

**STUDIES ON ANTHRACNOSE OF  
HORSEGRAM (*Macrotyloma  
uniflorum*[Lam.] Verdc.)**

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**B. Sc. (Ag.)**

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(PLANT PATHOLOGY)**



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**STUDIES ON ANTHRACNOSE OF  
HORSEGRAM**  
*(Macrotyloma uniflorum[Lam.] Verdc.)*

**By**

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**CHAIRPERSON: Dr. K.V.S. MEENA KUMARI**



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**2013**

**CERTIFICATE**

Mr. A UDAYA SANKAR has satisfactorily prosecuted the course of research and that thesis entitled “**STUDIES ON ANTHRACNOSE OF HORSEGRAM** (*Macrotyloma uniflorum*[Lam.] Verdc.)” 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.

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Place: Hyderabad

Chairperson

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This is to certify that the thesis entitled “**STUDIES ON ANTHRACNOSE OF HORSEGRAM (*Macrotyloma uniflorum* [Lam.] Verdc.)**” submitted in partial fulfilment 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 A UDAYA SANKAR** 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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## **DECLARATION**

I, **A UDAYA SANKAR** hereby declare that the thesis entitled “**STUDIES ON ANTHRACNOSE OF HORSEGRAM (*Macrotyloma uniflorum*[Lam.] Verdc.)**” submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of 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

**(A UDAYA SANKAR)**

Date:

**I. D. No: RAM/10-56**

## LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
°C	Degrees Celsius
@	at the rate of
±	Plus or minus
<	Less than
>	Greater than
=	is equal to
/	per
i.e.,	that is
h.	Hour
etc.	and so on
cv.	Cultivar
µm.	Micro meter
M ha.	Million hactares
viz.,	Namely
Kg ha <sup>-1</sup>	Kilograms per hactare
<i>et al.</i>	and others
ml.	Milli litre
S. No.	Serial Number
CD	Critical Difference
SE(m)	Standard Error of mean
No.	Number
min.	Minute
g	Gram
PSI	Pounds Square Inch

PDA	Potato Dextrose Agar
CAM	Carrot Agar Medium
PDI	Per cent Disease index
2,4 -D	2,4 dichloro phenoxy acetic acid
NaOCl	Sodium hypochlorite
MgSO <sub>4</sub>	Magnesium sulphate
CMIE	Centre for Monitoring Indian Economy
SMIE	Seed Management Information system
NBPGR	National Bureau of Plant Genetic Resources

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## **ABSTRACT**

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc. Syn. *Dolichos biflorus*) is a hardy, drought resistant, under exploited annual legume, grown extensively in peninsular India as poor man's pulse crop. Sporadic reports of occurrence of anthracnose disease in horsegram have been reported from India and it can cause infection to an extent of 68% in horsegram. Although anthracnose is considered as a minor disease of horsegram, it may attain the status of major disease in future due to the effect of climate change at global level. As there is meagre information available on etiology and sources of resistance against anthracnose of horsegram in Andhra Pradesh, the present studies were undertaken to understand the seed borne nature of the pathogen along with its preventive management.

*Colletotrichum dematium* was isolated from horsegram leaves suspected with anthracnose symptoms and pure culture of it was maintained for conducting further studies. The pathogenicity of *C. dematium* was proved by following seed inoculation, detached leaf technique and pot culture studies.

*C. dematium* infection was noticed in all parts of the horsegram seed, i.e., seed coat, endosperm (cotyledons) and embryo in Component plating technique with varied levels of intensity. Seed coat infection was the highest, ranged from 62-100%, followed by cotyledonary infection (36-72%) and embryo infection (30%). This indicates that the pathogen is both externally and internally seed borne. Seed to seedling transmission of the fungus, *C. dematium* was successfully proved using paper towel method, sand method and grow-out test methods. Paper towel method resulted in higher seed rot (34%) followed by sand method (12%) and grow-out test (10%). The Seedling decay symptoms were observed to a range of up to 20%.

Horsegram accessions (21 genotypes and 4 released varieties) were screened in the laboratory by seed inoculation using standard blotter method using AK-21 as resistant check. Three accessions (AK-21, IC 470275 and IC 470259) were found resistant by recording < 10% seed surface area infected. Four accessions (IC 343900, IC 343830, IC 526968 and Palem-2) were found highly susceptible with >50% of percent seed area infected with the fungus. Among the 25 accessions tested, 12% of the accessions fall under resistant category, 36% each under susceptible and moderately resistant categories and the remaining 16% under highly susceptible category.

Greenhouse studies of germplasm screening yielded one Immune accession, IC 470275 (NS/05-113) with zero Percent disease index (PDI). None of the accessions was found Resistant. Eleven accessions (IC 470259, IC 470255, IC 470272, IC 470311, IC 526968, IC 470171, IC 470263, IC 470278, IC 470176, IC 261287 and PALEM-1) were grouped under Moderately Resistant category and eight accessions under

susceptible category. The remaining five (AK-21, AK-26, IC 343830, IC 343900 and IC 257875) were Highly Susceptible with  $> 50$  PDI. It was observed that 4, 44, 32 and 20% of the accessions were grouped under immune, moderately resistant, susceptible and highly susceptible categories, respectively based on their PDI values.

The present investigation resulted in identifying an immune horsegram genotype, IC 470275 (NS/05-113), which can be used in the future crop improvement programmes to develop a suitable variety for growing in Andhra Pradesh region.

*Chapter I*

**INTRODUCTION**

## Chapter I

# INTRODUCTION

Horsegram (*Macrotyloma uniflorum* (Lam.) Verde. Syn. *Dolichos biflorus*), commonly known as kulthi, is one of the hardiest and drought tolerant crops, grown extensively in peninsular India as poor man's pulse crop. It is an under exploited grain legume with great potential in sustainable agriculture as it enriches soil considerably by fixing atmospheric nitrogen and increasing the organic matter of soil. It is the only choice crop of the farmers for delayed sowing due to late receipt of rains. Being a low input crop, it suits Indian farmers having small and marginal land holdings.

Horsegram seeds are rich in protein (23%) and its nutritional value is comparable with other pulses. Fodder of horsegram contains 10.5% protein, 44.8% crude fibre and is widely used as a feed to animals, especially horses. Besides its nutritional importance, it has several medicinal values and the medicinal potency of the crop was well reviewed by Prashanthi *et al.* (2011). Horsegram lipids were shown to be protective and promote healing effects on acute gastric ulceration produced by alcohol (Jayaraj *et al.* 2000) and also known to dissolve and dislocate the kidney stones in human beings (Chahota *et al.* 2005).

In India, horsegram is grown in an area of 0.51 M ha. and the major horsegram producing states are Karnataka, Orissa, Chhattisgarh, Tamil Nadu and Andhra Pradesh. During 2010-2011, Andhra Pradesh recorded an average seed yield of 432 kg ha<sup>-1</sup>, which is far below than that of other states, *viz.*, Bihar (980 kg ha<sup>-1</sup>), Jharkhand and Uttarakhand (820 kg ha<sup>-1</sup>), Karnataka (606 kg ha<sup>-1</sup>), Himachal Pradesh (510 kg ha<sup>-1</sup>) etc. as compared to the national average of 518 kg ha<sup>-1</sup> (CMIE, 2012). One of the main reasons for low yield may be attributed to diseases as the crop is susceptible to several diseases such as powdery mildew, anthracnose, leaf spots, dry root rot and yellow mosaic disease etc.

Anthracnose caused by *Colletotrichum* species, is one of the most important diseases in various leguminous crops, *viz.*, cowpea, soybean, french bean, faba bean etc including horsegram. The pathogen is known to infect leaves, stems and pods and can cause considerable yield losses. Earlier, several researchers reported the incidence of anthracnose infection to an extent of 68 per cent in horsegram (Rangaswami *et al.* 1991). Saharan (1979) reported that the disease is responsible for reduced seed

germination and crop stand upto 65 per cent in Himachal Pradesh, India. Although horsegram anthracnose is a disease of minor importance in Andhra Pradesh as of now, it may attain major status within no time due to the effects of global warming and climate change.

The causal organism responsible for anthracnose disease was coined differently by various workers all over the world as *Glomerella lindemuthianum* (Butler, 1918), *Colletotrichum dematium* (Neergaard, 1977), *C. truncatum* (Holliday, 1995) and *C. lindemuthianum* (Brink and Belay, 2006) etc. In India, *C. lindemuthianum* (Sharma, 1976), *C. capsici* (Pangtey and Sinha, 1980), *C. dematium* f.sp. *truncatum* (Bharadwaj and Singh, 1986), *C. dematium* (Murthy, 1997) etc. were identified as pathogens responsible for anthracnose disease by different workers. Thus, there is a gap in understanding the etiology of this disease in horsegram.

The pathogen is seed borne in chilli, soybean and many other leguminous crops. As this pathogen has wide host range, the disease spread among the commonly grown pulse crops is always possible. The fungus survives on infected seeds and on the crop debris for considerable length of time. Pangtey and Sinha (1980) observed seed infection upto 16-55 per cent in horsegram, while Murthy (1997) recorded seed infection up to 12.25 per cent in seeds collected from Karnataka. In beans, the primary infection is through infected seed and secondary infection by rain splash and wind within the crop (Minchinton, 1999). Thus, studying the seedborne nature and seed transmissibility, and identification of alternate pulse crop that can withstand the pathogen attack etc. play a key role.

It is a prerequisite for any crop improvement programme to identify location specific cultivars that possess superior traits such as disease resistance, higher yield etc. In India, research on identification of sources of resistance to anthracnose disease in horsegram is confined to northern region especially, Himachal Pradesh (Chahota *et al.* 2005), while Murthy (1997) identified few resistant lines of horsegram in Karnataka. Perusal of literature indicates that till date, research has not been oriented towards identifying a horsegram cultivar that is resistant/tolerant to anthracnose disease and suitable for growing in Andhra Pradesh and adjoining areas.

Keeping the above aspects in view, the present investigation was undertaken with the following objectives:

## **OBJECTIVES**

1. To isolate, identify and prove pathogenicity of fungus associated with anthracnose in horsegram.
2. To detect the location of pathogen infection in different parts of horsegram seed.
3. To determine the seed transmissibility of the fungus from infected horsegram seed to seedling.
4. To screen horsegram genotypes against anthracnose disease.

*Chapter II*

**REVIEW OF LITERATURE**

## Chapter II

# REVIEW OF LITERATURE

The literature available on anthracnose disease incidence on horsegram and related crops, isolation of pathogen and proving its pathogenicity, location of pathogen on different seed parts, extent of seed transmission, and varietal screening for disease resistance was reviewed and compiled in this chapter. Since the information on horsegram crop on the above aspects is very meagre, literature on related crops was also reviewed and compiled.

### 2.1 Status of disease incidence

Butler (1918) reported the occurrence of anthracnose disease on horsegram (*Dolichos biflorus*) for the first time and identified *Glomerella lindemuthianum* (Sacc. & Magn) as responsible pathogen. Sharma (1976) also found *Colletotrichum lindemuthianum* causing anthracnose disease on *D. biflorus* in Himachal Pradesh, India.

Neergaard (1977) reported that *Dolichos biflorus* was susceptible to anthracnose disease caused by *Colletotrichum dematium* (Pers. ex Fr.) Groove. Saharan (1979) studied the seed borne nature of *Colletotrichum truncatum* on horsegram seeds collected from Solan and Kangra districts of Himachal Pradesh. Both apparently healthy and abnormal seeds (wrinkled, shrivelled, discoloured with fungal growth, deformed and small sized) of horsegram were plated separately on moist blotters and on Potato dextrose agar plates to record the incidence of seed mycoflora. Apparently healthy seeds yielded higher counts of *C. truncatum* and some other fungi as compared to those abnormal seeds when placed both on potato dextrose agar (PDA) and blotter.

Pangtey and Sinha (1980) reported that *Colletotrichum capsici* (Syd.) Butler and Bisby was responsible for leaf spot disease on horsegram in Kumaun hills of Uttar Pradesh, India.

Bharadwaj and Singh (1986) conducted a disease survey of pulse crops in Kangra valley of Himachal Pradesh and recorded the incidence of leaf spot on horsegram due to *C. dematium* f.sp. *truncatum*. The symptoms appeared as water soaked circular spots with a greenish tinge. Later these spots turned into reddish brown with dark brown margins. The acervuli, which form abundantly were visible to naked eye and appeared mostly on lower surface.

## 2.2 Pathogen isolation and proving pathogenicity

Saharan (1979) studied different methods of seed health testing *viz.*, standard blotter method, standard agar plate method, deep freezing method and potato dextrose agar technique for the detection of seedborne pathogens on horsegram seeds. He found that standard blotter method was more useful in detecting pathogens.

Pangtey and Sinha (1980) studied the symptoms caused by *C. capsici* on horsegram that were evident as reddish brown, irregular leaf spots that were mostly confined to the upper surface of leaflets. The centre of the spots became yellowish brown and later turned necrotic. A large number of acervuli with long setae appeared on the under surface of leaflets in areas corresponding to the spots on the upper surface. Pathogenicity tests were conducted by sowing the naturally infected seeds in open field and glasshouse conditions. Also, the artificially inoculated seeds were sown in sterilized soil under glasshouse conditions. It was reported that under all the three conditions, the pathogen caused infection and the per cent disease incidence of 24.84, 10.26 and 7.18, respectively was recorded.

Kumaraswamy (1983) isolated *Colletotrichum* spp. from infected leaves of horsegram in Karnataka by using single spore isolation method. He described the conidia and setae of *Colletotrichum* spp. as curved or sickle shaped, hyaline, having guttule at the centre. Setae production was high, moderately longer, sparse, with a bulbous base and tapering ends, walls dark and thick with 3-5 septa and measured 20.5-33.8 x 4.7- 4.8  $\mu\text{m}$ . in size. Pathogenicity test was conducted by inoculation of spore suspension on horsegram leaves and the inoculated plants were covered with polythene bags to provide more humidity and kept at room temperature for 15 days.

Prasanna (1985) conducted studies on cowpea anthracnose caused by *C. lindemuthianum* and found that standard blotter method was most suitable as it provided sufficient moisture to the seed to imbibe and germinate. Detection of pathogen infection on or under the seed coat, cotyledons and embryo was easier with the production of more acervuli and meagre mycelial growth on infected seed. In agar plate method pathogen counts were lowest as the saprophytic and other fast growing fungi interfered in examination. The mycelial growth was more on potato dextrose agar without acervuli.

Renukeswarappa and Shethna (1985) found an improved blotter method of soaking chilli seeds in 5 per cent calcium chloride ( $\text{CaCl}_2$ ) for 24 hours before plating to detect *C. dematium*. By this method, 50 per cent more incidence of *C. dematium* was

recorded as compared to the untreated seeds. The incidence of saprophytic fungi i.e. species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Curvularia* was significantly reduced in the improved method. They suggested that this method could be followed for accurate scoring of chilli seeds as it had no deleterious effect on germination. The efficacy of  $\text{CaCl}_2$  in enhancing fungal growth and sporulation was also proved using agar plate method. It was opined that the traditional standard blotter method obscures the expression of the pathogen, *C. dematium* due to the growth of saprophytic seed borne fungi.

Bharadwaj and Singh (1986) conducted cross pathogenicity tests with four isolates of *C. dematium* f. sp. *truncatum* isolated from mungbean, horsegram, urdbean and soybean on the above hosts including cowpea (*Vigna unguiculata*) and adzuki bean (*Vigna umbellata*) in Himachal Pradesh. The results showed that all isolates differed from each other in their pathogenic behaviour. Horsegram and mungbean were found susceptible to all the four isolates and cowpea was found resistant to all the isolates. This differential pattern in the pathogenicity could be attributed to the existence of different pathogenic variants of *C. dematium* f. sp. *truncatum* in nature and their specific adaptation to the host species.

Kulkarni (2009) also conducted cross pathogenicity tests with *C. truncatum* isolate from greengram on eight other pulse crops including horsegram. It was revealed that only horsegram and blackgram showed positive reaction to the fungal isolate and all the remaining crops exhibited negative reaction indicating the host specificity nature of the isolate.

Rangaswamy *et al.* (1991) revealed that standard blotter method was an ideal option for detecting *Colletotrichum* spp. on horsegram and also to determine the seed health.

Nwachukwu and Umechuruba (1991) isolated *C. dematium* from African yam bean (*Sphenostylis stenocarpa*) seeds having varied seed coat types collected from two different places, viz., Afam and Kom-Kom in Nigeria, using standard blotter method developed by International Seed Testing Association. The recovery of *C. dematium* varied among the seed types from Afam and Kom-Kom, as the marble brown coloured seed had 4.8 and 2.3 per cent incidence, respectively, while the creamy white and smooth brown coloured seeds from both the areas did not yield the pathogen. In addition, the seeds pre-treated with 1% sodium hypochlorite for 5 minutes recorded very low incidence of 1 and 1.5%, respectively.

Asalmol *et al.* (2001) used standard blotter paper method to detect the seed borne fungi of chilli, especially *C. capsici* on 50 chilli seed samples of different cultivars, collected from Maharashtra State Seed Corporation, Akola. It was observed that four of the 7 cultivars recorded the *C. capsici* incidence and maximum of 74.75 % incidence was recorded on the local sample. Maximum seed mortality (88%) was caused by *C. capsici* as compared to the other seedborne fungi.

Singh *et al.* (2009) also found that standard blotter method was ideal for detection of *C. capsici* from seed, as the recovery was maximum over the other two methods *viz.*, agar plate method and deep freeze method. Maximum of 15% seed infection was recorded in untreated seeds as compared to 13% in seeds pre-treated with sodium hypochlorite. The fungus appeared as black acervuli on seed surface. Severely infected seeds, when split opened, showed deep infection, making the seed non-viable.

### **2.3. Location of pathogen infection in seed**

Hagborg *et al.* (1950) reported that 2, 4-dichlorophenoxy acetic acid was useful to prevent seed germination and facilitated evaluation and identification of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Sheer in bean seeds.

Puri *et al.* (1981) investigated the location of *C. dematium* in seven samples of chilli seeds with varied infection levels using component plating technique. Mycelium usually occurred in the seed coat including the thick walled epidermal cells and endosperm, while embryo invasion was seen only in heavily infected seeds. Abundant mycelium was seen in the parenchymatous cells of the seed coat and in the spaces between the endosperm and embryo, between the cotyledons and in the region of the comma stem of the endosperm.

Khare and Chacko (1983) confirmed the seed borne nature of *C. dematium* f.sp. *truncata* in soybean seed collected from diseased plants using blotter method, water agar method and sowing in pots with sterilized soil. The pathogen was isolated not only from diseased seed but also from hypocotyls of 5% - 8% of apparently healthy looking seedlings. They concluded that the pathogen occurred inside and outside the embryo leading to local and systemic transmission the disease.

Microtomy and component plating techniques conducted by Shivanna and Shetty (1988) revealed that *C. dematium* was located in the inner layer of the seed coat of cluster bean. Endosperm was found free of fungal mycelium and very few cotyledons showed the presence of the pathogen.

Haenglee and Supchung (1995) used component plating technique to investigate the location of *C. gloeosporioides* in infected red pepper seed. The infected seed collected from Korea were soaked in water for 24 hours and each seed was dissected into different parts, viz., seed coat, endosperm and embryo before incubation. Maximum colonisation (41%) was seen in seed coats followed by endosperm layers (36%). Seed transmission studies were conducted by placing infected seed in test tube containing 2% water agar. Histopathological observations of seedlings confirmed that *C. gloeosporioides* was transmitted from endosperm to the hypocotyls and radicles.

Basak (1998) conducted component plating technique to detect *C. capsici* infection on chilli seed collected from the Chittagong district, Bangladesh. The surface sterilized seed was dissected into different parts, viz., seed coat, endosperm and embryo and plated on water soaked blotters and incubated for 7 days. The fungus was recovered from all the three parts viz., seed coat (92%), endosperm (82%), and embryo (80%). Abundant fungal mycelium was found in the parenchymatous tissues of the endosperm and embryo, mostly concentrating on the intercellular spaces without disintegrating the cells.

Neergaard *et al.* (1999) artificially inoculated soybean plants of cv. SJ 5 with *C. truncatum* in Thailand and collected the seed samples and tested for seed infection. After soaking the seed in distilled water for 24 hours, the seed coat and embryo were separated and plated on a moist blotter paper. The plates were examined after 5 days for the presence of acervuli and found 89.1% infection on the seed coat alone and 10.9% in the embryo and seed coat. None of the seeds was infected exclusively in embryo and thus it was opined that the fungal colonisation of the seed had taken place through the seed coat by direct penetration and not through funiculus.

Component plating method conducted by Ravi *et al.* (1999) revealed that *C. lindemuthianum* was present mostly in seed coats and cotyledons, and rarely in embryonic axes of French bean.

Kumud Kumar *et al.* (2004) also conducted similar studies on chilli cv A-36, and reported that *C. dematium* was present on the seed coat of all the infected seeds. Embryo infection upto 31.25 per cent was recorded.

## 2.4. Seed transmission of the pathogen

Neergaard (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.

Shyam and Chakraborty (1985) studied the extent of seed transmission of *C. lindemuthianum* in four commercial cultivars of *Phaseolus vulgaris*, viz., White Panchamari, Dharmasala Local, Chamba Local and Kentucky Wonder from Himachal Pradesh using blotter paper method and grow-out test methods. In all the four cultivars, the seed transmissibility of the fungus was more in blotter paper method (11, 25, 18 and 13%, respectively) as compared to grow out test (6.6, 18.75, 12.5 and 7.69%, respectively).

Shivanna and Shetty (1988) collected seed samples from the infected cluster bean fields in Karnataka and observed mycoflora. The sample with 23.5% *C. dematium* incidence was used for transmission studies. Seed transmission was studied in controlled glasshouse conditions and in field. Transmission studies revealed that the seeds with 23.5% *C. dematium* showed 3% pre-emergence and 15% post-emergence mortality in sand method.

Kumud Kumar *et al.* (2004) conducted seed transmission studies of *C. dematium* in chilli cv A-36. The pathogen was transmitted to young seedlings through the seedborne inoculum by local contact giving a ratio of 8:1 in seed infection and seed transmission. The pathogen was never found systemic.

Singh *et al.* (2009) conducted transmission studies of *C. capsici* by sowing naturally infected chilli seeds with varied levels of infection in sterilized soil under controlled glasshouse conditions. They found that the pathogen got transmitted from infected seed to seedlings, which was evident through lack of germination or poor germination or development of seedlings with chlorotic lesions on cotyledons or on hypocotyl region. In severe cases, the stem got girdled and seedling got collapsed. The per cent seed infection was found to be positively correlated with the degree of pod area infected. Also, the infection was found to be transmitted from seed to seedling in an asymptomatic manner.

## 2.5. Screening for disease resistance

Tu (1986) used detached leaf technique for screening beans (*Phaseolus vulgaris* L.) in *in vitro* against anthracnose (*C. lindemuthianum*). The inoculum was brushed on the lower side of the excised leaves and leaflets. The inoculated leaves were placed,

inoculated side up, on several layers of wet paper towels in a tray and incubated at 22°C for 6 days for symptom development. The results showed that inoculation of excised primary leaves or leaflets of trifoliates provided differentiation between susceptible and resistant bean seedlings if spore concentration was higher than  $10^5$  spores  $\text{ml}^{-1}$ . Since disease severity in the susceptible cultivar increased with increasing spore concentration, the differentiation became more evident at higher spore concentrations. At  $10^7$  spores  $\text{ml}^{-1}$ , approximately 60% of vein area in the inoculated primary leaves or leaflets of trifoliolates was affected by the disease. On the contrary, virtually no disease lesions developed on the inoculated primary leaves or leaflets of trifoliolate of the resistant cultivar (Cornell 49-242) at any spore concentrations studied. The results also showed that leaflets taken from different growth stages of plants responded similarly to inoculation if they were excised immediately after being fully expanded.

Inglis *et al.* (1988) developed a simple method for screening beans against *C. lindemuthianum* under field conditions. Dry inoculum was prepared by from diseased leaves of greenhouse inoculated plants. The results showed that the dry inoculum was as effective as aqueous conidial suspension for inoculation on beans foliage.

Murthy (1997) screened 40 horsegram cultivars against *C. dematium* under field conditions in Karnataka and identified three cultivars AK-21, PHG-9 and CODB-2 as source of resistance to anthracnose. He also reported that 24 cultivars found moderately resistant, 12 cultivars showed as susceptible and one cultivar, D-634 as highly susceptible.

Rajan Sharma and Kaushal (1999) conducted host range studies on 11 isolates of *C. truncatum*, isolated from anthracnose infected urdbean (*Vigna mungo*) leaves from different areas of Himachal Pradesh. Of the two cultivars of horsegram tested, HPK 4 was found moderately susceptible to four isolates of the pathogen, while the local horsegram cultivar (Paharikulth) was moderately susceptible to all the isolates. Owing to the highly non specialized nature of pathogen, it was opined that there is a need to look for resistance not only in wide array of urdbean germplasm but also in other related legumes, which fall within the virulence spectrum of *C. truncatum*.

Chahota *et al.* (2005) evaluated 63 landraces of horsegram collected from different parts of Himachal Pradesh against 12 morpho-agronomical characters. Disease reaction against *C. truncatum* under field conditions revealed that the lines, viz., HPKC-39, HPKC-57 and HPKC-33 were found free from the disease and thus considered as potential.

Tullu *et al.* (2006) screened 574 accessions of wild lentil species for identifying sources of resistance to two races (Ct1 and Ct0) of anthracnose (*C. truncatum*) pathogen both under field and greenhouse conditions using a 1-9 scale (1, highly resistant; 2-3, resistant; 4-5, moderately resistant; 6-7, susceptible; and 8-9, highly susceptible). Although the accessions, Indianhead and PI 320937 were found to be resistant to race Ct1, turned out to be susceptible to race Ct0. Cultivars, Eston and Pardina were susceptible to both the races of *C. truncatum*. Results revealed that the highest frequency of resistance to the pathogen was exhibited among *Lens ervoides* accessions. The susceptible checks, Eston and Pardina wilted within 10-14 days after inoculation.

Prasath and Ponnuswami (2008) studied the differences in biochemical factors and enzymatic activities among different chilli genotypes against anthracnose disease. Seventeen genotypes (7germplasm lines and 10 hybrids) were screened for resistance to chilli anthracnose along with a susceptible variety (Arka Lohit) by detached fruit method using 0-5 scale. The genotypes were categorized as resistant (PBC-81), moderately resistant (S1, HY-1, HY-2, HY-3, HY-4, HY-5) and susceptible (Arka Lohit, MDUY, Arka Abir, Bydagi Dabbi, CO-4, HY-6, HY-7, HY-8, HY-9, HY-10). They also found that total phenols, peroxidase, polyphenol oxidase, phenylalanine ammonia- lyase were more in resistant varieties and less in susceptible varieties. Lot of variation in enzymatic activity was noticed in resistant and susceptible varieties and stated that enzymatic variation converts enzymes into quinines, which are toxic to pathogens.

Chandra Nayaka *et al.* (2009), in their compilation of technical bulletin on “Anthracnose disease of chilli pepper”, listed out the resistant varieties (IIHR 275-13-5, IIHR345-6, IIHR 332 -109, CC4, Ujwala, CA 87-4. S- 20-1, Lorai and BG-1) developed for anthracnose disease by IIHR, Bangalore, TNAU, Coimbatore and Punjab Agricultural University, Ludhiana.

Arjia Kulthi-21 (AK-21) variety of horsegram was released by the Central Variety Screening Committee in 1999 and was reported to be resistant to the attack of anthracnose as no disease attack was observed in this variety under field conditions (SIMS, 2011).

Deshmukh *et al.* (2012) screened five varieties and 39 genotypes of Indian bean (*Lablab purpureus* L.) against anthracnose (*Colletotrichum gloeosporioides* Penz. and Sacc.) in south Gujarat during Rabi 2008. The disease severity was recorded by observing three trifoliate leaves, (base, middle and upper portions) of selected plant

using 0-6 standard scale (0-Nil, 1-0.1-10%, 2-10.1-20 %, 3-20.1-30%, 4-30.1-40 %, 5-40.1-50 %, and 6- >50.1%). Observations were recorded at the initiation of the disease and at 15 days interval under field conditions starting from germination to harvesting. Three varieties, viz., Kapasi, JNP-4, Katargam and two germplasm accessions, viz., NWP8 and NWP21 showed resistant reaction, while fifteen genotypes, viz., NWP12, 19, 20, 22, 24, 25, 26, 27, 28, 29, 30, 32, 35, 37, 39 were found to be moderately resistant. NPS1 variety was found highly susceptible to anthracnose. Percent disease index (PDI) was calculated and the accessions were grouped as per the following scale given by Datar and Mayee (1981). The scale is as follows: 0.0-10%- Resistant (R), 10.1-20%- Moderately Resistant (MR), 20.1-40% Moderately Susceptible (MS), 40.1-60% - Susceptible (S), above 60% - Highly susceptible (HS).

Balbir Kour *et al.* (2012) screened seeds of 44 *Phaseolus vulgaris* cultivars collected from various areas of Jammu and Kashmir for anthracnose resistance using seed dip method. The inoculum of *C. lindemuthianum* at a concentration of  $1.2 \times 10^6$  spores per milliliter was used and the disease incidence and infection rate were determined by visual rating. The disease reaction was scored after 12 days of inoculation following 0-5 scale where 0 = no disease; 1 = pin point lesion; 2 = small lesion; 4 = large deep lesion and 5 = sunken lesions with rotten apex. Of the 44 accessions screened, 31 accessions could withstand the disease pressure and were devoid of disease symptoms and thus considered as Resistant.

*Chapter III*

**MATERIAL AND METHODS**

## Chapter-III

# MATERIAL AND METHODS

The present investigation on “Studies on anthracnose of horsegram (*Macrotyloma uniflorum* [Lam.] Verdc.)” was carried out at National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Hyderabad, and Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh.

### 3.1 General procedures

The general laboratory techniques described by Aneja (2003) were followed for preparation of media, sterilization, isolation and maintenance of fungal cultures with slight modifications wherever necessary.

Glassware of Borosil make was used throughout the investigation. The glassware was initially washed with a detergent followed by thorough cleaning with tap water before placing them in chromic acid mixture for about 24 h. It was rinsed three to four times with distilled water and air dried before sterilization. Finally, the glassware was sterilized in a hot air oven at 170 °C for two hours. Culture media and distilled water were sterilized in an autoclave at 121 °C temperature at 15 pounds square inch<sup>-1</sup> (PSI) pressure for 20 min.

In all the experiments, wherever surface sterilization of seeds is needed, the seeds were dipped in 1% chlorox (NaOCl) for two minutes and washed twice thoroughly using sterile distilled water and dried at room temperature (25±1 °C).

### 3.2 Isolation and identification of pathogen

Initial survey was undertaken in different horsegram growing areas of Andhra Pradesh, viz., Palem of Mahaboobnagar district, Buchhireddypalem of Nellore district, Rajendranagar mandal of RangaReddy district etc to look for the anthracnose incidence. Horsegram leaves suspected with anthracnose (*Colletotrichum* sp.) infection, were collected. After thorough washing with tap water, followed by surface sterilization by dipping in chlorox (1% sodium hypochlorite) solution for one minute and series of washings in sterile water, the suspected leaves were shade dried and incubated on 3- layered moist blotters in Plastic Petri dishes. The Petri dishes were incubated at 22±1 °C under alternate light and dark conditions for 4-5 days for the development of fungal growth. The plates were observed at regular intervals for the development of acervuli. Acervuli developed were transferred on to the Potato dextrose agar (PDA) medium in sterilized glass Petri dishes for isolating the fungus. The culture

thus grown was purified by transferring the individual acervulus into a sterile water blank of 5 ml capacity. The suspension was thoroughly mixed using a mechanical stirrer for proper dispersal of conidia and then poured on to Petri dishes containing PDA. The well isolated acervuli were transferred onto individual culture tubes and incubated for one week. The culture tubes with proper growth of the pathogen were stored under refrigerated conditions.

Different culture media, viz., Mathur's medium, Carrot agar medium and PDA were tested against the pathogen to obtain maximum sporulation and less mycelial growth. The composition of the media used for culture maintenance is given as under:

**Potato Dextrose Agar (PDA) (Source: HIMEDIA)**

Potato - 250.0 g

Dextrose - 20.0 g

Agar agar - 15.0 g

Distilled water - 1 litre

p<sup>H</sup>: 5.6 ± 0.2

**Mathur's medium**

Agar agar - 20.0 g

MgSO<sub>4</sub> - 2.2 g

Potassium phosphate - 2.2 g

Yeast - 2.0 g

Peptone - 2.0 g

Distilled water - 1 litre

p<sup>H</sup>: 5.6 ± 0.2

**Carrot agar medium**

Agar agar - 20.0 g

Carrot - 50.0 g

Tapioca Powder - 8.0 g

Distilled water - 1 litre

p<sup>H</sup>: 6.5 ± 0.2

The development of acervuli was observed under stereo-binocular microscope and the conidial characters were recorded by observing the culture slide under compound microscope. The culture was purified and maintained from time to time in Petri dishes containing medium for use in further experiments.

### **3.3. Pathogenicity**

Pathogenicity of the culture was proved using three different methods, *viz.*, seed inoculation method, detached leaf technique and sowing of infected seed in pots containing sterilized soil. The seven day old culture, growing on specific medium was taken and conidial suspension was prepared using sterile water. The conidial concentration was adjusted upto  $1 \times 10^6$  conidia per ml using haemocytometer. The popularly grown cultivar, Palem-2 was selected for pathogenicity tests.

**3.3.1. Seed inoculation method:** The surface sterilized seeds were soaked in conidial suspension in a beaker for 20 min. and placed on 3- layered moist blotters in the plastic Petri dishes after shade drying. Three replications were taken @ 10 seeds per replication. The plates were incubated at  $22 \pm 1$  °C for 3-5 days under alternate light and dark conditions and observed for the development of acervuli using stereo-binocular microscope. Conidial characters were confirmed by observing the acervuli under compound microscope. Seeds soaked in sterile water were plated in the control plates.

**3.3.2. Detached leaf technique:** Healthy and well developed leaves of each cultivar were collected, washed thoroughly with tap water, surface sterilized in chlorox (1% sodium hypochlorite) solution for one minute and washed three times in sterile water. Such leaves were used for proving pathogenicity. A drop of conidial suspension was put on each leaf and two leaves per plate were placed on three-layered moist blotters in the plastic Petri dish. The inner portion of the petri dish lid was also covered with moist blotters to maintain proper humidity. In the control plate, only sterile water drop was placed on the leaf. Three replications were maintained in each case. The plates were incubated at  $22 \pm 1$  °C for 3-5 days and observations on the development of acervuli were recorded as detailed above.

**3.3.3. Pot culture studies:** Surface sterilized seeds of cv. Palem-2 were soaked in the conidial suspension for 20 minutes and allowed for shade drying. The shade dried seeds were sown in the pots @ 10 seeds per pot, which were filled with sterilized soil and farm yard manure. Observations on seed germination, pre-emergence (seed rot) and post-emergence damping-off (seedling decay) were recorded. The seedlings were watered at regular intervals depending upon the requirement.

#### **3.4. Location of seed borne infection**

The location of pathogen in horsegram seed was determined by employing component plating technique (Maden *et al.*, 1975). Horsegram seeds of cv. AK-26 were used. The seeds were surface sterilized as indicated earlier and were subjected to 2, 4-D (2%) treatment for 10 minutes to hamper the germination. Hagborg *et al.* (1950) also reported that 2,4-Dichloro phenoxy acetic acid was useful to prevent seed germination

and facilitated evaluation and identification of *C. lindemuthianum* in bean seeds. The treated seeds were allowed for shade drying for 20 minutes. The 2, 4-D (2%) treated seeds (150 @ 50 seeds per observation) were soaked in pathogen inoculum ( $1 \times 10^6$  spores/ml.) for 20 minutes, while 30 seeds were soaked in sterile distilled water to serve as control. After shade drying, the inoculated seeds were placed on 3-layered blotters in plastic Petri dishes @ 10 seeds per plate. The plates were incubated at  $22 \pm 1^\circ\text{C}$  for seven days with 12 hours light and darkness alternatively. Three observations were recorded on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day using 50 seeds per each observation. While taking observations the seeds were dissected aseptically using sterile needle and forceps to separate the seed parts, viz., seed coat, cotyledons (endosperm) and embryo. The seed components were examined under stereo-binocular microscope for the presence of acervuli in different seed parts. The mean per cent seed infection was calculated for each part and each observation to understand the progress of infection.

### **3.5. Seed to seedling transmission**

The naturally infected horsegram seeds that were produced from the plants of cv. Palem-2 known to have anthracnose infection were collected and studied for their seed to seedling transmission under laboratory and glasshouse conditions. Healthy or disease free seeds of cv. Palem-2 from known source were used to serve as control.

#### **3.5.1. Laboratory studies**

Two methods, viz., paper towel method and sand method were used for studying seed to seedling transmission of the fungus.

**3.5.1.1. Paper towel method:** The anthracnose infected seeds (400 no.) were randomly picked up and surface sterilized as indicated earlier. These seeds were placed on two layers of moist germination papers that were placed on a polythene paper, at ten seeds per row. Four replicates of 100 seeds each were used and were covered with another moist germination paper and rolled carefully to avoid excess pressure on seeds. These towels were incubated in seed germinator at  $25^\circ\text{C}$  for 7 days and moistened as and when required. On the eighth day, observations were recorded on germination percentage and infection percentage (seed rot; seedling decay). Development of acervuli was noticed when the rotten seeds were observed under stereo-binocular microscope.

**3.5.1.2. Sand method:** Sterile sand medium was used for studying seed transmissibility of the fungus. Infected seeds were surface sterilized and sown in 30 x 15 cm plastic trays filled with sterilized sand. Four replications of 100 seeds each were maintained. The trays were watered regularly without disturbing the sand. Observations

on germination percentage and infection percentage (seed rot; seedling decay) were recorded upto 15 days after sowing.

In the above two methods, seeds exhibiting pre-emergence damping-off or rotting symptoms and seedlings with post-emergence damping-off and leaf anthracnose symptoms were taken out, washed and plated on 3-layered moist blotters in a plastic Petri dish after surface sterilization. After 3 days of incubation, plates were observed for acervuli development on rotten seeds or seedlings.

### 3.5.2. Glasshouse studies

Grow-out test was conducted for studying seed to seedling transmission of the fungus. Infected and healthy seeds of cv. Palem-2 were sown separately in earthen pots filled with sterilized soil + farm yard manure (FYM) @ 10 seeds per pot and were monitored for seedling growth and symptom expression till 30 days. Each pot is considered as one replication and three such replications were maintained. Observations on germination percentage and infection percentage (seed rot; seedling decay) were recorded at regular intervals.

### 3.6. Varietal screening studies against anthracnose

Horsegram accessions (21 genotypes and 4 released varieties) with phenotypic diversity were collected from NBPGR Regional station, Hyderabad, the details of which are given in Table 3.1 along with specific descriptors. These accessions were screened against anthracnose disease in the laboratory and greenhouse using the following methods.

- **Laboratory screening:** Seed inoculation and plating on moist blotters following Standard blotter method.
- **Greenhouse screening:** Spray inoculation on one month old seedlings

**Table 3.1.** Horsegram accessions selected for screening (Laboratory and Greenhouse) against anthracnose disease (Source: NBPGR Regional Station, Hyderabad).

IC number	Name of the accession	Major trait
IC 257875	S-6	Smooth leaf surface
IC 261287	NSM-147	Hairy leaf surface
IC 261298	NSM-202	Smooth leaf surface
IC 343830	SKN-21	Twining nature
IC 343897	SKN-88	Twining nature
IC 343900	SKN-91	Twining nature & medium leaf size

IC 426460	NS/04-11	Hairy leaf surface
IC 426503	NS/04-57	Pale green leaf
IC 426548	NS/05-102	Hairy leaf surface
IC 470171	NS/05-009	Twining nature
IC 470176	NS/05-14	Big leaf size
IC 470243	NS/05-81	Twining nature
IC 470255	NS/05-93	More 100 seed weight
IC 470256	NS/05-94	Stay green nature & small leaf
IC 470259	NS/05-97	Pale green leaf
IC 470263	NS/05-101	Small leaf size
IC 470272	NS/05-110	Low 100 seed weight
IC 470275	NS/05-113	More 100 seed weight & smooth leaf
IC 470278	NS/05-116	Moderate 100 seed weight
IC 470311	NS/05-149	Twining nature
IC 526968	PSR-11693-3	Stay green nature & moderate 100 seed weight

- ❖ AK-21, AK-26, Palem-1 and Palem-2 are the released varieties.
- ❖ AK-21 used as resistant check.

### 3.6.1. Laboratory screening: Seed inoculation and plating on moist blotters

Seeds of each accession were separated and surface sterilized by dipping in 1% chlorox solution for two minutes followed by washing twice in sterile water. The surface sterilized seeds were dipped in the 7-day old culture of *C. dematium* suspension ( $1 \times 10^6$  conidia/ml) for 15 minutes. The seeds were taken out and shade dried before plating on moist blotters using Standard blotter method. Singh *et al.* (2009) also found that standard blotter method was ideal for *C. capsici* detection from chilli seeds as the recovery of fungus was maximum over the other two methods, *viz.*, agar plate method and deep freeze method. Seeds for control plates were soaked in sterile water instead of inoculum. Ten seeds per plate were used and each plate was considered as one replication. Each accession was replicated thrice. The Petri dishes were incubated in the incubation room at  $22 \pm 1^\circ\text{C}$  under alternate light and dark conditions. Observations on seed germination and development of acervuli (per cent seed infection and per cent

seed area covered with infection) were recorded at different intervals i.e., 3<sup>rd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day after incubation. Average of per cent seed surface covered with acervuli on ten seeds was taken for one replication. Such data for three replications were compiled and the accessions were grouped into different categories as per 0-4 scale (Bansal and Grover, 1969), where in 0 = No acervuli (Immune); 1 = upto 10% area infected (Resistant); 2 = 11-25% (Moderately resistant); 3 = 26-50% (Susceptible); 4 = >50% (Highly susceptible).

### **3.6.2. Glasshouse screening: Spraying of inoculum on one month old seedlings**

**3.6.2.1. Standardization experiment:** Before taking up the screening studies, one preliminary experiment was conducted to standardize the methodology using two local cultivars, viz., Palem-1 and Palem-2. Five seeds of each accession were sown in each pot and 3 pots were maintained for each accession. Like this three sets were maintained for each accession. Conidial suspension of one week old culture at a concentration of  $5 \times 10^6$  conidia per ml was sprayed on one month old seedlings to ensure high inoculum pressure (Plate 3.1). Seedlings sprayed with sterile water served as control. All pots were kept in the incubation room ( $22 \pm 1^\circ\text{C}$  and 90% relative humidity) after covering with polythene sheets (Plate 3.2) to maintain optimum humidity required for disease development. The polythene sheets were removed at different intervals of 24, 48 and 72 hours in each set to determine the ideal time required for symptom expression. After removal of the polythene sheet, the pots were shifted to the greenhouse and regular spray with sterile water was given twice a day for one week to create ideal conditions for disease development. Observations on symptom development were recorded carefully from time to time.

### **3.6.2.2. Varietal screening by spraying inoculum on one month old seedlings**

Sowing of all accessions was taken up in the month of September, 2011. At the time of spraying on one month old seedlings, it was ensured that a minimum of 10 seedlings were maintained in each pot and three replications were maintained for each treatment. Cultivar, AK-21 was used as a resistant check. Another set of three accessions of AK-21 was also kept for spraying sterile water to serve as Healthy Control. The methodology followed was same as mentioned above, except that the polythene sheets were removed after 72 hours of inoculation. Pots were watered regularly and sterile water spray was given uniformly on all plants twice a day till the initiation of symptoms. Observations were recorded at weekly intervals on symptom development and seedling mortality and each seedling was given score based on 0-4 scale described earlier. The per cent disease index (PDI) per replication was calculated

as per the following formula and average of three replications was taken as the final figure.

$$\text{Per cent disease index} = \frac{\text{Sum of numerical disease rating values}}{\text{No. of plants observed} \times \text{Maximum disease rating}} \times 100$$

### **Statistical analysis**

Data obtained in different experiments were analyzed wherever necessary, using the statistical software, OPSTAT, developed by O. P. Sheoran, Programmer, Computer Section, CCS Haryana Agricultural University, Hisar.



**Plate 3.1. Spraying of inoculum on one month old seedlings in a special chamber**



**Plate 3.2. Covering the seedlings with polythene bags after spraying the inoculum for maintaining humidity**

*Chapter IV*

**RESULTS AND DISCUSSION**

## Chapter IV

# RESULTS AND DISCUSSION

The results obtained in various experiments such as isolation and identifying the pathogen responsible for anthracnose disease, location of seed borne infection, seed to seedling transmission of the pathogen and varietal screening of horsegram accessions are presented and discussed here under.

### 4.1. Isolation and identification of pathogen

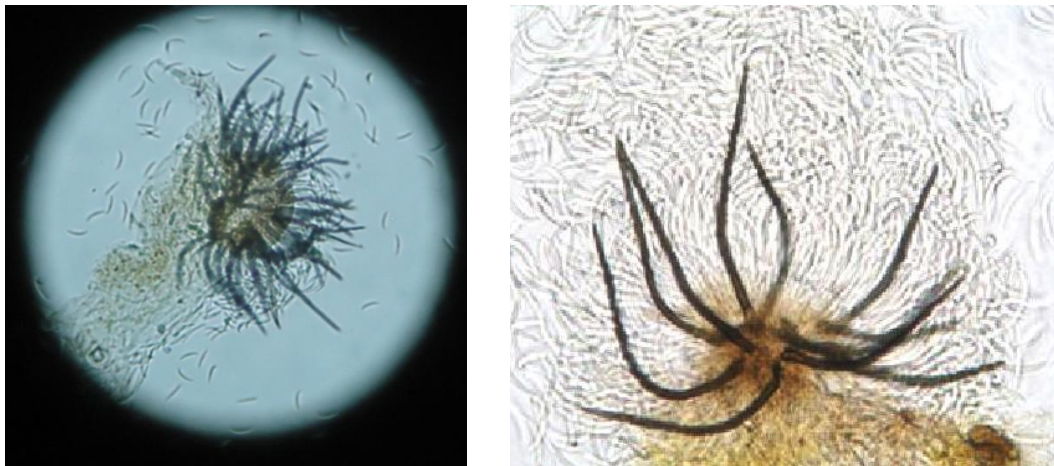
Horsegram leaves suspected with anthracnose (*Colletotrichum* sp.) infection were collected from different areas of Andhra Pradesh and tested for the presence of fungal association (Plate 4.1). Out of several sources of collection, leaves collected from the fields of NBPGR, Rajendranagar showed acervuli development. Acervuli developed were transferred on to the potato dextrose agar (PDA) and the culture was purified. On the PDA, colonies were brownish white and later turned dark grey in colour. The acervuli (Plate 4.2) were numerous, globose, scattered with large number of dark brown setae, 96-125  $\mu\text{m}$  long. Conidiophores were short, simple and hyaline. It produced falcate conidia of size 21.7 x 5.1  $\mu\text{m}$ . Conidia were aseptate, single celled, fusiform and sickle shaped (Plate 4.3). The specific features of acervuli and conidia were very much similar to the descriptions of Sutton (1980) and the culture was identified as *Colletotrichum dematium* (Pers. ex Fries) Grove. Murthy (1997) also identified *C. dematium*, as the organism responsible for anthracnose disease in horsegram. However, Brink and Belay (2006) revealed that anthracnose in horsegram was caused by *C. lindemuthianum*.

### 4.2. Inoculum maintenance

Initially, potato dextrose agar (PDA) medium was used for multiplication of inoculum. However, as the fungus was yielding more mycelial growth and taking more time (one month approx.) for acervuli development, two other media, viz., Mathur's medium and Carrot agar medium (CAM) were tested against the pathogen to obtain maximum sporulation and less mycelial growth. CAM was found ideal for obtaining quick growth with more number of acervuli and less mycelial growth and thus the same medium was used throughout the study to maintain the inoculum (Plates 4.4 and 4.5). Prasanna (1985) also observed more mycelial growth of *C. lindemuthianum* on PDA without acervuli.



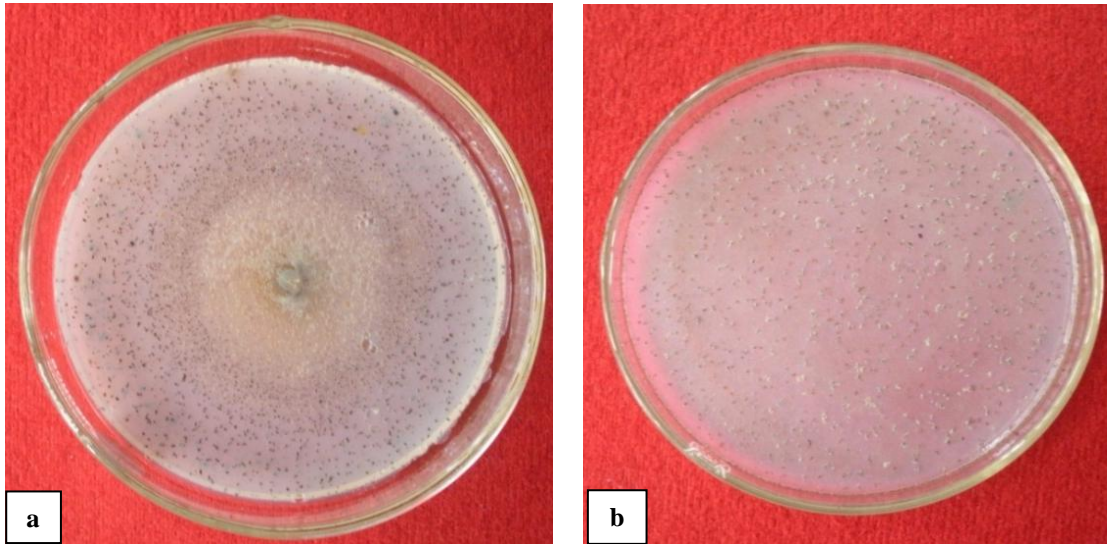
**Plate 4.1. Incubation of leaf samples suspected with anthracnose infection for isolation of pathogen**



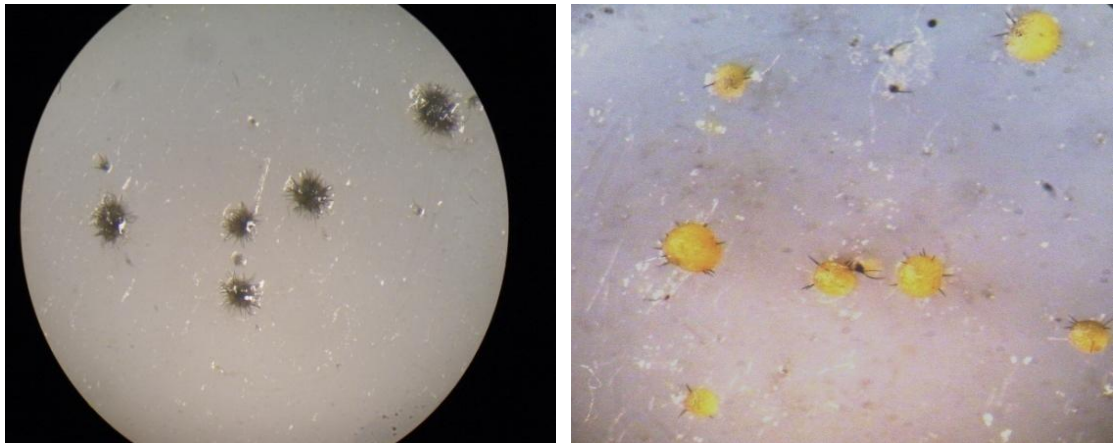
**Plate 4.2. Microscopic view of acervulus and setae of *Colletotrichum dematium* and oozing out of conidial mass (Left: 10X; Right: 40X)**



**Plate 4.3. Compound microscopic view of individual conidia of *C. dematium***



**Plate 4.4. Growth of *C. dermatium* on CAM with more acervuli and scanty mycelium: a. Disc inoculation method b. Pour plate method**



**Plate 4.5. Stereo-binocular microscopic view of *C. dermatium* acervuli (Left) and Oozing out of conidial mass from the acervuli (Right) on CAM**

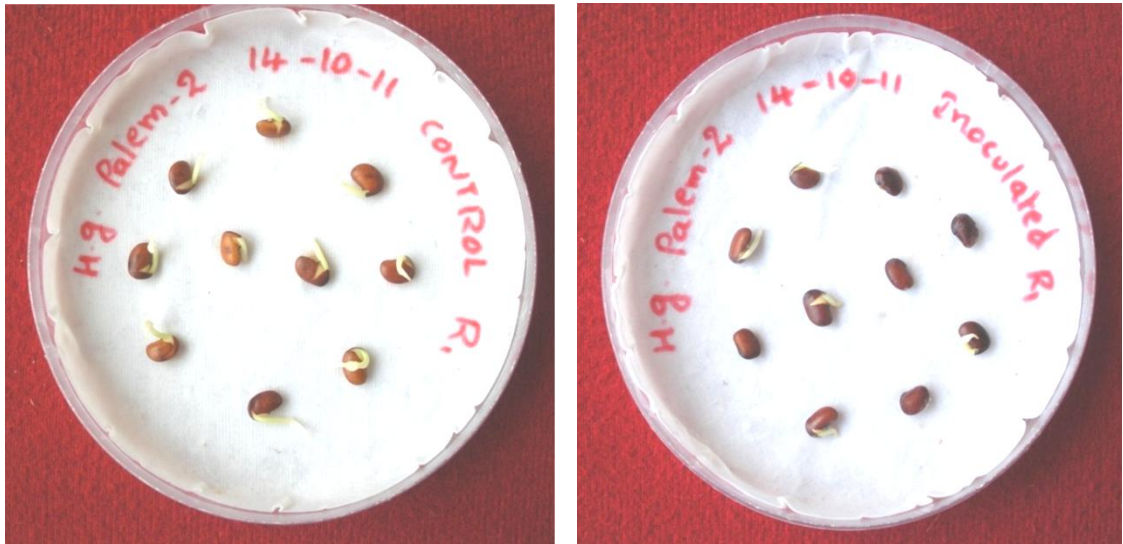
### **4.3. Pathogenicity**

Pathogenicity of the culture was proved using three different methods, *viz.*, seed inoculation method, detached leaf technique and sowing of infected seed in pots containing sterilized soil.

**4.3.1. Seed inoculation method:** Germination of the infected seeds was reduced to 46.7% (average of three replications) as compared with the control (100%), where uninoculated, healthy seeds were plated (Plate 4.6). Seed rotting (6.7%) of infected seeds was also observed. The colour of the inoculated seeds was transformed to dark brown to blackish brown due to the development of pathogen mycelium and acervuli, which was clearly visible under stereo-binocular microscope (Plate 4.7). Conidial characters, observed under compound microscope, were similar to *C. dematium* indicating the pathogenic nature of the fungus.

**4.3.2. Pathogenicity by Detached leaf technique:** The acervuli development was noticed within 3 days on the inoculated leaf and the whole leaf got disintegrated in one week due to the spread of infection (Plate 4.8). The disintegrated leaf portion showed numerous acervuli and the images of the same under stereo-binocular microscope at different magnifications are depicted in Plates 4.9 and 4.10. The conidial characters revealed the presence of *C. dematium*.

**4.3.3. Pot culture studies:** Germination of the pathogen inoculated seeds was drastically reduced to 23% (average of three replications) in pot culture studies when compared to the control (100%), where healthy seeds were sown (Plate 4.11). In addition, two of the germinated seedlings could not develop further and post-emergence damping-off symptoms were seen due to the pathogen infection. When these damped-off seedlings were taken out and plated on blotters after surface sterilization, acervuli development was seen, indicating the pathogenic nature of the fungus (Plate 4.12).



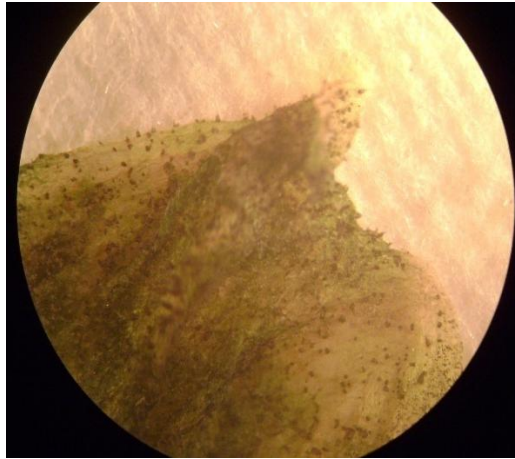
**Plate 4.6: Pathogenicity test by Seed inoculation method: Germination at 5 days after inoculation**



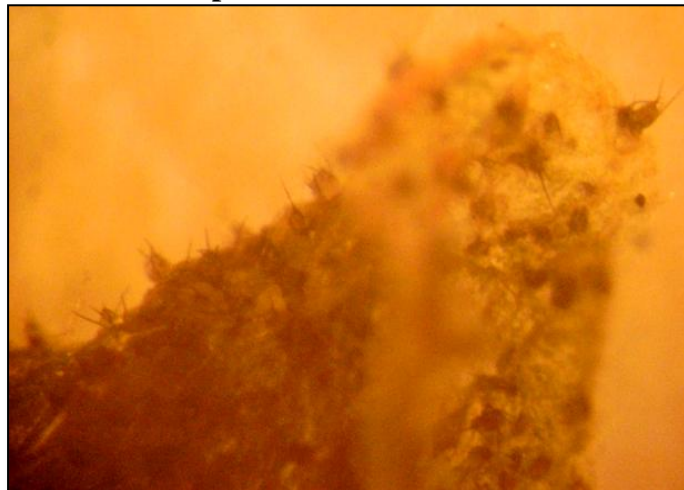
**Plate 4.7: Development of mycelial growth and acervuli on the infected seed**



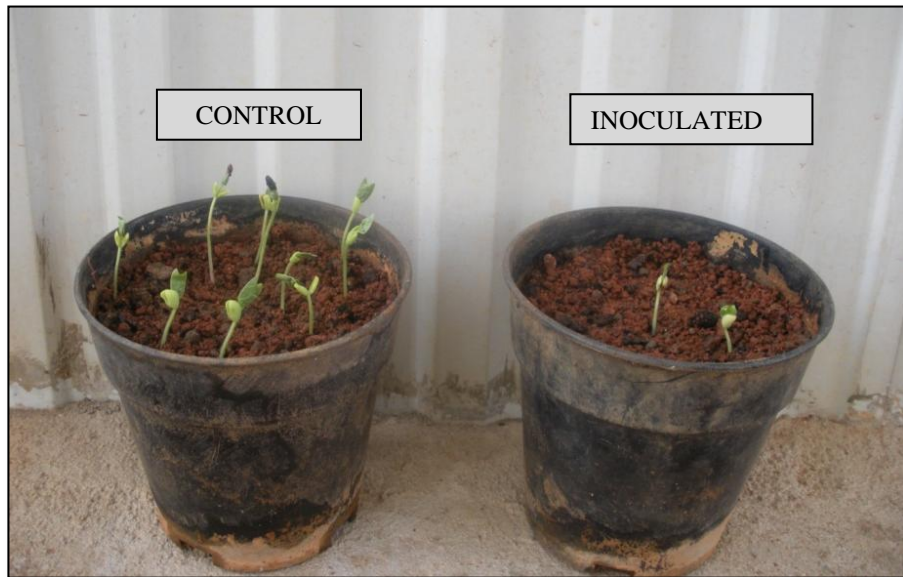
**Plate 4.8: Detached leaf technique showing uninoculated horsegram leaves (left) and *C. dematium* inoculated leaf (right) after one week**



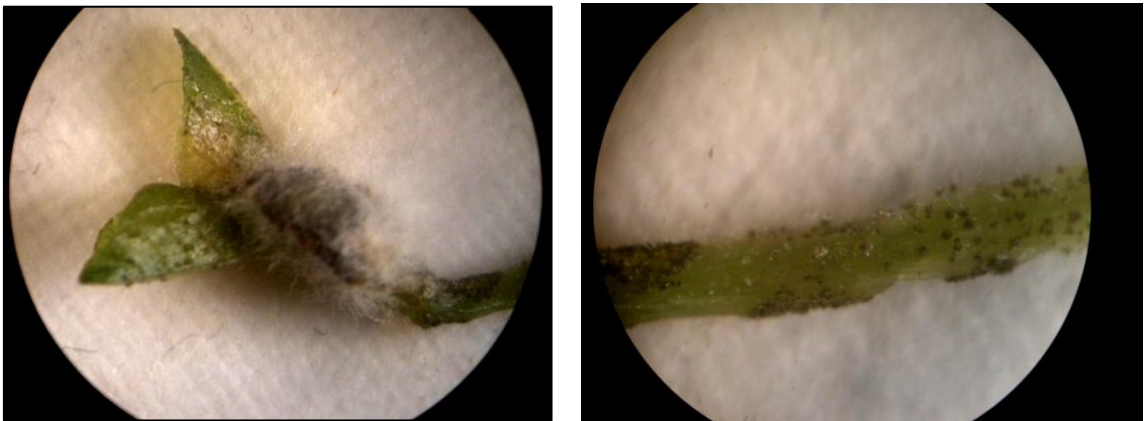
**Plate 4.9: View of severely infected leaf showing acervuli of *C. dematium* under stereo-binocular microscope**



**Plate 4.10: Enlarged portion of infected tip with prominent acervuli**



**Plate 4.11. Effect of *C. dematium* infection on seed germination and seedling development in horsegram**



**Plate 4.12: Presence of mycelium (left) and acervuli (right) of *C. dematium* on the damped-off seedling of horsegram**

#### 4.4. Location of seed borne infection

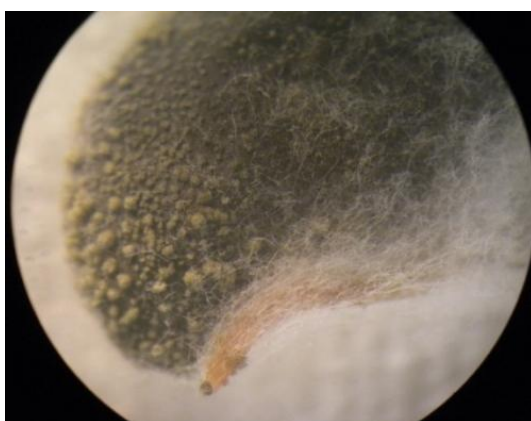
Results of the component plating experiment (Table 4.1) revealed that the *C. dematium* infection could reach all parts of the horsegram seed, though with varied levels of intensity. Seed coat infection was the highest on all the three observations of 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day of plating and ranged from 62-100%, followed by cotyledonary infection (36-72%) (Plates 4.13 a & b). The embryo infection was the least, which reached upto 30% on the 7<sup>th</sup> day from the initial 18% infection on the third day. The internally seed borne nature of the pathogen, *C. dematium*, was evident as the pathogen development in the form of acervuli was noticed on all parts of the seed. Ravi *et al.* (1999) reported that *C. lindemuthianum* was present mostly in seed coats and cotyledons, and rarely in embryonic axes of French bean. Mohammed Yesuf and Somsiri Sangchote (2005) also reported that the infection of *C. lindemuthianum* in the seeds of common bean was mainly located internally in the seed coat and rarely in the cotyledon and embryo axis. They had recorded 74.4, 21.3 and 4.3% infection of *C. lindemuthianum* in common bean seed components, the seed coat, cotyledon and embryo axis, respectively. Shivanna and Shetty (1988) used the same technique and revealed that *C. dematium* was located in the inner layer of the seed coat of cluster bean. Basak (1998) identified *C. capsici* infection on chilli seeds and recovered 92% infection from seed coat, 82% from endosperm and 80% from the embryo. Infection of *C. dematium* was present on the seed coat of all the infected seeds of chilli cv A-36, while embryo infection to an extent of 31.25% was reported (Kumud Kumar *et al.* 2004).

It was observed that on the third day of incubation, 14% (7 of 50) of seeds showed infection on all the seed components, i.e., seed coat, cotyledons (endosperm) and embryonic axis. The progress of disease infection was evident as 24% (12 of 50) on the 5<sup>th</sup> day and 30% on the 7<sup>th</sup> day was recorded. Although 2,4-D treatment was found effective in inhibiting the seed germination, in some cases, the radicle was emerged, but failed to develop further. In such cases, acervuli development was seen even on the radicle (Plate 4.13 c).

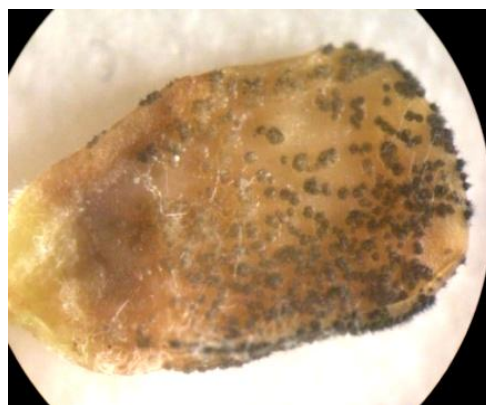
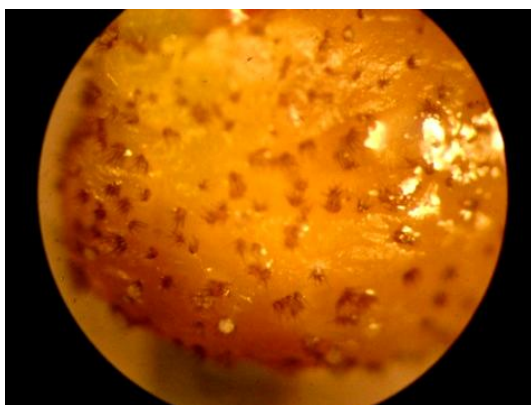
The seed coat was fully covered with mycelium and acervuli on the seventh day (Plate 4.13a). Puri *et al.* (1981) investigated the location of *C. dematium* in seven samples of chilli seeds using component plating technique and reported that abundant mycelium was seen in the parenchymatous cells of the seed coat.

**Table 4.1. Infection percentage of *C. dematium* in different parts of horsegram seeds by component plating method**

Day after incubation	No. of seeds observed	Per cent infection		
		Seed coat	Embryo	Cotyledon (Endosperm)
3 <sup>rd</sup>	50	62	18	36
5 <sup>th</sup>	50	84	24	50
7 <sup>th</sup>	50	100	30	72



**Plate 4.13 a. Location of pathogen on horsegram seed coat showing acervuli and mycelium**



**Plate 4.13 b. Development of acervuli on outer (left) and inner (right) surface of cotyledons (endosperm)**



**Plate 4.13 c. *C. dematium* acervuli development on the emerging radicle of horsegram**

## **4.5. Seed to seedling transmission**

### **4.5.1. Laboratory studies**

The naturally infected horsegram seeds that were harvested from the anthracnose infected plants of cv. Palem-2, were collected and studied for their seed to seedling transmission under laboratory and greenhouse conditions and results were presented here under.

#### **4.5.1.1. Paper towel method**

Observations recorded on the germination percentage revealed that the healthy seeds of Palem-2 showed 100% germination by 5<sup>th</sup> day, while the infected seeds recorded germination as low as 38% at the end of 3<sup>rd</sup> day, which reached upto 42% by the 5<sup>th</sup> day and didn't increase further (Table 4.2). Seed rot or pre-emergence mortality (20%) of infected seeds was noticed on the 3<sup>rd</sup> day and progressed to an extent of 34% by the end of 7<sup>th</sup> day. It was also observed that few seeds from the infected lot neither germinated nor showed seed rot, which may be attributed to the seed factor. Presence of acervuli on the seed coat which was attached to the cotyledonary leaves indicated the anthracnose infection. Some of the germinated seedlings of the infected lot showed post-emergence seedling decay (10%) on the 5<sup>th</sup> day, which reached upto 18% by the end of 7<sup>th</sup> day. The seedlings in the Control treatment were absolutely healthy without showing any post-emergence mortality (Plate 4.14).

The decayed seed and seedlings when plated again after surface sterilization, yielded acervuli of *C. dematium* indicating the transmission of fungus through seed to seedling. Higher seed transmission rates of *C. dematium* through cowpea seeds were recorded earlier by Cardona (1990).

**Table 4.2. Effect of *C. dematium* infection on seed germination and mortality of horsegram seeds in paper towel method.**

Observations after	Germination (%)		Mortality (%)	
	Control	Infected	Pre-emergence (seed rot)	Post-emergence (seedling decay)
3 days	92	38	20	0
5 days	100	42	28	10
7 days	100	42	34	18



**Plate 4.14. Seed to seedling transmission of *C. dematium* in horsegram using paper towel method**

#### **4.5.1.2. Sand method**

Results of sand method (Table 4.3) showed significant differences in the germination of infected and healthy seeds. Marked reduction in germination percentage of infected seeds (Plate 4.15) was observed on the 5<sup>th</sup> day (26%), which reached to a maximum of 30% on the 10<sup>th</sup> day and remained same even on the 15<sup>th</sup> day as compared to that of healthy control (70% on the 5<sup>th</sup> day and 80% on the remaining two observations). This was lower than the initial germination percentage (38%) of infected seeds in paper towel method as there are more ideal conditions in the soil that are

required by the fungus to proliferate and act. Pre-emergence seed rot of infected seeds to an extent of 6% was noticed on the 5<sup>th</sup> day, which reached to a maximum of 12% on the 10<sup>th</sup> day and remained constant even on the 15<sup>th</sup> day (Plate 4.16). The post-emergence seedling decay was also recorded on the 5<sup>th</sup> day (14%) and was increased in the subsequent observations (20%) on 10<sup>th</sup> and 15<sup>th</sup> day.

Seed transmission of *C. dematium* in cluster bean (23.5% disease incidence) was studied by Shivanna and Shetty (1988) under controlled glasshouse conditions and also in field. The infected seeds resulted in 3% pre-emergence and 15% post-emergence mortality in sand method. Mustafa (2009) also used sand method to determine seed transmission of several fungi in *Dalbergia sissoo* and obtained satisfactory results.

**Table 4.3. Effect of *C. dematium* infection on seed germination and mortality of horsegram seeds in sand method**

Observations after	Germination percentage		Mortality percentage	
	Control	Infected	Pre emergence (seed rot)	Post emergence (seedling decay)
5 days	70	26	6	14
10 days	80	30	12	20
15 days	80	30	12	20



**Plate 4.15. Seed transmission studies of *C. dematium* on seed germination and mortality of horsegram seeds using sand method.**

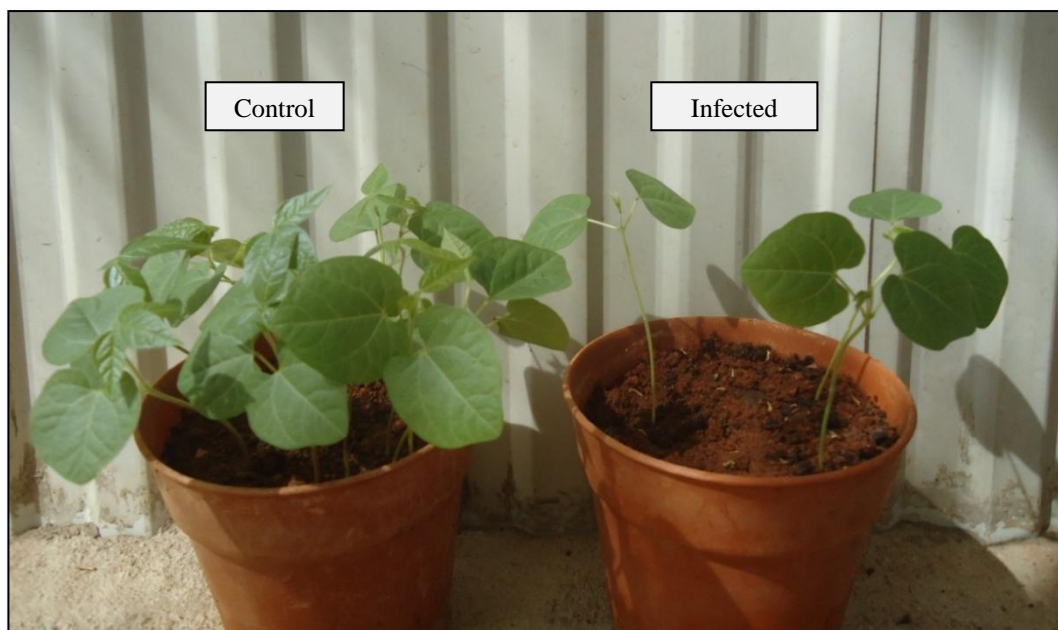


**Plate 4.16. Pre-emergence seed rot of horsegram seed due to the infection of *C. dematium*.**

### 4.5.2. Greenhouse studies

The germination percentage of anthracnose infected seeds was significantly less (30%) during the first observation (7<sup>th</sup> day after sowing) and remained unchanged even after a month (Plate 4.17). The control pot showed an initial germination of 80% on the 7<sup>th</sup> day and reached upto 90% on the 14<sup>th</sup> day of observation. The pre emergence seed rot of 10% intensity was recorded on the 14<sup>th</sup> day and no progress of disease observed up to 30 days. None of the infected seeds showed post-emergence seedling decay symptoms. Singh *et al.* (2009) conducted transmission studies of *C. capsici* by sowing naturally infected chilli seeds under controlled glasshouse conditions and reported that the infection was found to be transmitted from seed to seedling in an asymptomatic manner.

All the three methods successfully proved the seed to seedling transmission of anthracnose disease, *C. dematium*, which was evident with symptoms of poor germination and seedling decay, when infected seeds were used. However, paper towel method resulted in higher seed rot (34%) followed by sand method (12%) and grow-out test (10%). This may be due to the possibility that the seeds might have obtained congenial conditions in the sand method and grow-out tests so as to withstand the attack of the pathogen resulting in less seed rot as compared to the paper towel method. Seedling decay symptoms were observed in sand (20%) and paper towel (18%) methods, while nil report of seedling decay symptoms found in grow-out test method. Shyam and Chakraborty (1985) studied the extent of seed transmission of *C. lindemuthianum* in four commercial cultivars of *Phaseolus vulgaris*, viz., White Panchamari, Dharmasala Local, Chamba Local and Kentucky Wonder from Himachal Pradesh using blotter paper and grow-out test methods. In all the four cultivars, the seed transmissibility of the fungus was more in blotter paper method (11, 25, 18 and 13%, respectively) as compared to grow-out test (6.6, 18.75, 12.5 and 7.69%, respectively).



**Plate 4.17. Greenhouse studies on seed to seedling transmission of *C. dematium* in horsegram.**

**Table 4.4. Effect of *C. dematium* infection on seed germination and mortality of horsegram seeds in grow-out test**

Time period	Germination percentage		Mortality percentage	
	Control	Infected	Pre emergence (seed rot)	Post emergence (seedling decay)
7 days	80	30	0	0
14 days	90	30	10	0
21 days	90	30	10	0
30 days	90	30	10	0

## 4.6. Varietal screening studies against anthracnose

### 4.6.1. Laboratory screening: Seed inoculation and plating on moist blotters

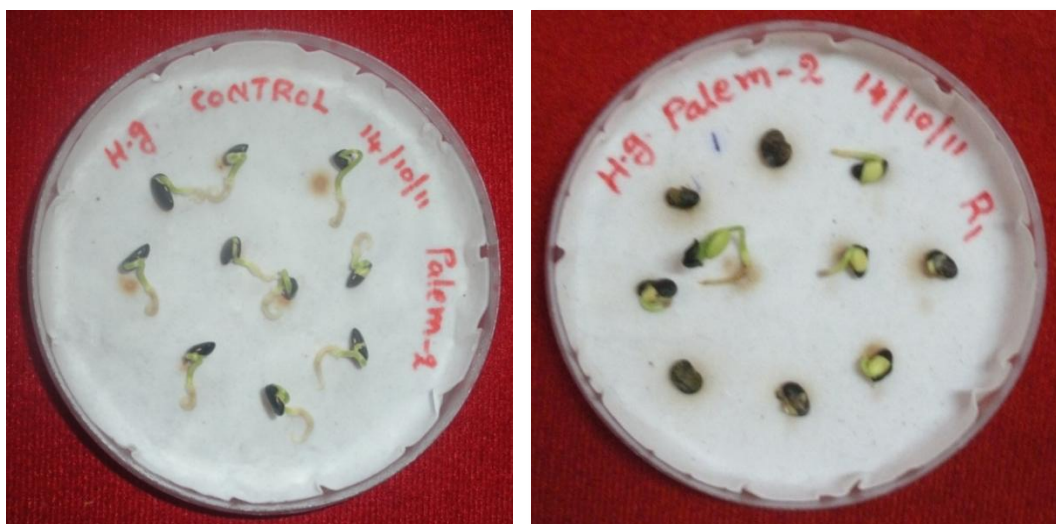
Observations (Table 4.5) on seed germination of inoculated and uninoculated horsegram seeds of 25 accessions revealed that there was no significant difference among the inoculated and uninoculated seeds of majority of the accessions except IC 470243, IC 261287, PALEM-2, IC 526968, IC 343830 and IC343900. Out of 25 accessions tested, inoculated and uninoculated seeds of 16 accessions, including the resistant check, AK-26, recorded equal germination percentage. Infected seeds of PALEM-2 recorded least germination (60%), followed by IC 343900(78.33%) as compared to their respective uninoculated controls (98.33 and 96.67%). Accessions AK-21, IC 426503, IC 470278, IC 470176, IC 470311 and IC 343897 recorded 100% germination both in control and inoculated seeds. This indicates the fact that the fungus requires certain period to get established and show its impact in the form of pre-emergence rotting.

Observations on per cent seed surface infected showed significant differences among accessions on all the three observations (Table 4.5). Accessions were grouped into immune (0%), resistant (0.1-10%), moderately resistant (10.1-25%), susceptible (25.1-50%) and highly susceptible (>50%) categories based on the per cent seed surface infected by following 0-4 scale. Among 25 accessions none of the accessions was found immune with 0% seed infection at the end of the 7<sup>th</sup> day of incubation. Three accessions (AK-21, IC 470275 and IC 470259) were considered as resistant as they have recorded < 10% seed surface infected (Fig 1). Nine accessions, viz., IC 426503, IC 470263, IC 426548, IC 470272, IC 470278, IC 470255, IC 261287, IC 261298 and PALEM-1 were categorized as moderately resistant as the percent seed area infected was in the range of 10.1-25%. Nine accessions AK-26, IC 426460, IC 470171, IC 470176, IC 470243, IC 470256, IC 470311, IC 257875 and IC 343897 were grouped under susceptible category as the per cent infected seed area was <50%. The remaining four accessions (IC 343900, IC 343830, IC 526968 and PALEM-2) were found highly susceptible as they have shown >50% of percent seed area infected with the fungus. The accession IC 343900, which recorded 'Highly Susceptible' reaction in the present investigation has black coloured seeds, while the accessions (AK-21, IC 470275 and IC 470259) that recorded 'Resistant' reaction have light brown coloured seeds. Nwachukwu and Umechuruba (1991), while conducting studies on *C. dematium* in

African yam bean, observed that the recovery of the fungus was more from the marble brown coloured seed and no recovery from creamy white and smooth brown coloured seeds. This indicates that the seed traits such as seed colour and texture also play a key role in imparting resistance to the pathogen attack.

It was observed that the seeds of resistant check, AK-21, used in the present study, did not get infection on the third day, while the infection was least (3.3%) on the fifth day compared to the remaining accessions and it could attain maximum infection of 7.67 by the end of seventh day. The remaining two accessions (IC 470275 and IC 470259) that recorded resistant reaction were at par with the resistant check used in the study as they could get a maximum infection of 7.67 and 9.33%, respectively. Majority of the moderately resistant accessions were significantly at par with each other. On the seventh day after inoculation, IC 343900 and PALEM-2 accessions (Plate 4.18) recorded Highly Susceptible reaction with seed infection as high as 81 and 74.33%, respectively, while IC 470275 and AK-21 accessions recorded least (7.67%) seed surface infected, followed by IC 470259 (9.33%).

Among the 25 accessions tested, 12% of the accessions fall under resistant category, 36% each under susceptible and moderately resistant categories and the remaining 16% under highly susceptible category.

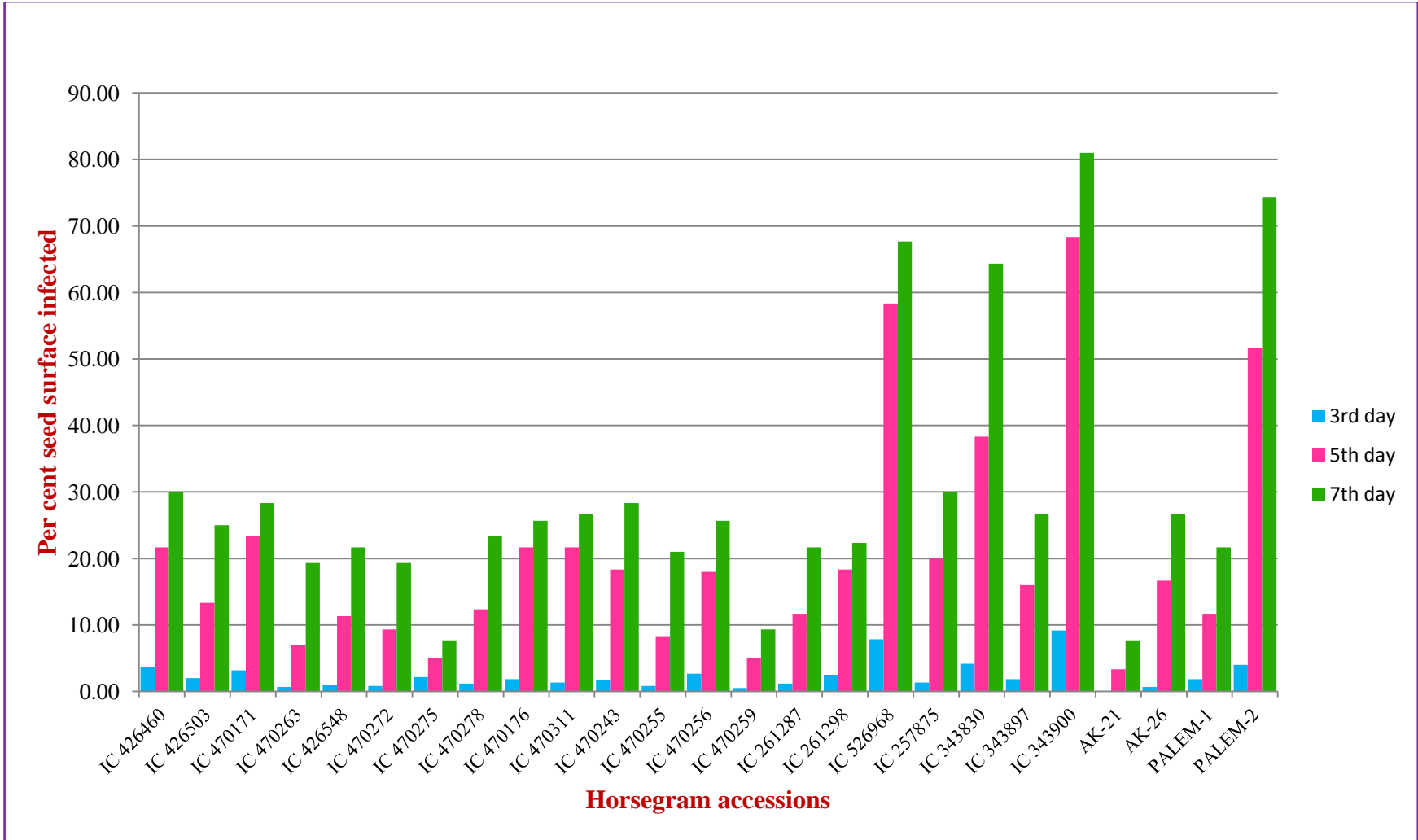


**Plate 4.18. Laboratory screening of horsegram accessions against *C. dematium* by seed inoculation method showing control and inoculated seeds of Palem-2.**

**Table 4.5. Laboratory screening of horsegram accessions against *Colletotrichum dematium* by seed inoculation using standard blotter method.**

IC numbers	Name of Accessions	Germination (%)		Per cent seed surface infected		
		Inoculated	Uninoculated	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
IC 257875	S-6	93.33 (81.14)	96.67 (83.84)	1.33 (6.60)	20.00 (26.44)	30.00 (33.14)
IC 261287	NSM-147	86.67 (68.83)	100.00 (90.00)	1.17 (6.17)	11.67 (19.87)	21.67 (27.69)
IC 261298	NSM-202	95.00 (77.05)	95.00 (77.05)	2.50 (9.06)	18.33 (25.29)	22.33 (28.17)
IC 343830	SKN-21	85.00 (71.75)	96.67 (83.84)	4.17 (11.77)	38.33 (38.23)	64.33 (53.33)
IC 343897	SKN-88	100.00 (90.00)	100.00 (90.00)	1.83 (7.76)	16.00 (23.36)	26.67 (31.03)
IC 343900	SKN-91	78.33 (62.26)	96.67 (83.84)	9.17 (17.60)	68.33 (55.75)	81.00 (64.20)
IC 426460	NS/04-11	93.33(81.14)	93.33 (81.14)	3.67 (11.03)	21.67 (27.69)	30.00 (33.19)
IC 426548	NS/05-102	96.67 (83.84)	100.00 (90.00)	1.00 (5.74)	11.33 (19.55)	21.67 (27.69)
IC 470171	NS/05-009	90.00 (71.54)	90.00 (71.54)	3.17 (10.24)	23.33 (28.65)	28.33 (32.12)
IC 470176	NS/05-14	100.00 (90.00)	100.00 (90.00)	1.83 (7.76)	21.67 (27.68)	25.67 (30.38)
IC 470243	NS/05-81	83.33 (70.05)	96.67 (83.84)	1.67 (7.39)	18.33 (24.99)	28.33 (32.00)
IC 470255	NS/05-93	96.67 (83.84)	100.00 (90.00)	0.83 (5.17)	8.33 (16.76)	21.00 (27.21)
IC 470256	NS/05-94	96.67 (83.84)	98.33 (85.68)	2.67 (9.35)	18.00 (25.07)	25.67 (30.38)
IC 470259	NS/05-97	96.67 (83.84)	96.67 (83.84)	0.50 (4.05)	5.00 (12.91)	9.33 (17.76)
IC 470263	NS/05-101	91.66 (79.99)	96.67 (83.84)	0.67 (4.61)	7.00 (15.25)	19.33 (26.06)
IC 470272	NS/05-110	93.33 (75.21)	93.33 (75.21)	0.83 (5.17)	9.33 (17.76)	19.33 (26.06)
IC 470275	NS/05-113	96.67 (83.84)	96.67 (83.84)	2.17 (8.45)	5.00 (12.92)	7.67 (15.92)
IC 470278	NS/05-116	100.00 (90.00)	100.00 (90.00)	1.17 (6.17)	12.33 (20.48)	23.33 (28.84)
IC 470311	NS/05-149	100.00 (90.00)	100.00 (90.00)	1.33 (6.60)	21.67 (27.69)	26.67 (31.05)
IC 426503	NS/04-57	100.00 (90.00)	100.00 (90.00)	2.00 (8.13)	13.33 (21.33)	25.00 (29.91)
IC 526968	PSR-11693-3	83.33 (65.92)	90.00 (71.54)	7.83 (16.24)	58.33 (49.78)	67.67 (55.33)
AK-21 (Resistant Check)		100.00 (90.00)	100.00 (90.00)	0.00 (0.00)	3.33 (8.61)	7.67 (15.92)
AK-26		96.67 (83.84)	96.67 (83.84)	0.67 (4.61)	16.67 (24.03)	26.67 (31.05)
PALEM-1		93.33 (81.14)	96.67 (83.84)	1.83 (7.76)	11.67 (19.87)	21.67 (27.69)
PALEM-2		60.00 (50.79)	98.33 (85.68)	4.00 (11.53)	51.67 (45.94)	74.33 (59.58)
CD at 5%		12.74	N.S.	0.55	5.76	5.74
SE(m)		1.17	2.47	0.19	2.02	2.01

\*Figures in parentheses are angular transformed values

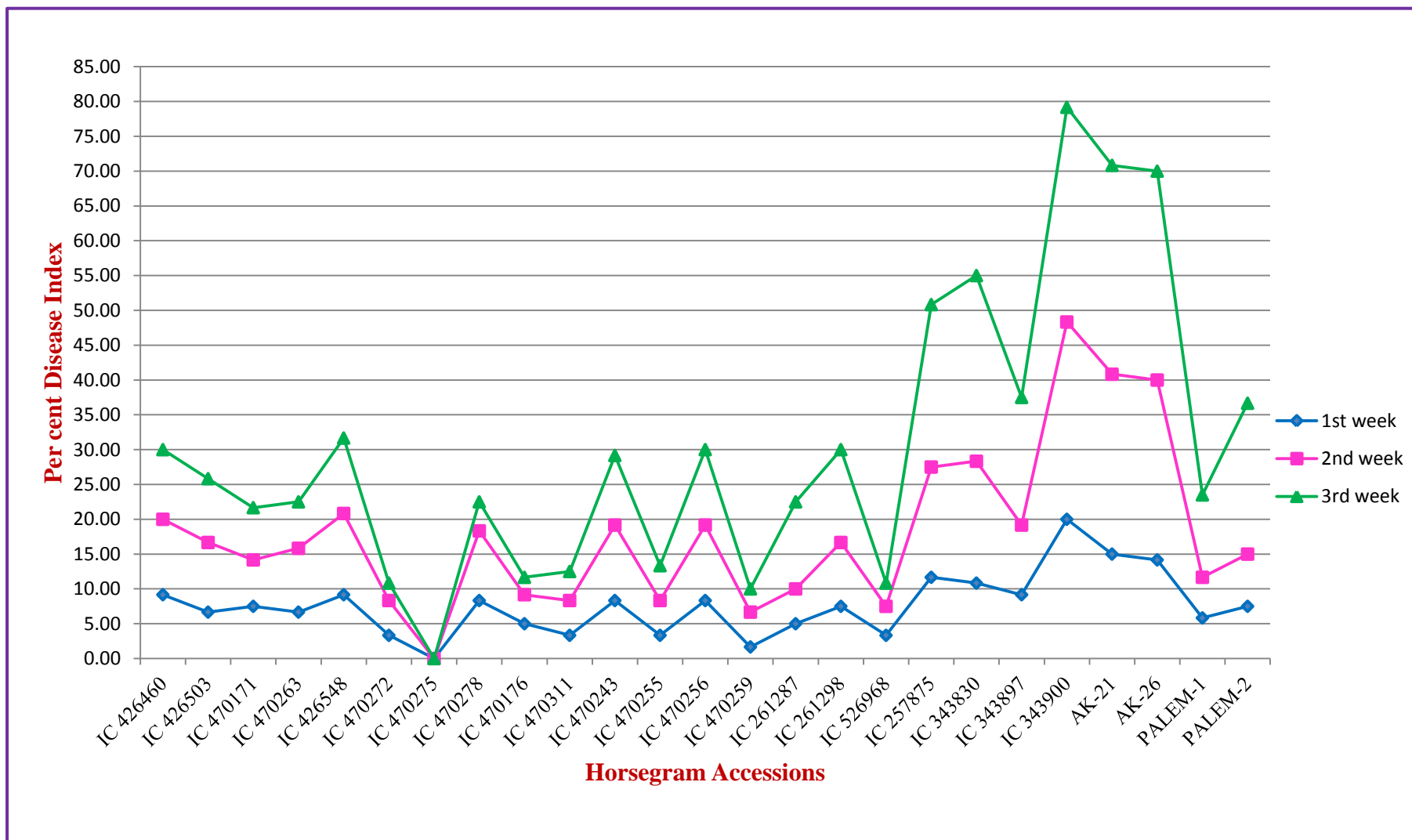


**Figure 1. Performance of horsegram accessions showing varied Per cent seed surface infected in the standard blotter test**

**Table 4.6. Screening of horsegram accessions for resistance against *C. dematium* under greenhouse conditions**

IC numbers	Name of accessions	Per cent disease index (PDI)		
		1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week
IC 257875	S-6	11.67 (19.94)	27.50 (31.60)	50.83 (45.46)*
IC 261287	NSM-147	5.00 (12.91)	10.00 (18.42)	22.50 (28.31)
IC 261298	NSM-202	7.50 (15.74)	16.67 (24.03)	30.00 (33.12)
IC 343830	SKN-21	10.83 (19.18)	28.33 (32.14)	55.00 (47.85)
IC 343897	SKN-88	9.17 (17.58)	19.17 (25.94)	37.50 (37.74)
IC 343900	SKN-91	20.00 (26.53)	48.33 (44.03)	79.17 (62.85)
IC 426460	NS/04-11	9.17 (17.58)	20.00 (26.46)	30.00 (33.18)
IC 426548	NS/05-102	9.17 (17.58)	20.83 (27.11)	31.67 (34.20)
IC 470171	NS/05-009	7.50 (15.74)	14.16 (22.08)	21.67 (27.67)
IC 470176	NS/05-14	5.00 (12.91)	9.17 (17.58)	11.67 (19.87)
IC 470243	NS/05-81	8.33 (16.73)	19.17 (25.94)	29.17 (32.67)
IC 470255	NS/05-93	3.33 (8.61)	8.33 (16.59)	13.33 (21.32)
IC 470256	NS/05-94	8.33 (16.73)	19.17 (25.94)	30.00 (33.18)
IC 470259	NS/05-97	1.67 (6.06)	6.67 (14.89)	10.00 (18.33)
IC 470263	NS/05-101	6.67 (14.89)	15.83 (23.37)	22.50 (28.28)
IC 470272	NS/05-110	3.33 (10.37)	8.33 (16.73)	10.83 (19.18)
IC 470275	NS/05-113	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
IC 470278	NS/05-116	8.33 (16.73)	18.33 (25.33)	22.50 (28.28)
IC 470311	NS/05-149	3.33 (10.37)	8.33 (16.73)	12.50 (20.63)
IC 426503	NS/04-57	6.67 (14.89)	16.67 (24.07)	25.83 (30.51)
IC 526968	PSR-11693-3	3.33 (10.37)	7.50 (15.74)	10.83 (19.03)
AK-21 (Resistant Check)		15.00 (22.65)	40.83 (39.68)	70.83 (57.36)
AK-26		14.17 (22.08)	40.00 (39.19)	70.00 (56.80)
PALEM-1		5.83 (13.91)	11.67 (19.87)	23.50 (28.95)
PALEM-2		7.50 (15.88)	15.00 (22.77)	36.67 (37.25)
CD at 5%		2.92	4.27	5.29
SE(m)		1.02	1.50	1.85

\*Figures in parentheses are angular transformed values



**Figure 2. Performance of horsegram accessions showing varied Per cent disease indices under greenhouse screening**

## **4.6.2. Glasshouse screening: Spray inoculation on one month old seedlings**

### **4.6.2.1. Standardization experiment**

This experiment was conducted primarily to standardize the optimum period of covering the plants with polythene cover after spray inoculation for disease development. It was evident that 72 hours of covering the seedlings with polythene cover after inoculation was ideal for disease development. Symptoms initiated with water soaked lesions followed by shrinking of primary leaves were observed 7 days after inoculation. Covering the seedlings for 24 and 48 hours with polythene cover did not show any effect on the seedlings in inciting the disease in spite of the heavy dose of inoculum. This may be due to the hardy nature of horsegram.

### **4.6.2.2. Varietal screening by spraying inoculum on one month old seedlings**

The symptomatic progress of disease development is depicted in Plate 4.19. On the diseased seedlings initial symptoms appeared as water soaked irregular lesions or blisters followed by shrinking of upcoming leaves. As the disease was progressed, the lesions got enlarged and covered the entire leaf lamina. At the end of third week the susceptible lines showed brown coloured, large lesions on the leaves along with leaf tip browning or burning. In highly susceptible accessions apart from above symptoms wilting and leaf drop symptoms were also observed. On the moderately resistant accessions, the symptoms were restricted without any spread exhibiting tolerance to disease. This may be due to the hypersensitive response exhibited by the host crop to minimize the disease development. Chahota *et al.* (2005) evaluated 63 landraces of horsegram against *C. truncatum* under field conditions and revealed that the lines HPKC-39, HPKC-57 and HPKC-33 were found free from the disease and thus considered as potential.

Spraying higher doses of inoculum on one month old seedlings of 25 accessions, followed by covering the plants with polythene bags for 72 hours resulted in disease incidence within seven days in all tested accessions except IC 470275 (Table 4.6). The Percent Disease Index (PDI) among all the accessions ranged from 0.00 (IC 470275) to 79.17 (IC 343900) at the end of third week after inoculation. The immune accession, IC 470275 was found completely free from the disease (PDI- 0.0) in all the three replications despite the usage of high inoculum concentration ( $5 \times 10^6$  conidia per ml) even after completion of three weeks (Plate 4.20). There was absolutely no difference between the pathogen inoculated and uninoculated (AK-21) plants (Plate 4.21). The laboratory screening studies also revealed that it was a resistant accession.

At the end of the third week, none of the accessions was grouped under “Resistant” category as all of them recorded PDI values of above 5.0. In all, 11 accessions were grouped under Moderately Resistant category. Among them, accessions IC 470259 (1.67 PDI) and IC 470255, IC 470272, IC 470311 and IC 526968 (3.33 PDI) exhibited tolerance to the disease by recording less PDI at the end of first week. Even at the end of 3 weeks also, low PDI in the range of 10.00-13.33 was recorded in these accessions and thus categorized under Moderately Resistant group. The other six accessions (IC 470171, IC 470263, IC 470278, IC 470176, IC 261287 and PALEM-1), though recorded slightly more infection in the first week, could restrict the infection (PDI) to < 25.0, thus falling under Moderately Resistant category.

Eight accessions, viz., IC 426460, IC 426503, IC 426548, IC 470243, IC 470256, IC 261298, PALEM-2 and IC 343897 were grouped under ‘Susceptible’ category and the PDI of these accessions was in the range of 25.83-37.50. The remaining five accessions AK-21, AK-26, IC 343830, IC 343900 and IC 257875 were found ‘Highly Susceptible’ with a PDI of > 50 (50.83-79.17) (Plate 4.22). Rajan Sharma and Kaushal (1999) conducted similar studies on horsegram using *C. truncatum* and found HPK- 4 and Paharikulth were moderately susceptible. Symptomatic comparison of horsegram accessions that recorded IMMUNE IC 470275 (NS/05-113) and HIGHLY SUSCEPTIBLE IC 343900 (SKN-91) reactions along with CONTROL during varietal screening studies was depicted (Plate 4.23).

It was observed that 4, 44, 32 and 20% of the accessions were grouped under immune, moderately resistant, susceptible and highly susceptible categories, respectively based on their PDI values. Accessions that were classified as resistant and moderately resistant can be used in future breeding programmes to combine this trait with other reproductive traits.

The resistant check, AK-21, used in the present investigation, was selected based on the reports of Murthy (1997) and SMIS (2011) wherein it was found resistant to the attack of anthracnose under field conditions in Karnataka. In the present study, the same accession, though found resistant under laboratory screening studies, recorded highly susceptible reaction in the greenhouse screening, which may be attributed to the use of heavy inoculum pressure under greenhouse conditions and also to the different pathogenic variants of anthracnose pathogen. Bharadwaj and Singh (1986) also observed the differential pattern in the pathogenicity of *C. dematium* f. sp. *truncatum* and attributed to different pathogenic variants of *C. dematium* f. sp. *truncatum* in nature.

On the contrary, IC 526968 was highly susceptible in laboratory conditions and found moderately resistant in glasshouse conditions. Palem-2 was found highly susceptible in laboratory conditions and susceptible in glasshouse conditions. Accessions IC 470176 and IC 470171 were found susceptible in laboratory conditions and moderately resistant in glasshouse conditions. This may be attributed to the fact that the laboratory studies were conducted only at seed level and thus dependent on the seed characters such as seed texture, seed colour, pubescence etc. The plant traits must have played a role in preventing the spread of the disease and thus performed better under greenhouse conditions. Schmit and Baudoin (1992) reported that *Phaseolus polyanthus* that showed high level of resistance to anthracnose (*C. lindemuthianum*), also possessed many desirable and useful agronomic traits, such as cold tolerance, lodging resistance due to the thick stem at the base of the plant, the presence of a tuberous or fibrous root system, long epicotyls, and a high number of pods per inflorescence.

The immune accession, IC 470275 possesses morphological traits like more 100 seed weight and smooth leaf. The highly susceptible and susceptible accessions IC 343900, IC 343830 and IC 343897 have twining nature in their morphology. Thus, we may consider more 100 seed weight as a positive trait for breeding resistant varieties to anthracnose in future.

Horsegram is known to be susceptible to the infection of wide range of *Colletotrichum* species (Sharma, 1976., Murthy, 1997 and Brink and Belay, 2006). It also acts as an alternate host to the *Colletotrichum* isolates that attack soybean, bean and urdbean genotypes as their isolates were found infecting horsegram. So the present findings of screening against anthracnose would help in selecting possible sources of resistance so as to use them in crop improvement programmes.



**Plate 4.19. Progress of anthracnose disease pattern in horsegram. a. Irregular water soaked areas soon turning to blistered areas b. Spread of lesions on the leaf lamina c. Shrivelling of top leaves due to more diseased areas d. Drying up of infected leaves and defoliation**



**Plate 4.20. Horsegram accession IC 470275 (NS/05-113) found IMMUNE with no disease symptoms during varietal screening studies**



**Plate 4.21. Horsegram uninoculated accession (CONTROL) during varietal screening studies**



**Plate 4.22. Horsegram accession IC 343900 (SKN-91) found HIGHLY SUSCEPTIBLE during varietal screening studies**



**Plate 4.23. Symptomatic comparison of Horsegram accessions that recorded IMMUNE IC 470275 (NS/05-113) and HIGHLY SUSCEPTIBLE IC 343900 (SKN-91) reactions along with CONTROL during varietal screening studies**

*Chapter V*

**SUMMARY AND CONCLUSIONS**

## Chapter V

### SUMMARY AND CONCLUSIONS

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc. Syn. *Dolichos biflorus*) is susceptible to anthracnose disease caused by different species of *Colletotrichum*. The present investigation was taken up to identify the specific causal organism responsible for this disease in Andhra Pradesh, study the location and seed transmission nature of the pathogen and identify horsegram genotypes that can withstand the anthracnose disease attack so as to make them available for future crop improvement programmes.

Horsegram leaves, suspected with anthracnose symptoms, were collected from fields of NBPGR, Rajendranagar and the fungal pathogen was isolated and identified as *Colletotrichum dematium* (Sutton, 1980). The pathogenicity of *C. dematium* was proved by following seed inoculation, detached leaf technique and pot culture studies.

Location of the seed borne infection was studied using component plating technique. Results revealed that the *C. dematium* infection could reach all parts of the horsegram seed, though with varied levels of intensity. Seed coat infection was the highest, which ranged from 62-100%, followed by cotyledonary infection (36-72%), and the embryo infection was the least, which reached upto 30%. The internally seed borne nature of the pathogen, *C. dematium*, was evident as the pathogen development in the form of acervuli was noticed on all parts of the seed.

In the third objective, naturally infected horsegram seeds were collected to study the seed to seedling transmission using paper towel method, sand method and grow-out test methods. All the three methods successfully proved the seed transmission nature of the fungus, *C. dematium*, which was evident with poor germination, seed rot and seedling decay symptoms. In Paper towel method, germination of infected seeds was reduced to 42%, while sand and grow-out tests recorded a germination of 30% in each method. In sand method, maximum of 12% seed rot recorded which was lower than the paper towel result of 34% and slightly higher than grow-out test result of 10%. Seedling decay symptoms observed in sand (20%) and paper towel (18%) methods, while nil report of seedling decay symptoms found in grow-out test method.

Horsegram accessions (25) with phenotypic diversity were collected from NBPGR Regional station, Hyderabad. These accessions were screened under laboratory and greenhouse conditions.

Laboratory screening was conducted by inoculating seeds of each accession with the pathogen followed by plating on blotters. The impact of pathogen was studied

by recording germination and seed infection percentages. Based on the per cent seed surface infected with acervuli of *C. dematium*, the accessions were grouped by following 0-4 scale. Among 25 accessions none of the accessions was found immune with 0% seed infection. Three accessions (AK-21, IC 470275 and IC 470259) were considered as resistant as they have recorded < 10% seed surface infected. Four accessions (IC 343900, IC 343830, IC 526968 and PALEM-2) were found highly susceptible with >50% seed area infected with the fungus. Among the 25 accessions tested, 12% of the accessions fall under resistant category, 36% each under susceptible and moderately resistant categories and the remaining 16% under highly susceptible category.

Greenhouse studies of germplasm screening resulted in identification of one Immune accession, IC 470275 with zero PDI. None of the accessions was found resistant. Eleven accessions (IC 470259, IC 470255, IC 470272, IC 470311, IC 526968, IC 470171, IC 470263, IC 470278, IC 470176, IC 261287 and PALEM-1) were grouped under Moderately Resistant category and eight accessions under susceptible category. The remaining five (AK-21, AK-26, IC 343830, IC 343900 and IC 257875) were Highly Susceptible with > 50 PDI. It was observed that 4, 44, 32 and 20% of the accessions were grouped under immune, moderately resistant, susceptible and highly susceptible categories, respectively based on their PDI values.

The present investigation yielded the following conclusions.

1. *Colletotrichum dematium* (Pers. ex Fr.) Groove was the organism found responsible for causing anthracnose in horsegram in Andhra Pradesh.
2. Presence of pathogen on all parts of the seed., viz., seed coat, endosperm and embryo was noticed and thus it is considered as both externally and internally seed borne.
3. The seed transmission nature of *C. dematium* was proved in all the three methods tested and was evident by seed rot (pre-emergence seed rot) and seedling decay (post-emergence damping-off) symptoms.
4. The horsegram genotype, IC 470275 was identified as 'Immune' and this accession can be used in the future crop improvement programmes to develop a suitable variety for growing in Andhra Pradesh region.

The accession, IC 470275, identified as a good source of resistance under greenhouse conditions can be further evaluated under field conditions for confirmation. It can also be tested against different isolates of *C. dematium* collected from other regions.

# **LITERATURE CITED**

## LITERATURE CITED

- Aneza, K.F. 2003. *Experiments in Microbiology, Plant Pathology and Biotechnology* (4<sup>th</sup> edition). New Age International Publishers. 66-73.
- Asalmol, M.N., Kale, V.P and Ingle, S.T. 2001. Seed borne fungi of chilli-incidence and effect on seed germination. *Seed research*. 29 (1): 76-79.
- Balardin, R.S., Jarosz, A.M and Kelly, J.D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central and North America. *Phytopathology*. 87: 1184-1191.
- Balbir Kour, Gurmeet Kour, Sanjana Kaul and Manoj, K.D. 2012. Screening of *Phaseolus vulgaris* cultivars growing in various areas of Jammu and Kashmir for anthracnose resistance. *International Journal of Scientific and Research Publications*. 2 (6): 40-49.
- Bansal, R.D and Grover, R.K. 1969. Reaction of chilli (*Capsicum frutescens*) varieties to *Colletotrichum capsici*. *Journal of Research. Punjab Agricultural University*. 6: 345-348.
- Basak, A.B. 1998. Studies on the location of *Colletotrichum capsici* (Syd.) Butler & Bisby in the infected chilli seeds. *Seed Reseach*. 26 (1): 101-104.
- Bharadwaj, C.L and Singh, B.M. 1986. Strain variation in *Colletotrichum dematium* f.sp. *truncatum* from four leguminous hosts. *Indian Journal of Mycology and Plant Pathology*. 16: 139-141.
- Brink, M and Belay, G. 2006. *Macrotyloma uniflorum* (Lam.) Verdc. In: Brink, M. & Belay, G. (Editors). PROTA 1: *Cereals and pulses/Céréales et légumes secs*. [CD-Rom]. PROTA, Wageningen, Netherlands.
- \*Butler, E.J. 1918. *Fungi and diseases in plants*. Thacker, Spink & Co. Calcutta.
- Cardona, J.A.O. 1990. Seed transmission and control of cowpea anthracnose (*Colletotrichum dematium*). *Revista Instituto Colombiano Agropecuario*. 25: 265-274.
- Chahota, R.K., Sharma, T.R., Dhiman, K.C and Kishore, N. 2005. Characterization and evaluation of Horsegram (*Macrotyloma uniflorum* Roxb.) germplasm from Himachal Pradesh. *Indian Journal of Plant Genetic Resources*. 18 (2): 221-223.

- Chandra Nayaka, S., Udaya Shankar, A.C., Niranjana, S.R., Prakash, H.S and Mortensen, C.N. 2009. Technical bulletin on Anthracnose disease of chilli pepper. *Published by the Asian Seed Health Centre (AsSHC)*, Department of Studies in Applied Botany & Biotechnology, University of Mysore, Manasagangotri, Karnataka, India.
- CMIE. 2012. Executive Summary – GDP Growth. Centre for Monitoring Indian Economy (CMIE) Pvt. Ltd. Mumbai. December, 2012. <http://www.cmie.com>.
- Datar, V.V and Mayee, C.D. 1981. Assessment of losses in tomato yield due to early blight. *Indian Phytopathology*. 34 (2): 191-195.
- Deshmukh, J., Mehta, B.P., Sabalpara, A.N and Patil, V.A. 2012. Screening of Indian bean (*Lablab purpureus* L.) varieties/ germplasms against anthracnose (*Colletotrichum gloeosporioides* Penz. and Sacc.) under field conditions. *JBiopest*. 5: 50-52.
- Haenglee, T and Supchung, H. 1995. Detection and transmission of seed-borne *Colletotrichum gloeosporioides* in redpepper, *Capsicum annuum*. *Seed Science and Technology*. 23: 533-541.
- Hagborg, W.A.F., Warner, G.M and Philipps, N.A. 1950. Use of 2,4-D as an inhibitor of germination in routine examination of beans for seed borne infections. *Science*. 111: 91.
- Holliday, P. 1995. *Fungus diseases of tropical crops*, Dover publications, Mineola, New York, USA. 624
- Inglis, D.A., Hagedorn, D.J and Rand, R.E. 1988. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. *Plant Disease*. 72: 771-774.
- Jayaraj, A.P., Toverly, F.I., Lewin, M.R and Clarck, C.G. 2000. Duodenal ulcer prevalence: Experimental evidence for the possible role of dietary lipids. *Journal of Gastroenterology and Hepatology*. 15: 610-616.
- Khare, M.N and Chacko, S. 1983. Factors affecting seed infection and transmission of *Colletotrichum dematium* f. sp. *truncate* in soybean. *Seed Science and Technology*. 11: 853-858.
- Kulkarni, A.S. 2009. Epidemiology and integrated management of anthracnose of green gram. *Ph.D. thesis*. University of Agricultural Sciences, Dharwad, Karnataka, India.
- Kumaraswamy, B.M. 1983. Comparative study of *Colletotrichum* spp. occurring on cowpea and horsegram in Karnataka. *M.Sc. thesis*. University of Agricultural Sciences, Bangalore, Karnataka, India.

- Kumud Kumar, Singh, J and Khare, A. 2004. Detection, location transmission and management of seed borne *Colletotrichum dematium* causing dieback and anthracnose in chilli. *Farm Science Journal*. 13 (2): 152-153.
- Maden, S., Singh, D., Mathur, S.B and Neergaard, P. 1975. Detection and location of seed borne inoculum of *Ascochyta rabiei* and its transmission in chickpea (*Cicer arietinum*). *Seed Science and Technology*. 3: 667-681.
- Minchinton, E. 1999. *Anthracnose of beans*. State of victoria, Department of primary industries, Knoxfield. 559.
- Mohammed Yesuf and Somsiri Sangchote. 2005. Seed transmission and epidemics of *Colletotrichum lindemuthianum* in the major Common bean growing areas of Ethiopia. *Kasetsart Journal of Natural Science*. 39: 34-35.
- Murthy, R. 1997. Studies on seed borne aspects and control of anthracnose of horsegram (*Macrotyloma uniflorum*) caused by *Colletotrichum dematium* (Pers. ex. Fr.) Groove. *M. Sc. Thesis*, University of Agricultural Sciences, Bangalore, Karnataka, India.
- Mustafa, A. 2009. Seed mycoflora of shisham (*Dalbergia sissoo* Roxb.) and their integrated management. *Ph. D Thesis*. Department of Plant Pathology, University of Agriculture, Faisalabad.
- Neergaard, E.D., Tornoe, C and Norskov, A.M. 1999. *Colletotrichum truncatum* in soybean: studies of seed infection. *Seed Science and Technology*. 27: 911-921.
- Neergaard, P. 1977. *Seed Pathology*. Volume I. The McMillan Press, London. 393-397.
- Nwachukwu, E.O and Umechuruba, C.I. 1991. Seed-borne fungi associated with African yam bean seeds in four Eastern states of Nigeria. *Seed Research*. 19 (1): 13-20.
- Pangtey, Y.P.S and Sinha, S. 1980. Two new seed-borne leaf spot diseases of horsegram in Kumaun hills. *Indian Journal of Agricultural Sciences*. 50: 502-504.
- Prasanna, K.P.R. 1985. Seed health testing of cowpea with special reference to anthracnose caused by *Colletotrichum lindemuthianum*. *Seed Science and Technology* 13: 821-827.
- Prasanth, D and Ponnuswami,V. 2008. Screening chilli genotypes against *Colletotrichum capsici* and analysis of biochemical and enzymatic activities in inducing resistance. *Indian Journal of Genetics*. 68 (3): 344-346.
- Prashanthi, G., Satish Kumar, D., Harani, A., Rao, K.N.V and David, B. 2011. A Medicinal potency of *Macrotyloma uniflorum* (Lam.)Verdc. *Inventi Rapid: Ethnopharmacology*. December 2012. <http://www.inventi.in>

- \*Puri, S., Singh, T and Singh, D. 1981. Location of *Colletotrichum dematium* in seeds of chillies (Abstract). *Third International Symposium on Plant Pathology, New Delhi, India*.
- Rajan Sharma and Kaushal, R.P. 1999. Characterization of pathogenic variability in *Colletotrichum truncatum* in Himachal Pradesh. *Indian Phytopathology*. 52 (4): 389-392.
- Rangaswami, K.T., Anilkumar, T.B and Yadahally, Y. H. 1991. Seed health of horsegram in relation to dates of sowing. *Plant Protection Bulletin*. 43: 3-4.
- Ravi, S., Sabitha, D., Valluvaparidasan, V and Jeyalakshmi, C. 1999. Impact of seed-borne *Colletotrichum lindemuthianum* on French bean seed quality. *Acta Phytopathologica et Entomologica Hungarica*. 34 (4): 283-291.
- Renukeswarappa, J.P and Shethna, Y.I. 1985. Improved blotter method to detect *Colletotrichum* on chilli (*Capsicum annum*). *Seed Research*. 13 (1): 86-88.
- Saharan, G.S. 1979. Seed mycoflora of horsegram (*Dolichos biflorus* L.) *Indian Phytopathology*. 32: 138-140.
- Schmit, V and Baudoin, J.P. 1992. Screening for resistance to *Ascochyta* blight in populations of *Phaseolus coccineus* L. and *P. polyanthus* Greenman. *Field Crops Research*. 30:155-165.
- \*Sharma, A.D. 1976. Two new diseases of *Dolichos biflorus* in India. *Indian Journal of Mycology and Plant Pathology*. 7: 10-12.
- Shivanna, M.B and Shetty, H.S. 1988. Seed-borne nature and transmission of *Colletotrichum dematium* in Cluster bean. *Seed Research*. 16 (1): 102-104.
- Shyam, K.R and Chakraborty, P.K. 1985. Seed transmission of *Colletotrichum lindemuthianum* in different cultivars of Bean (*Phaseolus vulgaris* L.). *Seed Research*. 13 (1): 211-212.
- Singh, K., Vishnuvat, K and Rashmi, T. 2009. Detection, transmission and management of seed-borne inoculum of anthracnose (*Colletotrichum capsici*) in chilli. *Seed Research*. 37(2): 143-146.
- SMIS. 2011. National informatics centre, Agricultural informatics division. Seed Management Information System (SMIS), SeedNet India Portal. September, 2011. <http://www.smis.dacnet.nic.in>
- Sutton, B.C. 1980. *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Commonwealth Mycological Institute, Kew, England.

- Tu, J.C. 1986. Detached leaf technique for screening beans (*Phaseolus vulgaris* L.) in *in vitro* against anthracnose (*Colletotrichum lindemuthianum*). *Canadian Journal of Plant Science*. 66: 805-809.
- Tullu, A., Buchwaldt, L., Lulsdorf, M., Banniza, S., Barlow B., Slinkard, A.E., Sarker, A., Taran, B., Warkentin, T and Vandenberg, A. 2006. Sources of resistance to anthracnose (*Colletotrichum truncatum*) in wild *Lens* species. *Genetic Resources and Crop Evolution*. 53: 111-119.
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