

**EPIDEMIOLOGY AND MANAGEMENT OF  
ANTHRACNOSE OF HORSE GRAM  
(*Macrotyloma uniflorum*)**

**THESIS**

*By*

**DEVANSHI PANDIT**

**(A-2008-30-21)**

*Submitted to*



**CHAUDHARY SARWAN KUMAR  
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA  
PALAMPUR – 176 062 (H.P.) INDIA**

*in*

partial fulfilment of the requirements for the degree  
*of*

**MASTER OF SCIENCE IN AGRICULTURE  
(DEPARTMENT OF PLANT PATHOLOGY)  
(PLANT PATHOLOGY)**

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*Since my birth to adulthood,  
you were there with me...*

*To bolster in all highs n lows  
of my life, you were there with me...*

*Dear PAPA & MAMASANY, I  
really owe all my success to you and  
thank you for shaping my future  
fabulously.*

*Dedicated to the Most  
Cherished Persons of my*

*Life...*

*My daughter*



**Dr. R.P. Kaushal**  
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## **CERTIFICATE – I**

This is to certify that the thesis entitled “**Epidemiology and management of anthracnose of Horse gram (*Macrotyloma uniflorum*)**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Devanshi Pandit (Admission No. A-2008-30-21)** daughter of Shri Suresh Pandit under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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

Place: Palampur  
Dated: 24 November, 2010

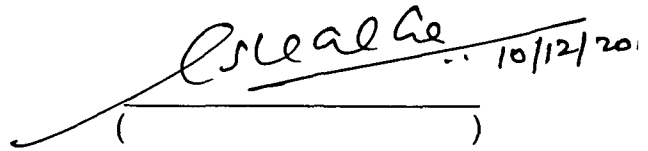
  
(Dr. R.P Kaushal)  
Major Advisor

## CERTIFICATE- II

This is to certify that the thesis entitled “**Epidemiology and management of anthracnose of Horse gram (*Macrotyloma uniflorum*)**” submitted by **Devanshi Pandit (A-2008-30-21)** daughter of **Shri Suresh Pandit** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.



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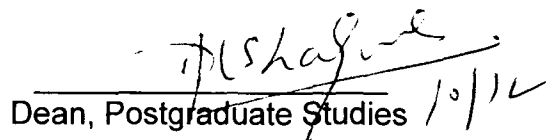
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*Needless to say, all omissions and errors are mine.*

Place : Palampur

Dated : 8<sup>th</sup> November, 2010

*Devanshi*  
(Devanshi Pandit)



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## LIST OF ABBREVIATIONS USED

Sr. no.	Abbreviation	Meaning
1	°C	Degree Celsius
2	/	Per
3	%	Per cent
4	μ	Micron
5	μm	Micro meter
6	cm	Centimeter
7	dia.	Diameter
8	<i>et al.</i>	et alii (and others)
9	Fig.	Figure
10	g	Gram
11	hr	Hour (s)
12	H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
13	<i>i.e.</i>	Id est (that is)
14	kg	Kilogram
15	mg	Milligram
16	min	Minute (s)
17	ml	Milliliter
18	mm	Millimeter
19	p.	Page
20	pp.	Pages
21	ppm	Parts per million
22	rpm	Revolutions per minute
23	<i>Viz.,</i>	videlicet (namely)

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**Department of Plant Pathology, COA  
CSK Himachal Pradesh Krishi Vishvavidyalaya  
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**ABSTRACT**

Investigations on anthracnose of horse gram were undertaken to study the pattern of disease development, agrometeorological factors affecting the disease and to work out its suitable management strategies. Among the epidemiological parameters studied in the laboratory, 20-25°C temperature, 95-100% relative humidity and 12 hour alternate dark and light photoperiod were found optimum for lesion development of anthracnose of horse gram. A field experiment was conducted on anthracnose development during the years 2009 and 2010 on susceptible horse gram cultivar HPK-4. The progress of disease exhibited sigmoidal pattern of the curve during both years. Disease severity was higher in early sown crop at minimum row to row spacing. Mean temperature, rainfall and bright sunshine hours showed positive correlation with disease severity during both the years. The correlation coefficient between disease severity and mean relative humidity was negative during 2009 and positive during 2010. One hundred five genotypes of horse gram were evaluated for resistance to anthracnose under field conditions during *Kharif* seasons of 2009 and 2010. None of the genotypes were resistant to disease. However, thirty five genotypes were found moderately resistant and twenty three genotypes were moderately susceptible. Bavistin @ 0.1% and Dithane-M-45 @ 0.25% were effective in management of anthracnose of horse gram under field conditions. Among botanicals tested in the laboratory, *Cannabis sativa* at 100 % was found best followed by *Chromolaena odorata* and *Adhatoda vasica*. Among biopesticides, Achook @ 0.5% was found most effective. Among biocontrol agents maximum inhibition of mycelial growth was observed with *Trichoderma viride*-H followed by *Pseudomonas fluorescens*.

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Student

Date: 8<sup>th</sup> Nov., 2010

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Major Advisor

Date: 8/11/10

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# INTRODUCTION

# 1. INTRODUCTION

---

Pulses are important food crops due to their high protein and essential amino acid content. Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen. Majority of population being vegetarian, pulses constitute an integral part of Indian diet. However, pulses are subjected to the attack of a variety of diseases and insect pests. Horse gram (*Macrotyloma uniflorum*), commonly known as *Kulthi* in north India, is an important edible legume, consumed throughout the country. It is primarily a crop of the dry and upland areas of the peninsular and eastern states of India.

In India horse gram is extensively grown in Karnataka, Andhra Pradesh, Tamil Nadu, Madhya Pradesh and parts of Maharashtra, Bihar, Orissa and to some extent in the hilly slopes of Himachal Pradesh and Uttar Pradesh. It accounts for about 7% of the total area under pulses and 4.5% of the total pulse production. In Himachal Pradesh, total area under cultivation is 2537 ha and average yield is 398kg/ha (Anonymous 2004) and is grown in Kangra, Hamirpur, Chamba, Mandi, Bilaspur and Sirmour districts of Himachal Pradesh.

Horse gram requires an average temperature of 20–30°C and does not tolerate frost. It is drought-resistant and can be grown with rainfall as low as 380 mm. It is mostly grown in areas with less than 900 mm annual rainfall. In high rainfall areas, it is grown on residual moisture in the dry season, e.g. after rice crop. Most horse gram cultivars are short-day plants. Horse gram grows on a wide range of soils with pH 5–7.5, including poor soils. It does not tolerate water logging.

Among diseases, leaf spotting fungi are a major constraint in realization of full genetic potential of a pulse crop. Horse gram too, suffers from both biotic and abiotic constraints. During the cropping season, wet and humid environment



conditions predispose the crop to the attack of many pathogens. The main diseases of horse gram in India are anthracnose (*Colletotrichum truncatum*), leaf spot (*Cercospora dolichi*, synonym: *Mycosphaerella cruenta*), rust (*Uromyces appendiculatus*), root rot (*Pellicularia filamentosa*, synonym: *Thanatephorus cucumeris*), dry root rot (*Macrophomina phaseolina*) and horse gram yellow mosaic virus (HgYMV).

Anthracnose and leaf spot are the major diseases of the crop under high rainfall conditions of Himachal Pradesh. No precise estimates of losses due to these diseases are known. About nine species of *Colletotrichum* have been recorded on legume crops worldwide, including *C. capsici*, *C. coccodes*, *C. crassipes*, *C. dematium*, *C. destructivum*, *C. gloeosporiodes*, *C. lindemuthianum*, *C. trifolii* and *C. truncatum* (Lenne 1992). Legume crops such as bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* L. Walp), soybean (*Glycine max* (L) Merr.), peanut (*Arachis hypogea* L.), lentil (*Lens culinaris* Medik.) and alfalfa (*Medicago sativa* L.) have been reported as hosts of *Colletotrichum* species (Bailey and Jeger 1992).

*C. truncatum* is a highly unspecialized pathogen, which attacks many grain and forage legumes including soybean (*Glycine max*), lentil (*Lens culinaris*), urdbean (*Vigna mungo*), mungbean (*V. radiata*), cowpea (*V. unguiculata*), horse gram (*M. uniflorum*), pea (*Pisum sativum*) and various weed hosts (Lenne and Sonoda 1978; Sharma and Kaushal 1999). Isolates of *C. truncatum* vary considerably in colony characteristics, size of fruiting structures and pathogenicity. Additionally, *C. truncatum* population from different hosts often exhibit different host preferences. Conidia of *C. truncatum* are described as hyaline and one celled, with slightly falcate shape and a size of 17.0-31.5 x 3.0-4.5µm (length x width). Dark brown to black setae are generally produced in abundance (Tiffany and Gilman 1954; Sutton 1992).

Anthracnose caused by *C. truncatum* is one of the most important *ex-eme* ryogenic fungal pathogens of horse gram. The disease causes a significant reduction of seed germination, seed quality thereby limiting its potential yield.

Anthracnose may also attack more mature plants during the later part of the growing season. The crop is vulnerable to attack at all growth stages depending upon the conditions favourable for initiation and development of the disease.

No plant pathological work on its epidemiology, losses due to the disease and its management has been done either in India at large or in Himachal Pradesh. Keeping these points in view, the present study was undertaken with the following objectives:

1. To work out epidemiology of the disease, and
2. to manage the disease through chemicals, cultural practices, host resistance, use of botanicals, bio-control agents and biopesticides.



**REVIEW**  
**OF**  
**LITERATURE**

## 2. REVIEW OF LITERATURE

---

The literature on anthracnose of horse gram is very scanty. The relevant literature pertaining to anthracnose of horse gram as well other crops is reviewed under the following sub heads:

- 2.1 Occurrence and distribution of anthracnose in India
- 2.2 The pathogen and its host range
- 2.3 Symptomatology
- 2.4 Mode of pathogen perpetuation
- 2.5 Factors affecting disease development
- 2.6 Epidemiological studies of the pathogen on horse gram and related legumes
- 2.7 Management
  - 2.7.1 Host resistance
  - 2.7.2 Chemical methods
  - 2.7.3 Biological control
  - 2.7.4 Botanicals (biopesticides)

### **2.1 Occurrence and distribution of anthracnose in India**

The genus *Colletotrichum* includes more than 900 species responsible for anthracnose disease. In India more than hundred species have been reported (Ramakrishnan and Subramanian 1952; Tondon and Chandra 1963; Tilak and Ramachandra 1968 and Sarbhoy *et al.* 1975, 1980, 1986 and 1996). Koch *et al.* (1989) collected the isolates of *Colletotrichum* from South Africa and tested the pathogenicity on soybean and observed that *Colletotrichum truncatum* was most pathogenic on soybean. Sharma and Kaushal (2001) collected eleven isolates of urd bean infected by *C. truncatum* from different locations of Himachal Pradesh and confirmed their pathogenic behaviour.

## 2.2 The pathogen and its host range

Tiffany and Gilman (1954) divided species of *Colletotrichum* from legumes into two categories. Those with straight, cigar shaped spores include *C. lindemuthianum*, *C. cajini* and *C. glycines* while species with curved (falcate) or crescent shaped species include *C. truncatum* and *C. capsici*.

Schneider *et al.* (1974) isolated *C. truncatum* from the seed coat of surface sterilized soybean seed. Graham *et al.* (1976) observed that Canadian isolates of *C. truncatum* was able to cause anthracnose of alfa-alfa (*Medicago sativa*). Saxena and Sinha (1977) obtained isolates of *C. truncatum* from the seed of mungbean (*Vigna radiata*) and urdbean (*V. mungo*) which had slightly smaller conidia and were slower to sporulate than more typical isolates.

Lenne and Sonada (1978) isolated *C. dematium* f. sp. *truncata* (*C. truncatum*) from leaf spot and stem canker on *Stylosanthes* spp. and *Desmodium* spp growing in field plots at Fort Pierce. This was first report of *C. truncatum* causing leaf spot and stem canker of *Stylosanthes* spp. Lenne and Sonoda (1978) also reported that *C. truncatum* is a highly unspecialized pathogen which also attacks many seed and forage legumes *viz.*, soybean, cowpea, horse gram, mungbean, cowpea, limabean, fababean and various weed hosts.

Hepperly *et al.* (1980) isolated *C. dematium* f. sp. *truncata* (*C. truncatum*) from *Abutilon theophrasti*, a common weed in soybean fields. Isolate of *C. truncatum* was pathogenic on the pods of Amsoy 71 soybean. Khan *et al.* (1980) observed anthracnose caused by *C. truncatum* on soybean, winged bean and lablab niger. Mathur and Mukherji (1981) observed *C. truncatum* on *Crotalaria juncea* in India. Wong *et al.* (1983) tested 7 media and found oat meal agar to be best for growth of *C. dematium* var. *truncatum* and Czapek Dox and potato dextrose agar for sporulation.

Nik and Lim (1984) reported that *C. dematium* f. sp. *truncata* (*C. truncatum*) was the most prevalent of 13 fungal spp. detected in seeds of 11 soybean cultivars. Mycelia occurred in all 3 layers of the seed coat while acervuli

were present in the palisade layer. Lenne *et al.* (1984) observed the ability of setae to act as conidiophores in several species of *Colletotrichum* including *C. truncatum*.

Mirza and Rehman (1985) described *C. dematium* var. *truncatum* (*C. truncatum*) as a pathogen of soybean. Bhardwaj and Singh (1986) observed cultural variation in four isolates of *C. truncatum* from mung, mash, horse gram and soybean. The isolates from urdbean and mungbean appeared as dirty white to dark colonies with abundant sporulation on PDA. However, the isolates from horse gram formed dirty white to steel grey colonies with sparsely formed acervuli whereas acervuli formation in soybean isolate was profuse. Hartman *et al.* (1986) recovered *C. truncatum* from 14 genera of weed hosts. Single conidium isolates of *C. truncatum* from 11 weeds and soybean cultivars.

Bhardwaj and Singh (1986) compared the pathogenic behaviour of isolates of *C. dematium* f. sp. *truncatum* from mungbean and horse gram with those of urdbean and soybean. The results of pathogenicity tests of four isolates on six leguminous host species showed that all isolates differed from each other in their pathogenic behaviour. Isolates from urdbean and mungbean were similar in their pathogenic behaviour on test species except adjukibean whereas isolates of horse gram and mungbean differed from each other in their pathogenicity on soybean and adjukibean. Mungbean and horse gram were susceptible to isolates from four host species whereas cowpea was resistant to all the four isolates of *C. dematium* f. sp. *truncatum*.

Singh and Shukla (1986) recorded the radial growth and mycelia dry weight of *C. truncatum* causing anthracnose of black gram. Mclean and Roy (1988) assayed field collections of prickly sida (*Sida spinosa*), spotted spurge (*Euphorbia maculata*), and smooth pigweed (*Amaranthus hybridus*) from various soybean fields in Mississippi for *Colletotrichum*. Isolates from weeds were identified as either *C. dematium* var. *truncatum* or *C. dematium*. These isolates caused infection of soybean seedlings.

Manandhar *et al.* (1988) found pathogenic variation in *C. truncatum* isolates on soybean. Weidemann *et al.* (1988) found *C. truncatum* pathogenic on species of six genera in two tribes, but was highly virulent only on *Lathyrus odoratus*, *Vicia ervillia*, and most cultivars of *Pisum sativum*. Pathogen also infected *Lupinus indigofera*, *Cicer* and *Lens* spp. Bains *et al.* (1989) observed red/brown lesions on leaves, stems, branches and pods of *V. mungo* and *V. radiata* in Gurdaspur whose causal organism was identified as *C. truncatum*. Patel *et al.* (1989) studied anthracnose of tobacco caused by *C. dematium*.

Boette (1991) reported anthracnose of hemp seedlings incited by *C. truncatum*. Walker *et al.* (1991) reported isolates of *C. truncatum* pathogenic to soybean from field infecting *Xanthium* sp. Mc Ghee (1992) described the *C. truncatum* as crowded, black acervuli on stomata. Acervuli were oval to elongate, hemispheric to truncate conical and erumpent, with numerous spiny setae measuring 60-300 x 3-8µm. Conidia were born on single conidiophores bluntly tapered, curved, unicellular and hyaline.

Kaushal and Singh (1993) studied the pathogenic behavior of two isolates of *C. truncatum* isolated from *Vigna mungo* and *V. radiata*. Two accessions of adjukibean EC15226 and HPU51 were resistant to both the isolates of *C. truncatum*. Isolates from *V. mungo* differed in pathogenicity from the isolate obtained from *V. radiata* on the basis of reaction on urdbean cultivars. All the urdbean cultivars were resistant to *V. radiata* isolates where as two out of eight cultivars, Pant U 26 and Kullu 4 were susceptible to *V. mungo* isolate.

Venette *et al.* (1994) reported lentil anthracnose caused by *C. truncatum*. Pathogen was isolated and its pathogenicity was confirmed. Kyungsook *et al.* (1995) isolated and identified fungi from soybean, mungbean and adjukibean. They compared different spp. of *Colletotrichum* including *C. truncatum*. Pathogenicity and susceptibility differed depending on the crop tested in cross inoculation studies. *C. truncatum* was the most virulent and predominant pathogenic species. Roy (1996) obtained field collections of falcate spored isolates of *Colletotrichum* sp. One was identified as *C. dematium* f. sp. *truncatum*. *C. truncatum* was extremely virulent on soybean seedling death.

Kaiser *et al.* (1998) also reported lentil anthracnose by *C. truncatum*. Sharma and Kaushal (1999) reported pathogenic variability in *C. truncatum* in Himachal Pradesh. The virulence of 11 isolates of *C. truncatum* was studied on 12 selected urdbean differentials. Reaction on related legumes like mungbean, arhar, adjukibean, soybean, cowpea, frenchbean and horse gram was also studied. Rana and Kaushal (2004) studied on the host range. Urdbean and horse gram were found to be susceptible to all the pathogens of *C. truncatum* isolates derived from different hosts.

### 2.3 Symptomatology

Andrus and Moore (1935) reported *C. truncatum* on garden pea and limabean. The initial stages of infection were seen first on pods as small reddish blotches which might have spread over entire surface. The fungus was also found pathogenic on the plant branches, causing girdling and killing in the same way as does pod spot caused by *Diaporthe phaseolorum*. Hemmi (1957) observed dieback or stem anthracnose of cowpea caused by *C. truncatum* resulting in considerable damage. This was the first report of disease occurrence in Japan.

Khan and Singh (1974) reported stem anthracnose of pigeon-pea due to *C. truncatum* causing blackening and drying of cortical region of stem. Singh *et al.* (1978) observed leaf spot of black gram caused by *C. truncatum*. Initial symptoms appeared in the form of small, water soaked, greenish spots on leaf. Morrall (1988) observed a disease of *Lens Culinaris* caused by *C. truncatum*. White to grayish spots developed on the leaf-lets turned brown. Brown lesions occurred on lower stems and pods of mature plants. Stem lesions coalesced causing general blighting and acervuli were observed on diseased stems. Survey showed that the disease occurred in 15 of 20 fields and on two different cultivars.

Simx (1990) observed *C. truncatum* on faba bean seeds Pathogen caused lesions on infected faba bean seeds and rotting of seedlings but was a minor pathogen in routine seed health testing. Mc Ghee (1992) found in his experiment



on leaf spot disease of mungbean caused by *C. truncatum*, the pre and post emergence damping off with dark brown sunken lesions formed on cotyledon and may extend upward and downward of the epicotyls, or extend toward the radical. Irregular blotches formed on the pods, stems and petioles with black acervuli at later growth stage.

Eken and Demirci (2000) reported the pathogen on alfa-alfa whose typical symptoms on stems of mature plants were large, sunken and irregularly shaped lesions. Rathaiah and Sharma (2004) conducted a field experiment on leaf spot disease of mung bean caused by *C. truncatum* both in *kharif* and *rabi* season during 1999. Characteristics symptoms in the form of blood red ring like spots of 8-11mm diameter occurred on the upper surface of leaf and also on pods measuring 2.5 cm in length.

#### **2.4 Mode of pathogen perpetuation**

Wrather and Elord (1990) reported that *C. truncatum* causal agent of anthracnose of soybean located in cotyledon and rarely developed on epicotyl region. Kaple (2000) studies the transmission of *C. truncatum* through infected soybean seed and observed the symptoms on cotyledons. Tiffany (1951) observed an important aspect of the ability of *C. truncatum* to penetrate certain cells of the host without appreciable disruption of their normal functions relates the role of latent mycelium in the survival of pathogen from one season to another. He investigated the role of seed borne inoculum of *C. truncatum* on soybean. Verma (1974) made perpetuation studies of four species of *Colletotrichum*; *dematium*, *graminicola*, *atramentarium* and *gleosporiodes*. Viability of conidia of almost all isolates of four species seems to have lost within 3-7 months. Mycelium can remain viable for 9 months or even after.

#### **2.5 Factors affecting disease development**

Wong *et al.* (1983) reported greater mycelial growth under 12 hour alternating light/dark and continuous light than in darkness. Sporulation was higher under 12 hr alternate light/dark period in PDA. Optimum temperature for

growth and sporulation was 25°C and for germination was 20°C. Lenne *et al.* (1984) observed the ability of setae to act as conidiophores in several spp. of *Colletotrichum* including *C. truncatum*.

Thakur and Khare (1993) studied the factors affecting sporulation and conidial germination of *C. dematium* from mungbean. In *in vivo* tests maximum sporulation was at 25°C and pathogen had 250 conidia per mm<sup>2</sup>. Relative humidity (RH) between 90-100 per cent was optimum for maximum sporulation (224.25/25 mm<sup>2</sup>) in the leaves of 30 days old plant. With increase in age of plant there was decrease in sporulation/mm<sup>2</sup>. *In vitro* tests of germination of conidia started at 25°C. Adebitan (1994) tested 6 media for the growth of *C. truncatum* from cowpea and found cowpea decoction agar best for mycelial production while PDA was best for sporulation. A temperature range of 15-30°C was suitable for the growth and 25°C was found suitable for sporulation. Sporulation was enhanced by continuous light and inhibited in complete darkness.

Egley (1994) studied substrate influences upon the germination of *C. truncatum* conidia. He reported germination of pathogen conidia less than 10 per cent in water or partially lignified agar and more than 70 per cent in firm agar. Mishra and Gupta (1994) studied the influence of environment on the growth and sporulation of *C. dematium*. Maximum radial growth, sporulation and spore germination of pathogen occurred at 27°C temperature as compared to 20, 25 and 30°C but same counts were highest at 100 per cent RH at room temperature (28±2°C). Maximum increase in lesion size was reported in continuous light and dark and total dark followed by room temperature with 100 per cent RH. Increased spores of the pathogen were trapped at drizzling rains, 28°C temperature and 82 per cent RH.

Kaushal *et al.* (1998) reported optimum temperature for germination and germ tube elongation of *C. truncatum* was 20°C. Three hr of light followed by an hour of dark was best for spore germination and germ tube elongation and high RH (100%) was required for the development of acervuli.

Chongo and Bernier (2000) reported optimum temperature for anthracnose development with day time temperature of 20-24°C, regardless of level of host resistance. Singh and Singh (2001) observed growth (mycelial dry weight) of *C. truncatum* from soybean leaves, most pronounced at 28°C (175 mg) and minimum at 5°C. Excellent sporulation was observed at 25, 28 and 30°C. Montazeri and Greaves (2002) found *C. truncatum* conidia washed twice in sucrose showing greater desiccation tolerance during storage at 15 per cent RH and 15°C than at 30 per cent RH and 15 or 25°C or at 15 per cent RH and 5, 10, 25°C.

## **2.6 Epidemiological studies of the pathogen on horse gram and related legumes**

Hirst and Steadman (1963) reported that rains promoted the release of many types of fungal spores and was particularly important in the release of anthracnose spores as they are embedded in a gelatinous substance on the acervuli. Thakur and Khare (1989) studied the influence of planting dates and varieties on the development of anthracnose of mungbean. Disease intensity in early planting was significantly higher as compared to later planting. Grain yield was also significantly influenced by planting dates and varieties.

Thakur and Khare (1991) studied epidemiology and aerobiology of mungbean anthracnose. Maximum trapping of the spores was recorded when there was moderate temperature between 26-29°C, RH between 91-96 per cent and rainfall from 0-21.6 mm. In laboratory, maximum increase in lesion size was recorded when RH was 100 per cent followed by temperature of 27°C and exposure to light for 72 hr.

Mishra *et al.* (1992) studied the effect of *Colletotrichum* spp. on black gram. Pre and post emergence mortality was counted in seed and soil infestation. Pre and post emergence mortality was maximum and significantly higher (55 and 49%) due to *C. dematium* followed by other pathogenic species of *Colletotrichum*. There was 22.4 – 61.4 per cent yield reduction in green gram and black gram.

Rehman *et al.* (1995) reported incidence of *C. dematium* higher in early sown soybean compared with late sown. Adebitan *et al.* (1996) found cowpeas at wider spacings (between and within rows) showing lower brown blotch disease (*C. truncatum*) incidence and severity than those at closer spacings. Adebitan and Ikotun (1996) observed that cowpea at wider spacing shows lower disease incidence and severity of cowpea anthracnose in both mono crop and intercrop as compared to close spacing. Mittal (1999) reported daily average maximum temperature of 26-28°C and minimum 18-20°C, RH of about 80 per cent and moderate, steady rainfall (40-120 mm in 4-5 rainy days/week) favoured the spread of anthracnose of black gram and late sown crop suffered less from disease.

Rana and Kaushal (2004) revealed that spores of *C. truncatum* isolated from urdbean germinated at optimum temperature range of 20-25°C and 90 per cent RH. Rana and Kaushal (2006) reported that temperature near 23°C, higher RH combined with more number of rainy days and 2.62 sunshine hours were favourable for *Colletotrichum* leaf spot development. Different sowing dates and cultivars influenced the yield and disease severity significantly. The highest terminal disease severity (0.48) was recorded in T9 while the lowest was in P 93. Kumar and Dubey (2007) assessed the losses in different cultivars of soybean due to anthracnose and the maximum reduction in yield per plant (34.15%), 1000-grain weight (47.36%) and number of pods per plant (37.42%) were recorded for Shilajeet followed by PK-416 (33.65, 41.26 and 34.44% respectively). Srivastava and Mishra (2008) reported incidence of *C. truncatum* in urdbean markedly reduced the yield.

## **2.7 Management**

### **2.7.1 Host resistance**

Wester (1960) reported that an introduction of *Phaseolus lunatus* from British Guiana (P.I. 199, 791) and another from S. Africa (P.I. 186, 984) showed resistance to *C. truncatum* which was lacking in all commercial varieties tested.

Machado and Carvalho (1975) observed that none of the 21 varieties of soybean inoculated with 30 isolates of *C. truncatum* was resistant, but low susceptibility (33-45%) was shown by SC 68-611 (22 isolates), Hill (19) and Parana (15). Chacko and Khare (1978) screened 47 varieties of soybean against *C. truncatum* using inoculation. Resistance was assessed on the basis of the per cent leaf area infected. Only Kalitur was classified as resistant. Under natural infection, Kalitur was free from the disease.

Hasan and Khan (1979) screened 49 pigeonpea lines against *C. truncatum*. Some were resistant and few (NPWR-015, Pant A-9, Prabhat, R-98 and 1234) were immune. When planted late, a few of the susceptible cvs. escaped severe disease. Oladiran and Oso (1983) observed that in field trial of nine varieties of *Vigna unguiculata* the severity of the disease caused by *C. truncatum* and *C. capsici* increased with age of the pods in all the varieties except Vital, which did not show any disease symptoms where as Kano 1696 and IAR 339-1 were moderately resistant.

Wong *et al.* (1983) conducted greenhouse tests for resistance in soybean against *C. truncatum*. They observed that Palmetto seedlings were highly resistant to *C. truncatum*. Rahman and Fakir (1985) reported that soil, stem and pod inoculation of 5 cultivars with *C. truncatum*, 3 were susceptible (Bragg, Clark-62 and Lee-74) and the other 2 highly susceptible. Stem inoculation of 2 week old seedlings caused upto 86 per cent mortality according to the cultivar but no plant died when inoculated at 4 week stage.

Kaushal and Singh (1988) tested 48 lines of urdbean in the seedling stage against the isolate C1 of the pathogen *C. truncatum*. Lines P7, 27, P53, P103, P115, P124, P130, P152, HPU431, HPU432, HPU434, HPU435 and HPU502 were found to be resistant to the pathogen. Manandhar *et al.* (1988) found that none of the 414 germplasm accessions of soybean of maturity groups 000-X inoculated at the VI growth stage and evaluated for anthracnose was immune. Several lines in maturity groups 00-IV, PI 96.680 (maturity group VI), and Tarheel Black (maturity group VII) were resistant.

Twumasi *et al.* (1989) reported that 44 lines of cowpea showing apparent field resistance to multiple diseases were inoculated with *C. truncatum* causing brown blotch, 16 were resistant, 15 moderately resistant and 13 susceptible. Adebitan *et al.* (1992) screened 12 cowpea cultivars for reaction to infection by *C. truncatum*. Three different inoculation techniques were used; spraying a spore suspension on leaves of seedlings, injecting a spore suspension into stems and wrapping wounded seedlings stems with inoculum. Wrapping of wounded seedlings with inoculum was the best method of inoculation because it produced optimal conditions for infection and disease development. Cultivars IT 82 E-62 and IT 8 D-699 were susceptible to brown blotch whereas TV X 3236, Vita-7 and IT 81 D-1137 were resistant and IT 82 E-16, IT 84 E-124, IT 82 E-32, IT 81 D-975. IT 845-2246-4 and IT 81D-994 were moderately susceptible.

Singh (1993) observed relative susceptibility of soybean cultivars to pod blight caused by *C. truncatum*. Forty eight soybean cultivars were evaluated by pod inoculation with a suspension containing 10<sup>4</sup> conidia/ml. None of the cultivars was immune, however, the degree of susceptibility varied among the cultivars. Ondrej (1994) reported high resistance to *Phoma* and *Colletotrichum* in Rita, 52, Cervin, Polanka and Maple Arrow varieties of soybean among others. Sharma and Kaushal (1999) reported that *C. truncatum* had a wide host range among the local edible legumes infecting all the species, however, the Parbhat cultivar of arhar (*Cajanus cajan*) was immune. Sharma and Kaushal (2000) studied horizontal resistance to *C. truncatum* in 12 selected lines of *Vigna mungo* on the basis of components of disease resistance *viz.*, incubation period, lesion size, lesion number per leaf and spore intensity. Based on these components, HPBU-71 was the most promising genotype possessing resistance to the majority of the pathogen isolates. HPBU 125 and Palampur 93 also showed comparatively more resistance to the eleven pathogen isolates.

Vandenberg *et al.* (2002) reported CDC Robin lentil cultivar, the first lentil breeding line to exhibit resistance to both Ascochyta and anthracnose. Galli *et al.* (2007) reported most resistant soybean cultivars *viz.*, MSOY 8001, Conquista,

MSOY 8400, Engopa and Vencedora where as CD 207, Embrapa 48 and BRS 183, the most susceptible to *C. truncatum*. Madhusudhan and Patil (2003) studied the reaction of different pulses to soybean isolate of *C. truncatum* and reported only *Phaseolus vulgaris* to be susceptible to the pathogen.

Chahota *et al.* (2005) evaluated 63 landraces of horse gram collected from different parts of Himachal Pradesh. Disease reaction against *C. truncatum* under field conditions revealed that most of the lines were found resistant to bean anthracnose. On the basis of evaluation, lines namely HPKC-1, HPKC-6, HPKC-7, HPKC-9, HPKM-51 and DMK-12 were found to be potential lines which can be exploited as a variety or can be used in future hybridization programme.

### 2.7.2 Chemical methods

Malhotra and Chaturvedi (1973) tested the efficacy of five fungicides *viz.*, Dithane-M-45 (0.2%), Cumin, Benlate (0.1%), Bordeaux mixture(0.1%) and Aureofungin (250 ppm) against pod blight of soybean caused by *C. truncatum*. Bordeaux mixture was highly toxic to the crop. Dithane M-45 (0.25%) and Aureofungin 200 ppm were significantly superior over cumin in reducing the disease under field condition. Singh *et al.* (1993) tested twelve fungicides *in vitro* from different chemical groups. Carbendazim (0.1%) gave a good control followed by Benomyl (0.1%) against *C. truncatum* on *Lablab purpureus*.

Singh *et al.* (1994) screened five fungicides for the control of anthracnose of mungbean caused by *C. dematium* and *C. lindemuthianum in vivo*. Maximum control of both the pathogen causing diseases were obtained by using two sprays of Carbendazim (0.15%). Mittal (1994) reported the most cost-effective control of *C. truncatum* in blackgram by seed treatment with Carbendazim followed by 3 foliar sprays of mancozeb. Ghawade *et al.* (1996) studied seven fungicides *in vitro* and found that the benomyl (0.1%) followed by Carbendazim (0.1%), Thiram (0.2%) and Captan (0.2%) were the most effective against *Colletotrichum* causing pod blight of soybean.

Reva *et al.* (1998) tested the efficacy of fungicides for the control of *C. lindemuthianum* *in vitro* and *in vivo* and concluded that Chlorothalonil (0.25%) was the most efficient for inhibiting spore germination. Singh *et al.* (1999) found Carbendazim as most effective followed by mancozeb against the anthracnose of soybean. Singh and Singh (2001) found Bavistin the best followed by Benlate and Topsin-M against *C. truncatum* in mung bean cultivar Jawahar 1. Gawade *et al.* (2009) evaluated five fungicides (Carbendazim, Chlorothalonil, Difenconazole, Hexaconazole, Propiconazole) *in vivo* against anthracnose of soybean. Fungicide Carbendazim (@ 0.1%) was found most effective and economical in controlling the disease, which recorded least mean foliage anthracnose (19.43%), mean defoliation (11.85%), mean pod blight (9.64%) with highest seed yield (2605 kg/ha). This was followed by the fungicides Hexaconazole and Propiconazole.

### 2.7.3 Biological control

Although research on exploitation of biocontrol agents in the management of major plant diseases has advanced considerably but the same has not been tried with horse gram anthracnose. Some species of fungi and microorganisms have been reported to be mycoparasites or antagonists to *C. truncatum*. Dennis and Webster (1971) found that many isolates of *Trichoderma* spp. produced volatile and non-volatile antibiotics active against wide range of fungi.

Bankole (1990) isolated an isolate of *Trichoderma viride* TH-31 from the cowpea phylloplane, which was found to hyperparasitize number of plant pathogenic fungi including *Colletotrichum*. Michereff *et al.* (1993) observed a significant reduction in the number of germinated spores with the culture filtrate of *T. viride*, *T. koningii*, *T. harzianum* and *Pseudomonas fluorescens*.

Successful exploitation of biocontrol agents (both fungal and bacterial) in the modern agriculture especially in the framework of integrated disease management in different crops has been reported by Jeyarajan *et al.* (1994). Bankole and Adebajo (1996) reported *T. viride* isolated from cowpea phylloplane hyperparasitised the mycelium of *C. truncatum*, causal agent of brown blotch disease *in vitro*.



Chandrasekaran *et al.* (2000) reported *Lawsonia inermis* leaf extract with *T. harzianum* increased seed germination, root and shoot length and reduced anthracnose disease incidence significantly. Gawade *et al.* (2009) evaluated two bioagents *in vivo* against anthracnose of soybean. Both bioagents (*T. viride* and *Verticillium lecanii*) were found significantly superior over unsprayed control.

#### 2.7.4 Botanicals (Biopesticides)

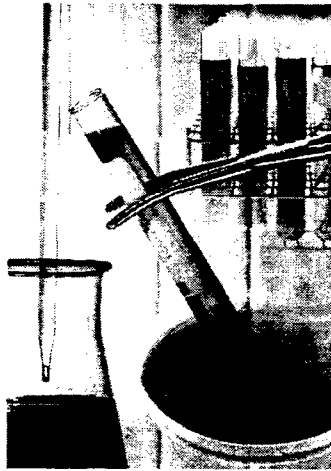
Kaushal and Paul (1989) studied inhibitory effects of some plant extracts (*Cannabis sativa*, *Pinus longifolia*, *Eupatorium* sp. and *Lantana indica*) on some legume pathogens and found that all the plant extracts inhibited *C. truncatum*. The most effective extract was *L. indica*. Akinbode and Ikotun (2008) evaluated some botanicals; tobacco (*Nicotiana tabacum*) and castor plant (*Ricinus communis*) *in vitro* for the control of *C. destructivum*. The extracts of *R. communis* at higher concentration acted as growth “promoter” to the pathogen; its mycelia had a better sporulation and fluffiness than that of the controlled plate. The extract of *N. tabacum* significantly controlled the growth of the pathogen at the highest concentration when compared with other extracts and the control.

Karande *et al.* (2007) studied effect of seven plant extracts on mycelia growth of *C. gloeosporioides* *in vitro*. All these were found significantly superior in controlling the fungus over control. Leaf extracts of tulasi (*Ocimum sanctum*) 10 per cent (63.8%), sadafuli (*Catharanthus roseus*) 10 per cent (57.1%) were quite effective in controlling *C. gloeosporioides*, followed by bougainvillea (*Bougainvillea spectabilis*) (45.2%), onion (*Allium cepa*) (32.2%), neem (*Azadirachta indica*) (29.6%) and glyricidia (*Glyricidia sepium*) (26.0%) showed moderate effect on the fungal growth.

Roat *et al.* (2009) studied inhibitory effects of some plant extracts (*Diospyros cordifolia*, *Datura stramonium*, *Cassia fistula*, *Solanum indicum*, *Santalum album*, *Annona squamosa*, *Justicia adhatoda*) *in vitro* and *in vivo* against fruit rot of chilli incited by *C. capsici*. Bitter temru (*D. cordifolia*) fruit and datura (*D. stramonium*) leaves inhibited the maximum mycelial growth and spore

germination of *C. capsici*, while minimum was recorded in sandal seed (*S. album*). The bitter temru (*D. cordifolia*) and datura leaves (*D. stramonium*) showed maximum reduction in the incidence of fruit rot.

Gawade *et al.* (2009) evaluated two botanicals against anthracnose of soybean *in vivo*. Both botanicals, Neem seed kernel extract (*Azadirachta indica*) and Mehndi leaves (*Lawsonia inermis*) were found significantly superior to unsprayed control in combating the foliage anthracnose, defoliation and pod blight and thereby increased the seed and test weight over unsprayed control.



# **MATERIALS AND METHODS**

## **3. MATERIALS AND METHODS**

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The present studies were carried out in the Department of Plant Pathology, CSKHPKV, Palampur. The details of material used and methodology followed during the course of study are given here under:

### **3.1 Collection and maintenance of disease samples**

Horse gram leaf samples showing typical disease symptoms were collected during 2009 crop season from the experimental farm of the university. These samples were washed under running tap water to remove soil particles, dried in blotting sheets and preserved for future studies.

### **3.2 Sterilization of glass/plastic wares**

'Borosil' brand glassware like test tubes, Petriplates, conical flasks, beakers and measuring cylinders etc. were dipped in chromic acid mixture (sodium dichromate 75 g, distilled water 500 ml and concentrated sulphuric acid 500 ml) overnight and washed in running tap water for 10 minutes and thrice in distilled water before use and sterilized in hot air oven at 160°C for 1 hour. Plastic wares were surface sterilized with 5 per cent ethyl alcohol or sodium hypochlorite (2%). Growth media were sterilized in autoclave at 1.05 kg/cm<sup>2</sup> pressure for 20 minutes.

### **3.3 Preparation of medium**

Potato dextrose agar (PDA) medium was used for culturing the pathogen. The peeled potatoes were cut into small pieces, boiled in water till softening, filtered through cheese cloth and the final volume was made to one litre with distilled water. Dextrose and agar agar were added and boiled for some time. In 250 ml flasks about 150 ml medium was filled and autoclaved at 1.50 kg/cm<sup>2</sup> pressure for 20 minutes. Mathur's medium was also used for effective sporulation of the pathogen.

### **3.4 Isolation and maintenance of pathogen**

Small bits of 2-3 mm size were cut from the junction of diseased and healthy leaf tissue from highly susceptible cultivar HPK-4 with the help of a sterilized scalpel. The leaf bits were surface sterilized by dipping in 0.1% mercuric chloride solution for 30 seconds and washed 3-4 times with sterilized distilled water. These bits were then placed on sterilized filter paper to drain off excessive moisture and then transferred to PDA slants under aseptic conditions and incubated at  $25\pm 1^{\circ}\text{C}$  in a BOD incubator. The cultures were purified by single spore method on PDA and maintained at  $25\pm 1^{\circ}\text{C}$ . Stock cultures were sub cultured at an interval of 25-30 days regularly.

For obtaining pure culture of the *C. truncatum* serially diluted spore suspension prepared from 10-11 day old culture was poured on sterilized water agar (2%) in Petriplate under aseptic conditions. The inverted plates were incubated at  $22\pm 1^{\circ}\text{C}$  for 24 hr. Single spores were located under microscope (40 x) and marked with fine pencil tip. The marked spores were then transferred to PDA slants.

### **3.5 Preparation of inoculum**

Inoculum of the pathogen isolate was prepared by harvesting spores from freshly sporulating cultures in sterilized distilled water by scrapping the surface of PDA slants with sterilized spatula. Seven to ten day old culture of the pathogen was used for preparing inoculum. The spores were harvested by adding small quantity of distilled water to agar slants of culture and gently shaking with a glass rod. The resultant spore suspension was filtered through double layer of cheese cloth to remove mycelial fragments and debris. Serial dilutions of the spore suspension were prepared and inoculum density was adjusted to  $7.5\times 10^5$ - $1.0\times 10^6$ /ml using a haemocytometer. It served as a standard inoculum for carrying out different studies.

### **3.6 Pathogenicity test**

To confirm the identity of the pathogen *C. truncatum*, pathogenicity test was performed with highly susceptible horse gram cultivar HPK-4. Seedlings of HPK-4 cultivar were raised in plastic pots. Seven day old seedlings bearing two primary leaves were sprayed with standard inoculum until runoff with the help of atomizer and incubated at  $25\pm 1^\circ\text{C}$  temperature and  $> 90\%$  RH in a growth chamber for 3 days. Inoculated plants after incubation were shifted to cage house, watered regularly and observed daily for the appearance of characteristic disease symptoms. The pathogen was reisolated from infected plants and identification was confirmed by microscopic examination.

### **3.7 Effect of different epidemiological parameters on disease development**

#### **3.7.1 Effect of temperature**

To study the effect of temperature, moisture chambers of Petriplates lined with wet filter paper were made. Three detached horse gram leaves were kept in each Petriplate. The leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette. This was replicated thrice and incubated at different temperatures i.e. 15, 20, 25, 30 and  $35^\circ\text{C}$  at 100% RH. Area ( $L \times B \text{ mm}^2$ ) was calculated by measuring the maximum length (L) and width (B) of the lesion at regular intervals i.e. after 48, 72 and 96 hr of inoculation.

#### **3.7.2 Effect of light**

To study the effect of light three different photoperiods were created i.e. complete dark (24 hr), complete light (24 hr) and 12/12 hr alternate light/dark period. Three detached horse gram leaves were kept in each Petriplate. Leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette. This was replicated thrice and incubated in three different photoperiodic conditions at  $25\pm 1^\circ\text{C}$  and 100% RH. Data were recorded on lesion development under different photoperiods at regular intervals as mentioned above.

### 3.7.3 Effect of relative humidity

Different humidity levels ranging from 75 to 100% were created by variable concentration of H<sub>2</sub>SO<sub>4</sub> in water as per method described by Dhingra and Sinclair (1985). Detached horse gram leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette and dried in the laminar air flow chamber. Three leaves were then kept in each moisture chambers made up of concentrated H<sub>2</sub>SO<sub>4</sub> and water. Data were recorded on lesion development at different humidity levels at regular intervals as mentioned above.

### 3.8 Disease progress under field conditions

Field trials on the effect of date of sowing and row to row spacing on disease severity were conducted at the experimental farm during *kharif* seasons of 2009 and 2010. Cultivar HPK-4 was selected for these studies. Agronomic procedures were applied as per recommended package of practices of the university. The experiments were laid out in randomized block design (RBD) and replicated thrice. Disease progress in time was studied by recording the severity of anthracnose from appearance of first disease symptoms at 10 day intervals. The details of the experiment are given as under:

#### 3.8.1 Effect of different date of sowing and spacing

To study the effect of different dates of sowing and row to row spacing, HPK-4 cultivar was sown in RBD on four different dates; D1 (8<sup>th</sup> June, 2009 and 2010), D2 (15<sup>th</sup> June, 2009 and 2010), D3 (22<sup>nd</sup> June, 2009 and 2010) and D4 (29<sup>th</sup> June, 2009 and 2010) during 2009 and 2010 crop growing periods in three different row to row spacing; 25 cm, 30 cm and 35 cm. A plot size of 2 X 1.5 m<sup>2</sup> was used.

### 3.8.2 Recording of data

Data on disease severity were recorded at 10 day intervals, starting with the appearance of disease. The disease was scored on 0-9 point scale described by Mayee and Dattar (1986) as under:

<u>Grade</u>	<u>Disease severity (%)</u>	<u>Reaction type</u>
0	0	Highly resistant
1	<1	Resistant
3	1-10	Moderately resistant
5	10-25	Moderately susceptible
7	25-50	Susceptible
9	>50	Highly susceptible

The disease was quantified by counting number of lesions per disease plant, on at least ten plants for each observation. Data were pooled at the end of the experiment to ascertain the effectiveness of each treatment against the disease. Per cent disease severity was determined by using McKinney (1923) formula:

$$\text{Disease severity} = \frac{\text{Sum of all disease ratings}}{\text{Total number of disease ratings} \times \text{Maximum disease grade}} \times 100$$

### 3.8.3 Role of weather parameters on disease development

Disease progress as a function of time was recorded at periodical intervals on susceptible cultivar HPK-4. The disease was correlated with meteorological factors such as temperature, relative humidity, bright sunshine hours and rainfall to determine the role of weather variables if any, on the development of disease. All these parameters were recorded from meteorological laboratory set up near by the Department of Agronomy.



The role of environmental factors in the development of disease was further established by multiple regression analysis of disease progress with weather parameters.

### **3.9 Disease management**

Different disease management strategies including host resistance, fungicide, biocontrol agents, biopesticides and botanicals were undertaken.

#### **3.9.1 Host resistance**

One hundred and five lines of horse gram procured from Department of Plant Breeding and Genetics were evaluated for disease reaction under natural epiphytotic conditions for two years in 2009 and 2010 during *Kharif* season.

Two rows of 1.5 m length of each horse gram line were sown in the field. Disease severity on these lines was calculated as per 0-9 scale (Mayee and Dattar, 1986) and horse gram lines were categorized into different reaction types based on disease severity as mentioned above.

#### **3.9.2 Fungicides**

To identify the suitable chemical(s) which could provide protection to the crop, eight fungicides *viz.*, four systemic; Bavistin 50-WP (carbendazim) @ 0.1%, Score (difenoconazole) @ 0.05%, Contaf (hexaconazole) @ 0.05%, Folicur (tebuconazole) @ 0.05%, and four protectant fungicides; Dithane-M-45 (mancozeb) @ 0.25%, Blitox 50-WP (copper oxychloride) @ 0.25%, Kavach 50-WP (chlorothalonil) @ 0.25% and Captan 50-WP (Captan) @ 0.25% were tested for their efficacy under field conditions.

The effectiveness of these fungicides as foliar sprays was conducted using horse gram cultivar HPK-4 in RBD with plot size of 2 X 2 m<sup>2</sup>. Spray of the fungicides was done three times after ten days of interval. Unsprayed check was kept for comparison. Three replications of each treatment were maintained. Data were recorded and disease severity was calculated as mentioned above.

### 3.9.3 Bio-control agents

Antagonistic activity of bioagents *viz.*, *Trichoderma viride*-H (Hydrabad strain) and *T. harzianum*-H (Hydrabad strain) against *C. truncatum* was tested using dual-culture technique (Huang and Hoes 1976). Two mm dia. culture discs of each of the pathogen and biocontrol agent were picked from the margin of 7 day old cultures with a sterilized cork borer and transfer under aseptic conditions to PDA plates in such a way that the distance between the pathogen and bioagent was about 5 cm. In case of bacterial antagonist *Pseudomonas fluorescens* (TNAU- Coimbatore strain) disc of 2 mm dia of the pathogen was placed at the centre of PDA plate and the bacterium was streaked around the disc (Laha *et al.* 1992). The disc of the pathogen grown alone on the PDA plates served as control. The cultures were incubated at 25±1°C. Each treatment was replicated thrice. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition of mycelial growth was calculated by Vincent (1947) formula:

$$I = \frac{(C - T)}{C} \times 100$$

I = % inhibition

C = growth in control

T = growth in treatment

### 3.9.4 Biopesticides

*In vitro* evaluation of neem based biopesticides was carried out by poisoned food technique. For this desired quantity of biopesticide *viz.*, neemban, neembenicide and achool were measured and different concentrations (0.05%, 0.1% and 0.5%) were made. Then double strength PDA medium was prepared and supplemented with the equal amount of different concentrations of biopesticides in the flasks under aseptic conditions. Twenty ml of this mixture was poured in sterilized Petriplates and inoculated with disc of 2 mm dia. taken

from the periphery of 7 day old culture of *C. truncatum* and incubated at  $25\pm 1^\circ\text{C}$ . Each treatment was replicated thrice. A control was also maintained for comparison where no biopesticide was added. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition on mycelial growth was calculated (Vincent 1947).

### **3.9.5 Botanicals**

Eight locally available plants which grow in abundance viz., basuti (*Adhatoda vasica*), bhang (*Cannabis sativa*), kali basuti (*Chromolaena odorata*), barein (*Acorus calamus*), butter-cup (*Ranunculus bulbosus*), safeda (*Eucalyptus globulus*), curry plant (*Murraya koenigii*) and pine needles (*Pinus longifolia*) were used for their efficacy against the pathogen.

#### **3.9.5.1 Preparation of extracts**

These plants were selected to estimate the antimicrobial activity against *C. truncatum* by poisoned food technique (Nene and Thapliyal 1965). Two hundred grams of fresh leaves from each plant were washed well and grounded in 200 ml of distilled water by using mixer and grinder. The macerate was filtered through double layered cheesecloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Whatman No. 41 filter paper. The supernatant (pure stock, 100%) was filter sterilized with filter syringes (0.2  $\mu$  pore size) under aseptic conditions and further dilutions were made to different concentrations of 20, 40, 60, 80 and 100 per cent.

Double strength PDA medium was prepared and then supplemented with the equal amount of different concentrations of plant extracts in the flasks under aseptic conditions. Twenty ml mixture of medium and extract was poured into sterilized Petriplate and inoculated with a disc of 2 mm dia. taken from the periphery of 7 days old culture of *C. truncatum* and incubated at  $25\pm 1^\circ\text{C}$ . Each treatment was replicated thrice. A control was also maintained for comparison where no plant extract was added. Data on mycelial growth were recorded when control Petriplate was fully covered. Per cent inhibition on mycelial growth was calculated (Vincent 1947).

### 3.10 Statistical analysis

Data collected during the course of these investigations were subjected to appropriate statistical analysis, wherever necessary using standard procedure (Gomez and Gomez 1984). The significance of difference was tested at 1 and 5 per cent level of probability. Simple correlation and multiple regression analysis were performed using computer facility, between disease (anthracnose of horse gram) and four independent variables *viz.*, mean temperature ( $X_1$ ), mean relative humidity ( $X_2$ ), bright sunshine hours ( $X_3$ ) and rainfall ( $X_4$ ). The multiple linear model:  $Y = a + b_i x_i + e$  was used to describe the functional relationship, where,

Y	=	Predicted mean severity
a	=	Intercept
$b_i$	=	Regression coefficient for $X_i$ ( $i = 1 \dots n$ )
$x_i$	=	Independent variables ( $i = 1 \dots n$ )
e	=	Random error



# **RESULTS AND DISCUSSION**

## 4. RESULTS AND DISCUSSION

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Horse gram anthracnose is a destructive fungal disease. The disease is predominant in Himachal Pradesh as crop season coincides with the onset of monsoon. The present studies were carried out on various epidemiological parameters and management practices to control the disease. Results of present investigation are described under the following sub heads:

4.1 Pathogen identification and pathogenicity

4.2 Effect of different epidemiological parameters in disease development

4.3 Effect of different date of sowing and spacing on disease development

4.4 Role of environmental factors on disease development

4.5 Disease management

### 4.1 Pathogen identification and pathogenicity

Horse gram leaf samples showing typical disease symptoms (Plate 1) were collected from the fields and the pathogen was isolated. The pathogen *Colletotrichum truncatum* was identified based on morphological traits (Plate 3) as per taxonomic status in the literature. Then the pathogen was inoculated on HPK-4 cultivar of horse gram. The typical anthracnose symptoms appeared on the cultivar after 7 days of inoculation (Plate 2). The pathogen was reisolated from these inoculated leaves and identification was confirmed by microscopic examination (Plate 4).

Pathogenicity was confirmed on detached leaves of highly susceptible horse gram cultivar HPK-4 under laboratory conditions at 25°C. Inoculated leaves also showed typical anthracnose symptoms. Single spore cultures of the pathogen were grown on PDA. Maximum mycelial growth of the fungus was observed at 25°C. Under microscopic examination acervuli and conidia of *C. truncatum* were observed.



**Plate 1: Typical symptoms of anthracnose on horse gram under field conditions**



**Plate 2: Pathogenicity test on susceptible horse gram cultivar HPK-4**

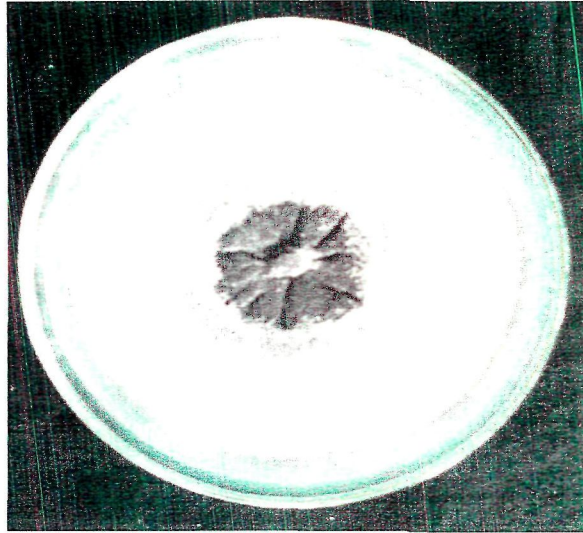


Plate 3: Culture characteristics of *C. truncatum*

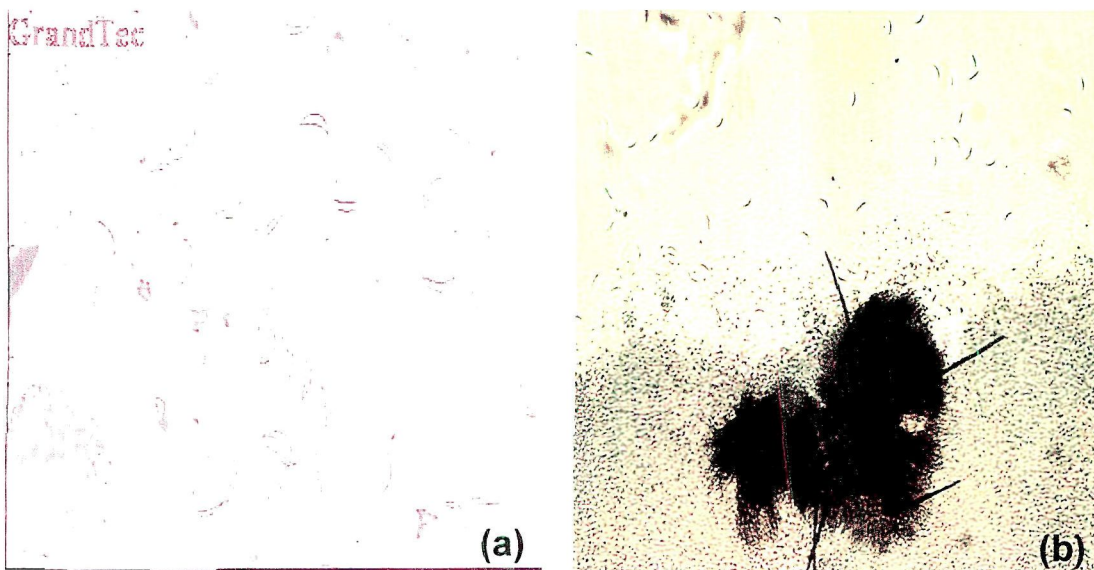


Plate 4: Conidia (a) and setae (b) of *C. truncatum*



Rana and Kaushal (2004) studied the host range of *C. truncatum* isolates of urdbean and horse gram. Urdbean and horse gram were found to be susceptible to all the isolates derived from different hosts. Bhardwaj and Singh (1986) observed cultural variation in four isolates of *C. truncatum* from mung, mash, horse gram and soybean. The isolates from urdbean and mungbean appeared as dirty white to dark colonies with abundant sporulation on PDA. However, the isolates from horse gram formed dirty white to steel grey colonies with sparsely formed acervuli. Mc Ghee (1992) described *C. truncatum* as crowded, black acervuli on stomata. Acervuli were oval to elongate, hemispheric to truncate, conical and erumpent, with numerous spiny setae measuring 60-300 X 3-8  $\mu\text{m}$ . Conidia were born on single conidiophores bluntly tapered, curved, unicellular and hyaline.

#### **4.2 Effect of different epidemiological parameters in disease development**

Effect of different ranges of temperature, RH and light was studied on the appearance of anthracnose of horse gram and the results obtained are presented below:

##### **4.2.1 Effect of temperature**

The data presented in Table 4.1 show the effect of different temperature regimes viz., 15, 20, 25, 30, 35 °C on the anthracnose development of horse gram under laboratory conditions (Plate 5). The lesion size was observed after 48, 72 and 96 hr after inoculation. Maximum lesion size was observed at 25°C (8.29 mm<sup>2</sup>) after 96 hr of inoculation. As the temperature increased and decreased from 25°C, there was decrease in the lesion size. After 96 hr of inoculation least lesion size was observed at 15°C (1.81 mm<sup>2</sup>). At 20°C and 30°C the lesion size was recorded 6.41 mm<sup>2</sup> and 5.57 mm<sup>2</sup> respectively, after 96 hr of inoculation. At 35°C, however the lesion size after 96 hr was reduced (4.56 mm<sup>2</sup>) indicating that 20°C and 25°C were the congenial temperatures for anthracnose development. At sub optimal temperatures (15, 30 and 35 °C) lesion size increased with extended incubation period of 96 hr.

**Table 4.1 Effect of temperature on the anthracnose development of horse gram**

Temperature(°C)	Lesion size (mm <sup>2</sup> )*		
	48hr	72hr	96hr
15	1.81	3.2	4.3
20	3.2	5.56	6.41
25	4.3	6.75	8.29
30	2.91	4.33	5.57
35	2.25	3.63	4.56
CD (P=0.05)	0.38	0.36	0.42

\*Mean of three replications

Each replication consists of observations on 3 leaves

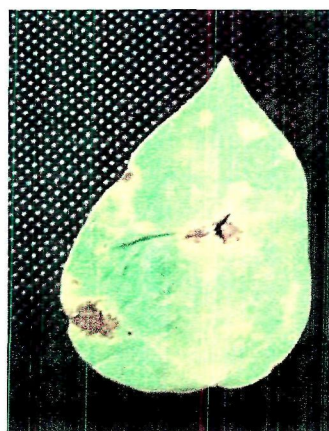
Temperature was found to play crucial role in disease development. The results show that under optimum conditions of temperature (25°C) disease severity is high and with increase in the period after inoculation, there is a corresponding increase in disease severity. Wong *et al.* (1983) reported 20°C temperature as optimum for spore germination in soybean. Temperature was found to be of critical importance in leaf spot development under artificial inoculation conditions. Maximum increase in lesion size was noticed in T9 (4.12 mm<sup>2</sup>) followed by 3.44 mm<sup>2</sup> 2.58 mm<sup>2</sup> in PDUI and P93 respectively at 25°C. Least increase in lesion size was noticed in P93 (1.34 mm<sup>2</sup>) at 35°C temperature. Mishra and Gupta (1994) reported minimum increase in lesion size at 35°C temperature and maximum (3.81 mm<sup>2</sup>) was noticed at 25°C temperature. Thakur and Khare (1991) reported maximum increase in lesion size of mungbean at 25°C temperature. These findings indicate that *Colletotrichum* leaf spot does not appear in plain areas where temperature is  $\geq 35^\circ\text{C}$  in July- August.



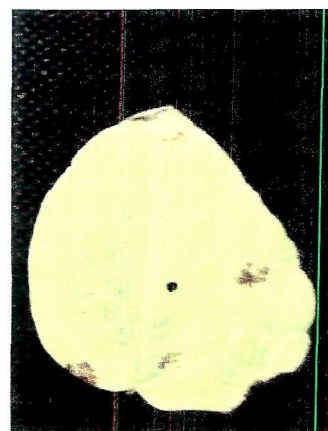
15 °C

20°C

25°C



30°C



35°C

**Plate 5:** Detached leaves showing the effect of different temperature regimes on anthracnose development

#### 4.2.2 Effect of relative humidity

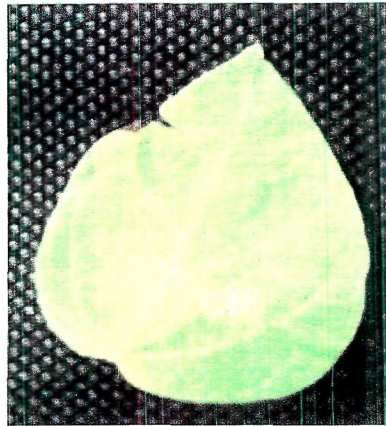
Data on the effect of relative humidity (RH) levels on the anthracnose development of horse gram are presented in Table 4.2. There was progressive increase in lesion size with an increase in relative humidity level (Plate 6). High RH (100%) favoured the lesion size development at 25°C temperature. Among different RH levels, maximum lesion size of 7.18 mm<sup>2</sup> was found at 100% RH after 96 hr of inoculation and minimum of 2.05 mm<sup>2</sup> after 96 hr of inoculation at 75% RH. With the increase in the duration after inoculation, increased lesion size was recorded at all humidity levels. The optimum RH requirement ranged between 95-100 per cent for anthracnose development.

**Table 4.2 Effect of relative humidity on the anthracnose development of horse gram**

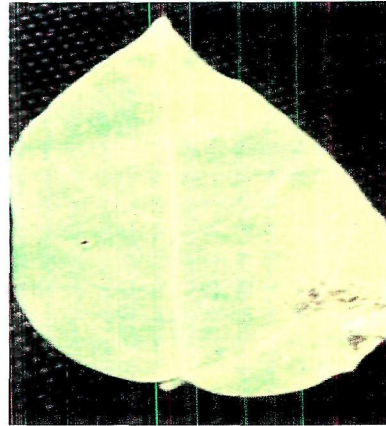
Relative humidity (%)	Lesion size (mm <sup>2</sup> )*		
	48hr	72hr	96hr
75	1.13	1.55	2.05
80	2.48	1.87	2.57
85	1.77	2.39	4.78
90	2.41	4.29	5.23
95	3.61	5.29	5.90
100	4.74	6.32	7.18
CD (P=0.05)	0.31	0.25	0.19

\*Mean of three replications

Each replication consists of observations on 3 leaves



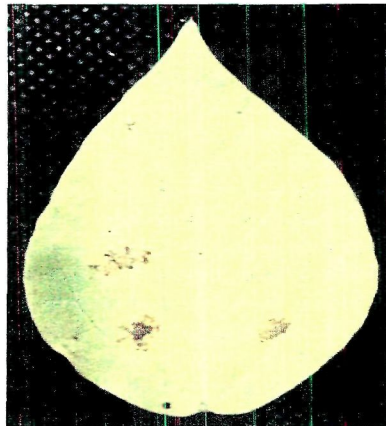
75 %



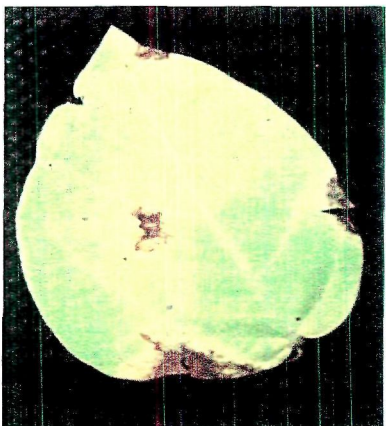
80 %



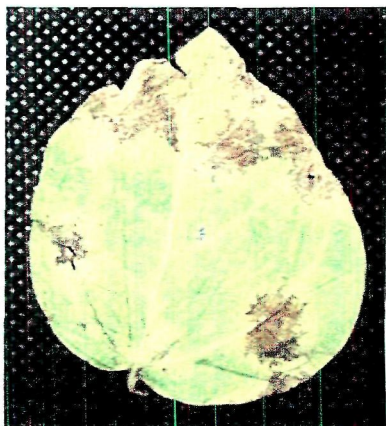
85%



90%



95%



100%

**Plate 6:** Detached leaves showing the effect of different humidity levels on anthracnose development

Hundred per cent RH was best for disease development. Disease severity was significantly higher at more than 90 per cent RH as compared to low levels of humidity. Sinclair and Backman (1989) support these findings by showing that leaf infections occur when free moisture is present on the leaves and RH is 80% or above. Mishra and Gupta (1994) reported maximum increase in lesion size (4.15 mm<sup>2</sup>) at 100 per cent RH in mungbean. However at 80 per cent RH increase in lesion size was 1.80 mm<sup>2</sup>. Thakur and Khare (1991) also reported maximum increase in lesion size at 100 per cent RH. When RH was decreased to 50 and 25 per cent, no increase in lesion size was recorded. It is inferred from these findings that the disease does not appear in areas with low or scanty rainfall i.e. in dry temperate zone of Himachal Pradesh.

#### 4.2.3 Effect of photoperiod

Data on the effect of photoperiod on the anthracnose development of horse gram are presented in Table 4.3. Different photoperiods showed significant variation in disease development (Plate 7). Maximum lesion size of 6.23 mm<sup>2</sup> was noticed at 12 hr alternating light and dark conditions after 96 hr of inoculation. Minimum lesion size of 4.51 mm<sup>2</sup> was noticed in complete dark conditions after 96 hr of inoculation.

**Table 4.3 Effect of photoperiod on the anthracnose development of horse gram**

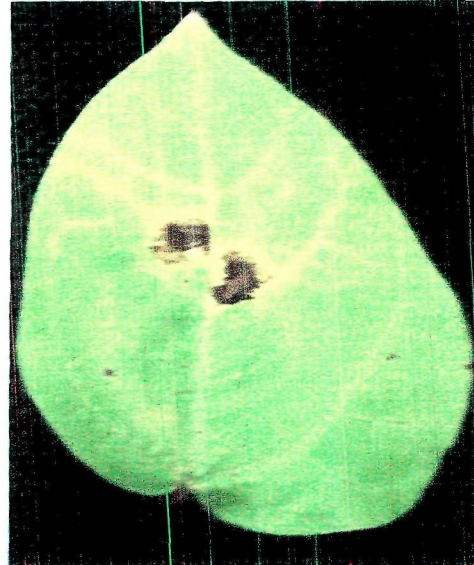
Photoperiod (hr)	Lesion size (mm <sup>2</sup> )*		
	48hr	72hr	96hr
12/12 alternate	2.25	3.44	5.39
Light (24)	2.04	3.35	5.39
Dark (24)	1.75	2.42	4.51
CD(P=0.05)	0.13	0.34	0.43

\*Mean of three replications

Each replication consists of observations on 3 leaves



**12/12 hr alternate light and dark**



**Complete light (24hr)**



**Complete dark (24hr)**

**Plate 7: Detached leaves showing the effect of different photoperiods on anthracnose development**

Faster disease development occurred at 12/12 hr alternate photoperiod where as complete dark or light induced slower disease development. According to Thakur and Khare (1991) lesion development was better in continuous light as well as in 12 hr alternate light and dark conditions as compared to dark conditions. Mishra and Gupta (1994) also reported increase in lesion size in alternate light and dark conditions as compared to continuous light and dark. Wong *et al.* (1983) reported 12 hr alternate light and dark best for disease development and sporulation of the pathogen in soybean.

### **4.3 Effect of date of sowing and spacing on disease development**

Field experiment was conducted in two consecutive years (2009 and 2010) to study the effect of date of sowing and row to row spacing on disease severity (Plate 8). Data are presented in Table 4.4 and Figure 4.1. Maximum disease severity of 52.4 per cent and 62.83 per cent was observed when the crop was sown on 8<sup>th</sup> June of 2009 and 2010 respectively, at 25 cm row to row spacing. The disease severity decreased with the delay in sowing and increasing row to row spacing. Interaction between these two factors was statistically significant. Crop sown on 29<sup>th</sup> June of 2009 and 2010 at 35 cm row to row spacing resulted in minimum disease severity of 25.28 per cent and 36 per cent, respectively. Effect of date of sowing and row to row spacing on grain yield was also observed only during 2009. The maximum grain yield (5.70 q/ha) was obtained from crop sown on 22<sup>nd</sup> June at 25 cm row to row spacing which reduced significantly when sowing was delayed irrespective of row to row spacing. At a spacing of 25 cm, the yield reduced to 4 q/ha in crop sown on 29<sup>th</sup> June. In early sown crop (8<sup>th</sup> and 15<sup>th</sup> June) the grain yield was low due to higher disease severity.

Different dates of sowing and row to row spacing influenced the disease severity and grain yield significantly. From these results it is clear that disease severity was higher in early sown crop at minimum row to row spacing which could be due to the dense growth leading to higher humidity. Therefore, it can be conjectured that late sowing and wider spacing between rows hinders the



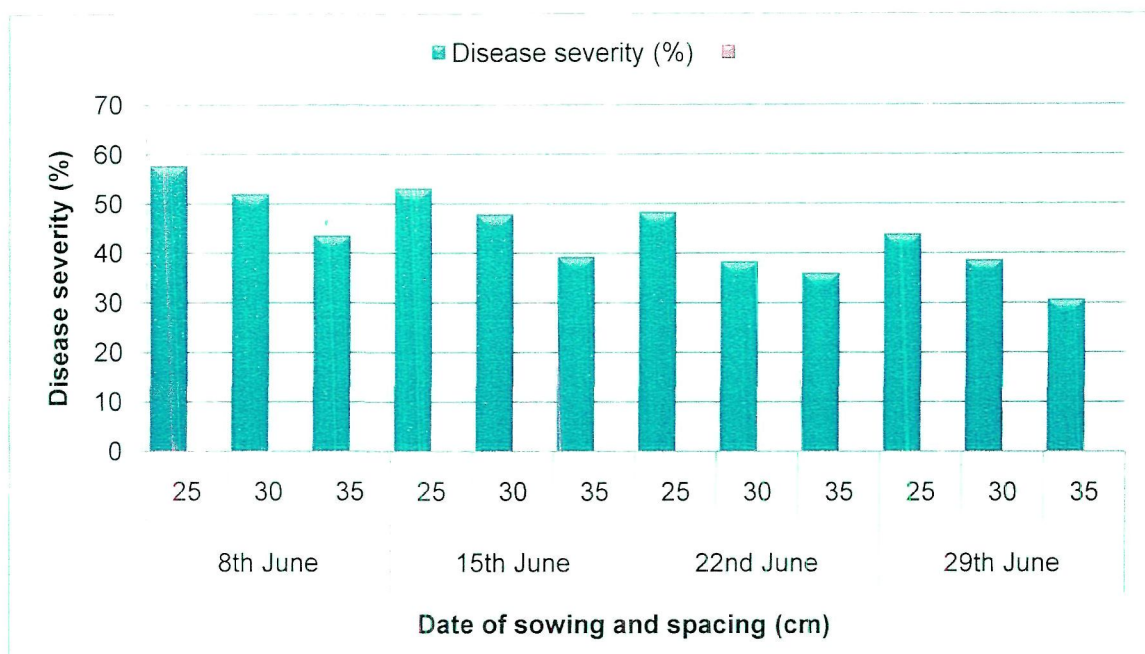
**Table 4.4** Effect of date of sowing and spacing on the disease severity of anthracnose of horse gram

Treatment		Disease severity (%)			Grain yield (q/ha)*
Date of sowing	Spacing (cm)	2009	2010	Mean	2009
8 <sup>th</sup> June	25	52.44 (46.38)	62.83 (52.42)	57.64	4.60
	30	47.10 (43.32)	56.83 (48.91)	51.97	4.50
	35	36.85 (37.35)	50.11 (45.05)	43.48	4.20
15 <sup>th</sup> June	25	48.22 (43.96)	57.89 (49.52)	53.06	4.70
	30	43.39 (41.18)	52.11 (46.19)	47.75	4.60
	35	33.56 (35.38)	44.90 (42.05)	39.23	4.40
22 <sup>nd</sup> June	25	42.48 (40.66)	53.89 (47.21)	48.19	5.70
	30	37.17 (37.55)	46.78 (43.13)	38.22	5.40
	35	29.66 (32.98)	42.11 (40.44)	35.89	5.20
29 <sup>th</sup> June	25	38.45 (38.30)	49.16 (44.50)	43.81	4.00
	30	35.28 (35.21)	41.78 (40.25)	38.53	3.70
	35	25.28 (30.17)	36.00 (36.85)	30.64	3.50
CD(P=0.05)	A	0.11	0.11	-	0.12
	B	0.97	0.92	-	0.24
	AB	0.19	0.18	-	0.10

The figures in parentheses are arc sine transformed values

A- date of sowing, B – row to row spacing

\* Grain yield in 2010 could not be obtained because of destruction of crop during harvesting period due to heavy rainfall.



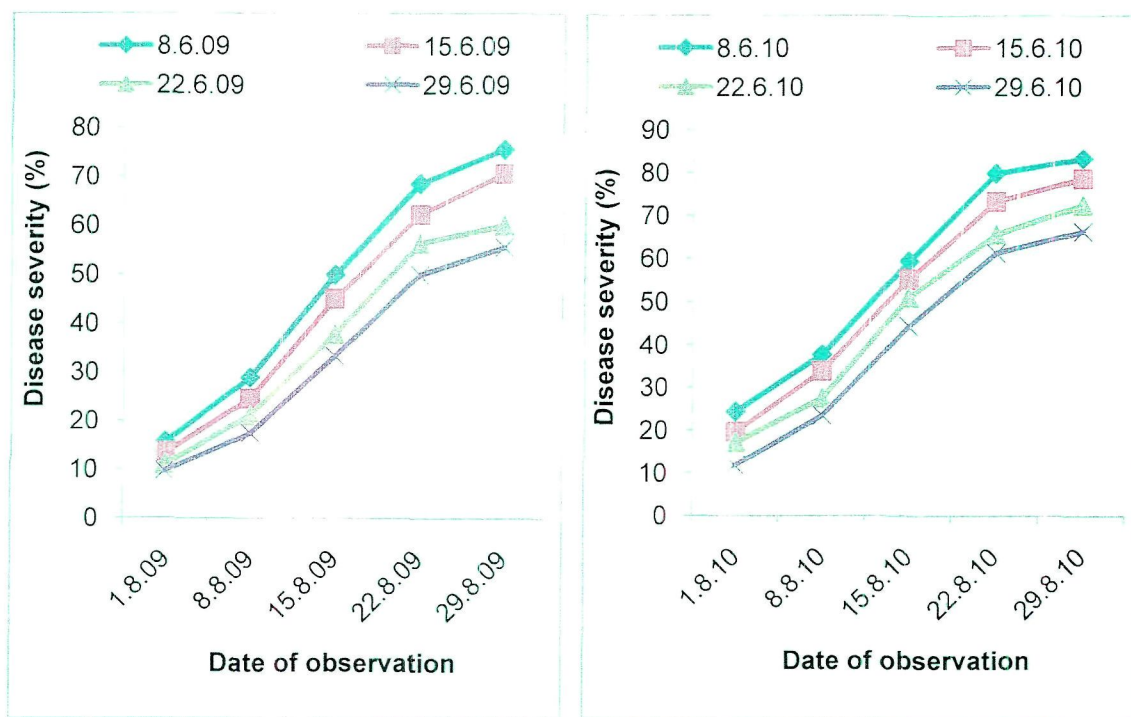
**Fig. 4.1** Effect of date of sowing and spacing on the disease severity of anthracnose of horse gram

disease development. Higher disease severity in crop sown during first week of June could be due to availability of sufficient mature tissue by the mid July when there is high humidity because of monsoon rains. Crop yield was significantly higher in timely sown crop. The yield was less in early sown crop due to high severity, whereas, in late sown crop, there was lesser flowering and reduced pod formation due to unfavorable weather conditions for crop growth which contributed to lower yields.

Data pertaining to progress of anthracnose of horse gram under different dates of sowing during 2009 and 2010 are presented in Table 4.5. The progress of disease exhibited sigmoidal pattern of the curve during both years (Figure 4.2). Initial amount of disease was more in 2010 as compared to 2009. The disease development was maximum (24.44 -83.33%) between 1<sup>st</sup> August to 29<sup>th</sup> August in 2010 under 1<sup>st</sup> date of sowing (8<sup>th</sup> June, 2010). Similar pattern of disease development was observed during 2009 from 19.78 - 75.55 per cent of the disease during 1<sup>st</sup> August to 29<sup>th</sup> August.

**Table 4.5** Progress of anthracnose of horse gram under different dates of sowing during 2009 and 2010

Date of sowing →	Disease severity (%)							
	8 <sup>th</sup> June		15 <sup>th</sup> June		22 <sup>nd</sup> June		29 <sup>th</sup> June	
	2009	2010	2009	2010	2009	2010	2009	2010
1.8.09/10	19.78	24.44	13.61	19.72	11.11	17.22	9.71	11.94
8.8.09/10	28.89	37.78	24.55	33.89	21.11	27.54	17.44	23.54
15.8.09/10	49.89	59.45	45.00	55.28	37.78	51.11	33.33	44.43
22.8.09/10	68.56	79.89	62.22	73.30	56.34	65.63	50.00	61.34
29.8.09/10	75.55	83.33	70.55	78.64	60.00	72.45	55.56	66.30



**Fig. 4.2** Disease progress curves under different dates of sowing during 2009 and 2010

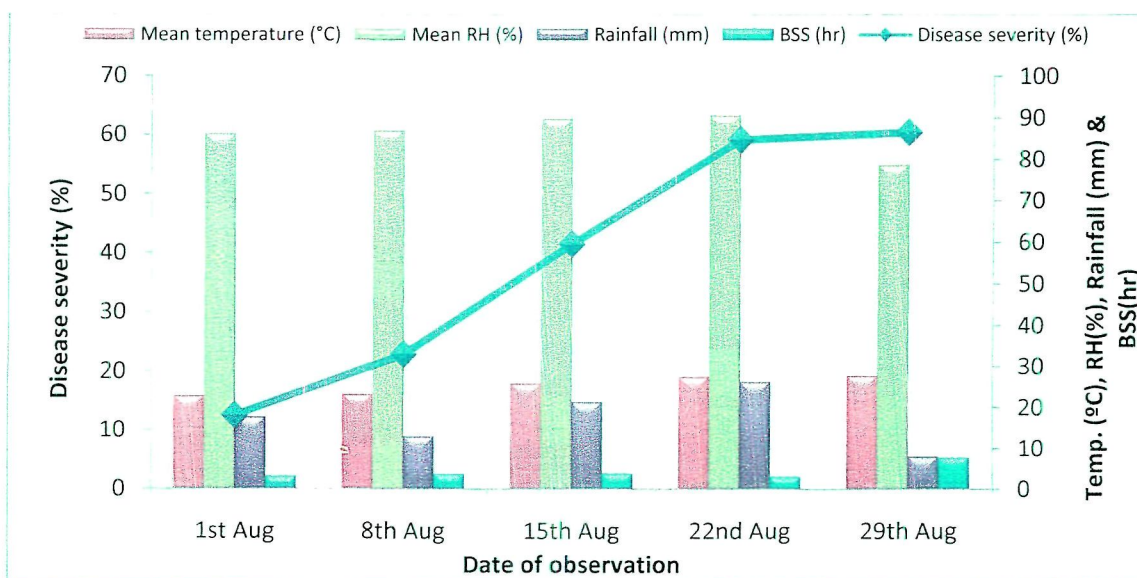


**Plate 8:** Field trial of effect of date of sowing and spacing on anthracnose development of horse gram

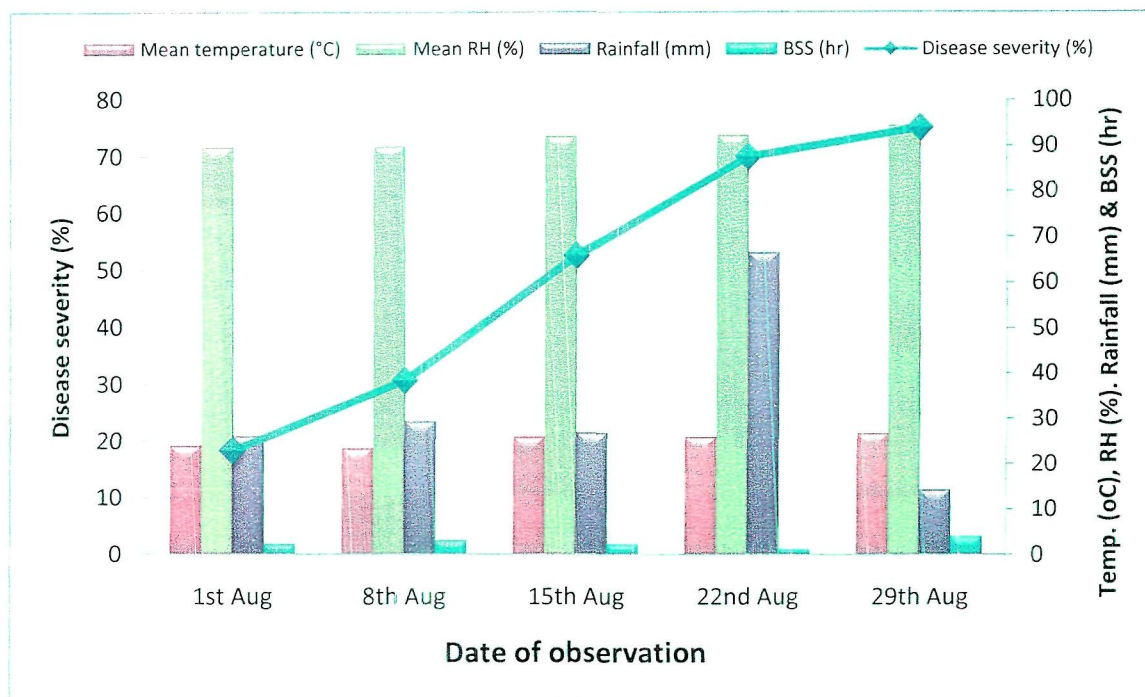
These findings are corroborated by Thakur and Khare (1989) in mungbean. They recorded higher disease intensity and less yield in early planted crop as compared to late planted crop. Rehman *et al.* (1995) recorded higher disease incidence in early sown crop of soybean as compared to late sown crop. Kumar (1992) also found significant results in his studies on pod blight of soybean. He recorded higher disease intensity and fewer yield in early planted crop of soybean as compared to late planted crop. The present findings on the effect of row spacing on disease severity are in agreement with earlier reports (Adebitan and Ikotun 1996; Adebitan *et al.* 1996).

#### 4.4 Role of weather variables on disease development

An experiment was conducted on anthracnose development during the years 2009 and 2010 on susceptible horse gram cultivar HPK-4. The anthracnose progress was recorded periodically and was plotted against time during both the years (Figures 4.3 and 4.4). The pattern of disease progress during both the years was similar. The progress of disease started picking up from the first week of August and maximum progress was observed in mid August, when the weather variables were most favourable.



**Fig 4.3** Role of weather variables on anthracnose development of horse gram in 2009



**Fig 4.4 Role of weather variables on anthracnose development of horse gram in 2010**

The disease progress was correlated with weather variables viz., mean temperature (°C), mean relative humidity (%), bright sunshine (hr) and rainfall (mm) (Table 4.6). There was a significant positive correlation between disease severity and mean temperature during both the years. Correlation coefficient ( $r$ ) 0.95 and 0.88 were highly significant during 2009 and 2010, respectively. The correlation coefficients between disease severity and mean relative humidity were negative ( $r=-0.24$ ) during 2009 and significantly positive ( $r=0.90$ ) during 2010. There was significant positive correlation between disease severity and bright sunshine hours ( $r=0.56$ ) during 2009 but was non significant during 2010. Rainfall though showed positive correlation (0.02 and 0.23) with disease severity during both years, but were non-significant. The correlation of disease severity with rainfall was non-significant during both the years. In pooled analysis, all factors are positively correlated with disease severity but only correlation with mean temperature was significant.

**Table 4.6 Simple correlation coefficients between disease severity and weather variables**

Weather variable	Simple correlation coefficient (r)		
	2009	2010	Pooled
Mean temperature	0.95*	0.88*	0.85*
Mean relative humidity	-0.24	0.90*	0.21
Bright sunshine hours	0.56*	0.05	0.19
Rainfall	0.02	0.23	0.24

\* Significant at 5% level of significance

Multiple correlation coefficients between disease severity and group of weather variables (mean temperature, mean relative humidity, bright sunshine hours and rainfall) were highly significant during both the years (Table 4.7).

During the year 2009, best fit regression equation for anthracnose of horse gram was found to be  $Y = -252.8890 + 13.2972X_1 + 0.2385X_2 - 6.844X_3 + 2.0686X_4$  with coefficient of determination ( $R^2$ ) 0.92. Coefficient of determination revealed that all the weather variables contributed upto 92 per cent towards disease severity. Similarly during the year 2010 best fit regression equation for anthracnose of horse gram was found to be  $Y = 4831.3744 + 132.9740X_1 - 93.8528X_2 + 124.3477X_3 + 4.8167X_4$  with coefficient of determination ( $R^2$ ) 0.93. Coefficient of determination revealed that all the weather variables contributed upto 93 per cent towards disease severity.

Regression equation was also developed from the pooled data of disease severity and weather variables of 2009 and 2010. The forecasting model for anthracnose of horse gram developed from pooled data was found to be,  $Y = -475.3750 + 6.5475X_1 + 3.3194X_2 + 11.6253X_3 + 0.8087X_4$  with  $R$  and  $R^2$ , 0.93 and 0.87, respectively. Multiple correlation coefficient between disease severity and weather variables was significant. Coefficient of determination revealed that all the weather variables contributed upto 87 per cent towards disease severity.

**Table 4.7 Multiple correlation coefficients between epidemiological parameters of anthracnose of horse gram during 2009 and 2010**

Year	Regression equation	R	R <sup>2</sup>
2009	$Y = -252.8890 + 13.2972X_1 + 0.2385X_2 - 6.844X_3 + 2.0686 X_4$	0.96*	0.92
2010	$Y = 4831.3744 + 132.9740X_1 - 93.8528X_2 + 124.3477X_3 + 4.8167X_4$	0.97*	0.93
Pooled	$Y = -475.3750 + 6.5475X_1 + 3.3194X_2 + 11.6253X_3 + 0.8087X_4$	0.93*	0.87

\* Significant at 5% level of significance

Y= Disease severity, X<sub>1</sub> = Mean temperature, X<sub>2</sub> = Mean RH, X<sub>3</sub> = Bright sunshine hours, X<sub>4</sub> = Rainfall, R= Multiple correlation coefficient, R<sup>2</sup> = Coefficient of multiple determination

Under field conditions a large number of biotic and abiotic factors influence ant host- pathogen interaction. During the present studies the overall disease severity was moderate to high during both the years. Disease progressed readily when environmental conditions were favourable.

Mean temperature and relative humidity were found to be positively correlated, therefore it is evident that disease severity progressed significantly with rise in mean temperature and mean relative humidity. Rainfall and bright sunshine hours were found to influence disease insignificantly. This could be due to desiccation and washing of spores by bright sunshine and heavy rainfall. However, more information on other weather variables needs to be studied.

Multiple regression analysis highlighted significant effect weather parameters on disease development. The effect of weather variables on anthracnose development has been studied by many workers. Mittal (1999) reported that daily average maximum temperature of 26-28°C and minimum 18



20°C, RH of about 80 per cent and moderate, steady rainfall (40-120 mm in 4-5 rainy days/week) favoured the progress of anthracnose of black gram. Rana and Kaushal (2006) reported that temperature near 23°C, higher relative humidity combined with more number of rainy days and 2.62 sunshine hours were favourable for *Colletotrichum* leaf spot development in urdbean.

#### **4.5 Management of disease**

##### **4.5.1 Host resistance**

One hundred five genotypes of horse gram were evaluated for resistance to anthracnose under field conditions during *Kharif* seasons of 2009 and 2010. On the basis of disease severity, genotypes were categorized as highly resistant (0%), resistant (<1%), moderately resistant (1-10%), moderately susceptible (11-25%), susceptible (26-50%) and highly susceptible (>50%). Data (Table 4.8) revealed that none of the genotypes was resistant to anthracnose. However, 35 genotypes were found moderately resistant and 23 genotypes were moderately susceptible.

Host resistance is an important component in plant disease management. In the present study, none of the genotypes showed a desired level of resistance to anthracnose. However, 35 genotypes were found moderately resistant whereas, remaining were either susceptible or highly susceptible. This indicated that there is narrow range of resistance against anthracnose within present evaluated germplasm.

However, Chahota *et al.* (2005) who evaluated 63 landraces of horse gram collected from different parts of Himachal Pradesh against *C. truncatum* under field conditions reported that most of the lines were found resistant to anthracnose. On the basis of evaluation, lines namely HPKC-1, HPKC-6, HPKC-7, HPKC-9, HPKM-51 and DMK-12 were found to be potential lines which can either be exploited as a variety for multilocation evaluation or can

**Table 4.8 Evaluation of horse gram germplasm for resistance to anthracnose**

Disease rating	Disease severity (%)	Reaction type	Genotypes
0	0	Highly resistant	NIL
1	< 1	Resistant	NIL
3	1-10	Moderately resistant	HPKC-1, HPKC-2, HPKC-3, HPKC-5, HPKC-7, HPKC-10, HPKC-14, HPKC-17, HPKC-19, HPKC-20, L-34-1, L-34-2, L-34-4, L-35, L-35-1, L-38-3, L- 44, L- 45-2, L-54, L-56, L-45-1, L-47, L- 48, L- 54- 3, L- 34- 3, L-54-2-1,HPKM-141, HPKM-51, HPKM-2, HPKM-118, HPKM-116, DMK-12, Kullu-13, Kullu-3, VL-Gahet.
5	11-25	Moderately susceptible	SKY/SNS-1654,SKY/SNS-510,RKS/KP-419, RKS/AKS-730, HPKM-24, HPKM-147, HPKC-18, HPKC-28, HPKM-149, DNK2P, VLG-1, L-38-1, L-38-2, L-41-1, L- 44-2, L-44-3, L-45, L-51, L-51-1, L-56-1, L-56-2, L -59-1, L-59-2.
7	26-50	Susceptible	IC-107214, IC-107314, IC-108078, IC-108079, IC-108080, IC-278824, IC-278826, IC-278827, IC-313369, IC-313370, IC-321237, IC-421954, IC-421960, IC-18925, NIC-17544, NIC-14351, NIC-14350, SKY/SNS-504, RKS/KP-418, RKS-730, Chamba, RICS/ASU-729, DHG-2, DHG-3, DHG-7, DHG-8, DHG-9.
9	> 50	Highly susceptible	IC-106911, IC-107098, IC-107114, IC-107188, IC-107205, IC-107337, IC-107446, IC-108076, IC-108077, IC-106910, IC-18344, NIC-17543, NIC-14410, NIC-14352, HPK-4, TRS/RKS-1101, AK-2, PVS /UKP- 420, KG-13, Himganga.

be used in future hybridization program. Present studies, however, show that there is acceptable level of resistance to the pathogen. The locally adapted highly susceptible genotypes can be improved by transferring resistance from the available moderate resistance sources in future breeding programme.

#### 4.5.2 Fungicides

An experiment was conducted to evaluate the fungicides as foliar spray under field conditions during 2009 and 2010 (Plate 9). The results are presented in Table 4.9 and Figure 4.5. Among all the fungicides, Bavistin recorded minimum disease severity (23.7%) followed by Dithane M-45 (28.34%) and Contaf (33.33 %) whereas, Blitox and Folicur resulted 41.48 per cent and 42.04 per cent disease severity, respectively. Maximum disease severity was recorded in Score (48.15%) followed by Captan (44.3%) and Kavach (43.15%).

Maximum disease control (63.62%) was recorded in Bavistin followed by Dithane M-45 (56.47%), Contaf (49.85%), Blitox (36.22%), Folicur (35.27%), Kavach (33.48%) and Captan (31.16%) whereas Score was least effective (25.95%). Maximum increase in grain yield was also recorded in Bavistin (94.6%) and minimum in Score (16.2%).

All the fungicides were effective in reducing anthracnose disease severity significantly, but spraying with Bavistin was found most effective.

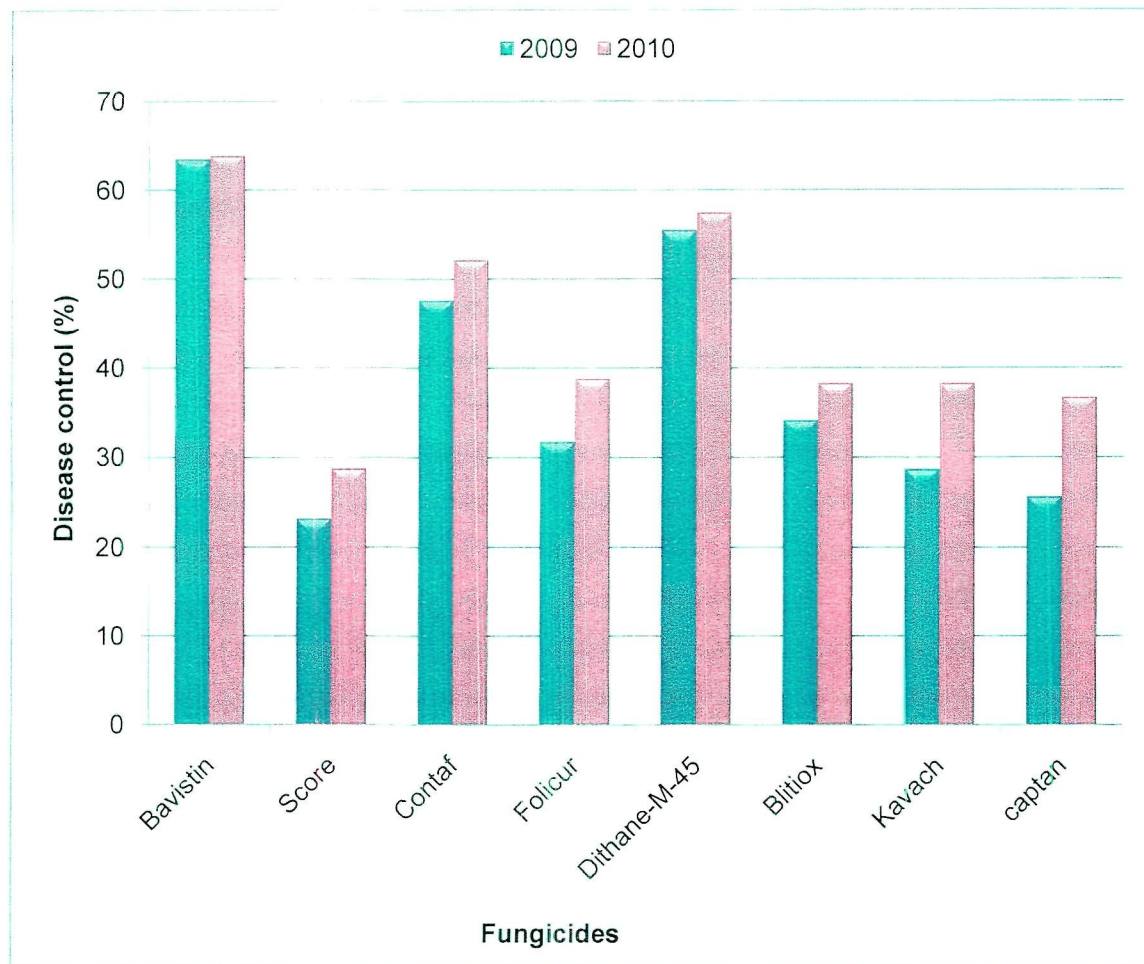
Results on the effectiveness of Bavistin and Dithane M-45 as foliar spray in the present study are in agreement with the findings of Malhotra and Chaturvedi (1973), who tested the efficacy of five fungicides viz., Dithane-M-45 (0.2%), Cumin, Benlate (0.1%), Bordeaux mixture(0.1%) and Aureofungin (250 ppm) against pod blight of soybean caused by *C. truncatum*. Dithane M-45 (0.25%) and Aureofungin (200 ppm) were significantly superior to Cumin in reducing the disease under field conditions. Singh and Singh (2001) found Bavistin as the best followed by Benlate and Topsin-M against *C. truncatum* in mungbean.

**Table 4.9 Evaluation of fungicides in the management of anthracnose of horse gram under field conditions**

Treatment (dose)	Disease severity (%)			Control (%)			Grain yield	
	2009 *	2010*	Mean	2009	2010	Mean	q/ha	% Increase
Bavistin(0.1%)	22.22 (28.09)	25.18 (30.08)	23.7	63.41	63.83	63.62	7.2	94.6
Score (0.05%)	46.67 (43.07)	49.63 (44.77)	48.15	23.17	28.72	25.945	4.3	16.2
Contaf (0.05%)	31.85 (34.33)	33.33 (35.29)	32.59	47.56	52.13	49.845	6.3	70.3
Folicur (0.05%)	41.48 (40.40)	42.59 (40.72)	42.04	31.71	38.83	35.27	5.3	43.2
Dithane-M-45 (0.25%)	27.03 (31.30)	29.63 (32.94)	28.34	55.49	57.45	56.47	7.0	89.2
Blitox (0.25%)	40.00 (39.20)	42.96 (40.96)	41.48	34.14	38.3	36.22	5.8	56.8
Kavach (0.25%)	43.33 (41.14)	42.96 (40.89)	43.15	28.66	38.29	33.475	4.7	27.0
Captan (0.25%)	45.18 (42.22)	44.07 (41.58)	44.63	25.61	36.7	31.155	4.5	21.6
Control (0.25%)	60.74 (51.18)	69.63 (56.56)	65.19	-	-	-	3.7	-
CD (P=0.05)	3.56	4.44					0.24	

\*Mean of three replications

Figures in parentheses are arc sine transformed values



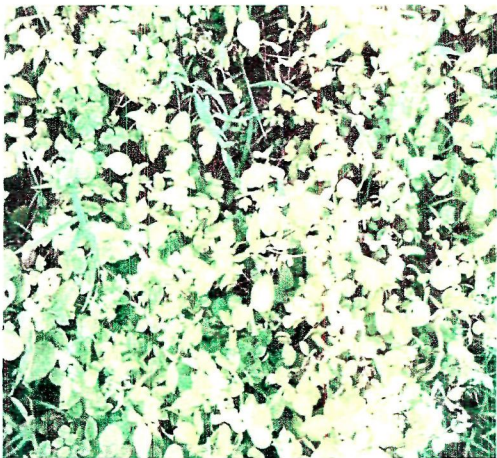
**Fig. 4.5** Evaluation of fungicides in the management of anthracnose of horse gram under field conditions



**(i) Bavistin**



**(ii) Dithane- M-45**



**(iii) Control**



**Field trial**

**Plate 9: Field evaluation of fungicides against anthracnose of horse gram**

#### 4.5.3 *In vitro* evaluation of bio-control agents

Results of evaluation of antagonistic effects of biocontrol agents *viz.*, *Trichoderma viride*-H, *T. harzianum*-H and *Pseudomonas fluorescens*-TNAU against *C. truncatum*, recorded as inhibition of mycelial growth in dual culture are presented in Table 4.10 and Figure 4.6. Maximum inhibition of mycelial growth was observed with *T. viride*-H (58.81%) followed by *P. fluorescens*-TNAU (46.03%) and *T. harzianum*-H (35.47%) (Plate 10).

**Table 4.10 Effect of biocontrol agents on mycelial growth of *Colletotrichum truncatum***

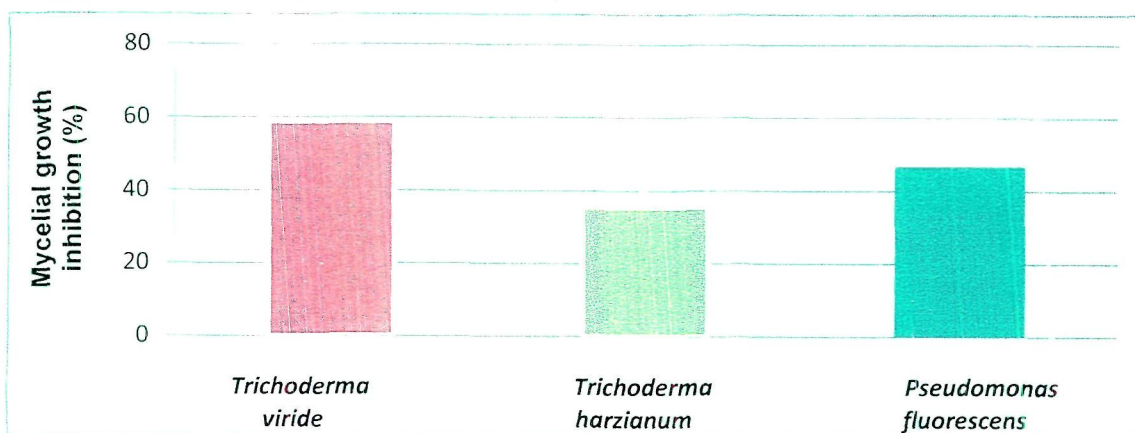
Treatment	Mycelial growth (mm)*	Inhibition (%)
<i>T. viride</i> - H	34.00	58.81 (50.06)
<i>T. harzianum</i> - H	53.33	35.47 (36.52)
<i>P. fluorescens</i> - TNAU	44.67	46.03 (42.70)
Control	82.67	-
CD (P=0.05)	6.45	4.48

Figures in parentheses are arc sine transformed values

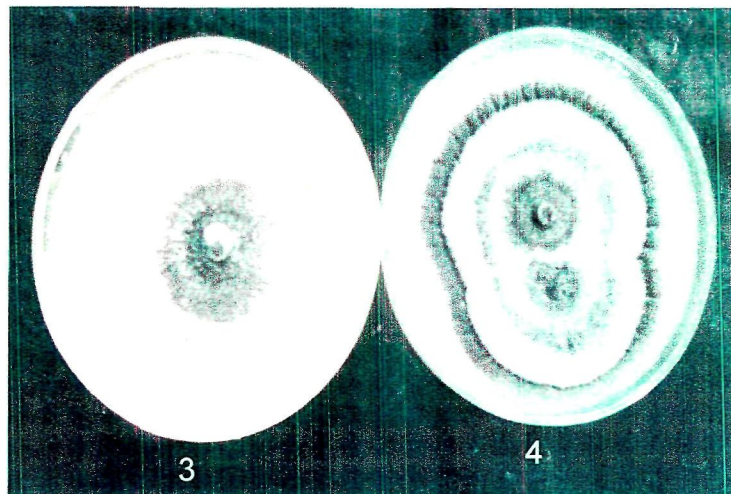
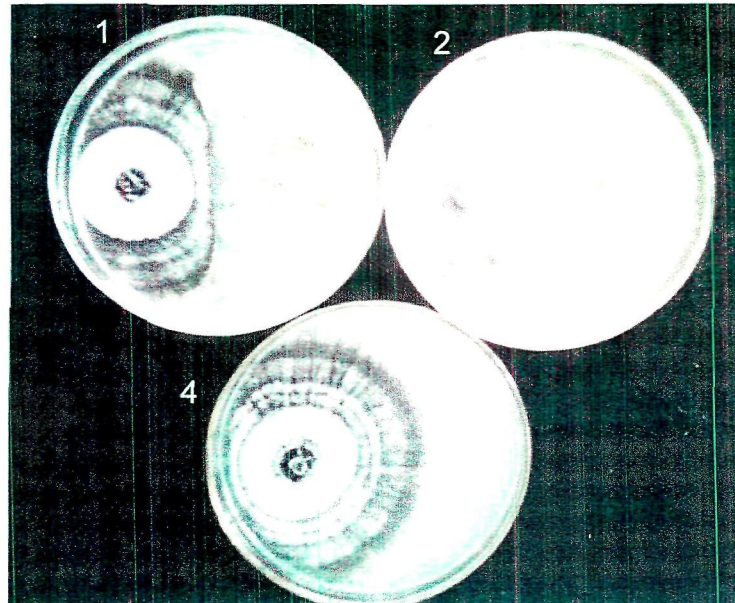
\*Mean of three replications

H- Hyderabad

TNAU- Tamilnadu Agriculture University



**Fig. 4.6 Effect of biocontrol agents on mycelial growth of *Colletotrichum truncatum***



**Plate 10: Antagonistic activity of biocontrol agents against *C. truncatum***

1= *Trichoderma harzianum*- H

2= *Trichoderma viride*- H

3= *Pseudomonas fluorescens* -TNAU

4=Control



Pollution problems in the environment and the toxic effect of synthetic chemicals on non target organisms have promoted investigations on exploiting biological control agents as one of the component of disease control. As many species of fungi and other microbes have been reported to be antagonists of *Colletotrichum*, only a few of them have been studied extensively. To reduce the harmful effects of pesticides in the ecosystem the use of biocontrol agents in plant disease management has been experimented. All the three bio-control agents used in the present study showed antagonistic activity under *in vitro* conditions by inhibition of mycelial growth of the pathogen. Bankole (1990) observed an isolate of *T. viride* TH-31 was found to hyperparasitize a number of plant pathogenic fungi including *Colletotrichum*. Gawade *et al.* (2009) evaluated two biocontrol agents *in vivo* against anthracnose of soybean. Both biocontrol agents (*T. viride* and *Verticillium lecanii*) were found significantly superior to unsprayed control.

#### 4.5.4 *In vitro* evaluation of biopesticides

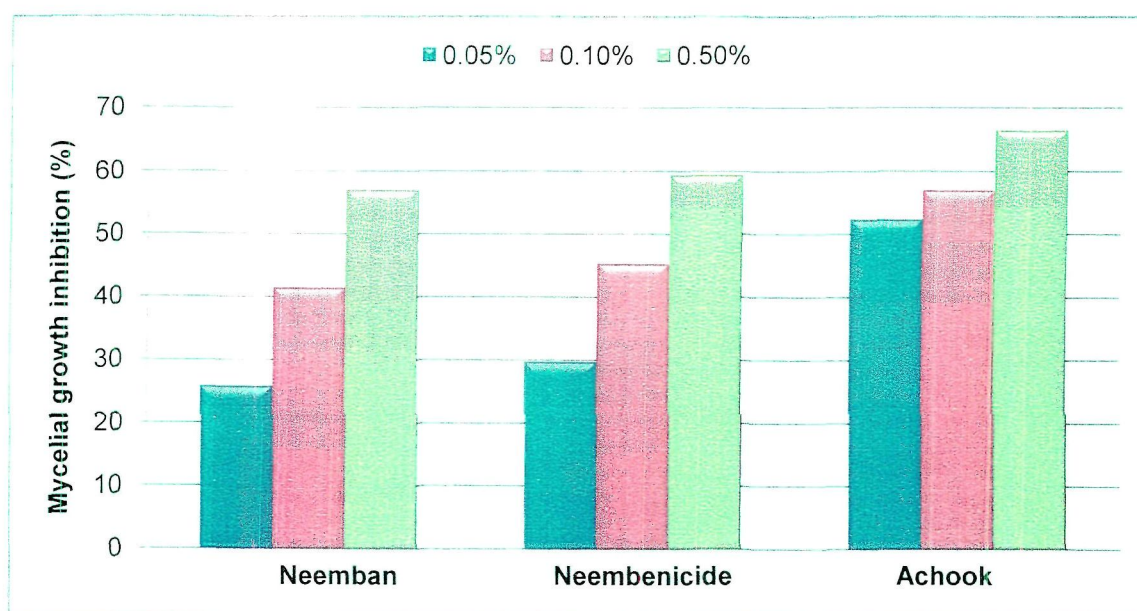
Three neem based biopesticides *viz.*, Neemban, Neembenicide and Ahook were tested at 0.05, 0.1 and 0.5 per cent concentrations using poisoned food technique (Plate 11). Data on per cent growth inhibition is presented in Table 4.11 and Figure 4.7. The data revealed that all biopesticides were found significantly effective against the *C. truncatum*. The per cent inhibition was increased with the increasing concentration from 0.05 to 0.5 per cent of all the biopesticides. However, Ahook was found most effective and resulted in maximum inhibition of 52.34, 57.03 and 66.40 per cent followed by Neembenicide which showed 29.66, 45.29 and 59.37 per cent inhibition at 0.05, 0.1 and 0.5 per cent concentrations, respectively. Minimum inhibition was recorded in Neemban 25.78, 41.40 and 57.01 per cent at 0.05, 0.1 and 0.5 per cent concentrations, respectively. However, Neemban (57.01%) and Neembenicide (59.37%) at 0.5 per cent concentration were at par with each other. During the past three decades, Neem has dominated the international literature on botanicals (NRC, USA 1992). In the present investigation, the results of evaluation of Neem based biopesticides showed that all the biopesticides inhibited mycelial growth of *C. truncatum* as compared to control.

**Table 4.11 Effect of neem based biopesticides on mycelial growth of *Colletotrichum truncatum***

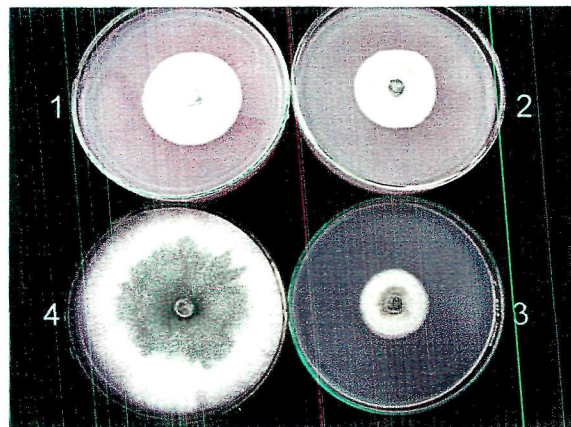
Concentration	0.05%		0.10%		0.50%	
	Mycelial growth (mm)*	Growth inhibition (%)	Mycelial growth (mm)*	Growth inhibition (%)	Mycelial growth (mm)*	Growth inhibition (%)
Neemban	63.33	25.78 (30.50)	50.00	41.40 (40.40)	36.67	57.01 (49.01)
Neembenicide	60.00	29.66 (32.96)	46.67	45.29 (42.28)	34.67	59.36 (50.37)
Achook	40.67	52.34 (46.32)	36.67	57.03 (49.03)	28.67	66.40 (54.55)
Control	85.33	-	85.33	-	85.33	-
CD (P=0.05)	2.66	2.48	3.77	3.18	2.17	2.23

Figures in parentheses are arc sine transformed values

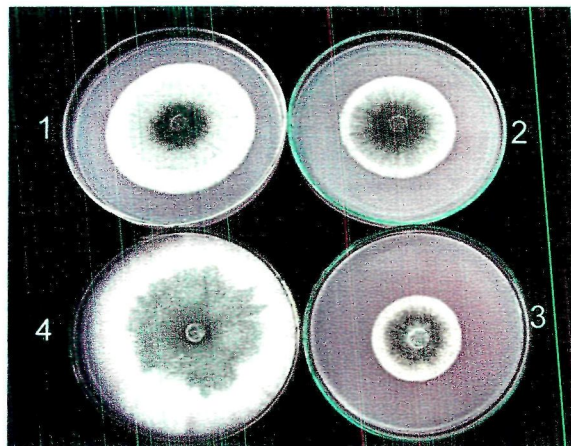
\*Mean of three replications



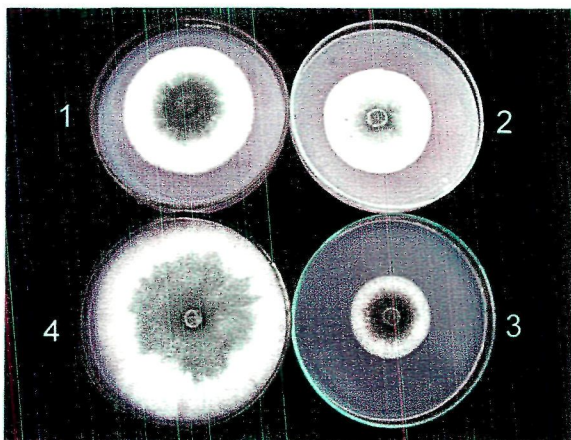
**Fig. 4.7 Effect of neem based biopesticides on mycelial growth of *Colletotrichum truncatum***



(i) Achook



(ii) Neemban



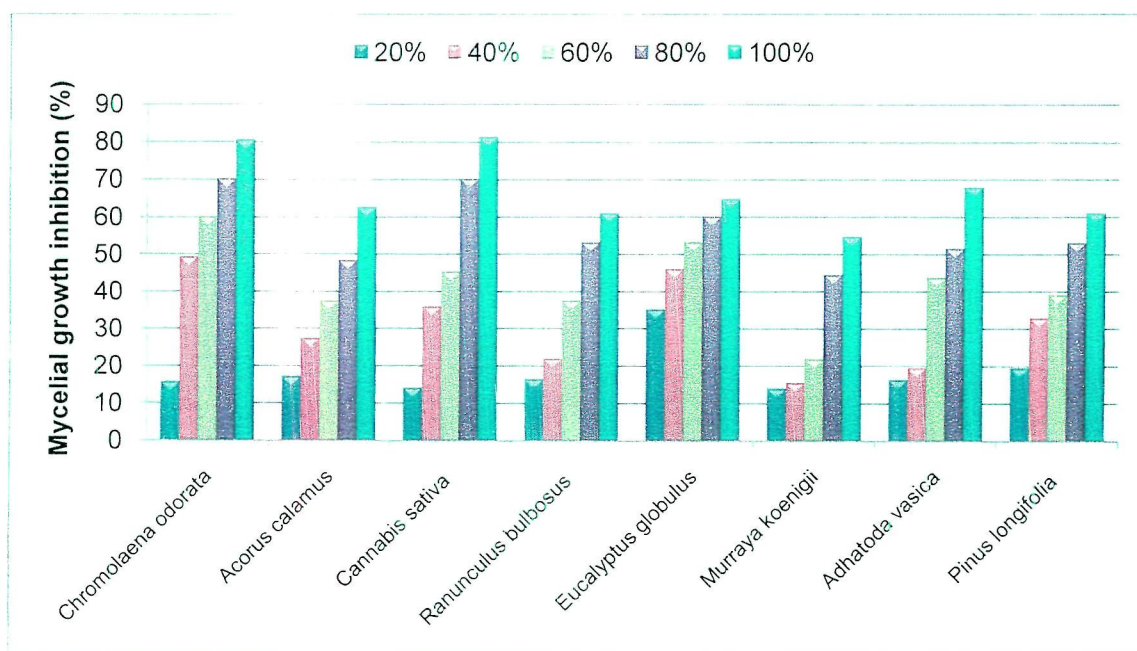
(iii) Neembenicide

1=0.05%    2=0.1%    3=0.5%    4=Control

Plate 11: Efficacy of different biopesticides against *C. truncatum*

#### 4.5.5 *In vitro* evaluation of botanicals

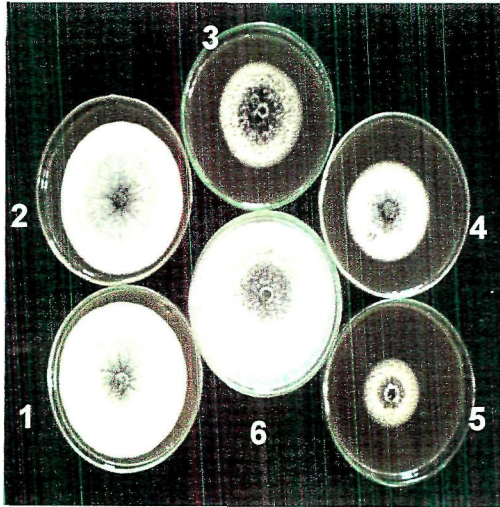
The data with respect to effect of eight plant extracts on mycelial growth of *C. truncatum* are presented in Figure 4.8 and Table 4.12. *Cannabis sativa* at 100 per cent was found best (81.23%) followed by *Chromolaena odorata* (80.46%) and *Adhatoda vasica* (67.96%) (Plate 12). At 20 per cent *Eucalyptus globulus* was found to be most effective (35.14%) followed by *Pinus longifolia* (19.51%) and *Acorus calamus* (17.14%). At 40 (49.24%), 60 (60.13%) and 80 (70.28%) per cent concentration *Chromolaena odorata* was found to be effective. Minimum mycelial inhibition was observed in *Murraya koenigii* at all the concentrations. *Murraya koenigii* (14.01%) and *Acorus calamus* (17.14%) were statistically at par at 20 per cent concentration. *Eucalyptus globulus* (46.07%) and *Chromolaena odorata* (49.24%) at 40 per cent concentration were found to be statistically at par. *Adhatoda vasica* (43.74%) and *Cannabis sativa* (45.30%) were statistically at par at 60 per cent concentration. Similarly, *Pinus longifolia* (60.95%) and *Eucalyptus globulus* (64.82%) were statistically at par with each other at 100 per cent concentration.



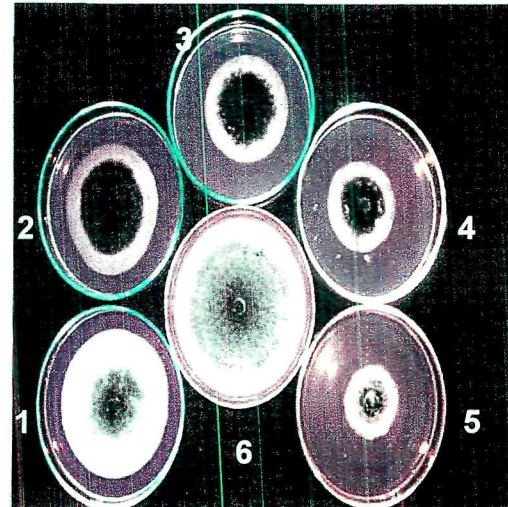
**Fig. 4.8** Effect of botanicals on mycelial growth of *Colletotrichum truncatum*

**Table 4.12 Effect of botanicals on mycelial growth of *Colletotrichum truncatum***

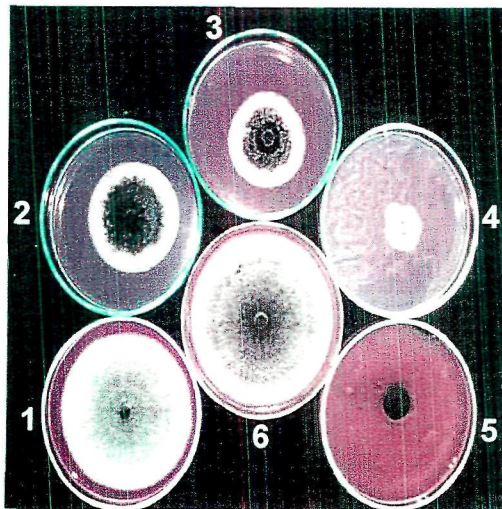
Treatment	Mycelial growth (mm)* at concentrations (%)										Mycelial growth inhibition (%) at concentrations (%)									
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100					
<i>Chromolaena odorata</i>	72.00	43.33	34.00	25.33	16.67	15.56	49.24	60.13	70.28	80.46	(23.19)	(44.55)	(50.83)	(56.96)	(63.75)					
<i>Acorus calamus</i>	70.67	62.00	53.33	44.00	32.00	17.19	27.37	37.48	48.39	62.48	(24.31)	(31.49)	(37.73)	(44.05)	(52.21)					
<i>Cannabis sativa</i>	73.33	54.67	46.67	25.33	16.00	14.04	35.93	45.30	70.28	81.23	(21.96)	(36.81)	(42.28)	(56.96)	(64.33)					
<i>Ranunculus bulbosus</i>	71.33	66.67	53.33	40.00	33.33	16.39	21.91	37.47	53.17	60.93	(23.84)	(27.84)	(37.71)	(46.81)	(51.30)					
<i>Eucalyptus globulus</i>	55.33	46.00	40.00	34.00	30.00	35.14	46.07	53.12	60.15	64.82	(36.34)	(42.73)	(46.78)	(50.84)	(53.61)					
<i>Murraya koenigii</i>	73.33	72.00	66.67	47.33	38.67	(14.01	15.61	21.87	44.50	54.65	(22.02)	(23.26)	(27.88)	(41.82)	(47.65)					
<i>Adhatoda vasica</i>	71.33	68.67	48.00	41.33	27.33	16.39	19.51	43.74	51.55	67.96	(23.84)	(26.21)	(41.39)	(45.87)	(55.51)					
<i>Pinus longifolia</i>	68.67	57.33	51.33	40.00	33.33	19.51	32.88	39.11	53.10	60.95	(26.17)	(34.88)	(39.11)	(46.76)	(51.30)					
Control	85.33	85.33	85.33	85.33	85.33	-	-	-	-	-	-	-	-	-	-					
CD (P=0.05)	3.36	4.43	3.02	5.44	3.43	3.68	3.26	2.56	4.16	2.89										



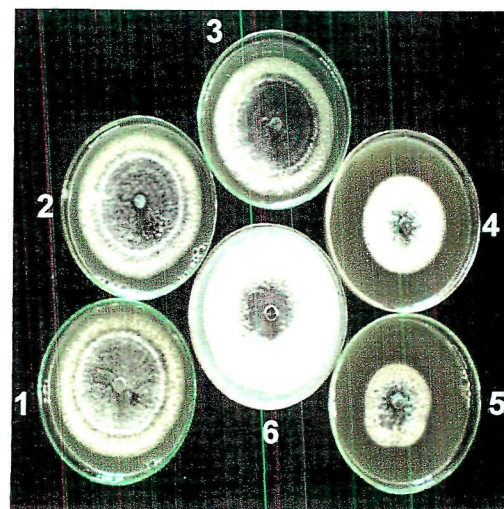
(i) *Adhatoda vasica*



(ii) *Acorus calamus*



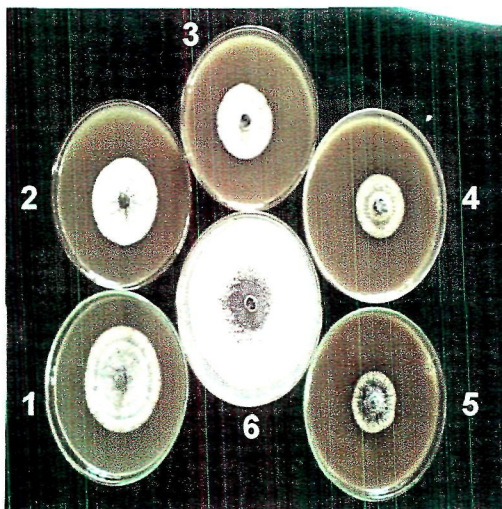
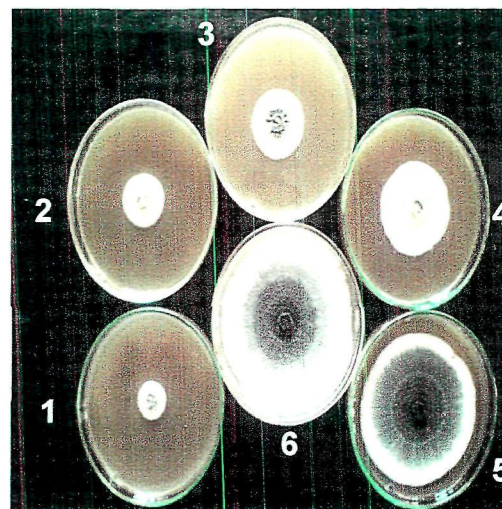
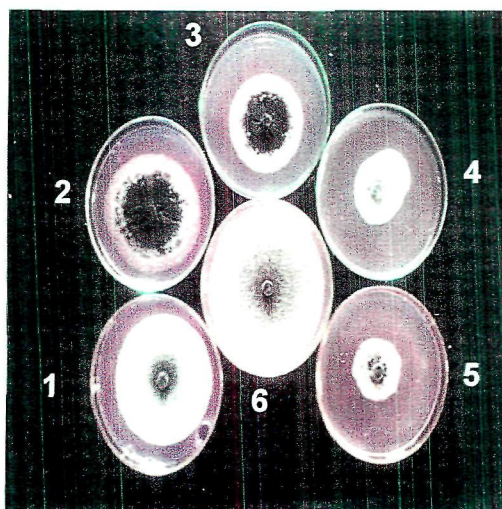
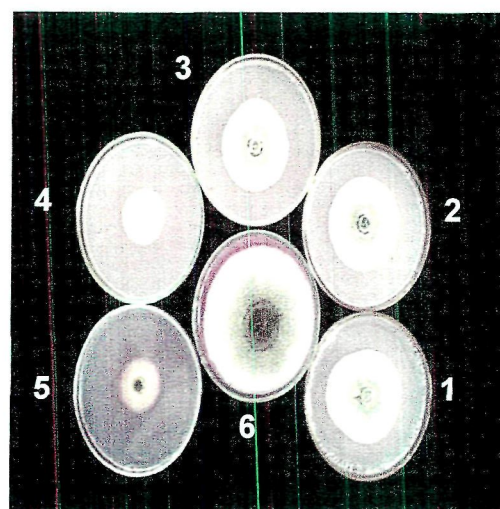
(iii) *Cannabis sativa*



(iv) *Murraya koenigii*

1= 20%      2=40%      3=60%

4=80%      5=100%      6=Control

(v) *Eucalyptus globulus*(vi) *Chromolaena odorata*(vii) *Pinus longifolia*(viii) *Ranunculus bulbosus*

1= 20%      2=40%      3=60%      4=80%      5=100%      6=Control

Plate 12: *In vitro* evaluation of botanicals against *C. truncatum*

The plant world is comprised of rich source of secondary metabolites which have a significant role in the primary physiological processes in the plants but are endowed with potential bioactivity. In the present study fairly good inhibitory effects against *C. truncatum* were obtained. The data revealed that the best inhibition of mycelial growth was observed at 100 per cent concentration of all botanicals significantly. An increased inhibitory effect of mycelial growth was observed with an increase in concentration from 20 to 100 per cent. A large number of plants are known to possess antifungal properties against *C. truncatum* as reported by various workers. Kaushal and Paul (1989) studied inhibitory effects of some plant extracts (*Cannabis sativa*, *Pinus longifolia*, *Eupatorium* sp. and *Lantana indica*) on some legume pathogens and found that all the plant extracts inhibited *C. truncatum*.

Laboratory screening of plant extracts in the present study has given encouraging results, indicating their potential use in the management of anthracnose of horse gram. However, their performance under field conditions needs further studies.





**SUMMARY**  
**AND**  
**CONCLUSIONS**

## 5. SUMMARY AND CONCLUSIONS

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Investigations on anthracnose of horse gram were undertaken to study the pattern of disease development, agrometeorological factors affecting the disease and to work out its suitable management strategies. The results obtained are summarized below.

Among the epidemiological parameters studied in the laboratory, 20-25°C was found optimum for anthracnose development. Maximum lesion size was observed at 25°C (8.29 mm<sup>2</sup>) after 96 hr of inoculation. As the temperature increased and decreased from 25°C, there was decrease in the lesion size. After 96 hr of inoculation least lesion size was observed at 15°C (1.81 mm<sup>2</sup>). The optimum RH requirement ranged between 95-100 per cent for successful infection. Among different RH levels, maximum lesion size (7.18 mm<sup>2</sup>) was observed, at 100 per cent RH after 96 hr of inoculation and minimum (2.05 mm<sup>2</sup>) after 96 hr of inoculation at 75 per cent RH. With the increase in the incubation period, there was increase in the lesion size at all humidity levels. Twelve hours alternate light and dark period influenced the anthracnose development while complete light and dark induced less disease. Maximum lesion size (6.23 mm<sup>2</sup>) was noticed at 12 hr alternating light and dark conditions after 96 hr of inoculation. Minimum lesion size (4.51 mm<sup>2</sup>) was noticed in complete dark conditions after 96 hr of inoculation.

Sowing dates and row to row spacing influenced the development of disease. The disease severity decreased with the delay in sowing date and increase in row to row spacing. Maximum disease severity (52.44% and 62.83%) was observed when the crop was sown on 8<sup>th</sup> June of 2009 and 2010 respectively, at 25 cm row to row spacing. Crop sown on 29<sup>th</sup> June (2009 and 2010) at 35 cm row to row spacing resulted in minimum disease severity of 25.28 per cent and 36.00 per cent respectively, however yield decreased. The maximum grain yield (5.70 q/ha) was obtained from crop sown on 22<sup>nd</sup> June at 25 cm row to row spacing which reduced significantly when sowing was delayed irrespective of row to row spacing and in early sown crop (8<sup>th</sup> and 15<sup>th</sup> June) the grain yield was low due to higher disease severity.

The disease progressed slowly during 2009 in comparison to 2010. The progress of disease exhibited sigmoidal pattern of the curve during both the years. The disease development was maximum (24.44 - 83.33 %) between 1<sup>st</sup> August to 29<sup>th</sup> August in 2010 under 1<sup>st</sup> date of sowing i.e. 8<sup>th</sup> June, 2010. Similar pattern of disease development was during 2009 when 19.78 - 75.55 per cent range of the disease was observed during 1<sup>st</sup> August to 29<sup>th</sup> August.

There was a positive correlation ( $r = 0.95$  and  $0.88$ ) between disease severity and mean temperature during both the years. The correlation coefficients between disease severity and mean relative humidity were non significant and negative ( $r = -0.24$ ) during 2009 and significantly positive ( $r=0.90$ ) during 2010. Positive correlation ( $r=0.56$ ) between disease severity and bright sunshine hours was observed during 2009. Rainfall also showed non significant correlation with disease severity during both the years. Multiple correlation coefficients between disease severity and all studied weather variables were highly significant during both the years.

During the year 2009, best fit regression equation for anthracnose of horse gram was found to be  $Y = - 252.8890 + 13.2972X_1 + 0.2385X_2 - 6.844X_3 + 2.0686 X_4$  with coefficient of determination ( $R^2$ ) 0.92. Coefficient of determination revealed that all the weather variables contributed upto 92 per cent towards disease severity. Similarly during the year 2010 best fit regression equation for anthracnose of horse gram was found to be  $Y= 4831.3744 + 132.9740X_1 - 93.8528X_2 + 124.3477X_3 + 4.8167X_4$  with coefficient of determination ( $R^2$ ) 0.93.

The best fit regression equation from pooled data was found to be,  $Y = - 475.3750 + 6.5475X_1 + 3.3194X_2 + 11.6253X_3 + 0.8087X_4$  with  $R$  and  $R^2$ , 0.93 and 0.87, respectively.

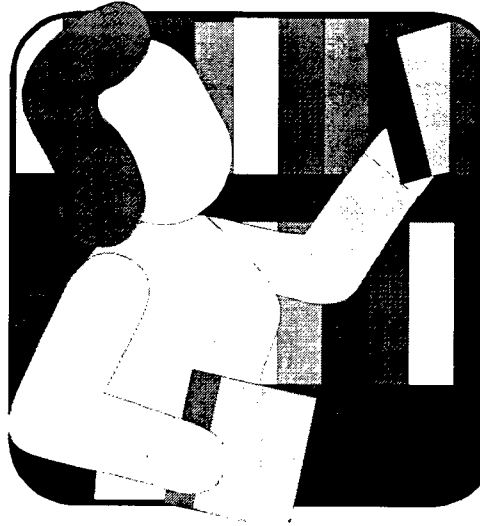
One hundred five genotypes of horse gram evaluated for resistance against anthracnose under field conditions during *kharif* seasons of 2009 and 2010, none of the genotype was resistant to the disease. However, 35 genotypes were found moderately resistant and 23 genotypes were moderately susceptible.

The results of studies conducted on the fungicidal management of anthracnose of horse gram revealed that under field conditions Bavistin gave maximum disease control (63.62%) followed by Dithane M-45 (49.85%), Contaf (52.13%), Blitox (36.22%), Folicur (35.27%), Kavach (33.48%) and Captan (31.16%) whereas Score gave minimum disease control (25.95%). Maximum increase in grain yield was also recorded in Bavistin (94.6%) and minimum in Score (16.2%).

Among the antagonists tested against the pathogen, *Trichoderma viride*-H was found most effective in inhibiting mycelial growth of *C. truncatum* (58.81%) followed by *Pseudomonas fluorescens*-TNAU (46.03%) and *T. harzianum*-H (35.47%).

During the present investigations three neem based biopesticides viz., Neemban, Neembenicide and Ahook were tested against *C. truncatum in vivo* at 0.05, 0.1 and 0.5 per cent concentrations. Ahook was found most effective and resulted in maximum inhibition of 52.34, 57.03 and 66.40 per cent followed by Neembenicide which showed 29.66, 45.29 and 59.37 per cent inhibition at 0.05, 0.1 and 0.5 per cent concentrations, respectively. Minimum inhibition was recorded in Neemban 25.78, 41.40 and 57.01 per cent at above mentioned concentrations, respectively.

Among all the plant extracts, *Cannabis sativa* at 100 per cent was effective (81.23%) in inhibiting mycelial growth of *C. truncatum* followed by *Chromolaena odorata* (80.46%) and *Adhatoda vasica* (67.96%). At 20% *Eucalyptus globulus* was found to be most effective (35.14%) followed by *Pinus longifolia* (19.51%) and *Acorus calamus* (17.14%). Minimum inhibition was observed in *Murraya koenigii* at all the concentrations.



# LITERATURE CITED

## LITERATURE CITED

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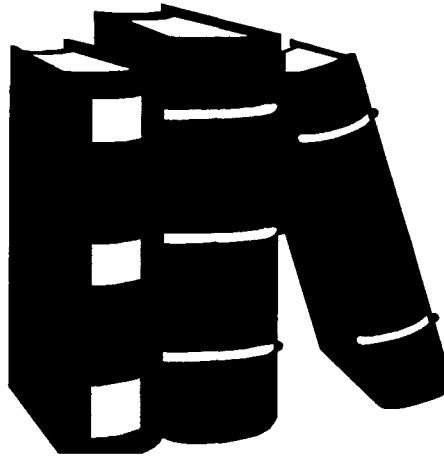
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# APPENDICES

## Appendix-I

### Composition of media used

#### Potato Dextrose Agar (PDA)

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

#### Mathur's media

Glucose	2.8 g
$\text{KH}_2\text{PO}_4$	2.72 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.23 g
Neopeptone	2.0 g
Agar	20 g
Distilled water	1000 ml

## Appendix-II

### Weather data at weekly interval during August, 2009 and 2010

Date (August)	Mean		Mean RH		BSS		Rainfall	
	Temperature (°C)		(%)		(hr)		(mm)	
	2009	2010	2009	2010	2009	2010	2009	2010
1	22.72	24.03	85.93	89.57	3.06	2.19	17.51	26.09
8	23.10	23.39	86.86	89.77	3.71	3.00	12.86	29.31
15	25.80	25.95	89.63	91.99	3.81	2.21	21.26	26.83
22	27.47	25.96	90.50	92.29	3.00	1.03	26.18	66.49
29	27.77	26.73	78.67	94.49	7.76	3.93	8.03	14.30

### Brief Biodata of student

**Name** : Devanshi Pandit  
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**Mother's Name** : Smt. Pratima Pandit  
**Date of Birth** : 17<sup>th</sup> Jan, 1987  
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#### Academic Qualifications:

Examination passed	Month	Year	School/Board/ University	Marks (%)	Division
10 <sup>th</sup>	March	2002	HPBSE, Dharamsala	75.9	1 <sup>st</sup>
10+2	March	2004	HPBSE, Dharamsala	67.8	1 <sup>st</sup>
B.Sc. Agriculture	June	2008	CSK HPKV, Palampur	72.3	1 <sup>st</sup>