati and Laxmi during winter and summer season. They isolated twenty six species of fungi from both the varieties, and found that blotter method was more efficient and effective than agar plate method in detecting the mycoflora. Prominent field fungi recorded during summer season were *Alternaria* sp., *Cochliobolous lunatus, Drechslera avenacea, Cladosporium cladosporiodes, Fusarium* sp., *Aspergillus* spp. and *Penicillium* spp. However, during winter season mostly the storage fungi viz. *A. niger, A. flavus, Trichoderma* and *Penicillium* were found.

Bilbao *et al.* (2000) while investigating mycoflora of red bean (*P. vulgaris*) and pea (*P. sativum*) seed samples in Cuba stored over a period of one year observed that in peas and beans, *Aspergillus* and *Penicillium*

prevailed over *Fusarium, Cladosporium* and *Alternaria* spp. They also found two aflatoxin producing strains of *A. flavus*. Chakravarthy *et al.* (2001) while analyzing seed mycoflora of different pigeon pea seed samples from different agro-climatic regions of Karnataka found the association of twenty one fungal species belonging to fourteen genera. The important genera encountered were *Aspergillus, Macrophomina* and *Fusarium*.

2.2 Factors Affecting Seed Mycoflora

Storage fungi have been reported to invade and destroy seeds of several crops (Arndt, 1946; Milner and Geddes, 1946; Christensen, 1969; Christensen and Kaufmann, 1969a). These fungi can colonize any kind of seed. However, their occurrence and distribution on seed is affected by various factors/conditions that prevail during storage after harvesting till sowing. These conditions mainly comprise of moisture, temperature, method of storage, duration and seed coat colour.

2.2.1 Moisture and temperature

Incidence of different *Aspergillus* spp. has been reported to vary with the increase/decrease in relative humidity/moisture content during storage (Thom and Raper, 1945). Wallace (1960) reported that damaged seeds were more susceptible to invasion of fungi than the undamaged seeds with intact seed coat that protect the seed from attack of micro-organisms. He also found that the damaged seeds having high moisture content deteriorated at a faster rate than that of the intact seeds during storage.

Christensen and Kaufmann (1969a) reported that all the field fungi required high moisture content in equilibrium with RH of 90 per cent or above for their activities. Harrington (1973) indicated that relative humidity of air affect the moisture content of different kinds of seeds. Sharma and Sharma (1978) reported that seed samples of lentil having 15-16 per cent moisture content harboured high fungal population along with less seed germination percentage whereas, seed samples having low moisture content (5.83-8.81%) exhibited less mycoflora with more germination

percentage. Emayavaramban and Ramabadran (1984) during their studies on seed borne fungi of rice observed that the incidence of storage fungi in the stored grains is influenced by various factors like seed moisture content, storage temperature, relative humidity of the storage atmosphere, period of storage and type of containers. Initially only field fungi like *Drechslera oryzae, Cladosporium* and *Fusarium* were recorded and no storage fungus was encountered but as the storage period increased the storage fungi dominated the population predominant being *Aspergillus flavus*. Jagdish *et al.* (1985) studied the effect of different moisture levels on seed damage caused during threshing and its impact on seed mycoflora on three cultivars of sunflower. They found that higher moisture level resulted in maximum visible seed damage, this in turn harboured high storage fungi during storage as compared to the undamaged seeds. *Alternaria, Penicillium* and *Rhizopus* spp. were more predominant on undamaged seeds threshed at highest moisture level. Das *et al.* (1998) in their experiments on effect of packing material, storage condition and duration of storage on seed viability and vigour of rajmash observed a significant increase in the seed moisture content of seeds stored in gunny bags as compared to plastic containers.

2.2.2 Storage duration and method of storage

The problem of biodeterioration of the agricultural commodities under tropical conditions is very common (Christensen and Kaufmann, 1965). The storage fungi may cause decrease in germination percentage, discolouration of seeds and may induce biochemical changes that reduce its economic value. Seed viability is largely dependent on age and nature of seeds (Harrington and Satyati-Harjadi, 1966) and method of storage (Popovska *et al.*, 1981).

Poor storage of agricultural commodities result in loss of quality and in some cases complete destruction due to invasion by storage fungi (Dwivedi and Tandon, 1975; Ramnath *et al.*, 1970; Singh and Chohan, 1974).

Prevalence of high relative humidity during storage hastens the process of seed deterioration under tropical conditions (Abdul-Baki and Anderson, 1972; Bass, 1980). Ageing of seed during storage resulted in decline of vigour and viability (Roberts, 1972; Singh and Gupta,1982)

Barrett (1973) and Pierre (1972, 1974) suggested that factors like seasonal influence, soil type, diseases and pests hampers the increased production of legumes and fosters deterioration during storage in Jamaica.

Kaul (1973) noticed that fungal flora from the french bean seeds was completely eliminated after four years of storage as none could be intercepted after repeated placement of seeds for isolation. Untreated seeds also outlined the seed borne mycoflora as only *Rhizopus* and *Penicillium* species were isolated occasionally.

Clemetson(1980) found that tradition of using seeds from one crop as planting material for the next, generates genetic deterioration, compounds disease problems and enhances deterioration in storage.

Arulnandhy *et al.* (1984) reported that the ambient storage of seeds in tropical and sub tropical environment with high temperature and humidity deteriorated vigour and viability of rajmash and soybean crop.

Emayavaramban and Ramabadran (1984) in their storage studies on rice observed that initially only field fungi like *Drechslera oryzae, Cladosporium* sp. and *Fusarium* sp. prevailed on rice seed and no storage fungus was encountered, however, as the storage period increased the storage fungi dominated the field fungi. *Aspergillus fumigatus* was noticed after 30 days whereas, *A. flavus* and *A. niger* appeared after 60 days of storage at 75, 80 and 85 per cent relative humidity. Sharma *et al.* (1985) observed thirty eight fungal forms isolated from seeds of *Vigna mungo* examined after storage of 12 months in gunny bags under ambient laboratory conditions. *A. flavus, A. terreus, F. roseum* and *Verticillium alboatrum* showed greater frequency, abundance and relative density in all the stored samples.

Chishlom and Coates-Becford (1997) noticed increased infestation of kidney bean, cowpea and groundnut seeds in Jamaica with *Aspergillus niger* after storage as the same was not observed before storage. They found that bean seeds stored in polyethylene bags at 5°C over a period of 12 months had higher seed germination with minimum fungal flora than those stored at room temperature (28°C).

Das *et al.* (1998) observed that storage condition, storage container and storage duration had drastic effect on seed viability of rajmash irrespective of container used. They found that storage of seed under cold storage maintained significantly higher percentage of germination as compared to seeds stored at ambient conditions. Similarly the survivability of seeds was least affected when stored in plastic containers and polythene bags in cold storage.

2.2.3 Effect of seed coat colour on mycoflora

Seed coat colour of different french bean cultivars has been reported to affect the seed mycoflora. Gonzalaze *et al.* (1982) noticed high selectivity of seed colour to some soil borne fungi and advocated the use of black seeded varieties of french bean, which harboured minimum number of fungi.

Singh *et al.* (1985) studied the effect of seed coat colour on germination and vigour of seedlings of okra and found seven species of fungi viz. *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Rhizopus nigricans, Cladosporium cladosporioides* and *Fusarium* spp. on seeds of four coloured varieties. *Alternaria alternata* was isolated from black seeds. Bright green coloured seeds yielded maximum number of *Aspergillus flavus* colonies followed by dull green. Alison *et al.* (1986) while studying the relationship between seed coat colour and field emergence in different dwarf french bean seed samples found that seed lots with black and brown testa exhibited maximum field emergence (91%) than in white seeded lots (67%). They also observed that rate of imbibition and seed quality was clearly associated with colour of testa as black and brown

seeded varieties had higher germination (94 and 93%) as compared with white seeded (82%).

Dhar and Gurha (1988) found wide variation in the mycoflora on different coloured seeds of kidney bean varieties, HUR 87 possessing black seed coat colour was least infested by the fungal flora as compared to white seeded varieties DDR 14 and HUR 15.

Dhar *et al.* (1988) observed that white coloured varieties of french bean harboured maximum mycoflora followed by deep red and black coloured varieties.

2.3 Pathological Significance of Isolated Mycoflora

French bean seeds harbour large number of mycoflora but only few of them are pathogenic. 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 them reduced germinability of seeds, caused seed and seedling rot (Baker and Smith, 1966).

Suryanarayana and Bhomba (1961) studied the fungal flora of french bean seeds and found that *Fusarium*, *Alternaria* and *Colletotrichum* spp. were pathogenic. Most of the legume diseases in Jamaica have been reported to be seed borne (Pierre, 1972, 1974; Richardson, 1979). Lokhande *et al.* (1986) reported impaired french bean seed germination and seedling mortality due to five seed borne fungi viz. *Alternaria alternata*, *Colletotrichum lindemuthianum*, *Drechslera australiensis, Fusarium semitectum* and *Macrophomina phaseolina.* However, maximum mortality was observed in seedlings infected with *M. phaseolina* and *D. australiensis.*

Aspergillus: *Aspergillus* species have been reported to cause seed decay and seedling mortality in number of legume crops (Gibson and Clinton, 1953; Chohan and Gupta, 1968). Clinton (1960) and Chohan (1971) highlighted the importance of *A. flavus and A. niger* in causing seed bed

diseases. However, *A. flavus* was considered as a weak pathogen by Rati and Ramalingam (1972).

Rao *et al.* (1985) observed heavy incidence of *Aspergillus* along with *Macrophomina phaseolina, Phoma* spp. and *Fusarium* spp. on different seed samples of bean and found that *A. flavus* caused seedling and seed decay. The decayed seeds were heavily colonized by yellowish-green fungal growth. Seedlings emerging from infected seeds started rotting and ultimately the whole seedling collapsed.

Lokhande *et al.* (1986) while studying the pathogenicity through seed inoculation of different *Aspergillus* spp. found that all the species caused complete seed decay and such seeds failed to germinate.

Cladosporium: Sinclair (1984) and McGee (1984) reported that *Cladosporium cladosporoides* produced small round, black scratchy type of brown spots on the seed coat and caused discolouration and splitting of seed coat. Krishnamurthy and Raveesha (1996) observed 2-53 per cent infection in soybean seed due to *Cladosporium* spp.

Colletotrichum: The pathological significance of *Colletotrichum lindemuthianum* causing bean anthracnose has been established in all the bean growing countries of the world including India (Voglino, 1892; Chaves, 1980; Peregrine, 1971; Crispin and compos, 1976). Seed borne infection of the fungus causes serious yield losses in different bean growing areas of the world particularly under cool and humid conditions (Masurat *et al.*, 1966, Mukunya, 1974).

Gram and Weber (1952) observed linear brown lesions or spots on the seedlings of bean plants raised from *Colletotrichum* infected seeds and such seedlings were ultimately killed in later stages. Seeds from heavily infected pods showed variable discolouration depending upon the colour of the seed testa (Zaumeyer and Thomas, 1950). In severely infected seeds, the lesions extended to the cotyledons.

Suryanarayana and Bhombe (1961) observed that seed inoculation of french bean with *Colletotrichum* isolate totally suppressed seed germination, however, the symptom expression varied with degree of infection. Similar effects of the pathogen were also observed by Lokhande *et al.* (1986). Seed borne infection had been found to induce longitudinal dark brown to black eye shaped lesions on the hypocotyle and cotyledons of the bean (Pastor-Corrales and Tu, 1989).

Drechslera: Chidambaram *et al.* (1973) and Neergaard (1977) reported the pathogenic behaviour of *D. sorokiniana* in different cereals and vegetables. Sati *et al.* (1993) found that bean seed infection with *Drechslera sorokiniana* reduced germination and caused rotting of seeds. The fungus caused seed rot inside soil and pre and post emergence damping off whereas, seedlings emerging from artificially inoculated bean seeds showed yellowing, blighting and wilt symptoms.

Fusarium: Burkholder (1919) reported that out of 16-17 per cent yield losses in bean due to various seed borne diseases in USA, one third was due to *Fusarium* species. Kendrick and Snyder (1942) reported that *F. oxysporum phaseoli* caused yellowing of french bean seedlings.

Suryanaryana and Bhombe (1961) observed that french bean seeds colonized by *Fusarium* spp. produced deformed and stunted sprouts. Dhingra (1978) found that *F. pallidoroseum* induced severe wilt symptoms in seedlings of french bean. Dhar and Gurha (1988) observed that *Fusarium oxysporum* caused typical wilt symptoms on bean variety HUR-75 in pathogenicity tests. They found that *Alternaria alternata* was not pathogenic on beans, whereas Gomes and Dhingra (1983) reported pathogenic nature of *Alternaria alternata*.

Tu and Tan (1991) demonstrated that severity of root rot caused by *Fusarium solani* was more significant in compact soils whereas Letourneau and Msuku (1992) found that root rot infection due to *Fusarium solani* f. sp. *phaseoli* was enhanced in plants which were infested by bean fly, *Ophionyia puparia*.

Macrophomina: The micro-sclerotia of *Macrophomina phaseolina* on or beneath the seed coat, in inner layers of seed coat, on cotyledons and embryos were observed by many workers. *M. phaseolina* detected from bean seeds was reported to cause rot and collar rot of beans and other host plants. Neergaard (1979), Dhar and Gurha (1988) also observed similar symptoms on HUR 75 variety of beans in pathogenicity tests.

Gupta *et al.* (1991) reported seed borne nature of *Macrophomina phaseolina* in bean seeds causing yellowing and wilting of plants, dark lesions on stem and dry rot symptoms on infected tissues. They also observed pycnidia on the surface of infected stem. The okra seedlings inoculated with *M. phaseolina* showed ashy black coloured rotting of the roots and collar region followed by shredding and ultimately death of plants within 10 days of inoculation (Pun *et al.*, 1989).

Macrosporium: Doyer (1938) observed *Macrosporium commune* on *Phaseolus* spp. as pinkish spots on the micropylar end of the bean seed. She found that infection was not deep seated.

Phoma: Shukla (1984) reported seed borne nature of *Phoma* species. Dhyani *et al.* (1989) studied pathogenicity of *Phoma medicaginis* on bean crop and observed that pathogen grew gradually and caused rotting of most of the non-germinated seeds. Rotting in young seedlings was noticed at collar and root region. Small dark-black pycnidia and mycelium were observed in diseased areas. Bhale *et al.* (1998) reported *Phoma* leaf spot as wide spread disease problem in major soybean growing areas of Madhya Pradesh. Later in 2001, they recorded *Phoma medicaginis* with soybean seed and observed seed rot, seedling decay and leaf spots symptoms. The association of the pathogen was recorded in the range of 2 to 17 per cent in 80 seed samples obtained from 5 agro-ecological zones of the state during 1999-2001.

Rhizoctonia: Watanabe (1972) observed that seed borne infection of *Rhizoctonia solani* caused considerable decrease in seed germination. Ahuja (1976) recorded root rot and web blight caused by *Rhizoctonia solani*

on number of hosts belonging to 287 genera. Symptoms appeared as reddish brown cankers on roots and stem above and below the surface of soil. The lesions enlarged rapidly and girdled the stem at the collar region, extending longitudinally downwards to the roots.

Gupta *et al.* (1991) also reported seed borne pathogenic nature of *Rhizoctonia solani* on french bean cultivars being grown in Himachal Pradesh. In the year 2000 while studying its seed transmission they observed that decrease in seed germination was more in cultivar Contender than in Kentucky Wonder.

2.4 Seed Quality Parameters as Affected by Mycoflora

Depletion and accumulation of important metabolites in seed borne infections is known in number of host- pathogen combinations (Goodman and Christensen, 1952; Ward and Diener, 1961; Lalithakumari *et al.*, 1971; Vidhyasekaran *et al.*, 1973).

Chemical composition of seed often changes during storage. Relationship of loss of seed viability and decrease in proteins, total sugars and increase in free fatty acids and reducing sugars in cereals has been reported (Zeleney, 1954; Bewley and Black, 1982). However, a significant change noticed in deteriorated seeds was increase in their acidity and free fatty acids (Zelney and Colmann, 1938 and 1939). Metabolic processes of various micro-organisms present on seed surface during storage remain operative on account of which the biochemical composition of the seeds gets altered (Dwarkanath *et al.*, 1969; Paul and Mishra, 1994). Some reports have shown that mycoflora had an adverse effect on the oil content of *Brassica campestris* and *Brassica juncea* seeds. Whereas, protein content showed either increase or decrease after infection (Nijhawan and Hussain, 1964; Degenhardt *et al.*, 1974; Singh and Negi, 1984). Chattopadhyay and Srimani (1972) reported that colonization of rice with fungi caused increase in fatty acid content of bran oil as compared to that of healthy seeds.

Vidhyasekaran and Govindaswamy (1968) observed accumulation of reducing sugars due to fungal invasion in rice seeds. Vidhyasekaran *et al.* (1973) also recorded increase in the concentration of several amino acids in rice grains during their deterioration by fungi.

Bilgrami *et al.* (1976) recorded marked decrease in the concentration of sucrose in mung and urdbean, however, decline in urd bean seed started after 10^{th} day of incubation. They also found an increase in L-cystine and L-leucine concentration whereas, some of the amino acids showed decline in diseased seeds. Decline was attributed to their consumption by *A. flavus*, while increase may be due to the break down of seed proteins.

Daniel *et al.* (1977) observed that the protein content of green gram increased from 23.7 to 24.2 per cent in the seed samples infested with mycoflora.

Singh and Bedi (1976) found that susceptible gram cultivars had higher sugar content as compared to resistant ones, however, difference in phenolic compounds were more conspicuous as compared to total sugars.

Kammar and Naik (1987) observed noticeable changes in the nutritive composition of green gram during storage. They found that proteins, fats and fiber content increased during storage whereas, the carbohydrate decreased along with increased storage.

Arulnandhy and Senanayake (1991) observed decrease in protein and ash content while oil, carbohydrate and free fatty acids increased in soybean seeds under storage and the viability of these seeds also dropped to almost zero after 9 months of storage.

Paul and Mishra (1994) while studying the effect of seed mycoflora of maize seeds on biochemical parameters reported that the fatty acid contents increased in *A. flavus* infected seeds, whereas the starch content was reduced in all the test varieties as compared to healthy seeds, however, the decline in starch content was slow in initial stages. The

decrease in starch content was attributed to its utilization by the mycoflora as substrate.

2.5 Management of Seed Mycoflora

Seed borne pathogens have been reported to cause significant losses both in terms of yield and quality of legumes (Shao and Teri, 1985; Shama *et al.*, 1988). Kaul (1973) studied the effect of fungicide treatment of bean seeds on mycoflora and seed health during prolonged storage. He found that five fungicides viz. Agrosan GN, Ceresan, Captan, Tillex and Thiram were most effective in controlling the mycoflora without impairing seed germination during 4 years of storage.

Ellis *et al.* (1976) found that treatment of infected seeds of french bean with Captan, Thiram and Benomyl @ 50, 75 and 100 mg/40 g seed increased the germination and helped in better emergence of seeds.

Dhingra and Maffia (1978) while studying the activity of different fungicides in treated seeds of *Phaseolus vulgaris* observed the activity of Benomyl and thiophanate-methyl in cotyledon, hypocotyle and in primary leaves of seedlings and that of carboxin in cotyledon only.

Khare *et al.* (1979) found that Dithane M-45 (mancozeb) as foliar spray was effective in controlling various foliar diseases of *Phaseolus vulgaris* and seeds stored from such plants were least affected by the storage fungi. They observed that treatment of seeds with Sulfex was also effective in reducing storage fungi.

Tanaka and Corea (1982) found beneficial effects of seed treatment with PCNB (quintozene) + captafol on all the cultivars of *P. vulgaris* seeds. They observed enhanced seed germination and emergence of poor quality seeds of bean cultivars after treating the seeds with carbendazim + streptocycline. Treatment of high quality seed with thiram, carbendazim + streptocycline improved the seed emergence.

Kore and Solanke (1982) studied the efficacy and longevity of seven fungicides viz. Thiram (0.2%), Bavistin (0.1%), Difolatan (0.2%), Vitavax

(0.2%), Captan (0.2%), MBC (0.1%) and Agrosan GN (0.2%) on french bean seeds using agar plate, blotter and rolled paper towel method and found that Agrosan GN, Vitavax, MBC and Bavistin improved germination as compared to Captan, Difolatan and Thiram. The fungal flora was also found to be decreased in treated seeds. Agrosan GN, Vitavax, MBC and Bavistin increased the germination percentage as compared to control.

Tsvetkov and Doner (1984) reported that bean seed treatment with fusamycine and sacomycine was effective against *Fusarium, Helminthosporium, Pythium* and *Rhizoctonia* resulting in increased yields. Khere (1985) reported that treatment of bean seeds with benomyl was very effective against some storage fungi like *Fusarium moniliformae*, *Aspergillus niger* and *Penicillium* spp. Treatment with fungicides like Thiram, carbendazim, mancozeb, benlate and Vitavax has been found most effective in controlling seed borne infection of *Colletotrichum lindemuthianum* (Sindhan and Bose, 1981; Estrada, 1989; Sharma and Sugha, 1995). Hegde and Hiremath (1987) while evaluating Captan, Thiram, Ceresen dry and Bavistin found that all the four fungicides used as seed dressers significantly improved germination, seed vigour and reduced seed mycoflora when applied @ 2.5 to 5.0 g/kg seed. However, when these fungicides were applied @ 10 g/kg seed, they were phytotoxic to all the seeds. Two fungicides Ceresen dry and Thiram proved better for all the characters and retained their efficacy even after 90 days of storage.

Vanangumdi and Karivartharaju (1987) observed that bean seed treatment with Captan and activated clay had higher percentage of field emergence. Whereas, red earth treatment was deletarious to the seeds as it reduced the percentage of field emergence and increased percentage of abnormal seedlings. Storage containers and seed treatment had significant effect on vigour index of stored seeds when estimated in terms of root-shoot length and dry weight of root, shoot and leaves.

Dhyani *et al.* (1989) while studying effect of 7 seed dressing fungicides viz. Thiram, captafol, Bavistin, Brassicol, Dithane M-45, Topsin

and Vitavax for the control of *Phoma medicaginis* on bean seed found that Thiram and captafol were most effective in controlling the seed infection and enhancing seed germination as compared to other fungicides

Jayasheela *et al.* (1998) studied the synergistic effect of Bavistin and *Bradyrhizobium japonicum* on soybean seeds. Plants raised from Bavistin treated seeds produced maximum number of nodules per plant, more nodule fresh weight and had higher leghaemoglobin content followed by treatment with Dithane M-45.

Pensalawar and Kore (1998) studied the effect of dry and slurry seed treatment with Thiram, Captan, Bavistin, Dithane M-45 and Aureofungin on seed mycoflora of urdbean seeds during storage and found that seeds treated with Thiram harboured less mycoflora and resulted in high vigour index and germination percentage.

Coutinho *et al.* (1999) studied the effect of anacardiaceous plant extracts and two fungicides Benomyl and Captan on seed mycoflora and physiological quality of common bean. They recorded the presence of *A. niger, A. flavus, Cladosporium* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizoctonia solani* on the seeds treated with dry and milled peels of aroeira (*Astronium urundeuva*), cajueiro (*Anacardium occidentale*) and the fungicides Benomyl and Captan. Benomyl was most effective in controlling fungal flora. Plant extracts were more effective when applied in combination with fungicides. Occurrence of *A. flavus* decreased by the application of plant extracts. Sinha *et al.* (1999) while studying seed mycoflora of french bean and its management by fungicides observed that Dithane M-45, Bavistin, Agrosen GN and PCNB reduced seed mycoflora significantly and improved germination. Krishnamurthy and Raveesha (2001) in their experiments on management of seed borne pathogens of pulse crops with seed dressing fungicides like captafol, Bavistin and Hardon found that captafol and Bavistin controlled seed borne pathogens (99%) as well as enhanced seed germination (up to 93%) more effectively as compared to Hardon.

MATERIAL AND METHODS

Chapter – III

MATERIAL AND METHODS

The present studies were conducted in the Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 1998-2001. The methodology followed during the course of investigations to fulfill the objectives is being described under following heads:

3.1 Varietal and spatial distribution of seed mycoflora

3.2 Pathological significance of mycoflora

3.3 Seed health parameters

3.4 Storage studies

3.5 Seed quality parameters

3.6 Management of seed mycoflora during storage

3.1 Varietal and Spatial Distribution of Seed Mycoflora

3.1.1 Collection and maintenance of seed samples

Seed samples of kidney bean comprising of local and improved varieties were collected from different commercial bean growing areas of Himachal Pradesh representing diverse agro-climatic situations. In all, 73 samples were collected from different locations of Chamba, Kangra, Kinnaur, Kullu, Lahaul- Spiti, Mandi and Shimla districts of the state. Sampling was done after harvesting of crop in October and after storage in May before sowing of the crop. At least 500 gms sample was collected from each location in accordance with rules formulated by International Seed Testing Association (ISTA, 1985). Information was recorded on place of collection, type of cultivar grown, date of collection and storage conditions etc. from the farmer of each location. The samples were

catalogued and kept in labelled cloth bags at room temperature for further investigations under laboratory conditions.

3.1.2 Grading of seed samples

The seed samples (500 seeds/ lot) were examined critically and grouped into three categories on the basis of their morphological traits/external symptoms according to the methods followed by Lokhande *et al.* (1986) with slight modifications as follows:

a) Apparently healthy seeds

b) Shrivelled seeds

c) Discoloured seeds

3.1.3 Detection of seed mycoflora

Bulk sample comprising of seeds of different grades was used for isolating fungi by standard procedures described by International Seed Testing Association (ISTA, 1976) with slight modifications. The mycoflora associated with kidney bean was detected immediately after acquisition of the samples with or without incubation of seeds.

a) Detection of mycoflora before incubation

   i) Visual examination

   ii) Washing test

b) Detection of mycoflora after incubation

   i) Rolled paper towel method

   ii) Blotter method

   iii) Agar plate method

   iv) Seed component plating technique

3.1.3.1 Detection of mycoflora before incubation

Visual examination: Five hundred seeds from each lot were examined under wild M5A stereoscopic binocular microscope for any discolouration, malformation or shrivelled appearance of seeds. Seed infection by bean pathogens was estimated by observing the diagnostic expressions of

various pathogen/ symptoms on the seed surface. Apparently healthy seeds present within the sample were also observed under stereoscopic binocular microscope for the presence of fungal fructifications.

Washing test: A sample of 100 seeds was treated/ soaked in 50 ml ethanol (25%) with constant shaking on rotary shaker for 25 minutes at 5000 rpm. The supernatant was decanted and the concentrated residue thus obtained was diluted to 1 ml with sterilized distilled water and examined under microscope for mycoflora. Spore count of micro-organisms associated with seed was determined with the help of haemocytometer.

3.1.3.2 Detection of mycoflora after incubation

Seed samples (100 seeds/sample) collected from various agro-ecological regions were subjected to incubation by employing different methods for the growth of associated mycoflora before its detection. The seed lots were treated with surface disinfectant sodium hypochlorite (1.0%) for 5 minutes prior to incubation.

Rolled paper towel method: The abundance of associated mycoflora was determined by rolled paper towel method (ISTA, 1985). 100 seeds of each sample, both surface sterilized and unsterilized, were lined on two layers of moist germination paper placed on wax paper sheets. There were four replications each having 25 seeds. The samples were incubated in BOD incubator at 25±1°C for 8-10 days and observations were recorded on frequency of mycoflora.

Standard blotter method: One hundred seeds of each sample, both sterilized and unsterilized, were plated in plastic Petri dishes (9 cm dia.) lined with three layers of blotting paper saturated with sterilized water. Ten seeds were placed in each plate equidistantly (Neergaard, 1977). These plates were incubated at 25 ± 1 °C in BOD incubator with 12 hours alternating cycles of light and dark and thereafter shifted to deep freezer (-20 °C) for 24 hours to prevent seed germination. The plates were then again incubated at 25 ± 2 °C for 7 days. Incubated seeds were examined

visually and under stereoscopic binocular microscope for the associated mycoflora.

Agar plate method (Neergaard, 1977): In this method unsterilized and surface sterilized seeds were plated on Malt Extract Agar (MEA) medium supplemented with 100 ppm streptocycline to avoid bacterial contamination. The plates were incubated under similar conditions as described in blotter method. The observations on seed mycoflora were recorded on 3rd, 5th and 8th day of incubation under stereoscopic binocular microscope.

Component plating technique: In order to detect the internally seed borne mycoflora in bean seeds, component plating technique was employed (Chisholm and Coates-Becford, 1997). 25 seeds were surface sterilized in 1.0 per cent sodium hypochlorite solution for five minutes, blot dried under two folds of blotting sheets and rinsed thrice with sterile distilled water. Seeds were then soaked in sterile water for 4-6 hours and different seed components viz. seed coat, cotyledons and embryonic axis were separated with the help of sterilized forceps under laminar air flow to avoid external contamination. The components were rinsed in sterile water, blot dried and placed on Petri dishes containing MEA. The plates were placed in sterile polyethylene bags and incubated at 25$\pm$1°C for 7 days. After incubation, each seed component was observed for the growth of fungi visually as well as under microscope.

3.1.4 Isolation, Identification and Maintenance of mycoflora

Mycoflora detected on kidney bean seed by different methods was isolated on PDA. Pure culture of each fungi was obtained by single spore isolation and maintained separately for further studies on PDA.

Isolated mycoflora was identified on the basis of colony colour, number of colonies, sporulation, conidial characters and fruiting structures using Leitz compound microscope with the help of literature (Booth, 1971; Barnett and Hunter, 1972; Raper and Fennell, 1977). In addition to PDA other specific media for different fungi were also used to establish the identity of their species (Appendix I).

3.2 Pathological Significance

The pathogenic behaviour of individual seed borne fungi isolated from kidney bean seed was studied by employing seed smearing, seedling dip and whole plant methods under pot culture conditions.

3.2.1 Seed smearing (Lokhande *et al.*, 1986)

Surface sterilized seeds and seedlings of susceptible cultivars `Jawala/ Kanchan' were smeared with spore suspension slurry prepared in sterilized distilled water from 14 days old sporulating cultures of the isolated fungi. The surface sterilized seeds were smeared with spore masses and processed as per rolled paper towel method. Observations were recorded on germination, root-shoot length and vigour of seeds and seedlings.

3.2.2 Seedling dip method (Champion *et al.*, 1973)

Rolled paper towel method was employed for the germination of seeds. Surface sterilized healthy seeds were placed in double layer of moistened germination paper at 26±2°C in the seed germinator. Seed coat of the germinated seeds was removed after three days. The germinating seedlings were dipped in standard spore suspension of each test fungus for about 5 minutes. The inoculated seedlings were placed on two layers of moist germination paper and kept for growth in seed germinator at 22±2°C. Data were recorded after 7-9 days of incubation on germination, root-shoot length, vigour index, seed and seedling mortality. Germinated seeds inoculated with distilled water were kept as control for comparison.

3.2.3 Effect of culture filtrate on seed and seedling health

The isolated fungi were grown individually on malt extract broth for 15 days at 25±1°C in BOD incubator. Culture filtrates were obtained by filtering the contents through bacteria-proof filter (Seitz filter). Surface sterilized kidney bean seeds and seedlings were soaked in the culture for six hours and then placed on two layers of moistened germination sheets

for growth (Reddy and Chaudhary, 1990; Ushamalini *et al.*, 1998) and observations were recorded on growth and viability traits.

3.2.4 Pathogenicity of non-sporulating fungi

The pathogenicity of non-sporulating fungal cultures was tested by following two methods of inoculations.

Seed inoculation: Surface sterilized seeds were placed on freshly growing culture in Petri-plates and data were recorded on seed colonization and germination after two weeks of inoculation.

Whole plant method: Healthy seedlings of cultivars `Jawala/ Kanchan' were raised in 10 cm plastic pots containing sterilized potting mixture. Five seedlings were inoculated at stem region by placing mycelial bits of test fungus obtained from 7-14 days old cultures. All the inoculated plants were covered with moist cotton swabs to provide humidity. The plants were kept in glass house for the appearance of disease symptoms, if any. Healthy seedlings maintained separately were kept for comparison.

3.3 Seed Health Parameters as Affected by Mycoflora

The status of seed health of each sample was assessed immediately after acquisition by determining the moisture content, germination percentage, seed and seedling vigour following standard methods with slight modification.

3.3.1 Moisture content

Moisture content of all the seed samples was determined on moisture testing machine `INDOSAW4047 digital' (Agro model). The seed sample (100 gm approx.) was put in the test cup (volume B, provided with moisture meter) and placed in the housing of the compression unit. The sample was then compressed to a specified thickness (363 mm) as per the specifications of moisture metre and the observations were recorded on moisture content of each sample by processing them separately.

3.3.2 Germination percentage

Germination of various seed samples used in different experiments was studied by employing rolled paper towel method as described in section 3.1.3.2(i) (ISTA, 1985). One hundred seeds replicated four times (25 seeds/replication) were used in each case. Data on per cent germination were recorded after 8-10 days of incubation. Observations were also recorded on root-shoot length of the 25 normally growing seedlings and their averages were taken to calculate vigour index by using the formula given by Abdul Baki and Anderson (1973) as:

Vigour index = % seed germination x (average root length + average shoot length)

The hypocotyls and radicals were then dried at 70 °C in hot air oven after recording their fresh weight for calculation of vigour on dry weight basis.

3.3.3 Seed vigour and viability

Seed vigour and viability of seed samples were tested by employing Tetrazolium test (TZ) described by Agarwal and Dadlani (1992). One hundred seeds of each sample were soaked overnight in sterilized distilled water at room temperature and their seed coats were removed before treating them with tetrazolium solution. Seeds devoid of seed coats were dipped in 1.0 per cent tetrazolium solution (pH 7.0) prepared in phosphate buffer, for 3-4 hours in dark at 30±1°C for the development of colour. After incubation, solution was drained off and seeds were rinsed thrice with distilled water before taking final observations on percentage of germinable and ungerminable seeds. In order to check the seed vigour embroynic axis of tetrazolium treated seeds were excised after taking readings on seed viability and washed thoroughly with distilled water according to the method given by Kittock and Law (1968). Excised embryos were then soaked in methyl cellusolve solution (10 ml/ 25 axis) for 4-6 hours with occasional stirring for the extraction of red coloured formazon till the axis become colourless. The colour intensity of the extract was observed at 480 nm in

Spectronic-20 calorimeter (Beckman DU 640 B). Methyl cellusolve was used for calibration of the instrument before taking final observations.

3.4 Storage Studies

3.4.1 Effect of different storage containers and duration of storage on mycoflora and seed health

Different storage containers viz., gunny bag (G), polylined bag (P) and metallic bin (M) commonly used by the farmers were evaluated to see their effect on seed mycoflora and seed health. Seed samples comprising of local and improved varieties collected from 5 locations viz. Barot (Mandi), Salooni (Chamba), Banjar (Kullu), Kukumseri (Lahaul) and Sangla (Kinnaur) were packed in respective packing material and stored under laboratory conditions over a period of 6 months. One lot of each sample was assessed for moisture content immediately after storage for comparison purpose. The observations were recorded on seed mycoflora and seed health parameters at an interval of 60 days. Data were recorded on these parameters at 0-level (i.e. before storage) for comparison purpose. Effect of various seed dressing fungicides and their doses was evaluated on variety `Kanchan' obtained from Kukumseri (Lahaul-Spiti) and stored in metallic bins. The data on maximum-minimum temperature and relative humidity recorded during the storage period are presented in Appendix II.

3.5 Seed Quality Parameters

3.5.1 Preparation of samples

Dry seeds of rajmash varieties/genotypes were cleaned and freed of any extraneous substances and dried in an electric oven at 60±5°C. The dried samples were ground in a willy mill to pass through 40 mesh sieve and stored in polyethylene bags. These samples were properly labelled and kept at room temperature for further analysis.

3.5.2 Crude proteins (AOAC, 1970)

Micro Kjeldhal method was adopted to determine percentage of nitrogen content and a conversion factor of 6.25 was used to calculate

crude protein content. One hundred mg dried sample was digested with concentrated sulphuric acid (20 ml) and digestion mixture (5.0 g) containing potassium sulphate and copper sulphate in the ratio of 10:1 in a Kjeldhal digestion flask till the contents of the flask became free from organic carbon. The contents were cooled, diluted with small amount of distilled water and transferred into a 100 ml volumetric flask. The volume was made up to the mark with addition of distilled water. Ten ml of aliquot was taken and transferred to a distillation assembly followed by addition of 10 ml of 40 per cent sodium hydroxide. On distillation ammonia librated was collected in 250 ml conical flask containing 10 ml of N/10 H_2SO_4 to which methyl red indicator (2-3 drops) was added. It was then titrated against N/10 NaOH solution. One blank containing concentrated sulphuric acid and digestion mixture was also run along with the experimental samples. Volume used in titration of alkali and amount of standard sulphric acid (N/100) used for ammonia absorption was recorded and protein contents were calculated as:

$$\% N = \frac{V \times 0.00014 \times D}{W \times A} \times 100$$

Where V = Volume of N/100 H_2SO_4 taken-volume of N/100 NaOH used for titration

D = Dilution factor (volume make in volumetric flask)

W = Weight of the sample (g)

A = Aliquot taken

% Crude protein = per cent Nitrogen x 6.25

3.5.3 Total carbohydrates

Phenol sulphuric acid method (Sadasivam and Manickam, 1996) was used for the estimation of total carbohydrates.

Reagents required:

- Phenol 5 per cent: Redistilled (reagent grade)
- Sulphuric acid 96 per cent (reagent grade)
- Standard glucose: Stock-100 mg in 400 ml of water. Working standard 10 ml of stock diluted in 100 ml with distilled water.

Procedure: One hundred mg of the representative sample was weighed in a test tube and hydrolysed by keeping it in boiling water bath for three hours with 5 ml of 2.5 N hydrochloric acid and cooled to room temperature. Solid sodium carbonate was added to hydrolysed product to neutralize acid till the effervescence ceased. The volume was made up to 10 ml with distilled water and contents were centrifuged at 5000 rpm for 10 minutes. The supernatant was collected in 200 ml volumetric flask and final volume was made 200 ml with distilled water. Then 0.1 ml of aliquot was taken and volume was made 1 ml with distilled water to which 1 ml of 5 per cent phenol and 5 ml of 96 per cent sulphuric acid were added. Solution was mixed well and placed in water bath at 25-30 °C for 20 minutes. Colour intensity was measured in Spectronic-20 (Beckman DU640B) at 490 nm and carbohydrate contents were calculated by using standard curve prepared with glucose.

Absorbance corresponding to 0.1 ml of test = X mg of glucose

100 ml of the sample solution contains = (X / 0.1) 100 mg of glucose

= % of total carbohydrate present

3.5.4 Fat content

Reagent required: Petroleum ether.

Apparatus: Soxhlets extraction apparatus, thimble with cotton swab, hot air oven, hot plate, analytical balance, weight box and desiccator.

Three gms of moisture free representative test samples was put in dried, pre weighed extraction thimble. The sample was dried by keeping overnight at 105°C in a hot air oven, followed by cooling in a desiccator. The thimble was placed in Soxhlets apparatus in a straight direction so that the condensed liquid fall on it at low rate of 5-6 drops/ second for extraction. After extraction, thimble was taken out and kept at room temperature for evaporation of ether and dried overnight in the oven at 100-105 °C followed by cooling in desiccators before weighing.

Calculations:

Weight of sample = (wt of thimble+ sample) - (wt of thimble)

Weight of fat = (wt of thimble+sample) - (wt of thimble+sample after extraction)

3.6 Management of Seed Borne Mycoflora

3.6.1 Effect of seed dressing fungicides

Efficacy of five commonly used fungicides viz. Bavistin (carbendazim), Bavistin + TMTD (carbendazim + thiram), Baylaton (triadimefon), Captan (captafol) and Thiram (thiram) was assessed to manage the mycoflora that bean seeds harbour during storage. One lot each (500 gms) was treated with each fungicide @ 2.0, 2.5 and 3.0 g/kg as dry seed treatment. Untreated seeds were also kept as check. The seeds were then stored in gunny bags, polylined bags and metallic bins over a period of six months under laboratory conditions.

A seed lot of variety `Kanchan' was first smeared with 72 hours old sporulating and non sporulating cultures of isolated mycoflora. After 24 hours of smearing five sub lots were made and each was treated with fungicide. Observations were recorded on seed mycoflora and other seed health and quality parameters at an interval of 60 days by subjecting 100 seeds/ lot to blotter and agar plate method and 25 seeds to seed component plating technique.

3.6.2 Effect of propionic acid on storage fungi (Dey *et al.*, 1992)

Different seed lots were treated with propionic acid @ 10 ml/kg seed and stored in gunny bags, polylined bags and metallic bins for six months. Data were recorded on various parameters as described in section 3.6.1.

3.7 Glassware and Plastic Ware Used

All the glassware and plastic ware used in different experiments was sterilized in hot air oven at 180°C for 2-3 hours or surface sterilized with rectified spirit to avoid contamination.

RESULTS

RESULTS

The present investigation entitled "Eco-pathological study of seed mycoflora and its impact on biodeterioration of *Phaseolus vulgaris* L. in Himachal Pradesh" was undertaken with a view to study the varietal and spatial distribution of seed mycoflora and its impact in relation to storage duration, containers and fungicides on seed health and quality parameters of kidney bean seed samples collected from various agro ecological zones of Himachal Pradesh.

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

4.1 Varietal and spatial distribution of seed mycoflora

4.2 Pathological significance of mycoflora

4.3 Storage studies

4.4 Seed quality vis-à-vis mycoflora

4.5 Management of seed mycoflora

4.1 Varietal and Spatial Distribution of Seed Mycoflora

4.1.1 Collection of seed samples

Seventy three kidney bean seed samples comprising of recommended varieties and local landraces (pulse type) were collected from three agro-ecological zones (II, III and IV) of Himachal Pradesh, where kidney bean is being grown on commercial scale (Figure 1). Seed samples were collected immediately after harvest and storage by the farmers and information collected on cultivars used, method of storage etc. is given in Table1. Forty five samples were obtained from Zone-III, whereas, number of samples collected from Zone-II and IV were 9 and 19, respectively. The

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

Agro-climatic zone	District/ Location	Line/ Variety	Storage condition	Sample number
1	*2*	*3*	*4*	*5*
Zone II	***Kangra***			
	Kandwari	Local	Gunny bag	KB 1
	Palampur	Baspa	Wooden bin	KB 2
		Triloki	Wooden bin	KB 3
		Kanchan	Wooden bin	KB 4
		Contender	Gunny bag	KB 5
		Laxmi	Gunny bag	KB 6
		SVM 1	Gunny bag	KB 7
		Kentucky Wonder	Gunny bag	KB 8
		Hans	Gunny bag	KB 9
Zone III	***Chamba***			
	Dayur	Local	Gunny bag	KB 10*
	Guner	Local	Gunny bag	KB 11
	Kathayari	Local	Gunny bag	KB 12*
	Kuntlehri	Local	Gunny bag	KB 13
	Lahal	Local	Gunny bag	KB 14
	Salooni	Local	Gunny bag	KB 15*
		Jawala	Gunny bag	KB 16*
		Kanchan	Gunny bag	KB 17*
		Jawala	Gunny bag	KB 18*
		Him 1	Gunny bag	KB 19*
		Triloki	Gunny bag	KB 20*
	Surgani	Jawala	Gunny bag	KB 21
	Kangra			
	Bara Bhangal-1	Local	Gunny bag	KB 22
	Bara Bhangal-2	Local	Wooden bin	KB 23
	Kothikohr	Local	Wooden bin	KB 24
	Lohardi	Local	Metallic bin	KB 25
		Local	Metallic bin	KB 26
		Local	Metallic bin	KB 27
	Multhan	Local	Metallic bin	KB 28

Continued

1	2	3	4	5
	Nalhata	Local	Metallic bin	KB 29
	Rajgundha-1	Local	Gunny bag	KB 30
	Rajgundha-2	Local	Gunny bag	KB 31
	Salar	Local	Wooden bin	KB 32
	Kullu			
	Drilahari	Local	Gunny bag	KB 33*
	Ghyagi	Local	Metallic bin	KB 34
	Kamand	Local	Gunny bag	KB 35
	Kao	Local	Gunny bag	KB 36
	Kotla	Local	Gunny bag	KB 37
	Naggar	Local	Gunny bag	KB 38
	Palchaan	Local	Gunny bag	KB 39
	Rujjag	Local	Wooden bin	KB 40
	Rohanda-1	Local	Metallic bin	KB 41
	Rohanda-2	Local	Gunny bag	KB 42
	Mandi			
	Banah	Local	Metallic bin	KB 43
	Barot	Local	Wooden bin	KB 44
	Koteru	Local	Gunny bag	KB 45
	Lassar	Him 1	Gunny bag	KB 46
	Nau	Local	Wooden bin	KB 47
		K 198	Wooden bin	KB 48
		Kanchan	Wooden bin	KB 49
		Local	Wooden bin	KB 50
	Shimla			
	Cheog	Local	Gunny bag	KB 51
	Kumarsain	Local	Gunny bag	KB 52
	Sawra	Local	Metallic bin	KB 53*
	Shilaru	Local	Gunny bag	KB 54
Zone IV	***Chamba***			
	Holi	Local	Gunny bag	KB 55*
	Parmas	Local	Metallic bin	KB 56*
	Purthi	Local	Metallic bin	KB 57*
	Shour	Local	Metallic bin	KB 58*
		Local	Metallic bin	KB 59*
		Local	Gunny bag	KB 60

Continued

1	2	3	4	5
	Lahaul and Spiti			
	Gompathang	Him 1	Gunny bag	KB 61
	Hinsa	Him 1	Metallic bin	KB 62*
		Local	Gunny bag	KB 63
	Jahlma	Him 1	Gunny bag	KB 64
	Kukumseri	Local	Gunny bag	KB 65*
		Local	Gunny bag	KB 66*
	Shakoli	Him 1	Gunny bag	KB 67
		Local	Gunny bag	KB 68
	Kinnaur			
	Ribba	Jawala	Gunny bag	KB 69
	Sangla	KRC 8	Wooden bin	KB 70
		Local	Gunny bag	KB 71
		Local	Gunny bag	KB 72
		Local	Gunny bag	KB 73

* Samples collected after harvest

potential kidney bean growing areas included Chamba, Kangra, Kinnaur, Kullu, Lahaul-Spiti, Mandi and some parts Shimla districts.

Information collected from farmers of different areas revealed that they generally use their own seed year after year and local landraces dominate the recommended varieties. Out of 73 seed samples, 49 were of local landraces and remaining 24 were of recommended varieties. Farmers generally use gunny bags for storage of seeds followed by metallic and wooden bins.

4.1.2 Grading of kidney bean seed samples for seed pathological studies

Different seed samples were categorized on the basis of their morphological appearance into three grades viz. apparently healthy, shrivelled and discoloured (Plate 1). Frequency of seeds in each category is presented in Table 2. The percentage of different graded seeds among 73 samples ranged between 60.0 - 99.6, 1.6 - 40.0 and 0.4 – 20.0 per cent, respectively, in different agro-climatic zones.

The percentage of apparently healthy seeds was highest in Zone III (99.6%), followed by Zone IV (99.2%). However, maximum number of shrivelled seeds were noticed in seed lots representing Zone III where samples KB 5, KB 51 and KB 23 showed 40 and 36.8 per cent shrivelled seeds and samples KB 51 had 20 per cent discoloured seeds. The frequency of discoloured seeds was highest in Zone III. Minimum percentage of abnormal seeds was observed in Zone IV, where some of the samples were absolutely free of any visible infection.

Seed samples of recommended varieties (Table1) revealed that cultivar Him 1 (Zone IV) showed the presence of minimum number of abnormal seeds whereas, Contender possessed the maximum. In general, the local landraces possessed more abnormal seeds in comparison to recommended ones.

Table 2. Grading of samples for seed pathological studies

Sample number	Proportion of seeds in different grades (%)		
	Apparently healthy	Shrivelled	Discoloured
1	2	3	4
KB 1	92.0	4.0	4.0
KB 2	98.3	1.6	1.0
KB 3	96.8	2.8	0.4
KB 4	97.8	2.0	0.4
KB 5	60.0	40.0	4.0
KB 6	96.0	4.0	2.0
KB 7	98.0	2.0	2.0
KB 8	96.0	10.0	2.0
KB 9	93.0	9.0	3.0
KB 10*	91.0	9.0	3.8
KB 11	77.0	18.6	1.1
KB 12*	86.2	13.4	4.0
KB 13	85.8	3.4	2.0
KB 14	87.6	5.6	4.6
KB 15*	78.4	18.4	3.2
KB 16*	86.4	9.6	3.2
KB 17*	98.3	1.6	1.0
KB 18*	96.8	2.8	0.4
KB 19*	97.8	2.0	0.4
KB 20*	83.6	16.0	1.1
KB 21	69.6	30.4	3.0
KB 22	75.6	24.4	6.0
KB 23	63.2	36.8	7.0
KB 24	73.8	26.2	5.4
KB 25	77.2	22.8	2.0
KB 26	77.6	22.4	1.6
KB 27	91.2	4.4	1.6
KB 28	68.8	31.2	2.4
KB 29	85.0	15.0	2.0
KB 30	89.6	7.0	0.4
KB 31	90.0	7.6	1.2
KB 32	82.0	16.0	1.0
KB 33*	93.6	4.0	2.4
KB 34	94.0	3.0	2.0
KB 35	81.2	18.0	0.8

Continued

Table 2. Grading of samples for seed pathological studies

Sample number	Proportion of seeds in different grades (%)		
	Apparently healthy	Shrivelled	Discoloured
1	*2*	*3*	*4*
KB 1	92.0	4.0	4.0
KB 2	98.3	1.6	1.0
KB 3	96.8	2.8	0.4
KB 4	97.8	2.0	0.4
KB 5	60.0	40.0	4.0
KB 6	96.0	4.0	2.0
KB 7	98.0	2.0	2.0
KB 8	96.0	10.0	2.0
KB 9	93.0	9.0	3.0
KB 10*	91.0	9.0	3.8
KB 11	77.0	18.6	1.1
KB 12*	86.2	13.4	4.0
KB 13	85.8	3.4	2.0
KB 14	87.6	5.6	4.6
KB 15*	78.4	18.4	3.2
KB 16*	86.4	9.6	3.2
KB 17*	98.3	1.6	1.0
KB 18*	96.8	2.8	0.4
KB 19*	97.8	2.0	0.4
KB 20*	83.6	16.0	1.1
KB 21	69.6	30.4	3.0
KB 22	75.6	24.4	6.0
KB 23	63.2	36.8	7.0
KB 24	73.8	26.2	5.4
KB 25	77.2	22.8	2.0
KB 26	77.6	22.4	1.6
KB 27	91.2	4.4	1.6
KB 28	68.8	31.2	2.4
KB 29	85.0	15.0	2.0
KB 30	89.6	7.0	0.4
KB 31	90.0	7.6	1.2
KB 32	82.0	16.0	1.0
KB 33*	93.6	4.0	2.4
KB 34	94.0	3.0	2.0
KB 35	81.2	18.0	0.8

Continued

1	2	3	4
KB 36	86.8	8.8	4.4
KB 37	83.2	4.8	3.0
KB 38	70.0	22.0	8.0
KB 39	81.2	2.0	4.8
KB 40	72.6	23.0	4.4
KB 41	62.0	25.0	5.6
KB 42	91.4	5.2	3.4
KB 43	99.6	4.8	0.8
KB 44	96.0	13.4	2.0
KB 45	97.4	4.6	0.8
KB 46	96.4	6.2	0.2
KB 47	90.0	6.0	5.0
KB 48	96.2	4.0	3.0
KB 49	82.0	18.0	1.0
KB 50	77.0	20.0	11.0
KB 51	62.0	40.0	20.0
KB 52	65.0	22.0	5.0
KB 53*	96.0	10.0	2.0
KB 54	93.0	9.0	3.0
KB 55*	93.6	2.8	2.4
KB 56*	92.8	6.8	2.0
KB 57*	95.6	4.8	2.0
KB 58*	69.6	30.4	5.8
KB 59*	87.6	6.2	6.0
KB 60	70.0	30.0	5.0
KB 61	89.6	8.4	2.0
KB 62*	79.5	20.0	1.5
KB 63	92.8	2.8	6.4
KB 64	77.0	23.0	1.5
KB 65*	94.0	4.6	1.4
KB 66*	99.0	10.0	0.4
KB 67	99.2	10.6	0.6
KB 68	98.4	6.2	1.2
KB 69	85.6	11.6	3.0
KB 70	92.0	10.0	2.0
KB 71	90.0	9.0	1.0
KB 72	72.2	20.6	7.2
KB 73	75.8	17.6	6.6

* Samples collected after harvest

4.1.3 Seed mycoflora through washing test

Various seed samples were analysed by washing test to detect the sporulating and non sporulating mycoflora immediately after their collection and data obtained are presented in Table 3. Perusal of data showed the presence of seven genera of field/pathogenic fungi viz. *Alternaria, Cercospora, Colletotrichum lindemuthianum, Fusarium, Isariopsis, Rhizoctonia* and *Sclerotinia*. Out of these, *C. lindemuthianum* was encountered in maximum number of samples (25) in low to high concentration followed by *Fusarium* and *Rhizoctonia*. However, *Sclerotinia, Isariopsis* and *Alternaria* were found in 14, 13 and 11 samples, respectively, whereas *Cercospora* was encountered only in 5 samples.

In Zone II, *Isariopsis griseola* was observed in 4 samples followed by *Alternaria* and *Fusarium* sp. in low to moderate concentration. In Zone III, *C. lindemuthianum* dominated other fungi as it was detected from 18 samples having moderate to heavy spore population. *Fusarium* and *Rhizoctonia* were recorded from 13 and 11 samples, respectively. Population of other fungi was low. *Isariopsis, Rhizoctonia, Sclerotinia* and *Fusarium* spp. were the main seed borne fungi in Zone IV with low to heavy incidence. However, in wet temperate area of the state, *Alternaria* and *Isariopsis* were observed in maximum number of samples.

4.1.4 Seed health parameters of kidney bean seed samples

Seed health parameters viz. germination percentage, seed moisture, seed vigour and seedling vigour index of kidney bean samples were analysed by using Rolled Paper Towel Method and Tetrazolium tests (as described in section 3.1) and data obtained are presented in Tables 4,5 and 6.

Perusal of data revealed that moisture content and seed vigour of all the seed samples collected after harvest ranged between 9.2-15.9 per cent and 1.860-4.500 whereas, it was 8.4 to 13.6 per cent and 2.356 to 4.500, respectively in stored samples of three agro-climatic zones of Himachal Pradesh. The germination percentage in unsterilized and sterilized seeds

Table 3. Seed mycoflora through washing test

Agro-climatic zone / Sample number	Mycoflora						
	Alternaria sp.	*Cercospora* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*Isariopsis griseola*	*Rhizoctonia solani* #	*Sclerotinia sclerotiorum* #
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Zone II							
KB 1	++	-	-	+	+	-	+
KB 2	-	-	-	-	-	-	-
KB 3	-	-	-	++	-	-	-
KB 4	-	-	++	-	-	+	-
KB 5	-	-	-	++	++	-	-
KB 6	+++	-	++	-	++	-	+
KB 7	++	-	-	-	++	+	-
KB 8	-	+	-	-	-	+	-
KB 9	-	-	-	-	-	-	-
Zone III							
KB 10*	-	-	-	++	-	-	-
KB 12*	-	-	-	+	-	-	-
KB 14	-	-	-	+++	-	-	++
KB 15*	-	-	-	-	-	-	-
KB 16*	-	-	-	-	-	++	-
KB 17*	-	-	++	-	-	+++	-
KB 18*	-	-	+++	-	-	+	-
KB 19*	-	-	-	-	-	-	+
KB 20*	-	-	++	+	-	-	-
KB 33*	-	-	-	-	+	++	-
KB 53*	+++	-	++	++	-	+	-
KB 11	-	-	-	-	-	-	-
KB 13	++	-	+	-	-	-	-
KB 21	-	-	+++	-	-	++	-
KB 22	-	-	-	++	-	-	-
KB 23	-	-	-	-	-	-	-

Continued

1	2	3	4	5	6	7	8
KB 24	-	-	++	-	-	-	+
KB 25	-	-	+++	++	+	-	-
KB 26	-	-	+	+	-	-	-
KB 27	-	-	++	+	-	-	-
KB 28	-	-	+	-	++	-	-
KB 29	-	-	-	-	-	-	++
KB 30	-	-	-	+++	-	-	-
KB 31	-	-	-	+	-	-	-
KB 32	-	-	++	-	-	++	-
KB 34	++	++	-	-	+++	-	-
KB 35	-	-	-	-	-	-	-
KB 36	-	-	-	++	-	-	-
KB 37	-	-	-	-	-	-	+
KB 38	-	-	+	-	-	+	-
KB 39	-	-	-	+	-	-	-
KB 40	-	-	-	-	-	+	-
KB 41	-	-	-	-	-	-	+
KB 42	-	-	++	-	-	++	-
KB 43	-	-	-	++	-	+	-
KB 44	+++	-	++	-	+	+	-
KB 45	-	-	-	-	-	-	-
KB 46	-	-	-	-	-	-	-
KB 47	-	-	+	-	-	-	-
KB 48	-	-	+	-	-	-	+
KB 49	-	-	+	-	-	-	-
KB 50	-	-	-	-	-	-	-
KB 51	-	-	-	++	-	-	-
KB 52	-	-	++	-	-	-	-
KB 54	-	-	-	++	-	+	-
Zone IV							
KB 55*	-	-	++	-	++	-	-
KB 56*	-	-	-	-	-	-	-
KB 57*	-	-	-	++	-	++	-
KB 58*	-	-	-	-	-	-	+
KB 59*	-	-	-	-	-	-	-
KB 62*	-	-	-	-	-	-	++

Continued

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
KB 65*	-	-	-	++	-	-	++
KB 66*	-	-	-	-	-	++	-
KB 60	-	-	-	++	-	-	-
KB 61	-	-	-	+	-	+	-
KB 63	-	-	-	-	-	-	-
KB 64	-	-	-	++	-	+	-
KB 67	-	-	-	-	-	++	-
KB 68	-	-	-	-	++	-	-
KB 69	-	-	++	-	-	-	-
KB 70	++	+	-	-	+++	-	-
KB 71	++	++	++	-	++	++	-
KB 72	+	+++	+++	-	+	-	++
KB 73	++	-	-	-	-	-	++

- Not observed
+ Low concentration (0.1 – 0.2x10^6 spores/ml)
++ Moderate concentration (0.5x10^6 spores/ml)
+++ High concentration (1.2x10^6 spores/ml)
# Mycelium observed

collected after harvest and storage was maximum in KB 16, 59 and KB 68, 69 and 27, respectively. In unsterilized seed samples collected after storage and harvest, seedling vigour index on fresh weight basis was maximum in KB 18 and minimum in KB 62. However, on dry weight basis it was highest in KB 58 and lowest in KB 62. Similarly, after surface sterilization these seed lots exhibited seedling vigour index of 9.75 to 52.86 in stored and 5.05 to 105.30 in freshly harvested samples.

Data on seed germination, seed moisture content , vigour and seedling vigour of Zone II presented in Table 4 showed that seed moisture ranged from 8.7 to 9.8 per cent, maximum being in KB 1 and minimum in KB 3 whereas, in remaining samples it was more or less similar. Seed vigour of 9 samples varied between 2.854 (KB 1) to 4.180 (KB 3). Similarly, the values of germination percentage and seedling vigour index on fresh and dry weight basis in sterilized seeds were highest in KB 3. However, in unsterilized seed samples, seedling vigour index on fresh weight basis was highest in KB 6. Statistical analysis of various health parameters revealed that KB 3 had superior values over other samples.

Moisture content of 10 samples collected after harvest in Zone III ranged between 9.2 to 15.3 per cent with maximum contents in KB 18 and minimum in KB 10 and 20 (Table 5). Three samples viz. KB 10, 19 and 20 had almost similar moisture levels whereas, all other samples possessed significantly higher values. Seed vigour in these samples ranged between 2.210 (KB 12) to 4.500 (KB 19). Statistically two samples (KB 18 and 19) were at par, though significantly superior over all other samples. Seed germination in unsterilized and sterilized seeds was minimum in KB 12 and maximum in KB 16.

Seedling vigour index of unsterilized seed collected after harvest on fresh weight basis varied between 534.73 to 3528.14 and 349.95 to 2604.28 in sterilized seeds. However, maximum vigour index on dry weight basis was recorded in KB 15 and KB 20 of unsterilized and sterilized seed lots, respectively, whereas, it was minimum in KB 19 in both the cases.

Table 4. Seed health status of samples collected from Zone II

Sample number	Seed moisture (%)	Seed vigour	Germination (%)		Seedling vigour index			
					Fresh weight basis		Dry weight basis	
			Unsterilised	Sterilised	Unsterilised	Sterilised	Unsterilised	Sterilised
KB 1	9.8	2.854	86.67 (68.70)	93.33 (76.09)	1106.83	1320.17	34.99	37.95
KB 2	9.0	3.580	86.67 (68.70)	96.66 (81.06)	1182.23	974.09	40.45	37.59
KB 3	8.7	4.180	98.65 (85.46)	95.32 (79.65)	1295.70	2278.56	38.22	43.36
KB 4	9.5	3.880	91.33 (73.54)	92.00 (73.83)	1473.26	1368.20	40.40	38.35
KB 5	9.2	3.126	88.00 (69.98)	93.00 (75.33)	1408.79	1459.99	35.15	38.28
KB 6	9.0	3.569	90.67 (72.44)	94.00 (76.66)	1485.10	1548.79	34.74	38.72
KB 7	9.3	3.269	90.67 (72.44)	93.33 (75.78)	1360.89	1265.07	30.48	31.37
KB 8	9.1	3.856	85.33 (67.75)	88.00 (70.41)	1482.99	1175.17	34.62	27.32
KB 9	9.3	3.861	85.33 (67.65)	92.00 (74.07)	609.07	1440.47	14.48	19.49
CD (0.05)	0.34	0.152	(7.996)	(NS)	263.97	276.75	6.53	6.53

Figures in parantheses are the angular transformed values

Table 5. Seed health status of samples collected from Zone III

Sample number	Seed moisture (%)	Seed vigour	Germination (%)		Seedling vigour index			
					Fresh weight basis		Dry weight basis	
			Unsterilised	Sterilised	Unsterilised	Sterilised	Unsterilised	Sterilised
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Samples collected after harvest								
KB 10	9.2	3.875	94.33 (77.45)	92.00 (74.07)	1896.01	2204.07	38.10	41.11
KB 12	11.8	2.210	37.00 (37.42)	35.33 (36.41)	769.70	349.95	17.79	17.84
KB 15	10.0	3.859	97.31 (83.82)	99.31 (86.59)	2583.67	2297.84	50.09	49.23
KB 16	11.5	3.762	98.65 (85.45)	99.31 (86.59)	2958.88	2267.05	37.52	46.25
KB 17	11.9	3.859	95.99 (80.78)	93.00 (75.29)	2359.02	1522.12	28.11	27.54
KB 18	15.3	4.488	97.97 (84.57)	97.97 (84.57)	3528.14	2604.28	31.94	44.29
KB 19	9.4	4.500	70.00 (56.82)	80.00 (63.53)	534.73	1273.03	13.91	27.56
KB 20	9.2	2.933	72.00 (58.09)	85.00 (67.38)	2005.03	2407.23	38.96	53.91
KB 33	13.9	4.011	78.67 (62.61)	75.33 (60.22)	2406.41	1206.55	37.58	39.82
KB 53	10.3	3.875	86.00 (68.22)	93.67 (76.70)	2278.13	2308.13	39.77	49.36
Samples collected after storage								
KB 11	12.2	3.001	92.33 (74.59)	94.66 (79.03)	1709.08	1912.20	34.57	48.32
KB 13	10.1	3.234	86.00 (68.22)	90.00 (71.91)	1935.85	1853.83	43.38	46.25
KB 14	11.1	2.893	77.67 (61.86)	80.00 (63.53)	978.68	1088.23	25.47	31.99
KB 21	9.2	3.779	94.00 (76.66)	94.00 (76.66)	2495.37	1826.09	41.02	44.73
KB 22	11.4	3.762	90.00 (71.91)	92.00 (74.07)	2589.13	2330.11	33.64	42.45
KB 23	10.3	3.508	86.00 (68.21)	94.00 (76.66)	2436.37	2301.73	24.59	43.46
KB 24	11.3	3.700	94.00 (76.66)	90.00 (71.91)	1011.11	978.13	43.98	37.31
KB 25	13.1	3.880	41.00 (39.78)	40.00 (39.19)	819.74	829.83	17.96	20.02
KB 26	12.1	4.000	91.00 (72.95)	95.00 (78.27)	1476.72	1824.58	27.02	51.34
KB 27	12.0	2.630	97.31 (83.83)	97.99 (83.08)	995.52	1713.11	31.40	50.78
KB 28	9.6	3.880	95.99 (80.78)	95.99 (80.78)	1931.86	2036.49	40.54	39.56

Continued

1	2	3	4	5	6	7	8	9
KB 29	11.2	3.742	93.00 (75.29)	95.99 (80.78)	2289.37	2193.92	39.33	48.57
KB 30	8.4	3.742	95.32 (79.85)	95.99 (80.78)	2679.40	2952.24	36.40	43.83
KB 31	12.3	3.596	76.00 (60.73)	81.00 (64.26)	1577.39	1755.46	23.45	35.68
KB 32	13.6	4.480	61.67 (51.75)	82.00 (65.01)	1518.95	1440.89	34.45	45.84
KB 34	11.4	4.180	86.00 (68.22)	86.00 (68.22)	1074.13	1036.29	37.23	41.70
KB 35	9.4	3.267	80.33 (63.79)	84.00 (66.57)	1097.82	1230.67	21.21	23.52
KB 36	12.2	3.856	81.67 (64.74)	86.00 (68.22)	1233.90	1337.29	24.86	30.70
KB 37	11.9	3.273	84.00 (66.57)	90.00 (71.91)	1818.67	2730.43	19.44	39.86
KB 38	9.8	3.732	90.33 (72.33)	95.99 (80.78)	736.29	1795.56	24.75	37.08
KB 39	11.3	3.742	90.00 (71.91)	88.00 (69.98)	2343.43	2397.03	49.63	52.86
KB 40	11.4	2.466	90.00 (71.91)	95.99 (80.78)	2079.73	2316.78	40.23	46.61
KB 41	11.8	3.543	94.00 (76.66)	97.98 (84.58)	1399.33	1527.74	33.22	44.10
KB 42	10.2	3.189	94.00 (76.66)	97.31 (83.83)	1521.53	1647.52	37.21	40.31
KB 43	10.5	2.357	94.00 (76.66)	94.00 (76.66)	2268.83	2851.63	25.95	35.25
KB 44	10.3	4.500	85.00 (67.38)	81.00 (64.26)	1822.43	820.72	18.02	35.44
KB 45	9.5	3.159	94.00 (76.66)	97.31 (83.83)	2290.45	2268.38	47.27	50.09
KB 46	11.2	3.839	70.00 (56.82)	76.00 (60.73)	2040.43	1717.99	17.23	31.09
KB 47	11.5	3.700	74.00 (59.39)	88.00 (69.98)	1057.93	901.03	34.30	39.89
KB 48	12.5	3.580	95.00 (78.27)	95.00 (78.27)	1619.38	1811.28	48.67	43.95
KB 49	12.4	4.000	84.67 (67.08)	86.67 (68.64)	1774.41	1837.33	27.19	31.84
KB 50	10.3	3.580	66.00 (54.35)	71.00 (57.45)	882.55	772.36	21.28	29.46
KB 51	10.4	2.923	86.00 (68.22)	92.00 (74.07)	1068.11	1207.71	21.62	31.18
KB 52	10.5	2.356	86.00 (68.22)	85.33 (67.75)	1093.05	1238.09	41.66	43.28
KB 54	10.6	2.561	74.00 (59.39)	78.00 (62.10)	1398.33	1334.11	25.39	32.63
CD (0.05)	0.33	0.144	(8.886)	(9.554)	305.326	295.246	6.330	6.837

Figures in parantheses are the angular transformed values

Significant differences were observed in seed vigour of both unsterilized and sterilized seed lots except KB 12 and KB 19 which were at par with each other.

In 35 stored seed samples procured from Zone III, the moisture content was in the range of 8.4 to 13.6 per cent. All the samples showed significant differences in moisture content, where KB 32 had significantly higher value over all other samples of this zone. Seed vigour in stored seeds varied from 2.356 to 4.500. Three seed samples viz. KB 52, 43 and 40 having lowest seed vigour were statistically at par and differed significantly from all other samples. Seed germination (Zone III) also varied significantly, where KB 27 showed statistically superior values in both unsterilized and sterilized seed lots (Table 5).

Highest seedling vigour index on fresh weight basis in unsterilized and sterilized seed lots was found in KB 30 (2679.40 and 2952.24) and lowest in KB 38 and 50 (736.29 and 772.36). Whereas, on dry weight basis it was maximum in KB 39 (49.63 and 52.86) and minimum in KB 25 (17.96 and 20.02) in unsterilized and sterilized seeds, respectively. Majority of the stored samples showed significantly higher seedling vigour over KB 38 which was at par with seven others.

Eight samples collected after harvest from Zone IV (Table 6) possessed moisture contents between 9.3 to 15.9 per cent and seed vigour ranging from 1.860-4.500. However, among stored seed lots these values were between 10.1-13.4 per cent and 3.461-4.500, respectively. Germination of unsterilized and sterilized seeds procured after harvest was maximum in KB 59 and 66 (95.99%) and minimum in KB 62 (40.00 and 44.00) whereas, these values in stored seeds varied between 44.00 to 99.97 per cent in unsterilized and 46.00 to 97.31 per cent in sterilized lots. Similarly seedling vigour on both fresh and dry weight basis in freshly harvested unsterilized seeds ranged between 472.77 – 2218.00 and 4.34 – 83.60, and in sterilized lots it was between 417.87 - 2207.73 and 5.05 – 105.30, respectively. Among stored seeds highest seedling vigour was

Table 6. Seed health status of samples collected from Zone IV

Sample number	Seed moisture (%)	Seed vigour	Germination (%)		Seedling vigour index			
					Fresh weight basis		Dry weight basis	
			Unsterilised	Sterilised	Unsterilised	Sterilised	Unsterilised	Sterilised
Samples collected after harvest								
KB 55	9.3	1.932	86.33 (68.52)	94.00 (76.66)	2218.00	2110.91	17.09	30.68
KB 56	10.4	4.488	72.00 (58.09)	76.00 (60.69)	1017.91	1958.71	27.91	28.44
KB 57	15.3	4.500	66.00 (54.35)	60.00 (50.76)	953.83	820.87	9.90	52.89
KB 58	11.4	4.187	80.00 (63.53)	78.00 (62.10)	865.83	1143.79	83.60	105.30
KB 59	15.9	4.187	95.99 (80.78)	94.00 (76.66)	2179.52	2207.73	32.25	30.40
KB 62	10.5	1.860	40.00 (39.19)	44.00 (41.53)	472.77	417.87	4.34	5.05
KB 65	11.1	4.187	52.00 (46.13)	62.00 (51.94)	845.31	760.73	15.64	20.81
KB 66	10.9	4.500	94.00 (76.66)	95.99 (80.78)	2122.19	1941.46	27.67	22.71
Samples collected after storage								
KB 60	10.4	3.839	90.00 (71.91)	88.00 (69.98)	2169.73	1810.07	13.40	14.82
KB 61	11.6	3.596	90.00 (71.91)	94.00 (76.66)	2075.23	2448.37	13.31	12.54
KB 63	11.1	3.742	97.31 (83.83)	95.99 (80.78)	1706.88	1821.48	24.84	28.25
KB 64	11.7	3.742	97.31 (83.83)	94.00 (76.38)	3061.48	2109.95	18.75	18.44
KB 67	10.7	4.500	72.00 (58.09)	74.00 (59.35)	1276.39	646.60	15.81	9.75
KB 68	10.1	3.742	99.97 (88.97)	97.31 (83.83)	2311.31	2221.67	26.69	31.79
KB 69	12.2	3.762	99.97 (88.97)	96.99 (81.81)	3097.07	3054.60	49.37	51.22
KB 70	11.1	4.888	97.31 (83.83)	95.99 (80.78)	2609.95	2491.09	29.32	27.01
KB 71	13.4	3.984	95.99 (80.78)	95.99 (80.78)	2871.61	2457.89	28.93	28.91
KB 72	11.9	3.461	86.00 (68.22)	90.00 (71.90)	2685.77	2656.63	18.89	34.42
KB 73	12.3	3.984	44.00 (41.52)	46.00 (42.68)	1138.19	1402.29	14.34	18.30
CD (0.05)	0.33	0.147	(9.22)	(9.69)	303.33	307.84	5.56	6.40

Figures in parantheses are the angular transformed values

recorded in KB 69 and minimum in KB 73 in both unsterilized and sterilized seed lots.

Statistically almost all the values for moisture content and seedling vigour varied significantly except two values of seed vigour (KB 55 and 62) and germination in unsterilized seeds (KB 62 and 65) which were at par with each other. In general, it was concluded from the data that sterilized seed lots exhibited higher values for various growth parameters in majority of the seed samples representing various agro-ecological zones of Himachal Pradesh.

4.1.5 Detection of seed mycoflora from bulk sample using Rolled Paper Towel Method

Rolled Paper Towel Method was employed to detect the mycoflora associated with kidney bean seeds collected from various bean growing areas of Himachal Pradesh by using 100 seeds each of unsterilized and surface sterilized ($NaOCl_2$) lots. The frequency (%) of fungi recorded on various samples is presented in Tables 7 and 8.

In unsterilized samples, 25 species of fungi belonging to 14 genera were found to be associated with seeds of different varieties being grown under varied agro-climatic zones. The mycoflora constituted both pathogenic as well as saprophytic fungi. Different fungi recorded were *Acremonium* sp., *Alternaria* sp., *Aspergillus* spp., *Botrytis* sp., *Cladosporium* sp., *Colletotrichum lindemuthianum, Fusarium* spp., *Gliocladium* sp., *Myrothecium* sp., *Penicillium* spp., *Phoma* sp., *Rhizoctonia solani, Rhizopus stolonifer, Sclerotium rolfsii* and *Trichothecium* sp. *Penicillium* species dominated all other storage fungi as these were detected from 25 samples whereas, *Colletotrichum lindemuthianum* (17) and *Fusarium oxysporum* (14) were the predominant field/ pathogenic fungi.

In Zone II, out of 9 seed samples, KB 3 possessed maximum mycoflora whereas, KB 2 had least number of fungi. *Aspergillus* and *Penicillium* species were found to colonize in majority of the samples,

Table 7. Per cent mycoflora of bulk sample (unsterilised seeds) through Rolled Paper Towel Method

Sample number	*Acremonium* sp.	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochracious*	*Botrytis* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Gliocladium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P.capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma* sp.	*Rhizopus stolonifer*	*Scleroltium rolfsii*	*Trichothecium* sp.	Unidentified	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
one II: *Samples collected after storage*																										
(B 1	-	8	3	4	-	2	-	-	2	-	-	-	-	-	2	-	-	-	6	-	-	2	-	-	2	9
(B 2	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
(B 3	8	-	-	-	-	-	-	11	16	3	1	1	2	-	-	10	6	-	5	-	-	-	-	-	-	10
(B 4	-	-	-	-	-	-	-	-	-	1	-	2	1	-	-	-	4	3	2	1	-	6	-	-	-	8
(B 5	-	6	-	-	2	-	-	-	4	-	-	-	-	-	-	-	2	-	2	-	-	2	-	-	-	6
(B 6	2	2	-	4	-	-	1	-	-	-	-	-	-	2	2	4	-	-	-	-	-	-	2	-	-	8
(B 7	-	-	2	-	-	-	-	-	2	-	-	-	4	-	-	-	-	-	6	-	-	-	1	-	6	6
(B 8	-	2	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
(B 9	2	-	-	2	-	-	-	-	-	-	-	-	-	-	2	4	-	6	-	-	-	2	-	2	-	7
ample ean	33.3	44.4	22.2	33.3	11.1	22.2	11.1	11.1	55.6	33.3	11.1	22.2	33.3	11.1	33.3	33.3	33.3	22.2	55.6	11.1	0.0	44.4	22.2	11.1	22.2	
one III: *Samples collected after harvest*																										
B 10	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	3
B 12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	12	-	-	-	12	-	-	2	4
B 15	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2
B 16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1
B 17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	4	-	-	-	3
B 18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
B 19	-	-	-	-	-	-	-	-	-	-	-	18	-	-	-	2	-	-	-	-	-	-	-	2	-	3
B 20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-	18	-	-	-	3
B 33	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2
B 53	4	-	-	2	-	-	-	-	1	-	-	-	-	1	2	-	-	2	-	2	-	2	-	4	-	9
ample ean	10.0	10.0	0.0	10.0	0.0	10.0	10.0	0.0	20.0	0.0	0.0	20.0	0.0	10.0	10.0	10.0	10.0	40.0	30.0	10.0	0.0	60.0	0.0	20.0	10.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Samples collected after storage																										
KB 11	-	5	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	4	-	1	-	4	-	2	6
KB 13	-	-	-	2	-	-	-	2	-	2	-	-	-	-	5	-	-	-	-	-	-	4	-	2	-	6
KB 14	3	4	-	-	-	-	-	-	-	2	2	4	-	-	-	-	-	-	-	-	-	-	-	2	2	7
KB 21	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	6	-	-	-	2
KB 22	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	2
KB 23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	1
KB 24	-	-	7	1	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	3
KB 25	1	5	-	-	-	-	-	-	-	-	-	10	-	-	-	2	-	-	5	5	-	3	-	-	-	7
KB 26	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	1	-	-	-	3
KB 27	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	2
KB 28	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	6	-	-	-	2
KB 29	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-	9	-	-	-	7
KB 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	1
KB 31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	8	-	-	-	7	-	-	-	4
KB 32	-	-	-	-	-	-	-	10	1	-	1	-	8	-	-	-	-	5	-	-	-	-	-	-	6	6
KB 34	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	3	4	-	-	-	1	-	-	-	4
KB 35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 36	4	8	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 37	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2
KB 38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 39	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2
KB 40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 41	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-	3
KB 42	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	4
KB 43	-	-	2	10	1	2	-	-	5	8	2	4	6	-	-	15	10	10	-	3	-	33	-	-	-	14
KB 44	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2
KB 45	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-	8	-	-	5	-	-	20	-	-	-	5
KB 46	50	-	52	-	-	-	-	-	-	-	-	-	-	-	-	-	56	-	-	-	-	-	-	-	-	3
KB 47	-	-	2	-	-	-	-	-	-	-	-	26	-	-	-	12	-	12	-	4	-	-	-	-	-	5
KB 48	-	4	5	-	-	-	-	-	-	-	-	3	-	-	-	-	6	-	-	-	-	-	-	-	-	4
KB 49	-	-	5	2	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	-	3
KB 50	-	2	-	-	-	4	-	-	-	-	-	-	-	-	-	2	2	-	2	-	-	-	2	-	-	6

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
KB 51	-	10	-	-	4	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	3
KB 52	-	5	3	2	-	-	-	-	1	2	-	-	4	-	-	-	8	5	-	-	-	-	-	-	1	9
KB 54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	4	-	-	-	8	-	-	-	3
Sample mean	11.4	22.9	28.6	20.0	8.6	8.6	0.0	8.6	14.3	14.3	11.4	22.9	14.3	0.0	5.7	22.9	40.0	25.7	22.9	14.3	2.9	37.1	5.7	5.7	14.3	
one IV: *Samples collected after harvest*																										
B 55	-	-	2	2	2	-	-	4	1	-	-	1	-	-	-	1	4	6	4	2	-	-	-	12	-	12
B 56	-	-	-	7	-	-	-	-	-	-	-	7	-	-	-	-	17	-	-	-	-	-	-	2	1	5
B 57	-	-	12	-	-	-	-	-	-	-	3	-	-	-	-	-	10	10	2	-	-	-	-	-	-	5
B 58	-	-	-	2	3	1	-	-	1	-	-	-	-	-	-	-	1	3	-	-	-	-	-	5	-	7
B 59	-	-	-	-	3	2	-	-	-	-	-	-	-	-	-	-	11	10	-	-	-	6	-	1	-	6
B 62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	1
B 65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	1	2
B 66	-	10	-	-	2	-	-	-	4	-	-	-	-	-	-	-	-	4	-	2	-	-	-	-	-	5
ample ean	0.0	12.5	25.0	37.5	50.0	25.0	0.0	12.5	37.5	0.0	12.5	25.0	0.0	0.0	0.0	12.5	62.5	62.5	25.0	25.0	0.0	25.0	0.0	62.5	25.0	
imples collected after storage																										
3 60	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	2
3 61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	1	-	-	-	-	-	5
3 63	-	-	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	6	-	-	-	5
3 64	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	2	4	-	-	-	-	-	-	-	-	3
3 67	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	2
3 68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	4	-	-	-	3
3 69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
3 70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	1
3 71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
3 72	-	2	-	-	-	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	8	1	-	-	5
3 73	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	1	3
mple ean	0.0	9.1	18.2	0.0	9.1	18.2	9.1	0.0	18.2	0.0	0.0	0.0	0.0	0.0	0.0	18.2	27.3	18.2	18.2	18.2	0.0	54.5	9.1	9.1	9.1	

however, *C. lindemuthiaum* was recorded on 55.6 per cent of the total samples tested with KB 3 showing heavy infection.

Ten seed samples procured immediately after harvest from Zone III having sub-temperate type of climate showed the association of 17 fungal species (Table 7). KB 18 was found free of any infestation whereas, KB 53 was colonized by maximum fungi, though their incidence ranged between 1-4 per cent. All other samples were colonized by 2 or 3 species individually. Out of 35 samples (Zone III) analysed after storage, KB 43 harboured 14 fungi and three sample viz. KB 35, 38 and 40 were free. The incidence of fungal flora was between 2.9-40 per cent with occurrence of *A. flavus* and *R. stolonifer* on 28.6 and 37.1 per cent samples, respectively.

The Zone IV samples, collected after harvest, showed the association of 16 species of both storage and field fungi with maximum infestation in KB 55. However, the stored seed samples of Zone IV showed the presence of 14 species and maximum being of storage fungi (Table 7). Highest number of fungi recorded on different samples was five. *R. stolonifer* was detected from 6 samples with an incidence of 2-10 per cent whereas, incidence of other fungi was between 0 - 5 per cent.

Seventy three seed samples, when analysed after surface sterilization, showed the association of only 13 fungal species (Table 8) as compared to 25 detected from unsterilized seed lots. Field fungi like *C. lindemuthianum, Fusarium* and *Rhizoctonia* were isolated from majority of the samples whereas, *Aspergillus* and *Penicillium* spp. dominated the storage fungi. Frequency of storage fungi was less as compared to field/pathogenic fungi in sterilized lots. Surface sterilized seed samples of Zone II revealed the occurrence of 6 species on KB 9 out of 12 species recorded on different samples. Whereas, among pathogenic seed borne fungi, *C. lindemuthianum* was detected from one sample (KB 8) only.

In Zone III, seed samples collected after harvest possessed 7 species of fungi on different lots (Table 8). However, seed samples analysed after storage were found to harbour 13 species. *Rhizopus* was detected from 13

Table 8. Per cent mycoflora of bulk seed sample (sterilized seeds)* through Rolled Paper Towel Method

Sample number	*Acremonium* sp.	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*F. solani*	*Penicillium* spp.	*Phoma* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Sclerotium rolfsii*	*Unidentified*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Zone II: *Samples collected after storage*															
KB 1	-	2	-	-	-	-	-	-	-	-	-	-	8	11	3
KB 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 3	-	-	-	-	-	-	-	-	2	-	-	3	-	-	2
KB 4	-	-	-	-	-	-	-	1	-	-	-	2	-	-	2
KB 5	1	-	-	-	-	-	-	-	-	2	-	1	-	-	3
KB 6	-	4	-	-	-	-	-	-	-	-	-	-	4	-	2
KB 7	-	-	4	-	-	-	2	-	-	-	1	-	-	-	3
KB 8	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1
KB 9	3	8	-	4	-	-	-	2	-	-	2	-	3	-	6
%Sample mean	22.2	33.3	11.1	11.1	0.0	11.1	11.1	22.2	11.1	11.1	22.2	33.3	33.3	11.1	
Zone III: *Samples collected after harvest*															
KB 10	-	-	-	-	-	-	-	-	-	-	-	14	-	-	1
KB 12	-	-	-	-	-	1	-	-	13	-	-	18	-	-	3
KB 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 16	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
KB 17	-	-	-	-	-	-	-	-	-	-	1	2	-	-	2
KB 18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 19	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1
KB 20	-	-	-	-	-	-	-	-	-	-	-	4	-	-	1
KB 33	-	-	2	-	-	-	-	-	-	-	-	2	-	-	2
KB 53	-	-	-	-	-	4	1	-	-	-	-	-	3	-	3
%Sample mean	0.0	0.0	10.0	0.0	0.0	20.0	20.0	0.0	10.0	0.0	20.0	50.0	10.0	0.0	
Samples collected after storage															
KB 11	-	-	-	4	-	-	2	-	-	-	-	-	-	-	2
KB 13	-	2	-	-	-	-	-	2	-	-	-	-	-	-	2
KB 14	1	-	-	-	-	-	-	-	-	-	4	-	-	-	2
KB 21	-	1	-	-	-	-	2	1	-	-	1	6	-	-	5
KB 22	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
KB 23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 24	-	-	4	-	1	1	-	-	-	-	-	2	-	-	4
KB 25	1	7	-	-	-	-	10	-	-	-	-	3	-	-	4
KB 26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 27	-	-	-	-	2	-	-	-	-	-	-	1	-	1	3
KB 28	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1
KB 29	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1
KB 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 31	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1
KB 32	-	-	-	-	-	-	-	8	-	-	-	-	-	-	1
KB 34	-	-	-	-	-	-	5	-	-	-	-	1	-	-	2
KB 35	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1
KB 36	3	-	-	-	-	-	-	2	-	-	-	-	-	-	2
KB 37	-	-	-	4	-	1	-	1	-	-	4	-	-	-	4

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
KB 38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 39	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1
KB 40	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
KB 41	-	1	-	-	-	1	-	-	-	-	-	-	-	-	2
KB 42	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
KB 43	-	-	-	5	-	2	-	8	1	-	-	19	-	-	5
KB 44	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1
KB 45	-	-	-	-	-	-	-	1	-	-	-	20	-	-	2
KB 46	50	-	1	-	-	4	8	-	-	-	-	-	-	-	4
KB 47	-	-	-	-	-	-	2	-	3	-	-	-	-	-	2
KB 48	-	4	-	-	-	-	4	-	-	-	-	-	-	-	2
KB 49	-	-	4	-	-	-	-	-	-	-	-	-	-	-	1
KB 50	2	-	2	-	-	-	-	-	-	-	-	-	3	-	3
KB 51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 52	-	4	-	1	1	2	1	-	1	-	-	-	-	-	6
KB 54	-	-	-	-	-	-	-	-	3	-	-	5	-	-	2
%Sample mean	14.3	17.1	11.4	14.3	8.6	20.0	22.9	22.9	11.4	0.0	8.6	37.1	2.9	5.7	
Zone IV: *Samples collected after harvest*															
KB 55	-	-	-	-	1	1	1	-	-	-	-	5	-	-	4
KB 56	-	-	-	-	-	-	1	-	-	-	-	-	-	3	2
KB 57	-	-	2	-	-	-	5	-	-	-	-	-	-	-	2
KB 58	-	-	4	5	-	1	2	-	3	-	-	8	-	-	6
KB 59	-	-	-	1	-	-	-	-	-	-	-	5	-	-	2
KB 62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 65	-	-	-	-	-	-	-	-	-	-	-	8	-	1	2
KB 66	-	1	-	-	-	-	3	-	-	-	2	-	-	-	3
%Sample mean	0.0	12.5	25.0	25.0	12.5	25.0	62.5	0.0	12.5	0.0	12.5	50.0	0.0	25.0	
Samples collected after storage															
KB 60	-	-	-	-	-	-	-	-	-	-	-	5	-	-	1
KB 61	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
KB 63	-	-	1	-	-	-	-	-	-	-	-	3	-	-	2
KB 64	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1
KB 67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 68	-	-	-	-	-	-	-	-	-	-	-	4	-	-	1
KB 69	-	-	-	-	-	-	1	-	-	-	-	1	-	-	2
KB 70	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1
KB 71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 72	-	1	-	-	-	-	-	-	-	-	-	8	1	-	3
KB 73	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1
%Sample mean	0.0	9.1	9.1	9.1	0.0	0.0	18.2	0.0	0.0	0.0	0.0	63.6	9.1	0.0	

* Sterilized with sodium hypochlorite

samples showing 1-20 per cent incidence. Among surface sterilized seed lots of Zone IV analysed immediately after harvest, KB 58 showed the presence of 6 fungal species whereas most of other samples were colonized by only 2-3 species with the dominance of *Fusarium oxysporum*. In stored seed lots of this zone, 2 samples were absolutely free and others were colonized by 1-2 species. Highest being on KB 72 viz. *Alternaria, Rhizopus* and *Sclerotium. Rhizopus* was observed on 63.6 per cent of the samples with an incidence of 1 to 8 per cent. Almost similar pattern of mycoflora was observed in seed samples of Zone III and IV tested after surface sterilization, least being in samples collected from Zone IV.

4.1.6 Detection of seed mycoflora from apparently healthy seeds

Blotter method

The mycoflora of apparently healthy kidney bean seed samples collected from various bean growing areas of Himachal Pradesh before and after surface sterilization detected by blotter method is presented in Tables 9 and10. Unsterilized samples harboured 17 species of storage/field fungi belonging to 12 genera with a range of 1-10 species in different seed lots (Table 9). However, on sterilized seeds only 10 species were observed (Table 10).

Seed samples of Zone II showed the presence of 10 species of fungi on KB 5, however, only *Penicillium capsulatum* was recorded on KB 4. Out of 9 samples procured from this Zone, *P. capsulatum* was recorded on 66.7 per cent of the samples followed by *A. flavus* colonizing 44.4 per cent samples and *Acremonium*, *Aspergillus fumigatus, A. niger* and *Fusarium* species colonizing 33.3 per cent samples with an incidence of 7-42 per cent (Table 9).

In seed lots of Zone III, collected after harvest, a maximum of 5 species were observed on KB 15 and minimum on KB 19 (2) whereas, KB 33 was absolutely free. *Rhizopus* was the dominating fungus with its presence on 90 per cent samples with an incidence of 7 to 36 per cent followed by *Aspergillus flavus*. Among samples collected after storage from

Table 9. Frequency (%) of seed mycoflora in apparently healthy seeds (unsterilised) through blotter method

Sample number	*Acremonium* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Cephalosporium* sp.	*Fusarium* spp.	*Fusarium moniliformae*	*Gliocladium* sp.	*Monosporium* sp.	*Penicillium* spp.	*P.capsulatum*	*P. simplicissimum*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Trichoderma* sp.	*Trichothecium* sp.	*Varicosporium* sp.	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Zone II: *Samples collected after storage*																		
KB 1	14	7	14	7	-	21	-	-	-	14	7	-	-	-	-	-	-	7
KB 2	29	14	-	-	29	-	-	-	-	-	-	-	71	-	-	-	-	4
KB 3	-	-	-	-	-	-	-	-	-	-	21	-	29	-	-	-	-	2
KB 4	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	1
KB 5	7	9	-	-	-	21	21	21	-	-	14	21	-	14	7	7	-	10
KB 6	-	-	-	-	-	-	-	-	14	14	42	-	-	-	-	-	-	3
KB 7	-	-	14	14	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 8	-	-	-	14	-	-	-	-	-	-	36	-	-	-	-	-	-	2
KB 9	-	14	14	-	-	14	-	-	7	-	-	-	-	7	-	21	-	6
%Sample mean	33.3	44.4	33.3	33.3	11.1	33.3	11.1	11.1	22.2	22.2	66.7	11.1	22.2	22.2	11.1	22.2	0	
Zone III: *Samples collected after harvest*																		
KB 10	-	-	-	-	-	-	14	-	-	-	-	-	14	-	-	42	-	3
KB 12	-	14	14	7	-	-	-	-	-	-	-	-	29	-	-	-	-	4
KB 15	-	29	7	-	-	-	-	-	-	7	-	-	36	-	21	-	-	5
KB 16	-	-	-	-	-	-	-	-	-	-	21	21	21	-	-	-	-	3
KB 17	-	-	-	-	14	-	-	-	-	86	-	-	21	-	-	-	7	4
KB 18	-	29	-	-	-	7	-	-	-	-	36	-	29	-	-	-	-	4
KB 19	-	21	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	2
KB 20	-	-	14	14	-	-	-	-	-	-	21	-	7	-	-	-	-	4
KB 33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 53	-	7	-	-	-	-	-	-	-	-	-	-	7	-	-	7	-	3
%Sample mean	0	50	30	20	10	10	10	0	0	20	30	10	90	0	10	20	10	
Samples collected after storage																		
KB 11	14	7	-	-	-	-	-	-	-	-	-	-	-	-	-	7	7	4
KB 13	14	-	-	7	-	21	-	-	-	21	-	-	-	-	-	-	-	4
KB 14	-	7	7	7	-	-	-	-	-	-	21	-	7	-	-	14	-	6
KB 21	-	50	-	-	-	-	29	-	-	-	-	-	21	-	-	-	-	3
KB 22	-	21	-	-	-	-	-	-	-	-	-	7	-	-	-	7	-	3
KB 23	-	7	7	-	-	-	-	-	7	-	42	-	-	-	-	-	7	5
KB 24	-	14	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	2
KB 25	21	-	-	-	-	-	-	-	-	14	-	-	21	-	7	-	-	4
KB 26	-	-	-	-	29	-	-	-	-	7	-	-	42	-	-	-	-	3
KB 27	-	-	-	14	-	-	-	14	-	-	21	-	-	-	-	-	-	3
KB 28	-	-	21	-	29	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	-	-	42	-	-	29	-	21	21	-	4
KB 31	-	14	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	2
KB 32	-	-	21	-	-	-	-	-	-	14	-	14	21	-	-	-	-	4
KB 34	-	-	-	-	-	-	-	21	-	-	-	-	14	-	-	-	-	2
KB 35	7	-	21	-	-	-	-	-	-	21	7	-	-	-	-	-	-	4
KB 36	-	-	14	-	21	21	-	-	-	7	-	-	-	-	-	-	-	4

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
KB 37	-	7	7	-	-	-	-	-	-	21	7	-	-	7	-	7	-	6
KB 38	21	-	-	-	-	-	-	-	-	-	14	-	14	14	-	-	-	4
KB 39	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	1
KB 40	-	-	-	-	-	-	-	-	-	29	14	-	99	-	-	21	-	4
KB 41	14	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 42	-	-	7	-	-	-	-	-	-	7	7	21	-	-	-	-	-	4
KB 43	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 44	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	1
KB 45	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	1
KB 46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 47	-	-	-	-	71	-	-	-	-	14	29	-	21	-	-	-	-	4
KB 48	-	57	29	-	-	-	-	-	-	-	-	-	14	-	-	29	21	5
KB 49	-	-	36	-	-	-	-	-	-	29	-	-	21	-	-	-	-	3
KB 50	-	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	1
KB 51	14	-	7	-	14	-	-	7	-	7	21	7	21	-	-	-	7	9
KB 52	14	21	-	-	-	-	-	-	-	-	-	7	-	-	-	-	14	4
KB 54	7	-	-	14	-	-	-	7	-	-	-	21	-	21	-	-	-	5
%Sample mean	25.7	31.4	34.3	11.4	14.3	5.7	5.7	11.4	2.9	40	34.3	20	40	8.6	5.7	20	14.3	
Zone IV: *Samples collected after harvest*																		
KB 55	-	-	-	-	-	-	7	-	-	21	-	-	21	-	21	-	-	4
KB 56	14	-	-	-	-	7	-	-	14	-	-	-	42	-	-	14	-	5
KB 57	-	-	14	-	-	-	-	-	-	-	29	-	21	-	-	-	-	3
KB 58	-	-	-	-	7	14	-	-	-	-	-	29	42	-	-	-	-	4
KB 59	-	-	29	-	-	-	-	-	-	-	29	-	21	-	-	-	-	3
KB 62	-	-	-	-	14	-	21	-	-	-	-	21	29	-	-	-	-	4
KB 65	-	42	-	7	-	-	-	-	-	-	-	-	21	-	-	-	-	3
KB 66	-	-	7	-	-	-	-	-	-	-	29	-	-	-	-	7	-	3
%Sample mean	12.5	12.5	37.5	12.5	25	25	25	0	12.5	12.5	37.5	25	87.5	0	12.5	25	0	
Samples collected after storage																		
KB 60	-	-	14	-	-	-	-	-	-	14	-	-	-	-	-	-	-	2
KB 61	-	-	-	-	-	14	-	-	-	21	-	-	-	-	29	-	21	4
KB 63	-	-	21	-	-	21	-	-	-	-	71	-	99	-	-	-	-	4
KB 64	-	-	21	-	-	-	-	-	-	-	86	-	-	-	-	-	-	2
KB 67	-	-	-	14	-	-	-	-	-	-	-	-	42	-	-	21	-	3
KB 68	-	-	-	-	-	-	-	-	-	-	71	-	-	-	-	-	-	1
KB 69	-	36	-	-	-	-	-	-	-	-	71	-	21	-	-	36	-	4
KB 70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 71	-	14	-	-	7	-	-	-	-	-	-	-	-	-	-	21	-	3
KB 72	-	-	-	-	-	-	-	-	-	-	-	-	42	-	21	21	-	3
KB 73	-	-	-	7	-	-	-	-	-	-	21	-	-	-	21	-	-	3
%Sample mean	0	18.2	27.3	18.2	9.1	18.2	0	0	0	18.2	45.5	0	36.4	0	27.3	36.4	9.1	

Table 10. **Frequency (%) of seed mycoflora in apparently healthy seeds (sterilized*) through blotter method**

Sample number	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Cephalosporium* sp.	*Cladosporium* sp.	*Fusarium* sp.	*Monosporium* sp.	*Penicillium* spp.	*P. capsulatum*	*Stemphylium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Zone II: ***Samples collected after storage***											
KB 1	-	-	-	-	-	-	-	7	-	-	1
KB 2	-	-	-	-	-	-	-	-	-	-	0
KB 3	-	-	-	-	-	-	-	-	-	-	0
KB 4	-	-	-	-	-	-	-	-	-	-	0
KB 5	-	-	-	-	-	-	-	-	-	-	0
KB 6	-	-	-	-	-	14	-	-	-	-	1
KB 7	-	-	-	-	-	-	-	-	-	-	0
KB 8	-	7	-	-	21	-	-	-	-	-	2
KB 9	-	-	-	14	21	-	-	-	-	-	2
%Sample mean	0	11.1	0	11.1	22.2	11.1	0	11.1	0	0	
Zone III: ***Samples collected after harvest***											
KB 10	-	-	-	-	-	-	-	-	-	-	0
KB 12	-	-	7	-	-	-	-	-	-	-	1
KB 15	-	-	-	-	-	-	-	-	-	-	0
KB 16	-	-	-	-	-	-	-	-	-	-	0
KB 17	-	-	-	-	-	-	-	-	-	7	1
KB 18	-	-	-	-	-	-	-	-	-	-	0
KB 19	-	29	-	-	-	-	-	-	-	-	1
KB 20	-	-	50	-	-	14	-	-	-	-	2
KB 33	-	-	-	-	-	-	-	-	-	-	0
KB 53	14	-	-	-	-	-	-	-	-	-	1
%Sample mean	10	10	20	0	0	10	0	0	0	10	
Samples collected after storage											
KB 11	-	-	-	-	-	-	-	-	-	-	0
KB 13	-	-	-	-	-	-	-	-	-	-	0
KB 14	-	-	-	-	-	-	-	-	-	-	0
KB 21	-	-	-	-	-	-	-	-	-	-	0
KB 22	-	-	-	-	-	-	-	-	-	-	0
KB 23	-	-	-	-	-	-	-	-	-	-	0
KB 24	-	-	-	-	-	-	-	-	-	-	0
KB 25	-	-	-	-	-	-	-	-	-	-	0
KB 26	-	-	-	-	-	-	-	-	-	-	0
KB 27	-	-	-	-	-	-	-	-	-	-	0
KB 28	-	-	-	-	29	-	-	-	-	-	1
KB 29	-	-	-	-	14	-	-	-	-	-	1
KB 30	-	-	-	-	-	-	-	-	-	-	0
KB 31	7	-	-	-	-	-	-	-	-	-	1
KB 32	-	-	-	-	-	-	-	-	7	-	1
KB 34	-	-	-	-	-	-	-	-	-	-	0

Continued

1	2	3	4	5	6	7	8	9	10	11	12
KB 35	14	-	-	-	-	-	7	-	-	-	2
KB 36	-	-	-	-	-	7	-	-	-	-	1
KB 37	-	-	-	-	-	-	-	-	-	-	0
KB 38	-	-	-	-	-	7	-	-	-	-	1
KB 39	-	-	-	-	-	-	-	-	-	-	0
KB 40	-	-	-	-	-	-	-	-	-	-	0
KB 41	-	-	-	-	-	-	-	-	-	-	0
KB 42	-	-	-	-	-	-	-	-	21	-	1
KB 43	-	-	-	-	-	-	-	-	-	-	0
KB 44	-	-	-	-	-	-	-	-	-	-	0
KB 45	-	-	-	-	-	-	-	-	-	-	0
KB 46	-	-	-	-	-	-	-	-	-	-	0
KB 47	7	-	-	-	-	-	-	-	-	-	1
KB 48	-	-	-	-	-	-	-	-	-	-	0
KB 49	-	-	-	-	-	-	-	-	-	-	0
KB 50	-	-	-	-	-	-	-	-	-	-	0
KB 51	-	-	-	-	-	-	-	-	-	-	0
KB 52	-	-	-	-	-	21	-	-	-	-	1
KB 54	-	-	-	-	-	-	7	-	-	-	61
%Sample mean	8.6	0	0	0	5.7	8.6	5.7	0	5.7	0	
Zone IV: *Samples collected after harvest*											
KB 55	-	-	-	-	-	-	-	-	-	-	0
KB 56	-	-	-	-	-	29	-	-	-	-	1
KB 57	-	-	-	-	-	-	-	-	-	-	0
KB 58	-	-	-	-	-	-	-	-	-	-	0
KB 59	-	-	-	-	-	-	-	-	-	-	0
KB 62	-	-	-	-	-	-	-	-	-	-	0
KB 65	21	-	7	-	-	-	-	-	-	-	2
KB 66	-	-	-	-	-	-	-	-	-	7	1
%Sample mean	12.5	0	12.5	0	0	12.5	0	0	0	12.5	
Samples collected after storage											
KB 60	-	-	-	-	-	-	-	-	-	-	0
KB 61	-	-	-	-	-	-	-	-	-	-	0
KB 63	-	-	-	-	-	-	-	-	-	-	0
KB 64	-	-	-	-	-	-	-	-	-	-	0
KB 67	-	-	-	-	-	-	-	-	-	-	0
KB 68	-	14	-	-	-	-	-	-	-	-	1
KB 69	-	-	-	-	14	-	-	-	-	-	1
KB 70	-	-	-	-	-	-	-	-	-	-	0
KB 71	-	-	-	-	-	-	-	-	-	-	0
KB 72	-	-	-	-	21	-	-	-	-	-	1
KB 73	-	-	7	-	-	-	-	-	-	-	1
%Sample mean	0	9.1	9.1	0	18.2	0	0	0	0	0	

* Sterilized with sodium hypochlorite

Zone III a maximum of 9 species were observed on KB 51 whereas, KB 29 and 46 were free. *Penicillium spp.* and *Rhizopus* were the major fungi recorded with a frequency range of 7-99 per cent. Eight samples collected after harvest representing Zone IV showed the presence of *Acremonium, Fusarium, Monosporium, Rhizopus and Trichothecium* sp. on KB 56 and three each on KB 57, 59, 65 and 66. However, 11 samples collected after storage revealed the occurrence of 4 species each on KB 61, 63 and 69. Overall perusal of the data showed that apparently healthy seed samples (collected after harvest and storage) of Zone IV were least affected by fungal flora as compared to other two zones.

Data recorded on mycoflora after surface sterilization of seeds (Table 10) revealed the occurrence of only one or two species with 92 per cent of the total samples being free of any infestation. Though in all 10 species belonging to 7 genera were detected from different samples with a maximum of 2 species in majority of samples.

Agar plate method (MEA)

Externally and internally seed borne mycoflora was detected by plating unsterilized and sterilized seeds on malt extract agar medium and its occurrence is given in Tables 11 and 12. Various seed samples were found to harbour only storage/ saprophytic fungi. Unsterilized seeds of 73 samples harboured 14 species belonging to 8 genera, whereas, only 12 species comprising of 9 genera were recorded on sterilized seed lots. In Zone II, a maximum of 4 species were detected from KB 3 with an incidence of 7-50 per cent. Out of various fungi *Aspergillus* species dominated the other fungi as they were detected from a maximum of 55.6 per cent of the total samples followed by *Penicillium perpuragenum* on 22.2 per cent samples. Almost similar trend of mycoflora was noticed in seed lots of Zone III and IV, where unsterilized apparently healthy seed samples collected after harvest and storage harboured up to 7 fungal species (Table 11). However, surface sterilized seeds of the respective samples when plated on malt extract agar showed the occurrence of 1, 2 or 3 species with

Table 11. Frequency (%) of seed mycoflora in apparently healthy seeds (unsterilized) through Malt Extract Agar

Sample number	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Cephalosporium* sp.	*Cladosporium* sp.	*Fusarium* spp.	*Myrothecium* sp	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizopus stolonifer*	*Stemphylium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Zone II: *Samples collected after storage*															
KB 1	-	-	14	-	-	-	-	-	-	-	-	-	-	-	1
KB 2	14	-	-	-	-	-	-	42	-	-	-	-	42	-	3
KB 3	14	-	-	-	-	-	-	-	-	21	-	14	50	-	4
KB 4	21	-	-	-	-	-	-	-	-	-	-	21	-	-	2
KB 5	-	-	21	-	-	-	-	-	-	-	-	-	-	-	1
KB 6	-	-	29	21	-	-	-	-	-	-	-	-	-	-	2
KB 7	21	21	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 9	21	21	-	14	7	-	-	-	-	-	-	-	-	-	4
%Sample mean	55.6	22.2	33.3	22.2	11.1	0	0	11.1	0	11.1	0	22.2	22.2	0	
Zone III: *Samples collected after harvest*															
KB 10	-	-	-	-	-	29	-	-	-	-	-	-	21	-	2
KB 12	-	-	21	-	-	-	-	-	-	-	-	-	-	-	1
KB 15	7	-	-	-	-	-	-	-	-	7	-	-	-	-	2
KB 16	14	-	-	-	-	-	-	-	-	-	29	-	14	-	3
KB 17	-	-	-	21	21	-	-	-	-	-	-	-	-	-	2
KB 18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 19	14	-	-	-	-	-	-	-	-	-	-	-	21	-	2
KB 20	-	-	21	7	-	-	-	-	-	-	21	-	50	-	4
KB 33	-	-	-	-	-	-	-	-	-	-	-	-	14	-	1
KB 53	-	-	29	-	-	-	-	-	-	-	-	-	-	-	1
%Sample mean	30	0	30	20	10	10	0	0	0	10	20	0	50	0	
Samples collected after storage															
KB 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 13	21	-	36	-	-	-	-	-	-	-	-	-	-	-	2
KB 14	-	-	36	-	36	-	-	-	-	-	-	-	-	-	2
KB 21	-	-	21	-	-	-	-	-	-	21	-	-	-	-	2
KB 22	21	-	14	-	-	-	-	7	-	-	7	7	21	-	6
KB 23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 24	-	-	-	-	-	-	-	-	-	-	-	-	21	-	1
KB 25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 26	21	-	-	-	-	-	-	14	-	14	14	14	-	-	5
KB 27	-	-	-	-	-	-	-	-	-	-	14	-	-	-	1
KB 28	21	-	-	-	-	-	21	-	-	-	21	-	29	-	4
KB 29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 31	-	-	7	-	-	-	21	-	-	-	-	-	-	-	2
KB 32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 34	-	-	-	-	-	-	-	21	-	-	-	-	-	-	1
KB 35	-	-	-	-	-	-	-	7	7	7	7	7	42	-	6

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
KB 36	-	-	-	-	-	-	-	-	-	-	-	-	-	29	1
KB 37	21	21	29	-	-	-	-	-	-	-	-	-	-	-	3
KB 38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 40	-	-	-	-	-	-	-	42	-	-	-	-	70	-	2
KB 41	7	-	-	21	-	-	21	-	-	-	-	-	-	-	3
KB 42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 43	-	-	-	-	-	-	-	-	-	-	-	-	21	-	1
KB 44	-	14	-	-	-	-	-	-	-	-	-	21	21	-	3
KB 45	-	21	-	-	14	-	-	-	-	-	-	-	-	-	2
KB 46	21	14	-	-	-	-	-	-	21	-	-	-	21	-	4
KB 47	21	-	-	-	-	-	-	-	-	7	14	-	14	-	4
KB 48	-	-	-	-	-	-	-	14	-	14	14	14	14	-	5
KB 49	-	7	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 50	21	-	-	-	-	14	-	-	-	-	-	-	-	-	2
KB 51	-	-	36	21	-	-	-	-	-	-	-	-	-	-	2
KB 52	-	-	21	-	7	-	-	-	-	-	-	-	-	-	2
KB 54	-	-	-	-	-	14	-	-	-	-	-	-	-	21	2
%Sample mean	25.7	14.3	22.9	5.7	8.6	5.7	8.6	17.1	5.7	14.3	20	14.3	28.6	5.7	
Zone IV: *Samples collected after harvest*															
KB 55	29	14	-	-	-	-	-	-	-	7	7	7	-	-	5
KB 56	-	-	-	-	7	-	-	-	-	7	21	7	-	-	4
KB 57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 58	21	21	-	-	-	-	-	-	-	21	-	-	-	-	3
KB 59	-	-	-	-	29	-	-	-	-	-	-	-	-	-	1
KB 62	-	-	-	29	42	-	-	-	-	-	-	-	-	-	2
KB 65	21	14	-	-	-	-	-	7	-	7	7	7	-	-	6
KB 66	21	-	-	-	21	-	-	-	-	-	-	-	-	-	2
%Sample mean	50	37.5	0	12.5	50	0	0	12.5	0	50	37.5	37.5	0	0	
Samples collected after storage															
KB 60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 63	-	-	-	-	-	-	21	-	21	-	-	-	-	-	2
KB 64	14	-	-	-	-	-	-	7	-	-	7	7	21	-	5
KB 67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 68	-	-	21	-	-	-	-	14	14	14	14	14	70	-	7
KB 69	-	-	-	-	-	-	-	14	-	-	14	7	70	-	4
KB 70	-	-	-	-	-	-	-	21	-	-	-	-	7	-	2
KB 71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 72	21	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 73	-	-	-	-	-	-	-	7	-	7	7	7	42	-	5
%Sample mean	18.2	0	9.1	0	0	0	9.1	45.5	18.2	18.2	36.4	36.4	45.5	0	16

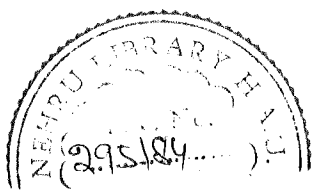


Table 12. Frequency (%) of seed mycoflora in apparently healthy seeds (sterilized) * through Malt Extract Agar

Sample number	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*A. ochraceous*	*Cephalosporium* sp.	*Cladosporium* sp.	*Fusarium moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*Rhizopus stolonifer*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Zone II: *Samples collected after storage*												
KB 1	-	-	-	-	-	-	7	-	-	-	-	1
KB 2	21	-	-	-	-	-	-	-	-	-	-	1
KB 3	-	-	-	-	-	-	-	-	-	-	-	0
KB 4	-	-	-	-	-	-	-	-	7	-	-	1
KB 5	14	-	-	-	-	-	-	-	-	-	-	1
KB 6	-	-	-	-	-	-	-	-	-	-	-	0
KB 7	-	-	-	-	-	-	-	-	-	-	-	0
KB 8	-	-	-	-	-	-	-	-	-	-	-	0
KB 9	-	-	-	-	-	-	-	-	-	-	-	0
%Sample mean	22.2	0	0	0	0	0	11.1	0	11.1	0	0	
Zone III: *Samples collected after harvest*												
KB 10	-	-	21	-	-	-	-	-	-	-	-	1
KB 12	-	-	-	-	-	-	-	-	-	-	-	0
KB 15	-	-	-	-	-	-	-	-	-	-	-	0
KB 16	-	7	-	-	-	-	-	-	-	7	7	3
KB 17	-	-	-	-	14	-	-	-	-	-	-	1
KB 18	-	-	-	-	-	-	-	-	-	-	-	0
KB 19	-	14	-	-	-	-	-	-	-	-	-	1
KB 20	-	-	-	-	-	-	-	-	-	-	-	0
KB 33	-	-	-	-	-	-	-	-	-	-	-	0
KB 53	-	-	29	36	-	-	-	-	-	-	-	2
%Sample mean	0	20	20	10	10	0	0	0	0	10	10	
Samples collected after storage												
KB 11	-	-	-	-	-	-	-	-	-	-	-	0
KB 13	-	-	-	-	-	-	-	-	-	-	-	0
KB 14	-	-	-	-	-	-	-	-	-	-	-	0
KB 21	-	-	-	-	-	-	-	-	-	-	-	0
KB 22	-	-	-	-	-	-	-	-	-	-	-	0
KB 23	-	-	-	-	-	-	-	-	-	-	-	0
KB 24	-	-	-	-	-	-	-	-	-	-	7	1
KB 25	-	-	-	-	-	-	-	-	-	-	-	0
KB 26	-	21	-	-	-	-	-	-	-	-	-	1
KB 27	-	-	-	-	-	-	-	-	-	-	-	0
KB 28	-	-	-	-	-	-	-	-	-	-	-	0
KB 29	-	-	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	-	-	-	-	0
KB 31	-	-	-	-	-	-	-	-	-	-	-	0

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13
KB 32	-	-	-	-	-	-	-	-	-	-	-	0
KB 34	7	-	-	-	-	-	-	-	7	7	-	3
KB 35	-	-	-	-	-	-	-	-	-	-	-	0
KB 36	-	-	-	-	-	-	-	-	-	-	-	0
KB 37	-	-	-	-	-	-	-	-	-	-	-	0
KB 38	-	-	-	-	7	7	-	-	-	-	-	2
KB 39	-	-	-	-	-	-	-	14	-	-	-	1
KB 40	14	-	-	-	-	-	-	-	-	-	-	1
KB 41	-	-	-	-	-	-	-	-	-	-	-	0
KB 42	-	-	-	-	-	-	-	-	-	-	-	0
KB 43	-	-	-	-	-	-	-	-	-	-	-	0
KB 44	-	-	-	-	-	-	-	-	-	-	-	0
KB 45	-	-	-	-	-	-	-	-	-	-	-	0
KB 46	-	-	-	-	-	-	-	-	-	-	-	0
KB 47	-	-	-	-	-	-	-	-	-	-	-	0
KB 48	-	-	-	-	-	-	-	-	-	-	-	0
KB 49	-	-	-	-	-	-	-	-	-	-	-	0
KB 50	-	-	-	-	-	-	-	-	-	-	-	0
KB 51	-	-	-	-	-	-	-	-	-	-	-	0
KB 52	-	-	-	-	-	-	-	-	-	-	-	0
KB 54	-	-	-	-	-	-	-	-	-	-	-	0
%Sample mean	5.7	2.9	0.0	0.0	2.9	2.9	0.0	2.9	2.9	2.9	2.9	
Zone IV: *Samples collected after harvest*												
KB 55	-	-	-	-	-	-	-	-	-	-	-	0
KB 56	-	-	-	-	-	-	-	-	-	-	-	0
KB 57	-	-	-	-	-	-	-	-	-	-	-	0
KB 58	-	-	-	-	-	-	-	-	-	-	-	0
KB 59	-	-	-	-	-	-	-	-	-	-	-	0
KB 62	29	-	-	-	-	-	-	-	-	-	-	1
KB 65	-	-	-	-	-	-	-	-	-	-	-	0
KB 66	-	-	-	-	-	-	-	-	-	-	-	0
%Sample mean	12.5	0	0	0	0	0	0	0	0	0	0	
Samples collected after storage												
KB 60	-	-	-	-	-	-	-	-	-	-	-	0
KB 61	-	-	-	-	-	-	-	-	-	-	-	0
KB 63	-	-	-	-	-	-	-	-	-	-	-	0
KB 64	-	-	-	-	-	-	-	-	-	-	-	0
KB 67	-	-	-	-	-	-	-	-	-	-	-	0
KB 68	-	-	-	-	-	-	-	-	-	-	-	0
KB 69	-	-	-	-	-	-	-	-	-	7	-	1
KB 70	-	-	-	-	-	-	-	-	-	7	-	1
KB 71	-	-	-	-	-	-	-	-	-	-	-	0
KB 72	-	-	-	-	-	-	-	-	-	-	-	0
KB 73	-	-	-	-	-	-	-	-	-	-	-	0
%Sample mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2	0.0	

Sterilized with sodium hypochlorite

maximum samples being free of any infestation (Table 12). The major fungi detected through plating of sterilized seeds were *Alternaria, Aspergillus, Cephalosporium, Cladosporium, Fusarium, Myrothecium, Penicillium, Phoma* and *Rhizopus* with an overall incidence of 0-36 per cent.

4.1.7 Detection of mycoflora from discoloured seeds

Agar plate method

Discoloured/infected seed lots of various samples were plated on MEA without surface sterilization and the mycoflora detected is given in Figure I. In all, 34 genera of field and storage fungi were recorded on seed samples analysed after harvest and storage. The occurrence of fungi on individual sample ranged between 2-11 species without any significant difference w.r.t. stage of analysis/detection. Samples analysed immediately after harvest showed the presence of a maximum of 10 species on sample KB 15 and 9 species on KB 58 of Zone III and IV, whereas among seed samples analysed after storage, three seed lots KB 9, 21 and 60, 63 and 73 from Zone II, III and IV, possessed a maximum of 6, 11 and 4 species, respectively. The storage fungi were found to be present in higher proportion with the dominance of *Aspergillus, Penicillium* and *Rhizopus*. Among field fungi *Alternaria, C. lindemuthianum* and *Rhizoctonia* were recorded frequently, colonizing 20.0 – 50.5 per cent of the total samples.

Among sterilized lots, various seed samples exhibited the presence of 1-4 fungal species. Some of the seed samples of Zone III and all samples collected after storage from Zone IV were free of any fungal infestation (Figure II). C. *lindemuthianum, F. oxysporum* and *R. solani* were the most prominent pathogenic fungi.

4.1.8 Detection of mycoflora in bulk seed sample

Blotter method

Kidney bean samples comprising of apparently healthy, shrivelled and discoloured/ infected seeds were analysed by blotter method for

Figure 1. Frequency (%) of seed mycoflora in discoloured seeds (unsterilised) through Malt Extract Agar

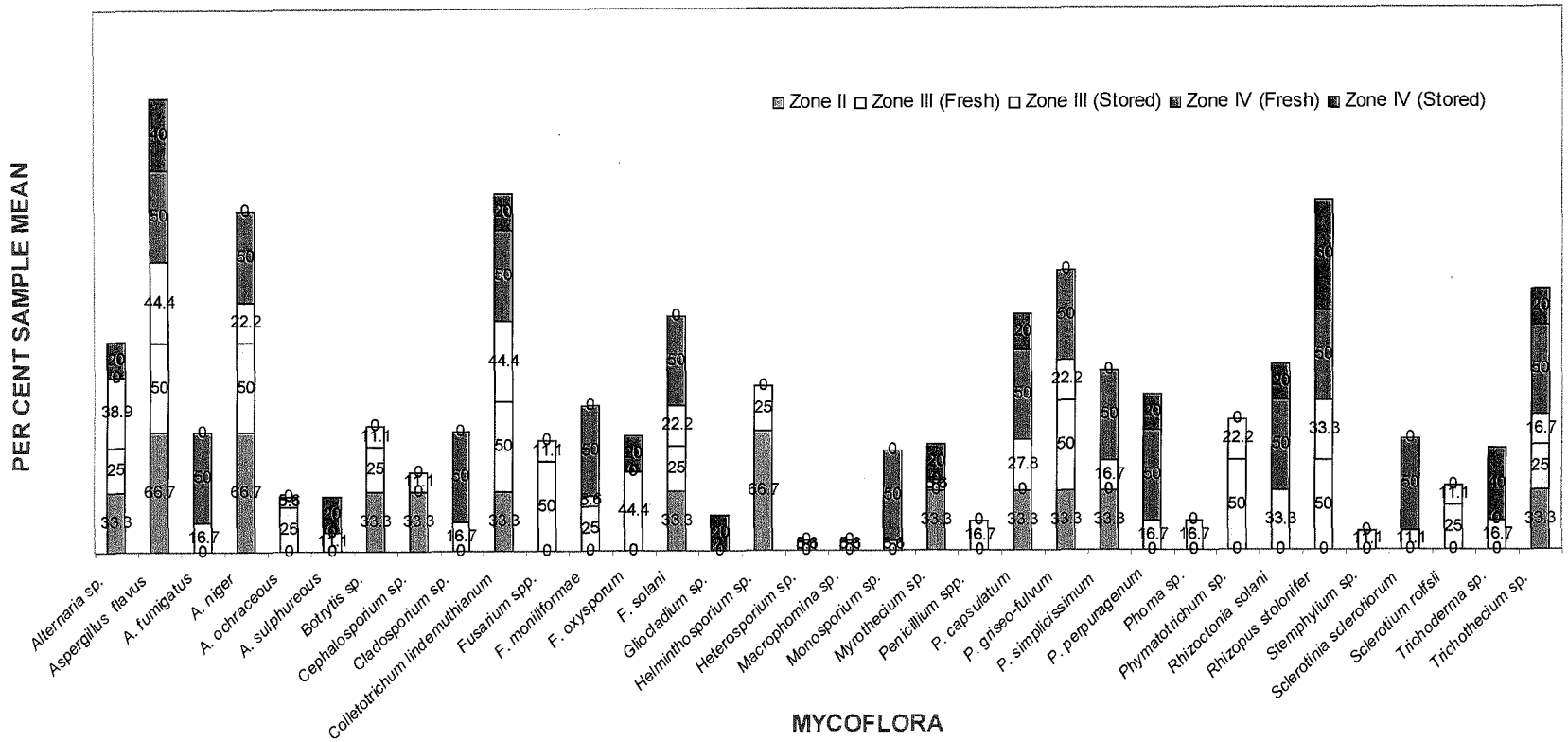
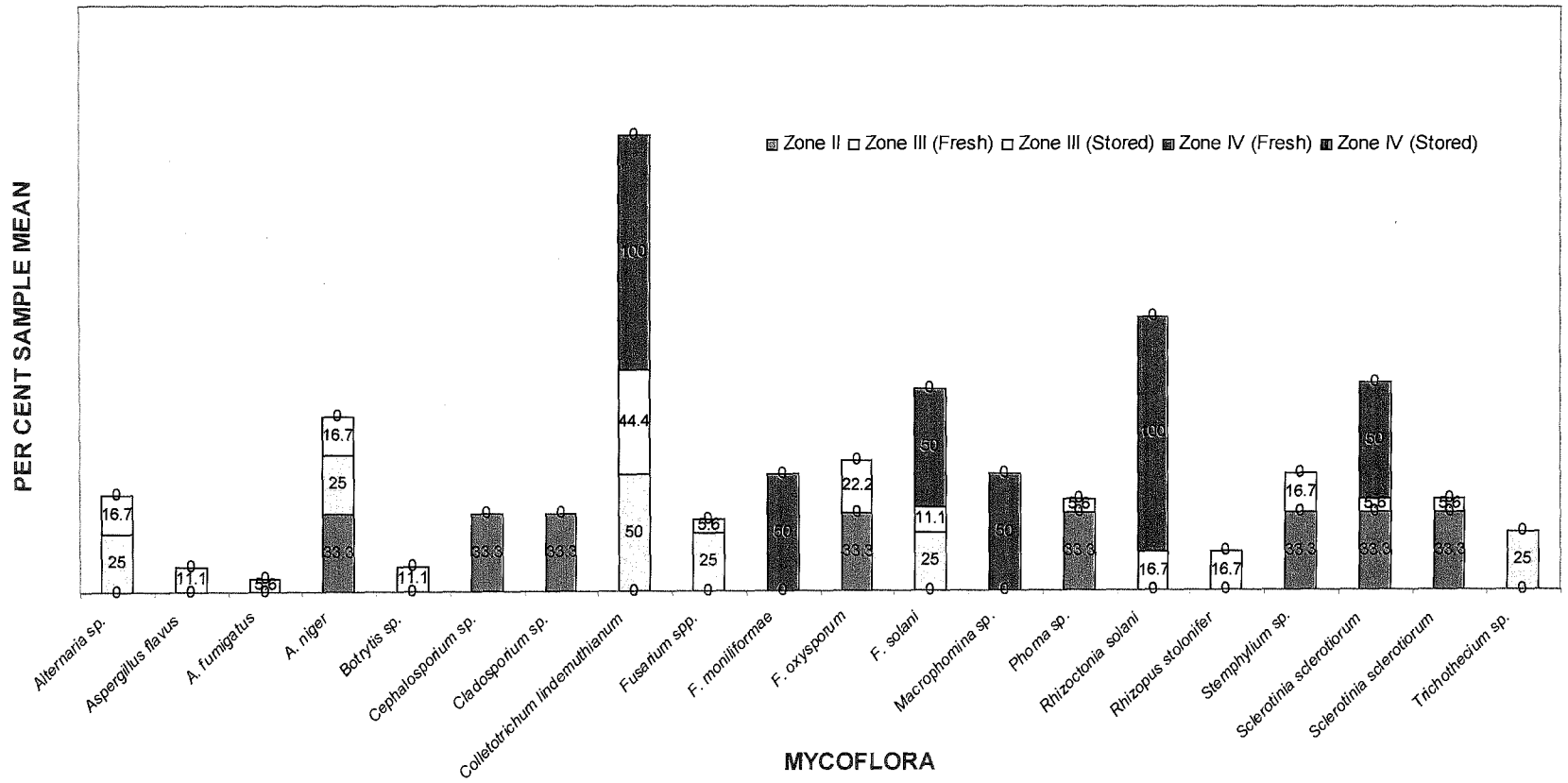


Figure 2. Frequency (%) of seed mycoflora in discoloured seeds (sterilised) through Malt Extract Agar



occurrence of mycoflora using unsterilized/ sterilized seeds. The fungal species detected are presented in Tables 13 and14.

Data clearly show that maximum mycoflora was observed on KB 48 and 51 (16) of Zone III followed by KB 5 , 9 and 49, all harbouring 15 species each in Zone II and III. Unsterliized seed samples from Zone IV possessed 14 species on KB 64, whereas in sterilized seed lots, 22 species comprising of 16 genera were encountered. Maximum fungal flora was recorded on KB 19 (8) of Zone III followed by KB 9 (7) of Zone II and KB 73 (6) of Zone IV (Table 13).

Fifteen fungi were found to be associated with unsterlized seed samples of Zone II. *Colletotrichum* and *Rhizoctonia* were observed on 77.8 per cent of the samples followed by *S. rolfsii* (44.4%), *Sclerotinia* and *Alternaria* (33.3%). The incidence of these fungi was between 7-29 per cent. *Myrothecium*, species of *Penicillium* and *Aspergillus fumigatus* were the dominant storage fungi (Plate 2).

Samples collected after harvest from Zones III and IV showed the presence of 14 and 10 fungi, respectively, which included *Alternaria*, *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Sclerotinia and S. rolfsii* among field fungi and *Aspergillus*, *Penicillium*, *Cephalosporium*, *Cladosporium*, *Myrothecium*, *Trichoderma* and *Trichotheciun* among storage fungi. Samples collected after storage showed the presence of more storage fungi as compared to field/ pathogenic fungi. However, in Zone III the occurrence of various fungi was higher on the seed samples which were analysed immediately after harvest as compared to that of stored samples. In Zone IV, *Penicillium capsulatum* was detected from 81.8 per cent of the analysed samples.

Sterilized seed samples of Zone II showed the association of 18 fungi with highest number on KB 9 (Table 14). Dominating mycoflora consisted of field fungi mainly *C. lindemuthianum*, *F. oxysporum* and *S. rolfsii*.

Analysis of samples collected after harvest from Zones III and IV showed that KB 19 was colonized by 8 fungal species whereas, remaining

ıle 14. Frequency (%) of seed mycoflora in bulk sample (sterilized)* through blotter method

Sample number	*Alternaria sp.*	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Botrytis sp.*	*Cephalosporium sp.*	*Cladosporium sp.*	*Colletotrichum lindemuthianum*	*Fusarium spp.*	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Heterosporium sp.*	*Macrophomina sp.*	*Penicillium spp*	*Phoma sp.*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium sp.*	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*
Zone II: *Samples collected after storage*																							
KB 1	-	-	-	29	14	-	-	-	21	-	-	-	-	-	-	-	14	-	-	14	-	21	6
KB 2	14	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 3	-	-	29	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	2
KB 4	7	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	2
KB 5	7	-	-	-	-	21	-	-	42	-	-	-	-	-	-	-	-	-	-	29	-	-	4
KB 6	-	-	-	21	29	-	-	-	-	42	-	-	-	29	-	-	-	-	-	-	-	-	4
KB 7	-	-	-	-	-	-	-	-	-	-	-	-	-	14	29	-	7	7	-	-	-	-	4
KB 8	-	-	-	21	-	14	-	-	29	-	-	-	21	-	-	-	-	-	-	-	-	21	5
KB 9	21	21	7	-	-	-	-	-	7	21	14	-	-	-	-	-	-	-	-	-	-	21	7
%Sample mean	44.4	11.1	22.2	33.3	22.2	22.2	11.1	0.0	44.4	22.2	11.1	22.2	11.1	22.2	11.1	0.0	22.2	11.1	0.0	22.2	0.0	33.3	
Zone III: *Samples collected after harvest*																							
KB 10	-	-	-	-	-	-	-	-	14	21	7	-	-	-	-	-	-	14	-	-	-	-	4
KB 12	-	-	-	7	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	2
KB 15	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	1
KB 16	-	14	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	7	-	-	7	-	4
KB 17	-	-	-	-	36	-	7	-	7	-	-	7	-	-	-	21	-	-	-	-	-	-	5
KB 18	-	21	-	-	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	2
KB 19	-	21	21	-	-	-	-	7	7	7	-	21	21	-	-	-	-	-	-	-	14	-	8
KB 20	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	1
KB 33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 53	7	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	2
%Sample *mean*	*10.0*	*30.0*	*10.0*	*10.0*	*10.0*	*0.0*	*10.0*	*10.0*	*40.0*	*20.0*	*10.0*	*60.0*	*20.0*	*0.0*	*0.0*	*10.0*	*0.0*	*20.0*	*0.0*	*0.0*	*20.0*	*0.0*	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
ples collected after storage									-	-	-	-	-	-	-	-		-	-	-	-	-	0
11	-	-	-	-	-	21	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	21	3
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
14	14	-	-	-	-	-	-	-	21	-	-	-	7	-	-	-	29	-	-	-	-	-	4
21	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	21	-	2
22	-	-	-	-	-	-	7	-	-	-	-	57	-	-	-	-	-	-	-	-	-	-	2
23	-	-	-	-	-	-	14	-	14	-	-	21	7	-	-	-	-	-	-	-	-	-	4
KB 24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 26	-	-	-	-	-	-	21	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	2
KB 27	-	14	14	7	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 28	7	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	29	-	-	3
KB 29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 31	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	1
KB 32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 34	7	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	2
KB 35	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	29	-	29	-	-	3
KB 36	-	-	-	-	-	-	21	-	21	-	-	-	-	-	-	-	21	7	-	21	-	-	5
KB 37	-	-	-	-	-	14	21	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	3
KB 38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	21	-	-	-	29	3
KB 39	14	-	-	-	-	7	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	3
KB 40	14	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 41	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	14	-	-	-	-	2
KB 42	29	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	21	-	-	-	36	4
KB 43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 45	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	1
KB 46	7	-	-	-	-	-	-	-	29	-	7	-	7	-	-	-	-	-	-	-	-	-	4
KB 47	-	-	-	-	-	-	36	21	14	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 48	-	-	-	-	-	-	7	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	2
KB 49	-	-	-	-	-	-	-	-	-	-	-	70	-	-	-	-	-	-	-	-	-	-	1
KB 50	29	-	-	-	-	-	-	21	-	-	-	14	-	-	-	-	-	-	-	-	-	-	3

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
KB 51	-	21	-	-	29	-	-	-	7	-	-	-	-	-	21	-	-	14	-	-	-	-	5
KB 52	50	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	14	-	-	-	-	3
KB 54	14	-	-	-	21	-	-	-	21	-	-	-	-	29	-	-	29	-	-	-	-	-	5
%Sample mean	28.6	5.7	2.9	2.9	5.7	8.6	22.9	11.4	34.3	2.9	2.9	22.9	14.3	2.9	5.7	0.0	11.4	22.9	0.0	8.6	2.9	8.6	
Zone IV: *Samples collected after harvest*									-	-	-	-	-	-	-	-	-		-	-	-	-	0
KB 55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 56	7	-	-	-	-	-	-	7	-	-	29	-	-	-	14	-	-	-	-	-	-	-	4
KB 57	-	14	-	-	-	-	-	-	29	-	-	36	-	-	-	-	-	-	-	-	7	-	4
KB 58	-	-	-	-	-	-	14	-	-	7	-	29	-	-	-	-	-	-	-	-	7	-	4
KB 59	-	-	-	-	-	-	-	-	7	-	-	29	-	-	-	-	-	-	-	-	-	7	3
KB 62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 66	7	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	2
%Sample mean	25.0	12.5	0.0	0.0	0.0	0.0	12.5	12.5	25.0	12.5	12.5	37.5	12.5	0.0	12.5	0.0	0.0	0.0	0.0	0.0	25.0	12.5	
Samples collected after storage									-	-	-	-	-	-	-		-	-	-	-	-	-	0
KB 60	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	1
KB 61	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	1
KB 63	-	-	-	-	-	-	-	-	-	-	-	70	-	-	-	-	-	-	-	-	-	-	1
KB 64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 68	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	1
KB 69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 70	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	7	-	-	7	-	3
KB 71	14	-	-	-	-	-	14	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	3
KB 72	-	-	-	-	-	-	-	-	-	-	-	7	29	-	-	-	-	-	-	-	-	-	2
KB 73	-	-	-	-	*29*	-	-	*7*	*21*	-	*7*	-	*29*	-	-	-	-	-	-	-	*21*	-	6
%Sample *mean*	9.1	0.0	0.0	0.0	9.1	9.1	9.1	9.1	9.1	0.0	9.1	54.5	18.2	0.0	0.0	0.0	0.0	9.1	0.0	0.0	18.2	0.0	

** Sterilized with sodium hypochlorite*

Table 13. Frequency (%) of seed mycoflora in bulk sample (unsterilised) through Blotter Method

Sample number	*Acremonium* sp.	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Botrytis* sp.	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Gliocladium* sp.	*Helminthosporium* sp.	*Heterosporium* sp.	*Macrophomina* sp.	*Monosporium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma* sp.	*Phymatotrichum* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	*Trichoderma* sp.	*Trichothecium* sp.	*Varicosporium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*
Zone II: *Samples collected after storage*																																					
KB 1	-	7	21	21	-	-	-	-	-	21	7	-	-	-	-	-	-	-	-	-	21	21	-	-	-	-	-	7	-	-	-	-	7	-	-	-	9
KB 2	21	7	-	21	-	-	-	-	14	-	-	-	-	-	-	21	-	-	-	-	-	43	43	-	-	-	-	-	-	36	-	-	-	-	-	7	9
KB 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	43	43	-	7	-	-	-	7	36	7	7	14	-	-	-	9
KB 4	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	14	-	4
KB 5	14	-	-	14	-	-	-	7	21	-	14	14	-	-	-	-	7	-	-	-	29	7	-	21	-	7	-	-	7	36	-	-	14	-	-	7	15
KB 6	-	21	-	7	-	-	14	-	21	-	21	-	-	-	7	-	29	-	7	-	-	-	-	-	21	-	14	-	21	-	-	-	-	-	7	-	12
KB 7	7	-	-	-	14	21	-	-	-	36	29	14	-	36	-	-	21	-	-	-	36	-	-	-	-	36	-	-	7	-	-	36	-	7	-	7	14
KB 8	-	-	7	-	-	-	-	7	-	-	21	-	-	21	-	-	-	14	-	-	7	-	-	-	-	-	-	-	14	-	-	7	-	21	-	-	9
KB 9	-	21	-	21	-	7	-	-	14	-	29	-	-	-	29	-	-	-	14	-	29	21	-	-	21	-	7	-	7	-	21	-	14	-	-	7	15
%Sample mean	33.3	44.4	22.2	55.6	11.1	22.2	11.1	22.2	44.4	22.2	77.8	22.2	0	22.2	22.2	11.1	33.3	11.1	33.3	0	66.7	55.6	22.2	11.1	33.3	22.2	22.2	11.1	77.8	33.3	22.2	33.3	44.4	22.2	22.2	44.4	
Zone III: *Samples collected after harvest*																																					
KB 10	-	29	7	-	-	-	-	-	14	43	14	57	-	-	-	-	-	-	-	-	7	21	7	7	-	-	-	-	-	-	-	7	-	-	-	-	11
KB 12	-	-	21	21	-	-	-	-	29	-	-	-	-	21	-	-	-	-	-	-	56	-	21	7	21	21	-	-	-	43	-	-	-	-	-	-	10
KB 15	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	7	7	-	21	14	-	-	14	-	-	-	14	-	-	8
KB 16	-	-	-	-	-	21	7	-	-	-	-	-	-	14	-	-	-	-	-	-	-	7	7	-	-	21	-	-	-	56	-	-	-	-	-	29	8
KB 17	-	-	-	-	29	-	-	-	-	7	-	-	14	14	-	7	-	-	-	-	-	7	14	-	7	14	-	-	-	-	-	-	-	-	-	-	9
KB 18	-	-	21	7	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	29	-	21	7	-	-	-	14	21	-	-	-	-	-	-	8
KB 19	-	-	*21*	-	-	-	7	-	-	7	-	-	-	14	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	5
KB 20	-	*7*	*21*	*21*	-	-	-	-	-	*21*	7	-	-	-	-	-	-	-	-	-	21	21	-	-	-	-	-	7	-	-	-	-	7	-	-	-	9
KB 33	-	*36*	-	-	-	-	7	-	-	7	-	-	7	-	-	-	-	-	-	-	64	7	7	-	-	-	-	-	-	7	-	-	36	-	-	-	9
KB 53	-	*14*	*21*	-	-	*21*	-	-	*29*	-	*21*	7	-	-	-	*14*	-	-	*29*	-	*21*	-	-	-	-	-	*21*	-	-	21	-	21	21	-	36	-	14
%Sample mean	10	40	60	30	10	20	30	0	30	50	30	20	20	50	0	20	0	0	10	0	50	70	70	40	30	40	20	10	10	60	0	20	30	10	10	10	

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Samples collected after storage																																					
KB 11	-	-	29	-	-	-	-	-	50	-	-	-	14	36	-	-	-	-	-	-	-	21	21	-	-	7	-	-	-	50	-	-	-	-	50	36	10
KB 13	-	14	-	-	-	-	-	-	-	14	-	-	-	14	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	14	-	-	14	-	-	-	6
KB 14	-	21	-	-	-	29	-	-	-	-	-	-	-	-	7	29	-	-	-	-	14	7	-	-	-	21	-	-	-	43	-	-	-	-	-	-	8
KB 21	21	-	-	-	-	-	-	-	-	-	-	-	21	21	-	14	-	-	-	-	-	21	21	-	-	7	-	7	-	7	-	-	-	-	-	-	9
KB 22	-	7	14	7	7	-	-	-	-	-	-	-	-	7	14	-	-	-	-	-	-	-	29	-	-	7	-	-	-	50	-	-	-	36	21	-	11
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KB 27	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	36	7	-	-	-	-	-	36	-	-	-	-	-	-	5
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KB 32	-	7	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	79	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	4
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KB 36	-	7	21	-	-	-	-	-	-	-	14	-	36	-	-	-	-	-	-	-	50	70	14	-	-	-	-	-	-	7	-	14	-	-	-	14	10
KB 37	-	-	-	-	-	-	-	43	-	-	-	14	7	36	-	-	-	-	-	-	29	64	-	-	-	-	-	-	-	56	-	-	-	-	21	-	8
KB 38	-	-	14	-	14	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	14	-	-	-	-	-	-	5
KB 39	-	-	7	-	7	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	21	7	-	-	-	-	-	21	-	-	-	-	-	7	7
KB 40	-	64	29	-	-	-	-	-	-	-	-	-	-	70	-	-	-	-	-	-	21	-	43	-	-	-	-	-	43	36	-	-	-	29	-	-	8
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KB 42	-	-	-	-	-	-	-	-	-	-	21	7	7	-	21	7	-	-	21	-	7	-	-	-	14	14	-	-	14	-	-	-	-	7	7	-	12
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KB 47	-	14	-	-	-	-	-	-	-	-	-	-	-	14	-	14	-	-	-	-	7	-	-	-	-	-	-	-	-	-	14	-	-	14	-	-	6
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KB 49	7	21	-	14	-	14	-	-	14	21	7	-	-	-	29	-	-	-	-	-	29	-	21	-	7	21	-	-	21	14	-	-	21	-	-	-	15
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ample ean		17.1	57.1	31.4	22.8	22.8	17.1	14.2	8.6	17.1	40	42.8	11.4	28.5	48.5	48.5	20	14.2	5.7	11.4	11.4	51.4	42.8	48.5	25.7	25.7	34.2	2.8	5.7	17.1	60	25.7	14.2	22.8	20	31.4	17.1	
e IV:	*Samples collected after harvest*																																					
KB 55		-	21	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	14	36	21	-	36	-	14	-	7	-	-	-	-	-	-	8
KB 56		-	-	-	14	-	-	-	-	-	-	-	-	-	21	-	-	-	-	7	-	-	-	36	-	-	-	-	7	-	50	-	-	-	-	-	-	6
KB 57		-	-	21	-	-	-	-	-	14	-	-	-	21	-	-	-	-	-	-	-	-	36	-	-	-	14	-	-	29	29	-	-	-	-	29	-	8
KB 58		36	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	7	-	-	21	-	-	-	29	-	-	-	-	-	43	-	7	-	-	-	-	7
KB 59		-	-	21	21	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	-	36	7	14	7	-	-	-	100	-	-	-	-	-	-	9
KB 62		-	14	21	7	-	-	-	-	-	-	-	-	7	-	21	-	-	-	-	-	-	-	7	36	7	-	-	-	-	-	-	-	7	7	-	-	10
KB 65		-	14	14	-	-	-	-	-	7	-	14	-	29	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	21	-	7
KB 66		-	-	28	-	-	-	-	-	-	14	-	-	21	-	43	-	-	-	-	-	-	21	21	-	-	-	-	-	-	14	-	-	29	-	21	-	9
%Sample mean		12.5	37.5	62.5	37.5	12.5	0	0	12.5	25	12.5	12.5	0	50	12.5	37.5	0	12.5	0	12.5	12.5	12.5	37.5	75	50	25	37.5	0	25	12.5	75	0	12.5	25	12.5	37.5	0	36
Samples collected after storage																																						
KB 60		-	-	21	-	-	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	36	-	-	-	-	-	7	50	-	-	-	-	-	-	7
KB 61		-	-	-	-	-	-	-	-	7	-	-	-	14	-	-	-	-	14	-	-	-	-	14	7	7	21	14	-	-	14	-	-	-	-	-	-	9
KB 63		-	21	-	-	21	-	-	-	-	29	14	-	-	-	-	-	-	-	-	-	-	-	36	7	7	-	-	-	-	21	-	-	-	-	21	-	9
KB 64		-	21	36	36	-	-	-	-	-	42	7	-	36	7	-	-	-	-	-	-	-	-	21	7	7	21	-	-	-	21	-	-	29	-	14	-	14
KB 67		-	-	-	29	21	-	-	-	-	21	-	-	-	14	21	-	-	-	-	-	21	-	-	21	-	-	-	-	-	-	-	-	-	29	21	-	9
KB 68		-	21	21	-	-	-	-	-	-	-	-	-	36	-	-	-	-	21	-	-	64	-	21	21	-	-	-	-	-	-	-	-	-	-	-	-	7
KB 69		-	14	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	21	7	-	7	21	21	-	-	29	-	-	29	-	21	-	7	11
KB 70		-	-	-	-	-	-	14	-	-	-	-	21	-	-	-	-	-	-	-	-	21	-	50	50	-	-	-	14	-	7	-	-	-	-	-	-	7
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KB 72		-	-	-	-	-	-	7	-	-	-	-	-	7	-	-	-	-	-	-	-	50	31	64	-	-	-	-	-	-	-	-	-	-	-	-	-	5
KB 73		-	-	-	-	-	-	-	-	-	14	-	-	-	29	-	-	-	-	-	-	15	-	21	-	-	-	-	-	-	21	-	-	-	7	-	-	6
%Sample mean		0	36.4	27.3	18.2	18.2	9.1	36.4	0	9.1	36.4	18.2	9.1	45.5	36.4	9.1	9.1	0	18.2	0	0	63.6	27.3	81.8	72.7	36.4	27.3	9.1	9.1	18.2	54.5	0	9.1	9.1	27.3	27.3	18.2	36

samples possessed 0-5 species. Some of the samples viz. KB 33, 55, 62 and 65 were free of any fungal infestation. *F. oxysporum* was detected from 60 per cent (Zone III) and 37.5 per cent of the total samples showing 7-42 per cent incidence. However, in samples collected after storage, *Cephalosporium, Fusarium oxysporum* and *Rhizoctonia* were recorded from majority of the samples, whereas *Colletotrichum* colonized 34.3 per cent samples in Zone III and 9.1 per cent in Zone IV. Sample number KB 36, 51 and 54 of Zone III were found to be colonized by 5 species, whereas, 6 fungi were observed on KB 73 (Zone IV).

Agar plate method

Kidney bean seed samples when analysed for mycoflora by agar plate method (MEA) revealed the occurrence of 36 fungal species belonging to 25 genera (Table 15) on unsterilized seeds whereas, sterilized seeds of same samples showed the presence of 24 species from 17 genera (Table 16).

Among unsterilized seed lots, *Rhizopus* was the most prevalent genera as it was isolated from 28 samples with an incidence ranging upto 95 per cent whereas, among pathogenic fungi, *Fusarium solani* was fairly dominant. In Zone II *Myrothecium* and *Fusarium moniliformae* were detected from 66.7 per cent samples. Major pathogenic fungi viz. *C. lindemuthianum, S. sclerotiorum* and *R. solani* were recorded on 22.2-44.4 per cent of the total samples with maximum incidence (29%) of *C. lindemuthianum* on KB 7 (Plate 3).

In Zone III, out of 10 samples collected after harvest maximum of 10 fungi were recorded on KB 53 comprising of 4 storage and 6 field fungi. Major storage fungi were *Rhizopus* (60.0%) and *Penicillium* spp. (40.0%) whereas, *F. moniliformae* was recorded on 40 per cent of the total samples with 7-14 per cent incidence. In Zone IV, out of 8 samples analysed after harvest, KB 66 harboured 14 fungi comprising of 12 storage and 2 pathogenic fungi. In remaining samples, the range of mycoflora was 2-9 fungi showing 7-95 per cent incidence.

ble 15. Frequency (%) of seed mycoflora in randomly selected seeds (unsterilised) through Malt Extract Agar

Sample number	*Acremonium* sp.	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Botrytis* sp.	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Gliocladium* sp.	*Helminthosporium* sp.	*Heterosporium* sp.	*Macrophomina* sp.	*Monosporium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma* sp.	*Phymatotrichum* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	*Trichoderma* sp.	*Trichothecium* sp.	*Varicosporium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*
one II: *Samples collected after storage*																																					
KB 1	-	-	29	-	29	-	-	-	-	-	-	-	7	-	21	7	-	-	-	-	14	-	-	14	-	14	-	-	-	-	21	-	14	-	36	-	11
KB 2	-	14	21	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	4
KB 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	21	-	21	-	-	-	-	-	-	-	-	-	-	-	3
KB 4	-	-	21	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	14	-	14	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 5	-	21	-	-	-	21	-	21	-	36	-	-	7	-	21	28	14	-	-	-	14	-	-	-	7	-	-	21	-	-	-	14	-	-	21	-	13
KB 6	-	-	22	22	-	-	7	-	-	-	21	28	14	-	-	-	-	-	14	-	-	-	-	7	-	-	-	21	-	-	21	-	-	-	-	-	10
KB 7	21	-	-	-	-	21	-	-	7	-	29	-	14	7	-	-	-	14	-	-	7	-	-	-	-	7	21	-	21	-	-	7	-	7	-	-	13
KB 8	-	-	-	14	7	21	-	7	-	-	7	21	21	-	-	-	-	-	7	-	-	-	-	7	-	-	-	21	-	-	21	-	7	-	-	7	13
KB 9	-	29	-	21	-	7	21	-	7	-	21	-	36	-	21	-	14	-	-	-	7	-	-	-	-	7	7	-	7	-	21	-	-	-	36	-	15
%Sample mean	11.1	33.3	44.4	33.3	22.2	44.4	22.2	33.3	22.2	11.1	44.4	33.3	66.7	11.1	33.3	22.2	22.2	11.1	22.2	0	66.7	11.1	11.1	44.4	11.1	44.4	22.2	33.3	22.2	0	44.4	22.2	22.2	11.1	33.3	11.1	
Zone III: *Samples collected after harvest*																																					
KB 10	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-	14	-	14	-	-	-	-	-	-	4
KB 12	-	-	-	-	21	14	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	7	-	21	-	14	-	-	-	-	-	-	14	-	7
KB 15	-	-	-	7	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	36	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 16	-	-	43	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	21	-	-	-	-	43	-	-	-	-	-	-	4
KB 17	-	7	-	-	-	-	-	-	21	14	-	7	-	14	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
KB 18	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	2
KB 19	-	-	-	-	-	-	21	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	7	29	-	7	-	-	-	-	6
KB 20	7	7	-	-	43	-	7	-	7	-	-	-	14	-	-	-	-	-	-	-	-	-	7	-	-	21	-	-	-	21	-	-	-	-	-	-	9
KB 33	-	-	-	-	-	-	-	-	-	-	-	-	7	21	14	-	-	-	-	-	-	-	7	7	7	-	-	-	-	43	-	-	-	-	-	-	7
KB 53	-	7	-	22	-	-	-	-	-	21	-	-	-	7	-	-	7	-	-	7	-	-	-	-	-	-	21	-	7	7	-	-	-	-	36	-	10
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Sample designation after storage																																				
KB 11	-	-	29	-	14	-	-	-	7	-	7	7	36	7	-	-	-	-	14	-	-	-	-	14	-	-	-	-	21	-	21	-	14	-	-	-	12
KB 13	-	-	-	-	-	-	-	7	-	21	21	-	21	-	21	14	-	-	-	7	-	-	-	-	14	-	-	14	-	7	-	-	-	7	-	7	12
KB 14	-	-	21	7	36	-	7	7	-	-	36	28	7	7	-	-	7	-	-	7	14	7	-	21	-	7	-	7	-	7	-	-	14	-	21	-	19
KB 21	-	-	36	-	21	-	-	-	-	-	43	21	7	21	21	-	-	-	-	-	-	-	-	7	7	-	43	-	-	-	-	-	36	-	-	-	11
KB 22	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	7	-	-	-	-	86	-	-	-	-	-	-	4
KB 23	-	-	-	-	-	-	-	-	-	-	7	14	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	14	43	-	-	-	-	-	-	5
KB 24	7	-	-	-	-	-	-	7	-	-	-	-	-	36	-	-	-	-	-	-	-	-	-	-	-	-	-	29	21	21	7	-	-	-	-	-	7
KB 25	-	29	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 26	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	-	7	36	-	14	-	-	-	-	-	-	-	-	5
KB 27	-	29	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 28	-	7	14	-	14	-	-	7	-	-	7	7	-	-	14	-	-	-	-	-	14	-	-	-	-	21	-	-	-	29	21	-	-	-	-	-	11
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KB 31	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	21	-	21	-	-	-	-	-	-	-	-	36	-	-	5
KB 32	-	-	-	14	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 34	-	29	-	-	-	-	-	-	-	-	14	-	7	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	57	-	-	-	-	-	-	5
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KB 39	-	7	-	-	-	-	-	-	-	-	-	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	-	-	-	-	7	-	5
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KB 42	-	21	-	-	-	-	-	-	-	7	-	14	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	-	-	-	73	-	-	6
KB 43	-	7	14	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	29	-	-	-	-	-	-	5
KB 44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 45	-	14	21	21	21	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	21	-	-	-	36	-	-	-	-	-	-	9
KB 46	-	-	-	-	-	-	-	-	29	-	-	-	-	-	21	-	-	-	-	-	-	7	7	7	-	21	-	-	-	14	-	-	-	79	-	-	8
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KB 49	-	-	21	-	-	-	-	-	-	-	7	7	-	-	14	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	5
KB 50	-	-	-	36	-	-	-	14	-	-	36	-	-	7	-	-	-	-	7	-	-	-	-	-	-	-	-	7	-	7	-	-	-	-	-	-	7
KB 51	-	-	7	36	-	21	7	-	14	-	7	21	7	-	-	-	7	-	-	-	14	7	-	-	7	-	-	-	7	-	-	7	-	-	-	7	15

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54	-	29	21	14	7	-	7	-	-	-	-	-	-	-	43	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	2
mple ean	5.7	31.4	28.6	20.0	20.0	8.6	14.3	22.9	8.6	11.4	31.4	25.7	25.7	20.0	25.7	2.9	8.6	0.0	5.7	5.7	17.1	14.3	22.9	20.0	14.3	20.0	2.9	14.3	20.0	42.9	8.6	2.9	11.4	14.3	5.7	8.6							6
: IV: *Samples collected after harvest*																																											
B 55	-	-	-	-	-	-	-	-	-	21	14	-	-	-	29	-	-	-	7	-	-	21	7	7	-	-	-	-	14	-	-	-	-	-	-	-							8
B 56	-	-	-	-	-	-	-	-	-	-	-	-	86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95	-	-	-	-	-	-							2
B 57	-	-	50	21	7	7	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	50	-	-	-	-	-	-	95	-	-	-	-	-	-							7
KB 58	-	-	-	-	-	-	-	-	-	14	14	7	43	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	21	-							7
KB 59	-	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	14	-	-	-							3
KB 62	-	-	14	14	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	43	14	7	7	7	7	-	-	-	-	-	-	-	-	-							9
KB 65	-	-	-	-	-	-	-	-	-	36	-	-	21	7	-	-	-	-	-	-	-	-	-	7	7	-	-	-	14	-	-	29	-	-	-							7
KB 66	-	21	-	-	7	-	21	-	-	21	-	-	21	-	21	7	-	14	-	-	14	-	14	-	-	14	14	-	14	-	-	14	-	-	-							14
%Sample mean	0	25	25	25	25	12.5	12.5	0	0	50	25	12.5	50	12.5	50	25	0	12.5	12.5	0	25	25	50	37.5	25	25	12.5	0	62.5	25	0	37.5	0	0	12.5	0						
Samples collected after storage																																										
KB 60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							0
KB 61	-	14	-	-	-	-	-	-	21	21	21	7	21	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							7
KB 63	-	21	7	14	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	21	21	-	-	-	-	-	-	-	43	-	-	-	-	-	-							7
KB 64	-	7	7	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	14	-	-	-	-	-	-	-							6
KB 67	-	-	-	-	-	-	-	-	29	7	-	-	7	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-							6
KB 68	-	71	-	-	14	-	-	-	-	-	-	21	21	21	7	-	-	-	-	-	-	21	21	7	7	21	-	-	-	79	-	-	-	-	-	-							12
KB 69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	96	-	-	-	-	-	-							2
KB 70	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	7	14	-	-	7	21	-	-	7	-	7	14	-	-	14	-	-	-	-	7	-							10
KB 71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							0
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KB 73	-	7	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	7	36	-	-	-	21	21	-	7						
%Sample mean	0	45.5	18.2	27.3	18.2	0	0	0	18.2	27.3	18.2	18.2	27.3	18.2	18.2	9.1	9.1	9.1	9.1	9.1	18.2	27.3	27.3	18.2	9.1	18.2	9.1	0	27.3	45.5	0	0	0	18.2	18.2	0						

Seed samples collected after storage showed the presence of 19 and 12 fungi in Zone III and IV, respectively. KB 29 in Zone III and KB 60 and 71 in Zone IV were free of any infestation. On other samples, the number of fungal species ranged between 2-15 in Zone III and 2-10 in Zone IV.

Out of 73 surface sterilized randomly selected seed samples tested through agar plate method for internally seed borne mycoflora, 11 samples were free and remaining exhibited the presence of 1-7 species of storage and field fungi (Table 16).

In Zone II, KB 7 harboured maximum mycoflora comprising of 3 pathogenic and 2 storage fungi viz. *C. lindemuthianum, S.sclerotiorum* and *S. rolfsii* and *A. flavus, A.ochraceous,* respectively. Whereas, in remaining samples the extent of mycoflora ranged between 0-5 species/sample. Among various storage fungi isolated from different samples, *A. flavus* showed an incidence of 21 per cent on 11.1 per cent samples.

In Zone III samples collected after harvest and storage exhibited the occurrence of 5 fungal species at the most. Major fungi isolated were *Colletotrichum, Fusarium oxysporum* and *Rhizoctonia.* However, in Zone IV highest number of 7 fungi were recorded on KB 62 followed by 5 on KB 67. Other samples colonized 0-4 species individually. Overall data revealed the presence of comparatively less mycoflora in seed samples collected before and after storage from Zone IV.

4.1.9 Detection of seed mycoflora from bulk sample using Seed Component Plating Technique

Seed component plating technique was employed to locate the position of seed borne mycoflora in different kidney bean seed samples collected from various agro-ecological zones of Himachal Pradesh. Twenty five seeds/ sample were plated on malt extract agar medium to locate the mycoflora on various seed components (seed coat, cotyledons and embryonic axis) and frequency of each is presented in Tables 17,18 and 19. Twenty species of fungi belonging to 12 genera were detected from the seed coat and cotyledons whereas, only 9 were recorded on embryonic

Table 16. Frequency (%) of seed mycoflora in bulk sample (sterilized)* through Malt Extract Method

Sample number	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Botrytis* sp.	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Helminthosporium* sp.	*Heterosporium* sp.	*Macrophomina* sp.	*Monosporium* sp.	*Phoma* sp.	*Phymatotrichum* sp.	*Rhizoctonia solani*	*Stemphylium* sp.	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Zone II: *Samples collected after storage*																									
KB 1	-	-	-	-	21	-	-	-	-	14	-	-	-	29	-	-	-	-	-	-	29	-	-	-	4
KB 2	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 5	-	-	-	-	-	-	-	21	-	-	-	-	-	-	21	-	-	-	-	-	21	-	-	21	4
KB 6	29	-	-	-	-	-	-	-	-	14	29	21	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 7	-	21	-	-	29	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	-	14	21	5
KB 8	21	-	-	-	-	21	-	-	-	-	-	-	-	-	-	21	-	-	29	-	-	-	-	-	4
KB 9	-	-	-	-	-	-	-	-	-	29	-	-	21	14	-	-	-	-	-	21	-	-	-	-	4
%Sample mean	33.3	11.1	0	0	22.2	11.1	0	11.1	0	44.4	11.1	11.1	11.1	22.2	11.1	11.1	0	0	11.1	11.1	22.2	0	11.1	22.2	
Zone III: *Samples collected after harvest*																									
KB 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 12	7	-	-	-	-	-	7	-	-	-	-	-	14	-	-	-	-	-	-	21	-	-	-	7	5
KB 15	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 16	-	14	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	2
KB 17	21	-	-	-	-	-	-	7	-	-	14	-	14	-	-	-	-	-	-	-	-	-	-	-	4
KB 18	-	-	-	-	-	-	-	-	-	7	-	21	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 19	14	-	-	-	-	-	-	-	-	21	-	21	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 20	21	21	-	-	-	-	-	7	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	4
KB 33	-	-	-	-	-	-	-	-	-	14	-	-	-	*29*	-	-	-	-	-	-	-	-	-	-	*2*
KB 53	-	-	-	-	36	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	2
%Sample mean	50	20	0	0	10	0	10	20	0	30	10	20	40	20	0	0	0	0	0	10	0	0	0	10	

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mples collected after storage																									
(B 11	-	-	-	-	-	-	-	-	-	-	21	-	-	29	-	-	-	-	-	-	29	-	-	-	3
KB 13	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	21	-	-	-	2
KB 14	-	-	-	21	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	14	-	-	-	-	3
KB 21	-	-	-	-	-	-	-	-	-	14	-	-	-	7	-	-	-	-	21	-	-	-	-	-	3
KB 22	-	14	-	7	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 23	-	-	-	-	-	-	-	-	-	21	-	-	-	21	-	-	-	-	-	-	21	-	-	-	3
KB 24	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	7	7	-	-	21	4
KB 25	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 26	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 27	-	-	7	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	2
KB 28	7	-	-	14	-	-	-	-	-	7	-	-	-	14	-	-	-	-	-	-	14	-	-	-	5
KB 29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	1
KB 31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 32	-	-	-	-	-	-	-	-	-	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 35	-	21	-	-	29	-	-	-	-	7	-	-	-	-	14	-	-	-	-	14	-	-	-	-	5
KB 36	29	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	21	-	-	-	-	-	-	-	3
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KB 38	29	21	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	7	-	7	-	5
KB 39	-	-	-	-	-	-	7	-	-	-	-	-	7	-	-	-	-	-	-	-	7	-	-	-	3
KB 40	14	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	2
KB 41	-	-	-	-	-	-	-	-	36	14	-	-	-	-	-	-	-	-	21	-	-	-	-	-	3
KB 42	-	-	14	-	-	50	-	-	-	21	-	-	-	-	-	-	-	-	-	-	14	-	-	-	4
KB 43	7	-	-	-	-	-	-	-	-	7	-	-	14	-	-	-	-	-	-	-	-	-	-	-	3
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KB 45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 46	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
KB 47	29	14	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	3
KB 48	-	-	-	-	-	-	-	-	-	7	-	-	-	14	-	-	-	-	-	-	7	-	-	-	3
KB 49	-	-	-	-	-	-	-	-	-	7	-	-	14	-	-	-	-	-	-	-	7	-	-	-	3
KB 50	-	-	-	-	-	-	-	-	-	7	14	-	-	7	-	-	-	-	-	-	-	-	-	-	3

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
B 51	-	-	-	-	21	21	-	-	-	-	21	-	21	-	-	-	-	-	-	-	-	-	-	-	4
B 52	14	-	-	-	29	-	-	-	-	-	-	-	21	-	-	-	-	-	-	14	-	-	-	-	4
B 54	-	-	21	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	29	3
Sample mean	31.4	11.4	8.6	8.6	8.6	5.7	5.7	0.0	2.9	48.6	11.4	0.0	20.0	22.9	5.7	0.0	2.9	0.0	5.7	11.4	31.4	2.9	2.9	5.7	
one IV: *Samples collected after harvest*																									
KB 55	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	1
KB 56	-	-	-	-	-	-	-	-	7	7	-	-	21	-	-	-	-	-	-	-	-	-	-	-	3
KB 57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 58	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	1
KB 59	-	-	-	-	-	-	-	-	14	-	-	-	14	-	-	-	-	14	-	-	-	-	-	-	3
KB 62	21	-	-	-	-	-	-	57	36	-	-	-	14	29	-	-	14	-	14	-	-	-	-	-	7
KB 65	7	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	3
KB 66	-	-	-	-	14	-	-	-	42	-	-	-	-	14	-	-	-	-	-	-	-	-	14	-	4
%Sample mean	25	12.5	0	0	12.5	0	0	12.5	50	12.5	0	0	50	37.5	0	0	12.5	12.5	12.5	0	12.5	0	12.5	0	
Samples collected after storage																									
KB 60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	1
KB 61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 64	14	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	2
KB 67	-	-	-	-	-	-	-	14	7	7	-	-	-	29	-	-	-	-	7	-	-	-	-	-	5
KB 68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 70	7	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	7	-	4
KB 71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	21	-	2
KB 72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 73	*21*	-	-	-	-	-	-	-	-	*7*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*2*
%Sample mean	*27.3*	*0*	*0*	*0*	*0*	*0*	*9.1*	*9.1*	*9.1*	*18.2*	*0*	*0*	*0*	*9.1*	*9.1*	*0*	*0*	*0*	*9.1*	*0*	*27.3*	*0*	*18.2*	*0*	

* Sterilized with sodium hypochlorite

axis. Field/ pathogenic fungi dominated the storage mycoflora in different components of seeds.

Seed coat: Mycoflora observed on seed coat resembled with the one detected by employing other methods of detection viz. blotter method and agar plate method with variable frequency. Data of mycoflora on seed coat in samples of Zone II revealed that seed coat of KB 4 harboured maximum mycoflora (12) followed by KB 2, 3 and 9 (Table 17) whereas, it was least on KB 1 (2). Incidence of various fungi ranged between 2-16 per cent. *Alternaria* and *C. lindemuthianum* were observed on maximum number of samples in Zone II. Four species of *Aspergillus* viz. *A. niger, A. flavus, A. fumigatus* and *A. ochraceous* were isolated from 22.22 to 44.44 per cent of the total samples with an incidence of 4-12 per cent (Plate 4).

Ten seed samples collected immediately after harvest from Zone III possessed more mycoflora as compared to those which were analysed after storage. Seed coats of KB 17, 19 and 53 showed the presence of 8 fungal species followed by KB 10 (7) whereas, minimum fungi were recorded on KB 12 and 15. *C. lindemuthianum* was found in KB 7 with 8 to 16 per cent incidence.

Seed samples collected after storage exhibited 2-8 fungal species, except KB 54 which was free of any fungal infestation. Frequency of *F. oxysporum* and *Alternaria* was higher as compared to other field fungi.

Seed coats of Zone IV samples showed similar trend as maximum number of fungal species were encountered in pre-stored samples, highest being in sample KB 62. However, stored seed samples possessed comparatively less mycoflora.

Cotyledons: Among 9 samples of Zone II, KB1, 8 and 9 possessed more mycoflora as compared to their seed coats followed by KB 4 (Table 18). However, KB 2 and 6 harboured minimum fungal flora with almost similar frequency as was observed on seed coats. *A. ochraceous* and *A. niger* were detected from 6 samples showing 4 to 12 per cent incidence.

ıble 17. Frequency (%) of seed mycoflora through seed component plating technique (seed coat)

Sample number	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Botrytis* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* sp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Macrophomina* sp.	*Penicillium simplicisimum*	*P. perpuregenum*	*Phoma* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Zone II: *Samples collected after storage*																					
KB 1	8	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 2	4	4	-	12	-	8	4	-	-	-	-	4	-	-	-	-	-	-	-	-	6
KB 3	4	-	-	12	-	-	-	-	-	-	-	-	12	-	-	4	4	-	8	-	6
KB 4	4	12	4	4	12	4	-	8	-	-	4	-	4	-	4	-	16	-	-	4	12
KB 5	12	-	12	-	-	-	-	16	-	-	-	16	-	-	-	-	-	-	-	4	5
KB 6	-	12	-	-	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 7	8	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	12	-	8	-	4
KB 8	-	-	12	-	-	-	-	-	-	-	-	-	4	-	-	4	4	-	-	12	5
KB 9	-	12	-	12	-	12	-	16	-	-	-	-	-	4	-	-	-	-	-	4	6
% Sample mean	66.7	44.4	33.3	44.4	22.2	33.3	22.2	55.6	0.0	0.0	11.1	22.2	33.3	11.1	11.1	22.2	44.4	0.0	22.2	44.4	
Zone III: *Samples collected after harvest*																					
KB 10	8	-	-	16	-	-	4	4	4	-	12	4	-	-	-	-	-	-	-	-	7
KB 12	4	-	-	-	-	-	-	4	-	-	8	-	-	-	-	-	-	-	-	-	3
KB 15	-	-	-	-	8	-	-	4	-	-	-	-	-	-	-	4	-	-	-	-	3
KB 16	8	8	-	-	-	-	-	4	-	4	-	-	-	-	-	-	16	-	-	4	6
KB 17	8	4	-	4	-	4	8	4	-	-	8	4	-	-	-	-	-	-	-	-	8
KB 18	-	8	-	-	-	-	-	-	-	-	-	8	4	4	-	-	-	-	-	-	4
KB 19	4	12	-	-	-	-	-	4	-	-	-	-	-	-	4	4	12	-	8	4	8
KB 20	-	-	-	-	-	-	-	4	-	4	4	4	-	-	-	-	-	-	-	-	4
KB 33	16	-	-	-	8	-	-	-	-	-	4	-	-	-	-	-	12	-	-	-	4
KB 53	4	-	-	4	4	-	-	-	-	4	8	4	12	-	-	-	8	-	-	-	8
% Sample mean	70.0	40.0	0.0	30.0	30.0	10.0	20.0	70.0	10.0	30.0	60.0	50.0	20.0	10.0	10.0	20.0	40.0	0.0	10.0	20.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Samples collected after storage																					
KB 11	12	-	-	-	-	-	-	-	-	-	-	-	12	-	-	4	-	-	-	-	3
KB 13	-	-	-	-	-	-	-	-	-	-	12	-	12	-	-	12	-	-	-	-	3
KB 14	16	-	-	12	-	8	-	-	-	-	12	-	-	-	-	-	-	-	-	-	4
KB 21	4	-	-	-	-	-	-	4	-	-	12	-	-	-	-	-	-	-	-	8	4
KB 22	-	-	-	8	-	4	-	12	-	-	-	8	-	-	-	-	4	-	-	-	5
KB 23	12	-	-	-	-	-	-	4	-	4	8	-	-	-	-	-	-	-	-	-	4
KB 24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-	-	2
KB 25	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	8	4	-	-	3
KB 26	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	3
KB 27	-	8	-	4	-	-	-	-	-	4	8	4	-	-	-	-	-	-	-	-	5
KB 28	4	-	-	-	-	-	-	4	-	-	4	-	-	-	-	-	4	-	-	12	5
KB 29	-	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 30	4	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	8	-	-	-	3
KB 31	4	-	-	-	-	-	-	-	4	4	4	4	-	-	-	-	-	-	-	-	5
KB 32	-	-	-	-	-	-	-	4	-	-	4	4	-	-	-	8	-	-	-	-	4
KB 34	4	-	-	8	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	3
KB 35	8	12	-	-	4	-	12	16	-	-	8	-	4	-	-	-	4	-	-	-	8
KB 36	-	-	-	8	-	12	-	16	-	-	-	16	-	-	-	-	-	-	-	-	4
KB 37	-	12	4	12	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 38	4	-	-	12	-	-	-	-	-	4	-	-	-	-	-	12	-	-	-	-	4
KB 39	-	8	-	-	-	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	3
KB 40	-	4	-	-	4	4	-	8	-	-	-	8	12	-	-	-	-	-	-	-	6
KB 41	8	-	-	-	-	4	-	-	-	-	8	-	-	-	-	-	12	-	-	-	4
KB 42	-	12	-	8	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 43	4	4	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	8	-	4
KB 44	8	-	-	12	4	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	5
KB 45	4	-	-	-	12	-	-	-	-	-	12	-	-	-	-	-	4	-	-	8	5
KB 46	-	-	4	-	-	-	-	-	-	-	12	4	-	-	-	-	-	-	-	-	3
KB 47	8	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 48	-	-	-	-	-	-	-	16	-	-	16	-	-	-	-	-	-	-	-	-	2
KB 49	-	-	-	-	-	-	-	4	-	-	4	-	-	-	-	-	-	-	-	-	2
KB 50	-	8	4	4	-	-	-	4	-	-	-	4	-	-	-	-	-	-	-	-	5

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
KB 51	-	-	4	-	4	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	3
KB 52	12	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
% Sample mean	48.6	25.7	11.4	34.3	20.0	20.0	5.7	40.0	2.9	17.1	42.9	28.6	11.4	0.0	0.0	14.3	22.9	5.7	2.9	8.6	
Zone IV: ***Samples collected after harvest***																					
KB 55	-	-	-	8	8	4	-	8	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 56	-	-	-	-	-	-	-	-	-	-	-	8	8	-	-	4	8	-	-	-	4
KB 57	8	-	-	-	-	-	-	16	-	-	8	-	-	-	-	-	16	-	-	-	4
KB 58	-	-	-	8	-	-	-	4	-	-	8	-	-	-	-	-	16	-	16	-	5
KB 59	-	16	8	-	-	8	-	-	12	-	-	-	-	-	-	-	8	-	12	-	6
KB 62	8	-	-	-	-	-	12	4	4	-	-	-	12	-	-	4	8	-	-	-	7
KB 65	8	-	-	8	8	-	-	4	-	-	8	-	-	-	-	-	8	-	-	-	6
KB 66	-	-	-	-	-	-	-	4	-	4	4	-	-	-	-	-	4	-	4	12	6
% Sample mean	37.5	12.5	12.5	37.5	25.0	25.0	12.5	75.0	25.0	12.5	50.0	12.5	25.0	0.0	0.0	25.0	87.5	0.0	37.5	12.5	
Samples collected after storage																					
KB 60	-	-	-	-	-	-	-	8	-	-	-	12	-	-	8	-	-	-	-	-	3
KB 61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	1
KB 63	-	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	4	-	4	4	5
KB 64	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	2
KB 67	-	8	-	-	12	-	-	-	8	-	-	-	-	4	-	-	-	-	-	-	4
KB 68	4	-	-	-	-	-	-	4	-	-	4	8	-	-	-	-	-	-	12	-	5
KB 69	-	16	-	8	-	4	-	8	-	-	-	-	-	-	-	-	4	-	4	-	6
KB 70	-	-	-	8	-	-	12	4	-	-	-	-	4	-	-	4	12	-	-	-	6
KB 71	-	-	-	-	-	-	-	-	-	-	12	8	-	-	-	-	-	-	-	-	2
KB 72	-	-	-	8	4	-	-	-	-	-	8	-	-	-	-	-	4	-	-	-	4
KB 73	-	-	-	12	12	-	-	-	-	-	-	-	4	-	8	-	-	-	-	-	4
% Sample mean	18.2	27.3	0.0	45.5	27.3	9.1	9.1	36.4	9.1	0.0	27.3	27.3	18.2	9.1	18.2	9.1	54.5	0.0	27.3	9.1	

Table 18. Frequency (%) of seed mycoflora through seed component plating technique (cotyledon)

Sample number	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Botrytis* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* sp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Helminthosporium* sp.	*Macrophomina* sp.	*Monosporium* sp.	*Penicillium* spp.	*Phoma* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Sclerotinia sclerotiorum*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Zone II: *Samples collected after storage*																					
KB 1	4	-	-	-	4	-	-	12	-	-	-	-	-	12	-	-	4	4	12	-	7
KB 2	16	-	-	-	-	4	-	-	-	-	4	-	-	-	-	-	-	-	-	-	3
KB 3	-	8	-	8	4	-	-	8	-	-	-	-	-	-	-	-	-	-	-	12	5
KB 4	-	-	-	4	4	-	8	-	-	-	4	12	8	-	-	-	-	-	-	-	6
KB 5	-	4	-	8	8	-	-	-	-	-	-	-	-	-	-	-	-	4	12	-	5
KB 6	4	-	-	12	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3
KB 7	8	8	-	8	4	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	5
KB 8	4	-	-	-	12	-	-	16	-	-	16	-	-	12	-	-	4	-	12	-	7
KB 9	12	-	-	4	-	12	-	4	-	-	-	8	4	-	-	-	-	8	-	-	7
% Sample mean	66.7	33.3	0.0	66.7	66.7	22.2	11.1	44.4	0.0	0.0	33.3	22.2	33.3	22.2	0.0	11.1	22.2	33.3	33.3	11.1	
Zone III: *Samples collected after harvest*																					
KB 10	-	-	-	-	-	-	-	8	-	-	12	-	-	-	-	-	-	-	-	-	2
KB 12	8	-	-	-	-	-	-	12	-	-	12	-	-	-	-	-	-	-	-	-	3
KB 15	-	-	-	-	-	-	-	4	-	-	8	-	-	-	-	4	8	-	-	-	4
KB 16	4	-	-	-	-	-	-	12	-	8	12	-	-	-	-	-	-	16	-	-	5
KB 17	8	4	-	8	-	-	8	8	-	-	8	4	-	-	-	-	-	-	-	-	7
KB 18	4	-	-	-	-	-	4	-	-	-	-	8	8	-	-	-	-	-	-	-	4
KB 19	8	-	-	-	8	-	-	12	-	-	-	-	-	12	-	-	-	12	-	12	6
KB 20	-	8	-	8	-	-	-	-	-	-	12	4	-	-	-	-	-	-	-	-	4
KB 33	-	-	-	-	-	-	-	4	-	-	12	-	-	-	-	-	-	-	-	-	2
KB 53	-	8	-	-	-	-	8	12	8	-	-	8	4	4	-	-	-	-	8	-	8
% Sample mean	50.0	30.0	0.0	20.0	10.0	0.0	30.0	80.0	10.0	10.0	70.0	40.0	20.0	20.0	0.0	10.0	10.0	20.0	10.0	10.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Samples collected after storage																					
KB 11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 14	-	4	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 21	-	-	-	-	-	-	-	4	-	-	42	-	-	-	-	8	-	-	-	-	3
KB 22	-	-	-	-	-	8	-	4	-	-	8	-	-	-	-	-	-	-	-	-	3
KB 23	4	-	-	-	-	-	8	4	4	-	-	4	-	-	-	-	-	-	-	-	5
KB 24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	12	-	-	2
KB 25	-	8	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	2
KB 26	-	-	-	-	4	-	-	-	-	-	8	4	-	-	-	-	4	-	-	-	4
KB 27	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	1
KB 28	12	-	-	-	-	-	-	12	-	-	12	-	-	-	12	-	-	12	-	-	5
KB 29	-	4	-	4	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 30	4	-	-	-	-	-	4	-	-	-	-	-	-	-	-	4	-	-	-	-	3
KB 31	8	-	-	-	-	-	8	-	-	4	8	-	-	-	-	-	-	-	-	-	4
KB 32	-	4	-	-	-	-	-	4	-	-	4	4	-	-	-	-	-	-	-	-	4
KB 34	4	12	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	3
KB 35	-	8	-	8	-	4	-	-	-	-	-	-	-	4	-	-	-	-	12	-	5
KB 36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 39	8	-	-	-	-	4	-	-	-	-	12	-	8	-	-	-	-	4	-	-	5
KB 40	8	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	4
KB 41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 43	-	4	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	2
KB 44	8	-	-	-	-	-	8	4	-	-	-	-	4	-	-	-	-	-	-	-	4
KB 45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	4	2
KB 46	-	-	-	-	-	-	-	4	-	-	12	-	-	-	-	-	-	-	-	-	2
KB 47	8	12	-	-	12	8	-	16	-	16	16	8	-	-	-	-	-	-	-	-	8
KB 48	4	-	-	-	-	-	-	12	-	-	12	12	-	-	-	-	-	-	-	-	4
KB 49	8	-	-	-	8	-	-	-	-	-	4	4	-	-	-	-	-	-	12	-	5
KB 50	-	8	-	8	-	-	-	8	-	-	4	4	-	-	-	-	-	-	-	-	5

Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
KB 51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
KB 52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	8	2
KB 54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	1
% Sample mean	31.4	28.6	0.0	11.4	8.6	11.4	11.4	40.0	2.9	5.7	37.1	22.9	5.7	2.9	2.9	8.6	5.7	11.4	11.4	5.7		
Zone IV: *Samples collected after harvest*																						
KB 55	-	16	-	-	12	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	4
KB 56	-	-	-	-	-	-	-	8	-	4	8	-	-	-	-	-	-	12	-	-	-	4
KB 57	-	-	-	-	-	-	-	8	-	-	12	-	-	-	-	4	-	8	-	-	-	4
KB 58	-	-	-	4	-	-	-	4	-	-	8	-	-	-	-	-	-	-	-	-	8	4
KB 59	-	-	-	-	-	-	-	4	-	-	-	-	-	4	-	-	-	4	-	4	4	4
KB 62	-	42	-	8	4	-	-	4	-	-	-	-	-	-	-	-	-	8	-	-		5
KB 65	12	8	-	-	-	4	-	8	-	-	8	-	-	-	-	-	-	4	-	-		6
KB 66	-	-	-	4	-	-	-	8	-	-	12	8	-	8	-	-	4	8	-	4		8
% Sample mean	12.5	37.5	0.0	37.5	25.0	25.0	0.0	100.0	0.0	12.5	62.5	12.5	0.0	25.0	0.0	12.5	12.5	75.0	0.0	37.5		
Samples collected after storage																						
KB 60	-	-	4	8	-	-	-	12	-	-	8	-	-	-	-	-	-	-	-	8	-	5
KB 61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-		2
KB 63	-	-	-	12	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-		2
KB 64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	4	-		2
KB 67	-	-	-	-	-	-	-	-	-	4	8	4	-	-	-	-	-	-	-	-		3
KB 68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0
KB 69	-	-	-	-	-	-	-	12	-	-	-	-	8	-	-	-	-	8	-	4		4
KB 70	-	-	-	12	-	8	8	4	-	-	-	-	-	-	-	-	8	16	-	-		6
KB 71	-	4	-	-	-	-	-	-	-	8	-	-	-	-	-	12	-	-	-	-		3
KB 72	-	8	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		2
KB 73	4	-	-	12	4	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-		4
Sample Mean	9.1	18.2	9.1	36.4	18.2	9.1	9.1	36.4	0.0	18.2	18.2	9.1	18.2	0.0	0.0	9.1	18.2	36.4	18.2	9.1		

Frequency of fungal flora on cotyledons of Zone III and Zone IV samples was almost similar. Eight samples collected after storage were free from any infestation in Zone III, whereas, only one sample was found free in Zone IV. KB 53 and 66 harboured maximum of 8 species in Zone III and IV, respectively. The storage fungi were more predominant on stored lots as compared to field/pathogenic fungi with almost equal frequency.

Embryonic axis: Embryonic axis of different seed samples of various agro-climatic zones were found to possess minimum mycoflora and the incidence ranged from 0 to 40 per cent (Table 19). Forty one samples were free of any infestation and remaining 32 harboured 1-6 fungal species. *C. lindemuthianum* was the major field fungi isolated from 16 samples followed by *F. oxysporum* whereas, *Aspergillus flavus* and *A. niger* dominated the storage fungi as they were recorded from12 and 11 samples, respectively. The frequency of mycoflora was more or less similar in pre-stored and stored samples of different zones.

4.2 Pathological Significance

Seed borne fungi, isolated from various seed lots of kidney bean (Plate 5,6), were evaluated for their pathogenic potential by inoculating or treating the seeds/ seedlings of the susceptible variety `Kanchan` with culture filtrates and spore suspension of the individual species. Different types of symptoms were observed on seeds and seedlings, when treated with spore suspension and culture filtrates of isolated seed mycoflora (Tables 20, 21, 22 and 23).

Seed smearing with the fungal spore suspension was found to reduce the germination by 5-50 per cent after 7-13 days of incubation and with culture filtrates showing 4-58 per cent decrease during same incubation period. Not only germination of the seeds was reduced or inhibited by spore suspension but necrosis and blight symptoms on germinating seedlings of such seeds were also observed. However, culture filtrates of all these fungi inhibited seed germination to variable extent.

Table 19. Frequency (%) of seed mycoflora through seed component plating technique (embryonic axis)

Sample number	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*A. ochraceous*	*Botrytis* sp.	*Colletotrichum lindemuthianum*	*Fusarium. oxysporum*	*Macrophomina* sp.	*Sclerotinia sclerotiorum*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Zone II: ***Samples collected after storage***										
KB 1	8	8	12	-	4	4	4	-	-	6
KB 2	-	4	-	-	-	-	-	-	-	1
KB 3	8	-	-	-	-	-	4	-	-	2
KB 4	-	-	-	-	-	-	-	-	-	0
KB 5	-	-	-	-	-	-	-	-	-	0
KB 6	-	-	8	-	4	12	-	8	-	4
KB 7	-	-	-	-	-	-	-	-	-	0
KB 8	-	-	-	-	-	16	-	-	-	1
KB 9	-	-	-	-	-	4	4	-	-	2
% Sample mean	22.2	22.2	22.2	0.0	22.2	44.4	33.3	11.1	0.0	
Zone III: ***Samples collected after harvest***										
KB 10	-	-	-	-	-	-	-	-	-	0
KB 12	-	-	-	-	-	-	-	-	-	0
KB 15	-	-	8	-	-	4	-	-	-	2
KB 16	4	-	-	-	-	4	4	-	-	3
KB 17	-	-	8	-	-	-	-	-	-	1
KB 18	-	-	-	-	-	-	4	-	-	1
KB 19	-	4	4	-	-	-	-	-	-	2
KB 20	-	-	-	-	-	-	-	-	-	0
KB 33	-	-	-	-	-	-	4	-	-	1
KB 53	-	-	-	-	-	-	-	-	-	0
% Sample mean	10.0	10.0	30.0	0.0	0.0	20.0	30.0	0.0	0.0	
Samples collected after storage										
KB 11	-	-	4	-	-	4	-	-	-	2
KB 13	-	-	-	-	-	-	-	-	-	0
KB 14	-	-	-	-	-	-	-	-	-	0
KB 21	-	-	-	-	-	-	-	-	-	0
KB 22	-	-	-	-	-	-	-	-	-	0
KB 23	-	-	-	-	-	-	-	-	-	0
KB 24	-	-	-	-	-	-	-	-	-	0
KB 25	-	-	-	-	-	-	-	-	-	0
KB 26	-	-	-	-	-	-	4	-	-	1
KB 27	-	4	4	-	-	-	8	-	-	3
KB 28	-	-	4	-	-	4	-	-	-	2
KB 29	-	-	-	-	-	-	-	-	-	0
KB 30	-	-	-	-	-	-	-	-	-	0
KB 31	-	8	-	-	-	4	-	-	-	2
KB 32	-	4	-	-	-	4	-	-	-	2
KB 34	-	4	8	-	-	-	-	-	-	2

Continued

1	2	3	4	5	6	7	8	9	10	11
KB 35	-	-	-	-	-	-	-	-	-	0
KB 36	-	-	-	-	-	-	-	-	-	0
KB 37	-	-	-	-	4	-	12	-	-	2
KB 38	-	-	-	-	-	-	-	-	-	0
KB 39	-	-	-	-	-	-	-	-	-	0
KB 40	-	-	-	-	-	-	-	-	-	0
KB 41	-	-	-	-	-	-	-	-	-	0
KB 42	-	-	-	-	-	-	-	-	-	0
KB 43	-	4	-	-	-	-	-	-	-	1
KB 44	-	-	-	-	-	4	-	-	4	2
KB 45	-	-	-	-	-	-	-	-	-	0
KB 46	-	-	-	-	-	-	-	-	-	0
KB 47	-	-	-	-	-	-	-	-	-	0
KB 48	-	-	-	-	-	4	-	-	-	1
KB 49	-	-	-	-	-	4	4	-	-	2
KB 50	-	-	-	-	-	-	8	-	-	1
KB 51	-	-	-	-	-	-	-	-	-	0
KB 52	-	-	-	-	-	-	-	-	-	0
KB 54	-	-	-	-	-	-	-	-	-	0
% Sample mean	0.0	14.3	11.4	0.0	2.9	20.0	14.3	0.0	2.9	
Zone IV: ***Samples collected after harvest***										
KB 55	-	12	4	8	-	8	-	-	-	4
KB 56	-	-	-	-	-	4	-	-	-	1
KB 57	-	-	-	-	-	-	-	-	-	0
KB 58	-	-	4	-	-	-	-	-	-	1
KB 59	-	-	-	-	-	-	-	-	-	0
KB 62	4	12	-	-	-	-	-	-	-	2
KB 65	-	-	-	-	-	-	-	-	-	0
KB 66	-	-	-	-	-	-	-	-	-	0
% Sample mean	12.5	25.0	25.0	12.5	0.0	25.0	0.0	0.0	0.0	
Samples collected after storage										
KB 60	-	-	-	-	-	-	4	-	-	1
KB 61	-	-	-	-	-	-	-	-	-	0
KB 63	-	-	-	-	-	-	-	-	-	0
KB 64	-	-	-	-	-	-	-	-	-	0
KB 67	-	4	-	-	-	-	4	-	-	2
KB 68	-	-	-	-	-	-	-	-	-	0
KB 69	16	-	-	-	-	40	8	-	-	3
KB 70	-	-	-	-	-	-	-	-	-	0
KB 71	-	4	-	-	-	-	-	-	-	1
KB 72	-	-	-	-	-	-	-	-	-	0
KB 73	-	-	-	-	-	-	-	-	-	0
% Sample mean	9.1	18.2	0.0	0.0	0.0	9.1	27.3	0.0	0.0	

Rhizoctonia, Sclerotinia and *S. rolfsii* induced stem necrosis and root rot symptoms (Table 20).

Healthy susceptible seedlings, when treated with spore suspension (Plate 7)and culture filtrates by seedling dip method showed that different fungi induced various types of abnormalities (Table 21) ranging from appearance of water soaked lesions to death of seedlings. Fungi like *C. lindemuthianum* caused severe necrosis and death of seedlings whereas, *F. oxysporum* induced wilting type of symptoms. However, all other pathogenic fungi induced slight to severe root rot symptoms after dip treatment with spore suspension within 7-16 days of incubation. Culture filtrates of such fungi invariably caused stem and root rot of seedlings and ultimately death of the seedlings (Plate 8,9).

Purified cultures of individual storage/field fungi detected from seed samples of different locations were also evaluated for their disease causing ability by inoculating seed/seedling with spore suspension and culture filtrates and the data are presented in Tables 22-23.

It was noticed that spore suspension of these fungi had least effect on seed germination except *Aspergillus sulphureous*, *Penicillium* sp. and *Cladosporium*. However, culture filtrates of *Aspergillus* and *Penicillium* species reduced the germination drastically as compared to other fungi.

Spore suspension of *Acremonium, Myrothecium, P. capsulatum, P. griseo-fulvum, P. simplicissimum* and *P. perpuragenum* had no effects on seeds but their culture filtrates caused seed rot except *Acremonium* and *Myrothecium* which induced slight necrosis of the hypocotyle and radical of the germinating seedlings (Table 22).

Spore suspension and culture filtrates of the fungi also induced variable symptoms on seedlings of healthy susceptible variety over a period of 7-16 days (Table 23). Out of 19 species tested, spore suspension of 10 species had no effect on seedling health, however, *Fusarium* and *Rhizopus* treated seedlings showed various types of abnormalities on all the seedlings.

able 20. Pathological significance of seed fungi using seed smearing method

Fungi	Germination (%)			Incubation period (Days)	Effect of	
	Spore suspension/ Mycelial suspension	Culture filtrate	Sterilized distilled water		Spore suspension/ Mycelial suspension	Culture filtrate
Botrytis sp.	95.0 (77.22)	96.0 (78.69)	100.0 (89.96)	10	Inhibition of seed germination and radical	Hypocotyls rot
Colletotrichum lindemuthianum	68.0 (55.53)	55.0 (47.85)	100.0 (89.96)	7	Seed and seedling rot	Seed and seedling rot
Fusarium moniliformae	50.0 (44.98)	55.0 (47.85)	100.0 (89.96)	9	Seed rot and seedling blight	Necrosis of germinating seed
F. oxysporum	50.0 (44.98)	42.0 (40.38)	100.0 (89.96)	8	Hypocotyls having sunken light brown lesions with dark margins, creamish fungal growth in centre	Partial sunken light brown lesions with dark margins showing creamish fungal growth in centre
F. solani	55.0 (47.85)	50.0 (44.98)	100.0 (89.96)	10	Inhibition of seed germination and radical necrosis showing reddish discolouration	Inhibition of seed germination
Macrophomina sp.	82.0 (64.89)	75.0 (59.99)	100.0 (89.96)	10	Inhibition of seed germination and Seedling rot	Seedling rot
Phoma sp.	62.0 (51.93)	65.0 (53.71)	100.0 (89.96)	13	Seed rot	Inhibition of seed germination
Rhizoctonia solani	55.0 (47.85)	42.0 (40.38)	100.0 (89.96)	11	Decreased seed germination	Stem necrosis and root rot
Sclerotinia sclerotiorum	70.0 (56.77)	72.0 (58.04)	100.0 (89.96)	10	Stem and root rot	Stem and root rot
Sclerotium rolfsii	75.0 (59.99)	70.0 (56.77)	100.0 (89.96)	7	Severe stem rot	Severe stem rot
Tricothecium sp.	90.0 (71.59)	80.0 (63.43)	100.0 (89.96)	13	No effect	Seedling rot
CD (P=0.05)	(2.53)	(2.51)	(NS)			

Figures in parentheses are the angular transformed values

Table 21. Pathological significance of seed fungi using seedling dip method

Fungi	Germination (%)			Incubation period (Days)	Effect of	
	Spore suspension	Culture filtrate	Sterilized distilled water		Spore suspension	Culture filtrate
Botrytis sp.	80.0 (63.43)	25.0 (29.98)	100.0 (89.96)	10	Slight stem rot	Stem and root rot
Colletotrichum lindemuthianum	100.0 (89.96)	100.0 (89.96)	100.0 (89.96)	9	Severe rotting of seedlings	Severe rotting of seedlings
Fusarium moniliformae	60.0 (50.75)	6/14 (30.0)	100.0 (89.96)	12	stem rot	stem rot
F. oxysporum	80.0 (63.43)	100.0 (89.96)	100.0 (89.96)	10	Severe rotting of seedlings	Severe rotting of seedlings
F. solani	15.0 (22.75)	100.0 (89.96)	100.0 (89.96)	12	Stem rot	Stem rot
Macrophomina sp.	40.0 (39.21)	50.0 (44.98)	100.0 (89.96)	8	Severe stem rot	Severe rotting of seedlings
Phoma sp.	30.0 (33.19)	65.0 (53.71)	100.0 (89.96)	9	Stem rot	Severe rotting of seedlings
Rhizoctonia solani	20.0 (26.54)	100.0 (89.96)	100.0 (89.96)	11	Root rot	Stem and root rot
Sclerotinia sclerotiorum	15.0 (22.75)	50.0 (44.98)	100.0 (89.96)	10	Severe stem rot	Stem and root rot
Sclerotium rolfsii	25.0 (29.98)	75.0 (59.99)	100.0 (89.96)	8	Severe stem rot	Severe stem rot
Tricothecium sp.	40.0 (40.0)	50.0 (44.98)	100.0 (89.96)	11	Slight stem rot	Slight stem rot
CD (P=0.05)	(2.21)	(1.65)	(NS)			

Figures in parentheses are the angular transformed values

Number of seedlings = 20

Table 22. Pathological significance of storage/ field mycoflora using seed smearing method

Fungi	Germination (%)			Incubation period (Days)	Effect of	
	Spore suspension	Culture filtrate	Sterilized distilled water		Spore suspension	Culture filtrate
1	2	3	4	5	6	7
Acremonium sp.	100.0 (89.96)	100.0 (89.96)	100.0 (89.96)	7	No effect	No effect
Alternaria sp.	95.0 (77.22)	90.0 (71.59)	100.0 (89.96)	8	Discoloration of hypocotyl region	Seed ,and seedling rot, redish brown lesions covered with creamish fungal mass
Aspergillus flavus	97.0 (80.42)	9.0 (17.38)	100.0 (89.96)	9	Seed ,and seedling rot	Seed rot
A. fumigatus	80.0 (63.43)	7.0 (15.23)	100.0 (89.96)	9	Extensive necrosis on cotyledons and stem	Seed rot
A. niger	100.0 (89.96)	10.0 (18.37)	100.0 (89.96)	7	Extensive sporulation on cotyledons, Slight necrosis on stem	Slight necrosis on stem,root rot of brownish colour
A. ochrareous	100.0 (89.96)	10.0 (18.37)	100.0 (89.96)	10	Slight necrosis on stem	Slight necrosis on stem
A. sulphureous	70.0 (56.77)	7.0 (15.23)	100.0 (89.96)	10	Slight necrosis on stem, inhibition of seed germination	inhibition of seed germination, Seed rot
Cephalosporium sp.	75.0 (59.99)	70.0 (56.77)	100.0 (89.96)	12	Root rot Light brown patches on stem	Root rot Light brown patches on stem
Cladosporium sp.	70.0 (56.77)	80.0 (63.42)	100.0 (89.96)	10	Root rot Light brown patches on stem	Root rot Light brown patches on stem
Fusarium spp.	98.0 (83.41)	95.0 (77.22)	100.0 (89.96)	16	Slight necrosis on stem	Slight necrosis on stem
Gliocladium sp.	80.0 (63.43)	85.0 (67.21)	100.0 (89.96)	11	Root rot	Root rot

Continued

1	2	3	4	5	6	7
yrothecium sp.	98.0 (83.41)	95.0 (77.22)	100.0 (89.96)	16	No effect	Root and stem necrosis (traces)
enicillium sp.	70.0 (56.77)	7.0 (15.23)	100.0 (89.96)	10	Slight necrosis on stem	Seed rot
?. capsulatum	100.0 (89.96)	9.0 (17.38)	100.0 (89.96)	7	No effect	Seed rot
P. griseo-fulvum	99.0 (85.34)	12.0 (20.21)	100.0 (89.96)	9	No effect	Seed rot
P. simplicissimum	98.0 (83.41)	8.0 (16.34)	100.0 (89.96)	12	No effect	Seed rot
P. perpuragenum	100.0 (89.96)	10.0 (18.37)	100.0 (89.96)	8	No effect	Seed rot
Rhizopus stolonifer	98.0 (83.41)	97.0 (80.42)	100.0 (89.96)	15	Root necrosis (traces)	Root necrosis (traces)
Stemphylium sp.	92.0 (73.62)	90.0 (70.59)	100.0 (89.96)	10	Discoloration of hypocotyl region	Seed ,and seedling rot, redish brown lesions covered with creamish fungal mass
CD (P=0.05)	(5.27)	(3.46)	(NS)			

Figures in parentheses are the angular transformed values

Table 23. Pathological significance of storage/ field mycoflora using seedling dip method

Fungi	Germination (%)			Incubation period (Days)	Effect of	
	Spore suspension	Culture filtrate	Sterilized distilled water		Spore suspension	Culture filtrate
1	2	3	4	5	6	7
Acremonium sp.	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	7	No effect	No effect
Alternaria sp.	0.0 (0.00)	100.0 (89.96)	0.0 (0.00)	8	Light discoloration of stem region	Rotting of stem, reddish brown sunken lesions, collar rot
A. flavus	0.0 (0.00)	10.0 (18.37)	0.0 (0.00)	9	No effect	Necrosis
A. fumigatus	0.0 (0.00)	5.0 (12.74)	0.0 (0.00)	9	No effect	Necrosis
A. niger	30.0 (33.18)	100.0 (89.96)	0.0 (0.00)	7	Root rot	Necrosis on stem, root rot
A. ochraceous	5.0 (12.74)	0.0 (0.00)	0.0 (0.00)	10	Root rot	Necrosis on stem, root rot
A. sulphureous	80.0 (63.42)	100.0 (89.96)	0.0 (0.00)	10	No effect	Seedling rot
Cephalosporium sp.	25.0 (29.97)	40.0 (39.21)	0.0 (0.00)	12	Slight rotting of roots	Slight rotting of roots
Cladosporium sp.	40.0 (39.21)	20.0 (26.53)	0.0 (0.00)	10	Root and stem necrosis (traces)	Stem necrosis in abnormal seedlings
Fusarium spp.	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	16	Stem necrosis	Stem necrosis and seedling rot
Gliocladium sp.	20.0 (26.53)	10.0 (18.37)	0.0 (0.00)	11	Slight rotting of roots	Slight rotting of roots

Continued

1	2	3	4	5	6	7
Myrothecium sp.	25.0 (29.97)	10.0 (18.37)	0.0 (0.00)	16	Root and stem necrosis (traces)	Stem necrosis in abnormal seedlings
Penicillium sp.	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	10	No effect	Very light necrotic spots on stem
P. capsulatum	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	7	No effect	Slight necrosis on stem, root rot
P. griseo-fulvum	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	9	No effect	Slight necrosis on stem, root rot
P. simplicissimum	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	12	No effect	Slight necrosis on stem
P. perpuragenum	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	8	No effect	Very light necrotic spots on stem
Rhizopus stolonifer	20.0 (26.53)	65.0 (53.71)	0.0 (0.00)	15	Root necrosis (traces)	Root necrosis in abnormal seedlings
Stemphylium sp.	0.0 (0.00)	100.0 (89.96)	0.0 (0.00)	10	Light discoloration of stem region	Rotting of stem, reddish brown sunken lesions
CD (P=0.05)	(1.59)	(1.82)	(NS)			

Figures in parentheses are the angular transformed values

Number of seedlings = 20

4.3 Storage Experiments

4.3.1 Effect of storage duration and containers on seed health parameters

Local cultivars of kidney bean seeds procured from five locations viz. Banjar (Kullu), Barot (Mandi), Kukumseri (Lahaul - Spiti), Salooni (Chamba) and Sangla (Kinnaur), representing various agro-climatic zones of Himachal Pradesh, were stored in containers commonly used by the farmers for storage, namely gunny bags, polylined bags and metallic bins. Stored seeds were analysed for their health parameters at different time intervals during storage and the information generated is presented in Tables 24-28.

A perusal of data given in Table 24 revealed that prolonged storage had adverse effect on germination of seeds in all the containers used for storage purpose. However, the mean germination was above 85.83 per cent in all the seed lots except for Barot (Mandi) area where it was 65.33 per cent. Seeds from Sangla (Kinnaur) showed significantly higher germination (98.66%) than others followed by Banjar (Kullu). It is also inferred from the data that germination declined gradually but significantly after 2, 4 and 6 months of storage and the storage containers had least effect on seed germination.

Seed moisture content was highest (10.27) in seed lots collected from Barot (Mandi) (Table 25) whereas, in all other samples, it was 9.17 per cent at 0-level of storage. It was observed that moisture content increased significantly after 2 months of storage and decreased thereafter. Storage containers had significant effect on seed moisture content, whereas gunny bags and metallic bins resulted in significantly more variation than the polylined bags.

The mean values for seed vigour during storage in various containers ranged between 3.722 to 4.479 (Table 26) corresponding to Banjar (Kullu) and Salooni (Chamba). The overall seed vigour was maximum in the beginning of storage in Salooni (Chamba) samples and minimum after 6 months with a lowest value of 2.900 in gunny bags stored seed samples of

Table 24. Effect of storage containers and duration on germination (%) of seeds

Seed source	Storage container	Time interval (Months)				
		0	2	4	6	Mean
Banjar (Kullu)	Gunny bag	100.00(89.31)	96.66 (81.18)	98.66 (84.33)	90.00 (71.59)	96.33 (81.60)
	Polylined bag	100.00(89.31)	97.33 (82.31)	95.33 (77.94)	92.00 (73.62)	96.17 (80.80)
	Metallic bin	100.00(89.31)	98.66 (84.33)	96.00 (78.69)	91.33 (73.01)	96.50 (81.33)
Barot (Mandi)	Gunny bag	72.67 58.47)	69.33 (56.36)	62.00 (51.93)	56.67 (48.82)	65.17 (53.89)
	Polylined bag	72.67 (58.47)	66.67 (54.72)	62.00 (51.93)	61.33 (51.53)	65.67 (54.16)
	Metallic bin	72.67 (58.47)	70.00 (56.77)	63.33 (52.72)	55.33 (48.05)	65.33 (54.00)
Kukumseri (Lahaul-Spiti)	Gunny bag	98.00 (83.19)	96.66 (81.18)	98.00 (83.19)	80.67 (63.90)	93.33 (77.86)
	Polylined bag	98.00 (83.19)	95.33 (77.81)	95.33 (77.94)	87.33 (69.21)	94.00 (77.04)
	Metallic bin	98.00 (83.19)	93.33 (75.25)	91.33 (73.01)	83.33 (65.93)	91.50 (74.35)
Salooni (Chamba)	Gunny bag	92.00 (73.62)	90.00 (71.59)	88.67 (70.31)	80.00 (63.43)	87.67 (69.74)
	Polylined bag	92.00 (73.62)	89.33 (70.98)	89.33 (70.98)	85.33 (67.52)	89.00 (70.78)
	Metallic bin	92.00 (73.62)	87.33 (69.18)	84.00 (66.42)	80.00 (63.43)	85.83 (68.16)
Sangla (Kinnaur)	Gunny bag	100.00(89.31)	98.66 (84.33)	98.00 (83.19)	98.00 (83.19)	98.66 (85.01)
	Polylined bag	100.00(89.31)	98.66 (84.33)	98.00 (83.19)	97.33 (82.31)	98.50 (84.79)
	Metallic bin	100.00(89.31)	97.33 (82.31)	97.33 (82.31)	95.33 (77.94)	97.50 (82.97)
Mean		92.53 (78.78)	89.69 (74.17)	87.82 (72.54)	82.27 (66.90)	88.08 (73.10)

Figures in parentheses are the angular transformed values

CD(P=0.05)

Seed source	1.680	Storage period	1.502
Seed source x Storage period	3.360		

Table25. Effect of storage containers and duration on seed moisture content (%)

Seed source	Storage container	Time interval (Months)				
		0	2	4	6	Mean
Banjar (Kullu)	Gunny bag	9.17	9.27	9.37	7.97	8.94
	Polylined bag	9.17	9.27	9.27	8.97	9.17
	Metallic bin	9.17	9.27	9.27	8.17	8.97
Barot (Mandi)	Gunny bag	10.27	10.57	10.57	8.07	9.87
	Polylined bag	10.27	10.27	10.07	9.88	10.12
	Metallic bin	10.27	10.27	10.37	8.47	9.84
Kukumseri (Lahaul-Spiti)	Gunny bag	9.17	9.57	9.37	7.97	9.02
	Polylined bag	9.17	9.27	9.17	8.90	9.13
	Metallic bin	9.17	9.47	9.37	8.47	9.12
Salooni (Chamba)	Gunny bag	9.17	9.57	9.37	8.07	9.04
	Polylined bag	9.17	9.27	9.37	9.17	9.24
	Metallic bin	9.17	9.37	9.37	8.27	9.04
Sangla (Kinnaur)	Gunny bag	9.17	9.37	9.17	8.27	8.99
	Polylined bag	9.17	9.17	9.17	9.00	9.13
	Metallic bin	9.17	9.17	9.17	8.07	8.89
Mean		9.39	9.54	9.49	8.51	9.23

CD(P=0.05)

Seed source A	0.071	Seed source x Storage period	0.144
Storage period B	0.063	Storage period x Storage container	0.111
Storage container C	0.055		

Table 26. Effect of storage containers and duration on seed vigour

Seed source	Storage container	Time interval (Months)				
		0	2	4	6	Mean
Banjar (Kullu)	Gunny bag	4.180	3.699	3.663	2.900	3.611
	Polylined bag	4.180	3.778	3.728	3.678	3.841
	Metallic bin	4.180	3.778	3.728	3.201	3.722
Barot (Mandi)	Gunny bag	4.180	3.875	3.792	3.201	3.762
	Polylined bag	4.180	3.875	3.694	3.532	3.820
	Metallic bin	4.180	3.875	3.792	3.500	3.837
Kukumseri (Lahaul-Spiti)	Gunny bag	4.620	4.500	4.392	3.875	4.347
	Polylined bag	4.620	4.500	4.265	3.778	4.291
	Metallic bin	4.620	4.500	4.392	3.875	4.347
Salooni (Chamba)	Gunny bag	4.680	4.500	4.492	3.875	4.387
	Polylined bag	4.680	4.599	4.462	4.176	4.479
	Metallic bin	4.680	4.500	4.462	3.778	4.355
Sangla (Kinnaur)	Gunny bag	4.580	4.500	4.464	4.004	4.387
	Polylined bag	4.580	4.500	4.264	3.875	4.305
	Metallic bin	4.580	4.500	4.364	4.000	4.361
Mean		4.448	4.232	4.130	3.683	4.123

CD(P=0.05)

Seed source	0.042	Seed source x Storage period	0.083
Storage period	0.037	Seed source x Storage container	0.072
Storage container	0.032	Storage period x Storage container	0.064
		Seed source x Storage period x Storage container	0.144

Banjar (Kullu). However, vigour of seeds from Salooni (Chamba) at different intervals remained higher which, inturn, was statistically at par with Sangla (Kinnaur) and Kukumseri (Lahaul - Spiti) seed samples. Seed vigour was found to decline significantly with increased storage period. It was also noticed that the decline in seed vigour was more in gunny bags stored seeds being statistically at par with metallic bins.

The seedling vigour index (fresh weight basis) of the seed samples from different localities ranged between 1582.23 to 2850.94 at 0-level (Table 27) and it declined significantly thereafter. Similar trend with respect to variation in seedling vigour on dry weight basis (Table 28) was observed, which was 27.42 - 44.10 at 0- level in various seed lots. However, after 6 months of storage, seedling vigour was significantly higher in seeds of Banjar (Kullu) followed by Kukumseri (Lahaul - Spiti), Salooni (Chamba) and Sangla (Kinnaur) as they were at par with each other. Storage containers alone and in combination with other factors did not influence the seedling vigour.

4.3.2 Effect of storage containers and duration of storage on seed mycoflora

Effect of different seed storage containers and duration of storage on mycoflora of kidney bean seeds was studied by employing blotter method, Agar plate method and seed component plating technique. Samples were drawn at two months interval for the presence of mycoflora.

Blotter method

Perusal of the data (Table 29) revealed the presence of 6,7,4,7 and 9 species of fungi on different samples in different locations before storage (0-level). The fungi were *Aspergillus flavus, A. fumigatus*, *Cephalosporium*, *Cladosporium*, *Colletotrichum*, *F. oxysporum, Gliocladium, Myrothecium, Penicillium, Rhizoctonia and Trichothecium* species.

During 6 months of storage period, maximum mycoflora was detected after four months in almost all the locations irrespective of container used

Table 27. Effect of storage containers and duration on seedling vigour index (fresh weight basis)

Seed source	Storage container	Time interval (Months)				
		0	2	4	6	Mean
Banjar (Kullu)	Gunny bag	2799.64	2409.59	2351.62	1574.93	2283.94
	Polylined bag	2799.64	2512.08	2020.17	1747.03	2269.73
	Metallic bin	2799.64	2599.26	1998.99	1665.04	2265.73
Barot (Mandi)	Gunny bag	1582.23	1513.16	827.97	837.00	1190.09
	Polylined bag	1582.23	1455.83	883.77	884.20	1201.51
	Metallic bin	1582.23	1502.73	1009.92	865.17	1240.01
Kukumseri (Lahaul-Spiti)	Gunny bag	2850.94	2804.94	1802.39	1409.75	2217.00
	Polylined bag	2850.94	2822.87	1812.35	1487.51	2243.42
	Metallic bin	2850.94	2717.97	1739.13	1481.13	2197.29
Salooni (Chamba)	Gunny bag	2738.79	2169.83	1665.66	1561.43	2033.93
	Polylined bag	2738.79	2245.05	1795.71	1776.90	2139.11
	Metallic bin	2738.79	2189.57	1637.87	1649.23	2053.87
Sangla (Kinnaur)	Gunny bag	2570.67	2671.29	1773.97	1454.51	2117.61
	Polylined bag	2570.67	2626.89	1788.67	1641.96	2157.05
	Metallic bin	2570.67	2469.25	2023.49	1604.52	2166.98
Mean		2508.45	2314.02	1675.45	1442.69	1985.15

CD(P=0.05)

Seed source	56.560
Storage period	50.589
Seed source x Storage period	113.120

Table 28. Effect of storage containers and duration on seedling vigour index (dry weight basis)

Seed source	Storage container	Time interval (Months)				
		0	2	4	6	Mean
Banjar (Kullu)	Gunny bag	38.23	32.23	34.86	25.29	32.65
	Polylined bag	38.23	36.04	35.63	28.35	34.56
	Metallic bin	38.23	33.11	33.15	26.09	32.65
Barot (Mandi)	Gunny bag	27.42	23.87	17.72	16.34	21.34
	Polylined bag	27.42	24.08	20.98	16.59	22.27
	Metallic bin	27.42	26.04	20.15	15.79	22.35
Kukumseri (Lahaul-Spiti)	Gunny bag	32.05	31.02	27.59	22.58	28.31
	Polylined bag	32.05	27.46	26.32	23.44	27.32
	Metallic bin	32.05	27.84	24.64	22.62	26.79
Salooni (Chamba)	Gunny bag	29.67	26.46	20.82	20.40	24.34
	Polylined bag	29.67	27.32	22.37	22.48	25.46
	Metallic bin	29.67	26.70	21.92	21.67	24.99
Sangla (Kinnaur)	Gunny bag	44.10	37.57	33.28	19.09	33.51
	Polylined bag	44.10	36.75	33.09	22.07	34.00
	Metallic bin	44.10	39.88	33.59	21.42	34.75
Mean		34.29	30.43	27.08	21.62	28.35

CD(P=0.05)

Seed source	1.21
Storage period	1.08
Seed source x Storage period	2.41

Table 29. Effect of storage containers and duration on seed mycoflora (%) of kidney beans using blotter method

Location	Storage container	Storage Period (Months)	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Gliocladium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Trichothecium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
Banjar (Kullu)	G	0	-	-	-	-	-	-	-	-	-	21	-	-	14	-	41	-	-	21	-	-	-	-	-	4
		2	-	-	-	-	-	-	-	-	-	-	-	-	36	-	70	-	-	-	-	-	-	-	-	2
		4	-	6	-	-	7	-	-	-	-	-	-	35	20	-	-	29	-	-	29	16	-	-	-	7
		6	-	8	7	5	-	-	-	-	-	7	-	20	-	-	-	-	-	-	-	-	-	-	-	5
		Mean	0	3.5	1.8	1.3	1.8	0	0	0	0	7	0	13.8	17.5	0	27.8	7.3	0	5.3	7.3	4	0	0	0	
	P	0	-	-	-	-	-	-	-	-	-	21	-	-	13	-	46	-	-	21	-	-	-	-	-	4
		2	-	-	-	-	-	-	-	-	-	-	-	35	36	-	70	-	-	-	-	-	-	-	-	3
		4	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	15	20	-	-	7	-	5
		6	-	16	16	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	3
		Mean	0	7	4	0	0	0	0	0	0	5.3	0	14	12.3	0	29	7.3	0	9	5	0	0	1.8	0	
	M	0	-	-	-	-	-	-	-	-	-	21	-	-	14	-	42	-	-	12	-	-	-	-	-	4
		2	-	-	-	-	-	-	-	-	-	-	-	35	29	-	85	-	-	-	-	-	-	-	-	3
		4	-	6	-	-	-	-	-	-	-	-	21	29	-	-	-	-	-	23	21	18	-	-	5	7
		6	-	8	5	-	-	-	-	-	-	7	-	20	-	-	-	-	-	-	-	-	-	-	8	5
		Mean	0	3.5	1.3	0	0	0	0	0	0	7	5.3	21	10.8	0	31.8	0	0	8.8	5.3	4.5	0	0	3.3	
Barot (Mandi)	G	0	-	-	-	-	-	-	30	33	24	-	-	49	-	10	66	-	-	-	-	-	-	-	-	6
		2	-	-	-	-	-	-	-	-	9	-	-	-	43	-	72	5	-	6	-	-	-	-	-	5
		4	-	19	5	13	12	-	-	-	15	14	-	7	-	-	-	-	21	20	8	15	-	5	15	13
		6	-	23	9	15	-	-	12	9	4	-	-	-	12	-	-	-	-	-	-	-	-	9	21	9
		Mean	0	10.5	3.5	7	3	0	10.5	10.5	13	3.5	0	14	13.8	2.5	34.5	1.3	5.3	6.5	2	3.8	0	3.5	9	

Continued

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
		0	-	-	-	-	-	-	36	33	7	-	-	36	-	12	70	-	-	-	-	-	-	-	-	6
		2	-	-	-	32	-	-	-	-	14	-	12	26	41	-	70	13	-	-	-	-	-	9	-	8
	P	4	-	19	-	-	14	-	-	-	10	21	-	30	-	-	-	32	36	14	-	-	-	14	29	10
		6	-	23	9	26	-	-	16	-	6	-	-	-	14	-	-	-	-	35	-	-	-	25	42	9
		Mean	0	10.5	2.3	14.5	3.5	0	13	8.3	9.3	5.3	3	23	13.8	3	35	11.3	9	12.3	0	0	0	12	17.8	
		0	-	-	-	-	-	-	38	34	6	-	-	18	-	12	65	-	-	-	-	-	-	-	-	6
		2	-	14	-	-	-	-	-	-	6	-	-	38	42	-	73	-	-	-	-	-	-	7	-	6
	M	4	-	22	-	7	15	-	-	-	4	35	-	-	-	-	-	20	22	21	14	36	-	-	50	11
		6	-	6	14	29	-	15	-	15	-	-	-	-	-	-	-	-	30	-	-	-	-	30	50	8
		Mean	0	10.5	3.5	9	3.8	3.8	9.5	12.3	4	8.8	0	14	10.5	3	34.5	5	13	5.3	3.5	9	0	9.3	25	
		0	-	21	7	-	-	-	-	10	-	-	-	31	-	-	29	-	-	-	-	-	20	-	7	7
		2	-	-	-	-	-	-	-	12	-	42	-	14	-	-	42	-	-	-	-	-	10	29	-	6
	G	4	64	-	-	-	-	-	-	7	-	-	-	9	-	-	-	32	-	-	-	20	12	-	32	7
		6	50	-	9	3	-	-	-	-	-	-	-	-	-	-	-	-	29	21	-	-	-	-	45	6
		Mean	28.5	5.3	4	0.8	0	0	0	7.3	0	10.5	0	13.5	0	0	17.8	8	7.3	5.3	0	5	10.5	7.3	21	
		0	-	22	7	-	-	-	-	5	-	-	-	28	-	-	29	-	-	-	-	-	24	-	7	7
		2	-	20	5	-	-	-	-	7	-	21	-	18	-	-	43	28	-	14	-	-	20	9	-	10
	P	4	24	15	-	-	-	-	-	-	-	-	-	13	-	-	-	32	-	-	-	-	20	14	17	7
		6	33	13	9	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	23	6
		Mean	14.3	17.5	5.3	2.5	0	0	0	3	0	5.3	0	14.8	0	0	18	15	0	3.5	0	0	16	10.5	11.8	
		0	-	20	7	-	-	-	-	7	-	-	-	29	-	-	29	-	-	-	-	-	18	-	7	7
		2	-	8	-	-	-	-	-	-	-	21	-	29	-	-	50	-	-	-	-	-	20	-	-	5
	M	4	40	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	13	-	50	4
		6	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	2
		Mean	21	7	1.8	0	0	0	0	1.8	0	5.3	0	16.3	0	0	19.8	0	0	0	0	0	12.8	0	24.8	
		0	-	7	-	-	-	-	21	-	14	-	-	70	-	23	7	-	-	18	-	-	-	-	-	7
		2	-	-	-	-	23	20	-	-	9	-	21	-	36	-	36	-	-	-	-	-	-	21	-	7
	G	4	6	-	-	-	-	-	-	-	5	-	-	29	21	-	-	-	7	10	5	-	-	-	-	7
		6	7	-	-	15	-	-	8	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	4
		Mean	3.3	1.8	0	3.8	5.8	5	7.3	0	7	1.8	5.3	24.8	14.3	5.8	10.8	0	1.8	7	1.3	0	0	5.3	0	

Kukumseri (Lahaul-Spiti)

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2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	0	-	7	-	-	-	-	22	-	7	-	-	70	-	20	6	-	-	21	-	-	-	-	-	7
	2	7	-	-	-	12	-	-	-	-	-	42	50	-	-	50	-	-	-	-	-	-	20	-	6
	4	-	8	-	24	-	-	6	-	10	-	-	7	14	-	-	29	-	18	-	15	-	29	-	10
	6	-	6	16	32	-	-	7	-	-	-	-	-	-	-	-	-	-	5	21	-	-	13	-	7
	Mean	1.8	5.3	4	14	3	0	8.8	0	4.3	0	10.5	31.8	3.5	5	14	7.3	0	11	5.3	3.8	0	15.5	0	
	0	-	7	-	-	-	-	18	-	-	-	-	69	-	21	7	-	-	20	-	-	-	-	-	6
	2	7	-	-	-	-	-	-	7	-	-	36	-	29	-	-	-	-	-	-	7	-	14	-	6
M	4	6	-	-	14	-	-	20	-	-	-	-	14	-	-	-	7	-	-	-	-	-	-	-	5
	6	8	-	14	14	-	-	25	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	5
	Mean	5.3	1.8	3.5	7	0	0	15.8	1.8	0	0	9	23.3	7.3	5.3	1.8	1.8	0	5	0	1.8	0	3.5	0	
	0	-	5	-	-	-	-	-	-	25	8	-	-	-	-	9	20	29	18	-	16	-	22	-	9
	2	-	-	-	-	-	-	-	-	4	-	-	-	14	-	66	-	-	-	-	-	-	29	-	4
G	4	14	8	14	6	-	-	-	-	10	-	-	-	-	-	-	50	46	23	-	23	9	-	21	11
	6	14	10	-	9	-	-	-	-	23	-	-	-	-	-	-	-	50	-	21	-	-	-	29	7
	Mean	7	5.8	3.5	3.8	0	0	0	0	15.5	2	0	0	3.5	0	18.8	17.5	31.3	10.3	5.3	9.8	2.3	12.8	12.5	
	0	-	7	-	-	-	-	-	-	14	7	-	-	-	-	7	12	33	8	-	21	-	29	-	9
	2	-	12	-	-	-	-	-	-	6	-	-	-	21	-	50	-	-	14	21	-	-	50	-	7
P	4	10	29	-	13	-	-	-	-	20	-	-	-	7	-	-	21	24	24	14	26	7	33	-	12
	6	16	9	23	30	-	-	-	-	35	-	-	-	-	-	-	-	-	-	-	-	-	26	7	7
	Mean	6.5	14.3	5.8	10.8	0	0	0	0	18.8	1.8	0	0	7	0	14.3	8.3	14.3	11.5	8.8	11.8	1.8	34.5	1.8	
	0	-	7	-	-	-	-	-	-	-	6	-	-	-	-	7	18	17	16	-	21	-	29	-	8
	2	-	-	-	-	-	-	-	34	36	-	-	-	14	-	50	-	12	-	19	-	-	50	-	7
M	4	-	29	-	-	-	-	-	-	-	-	-	-	-	-	-	25	36	34	29	-	-	70	-	6
	6	-	21	7	-	-	-	-	-	42	-	-	7	-	-	-	-	-	-	-	-	-	85	-	5
	Mean	0	14.3	1.8	0	0	0	0	8.5	19.5	1.5	0	1.8	3.5	0	14.3	10.8	16.3	12.5	12	5.3	0	58.5	0	

Sangla (Kinnaur)

G: Gunny bag
P: Polylined bag
M: Metallic bin

for storage. However, seeds stored in polylined bags exhibited the presence of maximum fungi except Banjar (Kullu) seed lots, with an average incidence of 6-70 and 4-70 per cent for storage fungi and field/pathogenic fungi, respectively.

Variable trend was noticed for increase or decrease of mycoflora in relation to duration of storage. Number of storage fungi increased with storage duration whereas, field/pathogenic fungi decreased after 4 months of storage in almost all the samples.

Agar plate method

Agar plate method yielded 30 species of storage and field fungi in different seed lots stored in three types of containers. Highest fungal flora (17 species) was recorded on Barot (Mandi) seed sample stored in different containers at almost all the stages of detection over all other locations (Table 30). However, seeds stored in polylined bags resulted in declined population of fungal flora after 4th month of storage whereas, this pattern was not found in any other storage containers. Analysis of seeds after 6 months of storage exhibited an increase in storage mycoflora in majority of samples. Seed samples of Salooni (Chamba) stored in metallic bins possessed least fungal infestation among other locations. The population of fungi in gunny bags was found to increase at faster rate in the later stages of storage in majority of the locations as compared to polylined bags. In general, decrease was noticed in the fungal population after 4th month of storage. The incidence of *C. lindemuthianum* and *Rhizoctonia* was maximum (36 and 31%) among field/pathogenic fungi whereas, *Aspergillus niger* (57%) dominated the storage fungi.

Seed component plating technique

Seed coat: Out of various seed components analysed for mycoflora, seed coats of different samples harboured more fungi as compared to their cotyledons and embryonic axis. In the beginning of storage (0- level) highest mycoflora was encountered in Barot (Mandi) where the number of species on seed coats, cotyledons and embryonic axis was 10, 7 and 7,

Table 30. Effect of storage containers and duration on seed mycoflora (%) of kidney beans using agar plate method

Location	Storage container	Storage Period (Months)	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Helminthosporium* sp.	*Macrophomina* sp.	*Monosporium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma* sp.	*Phymatotrichum* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	*Trichothecium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*
Banjar (Kullu)	G	0	36	13	9	-	-	-	-	-	-	-	-	14	14	-	-	-	-	-	-	-	-	-	7	-	14	14	-	-	-	-	8
		2	28	-	-	-	20	29	-	14	14	-	-	-	14	-	-	-	-	-	-	-	5	-	7	-	-	-	-	-	5	2	10
		4	13	-	6	29	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	42	13	-	-	12	5	-	-	-	-	9
		6	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	5	-	36	4
		Mean	19.3	3.3	3.8	7.3	6.8	7.3	0	8.8	3.5	0	0	3.5	7	0	0	0	0	0	0	1.5	11.8	3.3	3.5	0	8.3	4.8	0	1.3	1.3	9.5	
	P	0	35	12	5	-	-	-	-	-	-	-	-	14	14	-	-	-	-	-	-	-	-	-	5	-	16	14	-	-	-	-	8
		2	38	-	8	-	21	7	7	14	7	-	-	-	7	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	9
		4	-	-	20	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	7	-	-	-	7	21	-	-	-	2	42	8
		6	-	-	-	-	-	-	-	21	7	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	6	-	5
		Mean	18.3	3	8.3	1.8	5.3	1.8	1.8	8.8	3.5	0	0	3.5	7	0	0	0	0	0	3.5	1.8	0	0	7	1.8	13.3	3.5	0	0	2	10.5	
	M	0	34	13	7	-	-	-	-	-	-	-	-	14	14	-	-	-	-	-	-	-	-	-	7	-	14	14	-	-	-	-	8
		2	21	-	15	36	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	5	6
		4	36	16	6	-	-	-	-	-	-	-	-	14	7	-	-	-	-	-	25	-	-	-	-	5	12	-	-	7	-	-	9
		6	-	-	-	21	-	-	-	29	7	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	36	6
		Mean	22.8	7.3	7	14.3	0	0	0	7.3	1.8	0	0	7	14	0	0	0	0	0	6.3	0	0	0	3.5	1.3	11.8	3.5	0	1.8	0	10.3	
	G	0	6	12	-	7	20	12	-	7	14	-	-	29	29	-	-	-	-	-	-	-	-	-	12	-	10	2	-	-	7	11	14
		2	28	9	14	-	-	-	-	21	-	-	-	-	14	-	-	-	29	-	3	7	-	11	9	-	24	-	14	-	1	-	13
		4	7	14	-	29	-	-	-	-	-	7	7	14	7	7	-	-	-	-	10	12	13	14	-	-	-	-	-	-	-	27	13
		6	12	-	12	42	57	-	-	29	7	-	-	-	-	-	-	-	21	-	4	-	-	-	-	-	31	16	-	5	-	-	11
		Mean	13.3	8.8	6.5	19.5	19.3	3	0	14.3	5.3	1.8	1.8	10.8	12.5	1.8	0	0	12.5	0	4.3	4.8	3.3	6.3	5.3	0	16.3	4.5	3.5	1.3	2	9.5	

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2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
	0	8	12	-	7	21	16	-	7	14	-	-	29	29	-	-	-	-	-	-	-	-	-	13	-	14	4	-	-	7	18	14
	2	36	22	12	15	-	-	14	-	7	14	-	21	7	-	-	-	21	7	7	-	-	14	20	-	-	18	31	-	2	-	17
P	4	-	-	8	22	-	-	-	-	-	-	-	7	21	-	-	-	-	-	27	10	5	-	-	-	-	-	-	-	-	-	7
	6	-	-	10	32	-	-	-	14	-	-	-	-	-	7	-	-	29	-	-	-	-	-	-	-	9	-	-	-	5	-	7
	Mean	11	8.5	7.5	19	5.3	4	3.5	5.3	5.3	3.5	0	14.3	14.3	1.8	0	0	12.5	1.8	8.5	2.5	1.3	3.5	8.3	0	5.8	5.5	7.8	0	3.5	4.5	
	0	6	12	-	7	21	13	-	7	14	-	-	29	29	-	-	-	-	-	-	-	-	-	14	-	14	7	-	-	7	19	14
	2	16	23	8	-	-	-	-	21	7	-	-	7	14	-	-	-	21	-	-	14	7	-	6	-	-	-	7	-	-	-	12
M	4	-	9	-	20	28	-	-	-	-	-	-	21	21	-	-	-	-	5	7	16	14	20	-	-	-	-	1	-	-	-	11
	6	-	7	7	46	-	6	-	42	16	-	-	-	-	7	-	-	29	-	7	-	-	-	-	-	-	-	-	7	-	7	11
	Mean	5.5	12.8	3.8	18.3	12.3	4.8	0	17.5	9.3	0	0	14.3	16	1.8	0	0	12.5	1.3	3.5	7.5	5.3	5	5	0	3.5	1.8	2	1.8	1.8	6.5	
	0	21	6	22	7	-	-	-	-	7	-	-	29	14	-	-	-	7	-	-	-	-	-	7	-	29	-	-	-	-	-	10
	2	14	-	6	-	-	-	-	-	21	-	-	14	-	-	-	-	-	4	7	-	7	-	-	-	-	-	-	-	7	-	8
G	4	13	-	-	6	-	-	-	-	14	-	-	-	-	-	-	-	-	16	31	-	14	10	-	-	-	-	-	-	1	-	8
	6	-	-	7	14	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	4
	Mean	12	1.5	8.8	6.8	0	1.5	0	0	10.5	0	0	10.8	3.5	0	0	0	1.8	5	9.5	1.8	5.3	2.5	1.8	0	7.3	0	0	0	2	0	
	0	21	7	24	6	-	-	-	-	7	-	-	29	14	-	-	-	7	-	-	-	-	-	7	-	28	-	-	2	-	-	11
	2	7	-	7	7	-	-	-	7	7	-	-	-	-	-	-	-	7	20	-	6	-	7	-	-	-	-	-	-	-	-	9
P	4	34	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	7	-	7	15	14	10	-	12	20	-	12	7	-	-	11
	6	-	14	5	20	-	-	-	-	-	-	-	-	-	-	-	-	14	-	14	-	7	-	-	-	-	-	-	-	-	-	6
	Mean	15.5	5.3	9	8.3	0	0	0	1.8	5.3	0	0	7.3	3.5	0	0	0	8.8	5	5.3	5.3	5.3	4.3	1.8	3	12	0	3	2.3	0	0	
	0	21	6	23	7	-	-	-	-	7	-	-	29	14	-	-	-	7	-	-	-	-	-	7	-	29	-	-	-	-	-	10
	2	8	-	6	-	-	-	-	-	-	-	7	-	14	-	-	-	14	-	7	-	-	29	-	9	-	-	7	-	-	-	9
M	4	28	-	-	7	-	-	-	-	7	-	7	-	-	-	-	-	14	-	14	10	-	-	-	-	-	-	-	-	-	-	7
	6	-	12	5	18	7	5	-	-	7	-	-	-	-	-	-	-	7	-	-	-	14	-	-	-	-	-	-	-	-	21	9
	Mean	14.3	4.5	8.5	8	1.8	1.3	0	0	5.3	0	3.5	7.3	7	0	0	0	10.5	0	5.3	2.5	3.5	7.3	1.8	2.3	7.3	0	1.8	0	0	5.3	
	0	5	13	22	14	-	-	-	-	21	-	-	21	7	-	-	-	-	-	-	-	-	-	-	-	21	12	-	-	-	-	9
	2	16	-	-	-	-	-	-	-	-	-	-	-	7	-	21	-	7	3	-	-	7	10	-	-	-	-	5	-	6	-	9
G	4	29	-	6	6	6	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	7	14	-	-	-	-	-	20	21	18	10
	6	-	7	12	9	7	-	7	14	7	-	7	-	-	14	-	7	-	-	14	-	-	-	8	-	-	-	-	-	-	-	12
	Mean	12.5	5	10	7.3	3.3	0	1.8	3.5	7	0	1.8	5.3	3.5	3.5	5.3	1.8	1.8	2.3	3.5	0	3.5	6	2	0	5.3	3	1.3	5	6.8	4.5	

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...numseri (Lahaul-Spiti)

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
		0	6	15	24	14	-	-	-	-	21	-	-	21	7	-	-	-	-	-	-	-	-	-	-	-	22	14	-	-	-	-	9
		2	28	-	-	-	-	-	-	-	-	-	-	-	7	-	14	-	21	-	-	-	-	-	-	-	11	-	21	-	-	-	6
		4	37	8	12	-	-	22	-	-	29	-	6	4	-	-	-	-	-	-	-	6	-	13	-	-	-	-	-	-	-	-	9
		6	-	13	-	40	-	5	7	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
		Mean	17.8	9	9	13.5	0	6.8	1.8	0	16	0	1.5	6.3	3.5	0	3.5	0	5.3	0	0	1.5	0	3.3	0	0	8.3	3.5	5.3	0	0	0	
		0	7	16	21	14	-	-	-	-	21	-	-	21	7	-	-	-	-	-	-	-	-	-	-	-	21	11	-	-	-	-	9
		2	6	-	-	-	-	-	-	29	-	-	-	-	29	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	4
	M	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	31	-	-	-	-	-	-	-	-	2
		6	-	-	-	-	-	-	21	-	7	-	7	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
		Mean	3.3	4	5.3	3.5	0	0	5.3	7.3	7	0	1.8	5.3	12.5	0	0	0	3.5	0	3	0	0	7.8	0	0	5.3	2.8	0	0	0	0	
		0	21	7	6	50	-	-	-	-	36	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
		2	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-	-	-	-	-	-	3	3
	G	4	16	26	-	-	7	8	-	-	-	-	-	-	-	-	-	-	7	-	21	-	7	-	-	-	-	-	-	-	-	-	7
		6	-	16	5	29	-	-	-	0	-	-	-	-	-	-	-	-	-	21	14	5	-	-	-	-	7	-	-	-	-	18	9
		Mean	12.3	12.3	2.8	19.8	1.8	2	0	0	9	0	0	1.8	0	0	0	0	9	5.3	8.8	1.3	1.8	0	0	0	1.8	0	0	0	0	5.3	
		0	21	7	3	46	-	-	-	-	36	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
		2	31	-	-	-	-	-	-	7	-	-	-	-	14	-	-	-	14	-	14	-	12	-	-	-	-	21	-	-	-	-	7
	P	4	-	8	16	-	6	-	-	-	-	14	-	-	-	-	-	-	-	21	5	4	-	5	-	-	29	-	-	-	7	-	10
		6	19	6	12	57	23	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Sangla (Kinnaur)		Mean	17.8	5.3	7.8	25.8	7.3	1.5	0	1.8	9	3.5	0	1.8	3.5	0	0	0	3.5	5.3	4.8	1	3	1.3	0	0	7.3	5.3	0	0	1.8	0	
		0	18	7	7	50	-	-	-	-	36	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
		2	-	-	-	-	-	-	-	29	-	14	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	4
	M	4	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	7	-	14	6	-	-	-	20	-	-	-	-	-	-	5
		6	1-	35	12	7	-	-	7	14	-	-	-	-	7	-	-	-	-	-	7	17	-	-	-	-	-	-	-	-	-	7	9
		Mean	4.5	10.5	4.8	14.3	0	0	1.8	10.8	9	7	0	1.8	5.3	0	0	0	1.8	0	5.3	5.8	0	0	0	5	0	0	1.8	0	0	1.8	

G: Gunny bag
P: Polylined bag
M: Metallic bin

respectively (Tables 31, 32 and 33). Major pathogenic fungi recorded on various seed components consisted of *C. lindemuthianum, F. oxysporum* and *Rhizoctonia*. Among different storage containers, polylined bags possessed maximum fungi over gunny bags and metallic bins.

A decrease was noticed in the population of fungal flora after 2nd month of storage in majority of the samples except for Banjar (Kullu). On seed coats, main storage fungi recorded were species of *Aspergillus* and *Rhizopus* (Table 31).

Cotyledons: Cotyledons of Kukumseri (Lahaul - Spiti) seed sample exhibited comparatively less mycoflora over other locations at different stages of detection (Table 32). As the duration of storage increased, the storage fungi were found to replace the field fungi irrespective of storage containers. Out of various field fungi viz. *Colletotrichum* and *Fusarium* recorded on the seeds of five locations, *C. lindemuthianum* was present in all the samples except Kukumseri (Lahaul - Spiti) with mean incidence of 4 to 20 per cent.

Embryonic axis: Embryonic axis possessed least mycoflora as compared to other seed components, which comprised mainly of storage fungi whereas, among field fungi only *Colletotrichum* was detected with an average incidence of 4-28 per cent, followed by *Phoma* and *Macrophomina* being detected from 3 samples each showing 4 per cent incidence (Table 33).

4.3.3 Effect of storage containers and duration on seed quality parameters

Carbohydrate: Total carbohydrate contents (Table 34) in different local landraces of kidney bean ranged between 58.20-67.30 g/100 g of seed on dry weight basis before storage of the seeds. Kukumseri (Lahaul - Spiti) landrace possessed highest carbohydrate contents over all other locations, though statistically at par with Sangla (Kinnaur) sample and minimum values registered for the sample collected from Barot (Mandi). All the seeds stored in three containers showed gradual but significant decrease over

Table 31. Effect of storage containers and duration on seed mycoflora (seed coat) of kidney beans using Seed Component Plating Technique

Location	Storage container	Storage Period (Months)	*Alternaria sp.*	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphureous*	*Cladosporium sp.*	*Colletotrichum lindemuthianum*	*Fusarium spp.*	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Myrothecium sp.*	*Penicillium spp.*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma sp.*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium sp.*	*Sclerotium rolfsii*	*Trichothecium sp.*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
Banjar (Kullu)	G	0	-	12	12	-	-	-	-	4	-	-	-	-	-	8	12	-	-	-	-	24	-	-	-	6
		2	-	12	-	12	-	-	-	-	-	-	-	-	-	-	24	-	-	-	-	24	-	-	-	4
		4	-	-	-	8	12	-	-	4	-	-	-	8	-	-	16	-	-	-	-	12	-	-	-	6
		6	-	-	-	8	12	-	-	-	-	-	-	8	-	-	-	-	-	-	-	12	-	-	-	4
		Mean	0	6	3	7	6	0	0	2	0	0	0	4	0	2	13	0	0	0	0	18	0	0	0	
	P	0	-	16	12	-	-	-	-	4	-	-	-	-	-	8	12	-	-	-	-	24	-	-	-	6
		2	-	8	-	12	-	-	-	-	-	-	-	-	-	-	-	20	8	-	-	24	-	-	-	5
		4	-	-	-	8	8	-	-	12	-	-	-	4	-	4	-	-	-	-	-	12	-	-	-	6
		6	-	-	-	8	8	-	-	-	-	-	-	4	-	-	-	-	-	-	-	12	-	-	-	4
		Mean	0	6	3	7	4	0	0	4	0	0	0	2	0	3	3	5	2	0	0	18	0	0	0	
	M	0	-	8	12	-	-	-	-	4	-	-	-	-	-	8	12	-	-	-	-	24	-	-	-	6
		2	-	20	-	29	-	-	-	-	-	-	-	-	-	-	12	12	-	-	-	24	-	-	-	5
		4	-	12	-	8	-	12	-	12	-	-	-	4	-	-	16	-	-	-	-	16	-	-	-	7
		6	-	-	-	8	16	-	-	-	-	-	-	4	-	-	-	-	-	-	-	16	-	-	-	4
		Mean	0	10	3	11.3	4	3	0	4	0	0	0	2	0	2	10	3	0	0	0	20	0	0	0	
Barot (Mandi)	G	0	4	8	20	-	-	-	-	12	-	-	-	-	-	-	12	8	16	-	-	48	-	-	-	8
		2	-	4	4	-	-	-	-	12	-	-	12	-	28	-	8	-	16	8	16	24	-	-	-	10
		4	-	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	3
		6	4	12	-	24	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	4
		Mean	2	7	6	7	0	0	0	6	0	0	5	0	7	0	5	2	8	2	4	24	0	0	0	

Continued

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	0	-	28	20	28	-	-	-	12	-	-	-	-	-	-	12	8	16	-	-	42	-	-	-	8
	2	4	12	12	-	-	-	-	8	-	-	4	-	-	-	-	-	-	-	12	-	-	-	-	6
P	4	-	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	3
	6	12	12	-	24	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	4
	Mean	4	14	8	14	0	0	0	5	0	0	2	0	0	0	3	2	4	0	3	14.5	0	0	0	
	0	-	24	20	24	-	-	-	12	-	-	-	-	-	-	12	8	16	-	-	48	-	-	-	8
	2	4	12	-	-	12	-	16	4	-	-	4	4	-	-	-	-	-	-	12	-	-	-	-	8
M	4	-	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	3
	6	8	12	-	12	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	5
	Mean	3	13	5	10	3	0	4	4	0	0	2	2	0	0	3	2	4	0	3	17	0	0	0	
	0	4	12	12	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	48	-	-	-	5
	2	-	12	-	-	-	-	-	-	24	-	8	-	-	-	-	-	-	-	-	-	-	-	-	3
G	4	-	4	4	4	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	16	-	-	-	5
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	1
	Mean	1	7	4	1	0	0	0	0	6	0	4	0	0	0	0	6	0	0	0	20	0	0	0	
	0	4	8	12	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	48	-	-	-	5
	2	4	8	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	16	-	-	-	4
P	4	4	-	4	12	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	16	-	-	-	6
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	1
	Mean	3	4	4	3	0	0	0	0	0	0	3	1	0	0	0	6	0	0	0	25	0	0	0	
	0	4	12	12	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	48	-	-	-	5
	2	-	16	-	-	-	-	-	4	-	-	8	-	-	-	-	-	-	-	4	-	-	-	-	4
M	4	-	8	8	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	12	-	-	-	5
	6	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	2
	Mean	1	9	5	0	0	0	0	3	0	0	3	1	0	0	0	6	0	0	1	19	0	0	0	
	0	-	12	20	-	-	-	-	8	-	-	-	-	-	-	16	8	-	-	-	42	-	-	-	6
	2	4	-	-	-	-	-	-	12	-	-	12	12	-	-	-	-	-	-	-	-	-	-	-	4
G	4	4	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	2
	Mean	2	3	5	0	0	0	0	9	0	0	3	3	0	0	4	2	0	0	0	15.5	0	0	0	

Kukumseri (Lahaul-Spiti)

Salooni (Chamba)

Continued

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	0	-	20	20	-	-	-	-	4	-	-	-	-	-	-	16	8	-	-	-	48	-	-	-	6
	2	16	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	3
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	1
	6	12	-	-	-	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	3
	Mean	7	5	5	0	0	0	0	9	0	0	0	0	0	0	4	2	0	0	0	20	0	0	0	
	0	-	12	12	-	-	-	-	4	-	-	-	-	-	-	16	8	-	-	-	42	-	-	-	6
	2	16	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	3
M	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	2
	Mean	4	3	3	1	0	0	0	3	0	0	0	0	0	0	4	2	0	0	1	14.5	0	0	0	
	0	8	12	8	-	-	-	-	24	-	-	-	-	-	-	8	-	-	-	-	24	-	-	-	6
	2	-	-	-	20	-	-	-	12	-	4	8	12	-	-	-	-	-	-	-	16	-	-	-	6
G	4	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	1
	Mean	2	3	2	10	0	0	0	9	0	1	2	3	0	0	2	0	0	0	0	22	0	0	0	
	0	8	12	8	-	-	-	-	24	-	-	-	-	-	-	8	-	-	-	-	24	-	-	-	6
	2	-	-	-	16	-	-	-	-	-	-	-	-	-	-	-	4	-	-	12	24	-	12	-	5
P	4	-	16	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	3
	6	-	-	-	8	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	3
	Mean	2	7	2	10	3	0	0	6	0	0	0	0	0	0	2	1	0	0	3	24	0	3	0	
	0	8	12	8	-	-	-	-	24	-	-	-	-	-	-	8	-	-	-	-	24	-	-	-	6
	2	4	16	-	4	4	-	-	12	-	-	12	-	-	-	-	-	-	-	4	-	-	-	-	7
M	4	-	12	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	3
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	1
	Mean	3	10	2	3	1	0	0	9	0	0	3	0	0	0	2	0	0	0	1	16	0	0	0	

Sangla (Kinnaur)

G: Gunny bag
P: Polylined bag
M: Metallic bin

Table 32. Effect of storage containers and duration on seed mycoflora (Cotyledon) of kidney beans using Seed Component Plating Technique

Location	Storage container	Storage Period (Months)	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Macrophomina* sp.	*Myrothecium* sp.	*Penicillium* spp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Banjar (Kullu)	G	0	4	20	12	-	-	4	8	-	-	-	-	-	-	-	-	24	6
		2	-	-	-	4	4	-	4	-	-	4	8	-	-	-	-	-	5
		4	-	-	-	12	-	-	-	-	-	-	8	-	-	-	4	16	4
		6	12	-	-	4	-	-	4	-	-	8	-	-	-	4	-	8	6
		Mean	4	5	3	5	1	1	4	0	0	3	4	0	0	1	1	12	
	P	0	4	16	14	-	-	-	8	-	-	-	-	-	-	-	-	32	5
		2	-	-	-	4	4	-	12	-	-	8	4	-	-	-	-	-	5
		4	-	-	-	16	-	-	-	-	-	-	8	-	-	-	4	16	4
		6	12	-	-	12	-	-	8	-	-	12	4	-	-	16	-	16	7
		Mean	4	4	3.5	8	1	0	7	0	0	5	4	0	0	4	1	16	
	M	0	8	12	-	-	-	-	16	-	-	-	-	-	-	-	-	36	4
		2	-	8	-	-	4	-	4	-	-	4	-	-	-	-	-	-	4
		4	-	-	-	16	-	-	-	-	-	-	8	-	-	-	4	16	4
		6	4	-	-	16	-	-	4	-	-	4	-	-	-	8	-	4	6
		Mean	3	5	0	8	1	0	6	0	0	2	2	0	0	2	1	14	
Barot (Mandi)	G	0	12	20	12	12	-	-	16	-	-	-	-	-	-	-	-	20	6
		2	24	-	-	-	-	4	16	-	-	-	-	-	-	-	-	16	4
		4	12	-	-	-	-	-	4	-	-	4	-	-	-	4	-	-	4
		6	-	16	8	-	-	12	-	-	-	-	-	-	8	-	-	-	4
		Mean	12	9	5	3	0	4	9	0	0	1	0	0	2	1	0	9	
	P	0	14	16	8	16	-	-	20	-	8	-	-	-	-	-	-	32	7
		2	20	-	-	-	-	4	8	4	-	8	4	-	-	-	-	-	6
		4	4	-	-	-	-	-	4	4	-	4	4	-	-	-	-	-	5
		6	-	16	16	-	-	12	4	-	-	-	-	-	-	-	-	-	4
		Mean	9.5	8	6	4	0	4	9	2	2	3	2	0	0	0	0	8	
	M	0	12	16	14	24	-	-	16	-	-	-	-	-	-	-	-	28	6
		2	12	-	-	-	-	-	4	-	-	4	-	-	-	-	-	20	4
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	12	12	4	4	16	4	-	-	-	-	-	4	4	-	-	8
		Mean	6	7	6.5	7	1	4	6	0	0	1	0	0	1	1	0	12	
Kukumseri (Lahaul-Spiti)	G	0	8	24	-	12	-	-	-	-	-	-	-	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	1
		6	4	12	-	4	-	-	12	-	-	-	4	-	-	-	-	-	5
		Mean	3	9	0	7	0	0	3	0	0	0	1	0	0	0	0	0	
	P	0	12	20	-	20	-	-	-	-	-	-	-	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	8	4	12	4	-	4	-	-	-	-	-	-	-	-	-	5
		6	-	4	4	4	8	-	4	-	-	-	4	-	-	4	-	8	8
		Mean	3	8	2	9	3	0	2	0	0	0	1	0	0	1	0	2	

Continued

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	0	4	14	-	16	-	-	-	-	-	-	-	-	-	-	-	-	3
	2	4	-	-	-	-	8	4	-	-	12	-	-	-	-	8	-	5
M	4	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	1
	6	-	4	-	4	4	8	-	-	-	8	4	-	-	-	4	8	8
	Mean	2	4.5	0	5	1	4	1	0	0	5	1	1	0	0	3	2	
	0	12	4	16	14	-	-	12	-	-	-	-	-	-	-	-	12	6
	2	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	1
G	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	4	-	8	-	-	4	-	-	8	4	-	-	4	-	8	7
	Mean	3	2	4	5.5	0	0	5	0	0	2	1	0	0	1	0	5	
	0	-	4	-	12	4	-	4	-	-	-	-	-	-	-	-	4	5
	2	-	4	-	-	-	-	4	-	-	-	-	-	-	-	-	-	2
	4	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	1
	6	-	4	-	4	-	-	4	-	-	4	-	-	-	-	-	-	4
	Mean	0	3	0	4	1	0	6	0	0	1	0	0	0	0	0	1	
	0	-	12	-	8	4	-	4	-	-	-	-	-	-	-	-	16	5
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
M	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	1
	6	-	4	-	-	-	-	-	-	-	-	-	-	-	-	4	12	3
	Mean	0	4	0	2	1	0	1	0	0	0	0	0	0	0	2	7	
	0	8	12	-	16	-	-	24	-	-	-	-	-	-	-	-	20	5
	2	-	4	4	-	4	-	4	-	-	4	-	-	-	-	-	-	5
G	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	8	4	-	8	4	8	-	-	-	4	-	-	-	-	-	6
	Mean	2	6	2	4	3	1	9	0	0	1	1	0	0	0	0	5	
	0	20	40	-	12	-	-	20	-	-	-	-	-	-	-	-	20	5
	2	-	4	-	-	8	-	4	-	-	4	4	4	-	-	-	-	6
P	4	-	12	4	8	-	-	-	-	-	-	-	4	-	-	-	-	4
	6	-	4	4	-	8	-	8	-	4	-	-	-	-	-	-	-	5
	Mean	5	15	2	5	4	0	8	0	1	1	1	2	0	0	0	5	
	0	12	16	-	24	-	-	20	-	-	-	-	-	-	-	-	24	5
	2	-	8	-	8	-	4	4	-	-	4	-	-	-	-	-	-	5
M	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	4	4	-	4	4	8	-	-	-	4	-	8	-	-	-	7
	Mean	3	7	1	9	1	2	8	0	0	1	1	0	2	0	0	6	

Saloni (Chamba)

Sangla (Kinnaur)

G: Gunny bag, P: Polylined bag, M: Metallic bin

Table 33. Effect of storage containers and duration on seed mycoflora (Embryonic axis) of kidney beans using Seed Component Plating Technique

Location	Storage container	Storage Period (Months)	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*Macrophomina* sp.	*Penicillium* spp	*Phoma* sp.	*Rhizopus stolonifer*	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Banjar (Kullu)	G	0	-	12	8	8	4	-	-	-	8	-	16	6
		2	-	-	-	-	-	4	-	-	4	4	4	4
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	8	-	12	-	-	-	-	-	-	-	2
		Mean	0	5	2	5	1	1	0	0	3	1	5	
	P	0	-	8	16	12	-	-	-	-	8	-	20	5
		2	-	4	-	-	-	4	4	4	-	4	-	5
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	4	12	-	-	-	-	-	-	-	2
		Mean	0	3	5	6	0	1	1	1	2	1	5	
	M	0	-	16	8	12	-	-	-	-	8	-	24	5
		2	-	4	-	4	-	4	-	4	-	-	-	4
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	4	-	16	-	-	-	-	-	-	-	2
		Mean	0	6	2	8	0	1	0	1	2	0	6	
Barot (Mandi)	G	0	-	12	12	20	-	12	-	-	4	-	16	6
		2	-	-	-	-	4	8	4	-	-	-	-	3
		4	-	-	-	8	-	4	-	-	-	-	8	3
		6	-	12	-	4	-	-	-	-	-	-	16	3
		Mean	0	6	3	8	1	6	1	0	1	0	10	
	P	0	4	14	12	16	-	20	-	-	12	-	16	7
		2	-	-	-	-	-	4	4	-	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	16	1
		6	-	8	-	8	-	-	-	-	-	-	12	3
		Mean	1	5.5	3	6	0	6	1	0	3	0	11	
	M	0	-	12	12	14	-	16	-	-	8	v	20	6
		2	-	-	-	-	4	-	8	4	-	-	-	3
		4	-	-	-	4	-	-	-	-	4	-	16	3
		6	-	12	-	4	-	-	-	-	-	-	8	3
		Mean	0	6	3	5.5	1	4	2	1	3	0	11	
Kukumseri (Lahaul-Spiti)	G	0	-	12	8	8	-	-	-	-	12	-	12	5
		2	-	-	-	-	8	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	12	12	16	12	4	-	-	8	-	-	6
		Mean	0	6	5	6	5	1	0	0	5	0	3	
	P	0	-	16	20	8	4	-	-	-	12	-	12	6
		2	-	-	-	-	4	8	-	-	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	4	-	-	-	-	-	-	1
		Mean	0	4	5	2	3	2	0	0	3	0	3	

Continued

2	3	4	5	6	7	8	9	10	11	12	13	14	15
Salooni (Chamba)													
	0	-	12	12	8	-	-	-	-	12	4	12	6
	2	-	-	-	-	12	4	-	-	-	-	-	2
M	4	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	8	12	4	8	-	-	-	-	-	-	4
	Mean	0	5	6	3	5	1	0	0	3	1	3	
	0	4	12	14	4	-	4	-	-	-	-	12	6
	2	-	-	-	-	-	-	-	-	-	-	12	1
G	4	-	-	-	-	-	-	-	-	-	-	8	1
	6	-	8	4	8	-	4	-	-	4	-	4	6
	Mean	1	5	4.5	3	0	2	0	0	1	0	9	
	0	-	20	4	4	8	4	-	-	-	-	20	6
	2	4	-	-	-	-	4	4	-	-	-	8	4
	4	-	-	-	-	-	-	-	-	-	-	8	1
	6	-	4	-	8	-	4	-	-	-	-	-	3
	Mean	1	6	1	3	2	3	1	0	0	0	9	
	0	-	14	8	14	-	12	-	-	-	-	24	5
	2	-	-	-	-	-	-	-	-	-	-	-	0
M	4	-	-	-	-	-	-	-	-	-	-	8	1
	6	-	8	4	12	4	-	-	-	-	-	-	4
	Mean	0	5.5	3	6.5	1	3	0	0	0	0	8	
Sangla (Kinnaur)													
	0	-	12	12	-	-	16	-	-	-	-	16	4
	2	-	-	-	-	-	-	-	-	-	-	-	0
G	4	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	-	-	4	4	-	-	-	-	-	-	2
	Mean	0	3	3	1	1	4	0	0	0	0	4	
	0	-	24	20	-	-	28	-	-	-	-	40	4
	2	-	-	-	-	-	-	-	-	-	-	-	0
P	4	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	8	4	4	-	-	-	-	-	-	-	3
	Mean	0	8	6	1	0	7	0	0	0	0	10	
	0	-	12	12	-	-	16	-	-	-	-	28	4
	2	-	-	-	-	-	-	-	-	-	-	-	0
M	4	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	4	-	4	4	-	-	-	-	-	-	3
	Mean	0	4	3	1	1	4	0	0	0	0	7	

G: Gunny bag
P: Polylined bag
M: Metallic bin

increased storage duration. Various containers used for seed storage had no significant effect on carbohydrates in almost all the treatments, however, polylined bags stored seeds showed higher contents in almost all the locations.

Protein: Protein contents of various seed lots varied significantly at the initiation of experiment with maximum and statistically superior values recorded in Banjar (Kullu) seed sample (24.05 g/100 g). However, initial protein content in various seed samples ranged between 15.86-24.05 g. A significant increase in protein values was recorded after two months of storage till 6 months in all the genotypes, maximum being in Banjar (Kullu) sample and minimum in Kukumseri (Lahaul - Spiti). Various containers, however, had no effect on protein content throughout the storage. Interaction effects of all the treatments were also observed to be non-significant except for various locations and storage period (Table 34).

Fat: Fat contents ranged between 1.18-1.32 g in the seeds of all the locations with minimum and maximum in the seed samples of Salooni (Chamba) and Kukumseri (Lahaul - Spiti), however, the contents varied significantly with respect to seed source before storage (Table 34). Seeds stored in polylined bags showed significant increase in fat contents in almost all the locations after 6 months of storage, though maximum contents of 1.38 g were observed in Kukumseri (Lahaul - Spiti) sample stored in polylined bags. Mean values after storage exhibited uneven trend with respect to storage containers.

4.4 Management Studies

4.4.1 Effect of seed dressing fungicides on the seed health, mycoflora and seed quality parameters

Five seed dressing fungicides namely Bavistin, Bavistin + TMTD, Baylaton Captan and Thiram, used in the management studies of seed borne diseases, were evaluated to see their effect on seed health, mycoflora and quality parameters.

Table 34. Effect of storage containers and duration on seed quality parameters

Seed source	Storage container	Carbohydrate*				Protein*				Fat*			
		0**	2**	6**	Mean	0**	2**	6**	Mean	0**	2**	6**	Mean
Banjar (Kullu)	Gunny bag	65.30	61.90	59.50	62.23	24.05	24.96	25.68	24.90	1.29	1.28	1.30	1.29
	Polylined bag	65.30	63.60	61.30	63.40	24.05	24.32	25.06	24.48	1.29	1.30	1.33	1.31
	Metallic bin	65.30	62.30	60.80	62.80	24.05	24.10	25.23	24.46	1.29	1.30	1.32	1.30
Barot (Mandi)	Gunny bag	58.20	54.20	51.50	54.63	22.15	22.69	23.48	22.77	1.22	1.24	1.24	1.23
	Polylined bag	58.20	55.20	52.70	55.37	22.15	23.05	23.35	22.85	1.22	1.22	1.24	1.23
	Metallic bin	58.20	54.80	51.30	54.77	22.15	22.66	23.85	22.89	1.22	1.22	1.25	1.23
Kukumseri (Lahaul-Spiti)	Gunny bag	67.30	63.20	61.30	63.93	15.86	16.45	17.67	16.66	1.32	1.34	1.36	1.34
	Polylined bag	67.30	65.80	63.50	65.53	15.86	16.03	16.46	16.12	1.32	1.30	1.34	1.32
	Metallic bin	67.30	64.30	60.80	64.13	15.86	16.51	17.25	16.54	1.32	1.34	1.38	1.35
Salooni (Chamba)	Gunny bag	64.00	60.20	57.30	60.50	20.74	21.53	22.42	21.56	1.18	1.21	1.21	1.20
	Polylined bag	64.00	62.10	59.20	61.77	20.74	19.71	21.64	20.70	1.18	1.18	1.19	1.18
	Metallic bin	64.00	61.60	59.40	61.67	20.74	21.66	22.31	21.57	1.18	1.19	1.19	1.19
Sangla (Kinnaur)	Gunny bag	66.80	63.60	59.50	63.30	19.81	20.65	21.35	20.60	1.30	1.30	1.33	1.31
	Polylined bag	66.80	64.90	62.40	64.70	19.81	20.20	20.86	20.29	1.30	1.31	1.35	1.32
	Metallic bin	66.80	63.70	60.90	63.80	19.81	20.34	21.10	20.42	1.30	1.30	1.33	1.31
Mean		64.32	61.43	58.76	61.50	20.52	20.99	21.85	21.12	1.26	1.27	1.29	1.27
CD(P=0.05)		A Seed source			0.760	A	0.216	AxB	0.375	A	0.011	AxB	0.02
		B Storage container			0.590	B	0.168	AxC	NS	B	NS		
		C Storage period			0.590	C	0.168	BxC	0.290	C	0.008		

g/100 g of dry weight
Months of storage

4.4.1.1 Seed health

Data given in Table 35 revealed that initial seed germination was 100 per cent and declined gradually along with increased duration. However, seeds treated with Bavistin, Baylaton and Thiram did not affect the seed germination even up to 6 month storage. Thiram resulted in significantly high mean germination, irrespective of doses followed by Baylaton and Bavistin. Captan was found to have adverse effect on germination after 2 months of storage and thereafter it declined significantly but at lower rate.

Data pertaining to seed moisture content (Table 36) revealed that the variations in it were non-significant in treated seeds. However, significant differences were observed in moisture content with respect to storage period where seed moisture increased slightly after 2 months of storage ranging between 9.2-9.4 per cent and declined subsequently to 7.9-8.0 per cent after 6 months of storage. Interaction with respect to fungicides and period of storage was significant which revealed that after two months of storage untreated check resulted in minimum seed moisture, being at par with Bavistin and Bavistin+TMTD treated seeds.

At the initiation of experiment seed vigour was 4.500 which later showed gradual decrease up to 6 months of storage and varied between 3.580-4.350 in treated seeds as compared to that of 3.590 in untreated control (Table 37). Thereby, indicating that fungicidal treatment enhanced seed vigour. Among the various fungicides tested, Bavistin+TMTD resulted in significantly higher seed vigour as compared to control but was at par with Baylaton. Thiram and Captan resulted in significantly lower values. All the fungicides applied @ 2.5 g/kg seed improved the seed vigour during storage. Period of storage had significant effect on seed vigour which decreased slightly but significantly from the initial value.

Data with respect to seedling vigour index on fresh weight basis showed improvement with all the seed treatment fungicides significantly after 2 months of storage from the initial value of 2247.71, except in Baylaton and Captan (Table 38). Seedling vigour index in captan treated

Table 35. Effect of seed treatment with fungicides on germination (%) at different time intervals

Fungicide	*Dose* (g/kg seed)	Time interval (Months) 0		2		4		6		Mean	
Bavistin	2.0	100.00	(89.31)	100.00	(89.31)	96.00	(78.69)	90.00	(71.59)	96.50	(82.23)
	2.5	100.00	(89.31)	100.00	(89.31)	98.00	(83.19)	100.00	(89.31)	99.50	(87.78)
	3.0	100.00	(89.31)	96.00	(78.69)	98.00	(83.19)	100.00	(89.31)	98.50	(85.13)
Bavistin+TMTD	2.0	100.00	(89.31)	94.00	(75.92)	96.00	(78.69)	92.00	(73.62)	95.50	(79.39)
	2.5	100.00	(89.31)	94.00	(75.92)	100.00	(89.31)	98.00	(83.19)	98.00	(84.43)
	3.0	100.00	(89.31)	96.00	(78.69)	98.00	(83.19)	96.00	(78.69)	97.50	(82.47)
Baylaton	2.0	100.00	(89.31)	98.00	(83.19)	96.00	(78.69)	96.00	(78.69)	97.50	(82.47)
	2.5	100.00	(89.31)	98.00	(83.19)	98.00	(83.19)	100.00	(89.31)	99.00	(86.25)
	3.0	100.00	(89.31)	98.00	(83.19)	98.00	(83.19)	100.00	(89.31)	99.00	(86.25)
Captan	2.0	100.00	(89.31)	92.00	(73.62)	90.00	(71.59)	90.00	(71.59)	93.00	(76.53)
	2.5	100.00	(89.31)	96.00	(78.69)	94.00	(75.92)	92.00	(73.62)	95.50	(79.39)
	3.0	100.00	(89.31)	98.00	(83.19)	96.00	(78.69)	92.00	(73.62)	96.50	(81.20)
Thiram	2.0	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)
	2.5	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)
	3.0	100.00	(89.31)	100.00	(89.31)	100.00	(89.31)	98.00	(83.19)	99.50	(87.78)
Control		100.00	(89.31)	100.00	(89.31)	96.00	(78.69)	92.00	(73.62)	97.00	(82.73)
	Mean	100.00	(89.31)	97.78	(83.82)	97.00	(81.75)	95.56	(80.25)	97.58	(83.78)

Figures in parentheses are the angular transformed values

CD(p=0.05)

A Treatment	1.246	AxB	2.493	AxBxC	4.318	
B Stoage period	1.017	AxC	2.159			
C Dose	0.881	BxC	1.763			

Table 36. Effect of seed treatment with fungicides on seed moisture content (%) at different time intervals

Fungicide	Dose (g/kg seed)	Time interval (Months)				
		0	2	4	6	Mean
Bavistin	2.0	9.10	9.20	8.60	8.00	8.73
	2.5	9.10	9.20	8.60	7.90	8.70
	3.0	9.10	9.20	8.70	7.90	8.73
Bavistin+TMTD	2.0	9.10	9.20	8.50	7.90	8.68
	2.5	9.10	9.30	8.50	8.00	8.73
	3.0	9.10	9.20	8.40	7.90	8.65
Baylaton	2.0	9.10	9.30	8.50	8.00	8.73
	2.5	9.10	9.30	8.40	8.00	8.70
	3.0	9.10	9.30	8.50	7.90	8.70
Captan	2.0	9.10	9.30	8.30	8.00	8.68
	2.5	9.10	9.30	8.50	8.00	8.73
	3.0	9.10	9.30	8.50	7.90	8.70
Thiram	2.0	9.10	9.40	8.50	8.40	8.85
	2.5	9.10	9.40	8.30	8.00	8.70
	3.0	9.10	9.40	8.40	8.00	8.73
Control		9.10	9.10	8.60	7.90	8.68
Mean		9.10	9.26	8.50	7.97	8.71

CD(p=0.05)

A Treatment	NS	AxB	0.14
B Stoage period	0.03		
C Dose	NS		

*Months of storage

Table 37. Effect of seed treatment with fungicides on seed vigour at different time intervals

Fungicide	Dose (g/kg seed)	Time interval (Months)				
		0	2	4	6	Mean
Bavistin	2.0	4.500	4.106	3.770	3.580	3.989
	2.5	4.500	4.480	4.267	4.000	4.312
	3.0	4.500	4.184	3.890	3.580	4.039
Bavistin+TMTD	2.0	4.500	4.008	3.912	3.880	4.075
	2.5	4.500	4.500	4.410	4.350	4.440
	3.0	4.500	4.009	3.980	3.860	4.087
Baylaton	2.0	4.500	4.185	4.159	3.880	4.181
	2.5	4.500	4.008	3.962	3.580	4.013
	3.0	4.500	4.486	4.329	4.180	4.374
Captan	2.0	4.500	3.884	3.720	3.630	3.934
	2.5	4.500	4.009	3.982	3.920	4.103
	3.0	4.500	3.787	3.750	3.700	3.934
Thiram	2.0	4.500	3.883	3.879	3.780	4.011
	2.5	4.500	3.884	3.820	3.680	3.971
	3.0	4.500	3.787	3.710	3.620	3.904
Control		4.500	4.001	3.650	3.590	3.935
Mean		4.500	4.067	3.916	3.777	4.065

CD(P=0.05)

A Treatment	0.0416	AxB	0.0831
B Storage period	0.0339	AxC	0.0720
C Dose	0.0294	BxC	0.0588
		AxBxC	0.1441

Table 38. Effect of seed treatment with fungicides on seedling vigour index (fresh weight basis) at different time intervals

Fungicide	Dose (g/kg seed)	Time interval (Months)				
		0	2	4	6	Mean
Bavistin	2.0	2247.71	2507.67	1991.03	1689.23	2108.91
	2.5	2247.71	2666.65	2137.53	2057.73	2277.41
	3.0	2247.71	2476.79	2064.04	1677.78	2116.58
Bavistin+TMTD	2.0	2247.71	2506.95	2190.71	1868.47	2203.46
	2.5	2247.71	2730.67	2310.70	1935.66	2306.19
	3.0	2247.71	2986.55	2552.06	2240.63	2506.74
Baylaton	2.0	2247.71	1483.90	1358.67	1031.99	1530.57
	2.5	2247.71	1558.38	1512.32	1267.83	1646.56
	3.0	2247.71	1528.98	1563.28	1344.82	1671.20
Captan	2.0	2247.71	1981.63	1752.23	1620.83	1900.60
	2.5	2247.71	2025.59	1765.29	1652.27	1922.72
	3.0	2247.71	1965.06	1812.47	1712.07	1934.33
Thiram	2.0	2247.71	2746.64	2657.65	2263.70	2478.93
	2.5	2247.71	2719.65	2256.71	2007.74	2307.95
	3.0	2247.71	2685.65	2257.71	1875.65	2266.68
Control		2247.71	2028.74	1851.83	1502.31	1907.65
Mean		2247.71	2258.72	1985.44	1708.52	2050.10

CD(p=0.05)

A Treatment	44.241	AxB	88.482
B Storage period	36.123	AxC	76.628
C Dose	31.283	AxBxC	153.256

Table 39. Effect of seed treatment with fungicides on seedling vigour index (dry weight basis) at different time intervals

Fungicide	Dose (g/kg seed)	Time interval (Months)				
		0	2	4	6	Mean
Bavistin	2.0	27.54	28.19	26.31	23.60	26.41
	2.5	27.54	28.83	28.19	27.69	28.06
	3.0	27.54	26.65	24.73	23.59	25.63
Bavistin+TMTD	2.0	27.54	30.51	30.24	27.46	28.94
	2.5	27.54	29.92	30.44	27.32	28.81
	3.0	27.54	32.54	31.89	29.32	30.32
Baylaton	2.0	27.54	30.80	25.83	24.61	27.20
	2.5	27.54	29.71	29.26	28.29	28.70
	3.0	27.54	31.01	29.57	28.57	29.17
Captan	2.0	27.54	25.65	25.69	24.98	25.97
	2.5	27.54	27.76	28.65	28.77	28.18
	3.0	27.54	28.52	28.17	28.91	28.29
Thiram	2.0	27.54	32.85	31.84	30.54	30.69
	2.5	27.54	31.82	30.86	28.08	29.57
	3.0	27.54	28.84	28.27	26.78	27.86
Control		27.54	26.75	23.94	21.94	25.04
Mean		27.54	26.75	23.94	21.94	25.04

CD(p=0.05)

A Treatment	1.09	AxB	2.18
B Storage period	0.89	AxC	1.89
C Dose	NS		

seeds was at par with untreated check. Almost similar trend of decrease in vigour index values was observed up to 6 months of storage. After 6 months of storage, seeds treated with Thiram (2g/kg) were found to possess significantly higher vigour index (2263.70) as compared to other fungicides and untreated check. Higher doses of these fungicides viz. 2.5 and 3g/kg enhanced the vigour index significantly.

Similar trend was observed in the seedling vigour index on dry weight basis, where values ranged between 23.59-30.54 after six months of storage as compared to initial value of 27.54 (Table 39). All the fungicides had positive effect as compared to untreated check but Thiram and Bavstin + TMTD treated seeds resulted in statistically superior values. Contrary to vigour index on fresh weight basis. Baylaton treated seeds resulted in significantly higher values on dry weight basis.

4.4.1.2 Effect of seed dressing fungicides on seed mycoflora

Blotter method

Data presented in Table 40 revealed that seed dressing fungicides had significant effect against different seed borne fungi including storage ones. In all 14 species were recorded on the treated seeds, though the number of fungi on individual seed lots was 1-6 species. A maximum of 6 fungi were detected from Bavistin and Baylaton treated seeds after four months of storage whereas, in other treatments this frequency was 1-5 species of storage fungi.

A marked increase in the population of *Penicillium* species was recorded in various treatments after 4th month of storage. Seed treatment with Thiram and Baylaton resulted in maximum control of field and storage fungi after six months of storage followed by Bavistin, Bavistin+TMTD and Captan. Different doses of fungicides had no specific effect on the mycoflora in comparison to the recommended one.

Table 40. Effect of seed dressing fungicides on mycoflora (%) of kidney bean (Blotter method) at different time intervals

Fungicide	Dose (g/kg seed)	Storage duration (months)	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizopus stolonifer*	*Trichoderma* sp.	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
BAVISTIN	2.0	0	-	-	-	-	-	6	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	18	-	-	-	-	-	-	-	1
		4	9	7	-	-	-	-	-	12	6	-	-	9	7	-	6
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	2.3	1.8	0.0	0.0	0.0	1.5	4.5	3.0	1.5	0.0	0.0	2.3	1.7	0.0	
	2.5	0	-	-	-	-	-	5	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	9	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	12	9	7	5	9	41	-	6
		6	-	-	-	-	-	-	-	-	6	-	-	-	-	-	1
		Mean	0.0	0.0	0.0	0.0	0.0	1.3	2.2	3.0	3.8	1.8	1.3	2.3	10.3	0.0	
	3.0	0	-	-	-	-	-	11	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	19	-	-	-	-	-	2	-	2
		4	-	-	-	-	-	-	-	7	7	9	7	12	-	-	5
		6	-	-	-	-	-	-	-	-	8	-	-	-	9	-	2
		Mean	0.0	0.0	0.0	0.0	0.0	2.8	4.8	1.8	3.8	2.3	1.8	3.0	10.5	0.0	
BAYLATON	2.0	0	-	-	-	-	-	0	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	8	-	-	-	-	7	6	12	-	-	4
		4	16	-	-	-	-	-	-	-	8	5	-	6	-	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	4.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0	3.0	1.5	4.5	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	5	-	-	-	-	-	18	-	2
		4	14	-	-	-	-	-	-	6	9	6	12	-	16	-	6
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	3.5	0.0	0.0	0.0	0.0	0.0	1.3	1.5	2.3	1.5	3.0	0.0	8.5	0.0	
	3.0	0	-	-	-	-	-	7	-	-	-	-	-	-	-	-	1
		2	-	29	-	-	50	-	6	-	-	-	-	-	-	-	3
		4	-	14	-	-	-	-	-	8	6	-	7	12	-	-	5
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	10.8	0.0	0.0	12.5	1.8	1.5	2.0	1.5	0.0	1.8	3.0	0.0	0.0	
BAVISTIN + TMTD	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	-	7	-	1
		4	-	-	-	-	-	-	-	-	-	11	15	-	18	-	3
		6	-	-	-	6	-	-	-	-	-	-	-	-	-	-	1
		Mean	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	1.8	4.8	6.0	0.0	9.3	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	22	-	-	-	-	-	9	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	26	-	1
		6	-	-	-	7	-	-	-	-	-	-	-	-	-	-	1
		Mean	0.0	0.0	0.0	1.8	0.0	0.0	5.5	0.0	0.0	0.0	2.3	0.0	8.7	0.0	
	3.0	0	-	-	-	-	-	7	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	23	-	-	-	-	-	-	-	1
		4	6	-	-	-	-	-	-	-	-	5	6	-	11	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.5	0.0	0.0	0.0	0.0	1.8	5.8	0.0	0.0	1.3	1.5	0.0	2.8	0.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
CAPTAN	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	-	18	-	1
		6	-	12	-	-	-	-	-	-	-	-	-	-	26	-	2
		Mean	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.0	0.0	
	2.5	0	-	-	-	-	-	12	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	23	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	-	6	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	-	12	-	1
		Mean	0.0	0.0	0.0	0.0	0.0	3.0	5.8	0.0	0.0	0.0	0.0	0.0	4.5	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	27	-	-	-	-	19	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	20	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	6.8	0.0	0.0	0.0	0.0	4.8	5.0	0.0	
THIRAM	2.0	0	-	-	-	-	-	5	-	-	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	13	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	-	12	-	1
		6	-	10	-	-	-	-	-	-	-	-	-	-	-	-	1
		Mean	0.0	2.5	0.0	0.0	0.0	1.3	3.3	0.0	0.0	0.0	0.0	0.0	3.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	7	9	5	-	11	-	4
		4	-	-	-	-	-	-	-	7	6	-	8	-	16	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	3.3	2.3	3.3	0.0	6.8	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	14	-	-	-	-	-	-	-	1
		4	5	14	-	-	-	-	-	-	-	9	-	-	49	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.3	3.5	0.0	0.0	0.0	0.0	3.5	0.0	0.0	2.3	0.0	0.0	12.2	0.0	
UNTREATED CHECK		0	25	-	-	-	-	-	-	-	-	-	-	-	-	14	2
		2	-	-	6	-	-	-	10	-	-	-	-	-	-	-	2
		4	-	6	-	4	-	-	-	-	6	7	5	6	35	-	7
		6	-	-	-	-	-	-	-	-	5	8	9	6	29	-	5
		Mean	6.3	1.5	1.5	1.0	0.0	0.0	2.5	0.0	1.5	1.7	1.2	1.5	8.7	3.5	

Agar plate method

Fungicide treated seeds when analysed by agar plate method were found to be free of any infestation in the beginning of storage except Bavistin treated seeds where 4 species of storage fungi were recorded (Table 41). Majority of the fungicides were highly effective in controlling the mycoflora except Bavistin+TMTD at 2.0 g/kg. Bavistin+TMTD treated seeds were free of any infestation at all the storage intervals when applied @ 3.0 g/kg followed by Thiram and Bavistin.

Seed component plating technique

Seed coats: As compared to untreated check seed coats of treated seeds harboured almost negligible mycoflora at different stages of detection during storage. Only one or two species of storage fungi (Table 42) were observed showing 4-16 per cent incidence. Bavistin and Baylaton treated seeds were free of any infestation at almost all the doses of application.

Cotyledons: Cotyledons also harboured 1-2 species of storage fungi in one or two treatments as compared to untreated check where the number of species recorded was higher. At the end of storage almost all the treatments were free of any infestation whereas, number of fungi in untreated check was 11 species with an incidence ranging from 4-24 per cent (Table 43).

Embryonic axis: Embryonic axis of treated seeds were almost free of any fungal infestation, however, *Colletotrichum, Penicillium* and *Rhizopus* were detected with minor incidence in some treatments. No mycoflora was detected in any of the treatments after 6 months of storage (Table 44).

4.4.1.3 Effect of fungicide treatment on seed quality parameters

Carbohydrate: Carbohydrate contents (Table 45) before storage were similar in all the treatments including untreated check. However, a significant decrease in carbohydrate content was recorded at various stages of analysis during storage. The decrease was comparatively more in untreated check over all treatments at the end of storage. Maximum

Table 41. Effect of seed dressing fungicides on mycoflora (%) of kidney bean (Malt Extract Agar) at different time intervals

Fungicide	Dose (g/kg seed)	Storage duration (months)	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Trichoderma* sp.	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BAVISTIN	2.0	0	9	-	-	-	-	4	-	-	-	29	14	4
		2	5	-	-	-	-	-	6	-	-	-	-	2
		4	5	-	-	-	-	-	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	4.8	0.0	0.0	0.0	0.0	1.0	1.5	0.0	0.0	7.3	3.5	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	7	-	-	-	-	-	-	-	-	-	-	1
		4	7	-	-	-	-	-	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	21	-	-	-	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	7	-	7	-	-	2
		Mean	5.3	0.0	0.0	0.0	0.0	0.0	1.8	0.0	1.8	0.0	0.0	
BAYLATON	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	9	-	-	-	-	-	-	-	-	-	-	1
		4	9	-	-	-	-	-	9	6	-	-	-	3
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	4.5	0.0	0.0	0.0	0.0	0.0	2.3	1.5	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	15	-	-	-	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	14	-	-	-	7	-	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	3.5	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	
BAVISTIN + TMTD	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	56	-	-	1
		4	-	-	-	-	-	8	6	7	29	-	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	2.0	1.5	1.8	21.3	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	21	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	0
	3.0	4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
		2	7	-	-	-	-	-	-	-	-	-	-	1
	2.0	4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
CAPTAN		2	-	-	-		-	-	-	-	-	-	-	0
	2.5	4	-	-	-	-	-	-	-	-	7	-	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	56	-	-	-	21	-	-	-	2
	3.0	4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	14.0	0.0	0.0	0.0	5.3	0.0	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
		2	9	-	-	-	-	-	-	-	-	-	-	1
	2.0	4	14	-	30	-	-	-	-	-	-	-	-	2
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	5.8	0.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
THIRAM		2	7	-	-	-	-	-	-	-	-	-	-	1
	2.5	4	-	-	-	-	-	9	7	-	-	-	-	2
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.8	0.0	0.0	0.0	0.0	2.3	1.8	0.0	0.0	0.0	0.0	
		0	-	-	-	-	-	-	-	-	-	-	-	0
		2	5	-	-	-	-	-	-	-	-	-	-	1
	3.0	4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
UNTREATED CHECK		0	30	-	-	-	-	-	-	-	-	30	14	3
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	8	7	-	8	7	5	6	35	-	-	7
		6	-	-	-	-	9	8	9	6	29	-	-	5
		Mean	7.5	2.0	1.8	0.0	4.3	3.8	3.5	3.0	16.0	7.5	3.5	

Table 42. Effect of seed dressing fungicides on mycoflora (%) of kidney bean (Seed Coat) at different time intervals using Seed Component Plating Technique

Fungicide	Dose (g/kg seed)	Storage duration (months)	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*Cladosporium* sp.	*Fusarium solani*	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	*Penicillium* spp.	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BAVISTIN	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	7	-	-	-	-	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
BAYLATON	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	4	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
BAVISTIN + TMTD	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	8	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	16	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CAPTAN	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	8	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	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
		2	-	-	-	-	-	12	-	-	12	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	3.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	12	-	-	4	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	1.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	4	-	-	12	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	3.0	0.0	0.0	
THIRAM	2.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	4	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	12	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	4	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	
UNTREATED CHECK		0	-	-	-	-	16	-	-	8	8	4	-	4
		2	-	-	-	-	8	-	-	4	-	12	-	3
		4	-	-	-	4	12	-	4	8	-	8	4	5
		6	-	4	8	4	20	-	16	4	8	-	16	7
		Mean	0.0	1.0	2.0	2.0	14.0	0.0	5.0	6.0	4.0	6.0	5.0	

Table 43. Effect of seed dressing fungicides on mycoflora (%) of kidney bean (Cotyledon) at different time intervals using Seed Component Plating Technique

Fungicide	Dose (g/kg seed)	Storage duration (months)	*Alternaria* sp.	*Aspergillus flavus*	*A. niger*	*A. ochraceous*	*Cephalosporium sp.*	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*Rhizopus stolonifer*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
BAVISTIN	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	4	-	-	4	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	
	2.5	0	-	-	-	-	-	-	-	-	-	8	-	-	4	2
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	7	-	-	-	-	-	-	-	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	1.0	
	3.0	0	-	-	-	-	-	-	-	-	-	4	-	-	4	2
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	
BAYLATON	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	4	-	8	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	4	-	-	-	-	8	-	-	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	1.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	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	
BAVISTIN + TMTD	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	12	-	8	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	2.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	4	-	-	-	4	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.0	

Continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
CAPTAN	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	12	-	4	4	4	-	4
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	1.0	1.0	1.0	0.0	
	2.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	12	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	4	-	-	-	-	-	-	4	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	4	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	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	
THIRAM	2.0	0	-	-	-	-	-	-	-	-	-	8	-	-	-	1
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	4	-	-	4	2
		4	-	-	-	-	-	4	-	-	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	
	3.0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	4	-	-	4	2
		4	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	
UNTREATED CHECK		0	-	-	-	-	-	-	24	-	-	-	-	-	-	1
		2	4	-	12	4	24	4	8	-	4	-	8	4	16	10
		4	-	12	4	-	16	4	16	20	4	4	4	4	12	11
		6	-	12	8	4	12	8	-	16	4	4	4	4	16	11
		Mean	1.0	6.0	6.0	2.0	13.0	4.0	12.0	9.0	3.0	2.0	4.0	3.0	11.0	

Table 44. **Effect of seed dressing fungicides on mycoflora (%) of kidney bean (Embryonic axis) at different time intervals using Seed Component Plating Technique**

Fungicide	Dose (g/kg seed)	Storage duration (months)	*Aspergillus flavus*	*A. niger*	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*Penicillium* spp.	*P. capsulatum*	*Rhizopus stolonifer*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11
BAVISTIN	2.0	0	-	-	4	-	-	-	-	1
		2	-	-	-	-	-	-	-	0
		4	-	-	-	4	-	-	-	2
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	1.0	1.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
BAYLATON	2.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	4	-	1
		4	-	8	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	2.0	0.0	0.0	0.0	1.0	0.0	
	2.5	0	-	-	-	-	-	-	-	0
		2	4	-	-	-	-	-	-	1
		4	-	8	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	0
		Mean	1.0	2.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	4	-	-	-	-	-	-	1
		2	-	-	-	-	-	-	-	0
		4	-	4	-	-	-	-	-	1
		6	-	-	-	-	-	-	-	0
		Mean	1.0	1.0	0.0	0.0	0.0	0.0	0.0	
BAVISTIN + TMTD	2.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	4	-	1
		4	-	-	-	-	-	8	-	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	3.0	0.0	
	2.5	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	4	-	1
		4	-	-	-	-	-	4	-	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	2.0	0.0	

Continued

1	2	3	4	5	6	7	8	9	10	11
CAPTAN	3.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	8	4	-	2
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	2.0	1.0	0.0	
	2.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	4	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	1.0	
	2.5	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	4	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	1.0	
	3.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	8	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	2.0	
THIRAM	2.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	4	-	-	-	-	1
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	1.0	0.0	0.0	0.0	0.0	
	2.5	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	3.0	0	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	0
		6	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
UNTREATED CHECK			-	-	-	-	-	4	-	1
			-	-	-	-	-	-	-	0
			4	12	-	-	-	-	20	3
			-	-	-	-	-	-	-	0
			1.0	3.0	0.0	0.0	0.0	1.0	5.0	

decline was recorded in Bayletan treated seeds followed by Captan at 2.0 g/kg, though the values were significantly higher over control. Bavistin+TMTD and Thiram treated seeds showed superior values over other fungicides. Interaction between all the fungicides and period of storage was observed to be statistically significant.

Protein: Protein contents in fungicide treated seeds were found to increase with storage period in all the treatments (Table 45). A marginal increase in the protein content was recorded after two months of storage though statistically at par with 0-level of testing but the decreasing trend in some of the treatments continued even upto six months of storage. The contents were enhanced slightly but significantly in all treatments with storage but the increase was more in untreated seeds.

Fat: No significant variation for fat content was observed in all the samples after fungicide treatment except for untreated check where it increased from 1.28 g to 1.33 g/ 100 g after six months storage (Table 45). Fungicides and their doses also did not affect the fat content and the increase noticed was statistically at par with control. Interaction of fungicides with storage period was significant.

4.4.2 Effect of seed treatment with propionic acid and Bavistin on seed health, mycoflora and quality parameters at different time intervals

4.4.2.1 Seed health

Seed germination of treated and untreated seeds was 92 per cent in the beginning of storage, however, it declined gradually over a period of 6 months in propionic acid treated seeds, both alone and in combination with Bavistin. Bavistin treated seeds showed higher seed germination followed by untreated seeds. In Bavistin treated seeds, though a gradual decrease was observed but the values were significantly higher than those observed with propionic acid treatment and untreated check. When propionic acid was used with Bavistin, germination decreased drastically in all the containers, maximum decrease being in polylined bags (Table 46).

Table 45. Effect of fungicide treatments on quality parameters of kidney bean

Treatment	Dose (g/kg seed)	Carbohydrate*				Protein*				Fat*			
		0**	2**	6**	Mean	0**	2**	6**	Mean	0**	2**	6**	Mean
Bavistin	2.0	65.90	63.20	60.90	63.33	25.04	25.12	25.29	25.15	1.28	1.28	1.29	1.28
	2.5	65.90	64.20	62.20	64.10	25.04	25.06	25.12	25.07	1.28	1.28	1.29	1.28
	3.0	65.90	64.00	62.50	64.13	25.04	25.08	25.31	25.14	1.28	1.28	1.28	1.28
Bavistin+TMTD	2.0	65.90	63.80	61.60	63.77	25.04	25.06	25.19	25.10	1.28	1.28	1.28	1.28
	2.5	65.90	64.50	63.10	64.50	25.04	25.09	25.18	25.10	1.28	1.28	1.28	1.28
	3.0	65.90	65.20	62.70	64.60	25.04	25.01	25.26	25.10	1.28	1.28	1.28	1.28
Baylaton	2.0	65.90	63.20	60.30	63.13	25.04	25.03	25.21	25.09	1.28	1.28	1.29	1.28
	2.5	65.90	62.90	61.00	63.27	25.04	25.02	25.35	25.14	1.28	1.29	1.28	1.28
	3.0	65.90	63.70	60.70	63.43	25.04	25.00	25.19	25.08	1.28	1.28	1.29	1.28
Captan	2.0	65.90	63.40	60.70	63.33	25.04	25.26	25.51	25.27	1.28	1.29	1.29	1.29
	2.5	65.90	63.80	61.20	63.63	25.04	25.01	25.19	25.08	1.28	1.28	1.28	1.28
	3.0	65.90	63.80	61.50	63.73	25.04	25.11	25.23	25.13	1.28	1.28	1.29	1.28
Thiram	2.0	65.90	64.40	62.50	64.27	25.04	25.05	25.36	25.15	1.28	1.29	1.29	1.29
	2.5	65.90	64.80	61.90	64.20	25.04	24.98	25.15	25.06	1.28	1.28	1.28	1.28
	3.0	65.90	64.60	62.70	64.40	25.04	25.05	25.34	25.14	1.28	1.28	1.28	1.28
Untreated		65.90	62.80	58.30	62.33	25.04	25.62	26.06	25.57	1.28	1.29	1.33	1.30
Mean		24.84	63.77	61.13	63.60	24.84	25.16	25.39	25.20	1.28	1.28	1.29	1.29
CD(P=0.05)	A Fungicide		0.760	AxC	1.31	A	0.107	AxC	0.186	A	0.011	AxB	0.018
	B Dose		NS			B	NS			B	NS		
	C Storage period		0.540			C	0.076			C	0.008		

g/100 g of dry weight
Months of storage

Table 46. Effect of seed treatment with propionic acid and bavistin on germination (%) of kidney bean at different time intervals in different containers

Treatment	Storage container	Time interval (months)				
		0	2	4	6	Mean
Propionic acid (10 ml/kg)	Gunny bag	92.00 (73.62)	82.33 (65.13)	84.33 (66.67)	82.33 (65.13)	85.25 (67.64)
	Polylined bag	92.00 (73.62)	70.33 (56.98)	56.33 (48.62)	60.33 (50.96)	69.75 (57.55)
	Metallic bin	92.00 (73.62)	84.33 (66.67)	82.33 (65.13)	76.33 (60.87)	83.75 (66.57)
Bavistin (2.5 g/kg)	Gunny bag	92.00 (73.62)	90.67 (72.34)	92.67 (74.37)	84.00 (66.50)	89.83 (71.71)
	Polylined bag	92.00 (73.62)	90.67 (72.34)	90.67 (72.44)	88.67 (70.41)	90.50 (72.20)
	Metallic bin	92.00 (73.62)	92.67 (74.50)	88.67 (70.41)	82.67 (65.42)	89.00 (70.99)
Propionic acid + Bavistin	Gunny bag	92.00 (73.62)	92.00 (73.62)	79.67 (63.20)	66.00 (54.31)	77.75 (62.51)
	Polylined bag	92.00 (73.62)	92.00 (73.62)	58.67 (49.97)	37.33 (37.64)	59.42 (51.51)
	Metallic bin	92.00 (73.62)	92.00 (73.62)	85.33 (67.52)	75.66 (60.43)	83.66 (66.56)
Untreated check	Gunny bag	92.00 (73.62)	90.33 (72.05)	88.33 (70.14)	82.33 (65.19)	87.67 (69.74)
	Polylined bag	92.00 (73.62)	76.33 (60.87)	68.33 (55.75)	54.33 (47.47)	89.00 (70.78)
	Metallic bin	92.00 (73.62)	88.33 (70.14)	82.33 (65.15)	74.33 (59.54)	85.67 (68.16)
Mean		92.00 (73.62)	82.47 (65.97)	78.22 (68.72)	72.02 (58.67)	

CD(P=0.05)

A Treatment	0.806	AxB	1.396	AxBxC	2.793
B Storage container	0.698	AxC	1.612		
C Storage period	0.806	BxC	1.396		

Figures in parentheses are the angular transformed values

However, the values of the seeds kept in metallic bins were almost similar to those observed for propionic acid treated and untreated seeds stored in metallic bins. Interaction effect of treatment, storage containers and storage period was significant.

Seed moisture in samples before storage was 9.17 per cent (Table 47), however, after 2 months of storage a significant increase was noticed in all the treatments, highest being in gunny bag stored seeds. After that a discrete pattern of increase/ decrease was recorded in different storage containers. At the end of storage, moisture level of all the treatments decreased significantly in both gunny bag and metallic bins stored seeds, but seeds stored in polylined bags had comparatively more moisture content.

Seeds treated with Bavistin were found to have better seed vigour than propionic acid alone and in combination with it. However, with increased storage, the seed vigour declined in all the treatments (Table 48). Untreated seeds also showed higher seed vigour than the seeds treated with propionic acid and propionic acid + Bavistin. Interaction of storage containers, treatments and storage period was statistically significant. Seeds stored in metallic bins showed significantly higher and statistically superior values for seed vigour.

Seedling vigour index on fresh weight basis decreased sharply at all intervals of analysis in seeds treated with propionic acid and propionic acid + Bavistin. Different storage containers did not show any significant effect on seedling vigour as even trend of increase / decrease was observed (Table 49).

Seedling vigour index on dry weight basis of seeds treated with chemicals and stored in various containers also showed that Bavistin treated seeds had higher values and increase was noted up to 2nd month of storage. Combined application of propionic acid + Bavistin had more drastic effect on seedling vigour index, which was lower than untreated check (Table 50).

Table 47. ***Effect of seed treatment with propionic acid and bavistin on seed moisture content (%) of kidney bean at different time intervals in different containers***

Treatment	Storage container	Time interval (months)				
		0	2	4	6	Mean
Propionic acid (10 ml/kg)	Gunny bag	9.17	9.50	9.30	8.00	8.99
	Polylined bag	9.17	9.37	9.40	8.37	9.08
	Metallic bin	9.17	9.30	9.30	8.20	8.99
Bavistin (2.5 g/kg)	Gunny bag	9.17	9.67	9.47	8.17	9.12
	Polylined bag	9.17	9.37	9.27	9.13	9.24
	Metallic bin	9.17	9.37	9.37	8.37	9.07
Propionic acid + Bavistin	Gunny bag	9.17	9.50	9.30	8.00	8.99
	Polylined bag	9.17	9.37	9.37	9.10	9.25
	Metallic bin	9.17	9.30	9.30	8.20	8.99
Untreated check	Gunny bag	9.17	9.57	9.37	8.07	9.05
	Polylined bag	9.17	9.27	9.37	9.17	9.25
	Metallic bin	9.17	9.37	9.37	8.27	9.05
Mean		9.17	9.41	9.35	8.42	9.09

CD(P=0.05)

A Treatment	0.046	AxB	0.081	AxBxC	0.162
B Storage container	0.040	AxC	0.093		
C Storage period	0.047	BxC	0.081		

Table 48. Effect of seed treatment with propionic acid and bavistin on seed vigour of kidney bean at different time intervals in different containers

Treatment	Storage container	Time interval (months)				
		0	2	4	6	Mean
Propionic acid (10 ml/kg)	Gunny bag	4.680	3.742	3.574	3.594	3.898
	Polylined bag	4.680	3.596	3.511	3.433	3.805
	Metallic bin	4.680	3.839	3.778	3.710	4.002
Bavistin (2.5 g/kg)	Gunny bag	4.680	4.500	4.492	3.875	4.387
	Polylined bag	4.680	4.599	4.462	4.176	4.479
	Metallic bin	4.680	4.500	4.462	3.776	4.355
Propionic acid + Bavistin	Gunny bag	4.680	3.778	3.742	3.699	3.975
	Polylined bag	4.680	3.699	3.512	3.411	3.826
	Metallic bin	4.680	3.778	3.770	3.721	3.987
Untreated check	Gunny bag	4.680	3.907	3.861	3.016	3.866
	Polylined bag	4.680	4.009	3.984	3.599	4.068
	Metallic bin	4.680	4.486	4.386	3.884	4.359
Mean		4.680	4.036	3.961	3.658	4.084

CD(P=0.05)

A Treatment	0.033	AxB	0.057	AxBxC	0.113
B Storage container	0.028	AxC	0.065		
C Storage period	0.033	BxC	0.057		

Table 49. ***Effect of seed treatment with propionic acid and bavistin on seedling vigour (fresh weight basis) of kidney bean at different time intervals in different containers***

Treatment	Storage container	Time interval (months)				
		0	2	4	6	Mean
Propionic acid (10 ml/kg)	Gunny bag	2738.79	1827.84	1604.04	1454.05	1906.18
	Polylined bag	2738.79	1390.65	1007.54	1040.80	1544.45
	Metallic bin	2738.79	1743.19	1570.14	1363.42	1853.89
Bavistin (2.5 g/kg)	Gunny bag	2738.79	3411.71	3105.80	1716.91	2743.30
	Polylined bag	2738.79	3322.85	2686.43	2057.91	2701.50
	Metallic bin	2738.79	3195.03	2956.11	1814.61	2676.14
Propionic acid + Bavistin	Gunny bag	2738.79	1747.10	1479.63	1255.23	1805.19
	Polylined bag	2738.79	1117.89	949.74	795.67	1400.52
	Metallic bin	2738.79	1854.55	1705.98	1390.63	1922.49
Untreated check	Gunny bag	2738.79	2169.83	1665.66	1561.43	2033.93
	Polylined bag	2738.79	2245.05	1795.71	1776.90	2139.11
	Metallic bin	2738.79	2189.57	1637.87	1649.23	2053.87
Mean		2738.79	2184.61	1847.05	1489.73	2065.05

CD(P=0.05)

A Treatment	37.47	AxB	64.91	AxBxC	129.82
B Storage container	32.45	AxC	74.95		
C Storage period	37.47	BxC	64.91		

Table 50. ***Effect of seed treatment with propionic acid and bavistin on seedling vigour (dry weight basis) of kidney bean at different time intervals in different containers***

Treatment	Storage container	Time interval (months)				
		0	2	4	6	Mean
Propionic acid (10 ml/kg)	Gunny bag	29.67	21.65	21.06	19.55	22.98
	Polylined bag	29.67	18.30	14.69	16.42	19.77
	Metallic bin	29.67	23.41	20.74	19.59	23.35
Bavistin (2.5 g/kg)	Gunny bag	29.67	40.51	35.05	26.90	33.03
	Polylined bag	29.67	39.60	34.01	25.83	32.28
	Metallic bin	29.67	37.14	30.96	25.33	30.78
Propionic acid + Bavistin	Gunny bag	29.67	25.32	22.43	11.77	22.30
	Polylined bag	29.67	17.94	9.50	6.52	15.91
	Metallic bin	29.67	29.01	23.03	15.09	24.20
Untreated check	Gunny bag	29.67	26.46	20.82	20.40	24.34
	Polylined bag	29.67	27.32	22.37	22.48	25.46
	Metallic bin	29.67	26.70	21.92	21.67	24.99
Mean		29.67	27.78	23.05	19.30	24.95

CD(P=0.05)

A Treatment	1.40	AxB	2.43
B Storage container	1.22	AxC	2.81
C Storage period	1.40		

4.4.2.2 Seed mycoflora

Blotter method

Propionic acid treatment was found to have least effect on seed mycoflora of kidney bean (Table 51), as such seeds harboured different fungal species at various stages of detection during storage. Seed treatment with Bavistin alone and in combination with propionic acid checked the mycoflora to a greater extent in almost all the containers used for seed storage. Gunny bags possessed no mycoflora in the end of storage in propionic acid + Bavistin treated seed, however, an increase in number of *Penicillium* species was observed in both treatments.

Agar plate method

Almost similar trend was observed in the seeds when plated on Malt Extract Agar (Table 52) as propionic acid treated seeds possessed a maximum mycoflora whereas, application of propionic acid + Bavistin had least mycoflora. Number of fungi detected in the beginning was higher in most of the cases but declined with increased storage. Among different treatments samples treated with bavistin alone and in combination with propionic acid either suppressed mycoflora completely or very less mycoflora (2-5 species) was detected from such seeds.

Seed component plating technique

Plating of various components viz. seed coat, cotyledons and embryonic axis of the seeds treated with propionic acid and in combination with fungicide (Tables 53-55) showed almost similar trend of mycoflora occurrence on various parts of the seed. Propionic acid treated seeds possessed comparatively more mycoflora than other treatments in all the seed components except Bavistin treated embryonic axis at 0-level. Bavistin and Bavistin + Propionic acid treated seed parts exhibited less mycoflora than propionic acid treatment.

Table 51. Per cent mycoflora from kidney bean seeds after propionic acid and bavistin treatment (Blotter Method)

Treatment	Storage container	Storage Period (Months)	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cephalosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	*Rhizoctonia solani*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PROPIONIC ACID (10 ml/kg)	G	0	5	4	-	-	-	12	2	-	-	8	3	2	7
		2	3	3	-	-	4	-	-	-	4	1	2	-	6
		4	-	-	6	3	-	2	2	-	8	10	12	-	7
		6	3	2	2	-	-	-	-	4	-	2	-	-	5
		Mean	2.8	2.3	2.0	0.8	1.0	3.5	1.0	1.0	3.0	5.3	4.3	0.5	
	P	0	4	-	4	2	-	4	2	5	-	10	-	4	8
		2	2	-	-	-	-	-	-	3	7	6	-	-	4
		4	5	2	4	-	3	2	-	-	10	8	5	2	9
		6	6	6	5	5	-	-	-	2	-	-	2	-	6
		Mean	4.3	2.0	3.3	1.8	0.8	1.5	0.5	2.5	4.3	6.0	1.8	1.5	
	M	0	8	6	12	-	-	8	-	4	-	-	-	-	5
		2	-	8	2	-	-	-	1	5	9	8	6	-	7
		4	1	-	4	-	-	-	-	-	4	9	12	-	5
		6	4	5	6	-	-	-	-	-	-	-	4	-	4
		Mean	3.3	4.8	6.0	0.0	0.0	2.0	0.3	2.3	3.3	4.3	5.5	0.0	
BAVISTIN (2.5 g/kg)	G	0	5	-	-	-	7	-	-	-	-	-	6	-	3
		2	-	-	-	-	-	-	-	7	-	2	-	-	2
		4	2	-	-	-	-	-	-	-	4	-	2	-	3
		6	-	-	2	-	-	-	-	-	-	-	-	-	1
		Mean	1.8	0.0	0.5	0.0	1.8	0.0	0.0	1.8	1.0	0.5	2.0	0.0	
	P	0	6	-	7	-	-	-	8	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	14	-	-	-	1
		4	2	-	-	-	-	-	-	-	3	2	5	-	4
		6	-	-	4	-	2	-	-	5	-	-	-	-	3
		Mean	2.0	0.0	2.8	0.0	0.5	0.0	2.0	1.3	4.3	0.5	1.3	0.0	
	M	0	3	4	-	-	-	-	2	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	5	-	-	-	-	1
		4	-	2	2	-	-	-	-	4	4	2	-	-	5
		6	-	-	-	-	-	-	-	-	-	3	-	-	1
		Mean	0.8	1.5	0.5	0.0	0.0	0.0	0.5	2.3	1.0	1.3	0.0	0.0	
PROPIONIC ACID + BAVISTIN	G	0	2	-	2	-	-	-	-	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	-	1	-	1
		4	4	-	-	-	-	-	-	-	3	1	2	-	4
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.8	0.0	
	P	0	-	-	12	-	-	-	-	-	10	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	5	1	2	-	-	-	-	-	-	2	3	-	5
		6	-	-	-	-	-	-	-	-	1	-	2	-	2
		Mean	1.3	0.3	3.5	0.0	0.0	0.0	0.0	0.0	2.8	0.5	1.3	0.0	
	M	0	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	2	3	4	-	3
		6	-	-	2	-	-	-	-	-	-	2	1	-	3
		Mean	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	1.3	1.3	0.0	

G: Gunny bag; P: Polylined bag; M: Metallic bin

Table 52. Per cent mycoflora from kidney bean seeds after propionic acid and bavistin treatment (Malt Extract Agar)

Treatment	Storage container	Storage Period (Months)	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cephalosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	*Rhizoctonia solani*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PROPIONIC ACID (10 ml/kg)	G	0	12	4	-	-	-	12	4	6	-	8	3	-	7
		2	5	3	-	-	4	6	-	-	4	-	6	-	6
		4	-	-	6	3	-	2	2	-	8	10	12	-	7
		6	-	-	2	-	-	-	-	4	-	-	-	-	2
		Mean	4.3	1.8	2.0	0.8	1.0	5.0	1.5	2.5	3.0	4.5	5.3	0.0	
	P	0	4	8	4	5	-	6	-	5	-	10	-	4	8
		2	-	-	-	-	-	-	-	3	7	6	-	-	3
		4	5	-	4	9	3	8	-	-	12	16	15	2	9
		6	6	12	5	5	-	-	-	2	-	-	2	-	6
		Mean	3.8	5.0	3.3	4.8	0.8	3.5	0.0	2.5	4.8	8.0	4.3	1.5	
	M	0	16	12	20	-	-	9	-	4	-	-	-	-	5
		2	-	8	11	-	-	-	1	5	9	8	6	-	7
		4	1	-	4	-	-	2	4	5	4	9	12	-	8
		6	4	5	6	-	-	-	-	-	-	-	4	-	4
		Mean	5.3	6.3	10.3	0.0	0.0	2.8	1.3	3.5	3.3	4.3	5.5	0.0	
BAVISTIN (2.5 g/kg)	G	0	7	-	-	-	-	-	-	7	10	-	6	-	4
		2	-	-	1	-	-	-	-	1	-	2	-	-	3
		4	-	-	1	-	-	-	-	-	4	-	2	-	3
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	1.8	0.0	0.5	0.0	0.0	0.0	0.0	2.0	3.5	0.5	2.0	0.0	
	P	0	7	-	7	-	-	-	8	-	-	-	-	-	3
		2	-	-	4	-	-	-	-	-	14	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	2	5	-	2
		6	-	-	4	2	-	-	-	-	-	-	-	-	2
		Mean	1.8	0.0	3.8	0.5	0.0	0.0	2.0	0.0	3.5	0.5	1.3	0.0	
	M	0	3	4	-	-	-	-	2	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	5	-	-	-	-	1
		4	-	2	2	-	-	-	-	4	4	2	-	-	5
		6	-	-	-	-	-	-	-	-	-	3	-	-	1
		Mean	0.8	1.5	0.5	0.0	0.0	0.0	0.5	2.3	1.0	1.3	0.0	0.0	
PROPIONIC ACID + BAVISTIN	G	0	1	-	8	-	-	-	-	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	-	4	-	1
		4	-	-	-	-	-	-	-	-	-	2	2	-	2
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.3	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.5	0.0	
	P	0	-	-	12	-	-	-	-	-	10	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	2	1	4	-	3
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	3.0	0.3	1.0	0.0	
	M	0	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	2	-	3	-	2
		6	-	-	-	-	-	-	-	-	-	5	-	-	1
		Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.3	0.8	0.0	

G: Gunny bag; P: Polylined bag; M: Metallic bin

Table 53. Per cent mycoflora from kidney bean seeds (seed coat) after propionic acid and bavistin treatment

Treatment	Storage container	Storage Period (Months)	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cephalosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	*Rhizoctonia solani*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PROPIONIC ACID (10 ml/kg)	G	0	-	12	-	-	10	12	4	4	-	-	-	-	5
		2	4	-	-	-	8	4	-	-	4	-	4	-	5
		4	-	-	-	-	-	-	-	-	4	8	8	1	4
		6	10	8	8	-	-	-	-	2	-	-	1	-	5
		Mean	3.5	5.0	2.0	0.0	4.5	4.0	1.0	1.5	2.0	2.0	3.3	0.3	
	P	0	4	-	2	2	-	4	-	-	-	2	-	2	6
		2	-	-	-	-	-	-	-	-	-	-	-	-	0
		4	2	-	-	-	-	-	-	-	6	8	6	1	5
		6	6	2	8	-	-	-	1	-	-	2	4	-	6
		Mean	3.0	0.5	2.5	0.5	0.0	1.0	0.3	0.0	1.5	3.0	2.5	0.8	
	M	0	8	-	12	-	-	16	4	-	-	-	2	2	6
		2	-	-	-	-	-	-	-	-	9	-	8	-	2
		4	2	-	-	2	-	-	-	-	3	9	2	-	5
		6	10	2	10	-	-	-	-	2	-	-	-	-	4
		Mean	5.0	0.5	5.5	0.5	0.0	4.0	1.0	0.5	3.0	2.3	3.0	0.5	
BAVISTIN (2.5 g/kg)	G	0	7	-	-	-	-	-	14	2	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	14	2	5	-	3
		4	-	-	3	-	-	-	-	-	-	-	6	-	2
		6	-	-	2	-	-	-	-	-	-	-	-	-	1
		Mean	1.8	0.0	1.3	0.0	0.0	0.0	3.5	0.5	3.5	0.5	2.8	0.0	
	P	0	8	-	-	-	-	-	-	8	-	-	-	1	3
		2	-	-	1	-	-	-	-	-	5	2	-	-	3
		4	-	-	-	-	-	-	-	-	-	-	2	-	1
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	2.0	0.0	0.3	0.0	0.0	0.0	0.0	2.0	1.3	0.5	0.5	0.3	
	M	0	-	-	-	-	-	-	-	-	-	-	-	-	0
		2	-	-	3	-	-	-	-	-	-	-	-	-	1
		4	4	-	-	-	-	-	-	-	8	2	-	-	3
		6	5	2	1	-	-	-	-	-	-	-	-	-	3
		Mean	2.3	0.5	1.0	0.0	0.0	0.0	0.0	0.0	2.0	0.5	0.0	0.0	
PROPIONIC ACID + BAVISTIN	G	0	2	-	6	-	-	-	-	-	-	-	-	2	3
		2	-	-	-	-	-	-	-	-	-	-	2	-	1
		4	-	-	-	-	-	-	-	1	5	-	-	-	2
		6	-	-	2	-	-	-	-	-	-	-	-	-	1
		Mean	0.5	0.0	2.0	0.0	0.0	0.0	0.0	0.3	1.3	0.0	0.5	0.5	
	P	0	-	1	-	-	-	-	4	-	-	-	-	-	2
		2	2	-	-	-	-	-	-	-	-	-	-	-	1
		4	-	-	5	-	-	-	-	4	-	2	-	-	3
		6	-	-	-	-	-	-	-	-	-	-	-	-	0
		Mean	0.5	0.3	1.3	0.0	0.0	0.0	1.0	1.0	0.0	0.5	0.0	0.0	
	M	0	2	2	-	-	-	-	-	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	4	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	2	6	-	2
		6	8	-	2	-	-	-	-	-	-	-	-	-	2
		Mean	2.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	1.0	0.5	1.5	0.0	

G: Gunny bag; P: Polylined bag; M: Metallic bin

Table 54. Per cent mycoflora from kidney bean seeds (cotyledons) after propionic acid and bavistin treatment

Treatment	Storage container	Storage Period (Months)	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Colletotrichum lindemuthianum*	*F. moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	*Rhizoctonia solani*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PROPIONIC ACID (10 ml/kg)	G	0	-	-	12	-	16	-	-	-	-	-	-	2
		2	4	-	-	-	4	-	-	-	4	-	-	3
		4	-	-	-	-	-	-	-	-	6	12	-	2
		6	-	-	16	-	-	-	-	-	-	-	-	1
		Mean	1.0	0.0	7.0	0.0	5.0	0.0	0.0	0.0	2.5	3.0	0.0	
	P	0	-	-	8	-	10	-	-	-	4	4	-	4
		2	4	-	-	-	-	-	-	2	-	-	-	2
		4	8	-	-	-	-	-	-	12	8	4	-	4
		6	8	-	-	-	-	-	-	-	-	-	-	1
		Mean	5.0	0.0	2.0	0.0	2.5	0.0	0.0	3.5	3.0	2.0	0.0	
	M	0	-	-	-	12	10	-	-	-	-	4	-	3
		2	8	4	-	-	-	-	-	14	-	-	-	3
		4	-	-	-	12	-	-	-	-	10	12	-	3
		6	-	4	-	-	-	-	-	-	-	-	-	1
		Mean	2.0	2.0	0.0	6.0	2.5	0.0	0.0	3.5	2.5	4.0	0.0	
BAVISTIN (2.5 g/kg)	G	0	4	-	12	-	-	-	-	-	-	-	1	3
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	1	-	-	1
		6	4	-	4	-	-	-	-	-	4	-	-	3
		Mean	2.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.3	
	P	0	-	4	4	4	-	-	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	-	-	-	-	0
		6	8	-	-	-	-	-	-	4	8	-	-	3
		Mean	2.0	1.0	1.0	1.0	0.0	0.0	0.0	1.0	2.0	0.0	0.0	
	M	0	4	-	-	-	-	-	-	-	16	-	-	2
		2	-	-	-	-	-	4	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	8	8	-	-	2
		6	8	-	-	-	-	-	-	-	-	-	-	1
		Mean	3.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0	6.0	0.0	0.0	
PROPIONIC ACID + BAVISTIN	G	0	2	-	2	-	-	-	-	-	2	-	-	3
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	-	4	-	2	-	2
		6	2	-	-	1	-	-	-	-	-	-	-	2
		Mean	1.0	0.0	0.5	0.3	0.0	0.0	0.0	1.0	0.5	0.5	0.0	
	P	0	4	-	-	4	2	-	2	-	6	-	-	5
		2	-	-	-	-	-	-	-	2	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	2	-	1
		6	-	2	-	-	-	-	-	-	-	-	-	1
		Mean	1.0	0.5	0.0	1.0	0.5	0.0	0.5	0.5	1.5	0.5	0.0	
	M	0	4	-	-	-	-	-	-	-	-	2	-	2
		2	-	-	-	-	-	-	-	-	-	-	-	0
		4	8	-	-	-	-	-	-	5	-	8	-	3
		6	-	-	-	12	-	-	-	-	1	-	-	2
		Mean	3.0	0.0	0.0	3.0	0.0	0.0	0.0	1.3	0.3	2.5	0.0	

G: Gunny bag; P: Polylined bag; M: Metallic bin

Table 55. Per cent mycoflora from kidney bean seeds (embryonic axis) after propionic acid and bavistin treatment

Treatment	Storage container	Storage Period (Months)	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*Myrothecium* sp.	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. perpuragenum*	Total mycoflora
1	2	3	4	5	6	7	8	9	10	11	12	13
PROPIONIC ACID (10 ml/kg)	G	0	16	-	4	4	-	-	-	-	-	3
		2	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	5	-	6	2
		6	4	-	4	-	-	-	-	-	-	2
		Mean	5.0	0.0	2.0	1.0	0.0	0.0	1.3	0.0	1.5	
	P	0	24	-	4	16	-	-	-	-	-	3
		2	-	-	-	4	8	-	-	-	-	2
		4	-	-	-	-	-	-	4	4	12	3
		6	8	4	4	-	-	-	-	-	-	3
		Mean	8.0	1.0	2.0	5.0	2.0	0.0	1.0	1.0	3.0	
	M	0	14	2	14	12	-	-	-	-	-	4
		2	-	-	4	-	-	-	-	-	-	1
		4	-	-	-	-	-	-	-	-	-	0
		6	8	8	4	-	-	-	-	-	-	3
		Mean	5.5	2.5	5.5	3.0	0.0	0.0	0.0	0.0	0.0	
BAVISTIN (2.5 g/kg)	G	0	8	-	4	4	-	16	-	-	-	4
		2	-	-	-	2	-	4	-	-	-	2
		4	-	-	-	-	-	4	-	4	12	3
		6	4	-	8	-	-	-	-	-	-	2
		Mean	3.0	0.0	3.0	1.5	0.0	6.0	0.0	1.0	3.0	
	P	0	8	-	4	4	-	10	-	-	-	4
		2	-	-	-	4	-	4	-	-	-	2
		4	-	-	-	-	-	-	-	-	-	0
		6	8	2	8	-	-	-	-	-	-	3
		Mean	4.0	0.5	3.0	2.0	0.0	3.5	0.0	0.0	0.0	
	M	0	-	12	12	-	1	4	-	-	-	4
		2	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	8	-	-	-	1
		6	8	-	-	-	-	-	-	-	-	1
		Mean	2.0	3.0	3.0	0.0	0.3	3.0	0.0	0.0	0.0	
PROPIONIC ACID + BAVISTIN	G	0	4	-	4	-	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	2	5	-	2	3
		6	-	-	-	-	-	-	-	-	-	0
		Mean	1.0	0.0	1.0	0.0	0.0	0.5	1.3	0.0	0.5	
	P	0	2	-	-	2	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	2	-	1	-	2
		6	-	-	-	-	-	-	-	-	-	0
		Mean	0.5	0.0	0.0	0.5	0.0	0.5	0.0	0.3	0.0	
	M	0	2	-	-	2	-	-	-	-	-	2
		2	-	-	-	-	-	-	-	-	-	0
		4	-	-	-	-	-	-	2	-	-	1
		6	-	-	1	-	-	-	-	-	-	1
		Mean	0.5	0.0	0.3	0.5	0.0	0.0	0.5	0.0	0.0	

G: Gunny bag; P: Polylined bag; M: Metallic bin

4.4.2.3 Seed quality parameters

Seed quality parameters viz. carbohydrates, proteins and fat contents of the treated seeds (Table 56) were found to have similar effects as were noticed in earlier experiments. Carbohydrates showed an decreasing trend along with storage but this decline was minimum in Bavistin treated seeds followed by untreated check.

Protein content in all the treatments increased significantly with storage but at the end of storage, values were statistically at par with each other. No significant change was noticed in the fat content even at the end of storage.

4.4.3 Effect of seed dressing fungicides and isolated mycoflora on seed health, mycoflora and quality parametrers

4.4.3.1 Seed health

Data presented in Table 57 revealed that seed germination in the beginning was improved in almost all the treatments where fungicides were used to that of untreated and mycoflora treated seeds. The germination increased up to 4th month of storage and a subsequent decline was noticed in all the treatments though the percentage was higher than 0-level of testing. Maximum germination was recorded in Bavistin+TMTD treated seeds over all other fungicides.

Seed moisture content at 0-level of storage was 8.3 per cent (Table 58) and remained more or less the same during storage (8.4-8.5%). Significant changes in moisture level were observed at all the intervals of testing over 0- level, however, after an increase up to 4th month a decrease was noticed at the end of storage.

Seed dressing with fungicides alone improved the seed vigour, maximum being in Bavistin+TMTD treated seeds after two months of storage. Combined application of mycoflora + fungicides lowered the seed vigour values at various levels of testing (Table 59). Maximum improvement was noticed in seeds treated with Baylaton and Thiram after 6 months and

Table 56. Effect of propionic acid and bavistin on seed quality parameters

Treatment	Storage Container	Carbohydrate*				Protein*				Fat*			
		0**	2**	6**	Mean	0**	2**	6**	Mean	0**	2**	6**	Mean
Propionic acid (10 ml/kg)	Gunny bag	63.00	61.10	57.80	60.63	20.74	22.08	23.57	22.13	1.18	1.18	1.19	1.18
	Polylined bag	63.00	62.60	58.80	61.47	20.74	20.60	22.06	21.13	1.18	1.18	1.18	1.18
	Metallic bin	63.00	61.80	59.70	61.50	20.74	21.70	22.96	21.80	1.18	1.18	1.19	1.18
Bavistin (2.5 g/kg)	Gunny bag	63.00	61.90	59.20	61.37	20.74	21.62	22.85	21.74	1.18	1.20	1.20	1.19
	Polylined bag	63.00	63.00	61.50	62.50	20.74	21.54	22.49	21.59	1.18	1.18	1.18	1.18
	Metallic bin	63.00	62.40	60.70	62.03	20.74	21.04	22.23	21.34	1.18	1.17	1.17	1.17
Propionic acid + Bavistin	Gunny bag	63.00	61.90	60.20	61.70	20.74	21.28	21.49	21.17	1.18	1.19	1.19	1.19
	Polylined bag	63.00	62.40	61.10	62.17	20.74	20.72	21.02	20.83	1.18	1.18	1.18	1.18
	Metallic bin	63.00	62.10	60.00	61.70	20.74	21.73	21.97	21.48	1.18	1.18	1.18	1.18
Untreated check	Gunny bag	63.00	62.10	60.40	61.83	20.74	21.53	22.42	21.56	1.18	1.21	1.18	1.20
	Polylined bag	63.00	62.10	59.20	61.43	20.74	20.71	21.64	21.03	1.18	1.18	1.18	1.18
	Metallic bin	63.00	61.60	58.40	61.0	20.74	21.66	22.31	21.57	1.18	1.19	1.18	1.19
Mean		63.00	62.08	59.75	61.61	20.74	21.35	22.25	21.45	1.18	1.19	1.18	1.18
CD(P=0.05)	A Treatment				NS	A	0.108	AxB	0.188	A			NS
	B Container				NS	B	0.094	AxC	0.188	B			NS
	C Storage period				0.51	C	0.094	BxC	0.162	C			NS
								AxBxC	0.325				

* g/100 g of dry weight

** Months of storage

Table 57. Effect of seed dressing fungicides and mycoflora on germination (%)

Treatment	Time interval (months)				
	0	2	4	6	Mean
Bavistin	80.00 (63.43)	83.33 (66.03)	89.33 (71.24)	86.00 (68.22)	84.67 (67.23)
Baylaton	80.00 (63.43)	83.33 (66.03)	90.00 (71.91)	86.67 (68.83)	85.00 (67.55)
Captan	80.00 (63.43)	84.00 (66.59)	84.00 (66.57)	82.00 (65.01)	82.50 (65.39)
Thiram	80.00 (63.43)	86.00 (68.22)	90.67 (72.66)	84.00 (66.57)	85.17 (67.72)
Bavistin +TMTD	80.00 (63.43)	85.33 (67.65)	88.00 (69.98)	88.67 (70.65)	85.50 (67.93)
Untreated seed	80.00 (63.43)	79.33 (63.04)	70.67 (57.24)	74.67 (59.84)	76.17 (60.89)
Mycoflora+Bavistin	80.00 (63.43)	87.33 (69.37)	86.67 (68.83)	86.00 (68.22)	85.00 (67.46)
Mycoflora+Baylaton	80.00 (63.43)	84.00 (66.57)	92.00 (74.07)	88.00 (69.98)	86.00 (68.51)
Mycoflora+Captan	80.00 (63.43)	86.67 (68.83)	87.33 (69.37)	90.00 (71.91)	86.00 (68.38)
Mycoflora+Thiram	80.00 (63.43)	88.00 (69.98)	90.00 (71.91)	82.67 (65.55)	85.17 (67.72)
Mycoflora+ Bavistin +TMTD	80.00 (63.43)	86.00 (68.22)	91.33 (73.32)	86.00 (68.22)	85.83 (68.30)
Mycoflora treated seed	80.00 (63.43)	76.00 (60.73)	74.00 (59.39)	78.00 (62.10)	77.00 (61.41)
Mean	80.00 (63.43)	84.11 (66.77)	86.17 (68.87)	84.39 (67.09)	

CD(p=0.05)

Treatment	3.116
Period of storage	1.798
Treatmentx Storage period	NS

Figures in parentheses are the angular transformed values

Table 58. Effect of seed dressing fungicides and mycoflora on seed moisture content (%)

Treatment	Time interval (months)				
	0	2	4	6	Mean
Bavistin	8.3	8.4	8.7	8.4	8.4
Baylaton	8.3	8.4	8.7	8.5	8.4
Captan	8.3	8.5	8.8	8.5	8.5
Thiram	8.3	8.4	8.7	8.5	8.4
Bavistin +TMTD	8.3	8.4	8.6	8.4	8.4
Untreated seed	8.3	8.6	8.6	8.5	8.5
Mycoflora+Bavistin	8.3	8.5	8.7	8.4	8.4
Mycoflora+Baylaton	8.3	8.5	8.7	8.4	8.4
Mycoflora+Captan	8.3	8.5	8.8	8.4	8.5
Mycoflora+Thiram	8.3	8.5	8.7	8.4	8.4
Mycoflora+ Bavistin +TMTD	8.3	8.4	8.8	8.5	8.5
Mycoflora treated seed	8.3	8.7	8.7	8.5	8.5
Mean	8.3	8.5	8.7	8.4	

CD(P=0.05)

Treatment	NS
Period of storage	0.071
Treatmentx Storage period	NS

Table 59. Effect of seed dressing fungicides and mycoflora on seed vigour

Treatment	Time interval (months)				
	0	2	4	6	Mean
Bavistin	3.778	3.861	3.851	3.845	3.834
Baylaton	3.778	3.986	3.976	3.956	3.924
Captan	3.778	3.986	3.956	3.946	3.917
Thiram	3.778	3.986	3.976	3.956	3.924
Bavistin +TMTD	3.778	4.002	3.152	3.841	3.693
Untreated seed	3.778	3.508	3.499	3.399	3.546
Mycoflora+Bavistin	3.778	3.617	3.608	3.508	3.628
Mycoflora+Baylaton	3.778	3.861	3.841	3.821	3.825
Mycoflora+Captan	3.778	3.623	3.453	3.343	3.549
Mycoflora+Thiram	3.778	3.618	3.617	3.572	3.646
Mycoflora+ Bavistin +TMTD	3.778	3.861	3.851	3.142	3.658
Mycoflora treated seed	3.778	3.385	3.374	3.365	3.476
Mean	3.778	3.775	3.680	3.641	3.718

CD(P=0.05)

Treatment	0.072
Period of storage	0.042
Treatmentx Storage period	0.144

overall mean value was minimum in mycoflora treated and mycoflora+Captan treated seeds. Fungicide, mycoflora dressing and storage duration were found to influence seed vigour significantly over untreated check.

Seedling vigour index recorded on fresh weight basis decreased to 1306.81 from initial value of 1647.03 in the seeds smeared with mycoflora which, in turn, was at par with untreated seeds as well as seeds treated with mycoflora + Baylaton whereas, a significant increase in seedling vigour index was recorded upto 4^{th} month of storage in fungicide treated seed lots and thereafter, a decline was noticed in all the treatments except Bavistin+TMTD (Table 60). However, untreated seeds and seeds treated with mycoflora alone registered a constant decrease throughout the storage period.

Seedling vigour index on dry weight basis exhibited similar trend of increase/ decrease in vigour index and highest values were recorded in Bavistin + TMTD and Bavistin treated seeds though they were statistically at par with other treatments except Baylaton and mycoflora+Baylaton (Table 61).

4.4.3.2 Seed mycoflora

Seed samples of local variety treated with different seed dressing fungicides alone and in combination with sporulating and non-sporulating cultures of all the isolated fungi were analysed by different methods of mycoflora detection and data recorded on the development and recovery of fungi are listed in Tables 62-66.

Blotter method

It was observed that in the beginning of storage 7,6,7,8 and 8 species of fungi were recorded on fungicide treated seed lots with a total of 24 species on different treatments. Only storage fungi were noticed after 4 and 6 months of storage on fungicide treated seed lots. No fungal infestation was noticed on Thiram and Bavistin+TMTD treated seeds after 6 months of

Table 60. Effect of seed dressing fungicides and mycoflora on seedling vigour index (fresh weight basis)

Treatment	Time interval (months)				
	0	2	4	6	Mean
Bavistin	1647.03	2129.87	2229.39	1844.69	1962.75
Baylaton	1647.03	1635.70	1766.15	1598.87	1661.94
Captan	1647.03	1903.51	2276.47	1620.23	1861.81
Thiram	1647.03	1845.55	2823.40	2239.03	2138.75
Bavistin +TMTD	1647.03	2013.25	2764.87	1855.03	2070.05
Untreated seed	1647.03	1597.01	1433.11	1313.32	1497.62
Mycoflora+Bavistin	1647.03	2003.13	1990.07	1630.55	1817.70
Mycoflora+Baylaton	1647.03	1354.99	1707.13	1431.93	1535.27
Mycoflora+Captan	1647.03	1862.67	2107.93	1539.41	1789.26
Mycoflora+Thiram	1647.03	1737.03	2534.23	1647.31	1891.40
Mycoflora+ Bavistin +TMTD	1647.03	1955.63	2413.49	1855.03	1967.80
Mycoflora treated seed	1647.03	1532.55	1375.31	1306.81	1465.43
Mean	1647.03	1797.58	2118.46	1656.85	
CD(P=0.05)					
	Treatment			140.028	
	Period of storage			80.845	
	Treatmentx Storage period			280.056	

Table 61. Effect of seed dressing fungicides and mycoflora on seedling vigour index (dry weight basis)

Treatment	Months of Storage				
	0	2	4	6	Mean
Bavistin	21.62	21.60	19.59	21.37	21.05
Baylaton	21.62	17.52	16.16	15.35	17.66
Captan	21.62	19.66	16.89	20.09	19.57
Thiram	21.62	24.03	18.80	20.37	21.21
Bavistin +TMTD	21.62	20.46	17.50	21.83	20.35
Untreated seed	21.62	17.05	16.48	16.23	17.85
Mycoflora+Bavistin	21.62	18.49	20.27	18.70	19.77
Mycoflora+Baylaton	21.62	17.08	19.93	12.13	17.69
Mycoflora+Captan	21.62	19.58	16.22	20.05	19.37
Mycoflora+Thiram	21.62	19.45	17.95	19.56	19.65
Mycoflora+ Bavistin +TMTD	21.62	20.37	17.46	19.44	19.72
Mycoflora treated seed	21.62	14.05	13.23	16.17	16.27
Mean	21.62	19.11	17.54	18.44	
CD(P=0.05)					
	Treatment		2.286		
	Period of storage		1.319		
	Treatmentx Storage period		NS		

storage. Untreated control possessed maximum of 10 species in the beginning which decreased gradually to 7 at the end of storage and pathogenic fungi were replaced by storage fungi (Table 62).

Mycoflora smeared seeds exhibited different pattern at various stages of detection as seeds treated with mycoflora alone resulted in the recovery of a maximum of 15 species in the beginning and 3 species in the end of storage as that of 7 and 0 species, respectively in all fungicide treatments. Seeds treated with mycoflora+Thiram showed minimum infestation at the end of storage.

Agar plate method

Agar plate method revealed the presence of maximum of 10 species in Thiram treated seeds maximum being detected in the initial stages of storage. Minimum number of fungi (0-2) were recovered from Bavistin, Thiram and Bavstin + TMTD treated seeds after 4 and 6 months of storage (Table 63).

Majority of the pathogenic fungi perished and were replaced by storage mycoflora in all the treatments as compared to untreated check where 8 species were recorded after storage of 6 months. However, contrary to this mycoflora + fungicide treated seeds possessed more mycoflora, maximum being in the beginning of storage. The number of fungi though reduced with increased duration but the frequency of fungi was higher than that of those which were treated with fungicides alone.

Seed component plating technique

Seed coats: Seed coats contained maximum mycoflora as compared to cotyledons and embryonic axis. The frequency of fungi reduced significantly in all the treatments with duration of storage and minimum mycoflora was detected from Baylaton and Bavistin treated seeds. Similarly, in case of artificially inoculated seeds Captan and Bavistin were found to be most effective over other fungicides (Table 64).

Treatment	Storage duration	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cephalosporium* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. moniliformae*	*F. oxysporum*	*F. solani*	*Gliocladium* sp.	*Myrothecium* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	*Trichoderma* sp.	*Trichothecium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
Bavistin*	0	5	-	-	-	18	-	6	-	-	14	-	20	6	-	-	18	-	-	-	-	-	-	-	-	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	5	-	-	2
	4	9	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	18	-	-	-	-	3
	6	17	-	-	-	-	9	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	3
	Mean	7.8	0	0	0	4.5	2.3	1.5	0	1.8	3.5	0	5	1.5	3	0	4.5	0	0	0	4.5	1.5	1.3	0	0	
Bayleton*	0	4	-	-	-	-	-	6	-	-	22	-	20	6	-	-	18	-	-	-	-	-	-	-	-	6
	2	-	-	-	37	-	-	-	-	-	-	-	-	-	-	-	18	6	6	-	-	-	-	-	-	4
	4	-	-	-	-	-	-	-	-	7	6	-	-	-	-	-	-	5	-	-	18	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5	-	-	-	-	-	-	2
	Mean	1	0	0	9.3	0	0	1.5	0	1.8	7	0	5	1.5	0	0	9	4	2.8	0	4.5	0	0	0	0	
Captan*	0	9	-	-	-	20	5	-	-	-	7	-	22	6	-	-	20	-	-	-	-	-	-	-	-	7
	2	-	-	-	-	-	-	-	-	-	12	-	-	6	-	-	-	-	-	6	-	-	-	-	-	3
	4	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	2
	6	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Mean	4.3	0	0	0	5	2.8	0	0	0	4.8	0	5.5	3	0	0	5	0	0	1.5	8	0	0	0	0	
[illegible]	0	6	-	-	-	21	-	6	-	-	7	-	19	5	-	-	22	-	6	-	-	-	-	-	-	8
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	12	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	14	-	-	7	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	4.5	0	0	0	5.3	0	1.5	0	1.8	1.8	0	4.8	1.3	0	0	5.5	3.5	1.5	0	1.8	0	0	0	0	
	0	5	-	-	-	18	-	6	-	-	16	-	18	6	-	-	19	-	-	-	-	10	-	-	-	8
	2	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	6	-	-	2
	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	2.3	0	0	0	4.5	0	1.5	0	0	7.3	0	4.5	1.5	0	0	4.8	0	0	0	7.3	2.5	1.5	0	0	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Untreated Seed	0	6	-	4	-	18	-	-	-	-	66	3	20	6	-	-	19	-	14	-	-	12	-	-	-	10
	2	-	-	-	-	-	-	5	-	-	-	-	-	-	5	-	6	7	5	-	-	20	5	-	18	8
	4	12	-	-	-	12	-	-	-	14	-	-	-	-	-	6	7	-	-	-	15	-	6	-	-	7
	6	15	-	-	-	-	20	-	-	6	12	-	-	-	12	-	-	-	-	-	10	-	-	-	6	7
	Mean	8.3	0	1	0	7.5	5	1.3	0	5	19.5	0.8	5	1.5	4.3	1.5	8	1.8	4.8	0	6.3	8	2.8	0	6	
Mycoflora + Bavistin*	0	6	-	-	-	18	-	-	-	-	22	-	18	7	-	-	18	-	-	5	-	-	-	-	-	7
	2	-	-	6	8	-	-	-	-	-	-	-	-	-	-	-	12	12	12	-	-	5	-	-	20	7
	4	5	-	-	-	4	-	-	-	-	-	-	-	5	-	6	7	-	-	-	14	-	-	-	12	7
	6	6	-	-	-	-	-	-	-	-	-	-	-	-	-	6	7	5	-	-	12	-	-	-	-	5
	Mean	4.3	0	1.5	2	5.5	0	0	0	0	5.5	0	4.5	3	0	3	11	4.3	3	1.3	6.5	1.3	0	0	8	
Mycoflora + Bayletan*	0	8	-	-	-	20	-	-	-	-	24	-	19	5	-	-	22	-	-	-	-	-	-	-	-	6
	2	-	-	-	21	-	-	-	-	20	-	-	-	27	12	-	-	4	18	-	-	-	6	-	-	7
	4	-	-	-	-	-	12	-	-	-	-	-	-	12	-	-	-	9	-	-	12	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	7	-	-	-	-	2
	Mean	2	0	0	5.3	5	3	0	0	5	6	0	4.8	11	3	0	7	3.3	4.5	0	4.8	0	1.5	0	0	
Mycoflora + Captan*	0	7	-	-	-	19	-	-	-	-	19	-	20	6	-	-	20	-	-	4	-	-	-	-	-	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	29	-	-	-	20	-	-	-	15	4
	4	-	-	-	-	-	-	-	-	5	6	-	-	7	-	-	-	-	-	-	12	-	-	-	-	4
	6	-	-	5	-	-	-	-	-	-	-	-	-	-	5	-	7	5	6	-	12	-	-	-	-	6
	Mean	1.8	0	1.3	0	4.8	0	0	0	1.3	6.3	0	5	11.3	1.3	0	14	1.3	1.5	1	11	0	0	0	3.8	
Mycoflora+ Thiram*	0	6	-	-	-	20	-	-	-	-	21	-	20	5	-	-	20	-	-	-	-	-	-	-	-	6
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	16	12	11	-	21	-	-	-	-	5
	4	9	-	4	8	-	-	-	-	-	-	-	-	-	-	-	27	-	-	-	-	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	8	-	-	-	-	2
	Mean	3.8	0	1	2	5	0	0	0	0	5.3	0	5	1.3	1.3	0	18	3	2.8	0	7.3	0	0	0	0	
Mycoflora + Bavistin+ TMTD*	0	4	-	-	-	22	-	-	-	-	20	-	20	5	-	-	20	-	-	-	-	-	-	-	-	6
	2	-	-	-	12	-	-	-	-	34	-	-	-	-	-	20	22	8	-	-	-	-	-	-	-	5
	4	-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	6	-	5	-	12	-	-	-	-	4
	6	6	-	-	-	-	-	-	-	-	-	-	-	-	6	6	2	-	12	-	-	-	-	-	-	5
	Mean	2.5	0	0	3	5.5	5.3	0	0	8.5	5	0	5	1.3	1.5	6.5	12.5	2	4.3	0	3	0	0	0	0	
Seed + Mycoflora	0	5	-	-	-	17	18	7	7	15	68	-	22	7	-	-	21	-	20	12	7	7	20	-	-	15
	2	-	5	14	12	-	-	-	-	6	6	-	-	20	-	12	20	6	-	-	15	12	-	-	20	12
	4	-	-	20	-	-	7	-	-	6	6	-	-	6	21	-	12	12	-	-	24	-	-	-	-	9
	6	19	8	12	-	5	5	-	-	-	-	-	-	19	-	-	5	5	6	5	6	-	-	12	6	13
	Mean	6	3.3	11.5	3	5.5	7.5	1.8	1.8	6.8	20	0	5.5	13	5.3	3	14.5	5.8	6.5	4.3	13	4.8	5	3	6.5	

* Dose of fungicides: 2.5 g/kg seed

Table 63. Per cent mycoflora from naturally and artificially infected seeds after fungicide treatment (Malt Extract Agar)

Treatment	Storage duration	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Botrytis* sp.	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*F. oxysporum*	*F. solani*	*Macrophomina* sp.	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Phoma* sp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphyllum* sp.	*Sclerotinia sclerotiorum*	*Sclerotium rolfsii*	*Trichoderma* sp.	*Trichothecium* sp.	Total mycoflora
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Bavistin*	0	5	12	20	13	-	-	-	12	-	23	7	-	-	-	-	-	-	-	20	13	-	-	-	-	-	9
	2	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	21	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	4.3	8.3	5	3.3	0	0	0	4	0	5.8	1.8	0	0	0	0	0	0	0	5	3.3	0	0	0	0	0	
Bayleton*	0	6	12	18	12	-	-	-	22	-	20	5	-	-	-	-	-	-	-	21	14	-	-	-	-	-	9
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	6	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	-	13	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	2
	Mean	1.5	7.8	4.5	4.5	0	0	0	5.5	0	5	1.3	0	0	0	1.5	0	0	0	5.3	3.5	0	0	0	0	0	
Captan*	0	7	11	19	13	-	-	-	23	-	20	5	-	-	-	-	-	-	-	18	12	-	-	-	-	-	9
	2	-	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	2
	4	-	10	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	2
	6	-	7	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	2
	Mean	1.8	7	4.8	3.3	2.3	0	0	5.8	0	5	1.3	0	1.8	0	1.5	0	0	0	6.3	3	0	0	0	0	0	
Thiram*	0	6	16	21	12	-	-	-	22	-	20	6	0	-	-	-	-	-	-	20	12	-	-	-	-	-	10
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	22	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	1.5	9.5	5.3	7	0	0	0	5.5	0	5	1.5	0	0	0	0	0	0	0	5	3	0	0	0	0	0	
Bavistin + TMTD*	0	6	13	23	12	-	-	-	20	-	19	5	-	-	-	-	-	-	-	19	12	-	-	-	-	-	9
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	20	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	1.5	8.3	5.8	5.3	0	0	0	5	0	4.8	1.3	0	0	0	0	0	0	0	4.8	3	0	0	0	0	0	

Continued

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Untreated Seed	0	6	12	22	12	-	-	-	18	-	20	6	-	-	-	-	-	-	-	-	-	16	12	-	-	-	-	-	9
	2	-	-	-	32	7	6	8	-	12	-	-	-	-	-	-	7	-	-	-	48	-	-	6	-	22	9
	4	-	36	-	11	-	-	12	6	-	-	-	11	-	-	-	-	-	5	-	26	6	12	-	-	-	9
	6	-	14	-	12	-	-	-	-	-	-	-	-	12	-	-	-	-	-	6	29	6	-	18	12	-	8
	Mean	1.5	15.5	5.5	16.8	1.8	1.5	5	6	3	5	1.5	2.8	3	0	0	1.8	0	1.3	5.5	28.8	3	3	6	3	5.5	
Mycoflora + Bavistin*	0	7	12	19	13	-	-	-	4	-	16	7	-	-	-	-	-	-	-	-	-	21	13	-	-	-	-	-	9
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	1
	4	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5	18	-	-	-	-	-	-	-	-	-	4
	6	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	6	6	-	-	-	-	-	-	-	-	-	-	3
	Mean	1.8	9.5	4.8	3.3	0	0	0	1	0	4	1.8	0	0	1.5	2.8	1.3	4.5	0	5.3	8.5	0	0	0	0	0	
Mycoflora + Bayletan*	0	8	12	22	12	-	-	-	21	-	18	7	-	-	-	-	-	-	-	-	-	21	14	-	-	-	-	-	9
	2	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	2
	4	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	6	7	6	-	-	-	-	-	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	2	7	5.5	4.5	0	0	0	5.3	0	4.5	1.8	0	0	1.5	1.8	1.5	0	0	5.3	7	0	0	0	0	0	
Mycoflora + Captan*	0	6	13	23	12	-	-	-	2	-	22	6	-	-	-	-	-	-	-	-	-	21	14	-	-	-	-	-	9
	2	-	-	-	6	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	16	-	-	-	-	-	4
	4	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	2
	6	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	2
	Mean	1.5	8.8	5.8	4.5	4	0	0	0.5	0	5.5	1.5	0	0	1.3	0	0	0	1	8.3	7.5	0	0	0	0	0	
Mycoflora+ Thiram*	0	5	12	18	14	-	-	-	3	-	20	6	-	-	-	-	-	-	-	-	-	21	14	-	-	-	-	-	9
	2	-	-	-	20	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	4	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	6	7	6	-	-	-	-	-	-	-	-	-	4
	6	-	12	-	-	-	-	-	-	-	12	-	-	-	5	-	-	-	-	-	7	-	-	-	-	-	4
	Mean	1.3	10	4.5	8.5	3	0	0	0.8	0	8	1.5	0	0	2.8	1.8	1.5	0	0	5.3	5.3	0	0	0	0	0	
Mycoflora + Bavistin+ TMTD*	0	6	10	20	15	-	-	-	2	-	20	7	-	-	-	-	-	-	-	-	-	21	12	-	-	-	-	-	9
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	12	-	-	-	7	-	-	-	-	-	4
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	18	-	2
	Mean	1.5	5.8	5	3.8	0	0	0	0.5	0	5	1.8	0	0	0	3	3	0	0	5.3	6.5	0	0	0	4.5	0	
Seed + Mycoflora	0	6	13	18	12	-	-	-	20	-	21	7	-	-	-	-	-	-	-	-	-	18	12	-	-	-	-	-	9
	2	-	-	-	38	6	12	-	-	6	6	-	5	-	-	7	-	-	-	-	-	-	-	12	-	-	-	8
	4	-	36	-	10	-	-	22	6	-	-	-	-	6	-	-	6	7	-	-	14	23	20	9	-	-	11
	6	-	7	-	18	-	6	10	-	-	-	-	6	-	5	6	12	-	-	-	10	32	-	6	-	-	11
	Mean	1.5	14	4.5	19.5	1.5	4.5	8	6.5	1.5	6.8	1.8	2.8	1.5	1.3	3.3	4.5	1.8	0	4.5	9	13.8	8	3.8	0	0	

Dose of fungicides: 2.5 g/kg seed

Table 64. Per cent mycoflora from naturally and artificially infected seeds (Seed coat) after fungicide treatment using Seed Component Plating Technique

Treatment	Storage duration	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*F. oxysporum*	*F. solani*	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizopus stolonifer*	*Trichoderma* sp.	*Trichothecium* sp.	Total mycoflora
1	*2*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
Bavistin*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	4	-	-	-	-	8	-	-	-	-	-	-	-	-	-	2
	Mean	2	5	1	2	0	3	0	2	0	0	0	3	2	4	12	0	0	
Bayleton*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	4	-	-	16	-	-	-	-	-	-	-	-	4	-	-	4	-	-	3
	6	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Mean	2	5	8	1	0	3	0	0	0	0	0	4	2	4	13	0	0	
Captan*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	-	-	-	-	8	-	4	-	-	4	-	-	4	-	-	-	4
	Mean	2	5	5	0	0	5	0	1	0	0	1	3	2	5	12	0	0	
Thiram*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	4	-	3
	4	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	6	8	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	Mean	6	6	4	1	0	3	0	0	0	0	0	3	2	4	12	1	0	
Bavistin + TMTD*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	1
	6	-	-	-	-	-	4	-	8	8	-	-	-	-	-	-	-	-	3
	Mean	2	5	0	0	0	4	0	2	2	0	0	3	2	4	12	1	0	

Continued

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Untreated Seed	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	8	-	-	-	-	-	-	-	4	-	4	-	-	-	-	12	4
	4	-	-	24	-	-	-	-	-	-	-	-	-	-	-	8	-	-	2
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	Mean	2	5	8	0	0	3	0	0	0	1	0	4	2	4	14	0	3	
Mycoflora + Bavistin*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	8	-	-	-	-	-	-	-	-	8	-	-	-	-	-	2
	6	8	4	-	-	-	4	-	-	8	-	-	-	-	-	-	-	-	4
	Mean	4	6	2	0	0	4	0	0	2	0	0	5	2	4	12	0	0	
Mycoflora + Bayletan*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	-	-	-	-	4	-	4	4	-	-	4	-	12	-	-	5
	6	-	-	24	-	8	12	-	4	4	-	-	-	-	-	-	-	-	5
	Mean	2	5	7	0	2	6	1	1	2	1	0	3	3	4	15	0	0	
Mycoflora + Captan*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-	24	-	3
	4	-	-	8	-	-	-	-	-	-	-	8	-	-	4	-	-	-	3
	6	-	-	12	-	-	4	-	-	4	-	-	-	-	-	-	-	-	3
	Mean	5	5	8	0	0	4	0	0	1	0	2	3	2	5	12	6	0	
Mycoflora+ Thiram*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	8	-	4	4	-	-	-	-	4	4	4	4	-	-	-	7
	Mean	2	5	11	0	1	4	0	0	0	0	1	4	3	5	12	0	0	
Mycoflora + Bavistin+ TMTD*	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	8	4	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	6	-	-	4	-	4	8	-	4	-	-	-	-	-	-	-	-	-	4
	Mean	4	6	7	0	1	5	0	1	0	0	0	3	2	4	12	0	0	17
Seed + Mycoflora	0	8	20	-	-	-	12	-	-	-	-	-	12	8	16	48	-	-	
	2	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	4	-	-	-	-	-	-	-	-	-	-	4	12	-	-	3
	6	-	-	-	-	-	4	4	4	8	-	-	-	-	-	-	-	-	4
	Mean	2	5	3	0	0	4	1	1	2	0	0	3	2	5	15	0	0	

* Dose of fungicides: 2.5 g/kg seed

Cotyledons and embryonic axis: Evaluation of cotyledons and embryonic axis of seeds treated with different fungicides showed the presence of few storage fungi. However, artificially inoculated seeds possessed comparatively more mycoflora in all the treatments except seeds treated with Thiram and Bavistin which showed comparatively less mycoflora after 6 months of storage in cotyledons and embryonic axis, respectively (Tables 65, 66).

4.4.3.3 Quality parameters

Various quality parameters viz. carbohydrates, proteins and fats showed uniform values for their contents in the seeds of all treatments before storage of samples.

Carbohydrate: The carbohydrate contents were found to decrease with storage, though decrease was minimum in Thiram and Bavistin treated seeds whereas, mycoflora treated seeds showed maximum decline (Table 67).

Protein: Contrary to carbohydrates, protein content showed an increasing trend throughout the storage. Maximum protein content was found in mycoflora treated seeds (Table 67) followed by Bavistin + mycoflora and Bavistin+TMTD+mycoflora treated seeds. Among seed samples treated with fungicide alone, Bavistin+TMTD treated seeds possessed highest protein content. The untreated seed and artificially inoculated seeds dressed with Bavistin+TMTD had similar protein content.

Fat: Fat contents also showed almost similar pattern of increase/ decrease in various fungicide treatments both in artificially inoculated and uninoculated seeds (Table 67).

4.5 Effect of Seed Coat Colour on the Mycoflora of Kidney Bean Seeds

To observe the effect of various seed coat colours on the development of mycoflora, 12 varieties having different coloured seed coat were subjected to agar plate method (Malt Extract Agar, MEA) before and after

Table 65. Per cent mycoflora from naturally and artificially infected seeds (Cotyledon) after fungicide treatment using Seed Component Plating Technique

Treatment	Storage duration	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*F. oxysporum*	*F. solani*	*Penicillium* spp.	*P. capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Trichoderma* sp.	Total mycoflora
1	*2*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
Bavistin*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	4	-	4
	2	20	8	-	-	-	-	-	-	-	-	-	4	4	-	-	-	-	4
	4	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	1
	6	-	-	4	-	-	-	8	-	-	-	4	-	-	-	-	-	-	3
	Mean	6	2	3	0	0	1	2	1	0	0	1	1	1	0	0	1	0	
Bayleton*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	4	-	4
	2	12	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	8	-	-	-	-	4	4	-	-	-	-	-	-	-	-	3
	Mean	5	0	7	0	0	1	0	1	1	0	0	0	0	0	0	1	0	
Captan*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	4	-	4
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Mean	1	0	3	0	0	1	0	0	0	0	0	0	0	0	0	1	0	
Thiram*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	4	-	4
	2	12	8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	12	4	-	-	-	-	4	-	4	-	-	-	-	-	-	-	-	4
	Mean	7	3	3	0	0	1	1	0	1	0	0	0	0	0	0	1	1	
Bavistin + TMTD*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	4	-	4
	2	4	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	4	4
	4	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	8	2
	6	4	8	-	-	-	-	8	4	-	-	-	-	-	-	-	-	-	4
	Mean	3	2	4	1	0	1	2	1	0	0	0	0	0	0	0	1	3	

Continued

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Untreated Seed	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	12	-	4	4	-	4	-	4	-	-	-	-	-	-	-	4	-	-	6
	4	-	-	12	8	-	8	8	8	8	-	-	-	-	-	-	-	-	-	6
	6	-	-	-	-	-	-	-	-	-	-	-	8	-	8	-	8	-	3
	Mean	4	0	6	3	0	4	2	3	2	0	0	2	0	2	1	3	0	
Mycoflora + Bavistin*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	-	-	8	-	-	-	-	4	-	-	4	4	-	-	-	-	-	-	-	4
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	1
	6	4	4	4	-	-	-	-	4	8	-	4	-	-	4	-	-	-	7
	Mean	2	1	5	0	0	1	0	2	2	0	2	1	0	3	0	1	0	
Mycoflora + Bayletan*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	-	-	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	8	-	-	-	-	8	4	-	-	4	-	-	-	8	-	5
	6	8	-	-	-	8	-	-	8	-	-	-	-	12	-	-	-	-	4
	Mean	3	0	8	0	2	1	0	4	1	0	0	1	3	0	0	3	0	
Mycoflora + Captan*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	1
	4	-	-	8	-	-	-	4	8	8	-	8	-	4	4	-	16	-	8
	6	8	-	-	-	8	-	-	8	-	-	-	8	-	4	-	-	-	5
	Mean	3	0	4	0	2	1	1	4	2	0	2	2	1	2	0	5	6	
Mycoflora+ Thiram*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	2
	4	8	12	8	8	-	-	-	-	-	-	4	-	-	4	-	-	-	6
	6	-	-	12	-	-	-	-	12	-	-	-	-	-	-	-	-	-	2
	Mean	3	3	10	2	0	1	0	3	0	0	1	0	0	1	0	1	4	
Mycoflora + Bavistin+ TMTD*	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	8	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	4	-	8	12	-	-	-	4	8	-	8	-	8	-	-	-	-	-	6
	6	12	-	-	-	-	-	-	-	-	-	-	-	4	4	-	-	-	3
	Mean	6	2	7	0	0	1	1	2	0	2	0	2	1	1	0	1	0	
Seed + Mycoflora	0	4	-	8	-	-	4	-	-	-	-	-	-	-	-	-	-	-	4	-	4
	2	8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	4	-	-	24	16	-	-	-	-	-	-	-	8	-	-	4	4	12	-	6
	6	-	-	16	-	-	-	-	4	4	-	-	-	-	-	-	-	8	-	4
	Mean	3	1	12	4	0	1	0	1	1	0	2	0	0	1	1	6	0	

* Dose of fungicides: 2.5 g/kg seed

Fungi present mycoflora from naturally and artificially infected seeds (Embryonic axis) after fungicide treatment using Seed Component Plating Technique

Treatment	Storage duration	*Alternaria* sp.	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*Colletotrichum lindemuthianum*	*Fusarium moniliformae*	*F. oxysporum*	*F. solani*	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizopus stolonifer*	Total mycoflora
1	*2*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Bavistin*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	4	-	-	4	-	-	-	-	-	8	3
	6	-	-	-	-	-	-	-	4	4	-	-	-	-	-	2
	Mean	0	3	1	1	1	1	0	2	1	0	3	0	3	6	
Bayleton*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	-	-	4	-	-	-	-	-	-	-	-	4	-	2
	Mean	0	3	1	2	0	1	0	0	0	0	3	0	4	4	
Captan*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	4	-	-	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	-	4	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	-	4	-	-	-	-	-	-	-	-	-	-	1
	Mean	0	4	1	3	0	1	0	0	0	0	3	0	3	4	
Thiram*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	4	8	8	-	-	-	-	8	-	-	-	-	-	-	4
	Mean	1	5	3	1	0	1	0	2	0	0	3	0	3	4	
Bavistin + TMTD*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	12	-	-	-	-	-	-	-	-	-	-	-	-	1
	6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	1
	Mean	0	6	1	1	0	1	0	1	0	0	3	0	3	4	

Continued

	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Untreated Seed	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	8	-	8	-	-	-	-	-	-	-	-	-	-	2
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	6	-	-	-	-	-	-	-	-	-	-	-	4	4	8	3
	Mean	0	5	1	3	0	1	0	0	0	0	3	1	4	6	
Mycoflora + Bavistin*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	8	-	-	-	-	-	12	-	-	-	-	-	-	2
	6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	1
	Mean	0	5	1	1	0	1	0	4	0	0	3	0	3	4	
Mycoflora + Bayletan*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	-	-	-	-	8	4	-	-	-	-	12	3
	6	-	8	4	8	-	-	-	-	-	-	-	-	-	-	3
	Mean	0	5	2	3	0	1	0	2	1	0	3	0	3	7	
Mycoflora + Captan*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	8	8	-	-	-	-	4	4	-	-	-	4
	6	-	-	-	-	-	-	-	8	4	-	-	-	-	-	2
	Mean	0	3	1	3	2	1	0	2	1	1	4	0	3	4	
Mycoflora+ Thiram*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	20	-	20	8	-	-	-	-	-	-	-	-	-	3
	6	-	-	-	-	-	-	-	8	4	-	-	-	-	-	2
	Mean	0	8	1	6	2	1	0	2	1	0	3	0	3	4	
Mycoflora + Bavistin+ TMTD*	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	4	-	-	-	12	8	-	4	4	-	4	-	8	-	-	6
	6	-	-	-	12	-	-	-	4	-	-	-	4	-	-	3
	Mean	0	3	1	7	2	1	1	2	0	1	3	3	3	4	
Seed + Mycoflora	0	-	12	4	4	-	4	-	-	-	-	12	-	12	16	7
	2	-	-	-	4	-	-	-	-	-	-	-	-	-	-	1
	4	-	-	-	4	-	-	-	12	-	-	-	-	-	4	3
	6	-	-	-	12	-	-	-	4	-	-	-	-	-	12	3
	Mean	0	3	1	6	0	1	0	4	0	0	3	0	3	8	

* Dose of fungicides: 2.5 g/kg seed

Table 67. Effect of seed dressing fungicides and mycoflora on seed quality parameters

Treatment	Carbohydrate*				Protein*				Fat*			
	0**	2**	6**	Mean	0**	2**	6**	Mean	0**	2**	6**	Mean
Bavistin	63.00	62.40	60.70	62.03	20.74	21.04	22.23	21.34	1.18	1.17	1.17	1.17
Bayleton	63.00	61.20	59.80	61.33	20.74	20.93	21.8	21.16	1.18	1.19	1.19	1.19
Captan	63.00	61.80	60.30	61.70	20.74	20.82	22.05	21.20	1.18	1.18	1.19	1.18
Thiram	63.00	62.30	60.90	62.07	20.74	21.12	22.12	21.33	1.18	1.18	1.18	1.18
Bavistin+TMTD	63.00	61.90	58.60	61.17	20.74	21.42	22.1	21.42	1.18	1.18	1.18	1.18
Untreated Seed	63.00	61.60	58.40	61.00	20.74	21.66	22.31	21.57	1.18	1.19	1.19	1.19
Bavistin+Mycoflora	63.00	62.00	59.60	61.53	20.74	21.02	22.34	21.37	1.18	1.20	1.20	1.19
Bayleton+Mycoflora	63.00	61.40	59.20	61.20	20.74	21.33	22.02	21.36	1.18	1.19	1.21	1.19
Captan+Mycoflora	63.00	61.10	60.80	61.63	20.74	21.64	22.2	21.53	1.18	1.19	1.20	1.19
Thiram+Mycoflora	63.00	61.80	60.20	61.67	20.74	21.36	22.04	21.38	1.18	1.18	1.18	1.18
Bavistin+TMTD+Mycoflora	63.00	61.30	59.70	61.33	20.74	21.64	22.32	21.57	1.18	1.18	1.19	1.18
Mycoflora	63.00	60.20	55.40	59.53	20.74	21.52	23.08	21.78	1.18	1.20	1.26	1.21
Mean	63.00	61.58	59.47	61.35	20.74	21.29	22.22	21.42	1.18	1.19	1.20	1.19
CD(P=0.05)		Treatment		1.31				NS				0.049
		Storage period		0.66				0.188				0.025

Fungicide dose: 2.5 g/kg seed
* g/100 g of dry weight
** Months of storage

surface sterilization and data recorded on occurrence of mycoflora are presented in Tables 68-69.

Perusal of data showed significant variation in the mycoflora recorded on different coloured seeds. Seeds having purple maroon and maroon with dark striations were free of any fungal infestation whereas, white to brown coloured seeds harboured highest mycoflora. Majority of seed samples contained storage fungi, mainly *Rhizopus* and species of *Aspergillus* and *Penicilium*. When these varieties were analysed after surface sterilization, the mycoflora population was comparatively less on all the samples (0-4 species/ sample). These samples also exhibited the same pattern of mycoflora and presence of pathogenic fungi viz. *Colletotrichum* and *Rhizoctonia* with an incidence ranging from 4-24 and 24-36 per cent, respectively in addition to storage fungi (Plate 10).

4.6 Relative Occurrence of Seed Mycoflora on Vegetable and Pulse Type Kidney Bean Varieties

In order to have a comparative account of the seed mycoflora on vegetable and pulse type kidney bean varieties, 10 cultivars comprising of vegetable and pulse type were subjected to MEA before and after surface sterilization and data recorded are presented in Tables 70-71.

It is evident from the data that vegetable type varieties harboured more number of fungi as compared to pulse type on unsterilized seed samples. Maximum number of fungi were recorded on Kentucky Wonder (7) followed by SVM-1 (6) whereas, other 3 varieties viz. Contender, Hans and Laxmi were colonized by 4 species each. *Aspergillus, Cephalosporium, Cladosporium, Penicillium and Rhizopus* were the dominating storage fungi whereas, *Colletotriclum* and *Rhizoctonia* constituted major pathogenic flora. Overall incidence of mycoflora ranged between 4 to 64 per cent. *Aspergillus niger* and *C. lindemuthianum* were detected from 4 vegetable type varieties showing 8-24 and 32-48 per cent infestation, respectively.

Table 68. Effect of seed coat colour on the per cent mycoflora of kidney bean seed (Unsterilised)

Seed coat color	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. ochraceous*	*A. sulphurious*	*Fusarium* spp.	*F. oxysporum*	*F. solani*	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Trichoderma* sp.	Total mycoflora
White	-	-	-	-	-	-	-	16	24	8	8	36	-	64	-	6
Light Brown	-	-	-	12	-	16	-	-	-	12	-	-	-	48	16	5
Yellowish Brown	-	-	-	48	-	-	-	20	24	-	16	44	-	-	56	6
Orange Brown	-	36	16	-	16	24	-		8	-	-	12	-	48	-	7
Reddish Maroon	-	-	-	-	-	44	-	12	-	-	-	-	-	64	-	3
Maroon	-	-	4	-	-	-	-	-	-	24	-	-	-	-	-	2
Maroon with dark Stirations	-	-	-	-	-	-	-	-	-		-	-	-	-	-	0
Maroon with light Stirations	-	-	-	-	-	-	24	-	-	20	12	-	-	84	-	4
Purple Maroon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Black	44	-	-	-	-	-	-	-	52	-	-	-	-	64	-	3
Black with Brown Stirations	-	-	-	-	-	-	-	8	-	52	-	-	4	-	-	3
Black with light Stirations	32	24	-	-	-	-	-	-	-	-	-	-	-	52	-	3

Table 69. Effect of seed coat colour on the per cent mycoflora of kidney bean seeds (sterilised)

Seed coat colour	*Alternaria* sp.	*Cephalosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium oxysporum*	*F. solani*	*Heterosporium* sp.	*Monosporium* sp.	*Phymatotrichum* sp.	*Rhizoctonia solani*	Total mycoflora
White	-	-	24	8	20	-	-	-	24	4
Light Brown	24	-	4	-	-	16	-	-	24	4
Yellowish Brown	-	-	-	28	-	8	-	-	-	2
Orange Brown	-	-	24	32	-	-	-	12	36	4
Reddish Maroon	-	-	-	-	-	-	-	-	-	0
Maroon	-	-	-	-	-	-	-	-	-	0
Maroon with dark	-	-	-	-	-	-	-	-	-	0
Maroon with light Striations	-	-	-	-	-	-	-	-	28	1
Purple Maroon	-	-	4	-	20	-	56	-	-	3
Black	-	44	12	-	-	-	-	24	-	3
Black with Brown Striations	-	-	-	16	-	-	-	-	24	2
Black with light Striations	-	-	-	-	-	-	-	-	-	0

Table 70. Comparative mycoflora (%) on kidney bean seeds (unsterilised) of vegetable and pulse type cultivars

Cultivars	*Alternaria* sp.	*Aspergillus fumigatus*	*A. niger*	*Cladosporium* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. oxysporum*	*F. solani*	*Penicillium capsulatum*	*P. griseo-fulvum*	*P. simplicissimum*	*P. perpuragenum*	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Stemphylium* sp.	*Trichoderma* sp.	Total mycoflora
Vegetable Type																	
Contender	-	-	-	-	8	-	-	-	24	-	-	20	28	-	-	-	4
Hans	-	24	32	-	24	-	-	-	-	-	44	-	-	-	-	-	4
Kentucky Wonder	44	-	32	24	16	-	-	-	-	12	-	-	-	-	32	20	7
Laxmi	-	-	32	-	-	-	24	-	16	-	-	-	48	-	-	-	4
SVM-1	-	-	48	8	16	4	-	32	-	32	-	-	-	-	-	-	6
Pulse Type																	
Baspa	-		44	-	-	-	-	-	-	-	-	-	36	56	-	32	4
Him 1	-	36	-	-	20	-	-	-	-	-	20	-	48	64	-	-	5
Jawala	-	-	-	-	24	-	-	-	-	-	-	-	40	-	-	-	2
Kanchan	48	-	-	-	-	-	-	-	-	8	-	-	8	8	-	-	4
Triloki	32	-	-	-	-	-	-	-	8	-	-	-	8	12	-	-	4

Table 71. Comparative mycoflora (%) on kidney bean seeds (sterilised) of vegetable and pulse type cultivars

Cultivars	*Alternaria* sp.	*Colletotrichum lindemuthianum*	*Fusarium* spp.	*F. oxysporum*	*Rhizoctonia solani*	*Stemphylium* sp.	Total mycoflora
Vegetable Type							
Contender	-	-	48	-	32	24	3
Hans	-	-	-	-	24	-	1
Kentuky Wonder	-	-	20	44	32	-	3
Laxmi	-	12	-	-	16	-	2
SVM-1	24	-	20	-	20	24	4
Pulse Type							
Baspa	-	-	-	-	-	-	0
Him 1	-	16	-	-	8	-	2
Jawala	-	12	-	-	12	-	2
Kanchan	32	-	-	-	12	-	2
Triloki	16	-	-	-	12	-	2

Among pulse type varieties, Him-1 had 5 fungi viz. *Aspergillus fumigatus, Colletotrichum, Penicillium, Rhizoctonia* and *Rhizopus* sp. followed by Baspa, Kanchan and Triloki. The incidence of storage and field fungi on pulse type ranged between 8-64 and 8-48 per cent whereas, it was 4-48 and 16-48 per cent, respectively, on vegetable type varieties. *Rhizopus* was prevalent on almost all the varieties except Jawala showing 8-64 per cent incidence. Sterilized seeds of these two types of kidney beans yielded comparatively less fungi (Table 71). Cv SVM-1 had *Alternaria, Fusarium* spp., *Rhizoctonia* and *Stemphylium* with an incidence of 20-24 per cent. *C. lindemuthianum* was detected only from variety Laxmi and *Rhizoctonia* was found to be prevalent on both the beans, though the incidence on pulse type was lower as compared to vegetable type. Cultivar Baspa was free of any fungal infestation.

DISCUSSION

DISCUSSION

The experimental results derived from the present study entitled "Eco-pathological study of seed mycoflora and its impact on biodeterioration of *Phaseolus vulgaris* L. in Himachal Pradesh" are discussed here. Attempts have been made to discuss the findings of the experiments under the following heads in the light of available scientific literature and work done earlier by the other investigators on the subject.

5.1 Varietal and spatial distribution of seed mycoflora

5.2 Pathological significance

5.3 Storage studies

5.4 Management of mycoflora

5.5 Effect of seed coat colour

5.6 Relative occurrence of mycoflora on pulse and vegetable type beans

5.1 Varietal and Spatial Distribution of Seed Mycoflora

Seventy three kidney bean seed samples comprising of local landraces and recommended varieties were collected immediately after harvest and storage (by the farmers) from seven districts of Himachal Pradesh representing three agro-ecological zones. Information obtained from the farmers regarding storage and use of cultivars revealed that they generally cultivate local landraces year after year and store their produce in gunny bags.

Zone III and IV constitute the main bean producing areas of the state and wide variation in climatic conditions exists in these zones. Zone III has sub-tropical/ sub temperate type of conditions with comparatively high

rainfall whereas, Zone IV possess wet to dry temperate type of climate with an annual rainfall ranging between 250 - 400 mm, though both conditions are suitable for quality bean cultivation.

In order to assess the seed mycoflora on morphologically abnormal seeds, different seed samples were graded into three categories viz. apparently healthy, shrivelled and discoloured seeds. Maximum number of abnormal seeds was observed in the samples procured from Zone III and minimum from Zone IV. Different climatic conditions prevailing in these two zones might have accounted for such type of abnormalities as temperate conditions prevalent in Zone IV are more suitable for general host growth whereas, in Zone III frequent and high rainfall along with comparatively higher temperature during cropping season also favour the conditions (biotic and abiotic) which might lead to such type of abnormalities. Consequently, these conditions affect the general plant growth and seed health during and after the harvesting of crop. (Tarr, 1955; Frederiksen, 1974, Neergaard, 1977). Lokhande *et al.* (1986) also found various types of morphological abnormalities in french bean seeds harbouring variable number of mycoflora in Sitara district of Maharashtra.

5.1.1 Seed mycoflora

Washing test

Different samples when analyzed through washing test for the presence of seed mycoflora, immediately after its procurement showed the presence of *Alternaria*, *Colletotrichum*, *Fusarium, Sclerotium, Sclerotinia* and *Rhizoctonia* species along with *Isariopsis griseola* and *Cercospora*, which are difficult to isolate by other methods. Chandrani (1989) also detected these two fungi only through washing test in addition to other species. He also recorded *Phytophthora* species on bean seeds which was not noticed during present investigations. Presence of *I. griseola* on bean seed has also been reported by Orozco-Sarria and Cardona-Alvarez (1959) and Sohi and Sharma (1984). Whereas, other fungi recorded by this method have also been reported by many workers (Neergaard, 1943;

Suryanarayana and Bhombe, 1961; Watanabe, 1972; Gomes and Dhingra, 1983; Tylowska, 1984)

Blotter and agar plate method

Evaluation of bulk seed samples, comprising of apparently healthy, shrivelled and discoloured seeds collected from various locations representing varied agro-climatic zones, by blotter method for seed mycoflora revealed the presence of 36 species belonging to 24 fungal genera on unsterilized seeds. Freshly harvested samples of Zone III and IV carried upto 4 and 10 species of field and storage fungi viz. *Alternaria, Colletotrichum, Fusarium, Rhizoctonia, Sclerotinia* and *S. rolfsii* among pathogenic/ field fungi and *Aspergillus, Penicillium, Cephalosporium, Cladosporium, Myrothecium, Trichoderma* and *Trichothecium* among storage fungi.

Seed samples of various zones collected from farmers after storage possessed more storage mycoflora as compared to field/pathogenic fungi. However, in Zone III the occurrence of fungi was more on seeds which were analyzed immediately after harvest as compared to that of stored samples.

Similar mycoflora was noticed on unsterilized seed samples by agar plate method. However, individual seed sample was found to harbour more fungi as compared to that obtained by blotter method. The number of fungi recorded on sterilized seeds was much less as compared to unsterilized ones. Thereby, indicating the presence of *Colletotrichum, Rhizoctonia* and *Fusarium* species as internally seed borne through blotter and agar plate method. Unsterilized and sterilized seed samples of different zones exhibited the presence of various pathogenic fungi viz. *Colletotrichum lindemuthianum, Rhizoctonia, Sclerotinia and Fusarium solani* in varying proportion. Chisholm and Coates-Back ford (1997) detected 37 species belonging to 20 genera from kidney bean seeds in Jamaica. They also reported higher frequency of storage fungi in various samples which is in confirmity with the present findings. Occurrence of almost similar mycoflora

on bean seeds has also been reported from other countries including India (Abdel-Hafez, 1984; Lokhande, 1986; Sinha, 1999; Gupta, 2000; Chakravarthy, 2001). Dhingra (1978) reported *F. semitectum* as internally seed borne in 85 per cent of the samples tested, but this species was not detected in our samples, however, *Fusarium* species viz. *F. moniliformae*, *F. oxysporum* and *F. solani* were predominant in present studies. Internal seed borne infection of *Colletotrichum* was detected in 9-44 per cent samples as compared to 20 per cent reported from USA (Butler,1918). The frequency of storage fungi like *Aspergillus* and *Penicillium* was above 80 per cent in some of the stored samples but the number of species detected in present studies was low in comparison to those noted on several legumes by Weidenborner and Hinderof (1989) who isolated five species of *Aspergillus* viz. *A. flavus, A. fumigatus, A. niger, A. ochraceous and A. sulphureous* . Similarly Rao *et al.* (1985) recorded *A. vesicular* along with *A. flavus*.

It can be inferred from the above discussion that seed samples of Zone IV possessed less mycoflora as compared to other two zones. This variation in mycoflora population can be attributed to temperature conditions of Zone IV where in most of the locations the temperate during storage goes below sub-zero degree Celsius. Similar variation in the occurrence of mycoflora of kidney bean (dry bean) was recorded by Tseng *et al.* (1995). They found that the seeds produced in Taiwan having sub tropical type of climate had higher percentage of seed borne fungi as compared to Ontario produced seed under temperate and semi arid type of climate. Miller and Ray (1982) and Anderson (1985) found that seed lots of soybean produced in Ontario had fewer mycoflora than those produced in Mississippi, U.S.A. However, *Eurotium, Curvularia* , *Aureobasidium* and *Drechslera sorokiniana* were recorded on beans in addition to other fungi by Tseng *et al.* (1996), Ruiz *et al.* (1996) and Dhyani *et al.* (1989). These were not present in present studies. Therefore, the composition of the mycoflora

of bean produced in different regions is influenced by the prevailing climatic conditions in such regions.

Only storage mycoflora was detected from apparently healthy and shrivelled seeds by blotter and agar plate method. The number of fungi detected from individual seed samples ranged between 1-10. Freshly harvested seeds contained comparatively less mycoflora (5-6 spp.) to that of stored seeds which possessed 4-9 species, maximum being in Zone III whereas, sterilized lots of these samples were either free of any infestation or were colonized by 1-2 species. Mycoflora detected from discoloured seeds was similar to that of bulk seed sample analyzed by known methods. Similar observations on the mycoflora of apparently healthy, shrivelled and discoloured seeds were recorded by Lokhande *et al.*, (1986) on beans and Ushamalini *et al.* (1998) on cowpea.

Seed component plating technique

Seed component plating technique employed to detect the location of various seed borne fungi showed that seed coats harboured maximum mycoflora followed by cotyledons and embryonic axis. Most of field/ storage fungi were recovered only from the testa and cotyledons with almost similar percentage. Fungi like *Colletotrichum, Rhizoctonia*, *Fusarium* species, species of *Aspergillus* and *Penicillium* were detected from embryonic axis. Both storage and field fungi were located on different components of seed with variable frequency. These findings are in confirmation with those of Chisholm and Coates-Becford (1997) who detected 94.7, 68.4 and 31.6 per cent of fungi from testa, cotyledons and embryonic axis of bean seeds, respectively. Maximay (1984) and Prasanna (1985) also reported 92 per cent seed coat infection of cowpea seeds as compared to 27 per cent on cotyledons and 11 per cent in the embryos. Contrary to these results Shama *et al.*, (1988) noted that cotyledons were the main site of infection (40%)of various seed fungi in cowpea followed by seed coat and embryo showing 32 and 14 per cent infection, respectively. *M. phaseolina* detected on seed coats and cotyledons in the present

studies was also detected from embryonic axis of cowpea by Sinha and Khare (1977). However, Sati *et al.* (1993) found moderate to heavy incidence of *Drechslera sorokiniana* in seeds of bean in all the three components but the incidence was highest on seed coats and least on embryos. Rao *et al.* (1985) observed *A. flavus* in all the three components viz. seed coat, cotyledons and embryos. In the present investigation *A. flavus* as well as *A. niger* were the main storage fungi colonizing all the three components.

5.1.2 Seed health status

Seed health status of kidney bean seed samples collected from various agro-ecological zones of Himachal Pradesh indicated that seed moisture content in the samples collected after harvest was comparatively more than those collected after storage. While detecting mycoflora from these samples it was noticed that samples having high moisture levels possessed more mycoflora. It was also noticed that samples collected from Zone IV possessed comparatively less mycoflora in majority of samples tested and if the number of fungi detected was more, their frequency was low in comparison to other two zones. This may be attributed to the fact that low moisture and temperature conditions prevail in this zone during the storage period and these findings corroborates the observation of Christensen and Kaufmann (1966) who reported that all the field fungi require high moisture in equilibrium with RH of 90 per cent or above for their activities. Samples collected from Zone II and III harboured higher fungal flora and this can be due to high rainfall and temperature conditions prevailing in some locations of these two zones. Under humid conditions and with inadequate storage facilities an increase in moisture level of stored grains is expected until there is equilibrium with the ambient relative humidity.

Germination percentage, another important seed health parameter, was studied for all the samples by using unsterilized and surface sterilized seeds. The germination percentage in unsterilized samples collected after

storage in Zone II, III and IV ranged between 41.00 to 99.77 per cent whereas, in surface sterilized seeds these values were between 40.00 to 97.99 per cent. In samples collected after harvest these values for unsterilized and sterilized seeds ranged between 37.00 – 98.65 and 35.33 – 99.31 per cent. Germination percentage is a well studied parameter and the results of present studies are similar to those reported by Chisholm and Coates Becford (1997) in beans, Ushamalini *et al.* (1998) in cowpea and Das *et al.* (1998) in rajmash. Seed and seedling vigour were affected by moisture and germination percentage of sample. Higher values were recorded for unsterilized seeds but surface sterilized samples registered statistically similar values. Similar observations on these parameters have been recorded on various crops like beans, soybean, cowpea and lentil by many workers (Das *et al.,* 1998; Chisholm and Coates Becford, 1997; Ushamalini *et al.,* 1998).

5.2 Pathological Significance

Seed mycoflora isolated from different bean samples during the present investigation was tested for its pathological significance by using spore suspension and culture filtrates. Spore suspension of majority of the storage fungi did not affect seed germination whereas, culture filtrates of *Aspergillus* and *Penicillium* spp. caused drastic reduction in seed germination and the other storage fungi had less effect ranging from slight stem necrosis to root rot type symptoms under *in vitro* conditions. Similar type of seed and seedling abnormalities in number of legume crops due to these fungi have also been reported by many workers (Gibson and Clinton, 1953; Chohan and Gupta, 1968; Clinton, 1960; Chohan, 1971 and Rao *et al.*, 1985). Vishunavat and Shukla (1981) and Chisholm and Coates-Beckford (1997) observed that out of 25 species of fungi, the maximum reduction in seed germination was caused by the culture filtrates of *A. flavus, A. niger* and *A. terrus* which corroborates the present findings. However, Rati and Ramalingam (1972) considered *A. flavus* as a weak pathogen. *Penicillium* species have also been found to induce various

types of abnormalities on different legumes (Ushamalini *et al.*, 1998; Sinha, 1999 and Bilbao *et. al.*, 2000).

Spore suspension and culture filtrates of *Alternaria, Rhizopus, Cephalosporium, Gliocladium, Myrothecium, Stemphylium* and *Cladosporium* caused slight abnormalities on seeds and seedlings of kidney bean and their effects are similar to those observed by Suryanarayana and Bhombe (1961), Pierre (1972, 1974), Richardson (1979) and Lokhande *et al.* (1986).

Field fungi isolated during present studies when evaluated for their pathogenicity by using spore suspension and culture filtrate on seed and seedlings following smearing method caused identical symptoms or susceptible cultivar `Kanchan` which are in confirmation with other workers (Gram and Weber, 1952; Suryanarayana and Bhombe, 1961; Neergaard 1979; Agrawal and Jain, 1981; Shukla, 1984; Dhyani *et al.*, 1989; Dhar and Gurha, 1988; Gupta *et al.,* 1991; Bhale *et al.*, 1998 ; Pun *et al.,* 1998).

Watanabe (1972) observed that seed borne infection of *R. solar* decrease seed germination considerably and present findings strengthen this view. Root rot and web blight symptoms recorded in present studies were identical to those observed by Ahuja (1976) and Gupta *et al.* (1991) Pathogenic behavior of *Fusarium moniliformae*, *F. oxysporum, F. Solani* and *Fusarium* species corroborates the findings of Dhar and Gurha (1988) Tu and Tan (1991) and Letourneau and Msuku (1992) who observed deformed and stunted sprouts, severe wilt and root rot type of symptoms. The adverse effects of culture filtrates of *Fusarium* species on seed germination observed in the present studies are in agreement with earlier findings (Baldev and Amin, 1974; Jeswani *et al.*, 1977; Reddy and Chaudhary,1990).

5.3 Storage Studies

Seed being biological commodity, is influenced by various extern factors like temperature, moisture and method of storage during active phase and dormancy. Storage microflora as a whole and fungi in particul

have been reported to occur and influence the health of seed and their occurrence is greatly affected by storage conditions. Kidney bean seed harbour large number of seed borne pathogens in addition to storage fungi. The results pertaining to effect of various storage containers viz. gunny bags, polylined bags and metallic bins on seed health of local bean varieties representing various agro-ecological conditions revealed that seed germination, seed vigour, seedling vigour index on fresh and dry weight basis decreased with increased storage, irrespective of the type of containers used. Seed moisture showed an increase in first two months and decreased thereafter, up to 6 months storage. However, seed moisture contents were greatly influenced by the type of container used as gunny bag and metallic bin stored seeds possessed comparatively less moisture to that of polylined bags. Seed vigour was also higher in gunny bag stored seeds. The change in seed moisture content equilibrium has been attributed to easy transmission of moisture from the outer environment to inner in cloth/gunny bags and its restricted flow in polylined bags (Das *et al.*, 1998). The present results on seed moisture, vigour, germination percentage and seedling vigour index (fresh and dry weight basis) are in confirmation with those of Arndt (1946) Milner and Geddes, (1946), Christensen and Kaufmann (1969) and Das *et al.* (1998).

Arulnandhy and Senanayake (1984) also observed deterioration of vigour and viability of rajmash and soybean seeds under tropical and sub-tropical environments having high humidity and temperature. Storage of soybean seeds over a period of three months resulted in sharp decline of its viability (Arulnandhy and Senanayake, 1991). Chisholm and Coates-Becford (1997) observed that bean seeds stored in polythene bags at 5°C over a period of 12 months had higher seed germination with minimum fungal flora than those stored at room temperature (28°C). Decline in seed vigour in different types of containers can be attributed to the catabolic activities of the seed and presence/absence of microflora during storage.

Similar results have also been obtained in soybean and cowpea by Arulnandhy and Senanayake (1991) and Ushamalini *et al.* (1998).

Seed mycoflora

Seeds stored in different types of containers over a period of six months revealed the presence of 23 species of storage and field fungi by blotter method and 30 species by agar plating. The seed coats of various seeds harboured more fungi as compared to their cotyledons and embryonic axis. An uneven trend of increase or decrease in mycoflora was noticed in relation to storage duration, however, the storage fungi were found to replace the field fungi after 4 months of storage in various containers. Some of the pathogenic fungi like *C. lindemuthianum* and *Fusarium* species were recorded throughout storage and their absence or presence may be due to avoidance of seed borne infection under field conditions (Kaul, 1973; Emayavaramban and Ramabadran, 1984).

Dominance of storage mycoflora after prolonged storage of seeds has also been reported by Bilgrami *et al.* (1976), Sharma and Sharma (1978), Sharma and Gupta (1980) and Sharma (1984). Field fungi like *Drechslera oryzae*, *Cladosporium* sp. and *Fusarium* sp. were found on rice seed during storage studies, and as the storage period increased the storage fungi like *Aspergillus* and *Penicillium* dominated the total population (Sharma *et al.*,1985). These studies indicated that storage of seeds at ambient condition with higher moisture enhances the chances of seed infestation and survival of many fungi.

Seed quality

Evaluation of viability, vigour and chemical composition of any seed lot is of utmost importance to judge its quality as the chemical composition of seed often changes during storage. Since pulses are quiet vulnerable to biodeterioration by microflora due to their rich protein contents, they sustain considerable qualitative losses during storage. Depletion and accumulation of important metabolites due to seed borne infection is known in number of

host pathogen combinations (Goodman and Christensen, 1952; Ward and Diener, 1961; Lalithakumari *et al.*, 1971 and Vidhyasekaran *et al.,* 1973).

Seed storage upto six months duration showed reduction in carbohydrate contents of all local varieties collected from five districts of Himachal Pradesh., However, a significant increase in protein contents and no effect on fat contents was observed in general in the present studies during the storage duration. Samples collected from Lahaul - Spiti district possessed high carbohydrate and fat contents however, protein content was maximum in Kullu landrace. Various storage containers had no effect on carbohydrate, protein and fat contents. These findings are in confirmation with those of Bilgrami *et al.* (1976) who observed marked decrease in sucrose concentration and increase in amino acid concentration in mung and urdbean seeds. This increase may be attributed to breakdown of seed proteins due to micro-organism activity. Daniel *et al.* (1977) observed that the protein contents of green gram increased from 23.7 to 24.4 per cent in the seed samples infested with mycoflora. Similar increase in protein and fibre contents has also been recorded by Kammar and Niak, (1987).

Biochemical investigations with regard to the effect of the fungus on the protein and free fatty acid contents revealed an increase in infected seeds over apparently healthy seeds (Krishnamurthy and Raveesha, 2001). Fungi are known to cause changes that lead to deterioration of infected grains. Christensen (1967) has reported increase in total proteins after storage of three months followed by decrease in total proteins during storage for one year in groundnut. Pathan *et al.* (1989) reported increase in total proteins and free fatty acid in soybeans treated with different isolates of *C. kikuchii.* In the present studies similar increase in protein and free fatty acid contents were observed. Increase in protein content may be due to the formation of new proteins (Uritani and Stahmann, 1961). Such an increase may be due to the preferential utilization of non-protein substances like sugars and starch by the pathogens and might be entirely due to

abnormal metabolism owing to infection. Thus biochemical parameters studied indicate significant loss in the nutritional status of the infected seeds.

5.4 Management Studies

5.4.1 Efficacy of seed dressing fungicides

French bean seed harbour large number of fungi and many of them are pathogenic in nature whereas, some are responsible for biodeterioration of seed during storage. So, their management is of utmost importance and seed dressing fungicides provide an excellent and effective mean of eradicating the seed borne infection. In the present studies large number of storage and field fungi were recorded on seed lots of various bean growing areas of the state. Five fungicides namely Bavistin, Baylaton, Bavistin + TMTD, Captan and Thiram were evaluated for their effectiveness against seed borne fungi and the impact on seed health and quality parameters.

Various seed health parameters viz. seed moisture, vigour, germination and seedling vigour index were least affected by the application of fungicides. Seeds treated with Thiram, Baylaton and Bavistin showed higher germination, seed and seedling vigour as compared to that of untreated seeds. Seedling vigour index on fresh and dry weight basis also increased in fungicide treated seeds up to six months of storage. Thiram proved to be the best seed treatment fungicide as it improved all seed health parameters followed by Bavistin and Bavistin +TMTD. Seedling vigour index on dry weight basis was maximum in Baylaton treated seeds as it caused profuse rooting and stout shoots which increased the dry weight of both roots and shoots. The additive effects of Thiram and carbendazim on seed health parameters have also been reported by Tanaka and Coraea (1982), Kore and Solanke (1982) and Dhyani *et al.* (1989).

Seed mycoflora

Seed treatment with fungicides was found to have significant effect on different fungi associated with bean seeds as only 1-4 species were noticed on seeds after 6 months of storage, majority being storage fungi. However, some seed lots were free of any infestation. Thiram, Baylaton and Bavistin + TMTD were highly effective in controlling seed mycoflora. Effectiveness of these fungicides have also been reported by many workers on bean and other crops (Khare *et al.*, 1979; Sindhan and Bose, 1981; Khere, 1985; Estrada, 1989; Sharma and Sugha, 1995).

Effective control of seed mycoflora through seed treatment with Agrosan GN, Captan, Ceresan, Tillex and Thiram in long term storage of french bean was reported by Kaul (1973). Hegde and Hiremath (1987) found that four fungicides viz. Captan, Thiram, Ceresan dry and carbendazim used as seed dressers significantly improved germination, seed vigour and reduced seed mycoflora of cowpea when applied @ 2.5 to 5 g/kg seed. Contrary to the present studies, Vanangumdi and Karivartharaju (1987) observed that field bean seed treatment with Captan and activated clay had higher percentage of field emergence. Similarly Sinha *et al.* (1999) while studying seed mycoflora of french bean and its management by fungicides found that Dithane M-45 (mancozeb), Bavistin (carbendazim), Agrosen GN and PCNB reduced seed mycoflora significantly and improved germination. Krishnamurthy *et al.* (2001) observed Bavistin and Captafol as best fungicides for control of seed borne pathogens of pulses viz. *Alternaria, Colletotrichum, Fusarium, Rhizoctonia and Phoma* species. Therefore, it is imperative to treat bean seeds with fungicides for improving its viability and avoidance of mycoflora.

Seed quality of fungicide treated seeds

Carbohydrate content decreased in all the fungicide treated seeds however, decrease was more in untreated seeds. Maximum decline was observed in Baylaton and Captan treated seeds but the values were higher than untreated check. Protein contents increased slightly in fungicide

treated seeds but the highest values were recorded for untreated seeds, this may be attributed to the presence of more mycoflora in untreated seeds than fungicide treated seeds. No significant effect on fat content was observed however, a marginal increase was found being highest in untreated seeds (Bilgrami *et al.*, 1976; Pathan *et al.,* 1989, Krishnamurthy and Raveesha, 2001).

5.4.2 Efficacy of propionic acid as seed dresser

Apart from various seed dressing fungicides, propionic acid alone and in combination with Bavistin was evaluated to know its effect on seed health and quality parameters. Since propionic acid is specifically reported for the control of storage fungi like *Aspergillus* and *Penicillium*, it was combined with Bavistin with a view to have effective control of all seed borne fungi. It was found that seed germination decreased over 6 months storage period in all the treatments but it was higher in Bavistin treated seeds. Seed germination in propionic acid treated seeds was less than untreated check, and in combination it was even less than propionic acid alone. More drastic effect was observed on the seeds stored in polylined bags. The adverse effect of propionic acid on seed germination has been reported by Patkar *et al.*(1995) who observed marked decrease in seed germination of rice grains after 50 days of storage. Dhanraj *et al.* (1973) also observed negative effect of Liprosil (propionic acid formulation) application on seed germination of maize. Patkar *et al.* (1997) studied the effect of propionic acid application in stored rice, sorghum and groundnut under tropical conditions and observed that germination was dropped to zero in some of the treatments. Similar results were obtained in present investigations. Other seed health parameters like seed moisture, vigour and seedling vigour index were adversely affected in all the treatments except Bavistin treated seeds where significantly higher values were recorded.

Seed mycoflora detected by various methods in treated seeds revealed that propionic acid alone and in combination with Bavistin was not able to control storage mycoflora after six months storage. Though after two

months storage the number of species observed were less but after 4 months storage fungi particularly *Penicillium* species again appeared. After 6 months both *Aspergillus* and *Penicillium* species were observed. Bavistin alone effectively controlled the mycoflora. These studies are in confirmation with those of Dhanraj *et al.* (1973), Hegde and Hiremath (1987), Patkar *et al.* (1995,1997), Thippeswamy and Lokesh (1997) and White and Coates (1998). Seed quality parameters were not affected much by these treatments. Decreasing trend in carbohydrate, increase in protein and no change in fat content was observed.

5.4.3 Efficacy of fungicides on naturally and artificially infected kidney bean seeds

With a view to ensure mycoflora on seeds, seed samples treated with a slurry of isolated cultures of all the fungi and 5 seed dressing fungicides showed improved germination in seeds treated with fungicide alone up to 4 months of storage but it decreased in all mycoflora treated seeds up to six months storage. Seed moisture was not affected much by mycoflora treatment however, seed vigour and seedling vigour index increased in fungicide treated seeds and decreased in mycoflora treated seeds.

Seed quality

Carbohydrates decreased in all the treatments but decline was more in mycoflora treated seeds. Contrary to this, mycoflora treated seeds possessed more protein contents among all treatments however, fat content showed similar trend of increase/decrease in all the treatments as in other experiments. These changes may be attributed to the fact that increase in amino acids may be due to break down of seed proteins due to colonization by mycoflora as reported by Bilgrami *et al.* (1976), Kammar and Naik (1987) and Paul and Mishra (1994).

5.5 Effect of Seed Coat Colour

Twelve varieties having different seed coat colour were used to evaluate the effects of seed coat colour on mycoflora development. The

studies revealed that dark coloured varieties (black, maroon, purple) harboured less mycoflora as compared to white and brown coloured varieties which were colonized by many fungi. Among unsterilized seeds both field and storage fungi were observed but in surface sterilized seeds only pathogenic fungi were recorded. These findings were similar to those of Dhar and Gurha (1988) where white seeded variety HUR-15 harboured 15 fungi, PDR 14 (deep red with white striations) and HUR 87 (black seed coat) were colonized by 10 and 7 fungi, respectively. Gonzalez *et al.* (1982) have also reported high selectivity of seed colour in french beans to some soil borne fungi and advocated the use of black seeded varieties.

5.6 Relative Occurrence of Seed Mycoflora on Vegetable and Pulse Type Kidney Bean Varieties

The relative occurrence of mycoflora on certain cultivars of vegetable and pulse type of beans revealed that vegetable type harbour comparatively more seed borne fungi. Cultivars like Kentucky Wonder and SVM-1 were the most colonized. Baspa variety of pulse type bean possessed minimum mycoflora. *Rhizoctonia* was prevalent on both the beans though the incidence was less on pulse type. More carbohydrate content in vegetable type varieties may be ascribed for more mycoflora in these varieties. Similar observations were recorded on vegetable type beans like Contender, Kentucky Wonder and SVM 1 by Gupta *et al.* (1991, 2000).

SUMMARY

Chapter - VI

SUMMARY

Studies on seed borne micro-organisms and their impact on biodeterioration of kidney bean (*Phaseolus vulgaris*) were carried out with the objectives to investigate the occurrence and distribution of mycoflora in various agro-ecological zones of Himachal Pradesh. The effect of storage containers and duration on seed health, mycoflora development and seed quality parameters was also investigated. Seed dressing fungicides viz. Bavistin, Bavsitin + TMTD, Baylaton, Captan and Thiram and propionic acid alone and in combination with Bavistin were used for the management of the seed mycoflora and to see their impact on seed health and quality.

Seventy three seed samples comprising of local landraces and improved cultivars were collected from commercial bean growing areas of Himachal Pradesh to assess the distribution of seed mycoflora and their seed health status. Survey revealed that farmers' generally grow local land races year after year except few progressive farmers who cultivate improved varieties and store their produce invariably in gunny bags. Different samples from diverse agro-ecological situations representing 7 districts of Himachal Pradesh were catalogued and categorized into 3 grades i.e. apparently healthy, shrivelled and discoloured seeds on the basis of morphological appearance.The studies revealed that fungi associated with stored seeds caused seed deterioration and reduction in germination potential. Invasion of seed during storage by fungal flora also resulted in loss of viability, development of more mycoflora and loss in quality i.e. degradation of seed constituents like carbohydrate, protein and fats.

Prior to detection of mycoflora seed health status of all the collected samples was assessed and it was found that various seed health parameters like seed moisture, germination, seed and seedling vigour varied with location, storage conditions and cultivar. Freshly harvested samples possessed more moisture content (4.2 to 15.9 %) whereas, stored samples had lower values (8.4 to 13.6%). In majority of the samples germination percentage was above 80 per cent, but in some samples it was as low as 35.33% and as high as 99.7%. Seed and seedling vigour though varied with location and cultivar but was higher in stored seed samples.

Seed mycoflora was detected from all the samples individually, using Blotter, Agar plate, seed component plating technique and washing test. Washing test revealed the presence of maximum number of pathogenic fungi alongwith species of *Isariopis* and *Cercospora* which are difficult to culture. Blotter and Agar plate method yielded 36 species of fungi belonging to 24 genera in variable frequency with respect to location, cultivar and sample lot used. Storage and pathogenic fungi detected were the species of *Acremonium, Alternaria, Aspergillus, Botrytis, Cephalosporium, Cladosporium, Colletotrichum, Fusarium, Gliocladium, Helminthosporium, Heterosporium, Macrophomina, Monosporium, Myrothecium, Penicillium, Phoma, Phymatotrichum, Rhizoctonia, Stemphylium, Sclerotinia, Sclerotium, Trichoderma, Trichothecium* and *Varicosporium* comprising of both externally and internally seed borne fungi. The number of fungi detected from individual seed sample ranged between one to sixteen in unsterilized seeds whereas, among surface sterilized seeds this number was much less and many samples were free of any infestation. Freshly harvested seeds mainly possessed field fungi whereas, samples analysed after storage were dominated by storage mycoflora. Among various seed grades apparently healthy and shriveled seeds were mostly colonized by storage fungi whereas, discoloured samples possessed both storage as well as field fungi. Seed samples of zone IV were

found to harbour comparatively less mycoflora as the percentage of abnormal seeds in zone IV samples was very less. Among both methods agar plating was more efficient as even superficial infection could be detected after a period of 4 days incubation. Various seed borne fungi were found located on different parts of the seed, seed coats and cotyledons harboured more number and frequency of fungi as compared to embryonic axis. *Aspergillus flavus* and *A. niger* were recorded on all three components of the seed along with some pathogenic fungi like *Colletotrichum, Fusarium*, *Rhizoctonia* and *Sclerotinia.* However, species of *Aspergilii*, *Penicilli* and *Fusaria* were found frequent and dominant.

Isolated mycoflora when evaluated for its pathogenic behaviour by using spore suspension and culture filtrates of pathogenic and storage fungi exhibited various types of symptoms like seed rot, seedling rot, radicle necrosis, collar rot and wilting of seedlings and necrotic spots apart from inhibition of germination. Pathogenic fungi like *Colletotrichum, Fusarium, Rhizoctonia*, *Sclerotinia* and *Sclerotium* isolated from bean seeds reproduced identical symptoms on susceptible variety 'Kanchan'. However, spore suspension of storage fungi did not induce any kind of abnormality both in seeds and seedling. Culture filtrates of these fungi not only reduced germination drastically but also caused seed and seedling mortality.

Storage studies revealed that with increase in storage during seed health parameters like germination, seed vigour, seedling vigour index decreased but the values were statistically at par with the recommended standards of International Seed Testing Association. Storage containers had least effect on seed health parameters but affected the mycoflora significantly. Metallic bins proved to be the best containers for storage as seeds stored in such bins harboured less mycoflora to that of polylined and gunny bags. Seed quality parameters viz. carbohydrates, proteins and fats were influenced by mycoflora but not much by storage containers. Carbohydrates decrease in

almost all the treatments and proteins increased with storage, however, fat contents either remained constant or showed slight increase in some of the samples.

Five seed dressing fungicides namely, Bavistin, Bavistin + TMTD, Baylaton, Captan and Thiram were found to influence the seed health positively as majority of the treatments registered higher germination percentage, seed and seedling vigour index on fresh and dry weight basis. Thiram, Bavistin and Bavistin + TMTD were found to have more additive effect on seed health as compared to Captan and Baylaton. Baylaton however, induced profuse rooting which caused increase in dry weight of seedling, hence increase seedling vigour index on dry weight basis. Seed mycoflora was effectively controlled by almost all the fungicides used. Doses of the fungicide (2, 2.5 and 3 g/kg seed) had no significant difference, however, maximum control of mycoflora was obtained with recommended dose in almost all the treatments. Seed quality parameters showed decrease in carbohydrates and increase in proteins but decrease and increase was less than untreated check. Fat contents were not influenced much.

Propionic acid, recommended against storage fungi like *Aspergillus* and *Penicillium*, when evaluated against kidney bean seeds was found to have adverse effect on seed health as it decreased seed germination, vigour and seedling vigour index drastically. This effect was more pronounced on the seeds stored in polylined bags as compared to other containers. It was also not effective to check seed mycoflora. However, when it was combined with bavistin the seed health parameters got effected more adversely but mycoflora was controlled effectively than propionic acid alone. Seed samples smeared with cultures of isolated mycoflora followed by fungicide treatment also resulted in decreased germination, loss in vigour and viability and more mycoflora in such seeds as compared to that of those treated with fungicides alone.

Seed coat colour was found to cause wide variation in mycoflora development as Less fungal flora was recorded on black and dark coloured varieties as compared to white and brown seeded ones. It was also noticed that vegetable type varieties harboured more number and frequency of fungi as compared to pulse type varieties.

It can be concluded from the present investigations that occurrence of seed mycoflora and its effect on seed health parameters varied with location and cultivar. Environmental conditions prevailing in a particular agro-ecological situation had great impact on mycoflora development. Kidney bean harboured both pathogenic and storage fungi which can lead to severe seed biodeterioration under ambient environments and can render seed unfit for growing and consumption. Storage duration, condition and containers had significant effect on vigour and viability of seeds. In an ambient environment of storage metallic bins as a storage container proved to be the best. Thus, management of mycoflora and storage of seed in proper containers is of utmost importance and seed dressing fungicides can effectively ensure high vigour and viability of seed during storage.

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* Original not seen

APPENDICES

APPENDIX - I

COMPOSITION OF DIFFERENT TYPES OF MEDIA USED FOR DETECTION OF SEED BORNE MYCOFLORA

Media	Composition	Quantity
Potato dextrose agar	Potato	250 g
	Dextrose	20 g
	Agar	20 g
	Distilled water	1000 ml
Potato sucrose agar	Potato extract*	500 ml
	Sucrose	20 g
	Agar	20 g
	Distilled water	500 ml
Neopeptone glucose agar	Glucose	2.8 g
	Mg SO_4'$7H_2O$	1.23 g
	KH_2 PO_4	2.0 g
	Agar	20 g
	Distilled water	1000 ml
V-8	V-8 juice	8.3 g
	L – asperagine	10 g
	Calcium carbonate	2 g
	Glucose	2 g
	Yeast extract	2 g
	Agar	20 g
	Distilled water	1000 ml
Carrot decoction agar	Dry carrot leaves	300 g
	Agar	12 g
	Distilled water	1000 ml
Czapeck's agar medium	$NaNa_3$	3.0 g
	K_2 HPO_4	1.0 g
	Mg $SO_4.7H_2O$	0.5 g
	$FeSo_4.7H_2O$	0.01g
	Sucrose	30.0 g
	Agar	20.0 g
	Distilled water	1000 ml
Czapeck's yeast extract agar	K_2 HPO_4	1.0 g
	Czapeck's concentrate**	10.0 ml
	Yeast extract	50 g
	Agar	20.0
	Distilled water	1000 ml
Malt extract agar	Malt extract	20.0 g
	Glucose	20.0 g
	Becto-peptone	1.0 g
	Agar	20.0 g
	Distilled water	1000 ml

* **Potato extract** is prepared from 1800 g of potatoes peeled and sliced and suspended in muslin cloth in 4500 ml of water and boiled for 10 min. The potatoes are then discarded and the liquid placed in large glass containers and autoclaved at 15 pci for 20 minute.

** **Czepeck's concentrate** $NaNO_3$: 30g, $MgSO_4.7H_2O$: 50 g, KCl : 50 g and $FeSo_4$ $7H_2O$: 1g

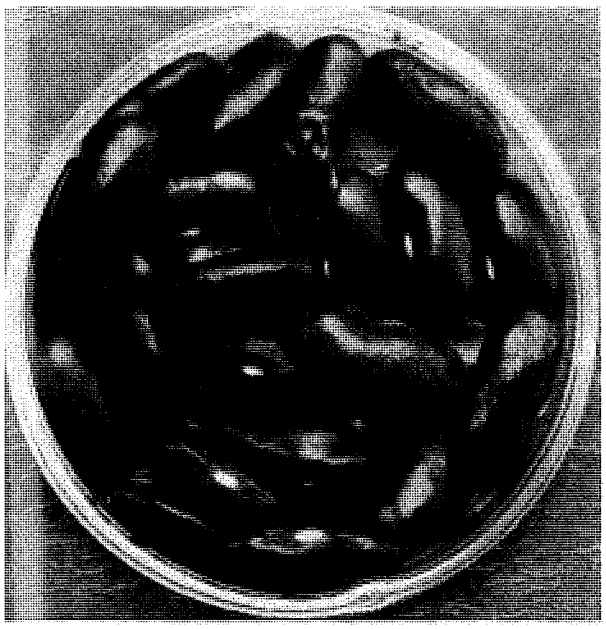
APPENDIX - II

WEEKLY MEAN TEMPERATURE AND RELATIVE HUMIDITY DATA DURING THE STUDY PERIOD (1999-2001)

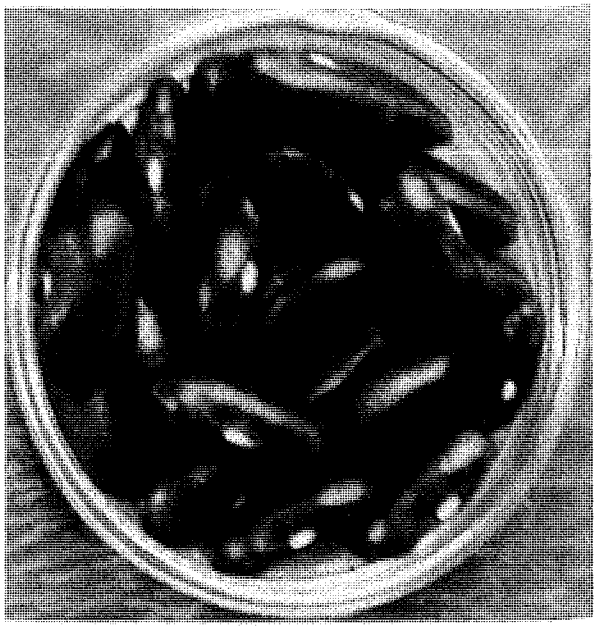
Standard Week	1999			2000			2001		
	Temperature (°C)		Mean RH (%)	Temperature (°C)		Mean RH (%)	Temperature (°C)		Mean RH (%)
	Max	Min		Max	Min		Max	Min	
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1	13.6	8.0	50	16.0	7.6	40	15.1	5.5	59
2	13.0	5.3	56	14.5	8.3	62	16.6	5.4	45
3	15.1	8.3	48	14.1	6.8	52	19.5	7.9	37
4	13.2	8.3	63	14.3	7.4	58	16.7	6.9	49
5	13.0	5.5	58	14.5	7.5	49	22.0	7.9	37
6	18.1	9.9	46	13.2	6.4	64	20.9	7.9	32
7	19.8	12.4	49	13.0	5.8	52	22.7	11.4	36
8	17.8	10.6	57	14.2	5.8	46	22.2	10.8	46
9	20.0	11.0	41	17.9	8.7	43	19.8	8.4	37
10	19.5	12.9	49	18.0	10.1	50	23.8	11.4	39
11	21.0	11.8	36	19.2	10.3	43	24.4	12.0	42
12	22.3	13.0	33	16.9	9.4	48	23.9	12.3	42
13	25.2	15.1	32	24.6	16.8	38	25.3	12.6	40
14	26.9	17.3	31	25.2	15.6	35	31.1	15.9	32
15	27.1	20.1	31	27.6	17.8	36	28.9	17.4	42
16	29.8	20.6	29	29.0	19.1	39	23.9	13.3	52
17	31.5	21.9	26	27.2	17.9	39	31.4	19.2	47
18	32.5	22.8	28	30.1	20.1	40	33.1	20.1	32
19	26.9	19.6	37	27.7	21.0	58	34.0	21.2	41
20	28.6	20.5	40	29.4	21.8	52	29.4	18.5	59
21	25.9	19.5	64	30.4	22.0	53	30.9	19.7	44
22	28.4	20.3	45	30.3	20.2	50	29.8	19.1	60
23	28.2	18.8	46	26.5	17.4	70	29.0	18.6	64
24	28.5	21.2	62	28.2	21.8	68	29.5	20.5	78
25	26.2	19.7	74	26.6	21.4	77	28.6	18.9	70
26	28.4	22.1	76	25.9	20.8	81	29.6	19.9	81
27	26.6	21.6	79	26.7	22.0	79	29.4	21.5	83
28	25.3	20.4	79	26.3	21.4	80	28.4	21.4	86
29	23.7	21.1	84	24.0	21.7	86	28.4	21.6	85
30	23.2	20.5	84	24.2	21.3	88	27.7	21.0	84
31	21.5	21.6	85	22.6	20.1	77	27.9	21.0	79
32	23.7	21.6	88	25.4	21.1	81	29.4	21.7	79
33	25.0	20.4	81	25.7	20.7	79	30.1	21.3	81

Continued

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
34	23.5	21.3	84	25.9	20.9	80	28.7	20.5	84
35	25.4	19.1	79	24.8	19.6	79	28.8	20.5	76
36	25.8	20.0	75	25.1	21.1	79	28.9	19.0	70
37	24.8	19.9	79	25.6	18.5	68	28.4	17.1	62
38	25.2	20.4	84	24.1	19.2	72	28.9	17.9	52
39	24.0	19.2	76	24.7	16.3	54	29.5	16.9	63
40	24.2	17.6	72	25.1	17.6	60	26.6	14.0	65
41	24.8	15.1	46	25.7	15.9	38	27.5	12.7	50
42	25.0	15.2	47	25.0	16.0	47	26.5	14.4	46
43	24.2	17.1	54	24.1	15.9	39	27.4	16.0	52
44	25.1	15.2	56	21.5	14.4	59	27.0	15.7	53
45	21.6	11.8	53	21.4	13.6	53	24.0	11.3	38
46	21.7	11.2	51	20.0	12.3	42	24.0	11.7	39
47	19.8	10.7	49	19.0	12.0	42	23.6	10.8	47
48	19.6	9.7	47	16.7	7.9	39	22.1	9.9	47
49	18.0	8.5	47	18.7	9.2	33	22.8	10.1	40
50	18.1	8.8	33	17.1	8.5	39	21.9	8.8	49
51	19.1	8.5	37	18.5	10.0	41	18.7	6.7	48
52	15.5	7.6	47	17.2	7.9	35	18.9	6.8	52

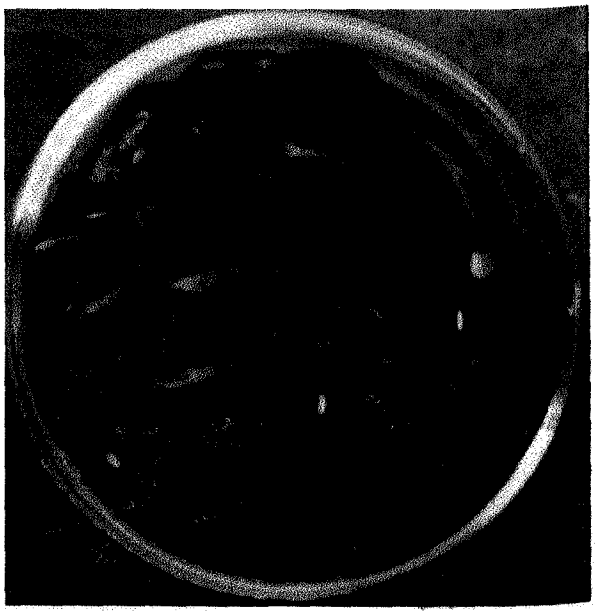


Bulk seed sample



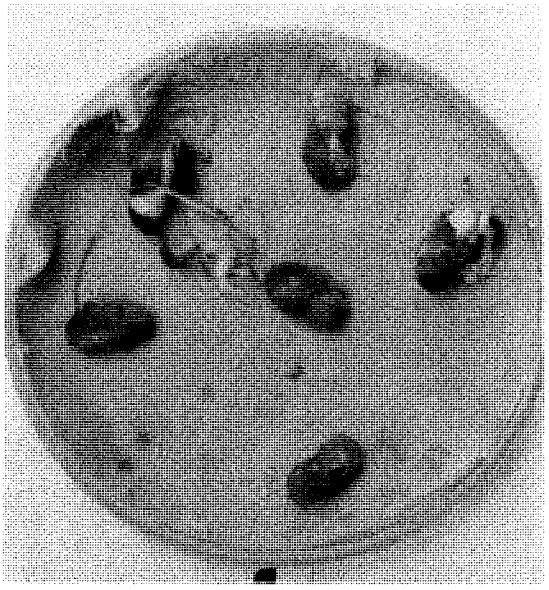
Apparently healthy

Shrivelled

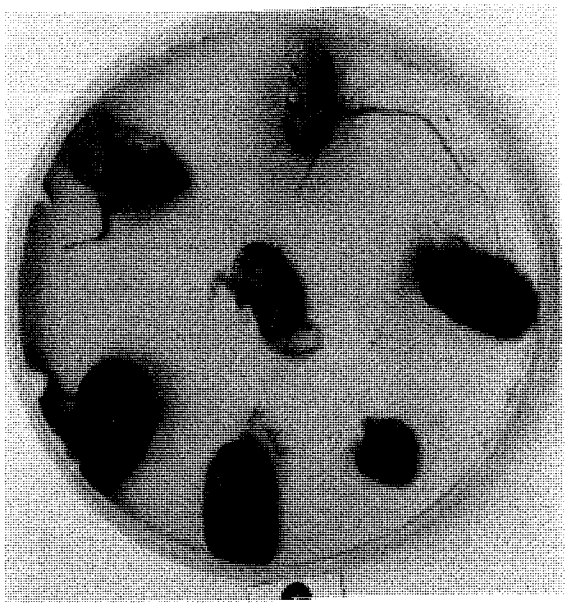


Discoloured

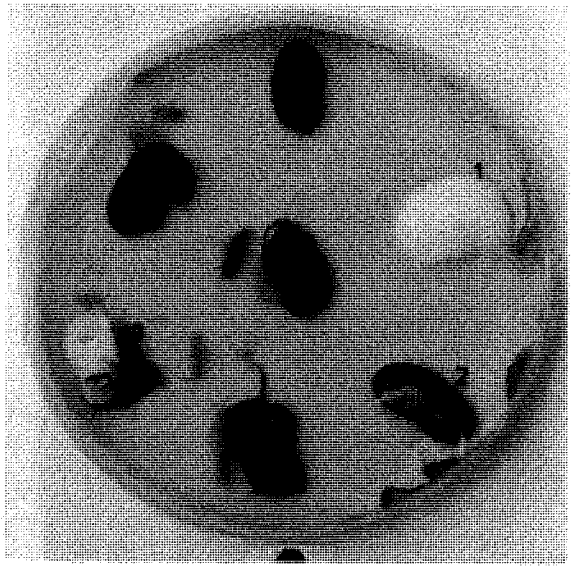
Plate 1. Grading of Kidney Bean Seed Samples for Seed Pathological Studies



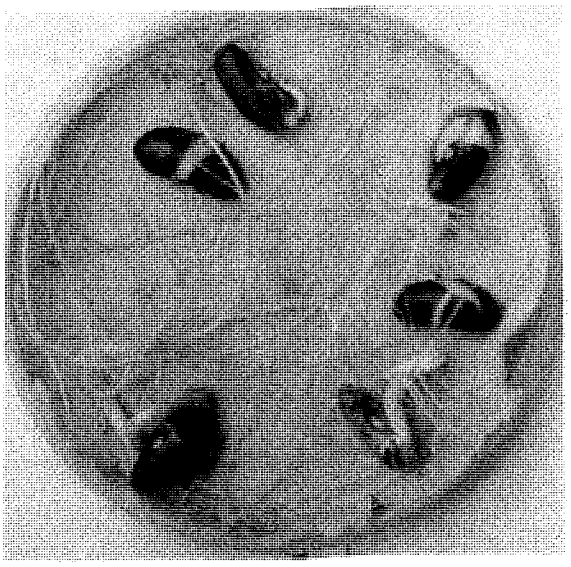
Species of *Penicillium*



Species of *Aspergillus*

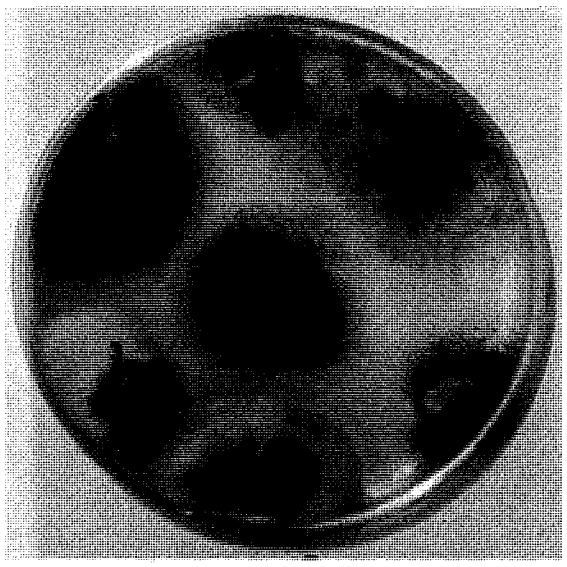


Species of *Fusarium (1)*, *Alternaria (2)* and *Colletotrichum (3)*

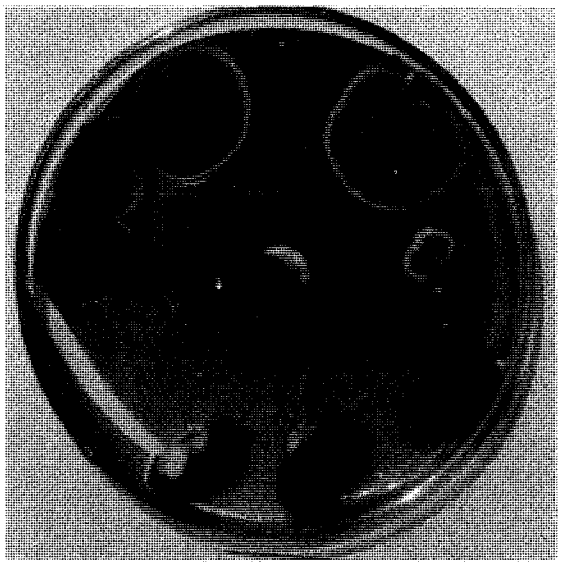


Healthy Seeds

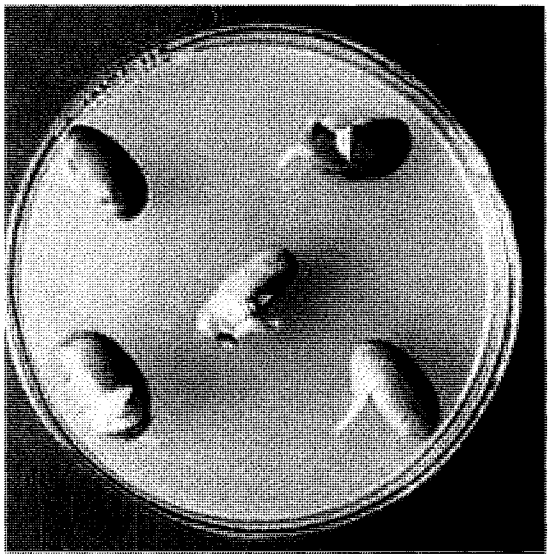
Plate 2. Detection of Seed Mycoflora by Blotter Method



Species of *Penicillium* (1), *Aspergillus* (2)and *Fusarium* (3)

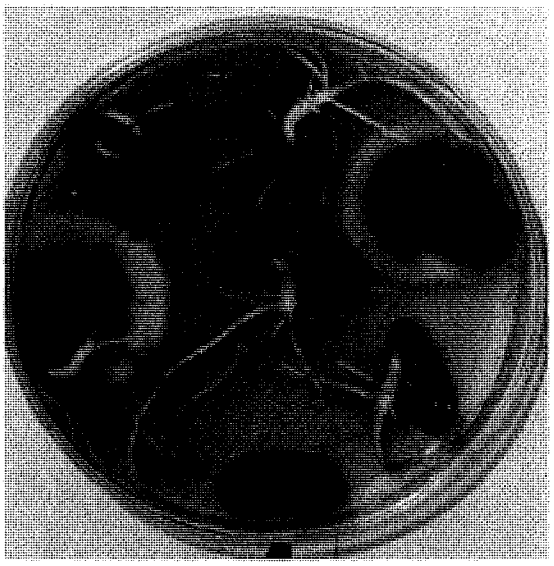


Species of *Penicillium* (1) and *Fusarium* (2)



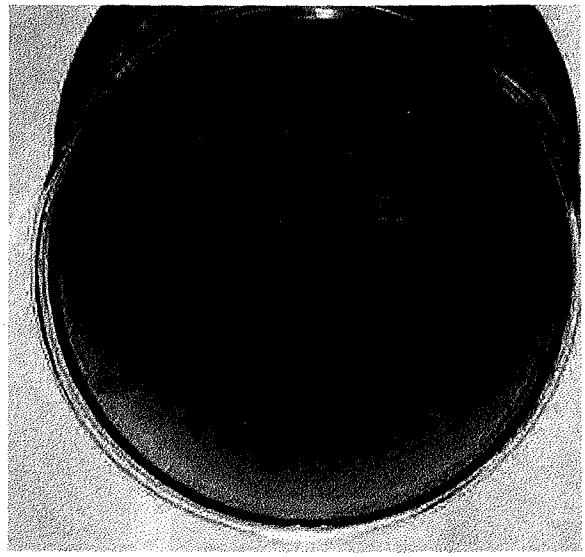
Healthy seed

Species of *Fusarium*

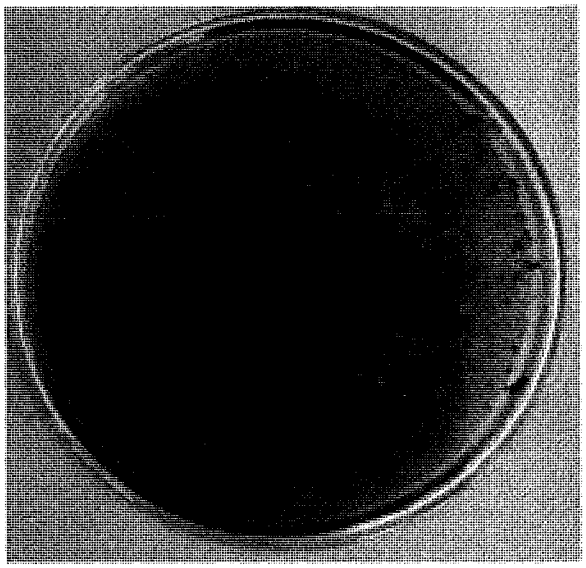


Species of *Colletotrichum*

Plate 3. Detection of Seed Mycoflora by Agar Plate Method

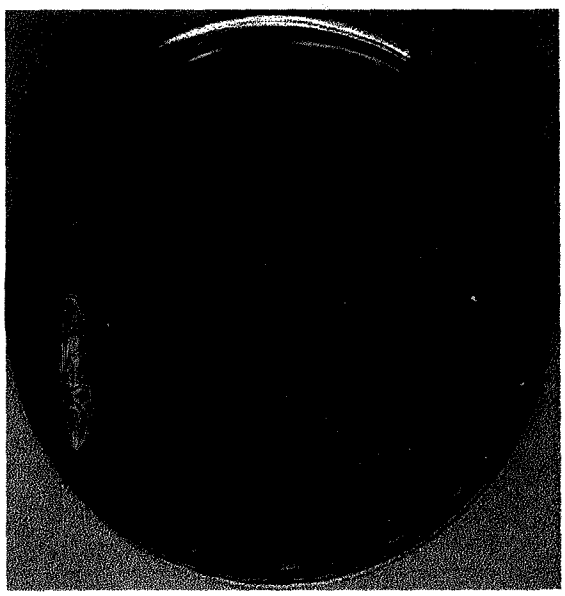


Rhizoctonia solani

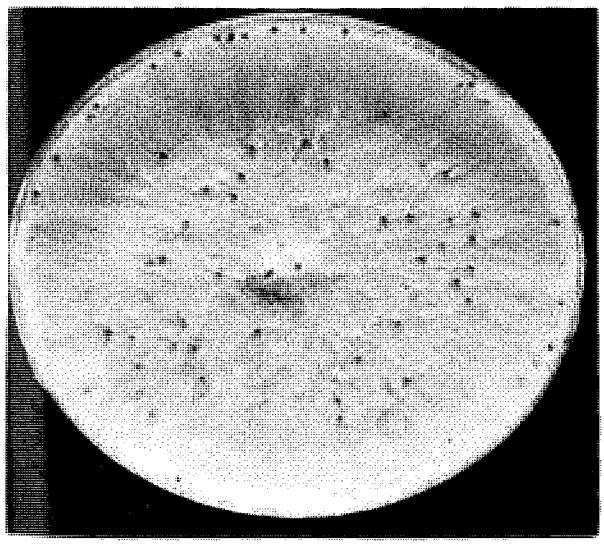


Fusarium solani

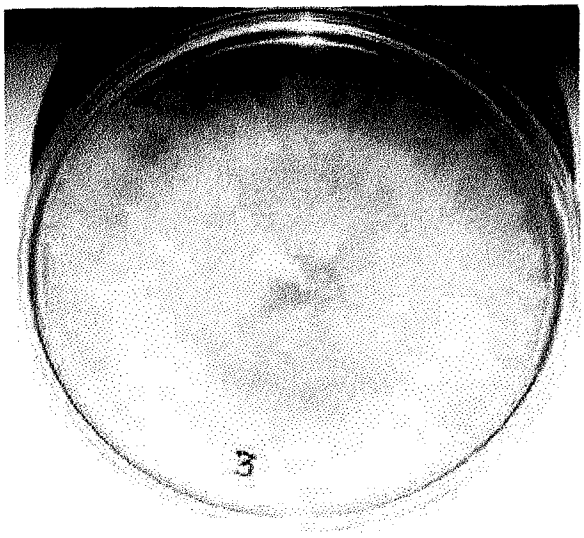
Fusarium spp.



Phymatotrichum sp.

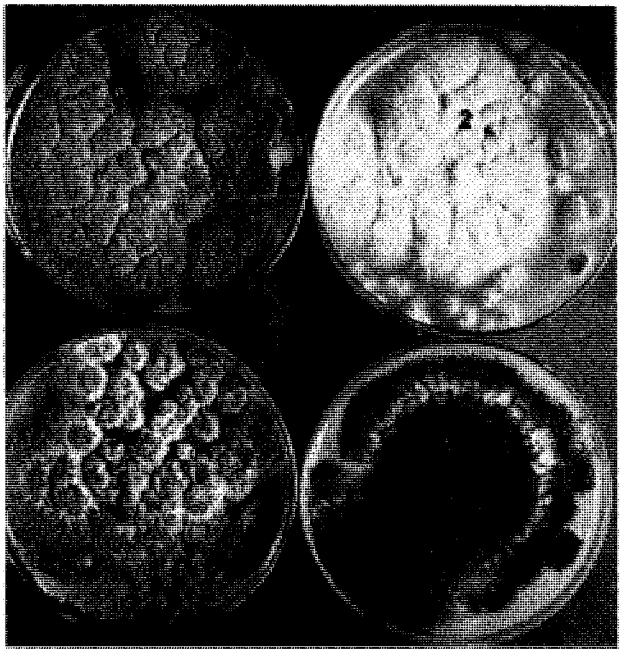


Sclerotium rolfsii

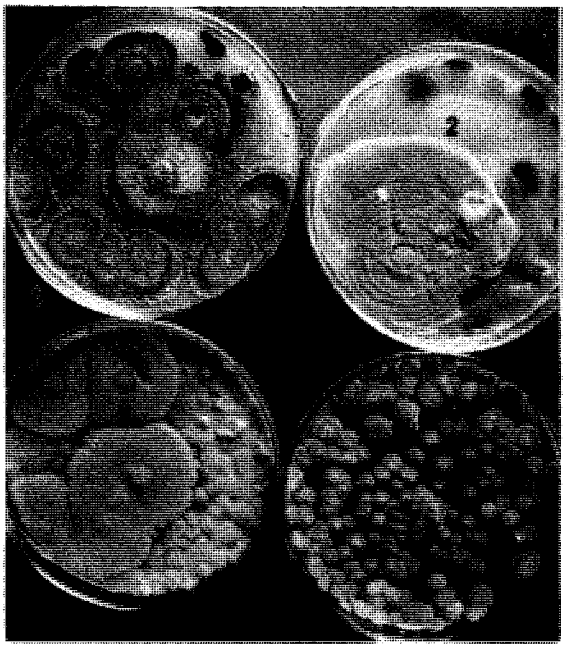


Fusarium oxysporum

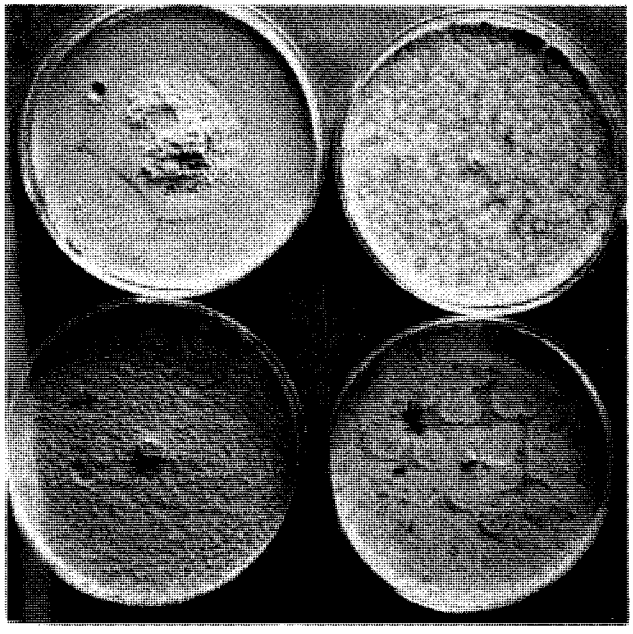
Plate 5. Pathogenic fungi isolated from kidney bean seeds

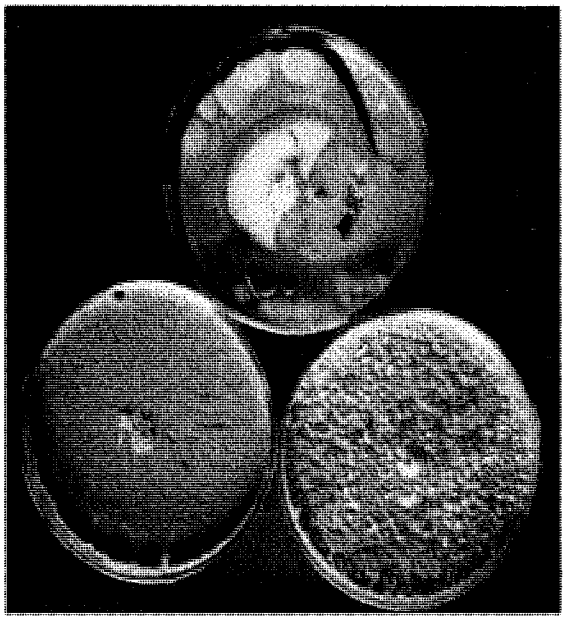


Aspergillus flavus (1), *A. ochraceous* (2), *A. fumigatus* (3) and *A. niger* (4)



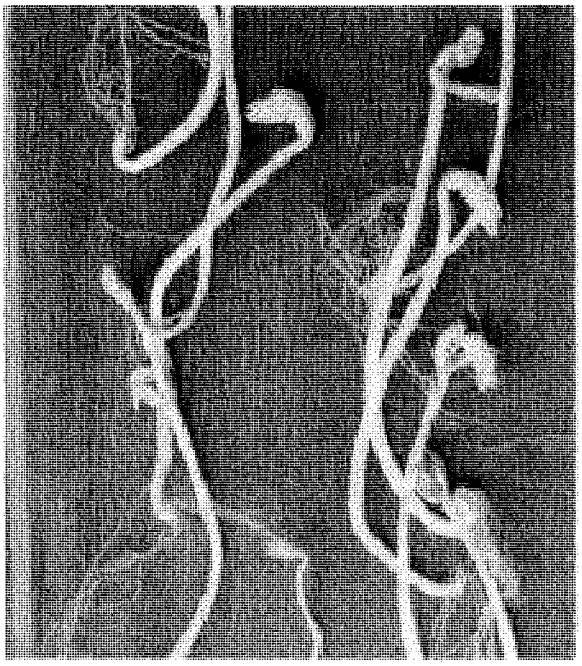
Penicillium perpuragenum (1), *P. simplicissimum* (2), *P. Griseo-fulvum* (3) and *P. capsulatum* (4)





Species of *Penicillium*

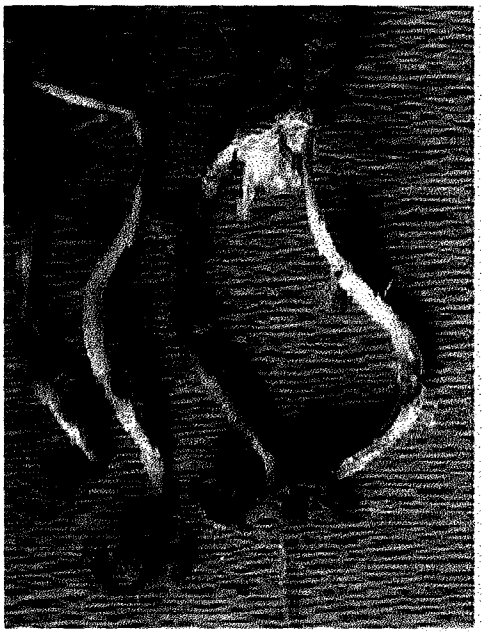
Plate 6. Storage Fungi Isolated from Kidney Bean Seeds



Healthy seedlings

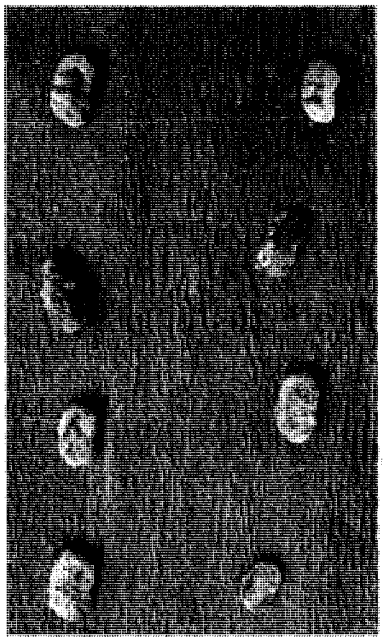
Sclerotium rolfsii

Rhizoctonia solani

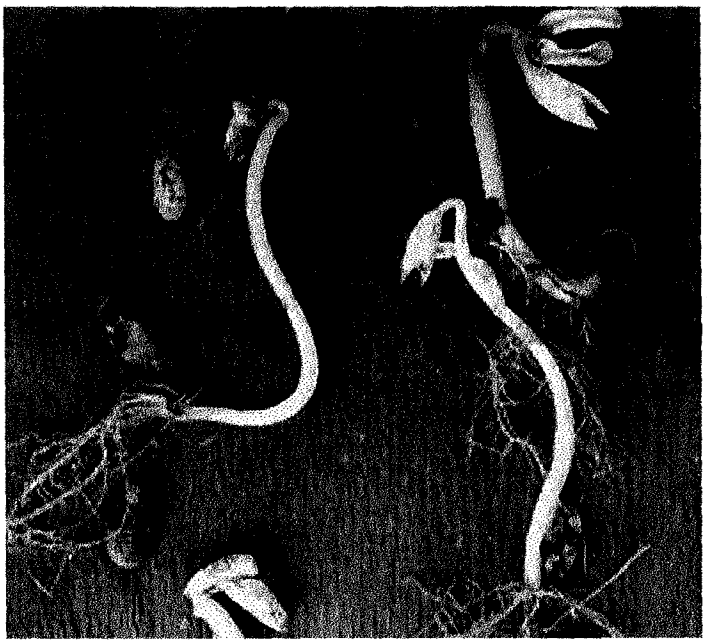


Alternaria sp.

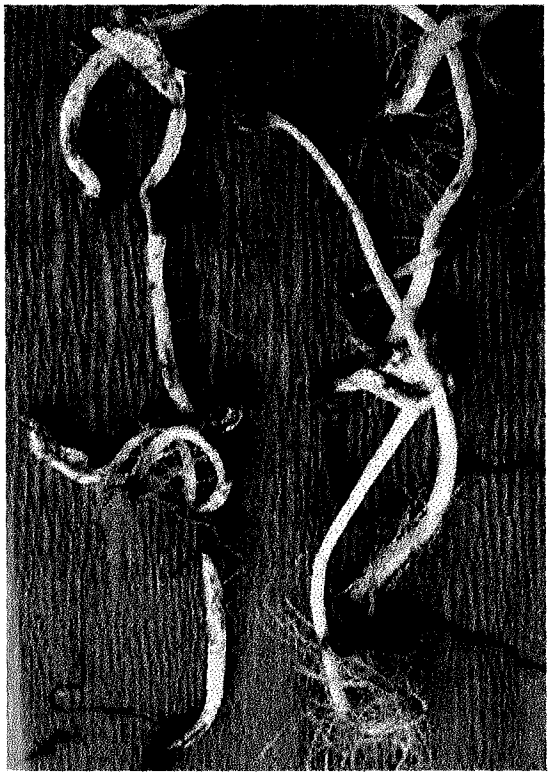
Plate 7. Effect of Spore/ Mycelial Suspension on Seedlings



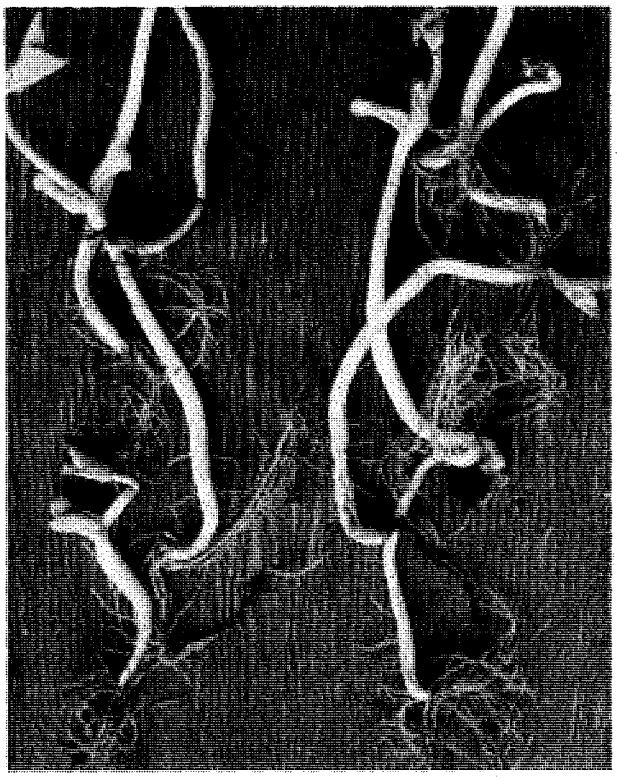
Species of *Aspergillus*



Species of *Penicillium*

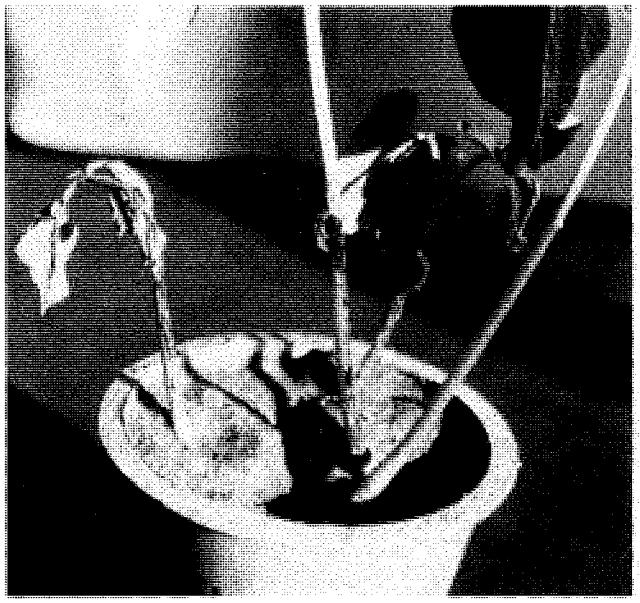


Species of *Aspergillus*

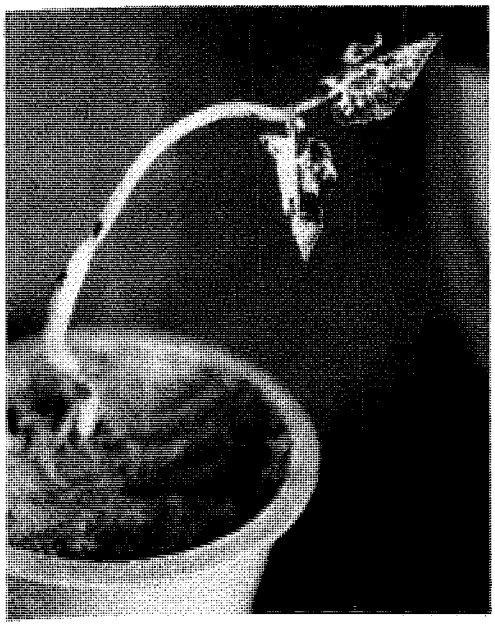


Species of *Penicillium*

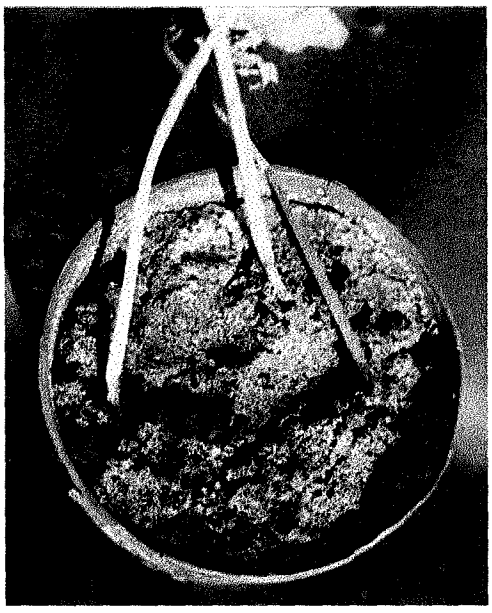
Plate 8. Effect of cultural Filtrate on seeds and Seedlings of Kidney Bean



Seed borne infection of *Colletotrichum lindemuthianum*

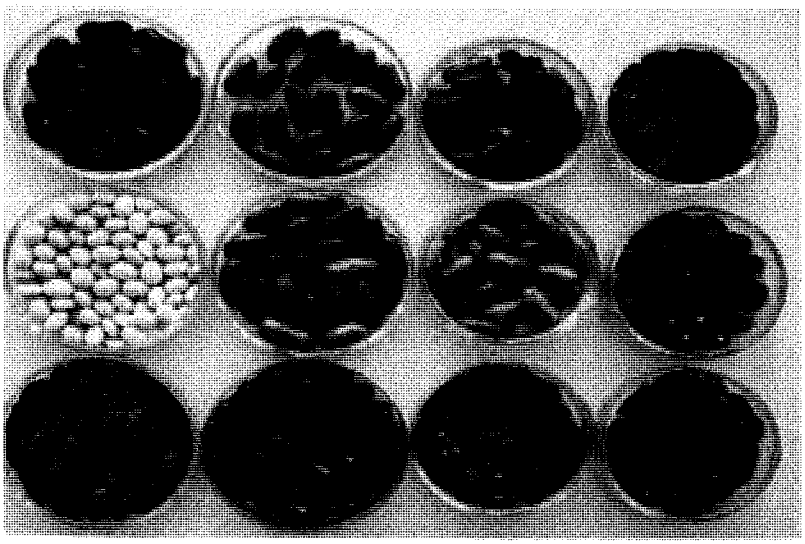


Web blight caused by *Rhizoctonia solani*



Stem rot caused by *Scleritium rolfsii*

Plate 9. Symptoms Induced by Seed Borne Pathogens



Variously coloured bean seeds

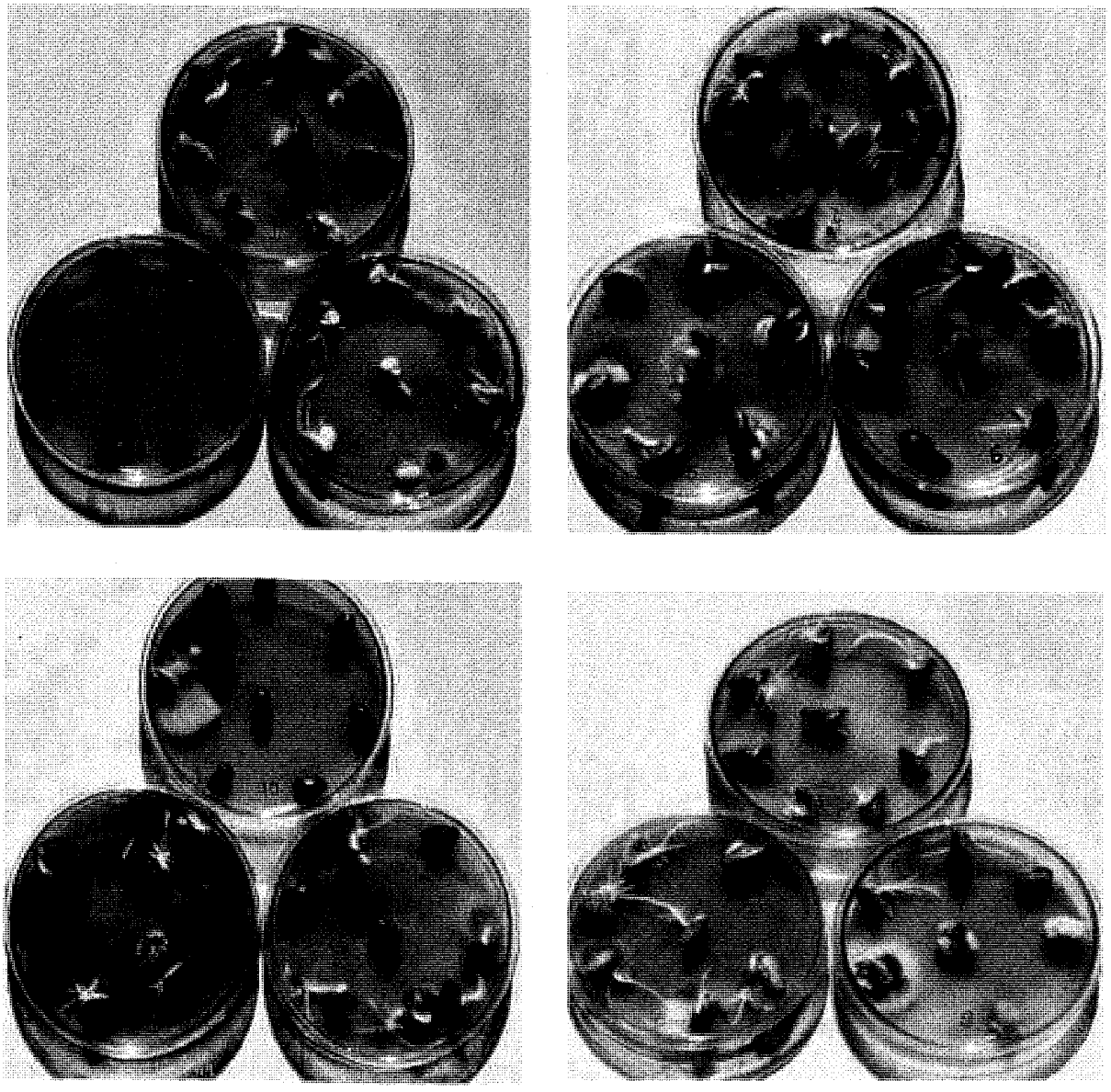


Plate 10. Occurrence of Mycoflora on Different Coloured

Title of the Thesis	:	**Eco-pathological study of seed mycoflora and its impact on biodeterioration of *Phaseolus vulgaris* L. in Himachal Pradesh**
Name of the Student	:	**Deepika Sud**
Admission No.	:	**A97- 40 -05**
Major Subject	:	**Plant Pathology**
Minor Subject(s)	:	**Plant Breeding**
	:	**Plant Physiology**
Degree	:	**Ph.D.**
Month and year of submission of thesis	:	**August 2002**
Total pages in thesis	:	**217**
No. of words in abstract	:	**430**
Major Advisor	:	**Dr O.P. Sharma**

ABSTRACT

Seventy three seed samples of kidney bean collected from various agro-ecolological situations when analysed for seed health, mycoflora and seed quality parameters revealed the presence of thirty six species of fungi belonging to twenty four genera detected by blotter, agar plate and seed component plating technique. Both storage and pathogenic fungi were recorded and included the species of *Acremonium, Alternaria, Aspergillus, Botrytis, Cephalosporium, Cladosporium, Colletotrichum, Fusarium, Gliocladium, Helminothosporium, Heterosporium, Macrophomina, Monosporium, Myrothecium, Penicillium, Phoma, Phymatotrichum, Rhizoctonia, Stemphylium, Sclerotinia, Sclerotium, Trichoderma, Trichothecium* and *Varicosporium*. The number of fungi detected from individual seed samples ranged between one to sixteen species whereas, some were free of any infestation. Various seed fungi were located on different parts of seeds, though maximum mycoflora was noticed on seed coat followed by cotyledons. Along with some pathogenic fungi *Aspergillus flavus* and *A. niger* were also recorded on embryonic axis. Freshly harvested seeds were found to harbour more mycoflora than stored seeds and samples collected from Zone IV possessed minimum fungi. Spore suspension and culture filtrates of pathogenic fungi induced identical symptoms, however, spore suspension of storage fungi did not affect seed germination and had no adverse effects whereas, their culture filtrates induced root and stem rot symptoms besides reducing germination. Storage studies revealed that storage containers had no significant effect on seed health however the seed mycoflora was affected. Metallic bins proved to be best storage containers as seed stored in them harboured less mycoflora in comparison to gunny bag and polylined bag stored seeds. The frequency of storage fungi increased with storage period and pathogenic fungi were replaced by storage ones. Biochemical analysis with regard to effect of the mycoflora on the seed revealed that protein and free fatty acid contents showed increase in infected seeds over control and decrease in carbohydrates. Thereby, indicating significant loss of nutrients in infected seeds. Management studies of seed mycoflora revealed that various fungicides applied @ 2, 2.5 and 3 g/kg seed resulted in significant reduction of

the health status of bean seeds. Bavistin, Thiram and Bavistin + TMTD were found to be most effective. However, application of propionic acid alone and in combination with Bavistin reduced the seed germination drastically including other seed health parameters without any significant effect on mycoflora. Seed coat colour was found to affect the occurrence of mycoflora during storage as dark coloured (black and maroon) varieties harboured less fungal flora as compared to brown and white seeded varieties. Vegetable type of beans were more liable to attack of various fungi as compared to pulse type varieties which are probably more hardy.

5/8/02

Signature of Student

5/8/02

Signature of Major Advisor