STUDIES ON SEEDBORNE FUNGI OF SOYBEAN AND ITS MANAGEMENT

BY

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B.Sc. (Ag.)

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2013

CERTIFICATE

Mr. THOTA VENUGOPAL RAO has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON SEEDBORNE FUNGI OF SOYBEAN AND ITS MANAGEMENT"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Date: **(Dr. B. RAJESWARI)**
Place: CHAIRPERSON

DECLARATION

I, **THOTA VENUGOPAL RAO** hereby declare that the thesis entitled **"STUDIES ON SEEDBORNE FUNGI OF SOYBEAN AND ITS MANAGEMENT"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of the original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Hyderabad
Date:

(THOTA VENUGOPAL RAO)
I.D No. RAM/11-44

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON SEEDBORNE FUNGI OF SOYBEAN AND ITS MANAGEMENT"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** of the Acharya N.G. Ranga Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Mr. THOTA VENUGOPAL RAO** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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(Thota Venugopal Rao)

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	at the rate
CD	:	Critical Difference
cm	:	Centimeter
CMIE	:	Centre for Monitoring Indian Economy
cv	:	Cultivar
et al.	:	and co-workers
EC	:	Emulsifiable Concentrate
g	:	Gram
ha^{-1}	:	per hectare
i.e.,	:	that is
ISTA	:	International Seed Testing Association
kg	:	Kilogram (s)
K Gy	:	Kilo Grays
l	:	Liter
M	:	Million
min	:	Minutes
m^2	:	Metre square
ml	:	Millilitre
Nos	:	Number of samples
PDA	:	Potato dextrose agar
SVI	:	Seedling vigour index
S.Em (±)	:	Standard error of mean
TFC	:	Total fungal colonies
Viz.,	:	Namely

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ABSTRACT

Charcoal rot / dry root rot caused by *Macrophomina phaseolina* is an economically important seed and soil borne disease causing significant yield losses in soybean. The pathogen causes charcoal rot, seedling blight, dry root rot, ashy stem blight and dry wilt diseases. Annual lossess due to charcoal rot in soybean was to an extent of 30 – 50%. Disease free quality seed production in soybean is utmost important to sustain the productivity and maintain the quality of the crop**.** Keeping this in view, detailed investigations were carried out to study the implication of seedborne fungi of soybean on seed quality parameters and to find out suitable detection methods for seedborne *M. phaseolina*. Studies on seed to seedling transmission of the pathogen and its management through seed treatments using fungicides, bioagents, botanicals and irradiations were made using soybean cv. JS-335.

A total of one hundred and twenty (120) seed samples comprising of soybean cv. JS-335 were collected from major soybean growing districts of Andhra Pradesh *i.e*., Nizamabad (60 Nos) and Adilabad (60 Nos) during *kharif* 2012 - 2013. The seed samples were analysed for seed health by standard blotter, 2, 4-D blotter, deep freeze blotter and agar plate methods as per ISTA (1996). Significant differences in occurrence of total number of fungal colonies due to location and source of seed samples were observed. Total per cent occurrence of seed mycoflora in Nizamabad and Adilabad districts of Andhra Pradesh was ranged from 30 to 49.2 % and 23.6 to 45.0 % by blotter method, 14.8 to 28.1% and 11.6 to 22.1% by 2, 4 - D blotter method, 11.8 to 19.3 % and 9.5 to 16.2 % by deep freeze blotter method, 13.1 to 37% and 15.4 to 26.4 % by agar plate methods, respectively. A total of nine fungal species belonging to eight genera were detected in all the seed samples tested in four detection methods. Nine fungal flora *viz., Macrophomina phaseolina, Colletotrichum dematium, Aspergillus flavus, Aspergillus niger, Rhizopus, Curvularia* sp. *Alternaria, Cladosporium* and *Fusarium* sp. were observed. Among them, pathogenic fungi *viz., Macrophomina phaseolina, Colletotrichum dematium, Curvularia* sp. *Alternaria, Cladosporium* and *Fusarium* sp. and storage / saprophytic fungi like *Aspergillus flavus, Aspergillus niger* and *Rhizopus* were observed.

Of the four detection methods adopted for isolation of seed mycoflora in soybean, standard blotter method was found superior in recording more number of total fungal

colonies in addition to predominant seedborne *M. phaseolina* over agar plate method, 2, 4 - D blotter and deep freeze blotter methods. Out of nine fungal species, *M. phaseolina* was found predominant in the seed samples analysed (8.5 to 28.5 %) in Nizamabad and Adilabad districts. The pathogen appeared as greyish mycelial growth on the incubated seeds. Pathogenicity of seedborne *M. phaseolina* was proved by seed inoculation method using soybean cv. JS-335. The pathogen was reisolated and compared with the original isolate.

The mycoflora associated with soybean seed samples were found to reduced seed quality parameters. Significant differences in mean seed germination (70.4 to 73.1 %), mean seed vigour index I (1697 - 1821) and mean seed vigour index II (68.8 - 82.2) was recorded. Among different Mandals, Ditchpally Mandal of Nizamabad district and Kubeer Mandal of Adilabad district recorded low seed germination (65.3 % and 67 %), seed vigour I (1394 – 1470) and seed vigour II (57.0 - 65.2) with increased seed rot (19.2 % and 14.1 %) and seedling blights (17.3 % and 12.5 %). Seedborne fungi present in soybean produced seed rots, seedling blights and decreased quality and quantity of soybean besides causing germination failures.

Seed transmission of *M. phaseolina* in apparently healthy soybean seeds (cv. JS 335) was 6 % and 8 % and in artificially inoculated soybean seeds (38.5 % and 49 %) and in naturally infected soybean seeds (32 % and 43.1 %). Germination in the above seed samples ranged from 75% to 72%, 55% to 46% and 59.3 % to 50.5 % in test tube water agar method (*in vitro*) and in glasshouse conditions.

The efficacy of seed treatments against seedborne *M. phaseolina* were evaluated under glasshouse conditions. The results indicated that soybean seeds treated with thiram + carbendazim @ 3 g kg^{-1} or vitavax power 200 @ 2.5 g kg^{-1} improved seed germination (91%, 89%) and reduced seed rot (5.7 %, 6.7 %) and seedling blights (4.0 % and 5.2 %). Seed treatment with *T. viride* @ 10 g kg^{-1} was also found on par with fungicide seed treatments in improving seed germination of 88 % and reducing seed rot and seedling blight of 7.3% and 6.3%, respectively. Seed treatment with bioagent (*P. fluorescens),* botanicals (neem seed kernel extract @ 5 % and neem leaf powder @ 5g kg^{-1}) and irradiations (1.5 k Gy and 2.5 k Gy) were also effective in improving seed germination and reducing seedling mortality as compared to untreated seeds (74%, 20.7 % and 15.0 %) and pathogen treated seeds (60 %, 26.3 % and 25.7%), respectively in soybean cv. JS-335.

Chapter I

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) the "Golden bean" is one of the fore most important oil seed crop known for its excellent protein (42-45%), oil (22%) and starch content (21%). The crop is one of the likely solutions for overcoming the world's protein hunger. It is also a good source of vitamin B complex, particularly thiamine and riboflavin. Soybean protein is rich in valuable amino acids like lysine (5%) in which, most of the cereals are deficient. Soybean can substitute for meat and to some extent to milk (Endres *et al*., 2013). Its oil is the largest component of the world's oils. Despite being a relatively new crop in India, soybean has shown a phenomenal growth both in area and productivity during the last three and a half decades. There is no other parallel to soybean as a new crop, from hardly any acreage in 1975; it has now become the largest oil seed crop in India with production of about 10.0 mt, surpassing even groundnut and rape seed mustard. In India, the crop is grown in an area of 93.03 lakh ha with 101.28 mt of production (CMIE, 2011). It is grown in the states like Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh and Andhra Pradesh. In Andhra Pradesh, the crop is grown in an area of 1.41 lakh ha with 1.51 mt of production (CMIE, 2011).The cultivation of soybean crop is gaining momentum at faster pace in Andhra Pradesh and it is extensively grown in Adilabad, Nizamabad, Kurnool, Prakasam, Guntur and Medak districts.

Inspite of phenomenal increase in area and production of soybean, its productivity remains low because of lack of quality seeds. Low yield and productivity of soybean in India is mainly due to various diseases and pests occurring in the field and causing yield losses. One of the major constraints in the endeavour of increasing productivity of soybean is its susceptibility to a large number of diseases caused by fungi, bacteria, viruses and nematodes. In India, although 40 fungal pathogens have been identified in soybean crop, but only a few of them are economically important (Sarbhoy and Agarwal., 1983).

The annual losses due to soybean diseases are estimated to the tune of 12 % of the total potential production in which fungal diseases alone can cause damage up to 6 to 8%. Occasionally the losses due to viral diseases may go up to 50 % (Sinclair., 1982) and reduce yield and quality. In field, the crop is affected by a number of pathogens *viz.*, Purple seed stain (*Cercospora kikuchi*), Anthracnose (*Colletotrichum dematium* var.

truncatum), Charcoal rot *(Macrophomina phaseolina*), *Fusarium* collar rot (*Fusarium semitectum*), *Alternaria* pod decay (*Alternaria alternata*), Bacterial blight (*Pseudomonas savastanoi* pv. *glycinea*), Soybean Mosaic Virus (SMV), Leaf Crinkle Virus (LCV), Peanut Bud Necrosis Virus(PBNV), Yellow Mosaic Virus (YMV). Many of these diseases were found to be seed borne in nature. Of these, charcoal rot caused by *Macrophomina phaseolina* is an economically important seed and soil borne disease causing significant yield losses. The pathogen causes charcoal rot, seedling blight, dry root rot, ashy stem blight and dry wilt diseases (Mengistu *et al.,* 2011). The estimated annual lossess due to charcoal rot in soybean was to an extent of 30 – 50% (Wrather *et al.,* 2003). Therefore, disease free quality seed production in soybean is utmost important to sustain the productivity and maintain the quality of the crop**.**

Seeds of soybean are known to harbour several species of seed borne fungi *viz*., *Cercospora kikuchi, Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp. and *Rhizopus stolonifer* were found in germinating seeds and seedlings of soybean (Shovan *et al*., 2008). Many fungi have been reported to be externally and internally seed borne which causes seed discolouration and rotting of seedlings and other diseases in mature plants. The pathogenic fungi associated with the seeds cause serious diseases in the field. These seed mycoflora reduces quality and quantity of soybean seed and infected seed can also transmit various diseases. Fungi associated with seed causes deterioration of seed quality and affects seed viability and reduce seed germination (Anuja and Aneja., 2000). The infected seeds failed to germinate or seedlings and plants developed in the field from infected seeds may escape the early infection but often may be infected at the later stages of the crop growth. Besides, pathogens can spread over a longer distance and uninfected field may be infected by the seeds in which different pathogens are present.

One of the important basic needs for higher agricultural production is quality seed which is characterized by high viability and vigour. To increase the production of soybean qualitatively and quantitatively, farmer requires healthy quality seeds with high percentage of germination and purity. Hence, it is imperative that seed must be tested before they are sown in the field. Seed health testing methods like blotter paper method, deep freeze blotter, 2, 4-D blotter and agar plate methods have been employed for detection of internal and external seedborne mycoflora of soybean, Solanke *et al*. (1997), Paul (1989) and Rajeswari and Meena Kumari, (2009). The frequency in

occurrence of such potentially pathogenic fungi on soybean cultivars poses a potential threat in crop production programme.

Transmission of the pathogen through seed is also known as a means of spread of disease into new areas and new countries. *M. phaseolina* in soybean transmits from seed to seedlings in a systemic manner. The reduction in seed germination and increase in seed rot and seedling mortality were noticed. Another adverse effect of seed borne pathogens is that it contaminate the areas which were disease free previously. So, it necessitates the eradication of seed borne inoculum through various seed treatments and through the enforcement of proper domestic and international quarantine acts and procedures (Arya *et al*., 2004).

For successful production of any crop the seed must be sound and free from seed mycoflora which interfere with seed germination and subsequent emergence of the crop. Seed treatment with bioagents and fungicides is an economical and viable approach to protect seed and seedlings from the early attack of pathogens (Ramanathan and Sivaprakasam., 1993; Rajeswari and Meena Kumari., 2009). Potential bioagents like *Trichoderma viride, Trichoderma harzianum, Bacillus subtillis* and *Pseudomonas fluorescens* are useful for management of the seed borne pathogens (Kulkarni *et al*., 2008). Botanicals and irradiations are also being used for effective elimination of the seedborne fungi and improvement of seed quality (Sahu and Kar., 2009, Alpa *et al*., 2010 and Ikram *et al*., 2010). However, information on seedborne fungi associated with soybean seeds and its detection by different methods, transmission of the pathogen from seed to seedlings and its management is meager. Keeping this in view, the present investigation was taken up with the following objectives:

1. Detection of seedborne fungi associated with seed samples of soybean and its effect on seed quality parameters.
2. To study the seed transmission of predominant seed borne fungi of soybean.
3. To find out the effect of seed treatments in the management of predominant seed borne fungi of soybean.

Chapter II

REVIEW OF LITERATURE

The available literature on assessment of seed mycoflora and its impact on seed quality evaluation of suitable detection methods and management of predominant seed borne fungi with fungicides, bioagents, botanicals and irradiations in the management of seed and seedling diseases of soybean were dealt in this chapter.

As the available information on these aspects is scanty, the literature pertaining to other crops has also been reviewed under the following heads.

2.1 Seed mycoflora of soybean

2.2 Effect of seed mycoflora on seed quality of soybean

2.3 Evaluation of seed health testing methods

2.4 Seed transmission studies of predominant seedborne fungi

2.5 Management of seed borne diseases under glasshouse conditions

2.5.1 Seed treatment with fungicides

2.5.2 Seed treatment with bioagents

2.5.3 Seed treatment with botanicals

2.5.4 Seed treatment with Irradiations

2.1 SEED MYCOFLORA OF SOYBEAN

Mishra and Kanaujia (1973) reported twenty nine fungal species from seed samples of seven oil seed crops. Among the screened mycoflora, *Rhizopus nigricans* and *Penicillium* were found to be the predominant.

Nik (1980) reported 27 species of seed mycoflora from16 soybean seed samples. The pathogenic fungi frequently isolated were *Botryodiplodia theobromae, Colletotrichum dematium, Diaporthe phaseolorum, Choanephora cucurbitarum, Fusarium moniliforme, F. semitectum, Macrophomina phaseolina, Myrothecium roridum,* and *Phoma sorghina, Aspergillus, Chaetomium, Cladosporium, Curvularia, Nigrospora, Nodulisporium, Pencillium, Rhizopus, Trichoderma* and *Zygosporium*.

Tripathi and Singh (1991) tested three soybean genotypes for presence of seed mycoflora which yielded sixteen fungal species *viz.*, *Alternaria alternata, Aspergillus flavus, Aspergillus terreus, Aspergillus sydowi, Pencillium oxalicum, Fusarium moniliforme, Mucor racemosus, Mucor subtilissimus, Curvularia lunata, Rhizopus*

arrhizus, Myrothecium roridum and *Nigrospora oryzae* in various localities of Uttar Pradesh.

Dawar and Ghaffar (1998) reported 38 species of fungal flora in sunflower seed samples collected from different parts of Pakistan *viz., Macrophomina phaseolina, Rhizoctonia solani, Fusarium moniliforme*, *F. solani, Aspergillus flavus* and *A. niger* were found predominant.

Anwar *et al.* (1995) recorded association of ten field fungi in soybean seeds *viz., Alternaria alternata, C. kikuchi, C. truncatum, C. dematium, F. moniliforme, F. oxysporum, F. solani, M. phaseolina and Fusarium equiseti* causing root diseases and damping off and reduced the seed germination and seedling emergence.

Murthy and Ravesha (1996) studied soybean seed mycoflora and reported 38 fungal species. Among them, *Aspergillus, pencillium* and *Rhizopus* sp. were commonly occurring storage fungi and *Alternaria, Chaetomium, Colletotrichum, Cladosporium, Diaporthe, Fusarium, Macrophomina, Myrothecium, Phoma* and *Trichothecium* were the most commonly occurring field fungi which reduced seed germination and seedling vigour and caused varied symptoms on seedlings.

Lal and Singh (1997) studied seed mycoflora of greengram and recorded fungal species of *Alternaria, Aspergillus* and *Pencillium* were found predominant and fungal species were found high in blotter method as compared to agar plate method. They have reported fungal populations were increased with increasing storage period.

Solanke *et al*. (1997) reported that occurrence of *Aspergillus niger* and *F. moniliforme* were found higher than *Curvularia lunata, Alternaria alternata* and *Pencillium* sp. in soybean cultivar PK-472.

Goulart (1997) observed 15 to 20 species of seedborne mycoflora in soybean seeds *viz., Phomopsis* sp. *Colletotrichum truncatum, Cercospora kikuchi, C. sojina, Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium semitectum, Aspergillus* sp. and *Pencillium* sp.

Grigaliuniate and Vitkus (1997) isolated *Fusarium* sp., *Thielaviopsis basicola, Botrytis cinerea, Alternaria alternata, Cladosporium cladosporioides, Diplodiscus* sp. *Trichothecium roseum, Stemphylium botryosum* and *Pencillium* sp. from soybean seeds.

Rauf (2000) reported twenty four seedborne fungi belonging to different genera were detected by blotter paper method from 145 seed samples of major leguminous crops in Pakistan. Among these fungi, *Alternaria alternata*, *Ascochyta* sp.

Colletotrichum sp. *Fusarium* sp. and *M. phaseolina* were the most frequent and known common pathogenic fungi in these crops. Highest seed mycoflora was detected in soybean (14) and chickpea (13) followed by mungbean, pea and lentil seed.

Dawar *et al*. (2007) reported seedborne mycoflora of 14 chickpea seed samples comprising of 21 species of fungi belonging to 13 genera *viz., F. moniliforme, F. oxysporum, M. phaseolina* and *Rhizoctonia solani* and saprophytic fungi like *A. niger* and *A. flavus.* The occurrence of *M. phaseolina* and *Rhizoctonia solani* were isolated from seed coat, cotyledons and seed axis. Blotter method of detection showed greater incidence of fungi followed by agar plate and deep freeze methods.

Shovan *et al*. (2008) reported that blotter method was found effective for detection of fungi in soybean seeds. Fungi *viz., Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp. and *Rhizopus stolonifer* was recorded in soybean seed samples.

Afzal *et al*. (2010) reported that a total of 13 phytopathogenic fungal species including *A. alternata, A. flavus, A. fumigatus, A. niger, C. lunata, Drechslera tetramera, Fusarium solani, Fusarium moniliforme, M. phaseolina, Mucor, Pencillium* and *Rhizopus* sp. were identified by using agar and blotter paper methods from seven cultivars of sunflower. The isolated fungi reduced seed germination by 10-20% and seedling mortality by 10-12%.

Ramesh *et al*. (2013) studied seed mycoflora of soybean and isolated 11 fungi by agar plate and blotter paper methods and six fungi were isolated from seed washing method. Pathogenic fungi frequently isolated were *M. phaseolina, F. oxysporum, A. flavus, A. niger, Phoma* sp. and *Sclerotinia sclerotiorum.* Less frequently isolated fungi were *F. solani, F. moniliformae, Rhizophus* sp. *Botrytis cinerea* and *Cercospora kikuchi.*

2.2 EFFECT OF SEED MYCOFLORA ON SEED QUALITY OF SOYBEAN

Dhingra and Sinclair (1975) reported that *M. phaseolina* present on the seed coat reduced seed germination and caused post-emergence damping off of cucumber seeds.

Sinclair and Shurtleff (1975) reported that seed borne fungi *viz., Diaporthe phaseolorum, Aspergillus* sp. *Cercospora kikuchi* and *M. phaseolina* reduced seed quality in soybean.

Sinclair (1977) observed 66 fungi, 6 bacteria and 8 viruses in soybean seed. These seedborne microorganisms have adverse effect on soybean seed and reduced seed germination, seedling emergence and enhanced seedling blights, leaf spots and other diseases on mature plants.

Anwar *et al.* (1995) reported ten field fungi in soybean seeds *viz., Alternaria alternata, C. kikuchi, C. truncatum, C. dematium, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, M. phaseolina* and *Fusarium equiseti* causing root rot diseases and damping off and reduced seed germination and seedling emergence.

Prasad and Kulshrestha (1999) reported that sunflower seeds naturally infected with *A. helianthi* showed 32.8 % reduction in seed germination and seed vigour index also decreased significantly.

Rahman *et al*. (2000) observed that artificially inoculated seeds of three mungbean varieties with *M. phaseolina* showed three fold lesser normal seedlings in comparison to the uninoculated seeds. The mean shoot length, root length and dry weight of seedlings, seedling vigour and storability were reduced.

Singh *et al*. (2004) reported that 10 fungal species from seven genera of groundnut seed. Among them, *Aspergillus flavus, A. niger, Fusarium oxysporum* and *F. moniliforme* were found predominant and pathogenic and reduced seed germination and root and shoot length of seedlings.

Lakshmeesha *et al*. (2013) studied seedborne fungi of soybean *viz*., *Fusarium* sp. *M. phaseolina, Pythium* sp. *Aspergillus* sp. *Phoma* sp. and *Phomopsis* sp. *M. phaseolina* the causal agent of charcoal rot of soybean caused post emergence damping-off of soybean seedlings leading to 50 % of crop losses.

2.3 EVALUATION OF SEED HEALTH TESTING METHODS

Ramnath *et al*. (1970) and Agarwal *et al*. (1972) confirmed that superiority of blotter method over agar plate method for assessing the seed borne fungi in sunflower seeds.

Neergaard (1977), Kushi and Khare (1978), Ellis (1979) and Arya *et al*. (2004) reported that standard blotter method was found superior over agar plate and deep freeze blotter methods for detection of *M. phaseolina* in soybean seeds.

Neergaard (1977), Bhale *et al*. (2000), Baliyan and Vishunavat (2007) reported that deep freeze blotter method was found superior over agar plate and standard blotter methods for detection of *Colletotrichum truncatum* in soybean seed.

Sundaresh and Hiremath (1978) reported that irrespective of the methods of isolation and variety used, unsterilized seeds gave more number of mycoflora than the sterilized seeds of soybean.

Michail *et al.* (1981) reported that blotter method was found superior for detecting *Cephalosporium, Fusarium* and *Myrothecium* species whereas PDA medium was preferable for *M. phaseolina* causing charcoal root rot of soybean. Both PDA and blotter methods were found effective for isolating other species of seed borne fungi of soybean.

Gill *et al.* (1983) proved that agar plate method was found superior over standard blotter paper method in detection of seed mycoflora of leguminous seeds.

Sirithorn and Boonchitsirikul (1988) reported seed mycoflora of sesame by blotter method and detected seed borne *M. phaseolina* which caused pre and post emergence mortality in sesame seeds.

Paul (1989) analyzed soybean seeds by standard blotter method, agar plate method and deep freeze blotter method to detect internal and external seed borne mycoflora. A total of 26 fungal species were found associated with soybean seeds.

Godika *et al.* (1999) found that agar plate method was more suitable for isolation of *M. phaseolina* in sunflower.

Solanke *et al*. (1997) reported that agar plate method yielded more seed mycoflora than blotter method in soybean.

Bhale *et al.* (2000) reported that deep freeze blotter method was found superior over agar plate and standard blotter method for detection of *C. dematium* var. *truncatum* in soybean seeds.

Nasreen (2003) reported that total number of 39 species of fungi belonging to 15 genera were isolated from 6 month old soybean seeds by four incubation methods. PDA method yielded the highest number of fungal members either with or without the treatment of disinfectant. Incubation of seeds on blotter paper method recorded lesser number of fungal species and the number of fungi gradually reduced on component plating and the least number of fungi appeared by deep freezing method.

Ramesh and Avitha (2005) reported that more fungi were isolated on blotter method as compared to agar plate method. But in contrary some workers observed that both the methods were equally valuable and supplementary to each other (Kumhar *et al*. 1987).

Singh *et al*. (2005) reported that standard blotter method was found best in terms of recording more number of fungal species isolated from chickpea seeds followed by agar plate method.

Tariq *et al*. (2005) observed that blotter and agar plate methods were suitable for detection of seed borne fungi in soybean.

Ahammed *et al*. (2006) found that eight fungal species were associated with soybean seeds in agar plate and blotter methods. Agar plate method was found better in yielding maximum number of fungal colonies than blotter method. In both the methods, unsterilized seeds yielded more number of colonies than the sterilized seed.

Dawar *et al*. (2007) reported that blotter paper method recorded greater incidence of fungi followed by agar plate and deep freeze methods in screening seed-borne mycoflora of 14 chickpea seed samples.

Nagaraja *et al*. (2009) reported that standard blotter method was found to be superior over potato dextrose agar method, water agar method and 2, 4 - D method for detection of seed mycoflora associated with castor.

Afzal *et al*. (2010) found that agar plate and blotter paper methods were effective in isolation of the sunflower seed mycoflora. Treated seed yielded less occurrence of seedborne fungi than the untreated seed indicating the partial elimination of some contaminating fungi.

Nagaraja and Krishnappa (2011) reported that standard blotter method was found to be superior over potato dextrose agar method, water agar method and 2, 4 - D method for detection of *Alternaria carthami* in safflower seed.

2.4 SEED TRANSMISSION STUDIES OF PREDOMINANT SEEDBORNE FUNGI

Neergard (1977) detected embryo infection of *C. lindemuthianum* in beans, which resulted in non-systemic seed transmission revealed by the detection of pathogen as hyphae on seed coat and embryo.

Raut (1985) reported the seedborne nature of *M. phaseolina* in sunflower seeds and infected seeds showed severe symptoms of pre and post emergence mortality of

seedlings and the ratio of seed infection to seed transmission were 1: 0.8. Seed borne inoculum caused infection of the primary root and cotyledons within 2 to 3 days of growth and subsequently under high temperature conditions resulted in pre emergence death of seedlings or post emergence mortality in the form of damping off, collar rot, root rot and stem rot and abundant pycnidia and sclerotia were often formed on infected stem and dead plant parts and the fungus did not cause any systemic infection.

Kunwar *et al*. (1986) observed that *M. phaseolina* was seed transmitted from infected seed to seedlings through microsclerotia present on or in seed coat and produced necrotic spot on hypocotyls and seedlings which were raised from infected seeds of soybean.

Shivanna and Shetty (1989) observed that cluster bean seed sample with 23.5% *C. dematium* infection showed 3% pre-emergence and 15% post-emergence mortality in sand method.

Anwar *et al*. (1995) reported that seed borne fungi of *M. phaseolina* was found to be seed transmitted from seed to seedlings of soybean.

Dawar (1996) reported that *M. phaseolina* was transmitted from seed to seedlings of sunflower**.**

Agarwal and Singh (2000) reported that seed borne infection of *M. phaseolina* in okra seeds resulted in seed rot, symptomatic seedlings and seedling mortality in water agar test. The pre emergence loss was significant in symptomatic (37.33, 48%), heavily discoloured seeds (42.66, 48%) than asymptomatic seed (1.33, 22.33%).

Kumar and Singh (2000) found that soybean seeds of K-60, Bragg, PK-262, PK 472, Alankar and Gaurav were found to carry 3 to 12% infection of *M. phaseolina* with maximum occurrence on the seed coat of all the infected seed and moved into the cotyledons (including embryonic axis) of the 40% infected seed. The pathogen transmitted from seed to seedlings during the germination by local contact. Out of 12 infected seeds of soybean the pathogen was able to transmit and cause infection in 4 seedlings, giving ratio of 1:0.33 in seed and seedling infection.

Arya *et al*. (2004) reported that *M. phaseolina* transmits from seed to seedling in a systemic manner. The germination in naturally infected soybean seeds was 52% and 45%, respectively as against 75% and 72% germination in healthy seed under laboratory and glasshouse conditions, respectively. In naturally infected seeds, 30% and 25% seed

rotting 18% and 8% seedling mortality were recorded in laboratory and glasshouse conditions, respectively.

Rakesh and Jain (2004) reported that seed inoculation with *M. phaseolina* resulted in pre and post emergence mortality and reduction in seed germination as compared to the untreated control. *M. phaseolina* infected seedlings showed seed rot, dark brown color patches on root and shoot transitional zone and brown or black circular spots on cotyledons in addition to root rot symptoms in cluster bean.

Mandhare *et al*. (2009) studied transmission of seed borne *M. phaseolina* causing charcoal rot in soybean using naturally infected and artificially inoculated seed with *M. phaseolina* in the laboratory and greenhouse conditions. Naturally infected seed resulted in abnormal seedlings, whereas severely infected seeds resulted in seedling mortality (14 and 10%, respectively) and seed rot (22 and 16%) and reduced germination. In case of artificially inoculated seeds, seed germination was lower in the greenhouse (54%) than in the laboratory (61%). Artificially inoculated seeds recorded seedling infection rates of 11% and 16% under laboratory and greenhouse conditions, indicating the transmission of *M. phaseolina* from infected seed to seedlings.

2.5 MANAGEMENT OF SEEDBORNE DISEASES BY SEED TREATMENTS

2.5.1 Seed treatment with fungicides

Ellis *et al*. (1975) reported that seed treated with fungicides had higher germination and emergence in vermiculite and field soil than untreated control. Further they reported that internally seed borne fungi were primarily located in the seed coat (testa) tissues and occasionally found in embryo tissues. Captan and thiram moved into seed coat tissues, but did penetrate the embryo and these were found effective only against the fungi present on the seed coat. The fungicide benomyl inhibited the pathogen present in the seed coat and embryo but it was phytotoxic in nature.

Sunderesh and Hiremath (1982) reported that increased germination and emergence of soybean due to fungicidal treatment.

Vyas and Khare (1986) obtained good control of soybean dry root rot by combined application of *T. harzianum* and carbendazim

Vyas (1987) further confirmed that dry root rot of soybean disease could be reduced by combination of thiram+carbendazim (2:1) followed by seed treatment with

fungicide tolerant strain of *Trichoderma* sp. or *Bacillus subtilis* in *Macrophomina* sick soils.

Singh and Agarwal (1988) tested different seed dressing fungicides and found that captafol and thiram resulted in the highest seedling emergence. While thiram, captafol and mancozeb increased yields as compared with control.

Agarwal and Sushma (1989) reported that seed and seedling mortality caused by *M. phaseolina* was reduced to an extent of 20 % in greengram due to seed treatment with carbendazim (1 g kg^{-1}).

Kawale *et al*. (1989) studied the efficacy of seed treatment fungicides viz., thiram, carbendazim and mancozeb and insecticide (disulfoton) and herbicide (prometryn) as seed treatments in soybean. All the seed treatments increased the seedling emergence to an extent of 96 to 98 % as compared with 70 % in untreated control and increased yields of 1052-1516 kg per ha was recorded as compared with untreated control (849 kg per ha).

Tripathi and Singh (1991) studied the efficacy of seed treatments with fungicides which gave significantly better plant stand and yield as compared to control. Seed treatment with captan, thiram, agrosan and mancozeb resulted in maximum plant stand and significantly improved yield of soybean.

Omar and Rahhal (1993) showed that seed coating with thiram (3 g kg^{-1}) increased the percentage of seedling survival in soybean seeds as compared to untreated control.

Ravi kumar *et al*. (1994) reported that seeds of soybean treated with fungicides reduced seed mycoflora and maintained higher germination and vigour index as compared with untreated seed.

Hall and Xue (1995) reported that carboxin + thiram (as vitaflo-280) increased seedling emergence, plant stand, seed yield and decreased the severity of stem infection when applied to the discoloured and shrivelled seeds of soybean.

Solanke *et al*. (1997) studied seed borne fungi *viz., Aspergillus niger, Aspergillus flavus, Fusarium moniliforme, Curvularia lunata, Alternaria alternata* from 2 soybean cultivars by different detection methods. Maximum occurrence of fungal flora was observed in PK-472 as compared with MACS-13. Seed treatment with thiram and inoculation with seed borne fungi improved germination percentage and controlled pre and post emergence mortality of seedlings.

Gaulart *et al.* (2000) reported the efficacy of fungicidal seed dressing in the control of soybean seedborne pathogens and noticed that fungicidal seed treatments reduced the incidence of *Phomopsis* sp. *Fusarium semitectum, Colletotrichum truncatum* and *Cercospora kikuchi*. Further they have reported that improvement in crop yield when seeds were treated with thiram and carbendazim.

Zorato and Henningh (2001) reported that soybean seeds treated with carboxin + thiram @ 2g kg^{-1} seed resulted significantly higher field emergence (83 %) over control (74 %).

Anuja and Aneja (2000) reported that seeds of soybean cv. JS 80 – 21 and Pusa – 16 treated with mancozeb @ 2.5g kg^{-1} (78.6%) and thiram @ 3g kg^{-1} (65.1%) effectively controlled seed mycoflora as compared to nimbecidine (10.1%) and bleaching powder (13%).

Muthuraj *et al.* (2002) noticed that soybean seeds treated with thiram (2g kg^{-1} of seeds) improved germination (80.75 %) and field emergence (70.72 %) as compared to control (79.10 % and 58.63 %, respectively).

Raj *et al*. (2002) reported that three fungi *viz., Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* were found predominant in 28 soybean varieties and reported that thiram seed treatment @ 3g kg^{-1} seed significantly improved seed germination and field emergence and reduced seed mycoflora.

Sunil Kumar (2004) reported that germination percentage, seedling vigour, field emergence and storability were high in seed treatments particularly thiram @ 3 g kg^{-1}, thiram + carbendazim @ 1:1 (3 g kg^{-1}) and *T. viride* @ 6 g kg^{-1} as a result of suppression of seed borne mycoflora and maintenance of strong membrane integrity.

Gawde *et al*. (2009) recorded efficacy of fungicides, bioagents and botanicals in the management of anthracnose/ pod blight of soybean and found that all five fungicides, two botanicals and two bioagents significantly reduced the disease intensity and thereby enhanced the seed yield over unsprayed or control. However, fungicide carbendazim @ 0.1% was found most effective and economical in controlling the disease.

Rajeswari and Meena kumarai (2009) reported that seeds of soybean cv. JS - 335 treated with *B. subtilis* (6 g kg^{-1}), *P. fluorescens* (6 g kg^{-1}), *B. subtilis* + *P. fluorescens* (6 g kg^{-1}), *T. viride* (6 g kg^{-1}), *T. harzianum* (6 g kg^{-1}), thiram + carbendazim (2 g kg^{-1}) along with untreated seed were tested for seed quality under laboratory conditions and

management of seed borne diseases under field conditions. The results revealed that significant increase in germination (91 %), seed vigour (2630) with less number of fungal colonies (Nil) was recorded in thiram+ carbendazim followed by *T.viride* (91 %, 2601 and 5 %) over untreated seeds (77 %, 2470 and 20 %), respectively under field conditions.

Rajeswari *et al*. (2012) studied the efficacy of seed treatments with bioagents and fungicides against seed mycoflora of safflower and the results showed that seed treatments with *T.viride* (6 g kg^{-1}), mancozeb (2.5g kg^{-1}) and neem oil (10 ml kg^{-1}) not only enhanced the seed quality but also effective in reducing total seed mycoflora and seedling mortality. Seeds of safflower cultivars Nira and Manjeera treated with *T. viride* @ 6g $kg^{-1}$was also recorded higher seed germination (90 % and 87 %) and seed vigour–I and II (1529,1383,4337,3432) with less number of total fungal colonies (7 % and 12 %).

2.5.2 Seed treatment with botanicals

Botanicals are antifungal agents with cost effective, non toxic, ecofriendly and eliminate the pathogens and prevent biodeterioration of the seeds.

Maraddi (2002) recorded that cowpea seeds treated with neem leaf powder @ 5 g kg^{-1} of seeds recorded higher germination (39.5 %) and vigour index (1072) as compared to control (34.2 % and 864, respectively) at the end of 10 months of storage period.

Neem leaf extract, Marigold leaf extract and garlic bulb extract at 5 % as seed treatments significantly reduced the charcoal rot incidence and increased yield (Sinha and Sinha, 2004).

Dubey *et al*. (2009) found that neem extracts like leaf (10 %), bark (10 %), oil cake (10 %) and neem oil (1 %) controlled mycelial growth and sclerotial survival of *M. phaseolina* by poisoned food technique. Neem oil was found to be most toxic followed by neem cake, leaf and bark extracts. Sclerotia treated with neem oil did not germinate and resulted in 100 % inhibition of plant pathogens.

Sahu and Kar (2009) reported that seed borne fungi *i.e., Fusarium moniliforme* infected black gram seeds were treated with botanicals and fungicides. Fungicides viz., carbendazim @ 0.2% and vitavax @ 0.2% followed by neem products like neemazal (0.3%) and neem oil (0.3%) were found significantly superior in reducing seed infection.

Devakumar and Usha Dev (2010) reported that causal agent of charcoal rot, seed rot, pre and post emergence damping off due to *M. phaseolina* was reduced by neem 5 EC and azadirachtin seed treatments.

Alpa *et al*. (2010) revealed that neem extract showed 93.7 % inhibition of the seed mycoflora there by enhancing the seed germination as compared to *Ricinus* plant extract (87.5 %) and *T. viride* (62.5 %).

Khatun and Bhuiyan (2011) evaluated neem leaf powder maintained the germination percentage of 88.7 % over control 70.7 % in chickpea seed.

2.5.3 Seed treatment with bioagents

Mukhopadhyay (1989) reported that biological seed treatments in tomato, potato, chickpea, lentil and peanut with *T. harzianum* and *Gliocladium virens* resulted in an excellent protection against a wide range of pathogens like *Sclerotium, Rhizotonia, Pythium aphanidermatum* and *Fusarium oxysporum* and these treatments were constantly found effective as or better than fungicidal seed treatments.

Shivanna and Shetty (1989) reported about the efficacy of bioagents and their combination with fungicides on the mycoflora of cluster bean. Among the biocontrol agents tested *Trichoderma* isolate and *T. viride* were found to be significantly effective in reducing the seedborne fungi of cluster bean. Biocontrol agents and their combinations not only increased seed germinability significantly but also seedling vigor.

Taylor and Harman (1990) reported that bioprotectants like *T.viride* and *Pseudomonas fluorescens* when applied to the seeds not only protects the seed but also colonize and protect roots and increases the plant growth.

Farzana *et al*. (1991) reported that significant reduction in *M. phaseolina* infection in soybean seed treated with *T. harzianum* @ 5 g kg^{-1}

Vyas (1994) reported that simultaneous application of *T.viride* or *T. harzianum* with carbendazim treatment were effective in reducing dry root rot in soybean.

Gupta and Ansari (1998) evaluated the efficacy of bioagents *viz*., *Pseudomonas fluorescence* and *T. viride* against seedling mortality in soybean. The results revealed that seedling mortality was reduced maximum with *T. viride* and *P. fluorescence* individually or in combination with fungicide and bioagent seed treatments enhanced seed germination over untreated control.

Rahman *et al*. (2002) reported that mungbean seed coated with conidial suspension of three *Trichoderma* sp. *viz., T. harizanum, T. hamatum* and *T. viride* in order to control seedborne *Macrophomina phaseolina*. The treated seed including control treatment were evaluated in blotter and in pot culture. All three species of *Trichoderma* showed excellent control of seedborne *M. phaseolina* and also increased germination significantly and there by production of healthy seedlings.

Gayathri and Indra (2003) reported that pre-emergence seedling rot was reduced to 88.05% in seed treatment with *T. viride* along with soil application of *T. viride* and neem cake followed by seed treatment with *T. viride* and carbendazim (70.6%) in groundnut affected by *Aspergillus niger*.

Sethuraman *et al.* (2003) conducted field experiments to test the efficacy of fungal and bacterial antagonistic formulations in controlling root rot disease caused by *M. phaseolina.* Seed treatment with *T. viride* (4 g kg $^{-1}$) and *P. fluorescens* (10 g kg $^{-1}$) significantly recorded less root rot incidence of 4.16 % and 4.59 %, respectively than control (14.04 %) in black gram.

Bioagents *viz., Aspergillus* sp. *Gliocladium virens, T. harzianum* and *T. viride* were tested against the root rot pathogen *M. phaseolina*. *T. harzianum* was proved highly effective in inhibiting the mycelial growth (71.85%) of *M. phaseolina* under green house conditions in green gram (Surender *et al.*, 2007).

Rajeswari and Meena Kumarai (2009) reported that significant increase in germination (91 %), seed vigour (2630) with nil fungal colonies in thiram + carbendazim seed treatment followed by *T. viride* (91 %, 2601 and 5 %) over untreated seeds (77 %, 2470 and 20 %), respectively in soybean cv. JS -335.

Vasebi *et al*. (2013) studied *M. phaseolina* of soybean, two bacteria, *Pantoea agglomerans* and *Bacillus* sp. fungus, *Trichoderma harzianum* T100, as potential biocontrol agents and fungicide mancozeb were evaluated against soybean charcoal rot disease in *in-vitro* and greenhouse conditions. All antagonists inhibited the growth of the pathogen. The overall results of this showed high capability of antagonists in reduction of initial inoculum for next season.

2.5.4 Seed treatment with gamma radiations

Ouf *et al.* (1999) found that irradiation of soybean seed for 3 min caused reduction in the number of seed borne fungi which was more pronounced as the time of irradiation was extended. Pathogens like *Rhizoctonia solani*, *Alternaria* sp. *C. kikuchii* and *C.*

truncatum were completely eliminated when the seeds were treated with irradiation for 10 minutes. Irradiation of pre sowing seeds greatly protected soybean stands against *Fusarium solani*. The reduction in disease incidence was accompanied by accumulation of high proline and phenol levels in the infected root tissues of soybean suggesting that these compounds have a certain role in the prevention of disease development.

Singh and Singh (2005) reported that number of fungal species associated with the seed as well as the per centage(%) fungal occurrence on the seed was significantly reduced due to irradiation treatment. Highest rate of seed germination in all the four rice cultivars was recorded among the seed irradiated with 0.10 k Gy.

Ikram *et al*. (2010) reported that infection of *M. phaseolina, R. solani* and *Fusarium* sp. were significantly decreased on greengram seeds treated with gamma rays and significantly increased the growth parameters and controlled the root rot fungi up to 90 days stored seed.

Chapter III

MATERIAL AND METHODS

The present investigation was carried out at the Department of Seed Science and Technology, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad, Andhra Pradesh in collaboration with Department of Plant Pathology, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad, Andhra Pradesh, India. Laboratory and glasshouse studies were conducted during 2012-13. The details of the material and methods are presented here under the following headings.

3.1 Collection of soybean seed samples

3.2 Isolation of seed mycoflora by different seed health testing methods

3.3 Seed quality studies

3.4 Seed transmission studies

3.5 Evaluation of seed treatments (fungicides, bioagents, botanicals and Irradiations) against predominant seedborne fungi under glasshouse conditions

3.1 COLLECTION OF SOYBEAN SEED SAMPLES

One hundred and twenty soybean seed samples (120) were collected from the major soybean growing districts of Andhra Pradesh *viz.*, Nizamabad (60 Nos) and Adilabad (60 Nos) for assessment of seed mycoflora and seed quality during 2012 – 13. The collected seed samples were shade dried and stored in paper bags at ambient storage temperatures of 28 $\pm$ 2 ^{0}C for further studies.

Table 3.1. Collection of seed samples from different districts of Andhra Pradesh.

S.No	Name of the District	Name of the Mandal	Number of samples
1.	**Nizamabad**	Kammarpalli	10
		Morthad	10
		Kotagiri	10
		Armur	10
		Bhodhan	10
		Ditchpally	10
2.	**Adilabad**	Muthol	10
		Thalamadugu	10
		Thanur	10
		Kubeer	10
		Nirmal	10
		Ichoda	10
		Total number of samples	120

3.2 ISOLATION OF SEED MYCOFLORA BY DIFFERENT SEED HEALTH TESTING METHODS

Four different seed health testing methods *viz.,* standard blotter method, 2, 4-D blotter paper method, deep freeze blotter method and agar plate method (ISTA, 1996) were employed for estimation of seed mycoflora associated with soybean seed samples. Seeds were surface sterilized with 0.1 per cent sodium hypochlorite solution for 1min. The seed were washed in three times of sterile water and placed in dry blotter paper to remove the excess moisture. Four hundred seeds were tested in different detection methods.

3.2.1 Standard blotter method (ISTA, 1996)

Sterilized blotter paper discs of 9 cm diameter were placed in sterile Petri plates (9 cm diameter) and moistened with sterile distilled water. The excess water was drained off from the plates. Soybean seeds were transferred to the plates containing the moist blotter paper. Ten seeds per plate were placed at equidistance in a circle. Four hundred seeds from each sample were placed in the plates in four replications. The plates were incubated at 25 ± 2^0C for seven days under alternate cycles of 12 h light and 12 h darkness for 7 days in BOD incubator. The plates were examined under stereo binocular microscope on 7^{th} day and total number of fungal colonies were counted and expressed in percentage (%).

The mycoflora associated with seed were further isolated, purified and identified. The per cent incidence of each fungus associated with the seed was recorded. The fungal colonies picked up from the seed were counted and expressed in percentage and pure cultures were maintained on agar slants. The various fungal cultures obtained were identified by observing their growth character on the slides under compound microscope by using standard key as described by Barnett (2003), Clements and Shear (1931).

3.2.2 2, 4 - D blotter paper method

Four hundred seeds were placed at the rate of 10 seeds per Petri plate with moistened blotter paper dipped in 0.2 per cent of sodium salt solution of 2,4 – dichloro phenoxy acetic acid. The Petri plates were incubated as described under standard blotter method. After eight days of incubation, the fungal growth on seeds was examined by using stereo-binocular microscope (Khare, 1996).

3.2.3 Deep freeze blotter paper method

This method was developed by Limonard (1968) to detect slow growing pathogens. Four hundred seeds were placed at the rate of 10 seeds per plate on moistened blotters in the way as described under standard blotter method. The Petri plates were incubated at 20± 2^{o}C for 24 h under alternate cycles of 12 h NUV light and darkness, for next 24 hours the plates were incubated at –20^{o}C in darkeness then kept back under original conditions for next five days. After eight days of incubation, the seed were examined under stereo-binocular microscope (Khare, 1996).

3.2.4 Agar Plate Method (ISTA, 1996)

PDA medium was prepared by using the following components for isolation of the mycoflora in the laboratory.

Potato Dextrose Agar (PDA)	
Potato	200 g
Dextrose	20 g
Agar	20 g
Water	1000 ml
pH	6.8

Peeled potato pieces were boiled in 500 ml distilled water in a 1000 ml beaker till the pieces get softened and the extract was collected in a beaker by sieving through a double layered muslin cloth. Agar – agar (20g) was melted in another 500 ml of distilled water in 1000 ml beaker into which 20g dextrose was added. The final volume of the medium was made up to 1000 ml by adding sterile distilled water. The pH of the medium was adjusted to 6.8 with 0.1 NaOH or 0.1 N HCl as the case may be with the pH meter. The medium was distributed to culture tubes and conical flasks at 8.0 ml and 100 ml each, respectively. The medium was sterilized in an autoclave at 15 Psi for 15 minutes. About 20 ml of the medium was distributed to each of the sterile petri plate under aseptic conditions. Soybean seed were transferred to the plates containing PDA medium. Ten seeds per plate were placed at equidistance in a circle. Four hundred seeds from each sample were placed in the plates in four replications. The Petri plates were incubated at 25 ± 2^{0}C in the incubator for 7 days and observed every day for the growth of fungi. Small quantity of streptomycin sulphate was added in each plate for the suppression of bacterial pathogens. The characteristic features of the isolated seed borne

fungi were tallied with the description given for identification (Ellis, 1976). The total fungal colonies were calculated and per cent infection was assessed.

$$\text{Total fungal colonies (\%)} = \frac{\text{No of seeds colonized in each plate by a particular species}}{\text{Total no of seed in each plate}} \times 100$$

3.3 SEED QUALITY STUDIES

3.3.1 Moisture content (%)

Moisture content of the seed was determined as per (ISTA, 1996). Five grams of seed was weighed and placed in aluminum cups. The seed material kept in aluminum cups was dried in hot air oven maintained at 103^{o}C temperature for 17 hours. The moisture content was determined on dry weight basis by using the following formulae.

Moisture content (%) = $(W_2\text{-}W_3/W_2\text{-}W_1)$ X 100

Where, W_1 - Weight of empty container with its cover (g)

W_2 - Weight of container with its cover and seeds before drying (g)

W_3 - Weight of container with its cover and seeds after drying (g)

3.3.2 Germination percentage (Rolled paper towel method)

Four replications of 100 seeds from each seed sample were randomly counted and placed on the germination paper at uniform spacing between the seeds in rows. The rolled paper towels with seeds are placed vertically in a cabinet of seed germinator by maintaining a constant temperature of 25 $\pm$ 1^{0} C and a relative humidity of 95 $\pm$ 2 per cent. The germination percentage was recorded on the 8th day based on normal seedlings. The germination per cent was calculated as per the following formulae.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

3.3.2 Root and shoot length (cm)

Ten normal seedlings selected at random from each of the replication from germination test were carefully removed on the 8th day (final count) and used for measuring shoot and root length. The shoot length was measured from the cotyledonary node to the tip of the apical bud. The root length was measured from the cotyledonary node to tip of the primary root. The mean root and shoot lengths were expressed in centimeters.

3.3.3 Dry weight of seedlings

Ten normal seedlings after measurement of root and shoot length were kept in butter paper and dried in a hot-air oven maintained at 80^0C temperature for 24 h. Later they were removed and allowed to cool in a desiccator for 30 min before weighing in an electronic balance. The dry weight of the seedlings was recorded and expressed in milligrams.

3.3.4 Seed Vigour Index (SVI-I)

The seeds were tested for germination using rolled paper towel method. The germination percentage was recorded on 8^{th} day. In each sample, 10 seedlings were taken separately for measuring seedling length in cm. The shoot length was measured from the cotyledonary node to the tip of the apical bud. The root length was measured from the cotyledonary node to tip of the primary root. The mean root and shoot lengths were expressed in centimeters.

The seedling vigour index was calculated as per the formula suggested by Abdul Baki and Anderson (1973).

Seedling vigour index (SVI - I) = Total seedling length (cm) x germination (%).

3.3.5 Seed Vigour Index (SVI-II)

The seedling vigour index II was calculated by taking average dry weight of 10 seedlings multiplied by germination percentage. Ten normal seedlings after measurement of root and shoot length were kept in butter paper and dried in hotair oven maintained at 80^0C temperature for 24 h. Later they were removed and allowed to cool in desiccators for 30 min before weighing in an electronic balance. The dry weight of the seedlings was recorded and expressed in milligrams.

Seedling vigour index (SVI-II) = Germination (%) x seedling dry weight (g)

3.3.6 Isolation of predominant seedborne fungi

M. phaseolina was found predominant among the seed mycoflora isolated from soybean seed samples by different detection methods. Pure cultures of seed borne *M. phaseolina* was maintained on PDA plates by regular sub culturing and used for further studies.

3.3.7 Pathogenicity test by seed inoculation method

Seeds of soybean (cv. JS - 335) were inoculated with spore suspensions of *M. phaseolina* @ 10^6 conidia per ml and sown in earthen pots each containing 5 kg sterilized soil. Five plants were raised for each pot with five replications. Equal number of untreated seeds sown in earthen pots served as control. Observations on seed germination, seed rot, seedling blight and symptoms on plants were recorded. Isolations were also made from the seeds that failed to germinate and from rotted seedlings to recover the fungus.

3.4 SEED TRANSMISSION STUDIES

3.4.1 Seed transmission of *M. phaseolina* under laboratory conditions

(Test tube water agar method)

Preparation of 2% water agar	
Distilled water	1000 ml
Agar	20 g

Twenty grams of agar was melted in 1000 ml of distilled water. The final volume was made up to 1 liter with distilled water and sterilized in an autoclave at 15 psi (121.6^0C) for 20 min. Fifteen ml of 2 % water agar was taken in each test tube and sterilized. Slanting was done at 60^o angle to provide required moisture and base for the seed to sprout and the fungus to develop. One hundred seeds each before and after sterilization with 2 % sodium hypochlorite solution for 1 min were transferred to the slants at the rate of one seed for each tube and incubated at 28 $\pm$ 1^oC. Healthy, artificially inoculated and naturally infected seeds of soybean (cv. JS – 335) with seedborne *M. phaseolina* was tested. The seed and seedlings were examined after 14 days for development of typical symptoms of the disease and per cent infection was assessed. Observations were taken when healthy control seedlings reach the rim of the tube and cotton plug were removed.

3.4.2 Seed transmission of *M. phaseolina* in pot culture under glasshouse conditions

Healthy, naturally infected and artificially inoculated seeds of soybean (cv. JS – 335) with seedborne *M. phaseolina* was sown in sterilized pots @ 5 seeds per pot in replicated trial. The data on per cent seed germination, seed rot (%) and seedling blight

(%) were recorded up to 35 days after sowing and per cent seed transmission in soybean due to *M. phaseolina* was calculated.

$$\text{Seedling mortality (\%)} = \frac{\text{Total number of dead seedlings}}{\text{Total number of germinated seedlings}} \times 100$$

3.5 EVALUATION OF SEED TREATMENTS WITH FUNGICIDES, BIOAGENTS, BOTANICALS AND IRRADIATIONS AGAINST SEED BORNE MACROPHOMINA PHASEOLINA UNDER GLASS HOUSE CONDITIONS

Seeds of soybean cv. JS – 335 were surface sterilized and artificially inoculated with *M. phaseolina* @ 10^6 conidia / ml. After 24h, seeds were again treated separately with thiram + carbendazim 1:1 @ 3 g kg^{-1}, vitavax power 200 @ 2.5 g kg^{-1}, *Trichoderma viride* @ 10 g kg^{-1}, *Pseudomonas fluorescens* @10 g kg^{-1} seed, NSKE (neem seed kernel extract) @ 5%, neem leaf powder @ 5g kg^{-1}seed, Irradiation dose 1.5 k Gy, Irradiation dose 2.5 k Gy along with untreated and pathogen treated seeds. Seeds after imposition of seed treatments were sown in sterilized soil filled in earthen pots containing 5kg soil @ five seeds per pot in replicated trial adopting CRD design under controlled glasshouse conditions. Observations were recorded on germination percentage, seed rot and seedling blight at 35 days after sowing. The details of the experiment were provided in Table 3.2

Table 3.2. Details of seed treatments in glasshouse experiment

T_1	Seed treatment with *Trichoderma viride* @ 10 g kg^{-1} seed
T_2	Seed treatment with *Pseudomonas fluorescens* @10 g kg^{-1} seed
T_3	Seed treatment with Irradiation dose 1.5 k Gy
T_4	Seed treatment with Irradiation dose 2.5 k Gy
T_5	Seed treatment with NSKE (neem seed kernel extract) @ 5%.
T_6	Seed treatment with neem leaf powder @ 5g kg^{-1}seed
T_7	Seed treatment with thiram + carbendazim 1:1 @ 3 g kg^{-1}
T_8	Vitavax power 200 @ 2.5 g kg^{-1}
T_9	Treated seeds (Pathogen)
T_{10}	Untreated seeds.

Note: Seeds were prior inoculated with *M. phaseolina* @ 10^6 conidia/ ml before imposition of seed treatments from T_1 to T_9 except T_{10} treatment.

3.6 STATISTICAL ANALYSIS

The data were statistically analyzed by using Completely Randomized Design (CRD) as suggested by Gomez and Gomez (1984). The data pertaining to percentages were angular transformed wherever necessary.

Chapter IV

RESULTS AND DISCUSSION

The results of the experiments conducted in the present investigation are presented here under the following headings

4.1 DETECTION OF SEED MYCOFLORA ASSOCIATED WITH SOYBEAN SEED SAMPLES

A total of 120 seed samples of soybean (cv. JS – 335) were collected from two major soybean growing districts of Andhra Pradesh *viz*., Nizamabad district (60 Nos) comprising of Kammarpalli, Morthad, Kotagiri, Armur, Bhodhan and Ditchpally Mandals and Muthol, Thalamadugu, Thanur, Kubeer, Nirmal and Ichoda Mandals of Adilabad district (60 Nos) during *kharif*, 2012 - 2013. The seed samples were analysed for seed health as per (ISTA, 1996) by standard blotter paper method, 2, 4 - D blotter paper method, deep freeze blotter paper method and agar plate methods. Seed mycoflora associated with soybean seed samples were isolated and identified.

4.1.1 Standard blotter method

Mycoflora associated with soybean seed samples collected from Nizamabad and Adilabad districts were detected following the standard blotter method (Table 4.1). Significant differences in occurrence of seed mycoflora in different districts of Andhra Pradesh were observed. The results indicated that irrespective of the locations and sources, a total of nine fungal species belonging to eight genera were detected from all the seed samples tested. Nine fungi *viz*., *Macrophomina phaseolina, Colletotrichum dematium, Aspergillus flavus*, *Aspergillus niger, Rhizopus*, *Curvularia* sp. *Alternaria*, *Cladosporium* and *Fusarium* sp. were observed. Total per cent occurrence of seed mycoflora in Nizamabad and Adilabad districts ranged from 30 % to 49.2 % and 23.6 % to 45.0 %, respectively. With respect to the mean total fungal colonies, seed samples collected from Ditchpally Mandal (49.2 %) of Nizamabad district and Kubeer Mandal (45.0 %) of Adilabad district recorded more total number of fungal colonies followed by Kotagiri Mandal (46.5 %) and Ichoda Mandal (41.7 %) of Nizamabad and Adilabad districts. Whereas least number of total fungal colonies were observed in Armur Mandal (30 %) of Nizamabad district and Muthol Mandal (23.6 %) of Adilabad district.

Out of nine fungal species recorded, the occurrence of *M. phaseolina* was found predominant in the seed samples analysed from two districts (8.5 to 28.5 %). The occurrence of *M. phaseolina* was found highest in the seed samples of Ditchpally

Mandal of Nizamabad (28.5 %) and Kubeer Mandal of Adilabad district (16.5 %). It was found to be the most predominant fungus followed by the *C. dematium* (2 % - 6.2%) in both the districts. While the occurrence of pod and seed rot fungi *Cladosporium* sp. ranged from 0.5 to 2.3 %. The occurrence of pathogenic fungi like *A. alternata* (1.5 to 4%), *Fusarium* sp. (2 to 5.3%) and *Curvularia* (1 to 4%) were observed in the samples analysed from both the districts, respectively. Where as storage fungi *viz*., *A. flavus, A. niger* and *Rhizopus* with a range of 1.0 % to 3.4 %, 0.5 % to 2.4 %, 2.3 % to 4 % were observed.

The differences in occurrence of seed mycoflora in soybean seed samples collected from different districts may be attributed to the variations in moisture content of the seed and storage conditions (Temperature, Relative humidity and Light) adopted by the farmers. Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. Seed mycoflora was highest in the seed samples of Ditchpally Mandal of Nizamabad district (49.2 %) while it was least in Muthol Mandal of Adilabad district (45 %). Storage fungi like *A.niger* and *Rhizopus* were not observed in Muthol Mandal of Adilabad district. The per cent incidence of the individual fungi ranged from 1.7 % to 52.9 %. Seed mycoflora *viz*., *M. phaseolina, C. dematium, A. flavus, A. niger, Rhizopus, Curvularia* sp, *Alternaria, Cladosporium* and *Fusarium* sp. were recorded in the soybean seed samples indicated their seedborne nature.

The present findings are in conformity with the earlier findings of Shovan *et al*. (2008) who reported that blotter method was found effective for detection of seedborne fungi in soybean and observed *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp. and *Rhizopus stolonifer* in soybean. Most of the fungal species detected in the present study was reported earlier in soybean by Gowde *et al.* (1987), Solanke *et al*. (1997), Goulart *et al*. (1997), Arya *et al*. (2004), Dawar *et al*. (2007), Afzal *et al*. (2010) and Ramesh *et al*. (2013) who reported seedborne fungi varied from one locality to another in the seed samples of soybean.

4.1.2 2, 4 - D blotter method

One hundred and twenty soybean seed samples collected from two major soybean growing districts *viz.*, Nizamabad and Adilabad were analysed following 2, 4 - D blotter method (Table 4.2). Significant variations in the occurrence of seed mycoflora in different locations of A.P were observed. The results indicated that irrespective of the

locations and sources, a total of nine fungal species belonging to eight genera were detected *viz., Macrophomina phaseolina, Colletotrichum dematium, Aspergillus flavus, Aspergillus niger, Rhizopus, Curvularia* sp, *Alternaria, Cladosporium* and *Fusarium* sp. were observed in the soybean seed samples. The fungal flora were similar as to that of blotter method but frequency of fungal flora were less in 2, 4 – D blotter method.

Total per cent occurrence of seed mycoflora in Nizamabad and Adilabad districts ranged from 14.8 % to 28.1% and 11.6 % to 22.1%, respectively. With respect to the mean total fungal colonies, Ditchpally (28.1 %) and Bhodan (24.8 %) Mandals of Nizamabad district and Kubeer (22.1%), Nirmal Mandals (18.6%) of Adilabad district recorded more total number of fungal colonies, where as least number of fungal colonies were observed in Armur Mandal (14.8%) of Nizamabad and Muthol Mandal (11.6 %) of Adilabad district. Out of nine fungal species recorded, the occurrence of *M. phaseolina* was found predominant (4 - 9.5 %) which was followed by *Colletotrichum* (3.3 - 9 %) in both the districts. The least occurrence of *A. flavus* (0.5 to 2.5%) and *Cladosporium* sp. (0.3 to 0.5%) were recorded in soybean seed samples analysed from Nizamabad and Adilabad districts.

Colonies of *A. flavus* and *Cladosporium* were not observed in Kammarpalli Mandal of Nizamabad district and Muthol Mandal of Adilabad district. Colonies of *Cladosporium* were not observed in Armur Mandal of Nizamabad district and Thanur Mandal of Adilabad district, Where as *Rhizopus* was not observed in Kotagiri Mandal of Nizamabad district. The pathogenic fungi like *Colletotrichum* (3.3 % to 9.0 %), *A. alternata* (0.5 % to 2. 5%), *Fusarium* sp (1 to 3.3%), *Curvularia* (1% to 2.5%) and *Cladosporium* (0.3 % to 1.5 %) were observed in the soybean seed samples of the two districts. Where as storage fungi *viz., A. flavus, A. niger, Rhizopus* with a range of (0.4 % to 1%), (0.5 % to 1.1%) and (0.5 % to 1.3 %) were observed. The present study indicated differential occurrence of seedborne fungi in soybean. Similar findings were also reported earlier by Rajeswari and Meena Kumari (2012) in soybean.

4.1.3 Deep freeze blotter method

Significant variation in occurrence of seed mycoflora was observed (Table 4.3). The results indicated that irrespective of the locations and sources, a total of 9 fungal species belonging to eight genera were detected from all the seed samples tested. Nine fungi *viz., Macrophomina phaseolina, Colletotrichum dematium, Aspergillus flavus, Aspergillus niger, Rhizopus, Curvularia* sp, *Alternaria, Cladosporium* and *Fusarium* sp. were observed in soybean seed samples. Fungal flora were similar but frequency of

occurrence in different detection methods was different. The per cent occurrence of fungal flora was less in deep freeze blotter method compared to blotter and 2, 4 - D blotter methods. Total per cent occurrence of seed mycoflora in Nizamabad and Adilabad districts ranged from 11.8 % to 19.3% and 9.5% to 16.2%, respectively.

With respect to mean total fungal colonies, Ditchpally and Morthad Mandals of Nizamabad district (19.3 % and 15.7%) and Kubeer (16.2 %) and Ichoda Mandals (12.3%) of Adilabad district recorded more total number of fungal colonies. Where as least number of fungal colonies were observed in Bhodhan (11.8 %) Mandal and Muthol (9.5 %) Mandals of Nizamabad and Adilabad districts. Out of nine fungal species recorded, the occurrence of *M. phaseolina* was found predominant (3 to 6.5 %) which was followed by *Colletotrichum* sp. in both the districts (1.2 to 3.5 %) while least occurrence of *Curvularia* (0.5 to 2%) and *Cladosporium* sp. (0.3 to 2.5%) were recorded.

Colonies of *A.niger* in Kammarpalli Mandal and colonies of *A alternata* and *Curvularia* were not observed in Morthad and Armur Mandals of Nizamabad district. Where as *A. niger,Cladosporium* were not observed in Nirmal and Ichoda Mandal of Adilabad district. The occurrence of pathogenic fungi like *Colletotrichum* sp. (1.2 to 3.5%), *A. alternata* (0.5 to 2.5 %), *Fusarium* sp. (1 to 2.6 %), *Curvularia* (0.5 to 2%) and *Cladosporium* (0.3 to 2.5%) were observed. Where as storage fungi *viz., A. flavus, A. niger, Rhizopus* with a range of 0.5 to 2.5%, 1 to 2% and 0.8 to 1.5% were observed. The present study revealed that occurrence of seed mycoflora may varied depending upon the location and source of seed samples collected from different farmers.

The present findings are in conformity with earlier reports of Tennee *et al*. (1974) who reported variation in the occurrence of seed mycoflora according to geographic location in soybean and Rajeswari *et al*. 2010 in safflower. The present study also indicated that deep freeze blotter method was found suitable for detection of *C. dematium* in soybean. The results are agreement with Neergaard (1977), Renukeswarappa and Shethana (1985) and Bhale *et al.* (2000) who observed that deep freeze blotter method was found superior to standard blotter method for detection of *C. dematium* in soybean. While Rajeswari and Meena Kumari (2010) reported that deep freeze blotter was suitable for detection of *M. phaseolina, C. dematium* and *Fusarium oxysporium* in infected soybean seed samples. The present study also revealed that deep freeze blotter method yielded less number of fungal species over blotter method. The present findings are in conformity with the earlier findings of Nasreen (2003).

4.1.4 Agar plate method

Significant differences in occurrence of seed mycoflora in different Mandals of Nizamabad and Adilabad districts of Andhra Pradesh were observed in agar plate method (Table 4.4). The results indicated that irrespective of the locations and sources, a total of nine fungal species belonging to eight genera were detected from all the seed samples tested. Nine fungi viz., *Macrophomina phaseolina, Colletotrichum dematium*, *Aspergillus flavus, Aspergillus niger, Rhizopus* sp, *Curvularia* sp, *Alternaria*, *Cladosporium* and *Fusarium* sp. were observed in soybean seed samples.

The fungal flora were similar as like in blotter, 2, 4 - D blotter and deep freeze blotter methods. Total per cent occurrence of seed mycoflora in Nizamabad and Adilabad districts ranged from 13.1 % to 37% and 15.4 % to 26.4 %, respectively. With respect to the mean total fungal colonies, Kotagiri (37 %) and Ditchpally (35.6%) Mandals of Nizamabad district and Ichoda (26.4 %) and Kuber Mandals (18.5%) of Adilabad district recorded more total number of fungal colonies. Where as least number of fungal colonies were observed in Armur (13.1%) and Muthol (15.4%) Mandals of Nizamabad and Adilabad districts. Out of nine fungal species recorded, the occurrence of *M. phaseolina* was found predominant (3.5% - 14 %) which was followed by *Fusarium* sp. in both the districts (2.5 % - 7.5 %). While least occurrence of *A. niger* (1 to 2 %) and *Cladosporium* sp. (0.5 to 1%) were recorded in seed samples of Nizamabad and Adilabad districts. Colonies of *A. niger* and *A. alternata* were not observed in Morthad and Armur Mandals of Nizamabad district. Where as *A. flavus* was not observed in Muthol Mandal of Adilabad district.

The occurrence of pathogenic fungi like *Colletotrichum* (1.2 to 3.5 %), *A. alternata* (1 to 3.6%), *Curvularia* (0.5 to 4.6 %) and *Cladosporium* (0.5 to 2.5 %) were observed in soybean seed samples of the two districts. Where as storage fungi *viz., A. flavus, A. niger* and *Rhizopus* with a range of (0 to 2 %), (0 to 2 %) and (0.5 to 3 %) were observed. The results revealed that per cent occurrence of seedborne fungi were found less in compared to blotter method where as the occurrence of seedborne fungi was more in this method as compared to 2, 4 - D blotter and deep freeze blotter method. Similar results were confirmed by Neergaard (1977), Kushe and Khare (1978), Ellis (1979), Solanke *et al*. (1997), Arya *et al*. (2004), Ahammed *et al*. (2006), Dawar *et al*. (2007) in chickpea and Ramesh *et al*. (2013) in soybean. The present study also indicated the predominant nature of *M. phaseolina* in soybean seeds. Michail *et al.*

(1981) also reported that PDA medium was preferable for isolation of *Macrophomina phaseolina* causing charcoal root rot of soybean.

4.1.5 Evaluation of different seed health detection methods

Four seed health testing methods *viz.,* blotter method, 2, 4 - D blotter, deep freeze blotter and agar plate methods were compared to know the efficacy of different detection methods.

The results (Table 4.5) indicated that among the four methods employed for detection of seed mycoflora, standard blotter method was found superior and recorded maximum total fungal colonies (40.9 % and 35.7 %), followed by agar plate method (26.4 % and 20.7 %), 2, 4 - D blotter (22.3 % and 16.2 %) and deep freeze blotter (14.4 % and 11.9 %). Where as mean total fungal colonies were found high in Nizamabad district (17.7 to 33%) followed by Adilabad district (18 to 26.4%) in four detection methods (Fig. 4.1). The results are in conformity with Rajeswari *et al*. (2009 and 2010) who reported that differences in seed mycoflora in different castor and safflower seed samples collected from different districts of Andhra Pradesh. Similar kind of variations in occurrence of seed mycoflora in different detection methods were also reported by several workers Tariq *et al*. (2005) in soybean, Dawar *et al.* (2007) detected more number of seed mycoflora in chickpea seed samples by blotter method followed by agar plate and deep freeze blotter method. The efficacy of blotter method was proved superior by various workers. Neergaard (1977), Kushe and Khare (1978), Ellis (1979), Rauf (2000), Arya *et al.* (2004) Singh *et al.* (2005) in chick pea and Dawar *et al*. (2007) and Shovan *et al.* (2008). On the contrary some of the workers reported that agar plate method was found superior in isolation of more number of fungal colonies over blotter method in soybean. (Gill *et al.,* 1983, Solanke *et al.,* 1997 and Godika *et al*., 1999).

Among the different detection methods, standard blotter method was found superior in the recovery of *M. phaseolina* in soybean seeds (Fig 4.2 & 4.3). The predominant nature of *M. phaseolina* in soybean seeds was detected earlier by Bhuiyan and Fakir (1982), Hartman *et al*. (1999), Kumar and Singh (2000), Arya *et al.* (2004), Shovan *et al.* (2008), Ramesh *et al.* (2013) who reported the seed borne nature of *M. phaseolina* in soybean.

4.2 SEED QUALITY STUDIES

4.2.1 Germination (%)

Significant differences in germination was observed in the soybean seed samples analysed from different Mandals of Nizamabad district. Irrespective of locations and sources, germination ranged from 65.3 % to 75 %. The per cent germination was below minimum seed certification standard (< 70 %) in the seed samples collected from Ditchpalli and Kotagiri Mandals of Nizamabad district Table 4.8. The per cent germination was highest in the seed samples of Armur Mandal (75 %) and it was least in Ditchpally of Nizamabad district (65.3%) (Fig. 4.4). Soybean seed samples which recorded significantly higher germination in the Mandals of Bhodhan, Kammarpalli, Morthad and Armur (> 70%) were also recorded less total fungal colonies (17.7%). In the remaining Mandals of Nizamabad district, the germination percentage was declined (65.3%) with increased total number of fungal colonies (33%)

Significant differences in germination was observed in the soybean seed samples collected from different Mandals of Adilabad district. The germination percentage ranged from 67 % to 78.3% Table 4. 9. The germination was below minimum seed certification standard (< 70%) in the seed samples of Kubeer Mandal of Adilabad district. Where as in the remaining Mandals of Adilabad district (Ichoda, Thalamadugu,Nirmal, Thanur and Muthol) recorded germination above minimum seed certification standard (>70%). The per cent germination was highest in Muthol Mandal (78.3 %) and it was least in Kubeer Mandal of Adilabad district (67%) (Fig. 4.5) Soybean samples with highest seed germination (78.3 %) recorded less total number of fungal colonies (15 %). With increased number of mean total fungal colonies (26.8 %) resulted in decreased seed germination (67 %). Mean germination was found less in samples analysed from different Mandals of Nizamabad district over samples of Adilabad district.

4.2.2 Seedling vigour index (SVI - I) (on seedling length basis)

Significant differences in seedling vigour index on length basis (SVI - I) were observed in the seed samples analysed from different Mandals of Nizamabad district. Irrespective of locations and source of collection, seedling vigour index - I decreased (1394) with increased number of total fungal colonies (33%). Among six Mandals, seed samples from Ditchpally Mandal of Nizamabad district recorded less mean seedling

vigour - I (1394) where as highest seedling vigour was recorded in the seed samples of Armur Mandal (2013) followed by Morthad Mandal (1861) of Nizamabad district. Seed samples analysed in the remaining Mandals of Nizamabad district *viz*., Kotagiri, Bhodhan and Kammarpalli, recorded seedling SVI - I with a range of 1514 to 1733 (Table 4.8).

Significant differences were also observed in SVI - I in the seed samples analysed from different Mandals of Adilabad district. Irrespective of locations and source of collection, SVI - I gradually decreased (1470) with increased number of total fungal colonies (26.8 %). Among the Mandals, seed samples analysed from Kubeer Mandal of Adilabad district recorded less mean seedling vigour - I (1470) where as highest seedling vigour was recorded in the seed samples of Muthol Mandal (2194) followed by Thanur Mandal (2032). In the remaining Mandals of Adilabad districts (Ichoda, Thalamadugu and Nirmal) recorded seedling vigour – I with a range of 1596 to 1859 (Table 4.9).

4.2.3 Seedling vigour index (SVI-II) (on seedling dry weight basis)

Significant differences in seedling vigour index on seedling dry weight basis (SVI-II) were observed in the seed samples analysed from different Mandals of Nizamabad district. Irrespective of seed samples analysed from different Mandals of Nizamabad district, SVI-II - II (on seedling dry weight basis) was gradually decreased (57) with increased total number of fungal colonies (33%). Among the Mandals, seed samples collected from Ditchpally Mandal of Nizamabad district recorded low mean seedling vigour – II (57) where as high seedling vigour was recorded in the seed samples of Armur Mandal (85) which was followed by Morthad Mandal (75.5) of Nizamabad district. Seed samples in the other Mandals of Nizamabad district *i.e.*, Kotagiri, Bhodhan and Kammarpalli recorded SVI-II - II with a range of 61.2 – 69 (Table 4.8).

Significant differences in Seedling vigour index (SVI-II) were observed in the seed samples analysed from different Mandals of Adilabad district. Irrespective of seed samples analysed from different Mandals of Adilabad district, seedling vigour index - II gradually decreased (65.2) with increased number of total fungal colonies (26.8%) Table (4.9). Among the Mandals, seed samples collected from Kubeer Mandal of Adilabad district recorded less mean seedling vigour - II (65.2) where as higher seedling vigour was recorded in the seed samples of Muthol Mandal (104.5) followed by Thanur Mandal (88.7) of Adilabad district. Seed samples collected from other Mandals of

Adilabad district *i.e.,* recorded Seedling vigour - II was ranged from 67.9 to 84.2 in other Mandals (Ichoda, Thalamadugu and Nirmal) of Adilabad district. Mean Seedling vigour index (on seedling dry weight basis) was found maximum in the seed samples analysed from Adilabad district (82.2) over seed samples of Nizamabad district (68.8). Decreased germination, seedling vigour I and seedling vigour II was observed in seed samples of Nizamabad and Adilabad districts with an increased total number of fungal colonies. Soybean seed samples with highest fungal flora were also recorded less germination and seedling vigour. Similar variation in germination and seedling vigour was reported earlier by Meena Kumari *et al*. (2002), Sunil kumar (2004) in soybean, Shovan *et al.* (2008), Rajeswari *et al*. (2012) in safflower and Ramesh *et al*. (2013) who reported that seedborne fungi associated with soybean seeds reduced seed germination, seedling vigour and other seed quality parameters.

4.2.4 Seed rot and seedling blight

Significant differences in seed rot and seedling blight were observed. Seed rot and seedling blight was high in Ditchpally Mandal of Nizamabad district (19.2 % and 14.1 %). Where as less seed rot and seedling blight were recorded in Armur Mandal of Nizamabad district (14.2 % and 8.8 %). Seed samples analysed from other Mandals recorded seed rot and seedling blight varied from 15% to 18.5 % and 9.3 % to 12.3 %, respectively (Table 4.8).

Significant differences in seed rot and seedling blight were also observed in the seed samples analysed from Adilabad district. Seed rot and seedling blight were found high in Kubeer Mandal of Adilabad district (17.3 % and 12.5 %), respectively. where as less seed rot and seedling blight was recorded in Muthol Mandal of Adilabad district (12.2 % and 7 %) , respectively. Seed samples analysed from other Mandals recorded seed rot and seedling blight which varied from 13.7 % to 16 % and 8.8 % to 10 %, respectively (Table 4.9).

4.2.5 Seed moisture

Significant differences in moisture content was observed in the seed samples analysed from different locations of Nizamabad and Adilabad districts. Moisture content in the seed samples of Nizamabad and Adilabad districts ranged from 7.7 % to 9%. Moisture content was high (9 % and 8.4 %) in the samples collected from Ditchpally Mandal of Nizamabad district and Kubeer Mandal of Adilabad district, where an increased number of total fungal colonies (33.0 % and 26.8 %) was observed.

4.3 SEED TRANSMISSION STUDIES

4.3.1 Isolation of the pathogen

Different seed health testing methods *viz.,* standard blotter paper method, 2, 4 - D blotter paper method, deep freeze blotter paper method and agar plate method were employed to detect the predominant seedborne fungi from soybean seed samples. Among the seedborne fungi, *M. phaseolina* was found predominant and it was used as test pathogen for further studies. The pathogen appeared as greyish mycelial growth on incubated soybean seeds in different detection methods.

4.3.2 Pathogenicity Studies

4.3.2.1 Seed Inoculation of *Macrophomina phaseolina*

Seeds of soybean (cv. JS – 335) were artificially inoculated with conidial suspension (10^6 conidia ml^{-1}) of *M. phaseolina* exhibited the symptoms of seed rot and seedling blight. First sign of symptoms were noticed in the form of seed rot (5 days) and seedling blights and charcoal rot 15 days after sowing. More than 90 to 95 % seedling mortality was observed. Control pots kept with healthy seeds in sterilized soil in isolation did not exhibit any symptoms up to 15 days after sowing. Similar observation was made by Kunwar *et al*. (1986) and Arya *et al*. (2004) who reported that similar type of symptoms on soybean seedlings inoculated with *M. phaseolina*. It was also confirmed earlier by Raut (1985) in sunflower seeds due to *M. phaseolina*.

4.3.2.2 Re isolation of the pathogen

The pathogen was re isolated from the infected seeds and seedlings and compared with the original isolate and confirmed as *M. phaseolina.*

4.3.3 Seed transmission studies

Seed transmission studies were carried out by test tube water method (seedling symptom test) under *in vitro* conditions and also in pot culture under glasshouse conditions.

4.3.3.1 Seedling symptom test (TWA)

Seeds of soybean cv. JS - 335 were tested by test tube water agar method under laboratory conditions. The results indicated that artificially inoculated soybean seeds with *M. phaseolina* showed reduction in seed germination (55 %) and increased in seed

rot and seedling blight (18 % and 20.5 %), respectively Table 4.10. Whereas naturally infected soybean seed samples recorded germination of 59.3 %, seed rot of 15.5 % and seedling blight of 16.5 %. On the contrary, apparently healthy seed samples recorded high germination (75 %), less seed rot (2.5 %) and seedling blight (3.5 %) (Fig. 4.6). The germinated seedlings from the infected seed sample exhibited the symptoms of seed rot, seedling blights, discolouration of roots and production of spots on cotyledons and true leaves after 15 days of incubation. Similar findings were reported earlier by Arya *et al*. (2004) in soybean and Raut (1985) in sunflower.

4.3.3.2 Seed transmission studies in pot culture under glasshouse conditions

The results indicated (Table 4.11) that artificially inoculated seeds of soybean cv. JS - 335 with seedborne *M. phaseolina* exhibited reduction in seed germination (46 %) and increased seed rot and seedling mortality (25.8 % and 23.2 %), respectively. Whereas naturally infected soybean seed samples recorded germination (50.5 %), seed rot (24 %) and seedling mortality (19.1 %). On the contrary apparently healthy seed samples recorded high germination (72 %), less seed rot (4.2 %) and seedling blight (3.8 %), respectively (Fig. 4.7) Seedlings raised from naturally infected and artificially inoculated seeds recorded seed rot and seedling blights and discoloration of basal stem at the above soil level with the numerous production of sclerotial bodies on the stem surface. The present findings are inconformity with the earlier findings of Arya *et al*. (2004) in soybean who reported that *M. phaseolina* transmits from seed to seedling in a systemic manner. The germination in naturally infected soybean seeds was 52 % and 45%, respectively as against 75 % and 72 % germination in healthy seeds under laboratory and glasshouse conditions, respectively. In naturally infected seeds, 30 % and 25 % seed rot, 18 % and 8 % seedling mortality were recorded in laboratory and glasshouse conditions, respectively. Similar findings was reported earlier by Kunwar *et al*. (1986) in sunflower, Anwar *et al*. (1995) and Mandhare *et al*. (2009) who reported that *M. phaseolina* causing charcoal rot in soybean transmits from infected seed to seedlings.

4.4 MANAGEMENT OF SEEDBORNE MACROPHOMINA PHASEOLINA UNDER GLASSHOUSE CONDITIONS USING SEED TREATMENTS

The results revealed that significant differences in different seed treatments were observed as compared to the untreated and pathogen treated seeds (Table 4.12). The germination percentage was significantly improved in all the treatments as compared to

the untreated seeds. Among the seed treatments, soybean seeds treated with thiram + carbendazim @ 3 g kg^{-1} recorded higher seed germination (91 %) followed by seed treatment with vitavax power 200 @ 2.5 g kg^{-1} (89 %) and it was found on par with seeds treated with *T. viride* @10 g kg^{-1} (88 %).The other seed treatments, *Pseudomonas fluorescens* 10 g kg^{-1} seed (84 %), botanicals NSKE (Neem Seed Kernel Extract) @ 5% (82 %), neem leaf powder @ 5g kg^{-1} (80 %), irradiation 2.5 k Gy (78 %), irradiation 1.5 k Gy (77 %) were also found effective in increasing seed germination over untreated seeds (74 %) and pathogen treated seeds (60 %) (Fig. 4.8)

Significant differences in seed rot and seedling blight in soybean due to different seed treatments were observed when compared with untreated seeds (20.7 % and 15 %) and pathogen treated seeds (26.3% and 25.7%) Table 4.12. However, seed treatment with thiram + carbendazim 1:1 @ 3g kg^{-1} resulted in maximum reduction of seed rot and seedling blight (5.7 % and 4 %) followed by seed treatment with vitavax power 200 @ 2.5 g kg^{-1} (6.7 % and 5.2 %) which was found on par with bioagents like *T. viride* @10 g kg^{-1} in reduction of seed rot and seedling blight (7.3% and 6.3%). The other seed treatments *viz., Pseudomonas fluorescens* 10 g kg^{-1} (9% and 8%), NSKE (Neem Seed Kernel Extract) 5% (12.3 % and 10.3 %) neem leaf powder @ 5g kg^{-1} seed (13 % and 11 %), irradiation 2.5 k Gy (14.7 % and 12.3 %) and irradiation 1.5 k Gy (15 % and 14 %) also recorded less seed rot and seedling blight over untreated seeds (20.7 % and 15 %) and were pathogen treated seeds (26.3% and 25.7%) (Fig. 4.9 & 4.10). The beneficial effect of seed treatments with bioagents and fungicides in minimizing the pre emergence mortality is in accordance with Sunil kumar (2004) in soybean.

Rajeswari and Meena Kumari (2009) also reported that significant increase in germination (91 %), seed vigour (2630) in seed treated with thiram + carbendazim followed by *T. viride* over untreated seeds in soybean cv. JS - 335. Similar findings were reported by Agrawal and Sushma (1989) and Solanke *et al.* (1997). Devakumar and Usha Dev (2010) who reported that causal agent of charcoal rot, seed rot, pre and post emergence damping off due to *M. phaseolina* was reduced by neem 5 EC and azadirachtin seed treatments. Ikram *et al*. (2010) and Raju (2012) reported that infection of *M. phaseolina, R. solani* and *Fusarium* sp. significantly decreased in greengram seeds treated with gamma rays and increased plant biometrics.

FUTURELINE OF WORK

Future research work should be focused in the following areas:

- More number of seed isolates of *M. phaseolina* could be collected from different locations representing all the soybean growing tracts of Andhra Pradesh.
- Efficacy of new seed dressing chemicals has to be tested to restrict the seed borne nature of the pathogen.
- Histopathological studies on location of the pathogen in different parts of the seed have to be carried out.
- Effect of native biocontrol agents / botanicals and low dosage of fungicides has to be tested as seed treatments against seed borne pathogens.
- For obtaining the reproducible results, studies on detection and management of seed borne fungi are to be conducted for the benefit of farmers and seed industry personnel.

Table 4.5. Detection of seed mycoflora by blotter, 2, 4- D blotter, deep freeze blotter and agar plate method methods

Name of the district	S.No	Name of the Mandal	Blotter method (%)	2,4 -D Blotter (%)	Deep freeze blotter (%)	Agar method (%)	Mean fungal colonies (%)
NIZAMABAD	1	Kammarpalli	41.4	20.2	14.3	26.2	25.5
	2	Morthad	39.2	22.4	15.7	16.6	23.4
	3	Kotagiri	46.5	23.8	12.3	37.0	29.9
	4	Armur	30.0	14.8	13.1	13.1	17.7
	5	Bhodhan	38.8	24.8	11.8	30.3	26.4
	6	Ditchpally	49.2	28.1	19.3	35.6	33.0
		Total fungal colonies (%)	**40.85**	**22.35**	**14.41**	**26.46**	
ADILABAD	1	Muthol	23.6	11.6	9.5	15.4	15.0
	2	Thalamadugu	35.6	15.2	11.1	22.1	21.0
	3	Thanur	35.4	12.6	11.6	18.5	19.5
	4	Kubeer	45.0	22.1	16.2	23.7	26.8
	5	Nirmal	32.9	18.6	10.5	18	20.0
	6	Ichoda	41.7	17.3	12.3	26.4	24.4
		Total fungal colonies (%)	**35.70**	**16.23**	**11.90**	**20.68**	

Table 4.1. Detection of seed mycoflora associated with soybean seed samples collected from Nizamabad and Adilabad districts of A.P following standard blotter method

District	S.No	Name of the Mandal	*M. phaseolina*	*Colletot-richum*	*Fusarium*	*Altern-aria*	*Curvularia*	*Rhizopus*	*A. flavus*	*A. niger*	*Cladospor-ium*	TFC (%)
NIZAMABAD	1	Kammarpalli	20.5 (27.1)	3.5 (10.7)	2.0 (8.12)	1.5 (7.03)	3.5 (10.7)	4.0 (11.5)	2.5 (9.09)	2.4 (8.91)	1.5 (7.02)	41.4
	2	Morthad	16 (23.5)	4.0 (11.5)	2.3 (8.72)	3.5 (10.7)	4 (11.5)	2.5 (9.09)	3.4 (10.6)	1.5 (7.02)	2.0 (8.12)	39.2
	3	Kotagiri	26 (30.6)	4.5 (12.2)	3.5 (10.78)	2.5 (9.08)	3.5 (10.7)	3.5 (10.78)	1.0 (5.61)	1.5 (7.03)	0.5 (4.18)	46.5
	4	Armur	14.4 (22.3)	2.0 (8.12)	2.5 (10.7)	1.5 (7.03)	1.0 (10.7)	3.5 (10.7)	1.5 (7.03)	1.3 (6.54)	2.3 (8.69)	30.0
	5	Bhodhan	24.5 (29.6)	2.5 (8.99)	1.5 (7.03)	3.0 (9.97)	2.0 (8.71)	2.3 (8.71)	1.5 (7.02)	0.5 (4.41)	1.0 (5.73)	38.8
	6	Ditchpally	28.5 (32.2)	3.5 (10.7)	3.3 (10.4)	2.5 (9.09)	3.4 (9.08)	2.5 (9.08)	1.5 (7.03)	2.5 (9.09)	1.5 (7.01)	49.2
		S.Em (±)	**0.80**	**0.44**	**0.167**	**0.165**	**0.18**	**0.18**	**0.37**	**0.21**	**0.29**	-
		CD at 5%	**2.49**	**1.36**	**0.514**	**0.510**	**0.57**	**0.57**	**1.16**	**0.67**	**0.89**	-
ADILABAD	1	Muthol	8.5 (16.9)	3.8 (11.2)	3.3 (10.4)	2.0 (8.12)	3.5 (10.7)	- (0.00)	1.5 (6.99)	- (0.00)	1.0 (5.73)	23.6
	2	Thalamadugu	13.2 (21.3)	3.5 (10.7)	2.5 (9.09)	2.3 (8.71)	3.1 (10.1)	4.0 (11.5)	2.5 (9.08)	4.0 (11.51)	0.5 (4.41)	35.6
	3	Thanur	9 (17.4)	4.5 (12.2)	3.4 (10.6)	4.5 (12.2)	2.5 (9.03)	4.0 (11.5)	2.5 (9.09)	2.5 (9.01)	2.5 (9.04)	35.4
	4	Kubeer	16.5 (23.9)	6.2 (14.4)	5.3 (13.3)	4.0 (11.5)	2.5 (9.09)	2.3 (8.72)	3.5 (10.7)	3.4 (10.6)	1.3 (6.53)	45
	5	Nirmal	10.5 (18.9)	4.5 (12.2)	3.0 (9.95)	3.3 (10.4)	3.3 (10.4)	2.5 (9.08)	1.5 (7.03)	3.0 (9.95)	1.3 (6.53)	32.9
	6	Ichoda	14.5 (22.3)	5.2 (13.1)	4.5 (12.4)	2.0 (8.12)	3.5 (10.7)	3.3 (10.4)	3.0 (9.97)	4.5 (12.2)	1.2 (6.28)	41.7
		S.Em (±)	**0.34**	**0.25**	**0.23**	**0.30**	**0.38**	**0.19**	**0.29**	**0.47**	**0.36**	-
		CD at 5%	**1.07**	**0.77**	**0.73**	**0.94**	**1.19**	**0.59**	**0.92**	**1.47**	**1.10**	-

Figures in parenthesis are angular transformed values. Mean of four replications. TFC: Total fungal colonies

Table 4.4. Detection of seed mycoflora associated with soybean seed samples collected from Nizamabad and Adilabad districts of A.P following potato dextrose agar method

District	S.No	Name of the Mandal	*M. phaseolina*	*Colletotri-chum*	*Fusarium*	*Alternaria*	*Curvularia*	*Rhizopus*	*A. flavus*	*A. niger*	*Cladosporium*	TFC (%)
NIZAMABAD	1	Kammarpalli	6.3 (14.5)	2.0 (8.12)	4.0 (11.4)	3.0 (9.97)	4.4 (12.0)	2.0 (8.12)	1.5 (7.02)	1.0 (5.58)	2.0 (8.12)	26.2
	2	Morthad	4.5 (12.2)	1.5 (7.03)	2.5 (9.09)	2.5 (9.08)	2.6 (9.26)	1.0 (5.58)	1.5 (7.03)	- (0.00)	1.5 (7.01)	16.6
	3	Kotagiri	12.5 (20.7)	2.5 (9.07)	6.5 (14.7)	1.5 (7.02)	5.0 (12.9)	3 (9.97)	1.5 (7.02)	2.0 (8.12)	2.5 (9.09)	37.0
	4	Armur	3.5 (10.7)	1.0 (5.67)	2.8 (9.63)	- (0.00)	4.3 (11.9)	1.0 (5.72)	0.5 (4.41)	- (0.00)	1.0 (5.73)	13.1
	5	Bhodhan	8.5 (16.9)	3.3 (10.46)	4.3 (11.9)	3.6 (10.9)	4.6 (12.3)	1.5 (7.03)	2.0 (9.09)	1.0 (5.79)	1.5 (7.02)	30.3
	6	Ditchpally	14 (24.4)	3.5 (10.7)	7.5 (15.8)	1.5 (7.03)	2.6 (9.24)	1.5 (7.02)	2.5 (9.08)	1.0 (5.72)	1.5 (6.87)	35.6
		S.Em (±)	**1.04**	**0.35**	**0.38**	**0.22**	**0.45**	**0.43**	**0.25**	**0.54**	**0.47**	-
		CD at 5%	**3.23**	**1.09**	**1.19**	**0.69**	**1.41**	**1.32**	**0.78**	**1.68**	**1.47**	-
ADILABAD	1	Muthol	5.5 (13.5)	1.2 (6.26)	3.5 (10.7)	1.0 (5.67)	1.2 (6.27)	1.5 (8.11)	- (0.00)	0.5 (5.72)	1.0 (8.10)	15.4
	2	Thalamadugu	8.5 (12.2)	1.3 (7.02)	5.5 (9.09)	1.3 (9.06)	1.2 (9.26)	1.0 (5.67)	1.0 (7.02)	1.3 (4.05)	1.0 (7.02)	22.1
	3	Thanur	7.8 (20.7)	1.0 (9.06)	4.0 (14.7)	1.5 (7.02)	1.0 (12.8)	0.5 (9.96)	1.0 (6.99)	1.3 (8.11)	0.4 (7.02)	18.5
	4	Kubeer	10.3 (10.7)	1.5 (5.69)	6.0 (9.58)	1.2 (4.05)	1.0 (11.9)	1.3 (5.72)	1.4 (4.51)	0.5 (4.05)	0.5 (5.67)	23.7
	5	Nirmal	6.5 (16.9)	1.3 (10.4)	4.5 (11.9)	1.2 (10.9)	0.5 (12.3)	0.8 (7.03)	1.0 (4.30)	1.2 (4.05)	1.0 (5.73)	18.0
	6	Ichoda	11.3 (19.6)	1.3 (10.7)	6.5 15.89	1.3 (6.54)	1.0 (5.73)	1.5 (7.03)	1.2 (6.28)	1.5 (7.03)	0.8 (5.73)	26.4
		S.Em (±)	**0.16**	**0.45**	**0.44**	**0.37**	**0.35**	**0.34**	**0.38**	**0.19**	**0.35**	-
		CD at 5%	**0.52**	**1.41**	**1.36**	**1.14**	**1.09**	**1.05**	**1.19**	**0.59**	**1.10**	-

Figures in parenthesis are angular transformed values. Mean of four replications. TFC: Total fungal colonies

Table 4.2. Detection of seed mycoflora associated with soybean seed samples collected from Nizamabad and Adilabad districts of A.P following 2, 4 - D blotter paper method

District	S.No	Name of the Mandal	*M. phaseolina*	*Colletotri-chum*	*Fusarium*	*Alternaria*	*Curvularia*	*Rhizopus*	*A. flavus*	*A. niger*	*Cladospo-rium*	TFC (%)
NIZAMABAD	1	Kammarpalli	5.8 (13.9)	5.8 (13.9)	3.3 (10.4)	1.0 (5.73)	1.5 (6.79)	1.3 (6.27)	- (0.00)	1.5 (7.02)	- (0.00)	20.2
	2	Morthad	5.5 (13.5)	5.0 (12.9)	1.3 (6.79)	1.8 (7.70)	1.3 (6.79)	2.2 (8.46)	2.5 (9.09)	1.3 (6.79)	1.5 (7.02)	22.4
	3	Kotagiri	8.8 (17.2)	8.5 (16.9)	1.0 (5.73)	1.5 (7.02)	1.0 (5.73)	- (0.00)	0.5 (4.18)	1.0 (5.73)	1.5 (7.02)	23.8
	4	Armur	5.0 (12.9)	3.5 (10.7)	1.5 (7.02)	1.0 (5.73)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	1.3 (6.79)	- (0.00)	14.8
	5	Bhodhan	6.8 (15.1)	6.5 (14.7)	1.3 (6.79)	1.5 (7.02)	2.5 (9.09)	3 (9.97)	0.5 (4.18)	1.5 (7.02)	1.2 (6.28)	24.8
	6	Ditchpally	9.5 (17.9)	9.0 (17.4)	1.6 (7.25)	2.5 (9.09)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	1.5 (7.02)	1.5 (7.02)	28.1
		S.Em (±)	**0.11**	**0.07**	**0.21**	**0.19**	**0.14**	**0.15**	**0.12**	**0.22**	**0.20**	-
		CD at 5%	**0.35**	**0.21**	**0.65**	**0.66**	**0.46**	**0.47**	**0.39**	**0.67**	**0.62**	-
ADILABAD	1	Muthol	4.0 (11.5)	3.5 (10.7)	1.0 (5.73)	0.5 (7.02)	1.0 (5.73)	0.5 (4.18)	- (0.00)	1.1 (6.00)	- (0.00)	11.6
	2	Thalamadugu	5.0 (12.9)	3.8 (11.2)	1.3 (6.53)	1.2 (6.28)	1.3 (6.53)	1.0 (5.73)	0.8 (5.12)	0.5 (4.18)	0.3 (4.05)	15.2
	3	Thanur	4.5 (12.2)	3.3 (10.4)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	0.8 (5.12)	1.0 (5.73)	0.5 (4.18)	- (0.00)	12.6
	4	Kubeer	6.8 (15.1)	5.5 (13.5)	1.5 (7.11)	2.3 (8.65)	3.0 (9.97)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	0.5 (4.18)	22.1
	5	Nirmal	6.5 (14.7)	4.5 (12.3)	1.3 (6.54)	2.1 (8.33)	1.8 (7.70)	0.7 (4.79)	0.4 (4.05)	1.0 (5.73)	0.3 (4.05)	18.6
	6	Ichoda	5.3 (13.3)	4.0 (11.5)	1.3 (6.53)	2.0 (8.12)	1.5 (7.03)	1.3 (6.54)	1.0 (5.73)	0.5 (4.18)	0.4 (4.18)	17.3
		S.Em (±)	**0.09**	**0.08**	**0.20**	**0.15**	**0.17**	**0.16**	**0.13**	**0.18**	**0.07**	-
		CD at 5%	**0.28**	**0.24**	**0.63**	**0.48**	**0.54**	**0.51**	**0.41**	**0.57**	**0.22**	-

Figures in parenthesis are angular transformed values. Mean of four replications. TFC: Total fungal colonies

Table 4.3. Detection of mycoflora associated with soybean seed samples from Nizamabad and Adilabad districts of A.P following deep freeze blotter paper method

District	S.No	Name of the Mandal	*M. phaseolina*	*Colletotr-ichum*	*Fusarium*	*Alternaria*	*Curvularia*	*Rhizopus*	*A. flavus*	*A. niger*	*Cladospo-rium*	TFC (%)
NIZAMABAD	1	Kammarpalli	3.5 (10.7)	1.5 (7.03)	1.3 (6.54)	2.5 (9.09)	1.4 (6.79)	1.3 (6.54)	1.5 (7.03)	- (0.00)	1.3 (6.54)	14.3
	2	Morthad	3.3 (10.8)	3.5 (10.7)	1.5 (7.03)	- (0.00)	- (0.00)	1.5 (7.03)	1.4 (6.54)	2.0 (8.12)	2.5 (9.09)	15.7
	3	Kotagiri	4.5 (12.2)	1.0 (5.73)	1.5 (7.03)	1.0 (5.73)	1.3 (6.54)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	0.5 (4.18)	12.3
	4	Armur	2.5 (9.09)	2.5 (9.09)	2.6 (9.27)	- (0.00)	- (0.00)	1.5 (7.03)	2.5 (9.09)	1.0 (5.73)	0.5 (4.18)	13.1
	5	Bhodhan	4.0 (11.5)	1.0 (5.73)	1.5 (7.03)	1.0 (5.73)	0.5 (4.18)	1.3 (6.54)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	11.8
	6	Ditchpally	6.5 (14.7)	1.5 (7.03)	3.0 (9.7)	2.5 (9.09)	2.0 (8.12)	1.0 (5.73)	0.5 (4.18)	1.0 (5.73)	1.3 (6.54)	19.3
		S.Em (±)	**0.14**	**0.13**	**0.12**	**0.11**	**0.10**	**0.15**	**0.13**	**0.13**	**0.13**	-
		CD at 5%	**0.43**	**0.42**	**0.39**	**0.35**	**0.33**	**0.46**	**0.42**	**0.42**	**0.42**	-
ADILABAD	1	Muthol	3.0 (9.96)	1.2 (6.28)	1.0 (5.73)	0.5 (4.04)	1.0 (5.73)	1.0 (5.73)	1.5 (7.03)	- (0.00)	0.3 (4.40)	9.5
	2	Thalamadugu	4.1 (11.6)	1.3 (6.54)	1.1 (6.01)	1.5 (7.03)	1.0 (5.73)	1.0 (5.73)	0.5 (4.04)	- (0.00)	0.5 (4.04)	11.1
	3	Thanur	4.0 (11.5)	1.5 (7.03)	1.0 (5.73)	0.5 (4.04)	1.0 (5.73)	0.8 (5.12)	2.5 (9.09)	- (0.00)	0.3 (3.30)	11.6
	4	Kubeer	5.1 (13.0)	1.8 (7.70)	2.3 (8.7)	2.5 (9.09)	0.5 (4.04)	1.0 (5.73)	0.5 (4.04)	1.0 (5.73)	1.5 (7.03)	16.2
	5	Nirmal	3.2 (10.3)	1.2 (6.28)	1.3 (6.54)	1.0 (5.73)	0.5 (4.04)	0.8 (5.73)	1.0 (5.73)	1.5 (7.03)	- (0.00)	10.5
	6	Ichoda	4.2 (11.8)	1.3 (6.54)	2.1 (8.3)	0.5 (4.04)	0.5 (0.00)	1.0 (0.00)	1.3 (6.54)	1.2 (6.28)	- (0.00)	12.3
		S.Em (±)	**0.08**	**0.14**	**0.18**	**0.19**	**0.18**	**0.15**	**0.17**	**0.10**	**0.45**	-
		CD at 5%	**0.26**	**0.44**	**0.56**	**0.60**	**0.56**	**0.48**	**0.55**	**0.33**	**1.40**	-

Figures in parenthesis are angular transformed values. Mean of four replications. TFC: Total fungal colonies

Table 4.6. Occurrence of seed mycoflora in blotter, 2, 4- D blotter, deep freeze blotter and agar plate method (Nizamabad district)

Fungal flora	Blotter method (%)	2, 4-D blotter (%)	Deep freeze blotter (%)	Potato dextrose agar (%)	TFC (%)
M. phaseolina	52.9	30.8	28.0	31.0	35.7
Colletotrichum	8.1	28.5	12.7	8.6	14.4
Fusarium sp	6.1	7.4	13.1	31.0	14.3
Alternaria	5.9	6.9	8.0	7.6	7.1
Curvularia	7.0	6.1	6.0	14.7	8.4
Rhizopus	7.4	5.5	8.7	6.2	6.9
A. flavus	4.6	4.1	8.5	5.9	5.7
A. niger	3.9	6.0	6.3	3.1	4.8
Cladosporium	3.5	4.2	8.2	6.2	5.5

TFC: Total fungal colonies

Table 4.7. Occurrence of seed mycoflora in blotter, 2, 4- D blotter, deep freeze and agar plate method (Adilabad district)

Fungal flora	Blotter method (%)	2, 4-D blotter (%)	Deep freeze blotter (%)	Potato dextrose agar (%)	TFC (%)
M. phaseolina	33.7	32.9	33.1	40.2	34.9
Colletotrichum	7.7	25.2	14.0	6.1	13.3
Fusarium sp	10.2	7.5	12.3	24.1	13.5
Alternaria	8.45	8.8	9.1	6.0	8.1
Curvularia	8.5	9.8	4.9	4.7	7.0
Rhizopus	8.8	5.4	7.8	5.3	6.8
A. flavus	6.76	3.7	10.2	4.5	6.3
A. niger	8.12	4.7	5.1	5.0	5.7
Cladosporium	3.64	1.7	3.6	3.7	3.2

Table 4.10. Seed transmission of *M. phaseolina* in soybean (cv. JS – 335) by test tube water agar method (TWA)

S.No	Treatment	*Germination (%)	*Seed rot (%)	*Seedling blight (%)
1.	**Apparently healthy seed sample**	75.0 (59.9)	2.5 (9.09)	3.5 (10.7)
2.	**Naturally infected seed sample**	59.3 (50.3)	15.5 (23.1)	16.5 (24.1)
3.	**Artificially inoculated seed sample**	55.0 (47.8)	18.0 (25)	20.5 (26.9)
	S.Em (±)	**0.62**	**0.18**	**0.22**
	CD at 5%	**2.19**	**0.64**	**0.80**

Figures in parentheses indicates angular transformed values. * Average of three replications

Table 4.11. Seed transmission of *M. phaseolina* in soybean (cv. JS – 335) under glasshouse conditions

S.No	Treatment	*Germination (%)	*Seed rot (%)	*Seedling blight (%)
1.	**Apparently healthy seed sample**	72.0 (58.0)	4.2 (11.8)	3.8 (11.2)
2.	**Naturally infected seed sample**	50.5 (45.2)	24.0 (29.3)	19.1 (25.9)
3.	**Artificially inoculated seed sample**	46.0 (42.6)	25.8 (30.5)	23.2 (28.7)
	S.Em (±)	**0.38**	**0.35**	**0.36**
	CD at 5%	**1.34**	**1.25**	**1.30**

Figures in parentheses indicates angular transformed values. *Average of three replications

Table 4.12. Evaluation seed treatments with fungicides, bioagents, botanicals and irradiations against seed borne *M. phaseolina* under glasshouse conditions

S.No	Treatment	*Germination (%)	*Seed rot (%)	*Seedling blight (%)
1.	*Trichoderma viride* (10 g kg^{-1} seed)	88 (70.3)	7.3 (15.6)	6.3 (14.5)
2.	*Pseudomonas fluorescens* (10 g kg^{-1}seed)	84 (66.6)	9.0 (17.4)	8.0 (16.4)
3.	Irradiation dose (1.5 kGy)	77 (61.7)	15.0 (22.7)	14.0 (21.9)
4.	Irradiation dose (2.5 kGy)	78 (62.2)	14.7 (22.4)	12.3 (20.5)
5.	NSKE (neem seed kernel extract) 5%.	82 (65.1)	12.3 (20.5)	10.3 (18.7)
6.	Neem leaf powder @ 5g kg^{-1} seed	80 (63.4)	13.0 (21.1)	11.0 (19.2)
7.	Thiram + carbendazim 1:1 @ 3 g kg^{-1}	91 (73.3)	5.7 (13.7)	4.0 (11.4)
8.	Vitavax power 200 @ 2.5 g kg^{-1}	89 (70.6)	6.7 (14.9)	5.2 (13.1)
9.	Treated seeds (pathogen)	60 (51.1)	26.3 (30.8)	25.7 (30.4)
10.	Control (untreated seeds)	74 (59.8)	20.7 (27.0)	15.0 (22.7)
	S. Em (±)	**0.96**	**0.46**	**0.62**
	CD at 5 %	**2.86**	**1.38**	**1.84**

*Mean of three replications. Figures in parenthesis are angular transformed values.

Table 4.8. Mean seed quality parameters (Germination (%), seed vigour I & II, moisture content (%) and seed rot (%) and seedling blight (%) in soybean samples of Nizamabad district.

S.No	Name of the Mandal	Number of samples	Germination (%)	Seed vigour index (SVI- I)	Seed vigour index (SVI- II)	Seed moisture content (%)	Seed rot (%)	Seedling blight (%)	Total fungal colonies (%)
1	Kammarpalli	10	71.7 (57.8)	1733	69.0	8.5 (16.9)	16.2 (23.6)	11.0 (19.3)	25.5
2	Morthad	10	73.0 (58.6)	1861	75.5	8.4 (16.8)	15.0 (22.7)	9.0 (17.7)	23.5
3	Kotagiri	10	67.3 (55.1)	1514	61.2	8.8 (17.2)	18.5 (25.4)	13.3 (21.4)	29.9
4	Armur	10	75.0 (59.9)	2013	85.0	8.2 (16.5)	14.2 (22.0)	8.6 (17.2)	17.7
5	Bhodhan	10	70.0 (56.8)	1665	65.0	8.60 (17.6)	17.3 (24.5)	12.3 (20.5)	26.4
6	Ditchpally	10	65.3 (53.9)	1394	57.0	9.00 (17.4)	19.2 (25.9)	14.1 (22.0)	33.0
	Mean		**70.4**	**1697**	**68.8**	**8.58**	**16.7**	**11.3**	**26.0**
	S.Em (±)		**0.82**	**37.5**	**1.99**	**0.06**	**0.27**	**0.25**	**-**
	CD at 5%		**2.56**	**116.8**	**6.21**	**0.21**	**0.84**	**0.80**	**-**

Figures in parenthesis are angular transformed values. Mean of four replications.

Table 4.9. Mean seed quality parameters (Germination (%), seed vigour I & II, moisture content (%) and seed rot (%) and seedling blight (%) in soybean samples of Adilabad district.

S.No	Name of the Mandal	Number of samples	Germination (%)	Seed vigour index (SVI- I)	Seed vigour index (SVI- II)	Seed Moisture content (%)	Seed Rot (%)	Seedling blight (%)	Total fungal colonies (%)
1.	Muthol	10	78.3 (62.2)	2194	104.5	7.7 (16.1)	12.2 (20.4)	7.0 (15.3)	15.0
2.	Thalamadugu	10	72.7 (58.4)	1773	79.9	8.2 (16.5)	15.8 (23.4)	10.0 (18.4)	21.0
3.	Thanur	10	76.0 (60.6)	2032	88.7	7.8 (16.2)	13.7 (21.6)	8.8 (17.2)	19.5
4.	Kubeer	10	67.0 (54.9)	1470	65.2	8.4 (16.8)	17.3 (24.5)	12.5 (20.60)	26.8
5.	Nirmal	10	74.3 (59.5)	1859	84.2	8.1 (16.4)	14.8 (22.6)	9.2 (17.6)	20.0
6.	Ichoda	10	70.0 (56.8)	1596	70.9	8.3 (16.7)	16.0 (23.5)	11.5 (19.8)	24.4
	Mean		**73.1**	**1821**	**82.2**	**8.08**	**14.9**	**9.83**	**21.1**
	S.Em (±)		**0.81**	**34.2**	**2.53**	**0.06**	**0.24**	**0.42**	**-**
	CD at 5%		**2.54**	**106.6**	**7.89**	**0.18**	**0.75**	**1.32**	**-**

Figures in parenthesis are angular transformed values. Mean of four replications.

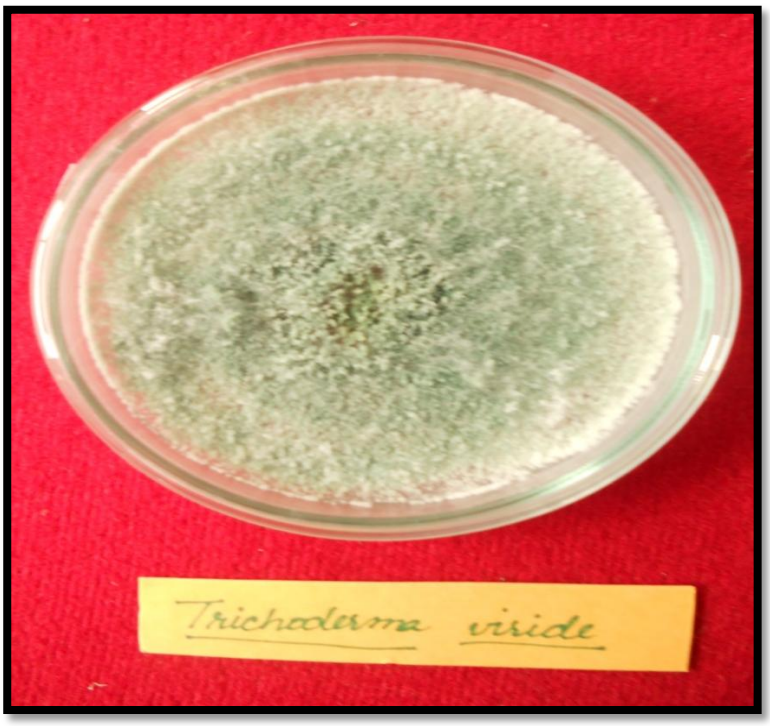

Plate 3.1. Pure culture of *T. viride* maintained on PDA medium

Plate 3.2. Pure culture of *Pseudomonas fluorescens* maintained on PDA medium

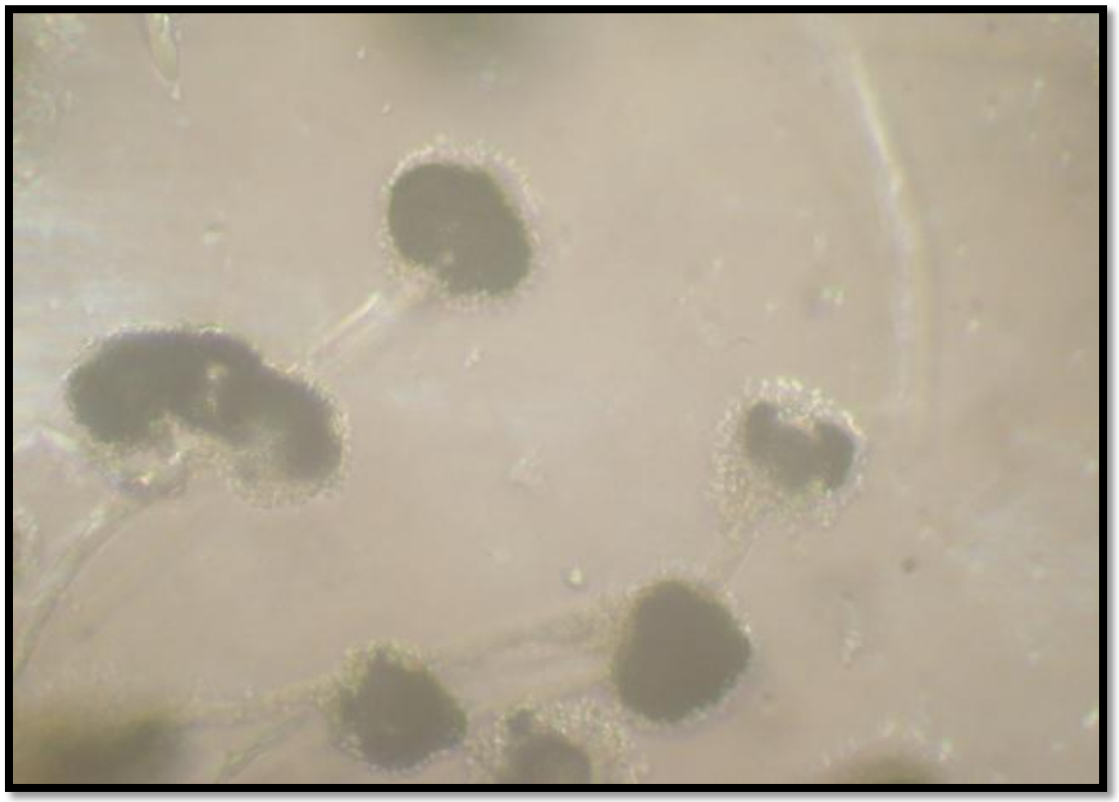

Plate 4.5. Photomicrograph of *Aspergillus niger* detected from seeds of soybean cv. JS- 335

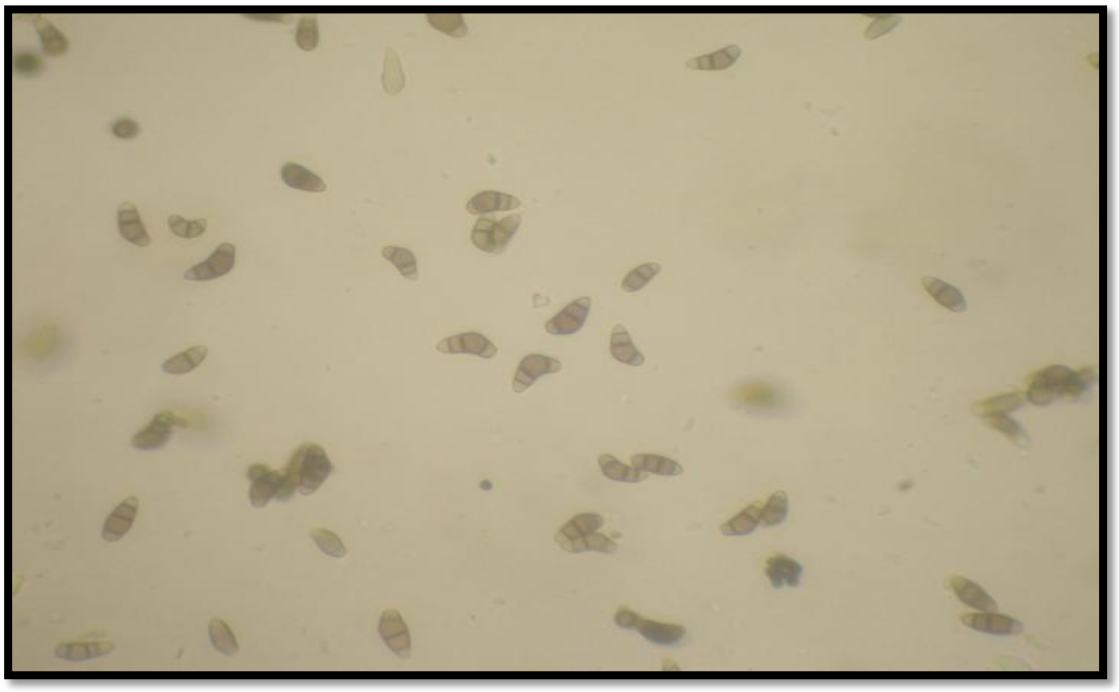

Plate 4.6. Photomicrograph of *Curvularia* sp. detected from seeds of soybean cv. JS-335

Plate 4.12. Germination studies of soybean cv. JS --335 (Paper towel method)

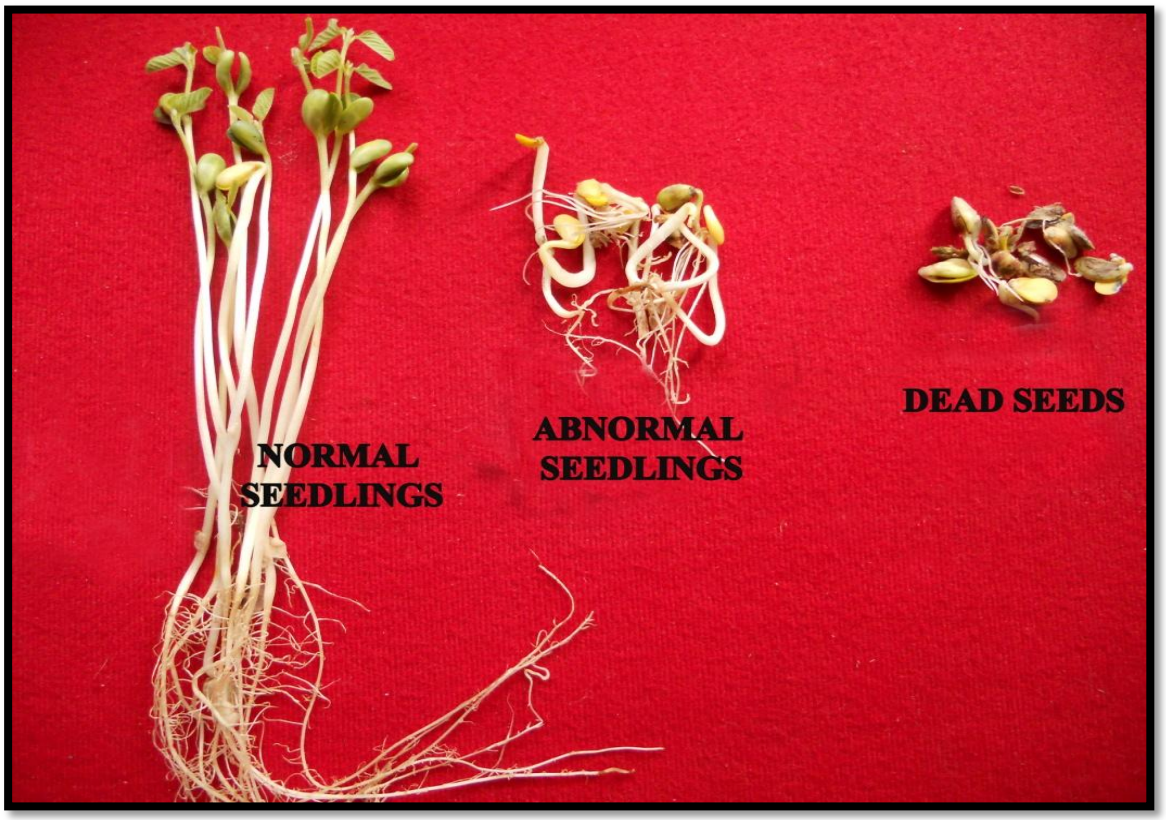

Plate 4.13. Evaluation of soybean seedlings in germination (Paper towel method)

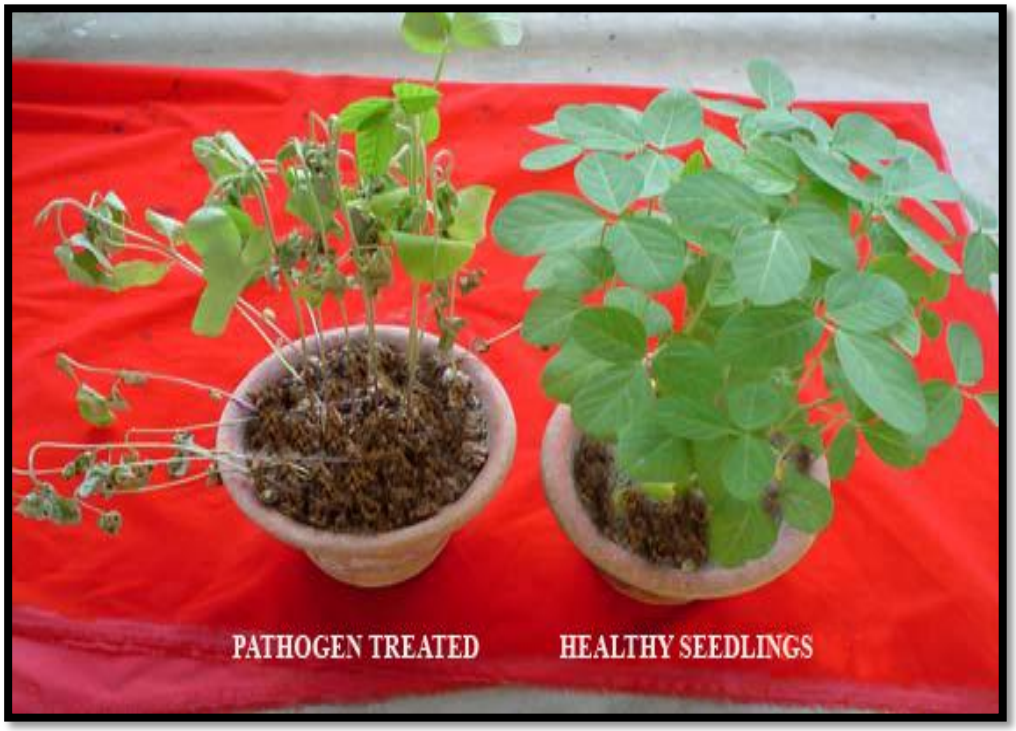

Plate 4.17. Pathogenicity test of *M. phaseolina* on soybean cv. JS - 335 by seed inoculation method

Plate 4.1. Seed mycoflora detected by standard blotter paper method in soybean

Plate 4.2. Seed mycoflora detected by 2, 4 - D blotter paper method in soybean

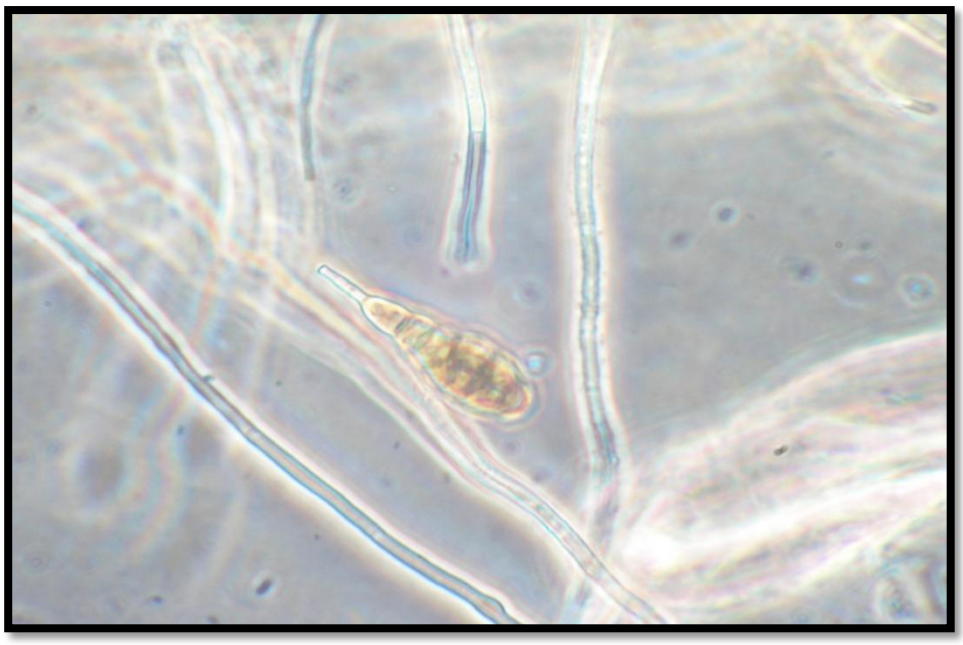

Plate 4.7. Photomicrograph of *Alternaria alternata* detected from seeds of Soybean cv. JS - 335

Plate 4. 8. Photomicrograph of *Rhizopus* detected from seeds soybean cv JS - 335

Plate 4.3. Seed mycoflora detected by Potato dextrose agar method in soybean

Plate 4.4. Seed mycoflora detected by blotter paper method, 2, 4-D blotter and deep freeze blotter method in seeds of soybean

Plate 4.9. Photomicrograph of *Colletotrichum* acervulus and setae detected from seeds of soybean cv. JS-335

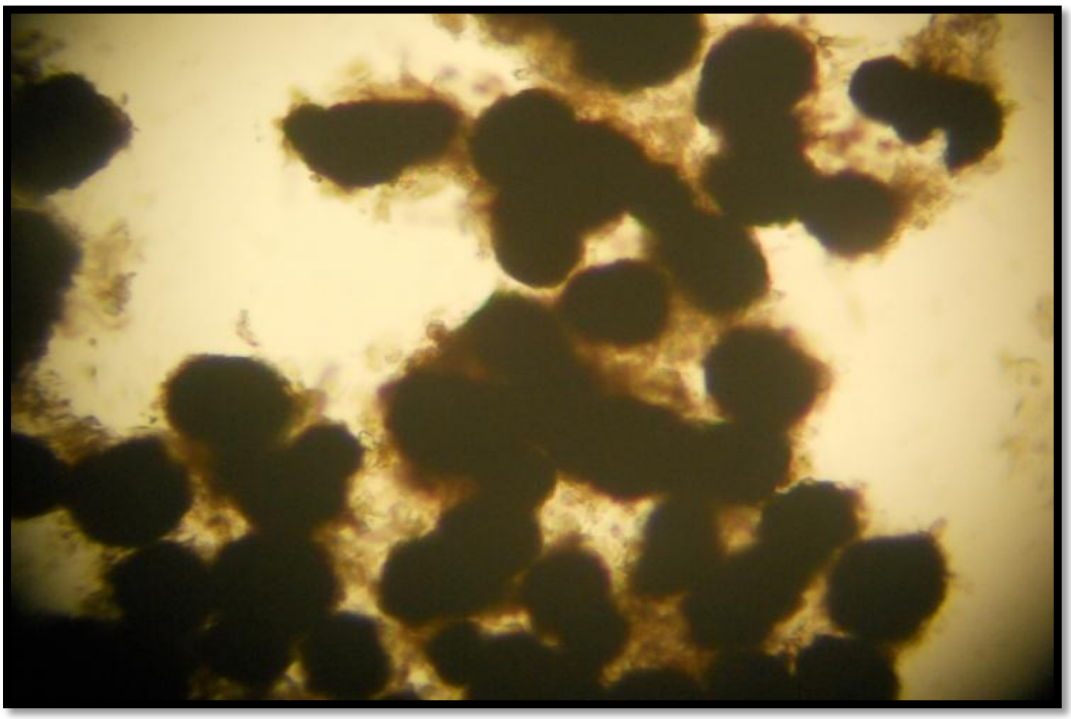

Plate 4.10. Photomicrograph of *M. phaseolina* sclerotial bodies detected from seeds of soybean cv. JS – 335

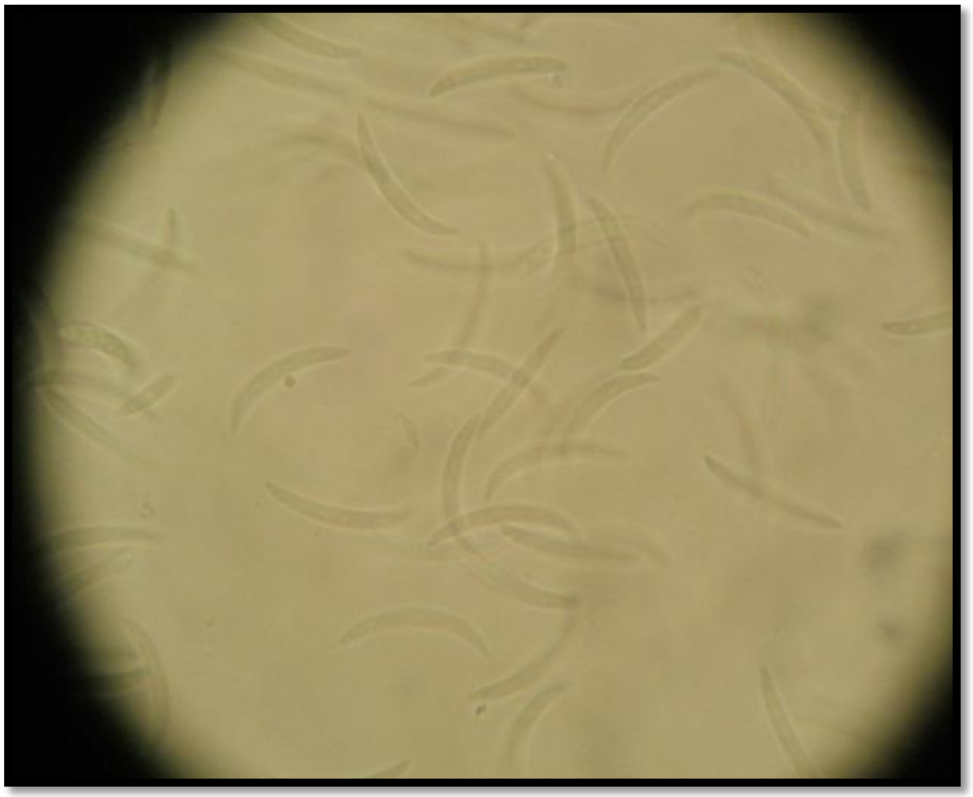

Plate 4.11. Conidia of *Fusarium* sp. detected from seeds of soybean cv. JS-335

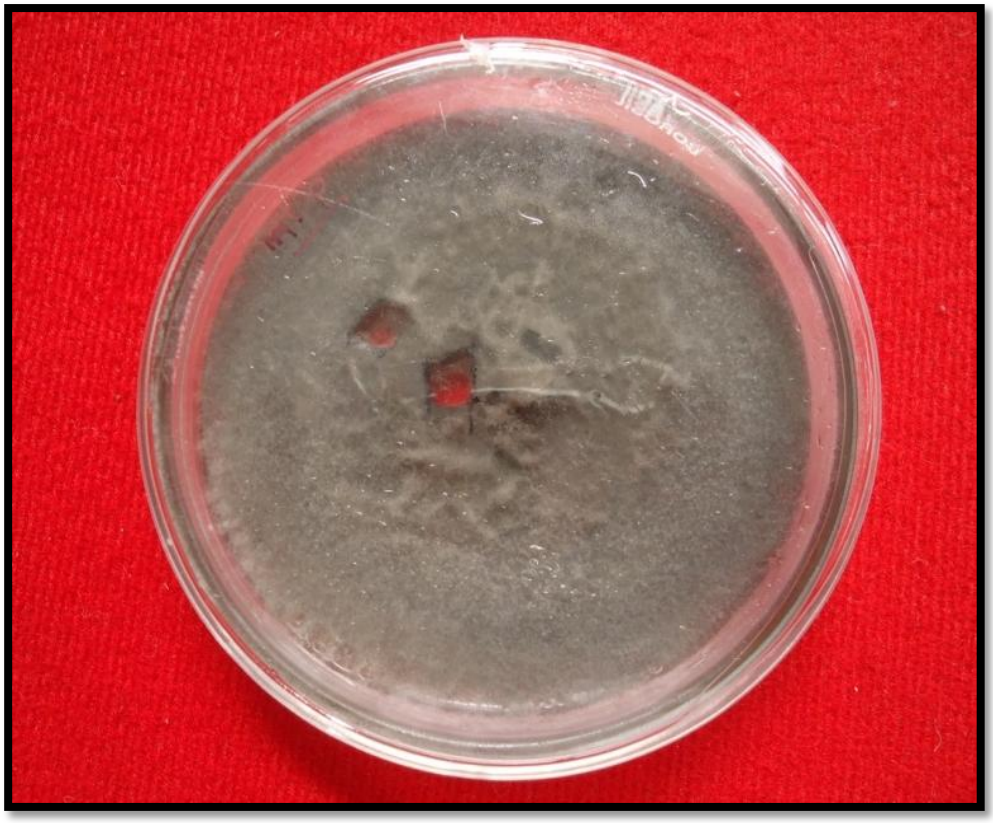

Plate 4.15. Pure culture of *M. phaseolina* isolated from soybean seed grown on PDA

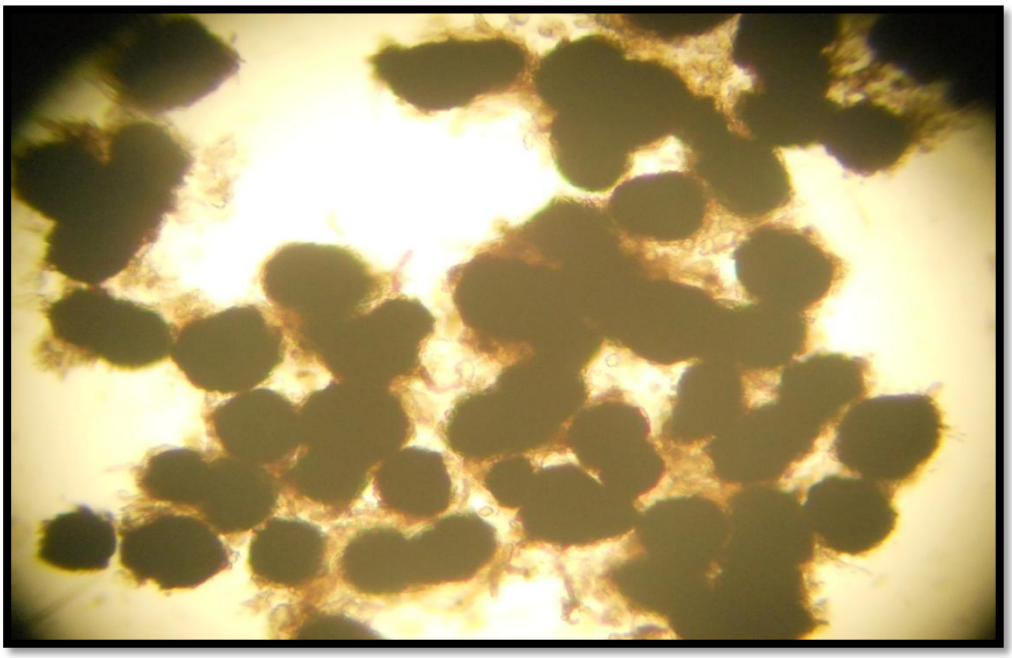

Plate 4.16. Sclerotial bodies of *M. phaseolina* detected from seeds of soybean cv. JS – 335

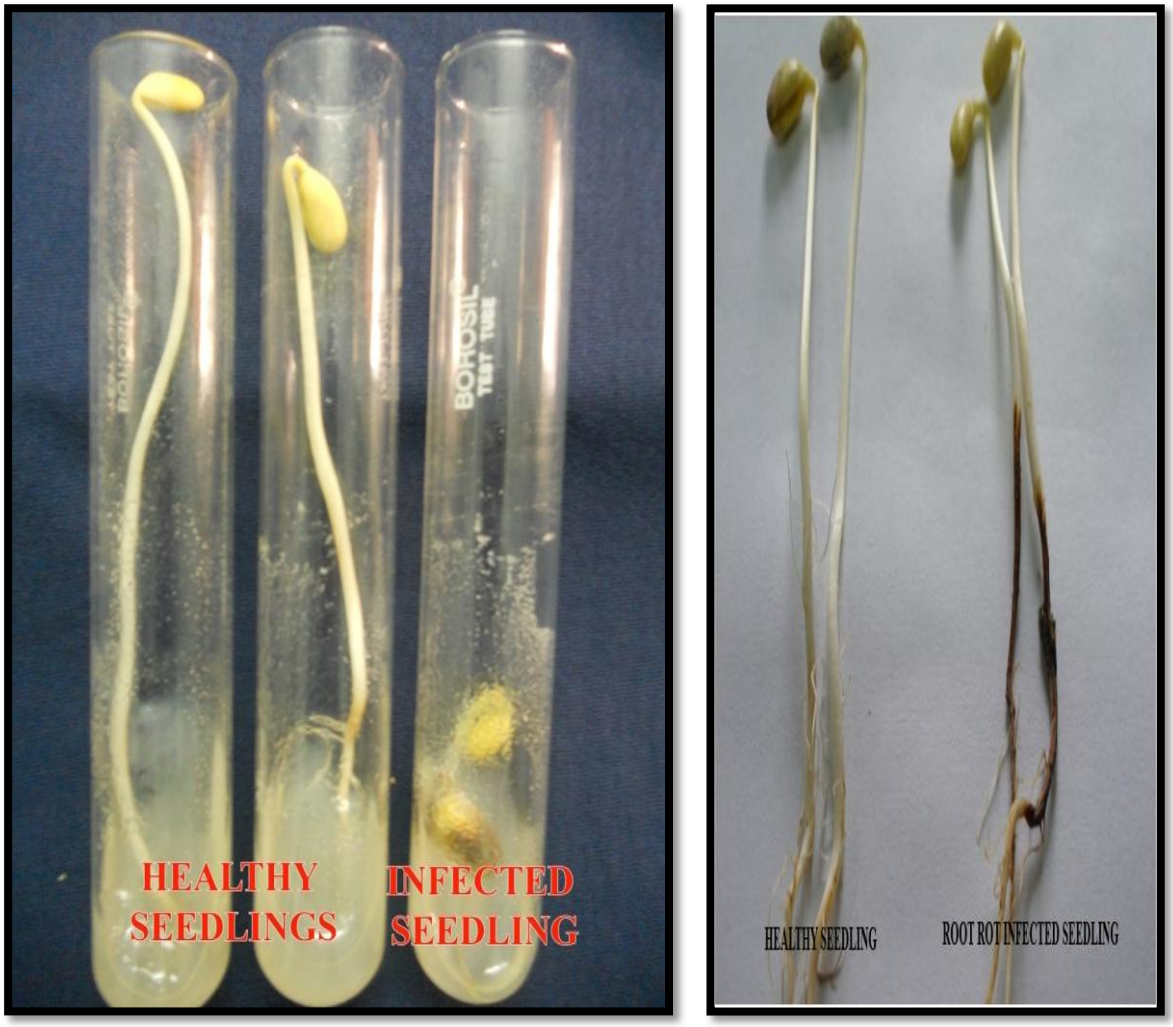

Plate 4.18. Testing of soybean seed samples by test tube water agar method (TWA)

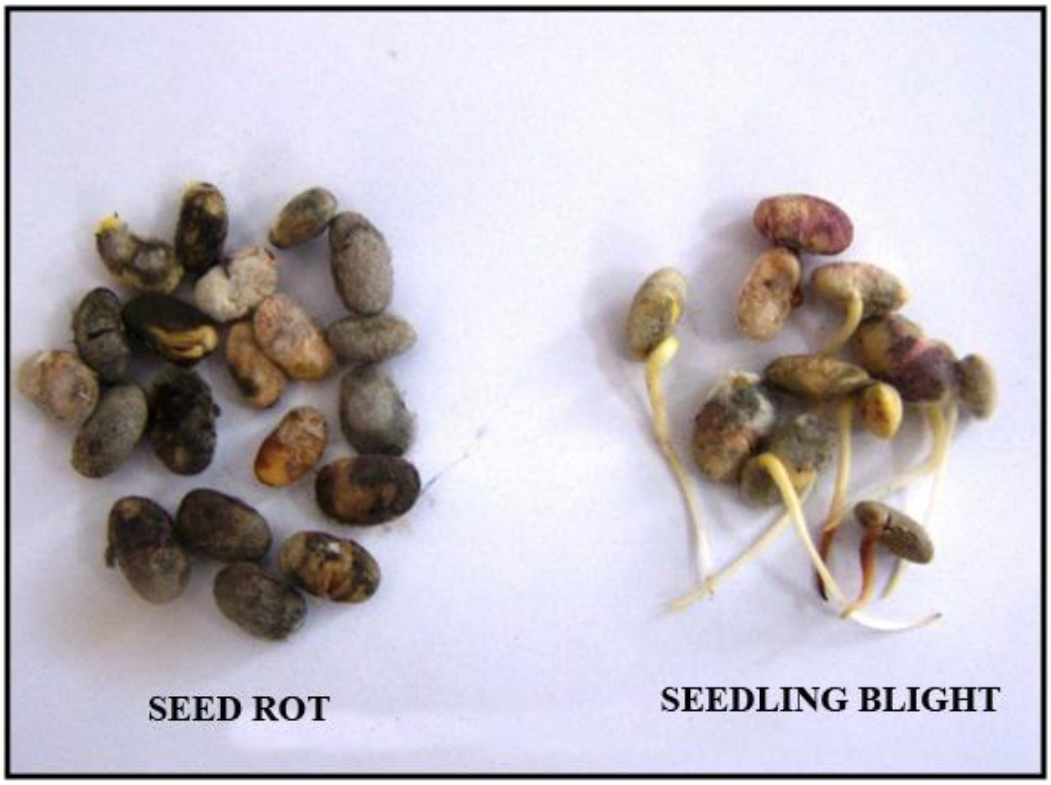

Plate 4.14. Seed rot and seedling blight of soybean cv. JS-335 due to *M. phaseolina*

Plate 4.19. Evaluation of seed treatments in the management of seedborne *M. phaseolina* in soybean cv. JS – 335 under glasshouse conditions

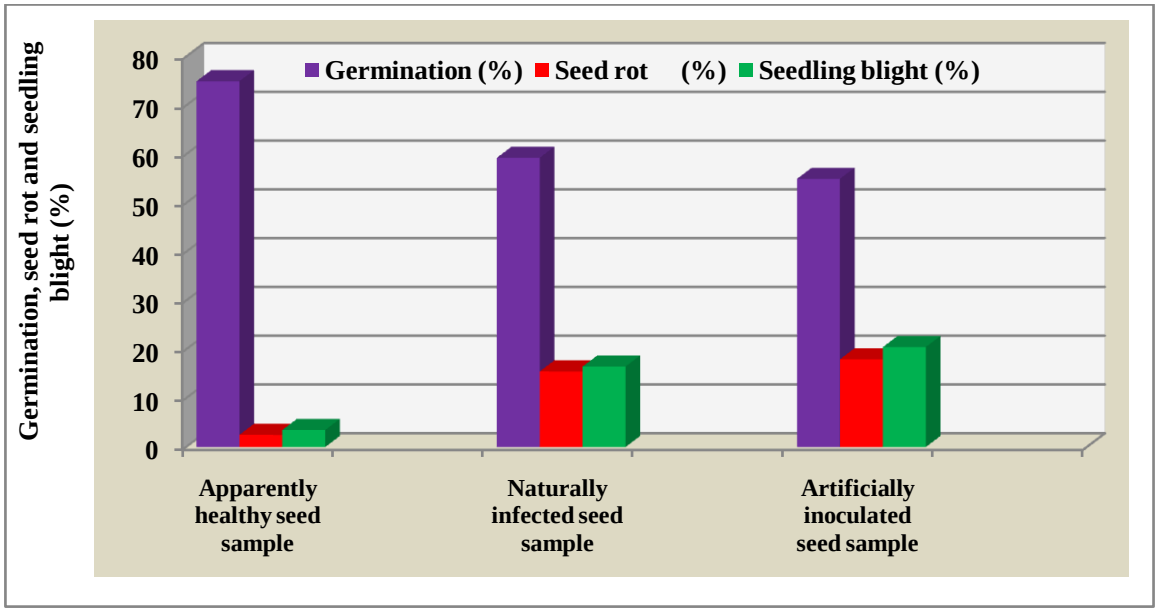

Figure 4.6. Seed transmission of *M. phaseolina* in soybean cv. JS-335 by test tube water agar method (TWA)

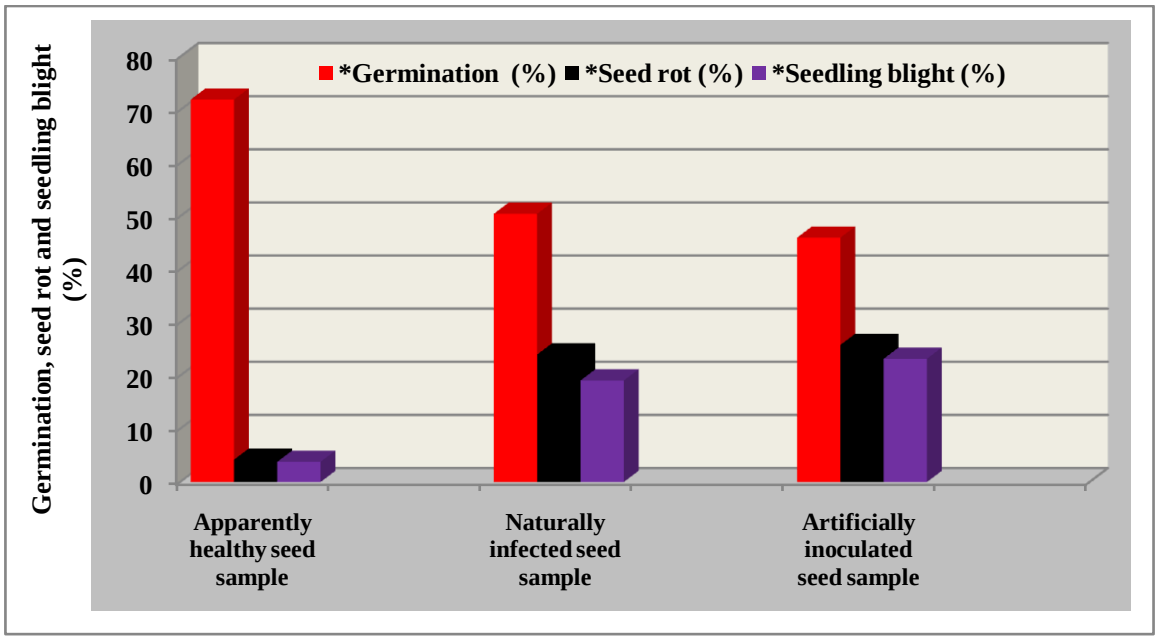

Figure 4.7. Seed transmission of *M. phaseolina* in soybean cv. JS-335 under glass house conditions

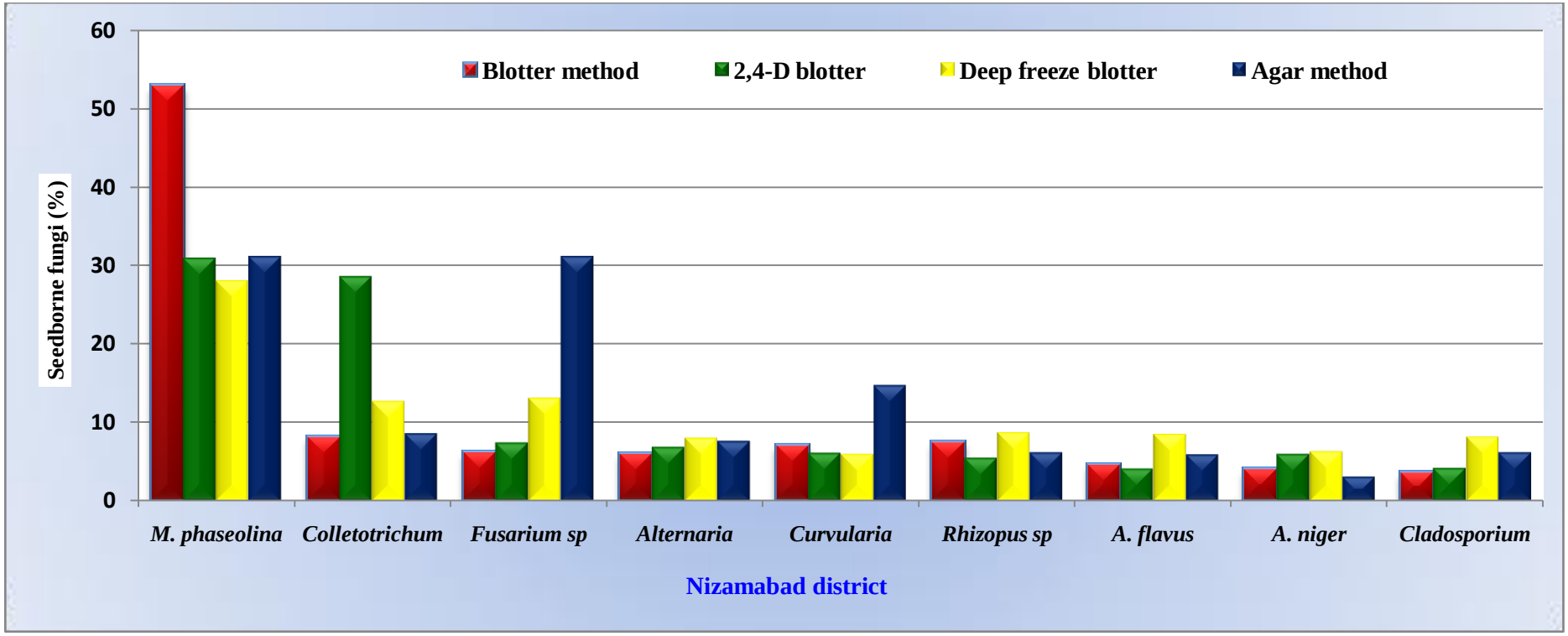

Figure 4.2. Per cent occurrence of seedborne fungi in soybean seed samples by different detection methods (Nizamabad district)

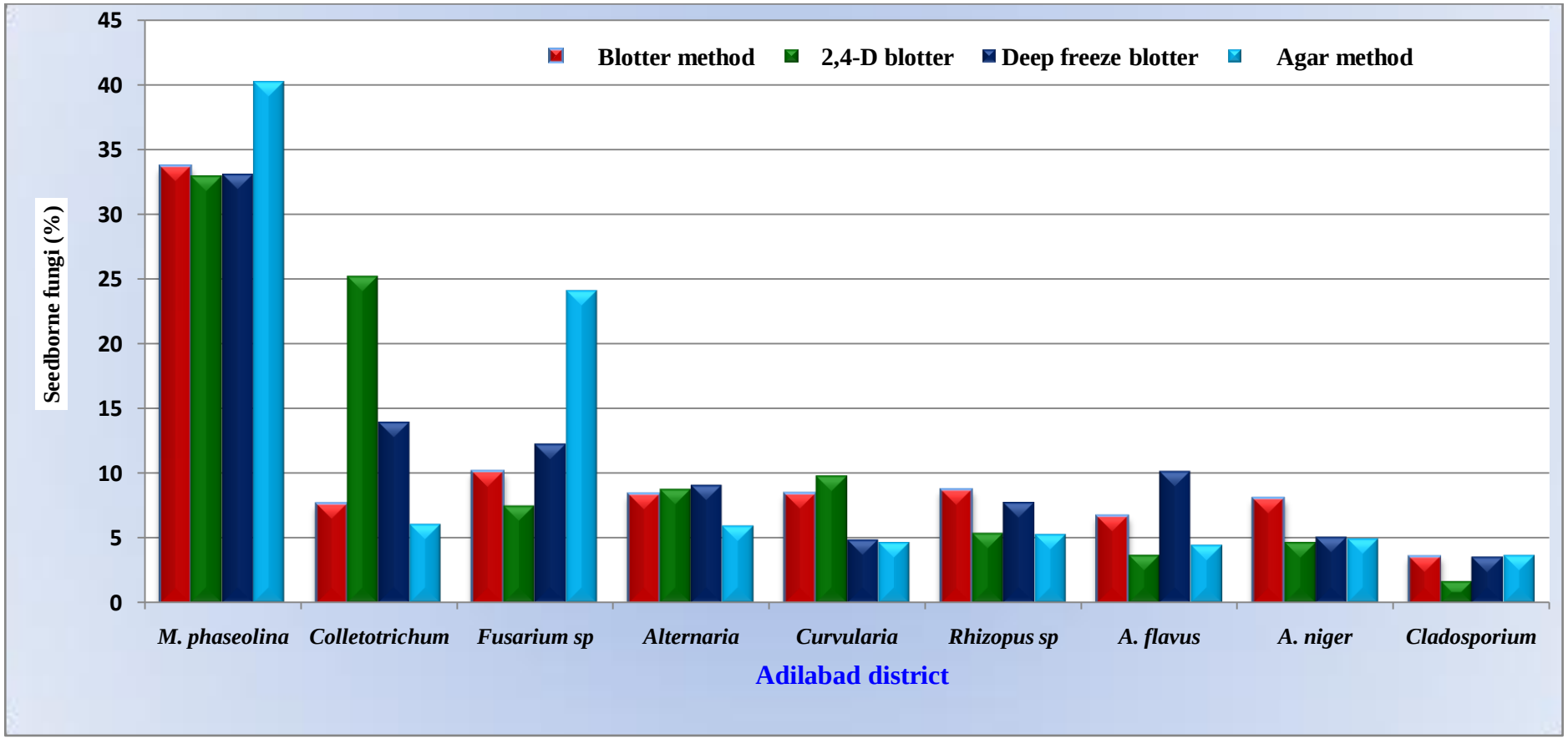

Figure 4.3. Per cent occurrence of seedborne fungi in soybean seed samples by different detection methods (Adilabad district)

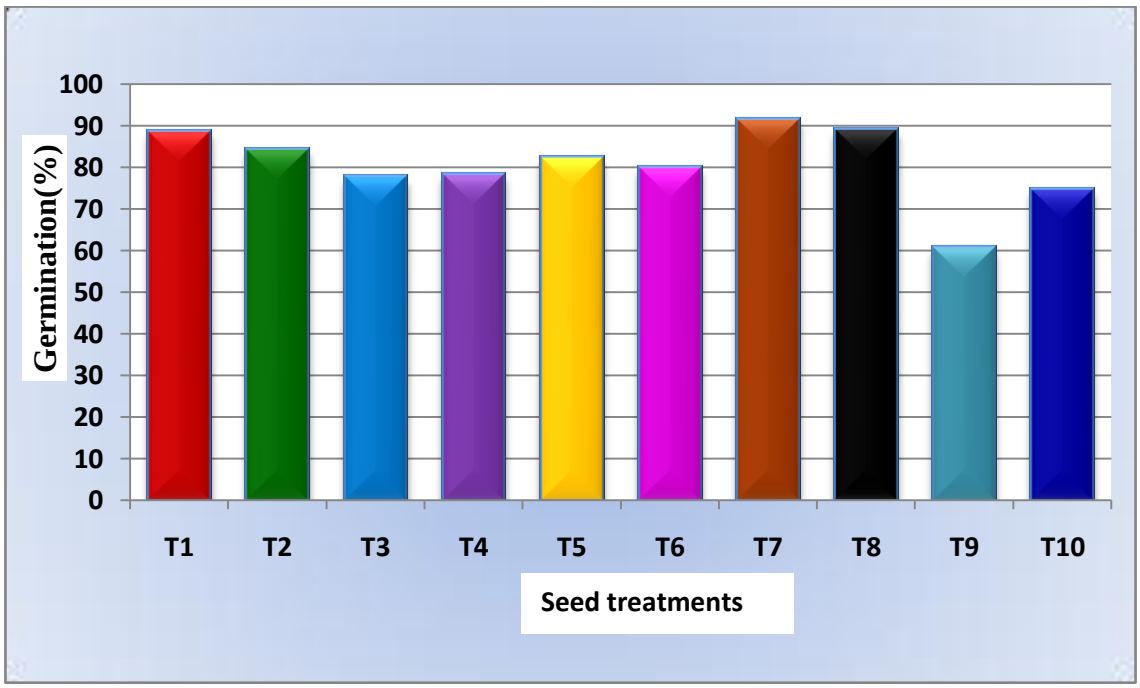

Figure 4.8. Evaluation seed treatments with fungicides, bioagents, botanicals and irradiations against seed borne *M. phaseolina* on germination of soybean under glass house conditions

T_1 - *Trichoderma viride* (10 g kg^{-1} seed)
T_2 - *Pseudomonas fluorescens* (10 g kg^{-1}seed)
T_3 - Irradiation dose (1.5 kGy)
T_4 - Irradiation dose (2.5 kGy)
T_5 - NSKE (neem seed kernel extract) 5%.
T_6 - Neem leaf powder @ 5g kg^{-1} seed
T_7 - Thiram + carbendazim 1:1 @ 3 g kg^{-1}
T_8 - Vitavax power 200 @ 2.5 g kg^{-1}
T_9 - Treated seeds (pathogen)
T_{10} - Control (untreated seeds)

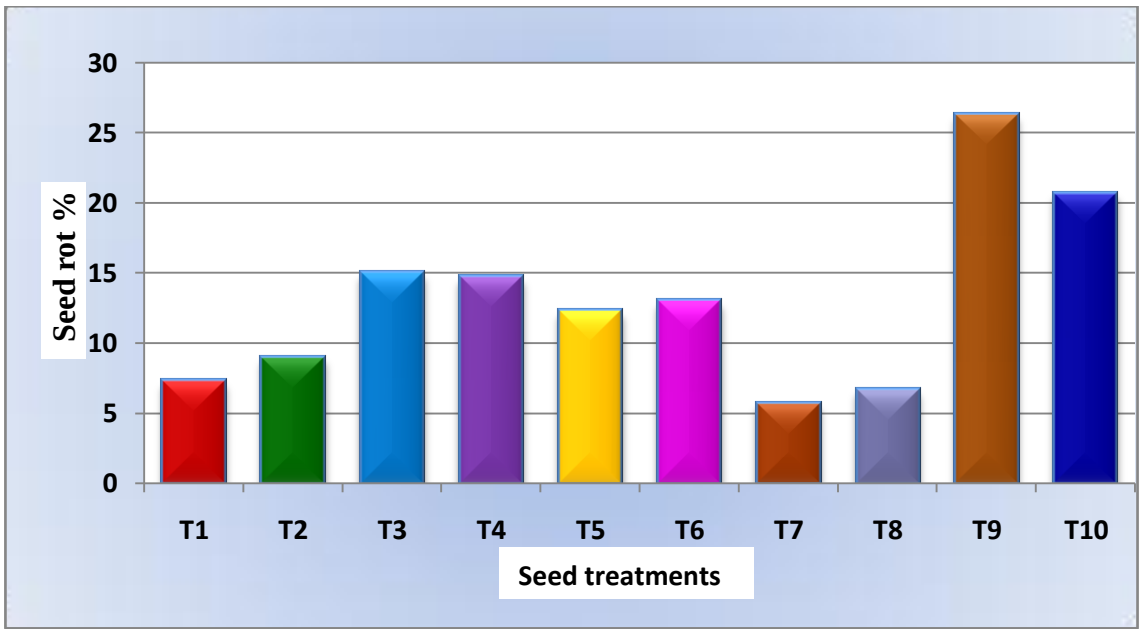

Figure 4.9. Evaluation of seed treatments with fungicides, bioagents, botanicals and irradiations against seed borne *M. phaseolina* on seed rot under glass house conditions

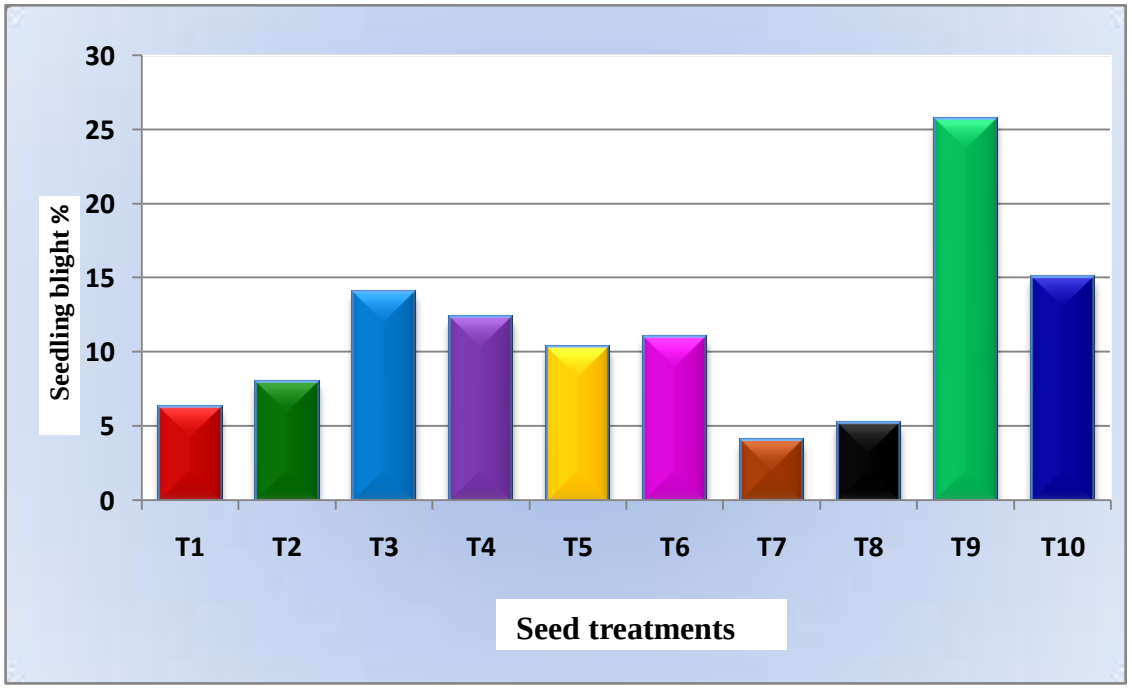

Figure 4.10. Evaluation seed treatments with fungicides, bioagents, botanicals and Irradiations against seed borne *M. phaseolina* on seedling blight under glass house conditions

T_1- *Trichoderma viride* (10 g kg^{-1} seed)
T_2- *Pseudomonas fluorescens* (10 g kg^{-1}seed)
T_3- Irradiation dose (1.5 kGy)
T_4- Irradiation dose (2.5 kGy)
T_5- NSKE (Neem Seed Kernel Extract) 5%.
T_6- Neem leaf powder @ 5g kg^{-1} seed
T_7- Thiram + carbendazim 1:1 @ 3 g kg^{-1}
T_8- Vitavax power 200 @ 2.5 g kg^{-1}
T_9 - Treated seeds (pathogen)
T_{1O}- Control (untreated seeds)

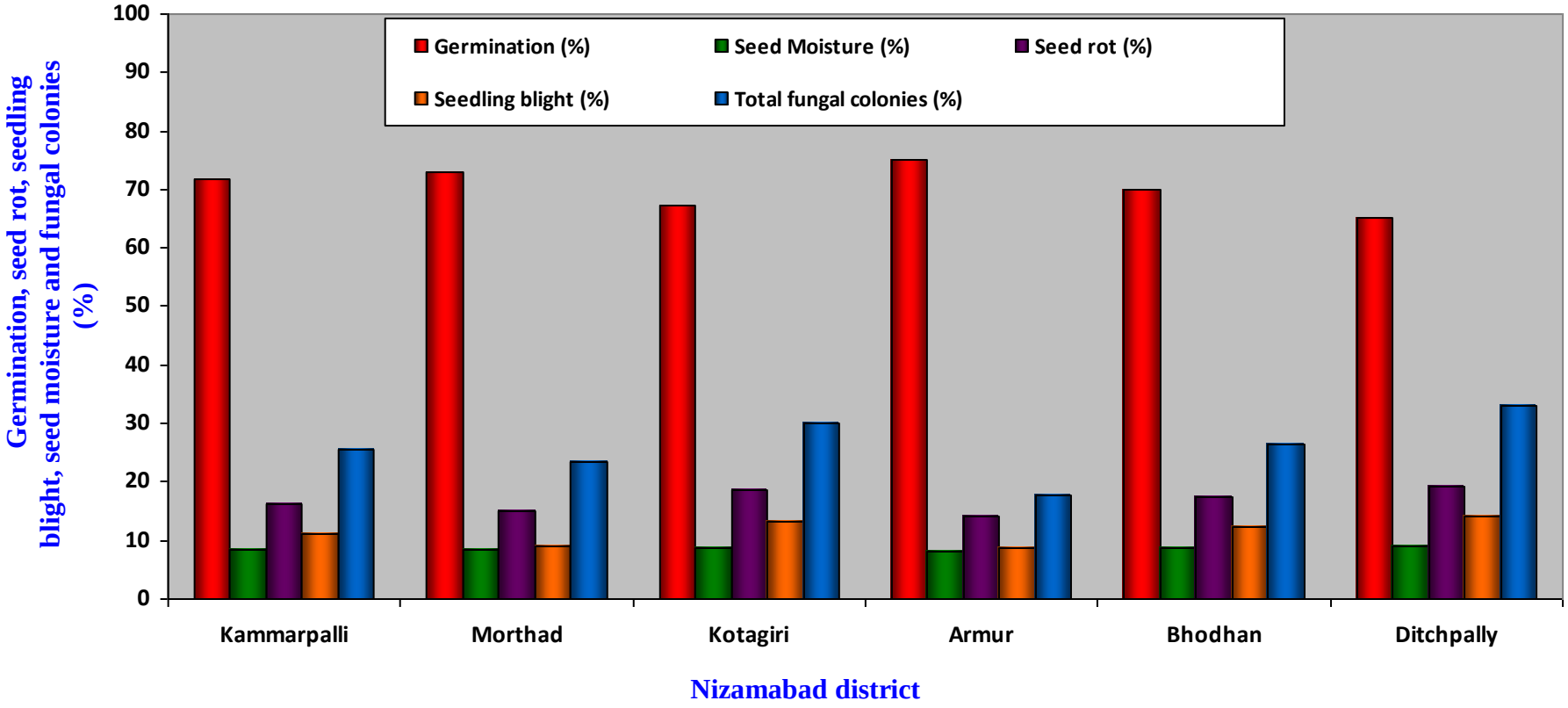

Figure 4.4. Assessment of seed quality parameters of soybean seed samples collected from different Mandals Nizamabad district, A.P during 2012 - 13

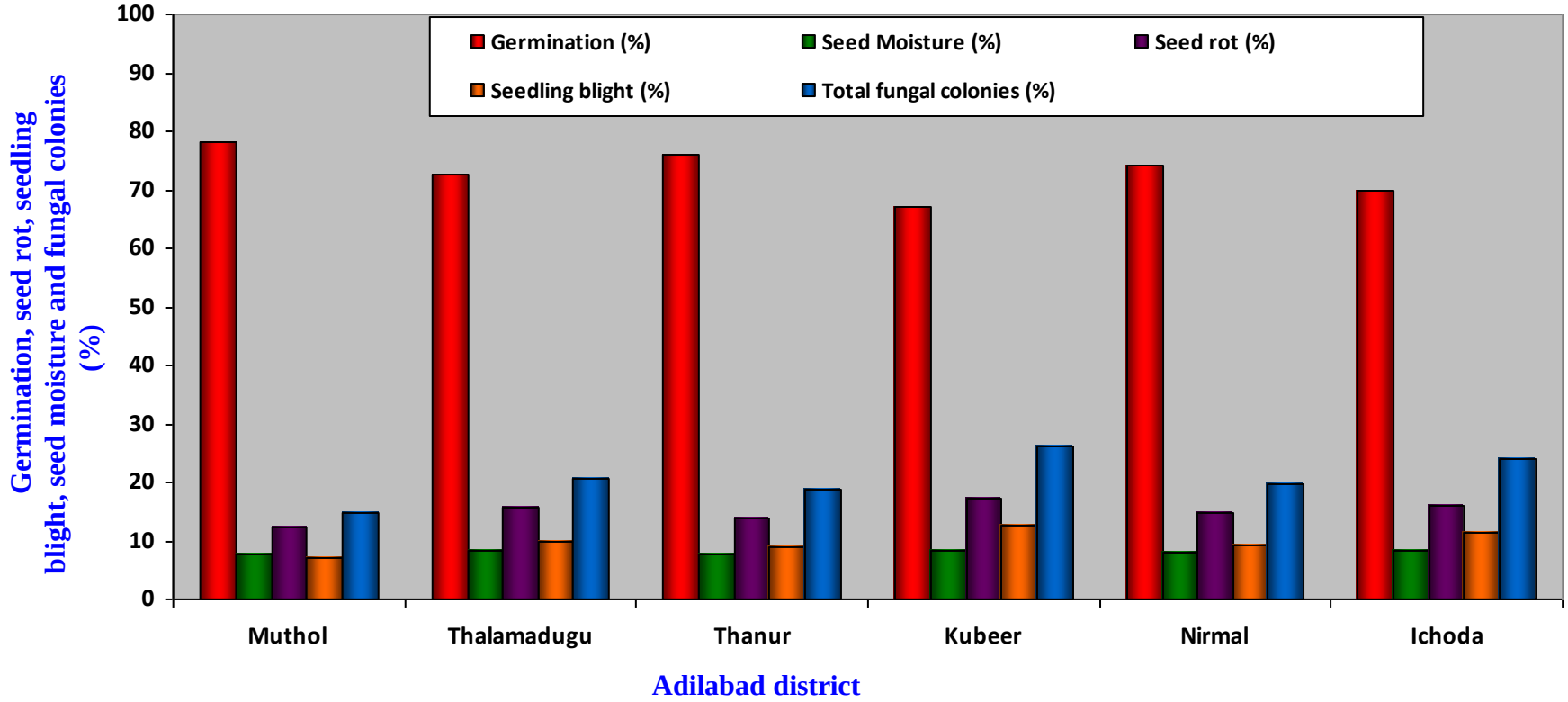

Figure 4.5. Assessment of seed quality parameters of soybean seed samples collected from different mandals of Adilabad district, A.P during 2012 - 13

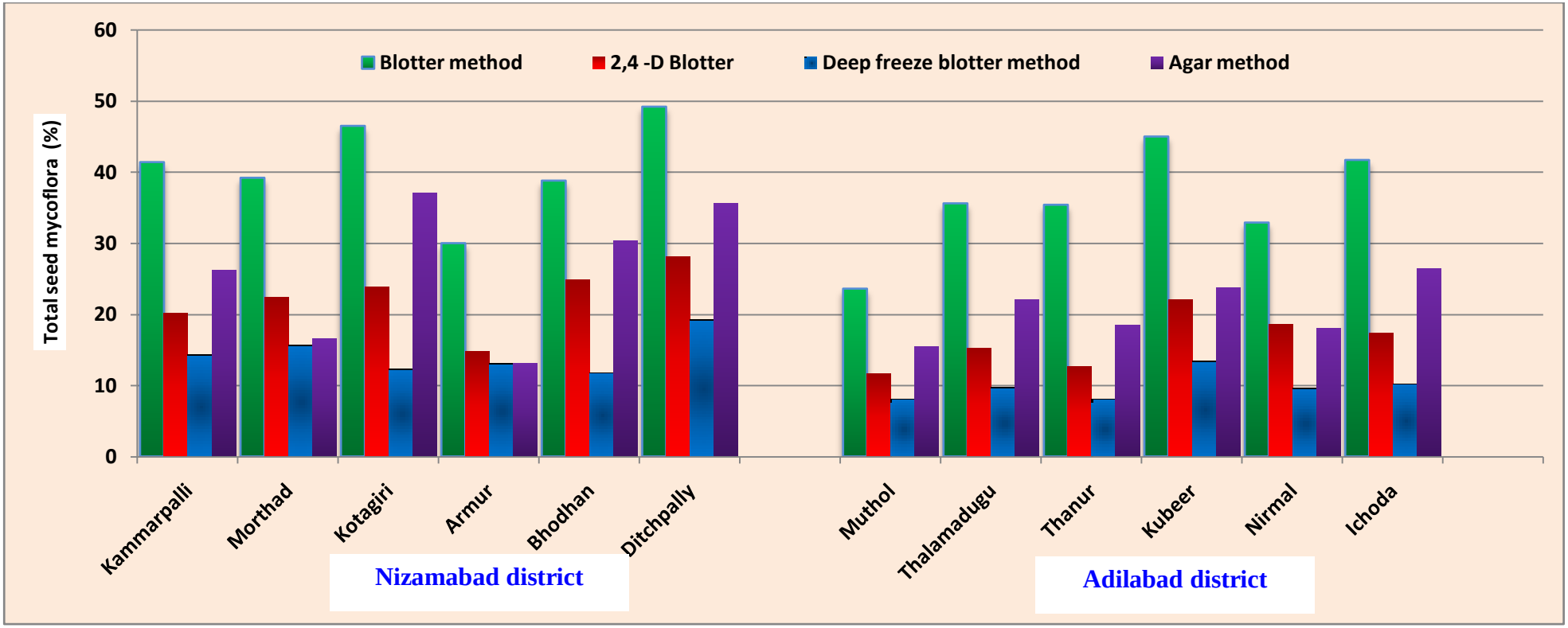

Figure 4.1. Total seed mycoflora detected in soybean seed samples collected from different Mandals of Nizamabad and Adilabad district of Andhra Pradesh

Chapter V

SUMMARY AND CONCLUSIONS

Soybean is an important oil seed and pulse crop and the most likely solution for overcoming the world's protein hunger. The major constraint in profitable soybean cultivation is the susceptibility to dry root rot / charcoal rot caused by *M. phaseolina*. It is one of the important diseases of soybean and is known to infect the crop at different growth stages from seedling to maturity. The pathogen is known to be a potential threat to soybean production because of its seedborne nature. Keeping this in view, the present investigation was taken up to assess the seed mycoflora and to study the implication of seedborne nature of *M. phaseolina* on seed quality and to find out the suitable detection methods for seedborne *M. phaseolina*. Studies on seed to seedling transmission were also made under *in vitro* and *in vivo* conditions. Evaluation of seed treatments involving fungicides, bioagents, botanicals and irradiations against seedborne *M. phaseolina* was tested under glasshouse conditions. The results of the present investigation are summarized below:

A total of one hundred and twenty (120) soybean seed samples of (cv. JS - 335) were collected from major soybean growing districts of Andhra Pradesh *viz.*, Nizamabad district (60 Nos.) comprising of Kammarpalli, Morthad, Kotagiri, Armur, Bhodhan and Ditchpally Mandals and sixty seed samples from Adilabad district comprising of Muthol, Thalamadugu, Thanur, Kubeer, Nirmal and Ichoda Mandals. The seed samples were analysed by standard blotter paper method, 2, 4 - D blotter paper method, deep freeze blotter paper method and agar plate methods as per ISTA (1996).

Soybean seed samples of Nizamabad and Adilabad district were tested by blotter method to record the prevalence of seedborne fungi. Significant differences were recorded in total fungal colonies with respect to location and source of seed samples. A total of nine fungi were observed. Pathogenic fungi *viz., Macrophomina phaseolina, Colletotrichum dematium, Curvularia* sp. *Alternaria*, *Cladosporium* and *Fusarium* sp. and storage / saprophytic fungi like *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* were observed. The occurrence of seedborne *M. phaseolina* was found predominant (8.5 to 28.5 %) while occurrence of *Cladosporium* (0.5 to 2.3%) was least in both the districts. Total seed mycoflora in the samples of Nizamabad and Adilabad districts ranged from 30 % to 49.2 % and 23.6 % to 45.0 %, respectively. Seed samples from Ditchpally Mandal of Nizamabad district and Kubeer Mandal of Adilabad district recorded more total fungal colonies (49.2 %) and (45.0 %), respectively. Whereas least

number of total fungal colonies were observed in Armur Mandal (30%) and Muthol Mandal (23.6 %) of Nizamabad and Adilabad districts.

Seed samples analysed by 2, 4 - D blotter method recorded similar type of seed mycoflora as like in the blotter method. The occurrence of *M. phaseolina* was found predominant (4 % to 9.5 %) while least occurrence of *A. flavus* sp. (0.5 to 2.5 %) and *Cladosporium* sp. (0.3 to 0.5%) from both the districts were observed. Seed mycoflora in Nizamabad and Adilabad districts ranged from 14.8 % to 28.1 % and 11.6 % to 22.1 %, respectively. Mean total fungal colonies were high in the samples collected from Ditchpally Mandal of Nizamabad district (28.1%) and Kubeer Mandal of Adilabad district (22.1 %). Whereas least number of fungal colonies were observed in Armur (14.8%) and Muthol (11.6 %) Mandals of Nizamabad and Adilabad districts.

The results of seed samples analysed by deep freeze blotter method were similar as observed in blotter and 2, 4 - D blotter methods. The occurrence of *M. phaseolina* was found predominant (3 % to 6.5 %). Total seed mycoflora in the samples of Nizamabad and Adilabad districts ranged from 11.8 % to 19.3% and 9.5% to 16.2%, respectively. Mean total fungal colonies were high in Ditchpally Mandal (19.3 %) of Nizamabad district and Kubeer Mandal of Adilabad district (16.2 %. The occurrences of *Curvularia* (0.5 to 2 %) were recorded least in the seed samples of Nizamabad and Adilabad districts.

Seed mycoflora associated with samples of Adilabad and Nizamabad districts were analysed by agar plate method. The occurrence of *M. phaseolina* was found predominant (3.5 % to 14 %) while least occurrence of *Cladosporium* sp. (0.5 to 1 %) was recorded. Total seed mycoflora ranged from 13.1 % to 37 % and 15.4 % to 26.4 %, respectively in both Nizamabad and Adilabad districts. The total fungal colonies were found high in Kotagiri Mandal (37%) of Nizamabad district and Ichoda Mandal (26.4 %) of Adilabad district**,** whereas least number of fungal colonies was observed in the samples of Armur (13.1 %) and Muthol Mandals (15.4 %) of Nizamabad and Adilabad districts.

Of the four methods adopted for detection of seed mycoflora, standard blotter method was found superior in recording the total number of fungal colonies of all the seed mycoflora than 2, 4 – D blotter, deep freeze blotter method and agar plate methods.

Irrespective of mandals and districts, significant variation in mean seed germination was observed. Mean seed germination ranged from 70.4 % - 73.1% and Mean seed vigour index I ranged from 1697 to 1821 and mean seed vigour index II

ranged from 68.8 to 82.2. Seed samples collected from Ditchpally and Kotagiri mandals of Nizamabad district and Kubeer Mandal of Adilabad district recorded germination below minimum seed certification standards of < 70 %. Irrespective of locations, seed samples exerted a significant variation in recording seed germination and seed vigour. The per cent seed germination, seed vigour I and seed vigour II was highest in the seed samples of Armur Mandal (75 %, 2013, 85) and Muthol Mandal (78.3 %, 2194, 104.5) and it was least in Ditchpally Mandal (65.3 %, 1394, 57) and Kubeer Mandal (67%, 1470, 65.2) of Nizamabad and Adilabad districts. Decreased seed germination and vigour was observed in seed samples with increased total fungal colonies. Seed samples which recorded higher fungal colonies recorded less germination and seedling vigour. Seed borne fungi in different soybean samples are known to produce seed rot, seedling blight and root rot diseases which decrease the quality and quantity of soybean seeds besides causing germination failures. Seed rot and seedling blight was recorded high in Ditchpally Mandal of Nizamabad district (19.2 % and 14.1 %) and Kubeer Mandal of Adilabad district (17.3 % and 12.5 %).

Among different detection methods, standard blotter method was found superior in the recovery of *M. phaseolina***.** Out of nine fungal species, *M. phaseolina* was found predominant which was used for further studies. The pathogen appeared as greyish mycelial growth on the incubated seeds. Pathogenicity test of seed borne *M. phaseolina* was proved by seed inoculation method. Seed inoculation of soybean cv. JS - 335 recorded 90-95% death of seedlings. The pathogen was reisolated and compared with the original isolate.

In test tube water agar method, mean seed transmission of *M. phaseolina* in soybean (cv. JS - 335) in apparently healthy seeds was 6.0%, artificially inoculated seeds (38.5%) and naturally infected seed samples (32%) and recorded germination of 75%, 55% and 59.3 % in the above samples, respectively.

Seed transmission of *M. phaseolina* in soybean (cv. JS - 335) under glasshouse conditions revealed that significant differences in seed rot, seedling blight and per cent emergence of seedlings was observed at 35 days after sowing. Mean seed transmission of apparently healthy seeds (8 %), artificially inoculated seeds (49%) and naturally infected seed samples (43.1%) and per cent seedling emergence of 72%, 46% and 50.5% was observed.

Seed treatments involving fungicides (thiram + carbendazim @ 3 g kg^{-1}, vitavax power 200 @ 2.5 g kg^{-1}), bioagents (*T. viride* @10 g kg^{-1} and *Pseudomonas fluorescens*

@10 g kg^{-1}), botanicals NSKE (Neem Seed Kernel Extract @ 5 % and neem leaf powder @ 5g kg^{-1}) and (irradiation dose of 1.5 k Gy and 2.5 k Gy) were evaluated against seedborne *M. phaseolina* under glasshouse conditions. The results revealed that, soybean seeds treated either with thiram + carbendazim or vitavax power 200 significantly superior in recording higher seed germination (91%, 89%) with less seed rot (5.7%, 6.7%) and seedling blight (4.0 %, 5.2 %) which was found on par with seeds treated with *T. viride* which recorded 88 % germination, seed rot 7.3% and seedling blight 6.3%. The remaining seed treatments were also effective in improving seed germination and reducing seed rot and seedling blight as compared to untreated seed (74%, 20.7% and 15.0%) and pathogen treated seed (60 %, 26.3 % and 25.7 %), respectively.

The following conclusions have been drawn from the investigations are as follows.

- Soybean seed samples collected from Nizamabad district recorded maximum total number of fungal colonies with reduced seed germination and seedling vigour as compared to the seed samples analysed from Adilabad district.
- Seedborne fungi present in soybean seed samples produced seed rots, seedling blights and decreased quality and quantity of soybean seed samples besides causing germination failures.
- Standard blotter method was found superior in recording higher number of fungal colonies than agar plate method 2, 4 – D blotter, deep freeze blotter methods.
- Standard blotter method was efficient in isolation of seed borne *M. phaseolina* over agar plate, 2, 4 - D blotter and deep freeze blotter method.
- Seed transmission of *M. phaseolina* in soybean (cv. JS-335) was found high in artificially inoculated and naturally infected seeds over apparently healthy seeds in test tube water agar and in glasshouse conditions.
- Soybean seeds treated with thiram + carbendazim or vitavax power 200 followed by bioagent *T. viride* were found effective in improving seed germination and reducing seed and seedling diseases due to seed borne *M. phaseolina*.

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