‘‘Studies on Anthracnose of Urdbean caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) and it’s eco-friendly management’’

THESIS

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Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya

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by

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CERTIFICATE – I

This is to certify that the thesis entitled **''Studies on Anthracnose of Urdbean caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) and it's eco-friendly management''** submitted in partial fulfilment of the requirements for the Degree of **MASTER OF SCIENCE** in **Department of Plant Pathology** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bona-fide research work carried out by **Mr. Aashish Deshmukh** I.D. No. 20111801 under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and Director of Instruction.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of this investigation has been acknowledged by the scholar.

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Place : Gwalior

Date: **Aashish Deshmukh**

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LIST OF ABBREVIATIONS AND SYMBOL

Symbol	Abbreviation	Stand for
%		Per cent
°C		Degree centigrade
±		Plus or minus
@		At the rate of
	Fig.	Figure
	Ha	Hectare
	i.e.	That is
	Gm	Gram
	Mg	Milligram
	Q	Quintal
	CV	Coefficient of variation
	CD	Critical difference
	cv.	Cultivar
	D.F.	Degrees of Freedom
	S.V.	Source of variation
	SEm±	Standard error of mean
	et. al.	Co-workers
	viz.,	Namely
	Mm	Milimeter
	Ml	Mililiter
	DAI	Days after inoculation
	DAS	Days after sowing

INTRODUCTION

Chapter-I

INTRODUCTION

Urdbean (*Vigna mungo* L. Hepper), which is also known as black gram, udad, urd and udid in India. Blackgram is native to India (De Candolle, 1986). It is one of the commercially important legume crop, domesticated from *V. mungo var. silvestris,* belongs to the sub-genus *Ceratotropis*, (Lukoki *et al.* 1980). It is extensively cultivated in the Indian sub -continent in all the three seasons such as in Kharif, Rabi, and Summer throughout the country and to a lesser extent in Thailand, Australia along with other Asian and South-Pacific countries.

As a tropical region crop, it requires warm weather, hot and humid climate for best growth. Due to low temperature during winter in Northern parts of the country it is cultivated generally during rainy and summer season. While in the Eastern regions, it is also grown during winter and in Central and Southern states, it is cultivated throughout the year because of almost no variation in the climate (Tiwari, Shivhare and Kumar, 2017).

The food value of urdbean lies in its high and easily digestible protein. Its seeds contains approximately 62 – 65% carbohydrates, 25 – 28% protein, 4.5 – 5.5% ash, 3.5 – 4.5% fiber on dry weight basis, while it also contains some macro and micro nutrients in minor quantity, which include calcium (154 mg/100 g), phosphorus (385 mg/ 100 g) and iron (9.1 mg/ 100 g). Additionally, they compose very good level of many B-complex vitamins such as vitamin-B6 (22 %), thiamin (23 %), pantothenic acid (18 %), riboflavin (20 %) and niacin (9 %) of daily recommended values. Most of these vitamins works as co-factors for the enzymes in carbohydrates, protein and fat metabolism. Being one of the important legume crop of India. It is edible in the form of ‘dal’ (split, whole, husked and un-husked) or perched. Apart from this, urdbean is very nutritive and useful for milch cattle in the form of green fodder. As a leguminous crop, it has the capacity to restore soil fertility by fixing atmospheric nitrogen. In terms of balanced human nutrition, urdbean is an excellent complement to rice due to high values of lysine. (Tiwari, Shivhare and Kumar, 2017).

Ninety five per cent of black gram production comes from 11 states of India *viz;* Madhya Pradesh, Andhra Pradesh, Rajasthan, Tamil Nadu, Uttar

Pradesh, Maharashtra, Jharkhand, Gujarat, Karnataka and West Bengal. India being the largest producer as well as consumer of urdbean, produces about 1.96 million tonnes of urdbean from about 3.55 million hectare of area annually with an average productivity of 552 kg/ha (Anon., 2021). In Madhya pradesh, it covers an area of about 1.44 mh with production of 829 thousand tonnes and productivity of 575 kg/ha (Anon., 2021).

Various pests and diseases are constant threat as well as limiting factor in sustainable production of urdbean. Disease is one of the major constraints to yield throughout most of the part of Asia. Among most serious fungal diseases of Urdbean are Anthracnose (*Colletotrichum lindemuthianum*), Cercospora leaf spot (*Cercospora canescens*), Root rot (*Macrophomina phaseolina*), Rust (*Uromyces appendiculatus*), Powdery mildew (*Erysiphe polygoni*), Wilts (*Fusarium spp.*), Web blight (*Rhizoctonia solani*), Sclerotium stem rot (*Sclerotium rolfsii*), Bacterial leaf blight (*Xanthomonas phaseoli*), Halo blight (*Pseudomonas phaseolicola*), Yellow mosaic virus, Leaf crinkle virus and Leaf curl virus (Agrawal, 1991).

The anthracnose is caused by *C. lindemuthianum* (Sacc. and Magn.) in urdbean, which was first reported in devastating form in Assam and caused severe loss in yield under suitable weather conditions. The disease is now widespread in India, which is caused by all *Colletotrichum spp* and occurs every year, but in severe form only in *Vigna mungo*. The symptoms mainly appear as brown blotches and leaf spots on leaves, pods and stem. The initial symptoms appear as small, circular, brown, spots, which on later enlarge to form dark brown concentric rings with greyish area in center, giving a appearance of target board effect. The spots may be circular or irregular or sickle in shape with greyish centre, which measures around 3 to 5 mm in diameter. At later stage, papery spotted portion falls off the from the leaves, which leads to shot holes and defoliation of the plant (Agrawal, 1991).

Objectives of investigation:

1. Isolation, purification and identification of pathogen.
2. Screening of urdbean genotypes against *C. lindemuthianum* to find out the resistant source of anthracnose.
3. *In vitro* evaluation of organic farm products against *C. lindemuthianum.*
4. *In vitro* evaluation of botanicals against *C. lindemuthianum*.
5. *In vivo* evaluation of selected organic farm products and botanicals for eco-friendly management of urdbean anthracnose.

REVIEW OF LITERATURE

Chapter-II

REVIEW OF LITERATURE

Urdbean [*Vigna mungo* (L.) Hepper], which is annual, semi erect to spreading herb, mainly cultivated as a kharif crop in tropical and subtropical countries. Throughout most of the urdbean cultivated part of Asia, disease are major constraints to yield. Most serious fungal disease is anthracnose caused by *C. lindemuthianum*. The initial symptoms appear as, brown blotches and leaf spots. Small, brown, circular spots appear on aerial part of the plant like leaves, stem and pods, at all growth stages of the plant, giving a target board appearance. Though pathogen was earlier considered to be of minor importance, but with the intensification of urdbean cultivation its severity has gradually increased.

History:

In 1875, the anthracnose of common bean was first identified in the fruit and vegetable garden of the Agricultural Institute of Popplesdorf, Germany by Lindemuth (Leach and Gibert, 1922).

In 1833, to describe a disease in grapes with symptoms possessing blackening of tissues caused by pathogen belonging to *Colletotrichum* species, term ‘Anthracnose’ was given by Fabra and Dunal, which literally means “coal like", (Kumar and Srivastava (1983).

The anthracnose was first time reported in India at Jorhat of Assam state in 1951 (Majid, 1953).

The infected leaves, stem and pods of green gram and black gram in different parts of the world have been found associated with the four species of *Colletotrichum* that (Saxena and Sinha, 1977).

Anthracnose is remarked as one of the most serious disease of urdbean throughout the world resulting maximum qualitative and quantitative losses (Gupta *et al*. 2007).

Wherever kharif pulses are cultivated including India, Nigeria, Thailand,

Philippines, Upper Volta, Zambia, Palmira and Columbia, there is infestation of anthracnose (Agarwal, 1991).

The number of infected pods per plant varied greatly, despite of *C. lindemuthianum* infection in nearly all plants in the study populations. In addition, infection was associated with a reduction of host fitness-related traits only in pods with sporulating lesions. The experimental data suggested that *C. lindemuthianum* may constitute a significant selection pressure in wild populations and impact of spore production on host fitness may influence virulence evolution in this fungus (Benard-Capelle *et al*. 2006).

Taxonomy:

Colletotrichum was originally described under the name *Vermicularia*, but later it was revised by Corda (1837) as *Colletotrichum,* which is being characterized by setose acervular fructifications from which hyaline, curved fusiform conidia were produced (Tode ,1790).

The classification of *Colletotrichum* in “Melanconiales” under “Coelomycetes” by (Hawksworth *et. al*., 1983) (The epithets *Colletotrichum* and *Vermicularia* were used indiscriminately during the 19th and early 20th centuries for a range of species, which are now classified in *Colletotrichum.* (Sutton, 1992).

The *Colletotrichum* was distinguished from *Vermicularia* by the presence of marginal setae as compared to the setae dispersed throughout the conidiomata in *Vermicularia* (Clements and Shear, 1931).

It was however had earlier demonstrated by Duke (1928) that the presence/ absence of setae, conidiomatal structure and form and their arrangement within the acervulus are extremely variable and of no taxonomic significance at the genus level. This results in transfer of large number of species from *Vermicularia* to *Colletotrichum* (Duke 1928, Cannon *et al*. 2012).

It was observed that distinguishing of *Colletotrichum* from *Gloeosporium* was tough, although *Gloeosporium* species did not produce setae, some could generate setae on certain substrates, which posed problems during identification because of morphologically similar anamorph (Baker *et al*. 1940).

In 1878, Saccardo and Magnus had made many observations on the cause of the anthracnose disease and recorded their results in *Michelia* 1:129. They concluded, it was caused by a fungus, which they named *Gloesporium lindemuthianum* (Stoneman and Bertha, 1898).

After several years, the discovery of presence of setae on the fungus by Briosi and Cavara (1889), helps in reclassification of genus *Gloeosporium* to *Colletotrichum*, where it remains today. Lately, *G. kaki* Hori has been transferred to *C. horii* (Weir and Johnston, 2010) and *G. pedemontanum* has been synonymized as *C. gloeosporioides* (Weir *et al*. 2012).

In 1911, the discovery of multiple fungal strains by Barrus, each of which differed in its ability to infect different varieties of bean plants, which started the work of Edgerton and Moreland, who found eleven different strains of the pathogen, but theorized more may exist. (Leach and Gilbert, 1922). Since then, numerous strains have been identified by use of the Greek alphabet, paired with numbers, but at the turn of the 21st century a naming system using binary code was adopted (Pynenburg and Martin, 2010).

Tode (1790) had interpreted the fructifications formed by *Vermicularia* as astomate ascocarps, an opinion upheld by Fries (1825), who validated the name. Saccardo (1884) doubted this interpretation and regarded the stroma-like fruiting bodies of *Vermicularia* as a further stage in the development of *Colletotrichum*. The matter had been further complicated by the description of *Gloeosporium* Desm. and Mont. in 1849 (Desmazieres and Montagne, 1849). Saccardo (1884) applied the name to. leaf-inhabiting fungi which were similar to *Colletotrichum* in most respects, except in their forming glabrous acervuli.

Arx (1957b) maintained that the type specimen of the genus had two-celled conidia and thus upheld its transfer to *Marssonia* by Saccardo (1884).

By 1957 the inseparability of *Colletotrichum* and *Gloeosporium* (Stoneman, 1898; Allescher, 1903; Shear & Wood, 1907; Taubenhaus, 1911; 1912; Diedicke, 1913; Schaffnit and Bohning, 1925; Small, 1926; Baker *et. al*. 1940; Tiffany and Gilman, 1954) and *Vermicularia* and *Colletotrichum* (Diedicke, 1913; Allescher, 1901; Wilson, 1914; Dickson, 1925; Duke, 1928) had been

firmly established. Many fungi, placed in *Gloeosporium* because they supposedly lacked setae, were found to produce setae on certain substrates (Allescher, 1903, Baker *et al*. 1940). In addition, the possibility was raised that the type species of *Vermicularia* and *Colletotrichum* had represented the same fungus (Duke, 1928). Some authors, however, continued to maintain the three genera separately (Grove, 1937).

In 1957, Arx (1957b) attempted to terminate the taxonomic uncertainty surrounding *Colletotrichum*. He concluded that many anamorphic fungi in the genera *Colletotrichum, Vermicularia and Gloeosporium* actually belonged in the same genus. Arx (1957a) accepted the decision that the name *Colletotrichum* Corda be conserved against *Vermicularia* Tode ex Fries (Duke, 1928) and he rejected the name *Gloeosporium* Desm. and Mont. on the grounds of its unacceptable heterogeneity. The generic concept of *Colletotrichum* was consequently broadened but the number of species was drastically reduced from several hundred to eleven, plus eleven host specific forms. The eight host specific forms of *Colletotrichum gloeosporioides* Penz. were left under their existing names on the grounds that they were distinct, if culture and had not yet been connected with the teleomorph of *C. gloeosporioides* with certainty. The name *C. gloeosporioides* , with nearly 600 synonyms, was selected to designate the extremely variable anamorph of *Glomerella cingulata* (Stonem.) Spauld. and Schr., not on the grounds of priority, but because it was the most widely used name which did not suggest a specific substrate.

Although Arx (l957a,b) drew attention to the variability of *Colletotrichum* species and their frequently wide host ranges and emphasized the necessity to avoid the unfounded description of new species, his work did not dispel the uncertainty complicating their identification.

Biology:

The isolation of *C. dematium f. sp. truncata* from infected mungbean plant formed dirty white to dark grey colonies with abundant sporulation on PDA, but colony of isolate from mungbean was found to be darker with leathery texture as compared to isolate from urdbean (Bharadwaj and Singh, 1986).

The description of morphological characteristics of *C. truncatum* as elongate to oval, hemispherical to truncate, conical and erumpent with black, needle like intermixed long and short setae, 60-300 x 3-8 µm acervulli on well developed stromata. Conidia formed were curved, unicellular, tapered and hyaline measuring 17-31 x 3-4.5 µm. (Sinclair and Backman, 1989).

The sexual form (teleomorph) of the pathogen was reported in the laboratory grown cultures 100 years ago. These cultures produced asci and perithecia, a typical characteristic of ascomycetes. Later named the perfect stage as *Glomerella lindemuthiana* (Shear and Wood, 1913).

The mating of two different isolates was done to rediscover of the perfect stage of the fungus, it was found that the ascospores were pathogenic to beans only, later they renamed it to *G. cingulata f. sp. phaseoli* (Kimati and Galli, 1970).

Study of morphological features of an isolate of *C. lindemuthianum* from greengram was done to conclude that mycelium was branched, septate, hyaline at first but then with age it became dark. The acervuli were saucer shaped, sub-cuticular and become erumpent. The conidia single celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed, appeared pink in mass, borne acrogenously on short conidiophores, which measures around 10 to 20 × 3 to 6 µm in size. The appressoria were sparse, pale to dark brown, clavate or circular in out line, regular and 8 × 6.7 µm in size (Quimio, 1975).

The spores of *C. lindemuthianum* were found to be dispersed by rain splash and gets quickly attach to the aerial parts of the plant in order to infect the host (Mercure *et. al*., 1994).

Morphological and cultural characteristics of 15 *Colletotrichum* isolates were studied, which belongs to *Colletotrichum capsici*, C. *dematium, C. falcatum [Glomerella tucumanensis], C. gloeosporioides [Glomerella cingulata] and C. lindemuthianum.* Data on the appressoria, setae, dimensions of the conidia and morpho-taxonomy of the different isolates were presented (Wijesekara and Agarwal, 2006).

The spore germinate on the new host and form a short germ tube which develops an appressorium, or ‘pressing organ’. As the germ tube grows, it pulls

the spore and the appressorium together, causing an indentation to occur in the cell wall. An infection peg is then able to protrude from the appressorium and penetrate through the cell wall. Once through the cell wall, an infection hypha grows and develops into an infection vesicle. (Leach and Gilbert, 1922 and Dean *et. al.*, 2012).

Conidiogenous cells of *Colletotrichum* spp. are usually aggregated in conidiomata but may form as side branches of the mycelium in culture in a hyphomycetous manner (Clausen, 1912; Schaffnit and Bohning, 1925; Arx, 1957a; Schmiedeknecht, 1957; Blakeman and Hornby, 1966; Stephan, 1967b; Hindorf and Muthappa, 1974). Setae and conidiogenous cells appear to be homologous (Negru, 1960). Seta formation is frequently variable and controlled by environmental factors (Schaffnit and Bohning, 1925; Frost, 1964; Stephan, 1967a; Chahal, 1978). The two types of acervulus development on host material (Arx, 1957a) are of no taxonomic significance, being dependant on both the strain of fungus and the nature of the substrate (Sutton, 1966).

Conidia collect in a mucilaginous matrix which is fibrillar (Griffiths and Campbell, 1972; Kozar and Netolitzk, 1978), composed of poly-saccharides and protein and IS water soluble (Nicholson and Moraes, 1980). The matrix possibly has two roles (Nicholson and Moraes, 1980), namely increasing germination efficiency, protecting conidia against desiccation and host penetration through the activity of the invertase and hydrolase.

Dispersal of conidia is brought about by water or occasionally insects (Arx, 1957a) and by wind, in dry particulate matter (Nicholson and Moraes, 1980). Germination takes place only in free water, or at a relative humidity approaching 100% (Riley, 1955; Frost, 1964; Nutman and Roberts, 1960). After germination aseptate, uninucleate conidia swell, their nuclei divide, septa form between the daughter nuclei and one cell, or both, produces a germ tube which may in tum produce an appressorium with an electron-dense outer wall (Politis and Wheeler, 1973). Appressoria may also be produced from hyphal tips in the mycelium (Sutton, 1968).

Conidia germinate on the host surface and the specialized penetration structure differentiates from germ tube known as appressorium. Melanized

appressorium penetrate the host surface directly by applying high turgor pressure to cell wall. From appressorium an infection peg emerges to form infection vesicles and primary hyphae (biotrophy). First biotrophic hyphae spread to a few adjacent cells, then the fungus produces secondary hyphae and switches to necrotrophy (O'Connell *et al.* 1985; Pastor-Corrales and Tu, 1989; Bailey and Jeger, 1992; Ishikawa *et al.* 2010).

Host resistance:

An experiment was conducted to evaluate 48 lines of urdbean against anthracnose, it was found that out of them 8 lines viz; T 65, UPU 79-2, UPU8 0-5-5, UG 201, PD 22, PDU 7, PDU 8 and PDU 10 were resistant (Kaushal and Singh, 1988).

Screening of six cultivars against anthracnose of urdbean *viz*. PU-94-1, TAU-1, PU-40, PU-38, PU-40, PU-30 and PU-31 and out of all genotypes PU-31 and PU-38 were found moderately resistant (Aggarwal *et al.* 2017).

Evaluation of 350 germplasm varieties against anthracnose of urdbean was done and it was found that 8 genotypes *viz;* T 65, UPU 79-2, UPU-80-5-5, UG 201, PDU 2, PDU 3, PDU 8 and PDU 10 were resistant (Kumar and Mukhopadhyay, 1987).

Screening of five varieties and thirty nine germplasms of Indian bean (*Lablab purpureus* L.) was done against anthracnose in *rabi* 2008 in south Gujarat conditions. Among all genotypes, three varieties Kapasi, JNP-4, Katargam and two germplasms NWP8 and NWP21 were resistant, while fifteen germplasms viz., NWP12, 19, 20, 22, 24, 25, 26, 27, 28, 29, 30, 32, 35, 37, 39 were found to be moderately resistant, whereas variety NPS1 was found highly susceptible (Deshmukh *et al*. 2012).

Widely grown 27 urdbean genotypes were evaluated against anthracnose. Only two varieties (T-9 and TPU-4) were resistant (Santosh *et al*. 2015).

An experiment was conducted at 6 locations of Rhodope Mountain to study about virulence diversity of causal agent of the bean anthracnose (*Colletotrichum lindemuthianum*). Virulence of the isolates was established after

inoculation of 10 day-old seedlings from 12 bean differential cultivars for that plants were placed in moist chamber for 72 hours in the greenhouse at 20-22/16-19 ^{o}C day/night. Maintained the same temprature after removing the chamber. Evaluation for disease reaction of the cultivars was done, 10 day after inoculation by following a 9-degree scale. Race 22 was the most frequent (45.8% of the isolates). This race was virulent to the cultivars 'MDRK', 'Perry Marrow' and Widusa'. Races 6 (26.5%) and 2 (17.6%), came next in frequency. The first race overcame the resistance of 'MDRK' and the 'Perry Marrow', and race 2 was virulent only on 'MDRK'. Race 54 was the least frequent (8.8%) (Kiryakov, 2009).

Two hundred sixty eight genotypes of urdbean was evaluated after screening them against anthracnose. It was found that two (T9 & TPU-4) genotypes were resistant, eight genotypes (SKNU, KU-09, UB-7, UB-10, UB-12, UB-13, UB-14 & UB-18) were moderately resistant (Kotgire *et al*. 2010).

After investigation of pathogenic potential of 9 isolates of *C. capsici* on chilli cultivars Sadabahar. The inoculated fruits of Hisar Vijay, Hisar Shakti and Kiran revealed variations about incubation period and lesion size. The isolates Cc-1, Cc-2, Cc-3, Cc-4, Cc-5 and Cc-7 exhibited resistant reaction on all cultivars, while Cc-9 showed susceptible reaction in all cultivars. Isolate Cc-8 induced susceptible reaction in Hisar Shakti and resistant reaction in Hisar Vijay, whereas, Cc-6 gave susceptible reaction in Hisar Vijay and resistant reaction in Hisar Shakti. (Vanan *et al.* 2005).

The leaves from 12 varieties of detached bean (*P. vulgaris*) was inoculated by *C. lindemuthianum* race 64, which was grown at two moisture levels in soil (oversaturated and field capacity) and the response of plants were recorded. It was found that the effect was stronger in Michelite, Cornell 49242, PI207262, To and Tu. Resistance of some varieties such as Michelite, Cornell 49242, PI207262, To and Tu developed in oversaturated soil and decreased as plant age increased (Otero and frias, 1996).

Screening of fifteen cluster bean cultivars was done to evaluate them against anthracnose caused by *Colletotrichum capsici* at different stages. It was reported that at flowering stage, one entry was immune, four entries were

resistant, nine entries were found moderately resistant and one entry was susceptible, while at pod formation stage eleven entries were found moderately resistant, two entries were susceptible and one entry were found to be resistant (Jat, 2017).

Screening of 240 genotypes of urdbean against anthracnose at two completely different agroclimatic locations *i.e.* Palampur and Dhaulakaun of Himachal Pradesh was conducted under natural epiphytotic conditions. It was found that 44 entries were resistant while 13 were moderately resistant (Sharma, 2011).

Symptomatology:

Colletotrichum, was described as one of the major plant pathogenic genus responsible to anthracnose, which cause plant disease on a variety of hosts from trees to grasses. (Dean *et al*. 2012).

The pathogen requires warm and humid climate to infect different host, including gymnosperms, angiosperms, ornamentals and fruit plants, vegetables, field crops or even grasses. Primary infection is disseminated by wind or rain, the pathogen is cosmopolitan in distribution (Farr *et al*. 2006).

The penetration and infection of tissues by *C. truncatum* produced lesions on lentil which caused tissue collapse and death of the upper portion of the plant which significantly reduced yield. Damage to soyabean tissue by by *C. truncatum* was associated with peg formation on plant surfaces. The extent and duration of favourable environmental factors influences appressorial development, conidial germination and infection peg penetration of the host tissues (Manandhar *et al*. 1985).

On different growing areas of common bean (*P. vulgaris)* in Himachal Pradesh (India), eighty five isolates of *C. lindemuthianum* were characterized on the basis of their reaction types on International and CIAT differentials. On international differentials, 12 races were characterized, specifically Alpha-Brazil, Beta, Gamma and Ind I to Ind IX. The races designated as Ind I to Ind IX were different from those identified in Europe and USA, thus forming a new race group from the Indian subcontinent. On the CIAT differential set the 85 isolates

were grouped into 19 races. Of these, only races 65 and 73 resembled the North American races. Exotic P. vulgaris accessions AB 136 and G 2333 were found resistant to all the Indian races (Sharma *et al*. 1999).

In *Vigna radiata* and *V. mungo*, reddish brown lesions was observed on leaves, stems, branches and pods as initial symptoms of anthracnose (Agarwal, 1991).

Symptoms produced by *C. lindemuthianum* on mungbean and urdbean as leaf spots and blotches (Bains *et al*. 1989).

The anthracnose and leaf spot symptoms was appeared as irregular brown spots in mungbean, which later turn to black. The disease was characterized by brown colour irregular spot on leaves, generally appear after 40-60 days of sowing and go up to pod formation stage (Devi, 2014).

Management:

1. Organic farm products:

Organic farming products emerging as a new eco-friendly input beneficial for both plant and environment. They are proving to the best way in preventing the spread of the disease without affecting the health of the plant as they do not have any residual effect.

The effect of different organic inputs was studied *viz;*. cow urine, fermented butter milk, panchgavya, vermiwash and cow milk on spore germination of *C. truncatum* and it was observed that maximum percent inhibition of spore germination was observed in cow urine (75.57%) followed by fermented buttermilk (64.59%), panchgavya (62.70%) and vermiwash (59.73%) while, least inhibition (56.10%) was recorded in cow milk (Kulkarni, 2009)

Efficacy of five organic and natural farming inputs viz; cow urine, panchgavya, vermiwash, biosol and buttermilk was studied against *C. capsici* causing anthracnose in bell pepper under both *in vitro* and *in vivo* at different concentration, They observed that under *in vitro* cow urine showed 100% mycelial growth inhibition @ 10 per cent conc., followed by fermented butter milk (98.22%), panchgavya (97.91%), vermiwash (97.40%) and biosol (93.91%).

They also observed that cow urine was the most effective against anthracnose with 74.59 per cent disease control followed by panchgavya (70.46%), biosol (68.94%), buttermilk (64.39%) and vermiwash (63.45%) under field conditions (Ashlesha and Paul, 2014).

Five neem-based products *viz*; Neemgold, Achook, Biotas, Tricure and Neemazel was studied for their efficacy against *C. capsici* under *in vitro* condition. It was reported that biotas was most effective among all the products, which completely inhibited the growth of *C. capsici* (Tiwari *et al.* 2008).

Efficiency of cow urine was studied at 5, 10, and 15 % concentration against *C*. *capsici* through poisoned food technique. It was reported that cow urine showed different inhibition at different concentration. It was recorded that >50 % inhibition was observed at 5 % concentration. In the end, they concluded that use of cow urine can be the cost effective and eco-friendly approach for controlling anthracnose in chilli. (Kambhar *et al.* 2013).

The efficiency of 3 neem-based formulations viz; Neemgold (300 ppm), Achook (1500 ppm) and Neem fighter (10000 ppm) *in vitro* was studied and it was found that Neemgold and Achook were successful in complete inhibition of *C. capsici* mycelial growth, while, neem fighter exhibited 75.6 percent growth inhibition as compared to control. (Rajput and Palakshappa, 2014).

The study of eleven organic products viz.; fresh sugarcane bagasse, neem cake, tea waste, cow urine, cow dung, banana peeled skin, vermicompost, compost, green manure (Dhaincha), wheat straw and poultry manure was done for screening against the test pathogen, which have been widely used as composts and as amendment in soil for management of soil borne diseases (Rekha and Dubey, 2014).

The antifungal efficacy of cow urine-based extracts from four plants *viz*; *Anacardium occidentale* (leaf and bark), *Pimeta dioica* (leaf and bark*), Alpinia galangal* (leaf and rhizome) and *Anisomeles indica* (leaf) against *C. capsica* was studied at 5 per cent concentration under *in vitro*. It was observed that all the preparations inhibited the mycelial growth of the fungus, among them highest inhibition (61.29%) was recorded in *P. dioica* (bark) and *A. galangal* (leaf)

followed by 32.25% in *A. occidentale* (leaf) and *A. indica* (leaf). Minimum percent mycelial inhibition (9.67%) was recorded in cow urine-based extract of *P. dioica* (Prashith *et al.* 2014).

2. Botanical extract:

The oil extract of *Azadirachta indica* (Neem) and *Xylopia ethiopica* was studied before and after infection of cowpea with *C. lindemuthianum.* It was found that the oil extract of *Azadirachta indica* (Neem) significantly reduced the spore germination and growth of *C. lindemuthianum in vitro* and were also effective in reducing size of the pathogen induced lesions (Amadioha and Obi, 2008).

Botanicals *viz;* Garlic (clove), Soap nut (fruit), Neem (leaves), Eucalyptus (leaves), Castor (fruit) and Onion (bulb) were tested for their antifungal efficacy at 10 per cent concentration against *C. gloeosporioides* causing leaf blight of Sarpagandha under *in vitro* condition. It was found that Garlic completely inhibited the mycelial growth of the test pathogen followed by Soap nut (81.11%), Neem (80.0%), Eucalyptus (74.44%), Castor (65.5%) and Onion (65.3%), after evaluating. (Gurav *et al.* 2013).

The extracts of Garlic (clove), Neem (leaf), Ginger (rhizome), Dhatura (leaf) and Mehandi (leaf) were evaluated against *C. lindemuthianum* in greengram at 10 per cent concentrations both under in vitro and field conditions. Results showed, Garlic exhibited highest growth inhibition (80.56%), followed by Neem (78.83%), Ginger (74.38%), Dhatura (70.91%) and Mehandi (64.60%) under in vitro studies, while, Neem showed minimum disease intensity 17 (23.57%) under field conditions followed by Garlic (26.86%), Ginger (28.85%), Dhatura (32.31%) and Mehandi (34.83%) as compared to 37.60 per cent in control, after evaluating (Choudhary *et al.* 2017).

In vitro evaluation of aqueous extracts were done at different concentrations against *C. truncatum* causing anthracnose of urdbean. It was found that at 100 per cent concentration bhang showed highest inhibition of 81.23 per cent followed by kali basuti (80.46%) and basuti (67.96%), however at the lowest test concentration (20%) safeda gave 35.14 per cent mycelial

inhibition followed by pine needles (19.51%) and barian (17.14%) (Pandit and Kaushal, 2011).

The efficacy of wild, cultivated and medicinal plants extracts against *C. capsici*, causing anthracnose of urdbean were investigated against *C. capsici*, causing anthracnose of urdbean under both *in vitro* and field. The leaf extract of neem (*Azadirachta indica*) showed significantly the highest reduction in mycelial growth of the pathogen. *Prosopis juliflora* and *Ocimum sanctum* were the next best treatments. In the field, all the tested plant extracts (neem, *P. juliflora*, garlic, *Ocimum sanctum*, *Datura metel* and *Tagetes erecta*) showed inhibitory effects against anthracnose disease of urdbean compared to the control. The leaf extracts of neem and *P. juliflora* recorded higher (59.9-63.0%) disease control efficacy compared to the control. The garlic extract also showed good (52.1%) efficacy in controlling disease severity (Mishra *et al.* 2011).

Antifungal efficacy of crude plant extracts *viz*; neem (*Azadirachta indica*), garlic (*Allium sativum*) and tagak-tagak (*Rhinocanthus nasuta*) at 5000 ppm were evaluated against chilli anthracnose. It was found that Garlic extract performed well under room humidity, while Tagak-tagak extract showed good control of under high moisture conditions and Neem extract minimized the ‘ripe-chilli fruit rot and was comparable with the fungicide carbendazim (Bavistin) at 100 ppm conc. and they reported them as an alternative commercial fungicides in the control of *Capsicum anthracnose* (Singh and Korpraditskul, 1999).

After evaluating the efficacy of neem extract, the percentage inhibition of neem extract (*A. indica*) was distributed in a decreasing order of potency among the seed, fruit, leaf, root and bark. Neem. It was found directly inhibitory to *Colletotrichum lindemuthianum* in laboratory tests and reported the antifungal efficacy of the extract increased with decreased concentration also fungal conidia were ruptured and completely prevented from germinating in 90 and 100 g/litre aqueous neem seed extracts (Onifade, 2000).

Efficacy of nine aqueous leaf extracts viz; neem, mehandi, nilgiri, bouganveilia, parthenium, garlic, onion, ginger and eucalyptus at 10 and 15 per cent concentration were tested against *C. truncatum* by poisoned food technique and It was reported that garlic gave maximum growth inhibition of

81.82 per cent followed by tulsi (65.17%), onion (60.31%), ginger (55.25%), neem (49.72%), parthenium (47.07%), bouganveilia (42.90%), eucalyptus (41.11%) and mehandi (40.36%) (Jagtap *et al*. 2014).

The inhibitory properties of leaf extracts of plants viz; Neem oil, Ashok tree, Tulsi and Bel were evaluated both *in vitro* and *in vivo* against *C. truncatum* and it was observed that Neem oil gave highest growth inhibition (46.2 %) followed by Ashok leaf extract (34.1%), Tulsi (31.0%) and Bel (26.7%). Under field conditions, Neem oil at 2% conc. gave 24.2 % disease severity followed by Ashok at 15% conc (24.8%), Tulsi at 5% conc. (26.0%) and Bel leaf extract at 5 % conc. (29.8%) as compared to control (34.1%) (Meena *et al.* 2017).

The aqueous and acetone leaf extracts of five botanicals were evaluated *in vitro* for their antifungal activity against the pathogenic fungi of cotton *Colletotichum gossypii* using poisoned food technique. It was observed that among five botanicals aqueous and acetone leaf extracts of *Catharanthus roseus* (*Vinca rosea*) was most promising followed by *Eucalyptus globulus, Lantana camera*, *Azadirachta indica* and *Withania* (Kanherkar, 2013).

MATERIAL AND METHODS

Chapter-III

MATERIAL AND METHODS

Sustainable cultivation of black gram [*Vigna mungo* (L.) Hepper] is continuously challenged by diseases that cause both quantitative and qualitative losses in yield. Out of the major diseases of urdbean, anthracnose is the most prominent one, which infest the crop particularly under cool and humid environmental conditions and cause 80 to 100 per cent yield losses. The yield losses caused by black gram are proportional to the disease severity and vary remarkably depending upon the stage of infection, genotypes and environmental conditions.

The experiments were carried out in the laboratory and field of Department of Plant Pathology, College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) and details of methodologies followed during the course are described as follows.

3.1. TECHNICAL PROGRAMME OF WORK

3.1.1. Location:

The field experiment was conducted at the experimental field of Department of Plant Pathology, College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidhyalaya, Gwalior (M.P) during the *kharif* 2021 and lab work was carried out at Plant Pathology Lab, Department of Plant Pathology, RVSKVV, Gwalior. The Gwalior is situated at 260.130 N Latitude and 780.130 E longitudes and altitude 211.52 m at above the mean sea level. This region has subtropical, semi- arid climate with hot and dry summers and cold winters with occasional showers. The soil is sandy loam, low in available nitrogen, medium in phosphorous and high in potash with pH of 8.5.

3.2. Materials

3.2.1. Glassware:

Borosil® make glassware was used throughout the experimental study. Glassware was cleaned with detergent and finally rinsed with the tap water.

After drying glassware was sterilized in hot air oven at 180^{o} C for 2 hours. The metallic equipments like foreceps, needle and cork borer were sterilized by dipping in alcohol and heating to red hot on the flame of a spirit lamp. Surface sterilization of plant parts and diseased materials were done by dipping them in 0.1% $HgCl_2$ solution for 30 seconds and washing in sterilized water 3 times. The culture media was sterilized in autoclave at 121.6^{o} C and 15 lbs pressure per square inch (1.05 kg/ cm^{2}) for 20 minutes.

3.2.2. Culture Media:

The ingredients of the media used during the course of investigation are as follows:

Medium	Ingredients	Quantity
Potato dextrose agar	Peeled and sliced potato	200.0 g
	Dextrose	20.0 g
	Agar-agar	20.0 g
	Distilled water	1000 ml

3.2.3. Botanicals:

During the course of investigation six botanicals were evaluated against test pathogen for their fungicidal properties which were collected locally and from the vicinity of the college of Agriculture campus. The evaluated botanical are given in the Table. 1 (List).

3.2.4. Organic farm products:

During the course of investigation six farm products viz; Jivamrut, Bijamrut, Neemastra, Buttermilk, Vermicompost, FYM were evaluated against test pathogen.

Table 1 : List of botanicals

S.No.	Botanical Name	Plant Part	Per cent/ 100 ml	Common Name	Family	Constituents
1.	*Tagetus erecta*	Flower	20	Marigold	Astereacea	Essential oils, Thiophenes, Flavanoids, Carotenoids, Phenolic compounds
2.	*Cymbopogon citratus*	Leaves	20	Lemon grass	Gramineae	Terpenes, Alcohols, Ketones, Aldehydes, Esters, Essential oils, Citral-α, Citral-β, Citranellal, Terpinolene, Myrecene, Flavonoids
3.	*Allium sativum*	Cloves	20	Garlic	Amaryallidaceae	E-ajoene, Z-ajonene, Thiosulfinates (allicin), Vinyldithins, Diallyl disulphide, Diallyl tri/ poly sulphides, Allyl propyl disulphides, Diallyl dipropyl disulphides
4.	*Allium cepa*	Bulbs	20	Onion	Amaryallidaceae	Allicin, Quercetin, Allyl propyl disulphide, calcium, phosphorus, Protein, Vitamin C, Fisetin, Diallyl disulphide, Diallyl tri/ poly sulphides, Ascorbic acid
5.	*Aloe barbadensis*	Leaves	20	Aloe vera	Liliaceae	Vitamin, Mineral, Enzymes, Sugars, Lignin, Saponins, Salicylic acids, Amino acids, Amino acids, Vitamin A (β-carotene), C, E, B12, Folic acid, Choline
6.	*Parthenium hysterophorus*	Leaves	20	Parthenium	Astereaceae	Caffeic acid, Vanillic acid, Ferulic acid, Chologenic acid, Neochlorogenic acid, Anisic acid, P-coumaric acid, Protocatechoic acid, P- hydroxybenzoic acid

3.2.4.1.Preparation method of Indigenous Technical Knowledge (ITKs):

Indigenous Technical Knowledge (ITK) is the actual knowledge of a given population that reflects the experiences based on tradition and includes more recent experiences with modern technologies. It is held in different brains, languages and skills in as many groups, cultures and environments as are available today (Atte, 1989). Hence, there is immense pressure on the people of India to collect, preserve, validate and adopt IAPs so as to reduce dependence on external inputs, to reduce the cost of cultivation and to promote eco-friendly agriculture (Sundramari and Ranganathan, 2003).

1. Bijamrut:

Bijamrut solution was prepared by using locally available ingredients as listed in Table 2. 5 Kg of local cow dung was taken in a cloth and bound by tape and was submerged in 20 liters of water for 12 hrs. Simultaneously, 50 g of slaked lime was dissolved in 1 liters of water in separate container and kept stable for overnight. After 12 hrs, this bundle of cow dung was squeezed thrice, thereby: all the essence of cow dung will be drawn to water phase (cow dung extract). 50 g of soil was dissolved in cow dung extract by stirring it well. To this, 5 liters of wild cow urine and lime water was added and mixed well (Palekar, 2007).

Table 2 : Ingredients used for preparation of Bijamrut

Ingredients	Quantity
Water	20 L
Cow dung	5 kg
Cow urine	5 L
Lime	50 gm
Soil from bund	Handful

2. Jivamrut:

Take 200 L water in barrel, take 10 kg local cow dung (Indian breed) and 5 – 10 litre cow urine (Gomutra) and add it in water. Add 1 kg jaggery (gud), 1 kg gram flour and handful soil under Banyan tree in barrel. Then stir the solution well and need to keep it for 48 hours in the shadow. The mixture needs to be

stirred couples of times for minimum 10 minutes. It gets fermented. After 48 hours Jiwamrut is ready to use. It can be used for 7 days (Kumar *et. al*, 2021). Ingredients are listed in Table 3.

Table 3 : Ingredients used for preparation of Jivamrut

Ingredients	Quantity
Fresh cow dung	10 kg
Water	200 L
Cow urine	5-10 L
Gram flour	1 kg
Soil under Banyan tree	Handful
Jaggery	1 kg

3. Neemastra :

According to NCOF, crush neem leaves in 50 litres of water properly. In a plastic drum or earthen pot mix this crushed neem leaves and water in cow dung and urine.

Leave this solution under shade for 24 hours for fermentation. In the meanwhile stir the solution 5 to 6 times in a day with the help of a wooden stick. During winters, keep this solution for 48 hours for fermentation.

After 24 hours filter this solution with the help of a cotton cloth. Dilute this filtered solution in 100 litres of water and now you can use it on your plants. You can use this solution for one acre of farmland (Anon., 2021). Ingredients are listed in Table 4.

Table 4 : Ingredients used for preparation of Neemastra

Ingredients	Quantity
Neem leaves	5 kg
Cow dung (fresh)	2 kg
Cow urine (fresh)	5 L
Water	50+100 L

Plate 1 : Ingredients used for preparation of Bijamrut

Plate 2 : Ingredients used for preparation of Jivamrut

Plate 3 : Ingredients used for preparation of Neemastra.

3.2.5. Seeds:

Seeds of urdbean genotypes were evaluated against anthracnose for their reaction, The seeds were obtained from Department of Plant Pathology, College of Agriculture, Gwalior (M.P.). The list of evaluated urdbean genotypes is given in Table 5.

Table 5 : List of urdbean genotypes

S.No.	Genotypes	S.No.	Genotypes
1.	Azad – 3	11.	IPU 172
2.	VBG 12-034	12.	SBC 50
3.	KPU 520-69	13.	KU 17-08
4.	AKU 1608	14.	PU 1541
5.	PU 31	15.	TPU 4
6.	LBG 752	16.	Uttara
7.	PU 14-19	17.	KU 96-3
8.	TBG 125	18.	TU 94-2
9.	NGU 24	19.	PU 1617

3.2.6. Test organism:

Colletotrichum lindemuthianum is the causal organism of anthracnose of urdbean, which was isolated from the infected leaves of urdbean, collected during experimentation.

3.3. METHODS

3.3.1. Symptomatology:

The urdbean plants grown on experimental field were regularly observed for their symptoms. The symptoms were then recorded and described.

3.3.2. Isolation, purification and identification:

The infected leaves and pods of urdbean showing typical symptoms of anthracnose were collected from experimental field, which were properly marked, packed in the polybags and brought to the laboratory and washed

thoroughly with distilled water. These leaves and pods were dried by placing them in between sterilized blotting paper. Small bits of 5 mm were cut from the sharp sterilized blade from the intermittent zone of healthy and diseased tissue under laminar air flow. These bits were surface sterilized with 0.1 per cent of sodium hypochlorite solution for 30 seconds and subsequently washed thrice with sterile distilled water to remove the traces of disinfectant and placed in between sterilized blotting paper. The surface sterilized bits were then transferred to the PDA (Potato dextrose agar) medium with the help of inoculation needle under aseptic conditions. The inoculated Petri plates were then incubated in the BOD incubator at 25±2 °C temperature for three to four days.

The pure culture of the isolate was obtained by using hyphal tip techniques (Choi *et al*. 1999) from the actively growning mycelium in Petri plate then the fungus were transferred on the PDA slants in culture tubes by sub culturing. The purified fungus were then maintained on agar slants culture tubes by placing them in the refrigerator for further studies. The cultural characters of the isolated fungus were examined and identified on the basis of detailed morphological and reproductive characters (Barnett and Hunter, 1972).

3.3.3. Screening of urdbean genotypes against *C. lindemuthianum* to find out the resistant source of anthracnose:

Twenty genotypes of urdbean were evaluated in the kharif 2021 against anthracnose of blackgram caused by *Colletotrichum lindemuthianum* in the field. Cultivars were sown with row to row and plant to plant spacing of 30 and 10 cm respectively. The experiment was conducted in randomized block design with two replications with ferlilizer dose of Nitrogen 20 Kg/ha and P 50 Kg/ha. The disease severity of anthracnose on genotypes were recorded at 70 days after planting.

The data on the disease severity were recorded and calculated as follows:

$$\text{Disease Severity} = \frac{\text{Sum of individual rating} \times 100}{\text{Number of leaf observed x Maximum rating value}}$$

The incidence of disease severity were recorded with the help of 0-9 disease

rating scale (Mayee and Datar, 1986), which are as follows:

Table 6 : Disease severity rating scale

Grade	Per cent Disease Severity	Reaction
0	No infection	Highly Resistant
1	0.1 – 1.00	Resistant
3	1.1 – 10.00	Moderately Resistant
5	10.1 – 25.00	Moderately Susceptible
7	25.1 – 50.00	Susceptible
9	More than 50	Highly Susceptible

3.3.4. *In vitro* evaluation of organic farm products against *C. lindemuthianum* :

The six organic farming input *viz*; Jivamrut, Bijamrut, Neemastra, Buttermilk, Vermicompost and FYM were evaluated @ 20 % concentration by poisoned food technique (Nene and Thapliyal, 1979) under aseptic condition. The organic farm products were procured from Department of Plant Pathology, College of Agriculture, Gwalior. The procured material were then filtered through double layer muslin cloth and the filtrate obtained was used as 100 per cent stock solution. These solutions were sterilized in conical flask at 121 °C and 15 lb pressure per square inch in autoclave for 20 minutes. The required quantity of organic farming inputs was added to the melted PDA medium separately in a conical flask thereby making it 20 per cent concentration, mixed thoroughly and poured into sterilized Petri plates. After solidification of the mixed media, the 7 mm disk of 7 days old actively growing culture of *C. lindemuthianum* were placed in Petri plates under aseptic condition. These Petri plates were then transferred in BOD incubator incubated at 25±2 °C temperature. The data was observed at 3rd, 5th and 7th day after inoculation. The experiment was carried in completely randomized design (CRD) with 3 replications. The inhibition percentage was calculated by comparing the measurement of the mycelial

growth on farm products with PDA mixed plates with the control (only PDA without farm products) plates.

The formula for calculating the per cent growth inhibition of the test pathogen was given by Vincent (1947), which are given as under:

$$PGI = \frac{C-T}{C} X\ 100$$

Where,

PGI = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.3.5. *In vitro* evaluation of botanicals against *C. lindemuthianum* :

The Plants extracts from five locally available plant *viz*; *Tagetes erecta, Cymbopogan citratus, Alium sativum, Alium cepa, Aloe barbadensis* and *Parthenium hysterophorus* were evaluated against test pathogen @ 20 % concentration by poisoned food technique (Nene and Thapliyal, 1979) under *in vitro* conditions to know their antagonistic effect on the growth of *C. lindemuthianum*. The phytoextracts along with their constituents are given given in Table 1.

The experiment was conducted in completely randomized design with 3 replications. Collected leaves of each plant were washed thoroughly with water to remove dirt and dried between sterilized blotting paper in room. Properly dried leaves were grinded in blender to obtain fine powder and stored in clean plastic bag. Hundred gram of grinded fine powder mixed with 100 ml of distilled water thereby making 1:1 ratio of 100 % stock solution. The stock solution were then filtered through double layer muslin cloth in 100 ml conical flask. These solution were sterilized in autoclave at at 121 °C and 15 lb pressure per square inch in autoclave for 20 minutes. The 80 ml of melted PDA was added to the 20 ml of sterilized phytoextract separately in a conical flask thereby making it 20 per cent concentration. These were mixed thoroughly and poured into sterilized Petri plates. After solidification of the mixed media, the 5 mm disk of 7 days old actively growing culture of *C. lindemuthianum* was placed in Petri plates under

aseptic condition. These Petri plates were then transferred to BOD incubator and incubated at 25±2 °C temperature. The data was observed at 3rd, 5th and 7th day after inoculation by measuring the mycelial growth of the pathogen. The inhibition percentage was calculated by comparing the measurement of the mycelial growth in phytoextract mixed plates to the control (only PDA without phytoextract) plates.

3.3.6. *In vivo* evaluation of selected organic farm products and botanicals for eco-friendly management of urdbean anthracnose.

Seven selected botanicals and organic farm products *viz*., *Tagetus erecta, Aloe barbadensis, Alium sativum, Alium cepa*, Jivamrut, Bijamrut and Neemastra, found effective for their efficacy against *C. lindemuthianum* under *in vitro* condition were evaluated for the management of anthracnose of urdbean under field condition.

The seeds of the susceptible cultivar Azad-3 were sown in the field. The size of the experimental plot was 3 x 0.9 m^2 with row to row and plant to plant spacing of 30 and 10 cm respectively. The fertilizer dose @ N:P:K 30:60:30 were applied in the form of urea, SSP and MOP in furrows as basal dressing at the time of sowing. The experiment was carried out with 8 treatments (including control) having three replications in randomized block design during kharif 2021 by randomly tagging 8 plants with treatment. Plants were then artificially inoculated with mycelial suspension of *C. lindemuthianum* after 30 days of sowing. The data on the disease severity was recorded after 15 days of spraying on the 8 randomly tagged plants.

RESULTS

Chapter-IV

RESULTS

Urdbean [*Vigna mungo* (L.) Hepper] is an annual, semi-erect to spreading crop. Mostly grown as a kharif crop in subtropical and tropical countries. Production of urdbean is largely impaired by the wide range of diseases. Anthracnose is one of the serious disease of urdbean caused considerable yield damage upto 80 to 100 per cent.

The present investigation on **Studies on Anthracnose of Urdbean caused by Colletotrichum lindemuthianum (Sacc. and Magn.) and it's eco-friendly management** consist of isolation, purification and identification of *C. lindemuthianum*. Screening of urdbean genotypes against anthracnose. Evaluation of botanicals and organic farm products against *C. lindemuthianum,* causing anthracnose of urdbean both *in vitro* and *in vivo* condition during Kharif 2021. The results obtained on various aspects are presented and discussed as follows.

4.1. Isolation, purification and identification of the pathogen

4.1.1. Isolation and purification of the pathogen:

The leaves, pods and stem exhibiting typical symptom of anthracnose were collected from the experimental field in kharif crop season 2020-21 and brought to the laboratory. The infected region was cut taking healthy part of the plant. The diseased plant parts were washed in running tap water to remove the attached dirt. The infected plant parts were cut (20-30 mm) into small pieces with sterilized scalpel. These infected pieces with healthy part were surface sterilized with $HgCl_2$ (0.1%) for 30 seconds. After that these pieces were washed with sterilized distil water 3 times to eliminate traces of $HgCl_2$. These sterilized pieces were transferred in between sterilized filter paper and then to PDA media in Petri plate. These inoculated plates were incubated at 25±2°C and examined regularly to see the growth of the fungus from different pieces and repeated culturing of the isolates (*C. lindemuthianum*) was done until clear growth was observed. Once the clear growth of the mycelium was observed anthracnose fungus was further sub cultured with the help of fungal hyphal tip

(A)

(B)

(C)

Plate 4 : Anthracnose of urdbean on (A) Leaves (B) Pods (C) Stem

method and transferred into new Petri plates containing PDA aseptically every 7 days interval. The pure culture was maintained at 25±2°C under BOD incubator for further studies.

4.1.2. Identification of the pathogen:

The pathogen was identified with the help of compound microscope. It produced hyaline, single celled, oblong, sickle shaped, sometimes with one end slightly pointed spores. The length of conidia ranged between 10.5 – 15.5 µm and with a breadth of 3.5 – 4.5 µm, it was produced from conidiophores which was arranged in acerculi. The acervuli were numerous, rounded with numerous setae and 60 – 270 µm across. These appear as brownish white, uniform, circular, fluffy growth in center with concentric rings and appressed growth at periphery on potato dextrose agar (PDA) media. The cultural characters of the isolated fungus were examined and identified on the basis of detailed morphological and reproductive characters (Barnett and Hunter, 1972).

4.1.3. Symptomatology:

The symptoms of anthacnose on urdbean were observed from appearance of disease to harvest of the crop by regularly visiting the experimental field of Department of Plant Pathology, COA Gwalior. It was found that the fungus attacks all the aerial parts plant parts at any stage of the plant growth but the leaves and pods are more vulnerable to infection.

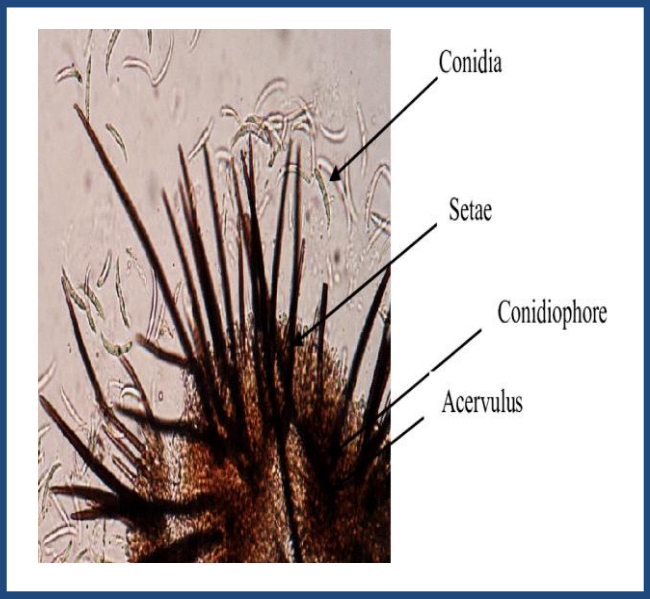
Symptoms appear as regular or irregular dull violet or black sunken spots with dark centers and bright red orange margins on leaves, mostly adjacent to veins and pods. In severe infections, the affected parts wither off. Infection soon after germination of seeds often causes blightening of the seedlings.

On the seedling cotyledons show small, brown to black sunken spots. In wet season these spots may bear pink spore masses of the fungus. Later, the young plant suffer from necrosis of veins and adjoining tissues.

On the pods the spots appear as black sunken cankers with gray central area. The central portion of these spots show pinkish mass, especially in wet weather. Later, the side of these spots appear raised. Seedlings raised from

(A)

(B)



(C)

(D)

Plate 5 : (A) Culture of *C. lindemuthianum* (B) Mycelium (C) Acervuli (D) Conidia

diseased pods may show typical lesions on the cotyledons. The spores are almost entirely water borne. Primary leaves and hypocotyl are faci of secondary infection. Spattering rains associated with wind currents are the main source for the local dissemination of the pathogen.

On adult plant stem, the spots are eye shaped and longitudinal along the stem.

4.2. Screening of urdbean genotypes against *C. lindemuthianum* to find out the resistant source of anthracnose:

Twenty genotypes of urdbean were evaluated against anthacnose caused by *C. lindemuthianum* under field condition to find out the resistance source of anthracnose. The data presented in Table 7 screened in Kharif 2021.

Out of the screened 20 cultivars two cultivars *viz*; PU 1617 and PU 1514 were found moderately resistant. Three cultivars *viz*; VBG 12-034, KPU 520-69 and KU 96-3 were found moderately susceptible. Thirteen cultivars *viz*; Azad – 3, PU 31, LBG 752, PU 14-19, TBG 125, NGU 24, MU 52, IPU 172, KU 17-08, TPU 4, Uttara, TU 94-2 and T-9 were found susceptible and 2 cultivars namely; AKU 1608 and SBC 50 showed highly susceptible reaction.

Table 7 : **Field evaluation of urdbean cultivars against anthracnose under field condition.**

Genotypes	Scale grade	Per cent disease severity	Disease Reaction
Azad – 3	7	43.20 (41.07)	Susceptible
VBG 12-034	5	24.61 (29.73)	Moderately Susceptible
KPU 520-69	5	22.42 (28.24)	Moderately Susceptible
AKU 1608	9	57.65 (49.38)	Highly Susceptible
PU 31	7	34.27 (35.82)	Susceptible
LBG 752	7	31.80 (34.31)	Susceptible
PU 14-19	7	31.62 (34.20	Susceptible
TBG 125	7	33.91 (35.60)	Susceptible
NGU 24	7	35.41 (36.50)	Susceptible
MU 52	7	28.28 (32.12)	Susceptible
IPU 172	7	31.57 (34.17)	Susceptible
SBC 50	9	51.76 (45.99)	Highly Susceptible
KU 17-08	7	27.59 (31.67)	Susceptible
PU 1514	3	9.97 (18.38)	Moderately Resistant
TPU 4	7	28.93 (32.52)	Susceptible
Uttara	7	29.76 (33.04)	Susceptible
KU 96-3	5	17.38 (24.62)	Moderately Susceptible
TU 94-2	7	29.00 (32.57)	Susceptible
PU 1617	3	5.09 (12.97)	Moderately Resistant
T – 9	7	28.86 (32.48)	Susceptible
SEm.±		0.62	
C.D. at 5 %		1.84	

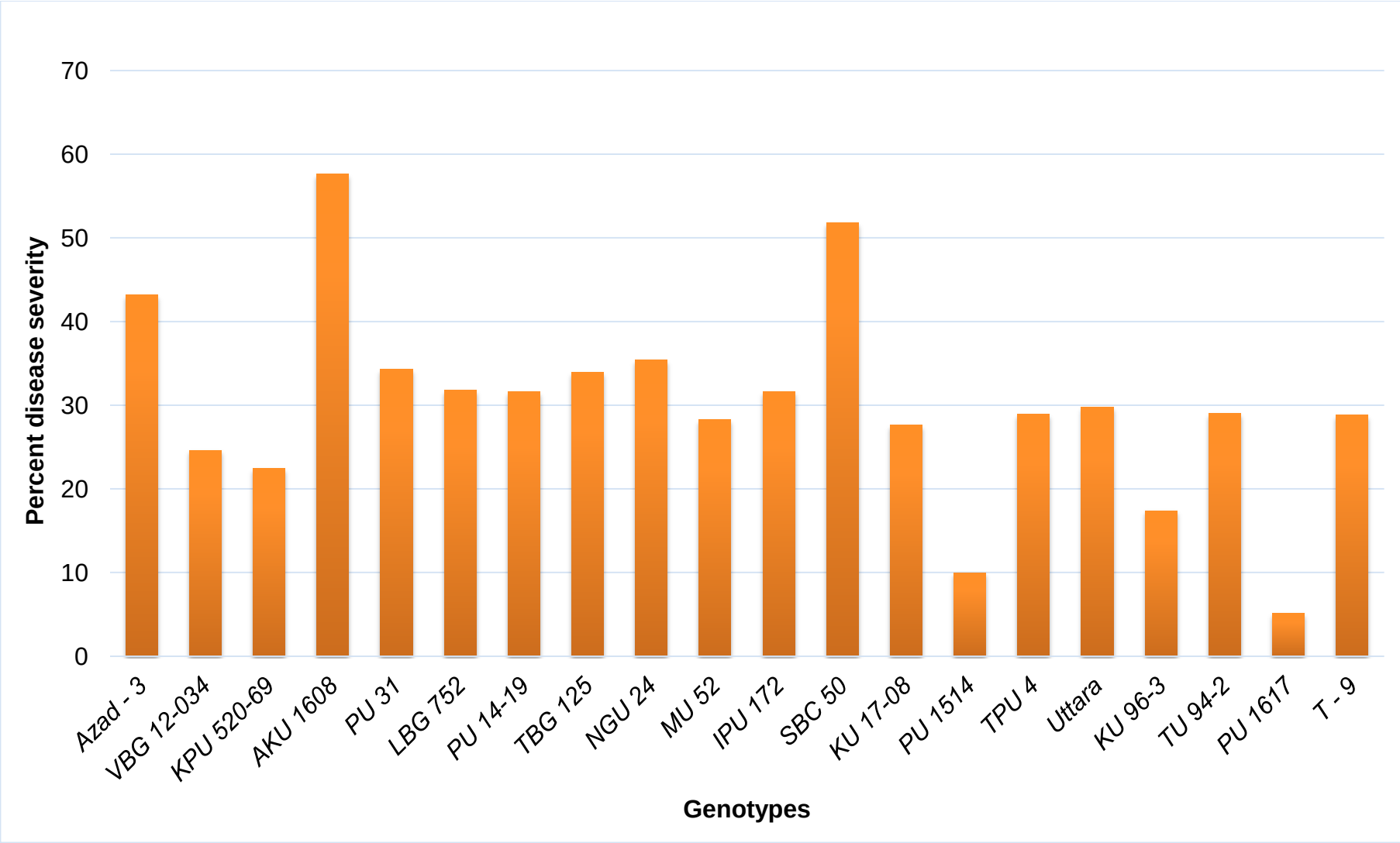


Fig. 1 : Field evaluation of urdbean cultivars against anthracnose under field condition.

Plate 6 : Screening of urdbean genotypes against anthracnose.

4.3. *In vitro* evaluation of organic farm products against *C. lindemuthianum.*

The experiment was conducted in the lab of Department of Plant Pathology, College of Agriculture, Gwalior to evaluate the inhibitory action of organic farm products against anthracnose disease of urdbean caused by *C. lindemuthianum* by poisoned food technique.

The Six organic farm products those who were tested for their efficacy *viz*; Jivamrut, Bijamrut, Neemastra, Buttermilk, Vermicompost and FYM at 20 % concentration in completely randomized design (CRD) having 3 replications. The data presented in table 8 summarized after the mean colony diameter recorded in all 7 treatments at 3^{rd}, 5^{th} and 7^{th} day of inoculation.

The mean colony diameter of the pathogen at 3 days after inoculation varied from 10.20 mm to 28.20 mm as compared to control (30.30 mm). Neemastra was found was most effective and significantly superior to other organic farm products with mean colony diameter of 10.20 mm and highest per cent inhibition of 66.34 % followed by Jivamrut (48.18%), Bijamrut (38.71%), Fermented butter milk (26.37%) and Vermicompost (13.63%), while minimum per cent inhibition was observed in FYM (6.93%).

The mean colony diameter of the pathogen at 5 days after inoculation varied from 15.70 mm to 46.43 mm as compared to control (51.03 mm). Neemastra was found was most effective and significantly superior to other organic farm products with mean colony diameter of 15.70 mm and highest per cent inhibition of 69.23% followed by Jivamrut (41.80%), Bijamrut (33.24%), Fermented butter milk (21.22%) and Vermicompost (13.25%), while minimum per cent inhibition was observed in FYM (9.01%).

The mean colony diameter of the pathogen at 7 days after inoculation varied from 22.23 mm to 71.97 mm as compared to control (76.50 mm). Neemastra was found was most effective and significantly superior to other organic farm products with mean colony diameter of 22.23 mm and highest per cent inhibition of 70.94% followed by Jivamrut (33.16%), Bijamrut (23.18%), Fermented butter milk (18.52%) and Vermicompost (13.50%), while minimum per cent inhibition was observed in FYM (5.92%).

Table 8 : *In-vitro evaluation* of organic farm products for the management of *C. lindemuthianum.*

Organic farm products (20 %)	3 DAI		5 DAI		7 DAI	
	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control
Jivamrut	15.70	48.18	29.70	41.80	51.13	33.16
Bijamrut	18.57	38.71	34.07	33.24	58.77	23.18
Neemastra	10.20	66.34	15.70	69.23	22.23	70.94
Fermented butter milk	22.31	26.37	40.20	21.22	62.33	18.52
Vermicompost	26.17	13.63	44.27	13.25	66.17	13.50
FYM	28.20	6.93	46.43	9.01	71.97	5.92
Control	30.30	0.00	51.03	0.00	76.50	0.00
SEm.±	0.77		0.90		0.75	
C.D. at 5 %	2.35		2.75		2.29	

DAI = Days after inoculation

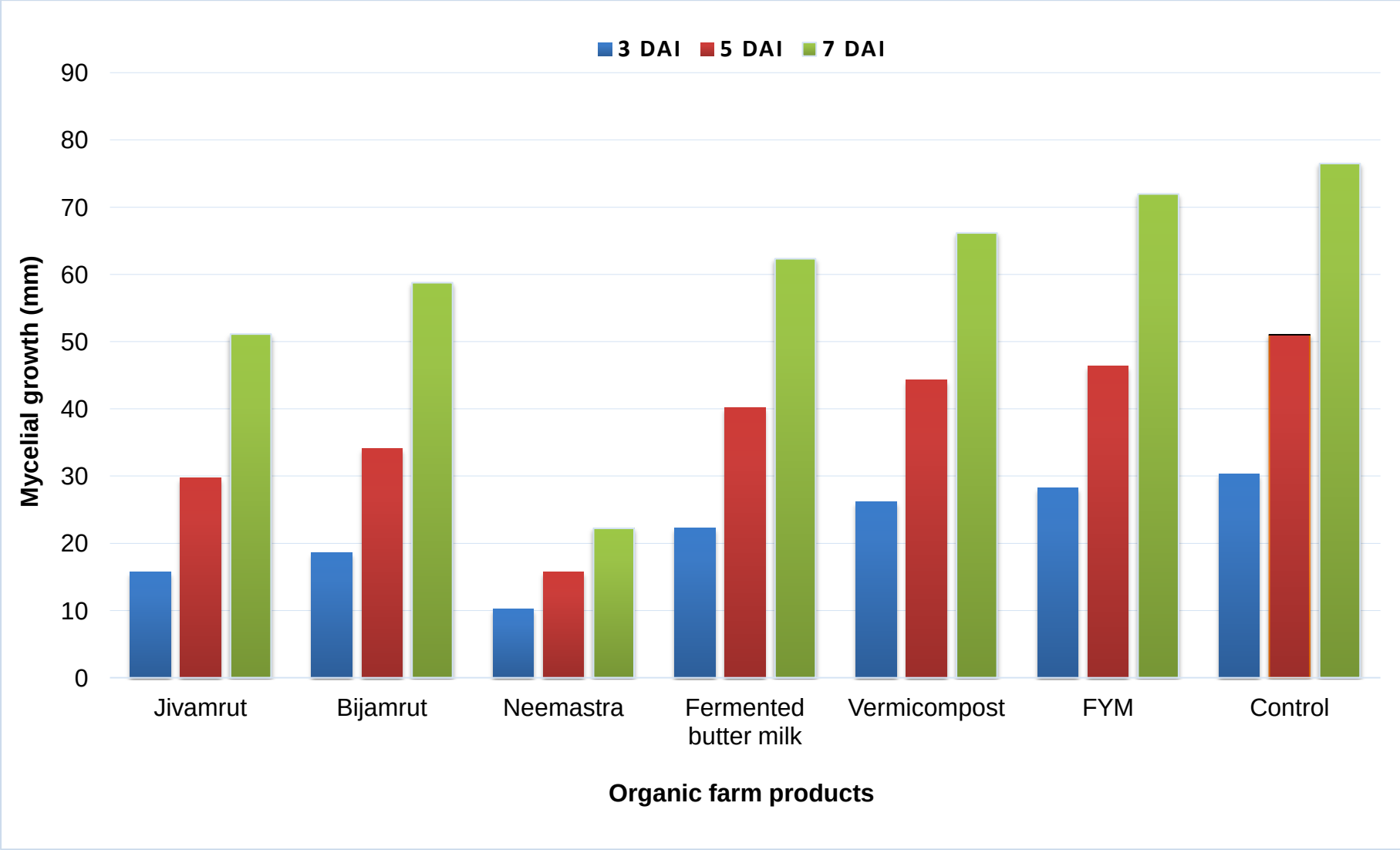
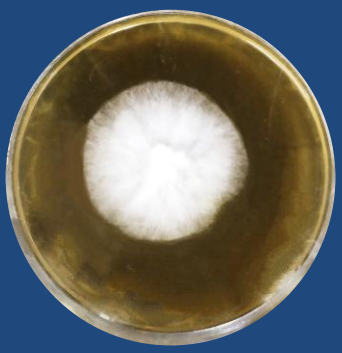
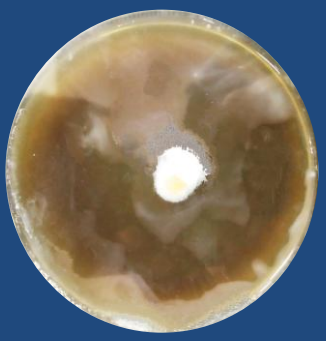


Fig. 2 : *In-vitro* evaluation of organic farm products for the management of *C. lindemuthianum*

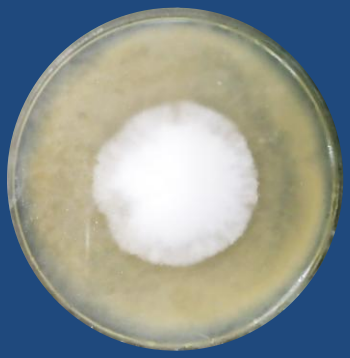
Jivamrut



Bijamrut



Neemastra



Fermented butter milk

Vermicompost

FYM

Control

Plate 7 : Evaluation of organic farm products (20 %) against *C. lindemuthianum*.

4.4. ***In vitro*** **evaluation of botanicals against *C. lindemuthianum*:**.

In vitro evaluation of the six botanicals to check their inhibitory properties over *C. lindemuthianum* was conducted at the laboratory of Department of Plant Pathology, College of Agriculture, Gwalior. The botanicals opted *viz*; *Tagetes erecta, Cymbopogan citratus, Alium sativum, Alium cepa, Aloe barbadensis* and *Parthenium hysterophorus* were evaluated at 20 % concentration by poisoned food technique in completely randomized design (CRD) having three replications. The data presented in table 9 summarized after the mean colony diameter recorded in all 7 treatments at 3rd, 5th and 7th day of inoculation.

The radial growth of the test pathogen at 3 days after inoculation varied from 10.40 mm to 28.77 as compared to untreated control (32.10 mm). The maximum radial growth of *C. lindemuthianum* was inhibited by *Tagetes erecta* (10.40 mm) and found to be significantly superior over other treatments with maximum per cent inhibition (67.60%) followed by *Aloe barbadensis* (40.50%), *Alium sativum* (33.24%), *Alium cepa* (23.89%) and *Cymbopogan citratus* (19.71%), while minimum per cent inhibition was observed in *Parthenium hysterophorus* (10.37%).

The radial growth of the test pathogen at 5 days after Inoculation varied from 25.07 mm to 59.93 mm as compared to untreated control (63.67 mm). The maximum radial growth of *C. lindemuthianum* was inhibited by *Tagetes erecta* (25.07 mm) and found to be significantly superior over other treatments with maximum per cent inhibition (60.63%) followed by *Aloe barbadensis* (33.36%), *Alium sativum* (24.45%), *Alium cepa* (17.75%) and *Cymbopogan citratus* (11.89%), while minimum per cent inhibition was observed in *Parthenium hysterophorus* (5.87%).

The radial growth of the test pathogen at 7 days after inoculation varied from 35.53 mm to 81.40 mm as compared to untreated control (86.63 mm). The maximum radial growth of *C. lindemuthianum* was inhibited by *Tagetes erecta* (35.53 mm) and found to be significantly superior over other treatments with maximum per cent inhibition (58.99%) followed by *Aloe barbadensis* (27.24%), *Alium sativum* (19.58%), *Alium cepa* (15.54%) and *Cymbopogan citratus* (10.42%), while minimum per cent inhibition was observed in *Parthenium hysterophorus* (6.04%).

Table 9 : *In-vitro* evaluation of botanicals for the management of *C. lindemuthianum*.

Botanicals (20 %)	3 DAI		5 DAI		7 DAI	
	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control
Tagetus erecta	10.40	67.60	25.07	60.63	35.53	58.99
Cymbopogan citratus	25.77	19.71	56.10	11.89	77.60	10.42
Alium sativum	21.43	33.24	48.10	24.45	69.67	19.58
Alium cepa	24.43	23.89	52.37	17.75	73.17	15.54
Aloe barbadensis	19.10	40.50	42.43	33.36	63.03	27.24
Parthenium hysterophorus	28.77	10.37	59.93	5.87	81.40	6.04
Control	32.10	0.00	63.67	0.00	86.63	0.00
SEm.±	0.87	-	0.68	-	0.85	-
C.D. at 5 %	2.66	-	2.09	-	2.61	-

DAI = Days After Inoculation

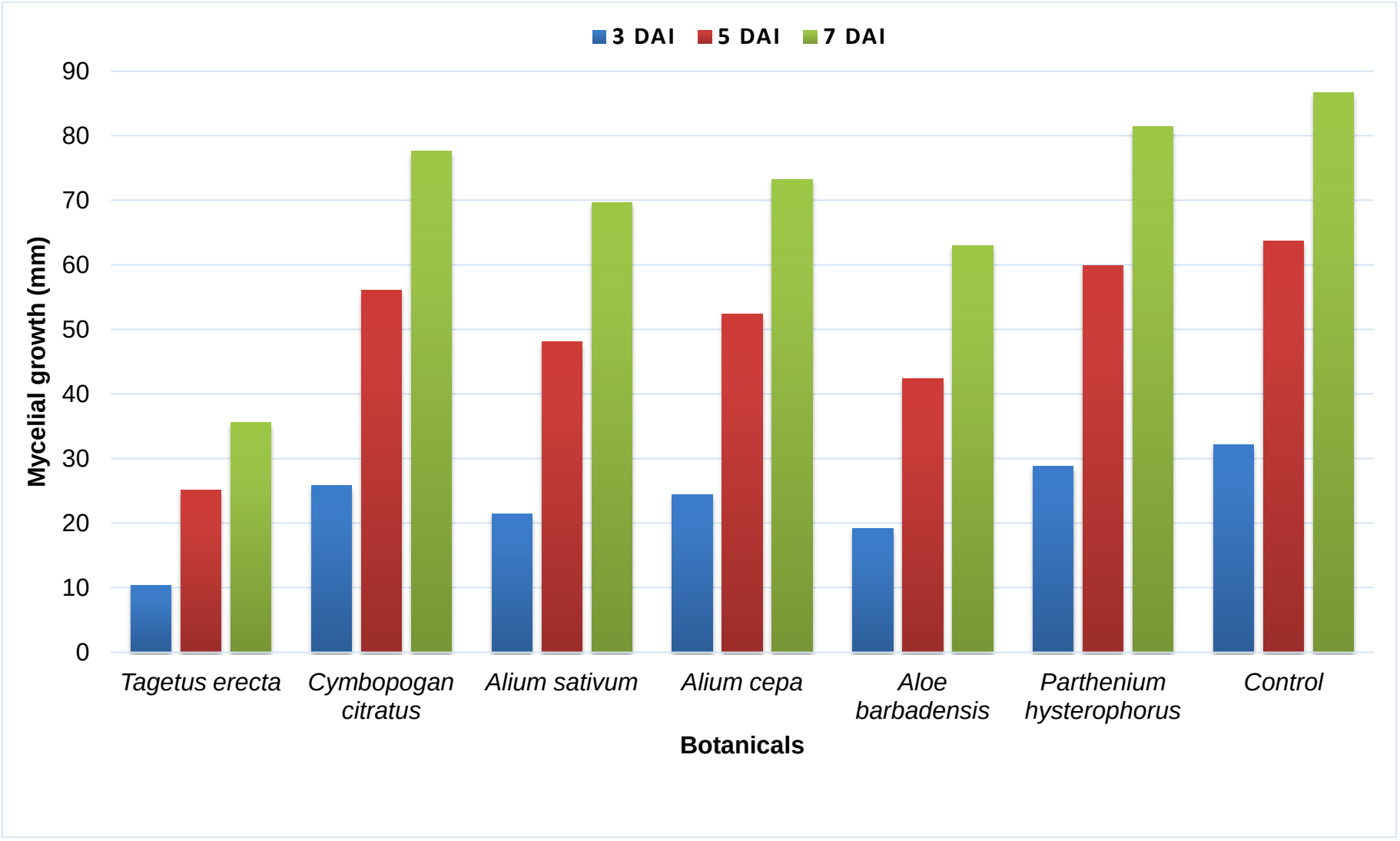
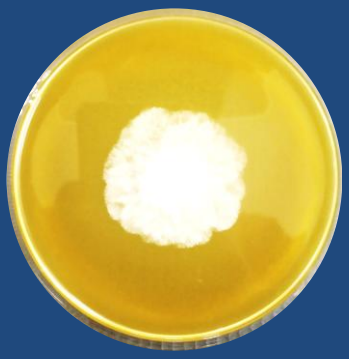
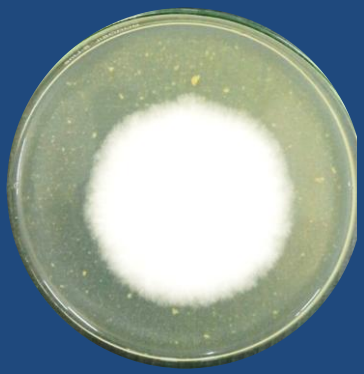


Fig 3 : *In-vitro* evaluation of botanicals for the management of *C. lindemuthianum*



Tagetus erecta

Cymbopogan citratus



Alium sativum

Alium cepa

Aloe barbadensis

Parthenium hysterophorus

Control

Plate 8 : Evaluation of botanical extracts (20 %) against *C. lindemuthianum*.

4.5. *In vivo* evaluation of selected organic farm products and botanicals for eco-friendly management of urdbean anthracnose:

The experiment was conducted on field in kharif 2021 in order to evaluate the selected botanicals and organic farm products for the eco-friendly management of anthracnose of urdbean.

Four botanicals viz; *Tagetus erecta, Aloe barbadensis, Alium sativum, Alium cepa* and three organic farm products viz; Jivamrut, Bijamrut and Neemastra were selected after *in vitro* screening and evaluated against urdbean anthracnose caused by *C. lindemuthianum*.

Data summarized in Table 10 indicated that per cent disease was inhibited in response to treatments as compared to control. The variation in per cent disease control was observed from 58.53 % to 7.77 %. Among all the treatments Neemastra was found to be most significant with maximum Percent Disease Control (PDC) (58.53 %) followed by *Tagetus erecta* (46.19 %), *Aloe barbadensis* (37.22 %), *Alium sativum* (28.65 %), Jivamrut (18.06 %) and Bijamrut (13.35 %), while minimum Percent Disease Control (PDC) was observed in *Alium cepa* (7.77 %), which was at par with Bijamrut (13.35 %).

Table 10 : **Effect of eco-friendly treatments on disease severity of anthracnose.**

Treatments (20 %)	Per cent disease severity	Per cent disease control (PDC)
Tagetus erecta	32.62 (34.80)	46.19
Bijamrut	52.53 (46.43)	13.35
Aloe barbadensis	38.06 (38.05)	37.22
Jivamrut	49.67 (44.79)	18.06
Alium cepa	55.91 (48.38)	7.77
Alium sativum	43.25 (41.10)	28.65
Neemastra	25.14 (29.95)	58.53
Control	60.62 (51.13)	0.00
SEm.±	1.15	-
C.D. at 5 %	3.53	-

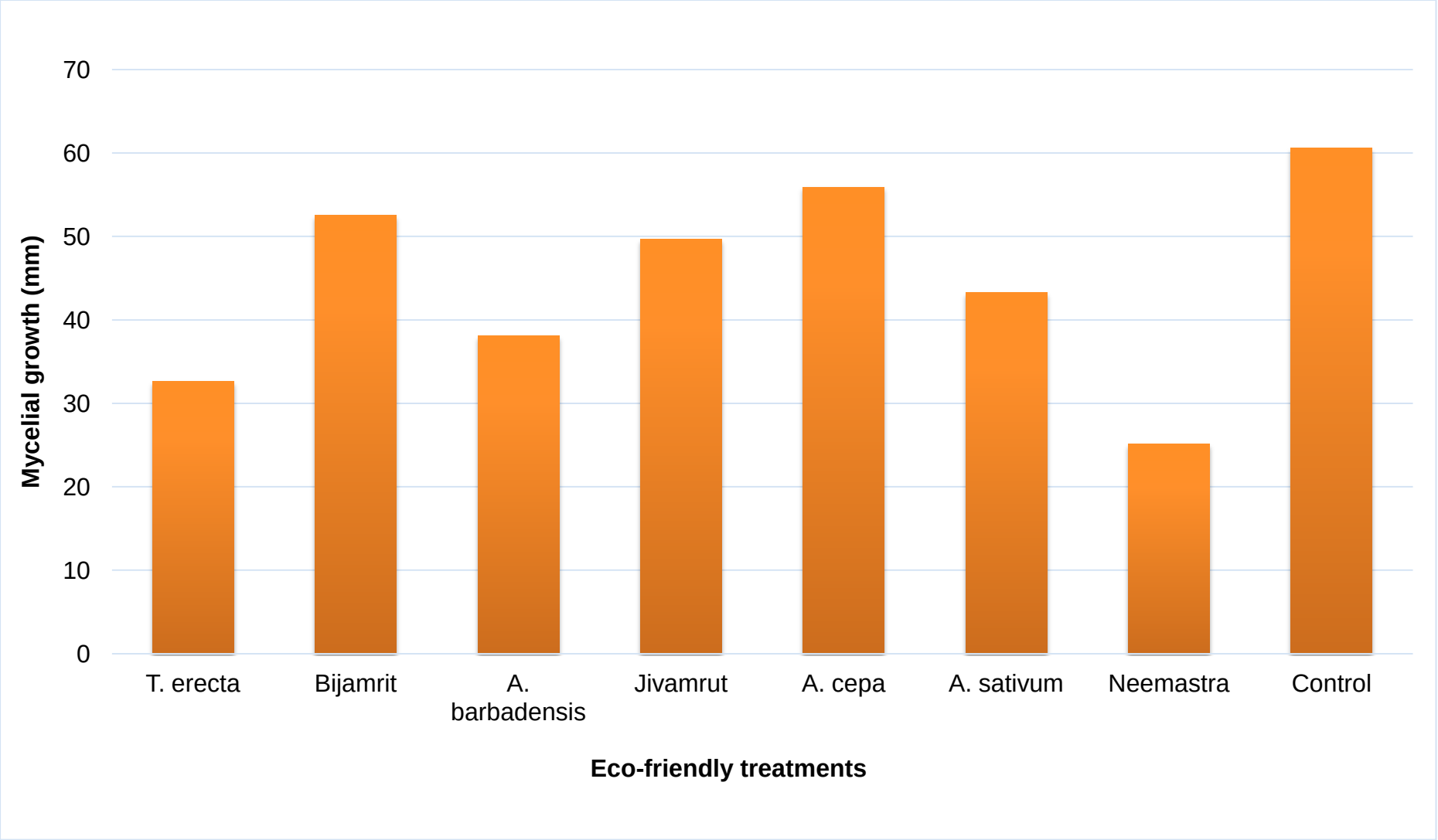


Fig. 4 : **Effect of eco-friendly treatments on disease severity of anthracnose.**

Plate 9 : Field evaluation of eco-friendly products against anthracnose of urdbean.

DISCUSSION

Chapter- V

DISCUSSION

Blackgram or urdbean [*Vigna mungo* L. Hepper] is a primitively cultivated crop of India. Black gram is densely hairy, annual and having erect to spreading growing habit. The pods are narrow, cylindrical and upto six cm long with a terminal, hooked beak. Each pods contains 6 to 10 seeds, which are square or oblong in shape and black with a pronounced white concave hilum. The nutritive value of urdbean lies is very high as it contains approximately 25-28% protein,1.0% oil, 3.5-4.5% fiber, 4.5-5.5% ash and 62-65% carbohydrates on dry weight basis. High values of lysine make urdbean an excellent complement to rice in terms of balanced human nutrition.

The low productivity of commercially cultivated urdbean cultivars is due to many biotic and abiotic factors. The crop is attacked by several pathogens *viz*; anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Cercospora canescens*), bean common mosaic virus, web blight (*Rhizoctonia solani*), halo blight (*Pseudomonas phaseolicola*), root rot (*Macrophomina phaseolina*) etc. Bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams.- Scrib, is a serious seed borne pathogen throughout the world, which cause considerable losses to the crop during rainy season right from seed germination to pod formation stage. The disease is seed borne and also survives through infected crop debris and causes severe infection at seedling stage.

In the present investigation, the symptoms were seen mainly on the leaves but in severe infection, it also appeared on other aerial parts of the plant like stem and pods. Initially the irregular shaped, dull violet or black sunken spots with dark centers and bright orange margins were produced on the margin of leaflets which were small and measuring around 2-5 mm in diameter. At later stage the spots enlarged, became irregular in shape, variable in size and gave a scorched appearance. After rain splashes spots coalesced, giving papery appearance to the leaves. Later on during last stage, necrotic portion fall off from the leaves causing formation of the shot holes. Young leaves were found relatively resistant to disease but they became susceptible with age. Infection

appeared on stem as light brown, irregular shaped necrotic spots followed by drying of the stem. While on pods the spots appear as black sunken cankers with grey central area. Pods which became infected develops less number of seeds as compared to the healthy ones. Form large 5-6 mm necrotic, circular to rectangular areas covering the whole midrib. On seedlings cotyledons, fungus appeared as small, brown to black, sunken spots and on adult plant stem, the spots are eye shaped and longitudinal along the stem. In advance stage of the disease large number of acervuli was found scattered all over the necrotic surface of the affected twigs. This result is in agreement with the observation done by Devi (2014), Bains *et. al.* (1989) and Majid (1953) which reported symptoms in mungbean (*Vigna radiata* and *V. mungo*) by anthracnose as irregular brown spots leaf spots, which looks like shape of a horse shoe, lesions eventually turned straw colored with dark brown to reddish brown margin which later turned to black. The spots appeared after 40-60 days of sowing and upto pod formation stage.

Like wise, Sardhara *et. al*. (2016) and Bindra *et. al*. (2016) observed yellow to brown sunken lesions on infected seed by *C. lindemuthianum*. Further, symptoms of *C. lindemuthianum* was observed as small, circular, brown spots on leaves, which later enlarge and form concentric rings which resembles target board. The spotted portion became papery and falls off producing shot holes, while on pods infection appeared as circular spots (Agarwal, 1991).

Dean *et. al.* (2012) described *Colletotrichum* as one of the important plant pathogenic genus responsible for anthracnose, which causes plant disease on variety on host from plants to grasses. The disease is characterized by sunken spots on leaves, stems, fruits and flowers. These spots often enlarge which leads to wilting, withering and drying of infected plant tissues (Hiremath *et. al*., 1993).

Similarly, Saettler, (1983) observed black spots on the surface and underside of the leaf. As the lesions grow they became indented in the center, where conidia begins to develop. These conidia were colourless at first, but developed into light pink, flesh coloured pustules which spread on new host by rain.

The pathogen requires warm and humid climate to infect different plant hosts, including gymnosperms, angiosperms, ornamentals and fruit plants,

vegetables, field crops or even grasses. As the primary inoculum is disseminated by wind or rain, the pathogen is cosmopolitan in distribution (Farr *et. al*., 2006). Most crops grown throughout the world are susceptible to one or multiple species of *Colletotrichum* (Weir *et. al*., 2012).

The morphological and cultural characteristic of *C. lindemuthianum* in present investigation was identified using mycelial bit from the margins of actively growing culture in PDA plates on slide with the help of compound microscope using morphological and reproductive characters up to species level with the help of standard description (Barnett and Hunter, 1972) and it was found that the pathogen produced hyaline unicellular, sickle shaped spores and uniform, circular, creamish white fluffy colony in culture plate. The mycelium was branched, septate, hyaline at first then became dark with age. The conidia were single celled hyaline, curved and ranged between 10.5 to 15.5 µm x 3.5 to 4.5 µm in size.It germinate to form appressorium, which penetrates the host surface, then infection peg emerges from appressorium to produce infection vesicles and primary hyphae. The primary hyphae spread to adjacent cells then fungus switches to necrotic phase by producing secondary hyphae. The acervuli was numerous, oval to round in shape and black in colour, measuring across 60 x 270 um. This result is in corroboration with earlier reports of Quimio (1975) reported similar observation about morphological features of *C. lindemuthianum* from mungbean, as branched, hyaline and septate mycelium which becomes dark with age. The acervuli were found to be saucer shaped, sub-cuticular and become erumpent. The conidia appeared pink in number which were borne acrogenously on short conidiophores. They were single celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed and measure 10 to 20 × 3 to 6 µm in size. similarly, mycelium of *C. truncatum* was branched, hyaline and septate. Acervuli was found to be numerous, oval to conical in shape, black to dark brown in colour, measuring 181.0 x 275.5 µm in size. Condia measured across 21.0 – 23.5 x 3.80 – 4.10 µm in size and found to be unicellular, smooth, hyaline and curved (Madhusudan, 2002).

Further it was observed that *Colletotrichum* spore germinate on the new host and form a short germ tube called an appressorium, or ‘pressing organ’. The germ tube grows and pulls the spore and the appressorium together, causing an indentation to occur in the cell wall, then infection peg protrude from

the appressorium and penetrate through the cell wall to form hypha which develops into an infection vesicle (Leach and Gilbert, 1922) and (Dean *et. al*. 2012).

A field trial was conducted for evaluation of 20 genotypes of urdbean against anthracnose caused by *Colletotrichum lindemuthianum*. Out of 20 genotypes evaluated, none of the them were found to be highly resistant. Two cultivars (PU 1617 and PU 1514) were found moderately resistant. Three cultivars (VBG 12-034, KPU 520-69 and KU 96-3 were found moderately susceptible. Thirteen cultivars (Azad -3, PU 31, LBG 752, PU 14-19, TBG 12, NGU 24, IPU 72, KU 17-08, TPU 4, Uttara, TU 94-2 and T-9) were found to be susceptible, while two cultivars (AKU 1608 and SBC 50) were found to be highly susceptible.

The present findings is in agreement with, Santosh *et al.* (2015) who evaluated 27 most widely grown varieties of urdbean against anthracnose and they found that only two varieties (T-9 and TPU-4) were resistant and two hundred and sixty eight genotypes of urdbean were evaluated against anthracnose. They found that two (T9 & TPU-4) lines were resistant, eight lines (SKNU, KU-09, UB-7, UB-10, UB-12, UB-13, UB-14 & UB-18) were moderately resistant (Kotgire *et. al.* 2010).

Similarly, Sharma (2011) screened 240 genotypes of urdbean against anthracnose and recorded 44 genotypes as resistant while 13 genotypes as moderately resistant.

In addition, Kumar and Mukhopadhyay (1987) evaluated 350 germplasm lines and found that 8 genotypes *viz;* T 65, UPU 79-2, UPU-80-5-5, UG 201, PDU 2, PDU 3, PDU 8 and PDU 10 were resistant to urdbean anthracnose.

Correspondingly, six cultivars *viz*; PU-30, PU-31, PU-38, PU-40, PUI-94-1 and TAU-1 were evaluated. Two cultivars PU-31 and PU-30 were moderately resistant with PDI of 28.40 % and 31.40 % respectively. Two cultivars PU-38 (39.60%) and PU-40 (42.90%) were moderately susceptible and Two cultivars TAU-1 (47.20%) and PUI-94-1 (51.40%) were found to be susceptible (Aggarwal *et. al*. 2019).

Equivalently, forty nine genotypes were evaluated against different races of *C. lindemuthianum*. Some of the genotypes *viz*; G 2333, Cornell 49242, PI

207262, Mexique 222, TO, Perry Marrow, Kaboon and Widusawere were found to be resistant against more than five Indian races, while two genotypes *viz*; KRC-5 and Hans were observed to show resistant against six and four races respectively (Pathania *et. al.* 2006).

A field trial was conducted in similar manner to elucidate the reaction of Dolichos bean genotypes against *C. lindemuthianum* under natural infected condition (Rajesha *et. al*. 2010).

Like wise, same experiment was conducted at two completely different agroclimatic locations *i.e.* Palampur and Dhaulakaun of Himachal Pradesh under natural epiphytotic conditions to screen 240 lines of urdbean against anthracnose and recorded 44 lines as resistant while 13 as moderately resistant (Sharma, 2011).

Further, Deshmukh *et. al.* (2012) evaluated five varieties and thirty nine germplasms of Indian bean against anthracnose in Rabi 2008. They reported out of three varieties (Kapasi, JNP-4, Katagram) two germplasms (NWP 12, 19, 20, 22, 24, 25, 26, 27, 28, 29, 30, 32, 35, 37, 39) were found to be moderately resistant, whereas variety NPS 1 was found to be highly susceptible to anthracnose of Indian bean (*Lablab purpureus* L.) under south Gujrat conditions.

In the present investigation, *in vitro* experiment were conducted to evaluate six botanicals against *Colletotrichum lindemuthianum* and it was found that maximum percent inhibition of the pathogen was recorded in *Tagetus erecta*, which was followed by *Aloe barbadensis, Alium sativm* and *Cymbopogan citratus*, Whereas the least percent inhibition was recorded in *Pathenium hysterophorus* and found to be relatively non-effective. The experiment is in conformity with, Gurav *et al.* (2013) which evaluated extracts of Garlic (clove), Soap nut (fruit), Neem (leaves), Eucalyptus (leaves), Castor (fruit) and Onion (bulb) at 10 per cent concentration against *C. gloeosporioides* causing leaf blight of Sarpagandha under *in vitro* condition. It was found that Garlic completely inhibited the mycelial growth of the test pathogen followed by Soap nut (81.11%), Neem (80.0%), Eucalyptus (74.44%), Castor (65.5%) and Onion (65.3%).

Similar work was done on neem oil extract of *Azadirachta indica* (Neem) and *Xylopia ethiopica*, before and after infection of the plants of cowpea with *C.*

lindemuthianum and it was found that the neem oil extract significantly reduced the growth of *C. lindemuthianum in vitro* and were also effective in reducing size of the pathogen induced lessions (Amadioha and Obi, 2008).

Corresponding experiment on effect of aqueous extracts of nine botanicals at 10 and 20 % conc. against *Colletotrichum graminicola* causing anthracnose of sorghum were done and it was observed that maximum growth of the pathogen was inhibited by *A. indica* (70.73%), followed by *Z. officinale* (62.58%), *A. cepa* (54.43%), *P. hystrophorus* (49.81%) and *P. pinnata* (42.95%) (Rewale *et. al.* 2018).

Like wise, Jagtap *et. al.* (2014) evaluated nine botanicals against *C. truncatum in vitro* and reported that garlic significantly inhibited the pathogen with highest growth inhibition (81.82%) followed by tulsi (65.17%), onion (60.31%), ginger (55.25), neem (49.72%), parthenium (47.09%), bogunveilia (42.90%), eucalyptus (41.11%) and Mehandi (40.36 %).

In other experiment, *Lawsonia inermis* was found superior with maximum percent inhibition of 88.55 %, followed by *Zingiber officinale* (65.55 %), *Pongamia pinnata* (56.00 %), *Azadirachta indica* (37.04 %), *Eucalyptus globules* 32.22 % and *Oscimum sanctum* (26.33 %) (Bagade *et. al*. 2020). Khan and Nasreen (2010) also reported maximum percent inhibition of *C. lindemuthianum* mycelium by Lawsonia inermis (81.81 %). Similarly Choudhary *et. al*. (2017) observed growth inhibition of *C. lindemuthianum* mycelium by Mehandi (64 %).

The effect of twelve plant extracts viz., *Acalypha indica, Ocimum sanctum, Coleus amboinicus, Phyllanthus niruri, Tribulus terrestris, Allium sativum, Parthenium hysterophorus, Lawsonia inermis, Senna alexandrina, Azadirachta indica, Anisomeles malabarica* and *Zingiber officinale* were evaluated at 5% and 10% concentration, against anthracnose of urdbean under *in vitro* condition through poison food technique. It was found that at 5% and 10% concentration, *Anisomeles malabarica* recorded lowest mycelial growth of 3.2 cm and 1.0 cm and highest mycelial inhibition of 64.4% and 88.8% followed by *Allium sativum* recorded the mycelial growth of 3.5 cm and 3.0 cm and mycelial inhibition of *61.1%* and *66.6 %* against control respectively (Vasuki *et. al.* 2020).

Equivalent experiment was done to evaluate the effect of six plant

extracts on growth, sporulation and conidial germination of *C. lindemuthianum* under *in vitro*. Six plant extracts *viz*; *Tridax procumbens, Jatropha gossypifolia, Sida acuta, Blighia sapida, Ricinus communis* and *Datura stramonium* were evaluated against test pathogen at three concentrations (30, 50 and 65 %). It was observed that at 30, 50 and 65% concentrations, *D. stramonium* was the most effective with per cent inhibition of 10, 16 and 33% respectively, followed by R. communis and J. gossypifolia while *B. sapida* caused the least inhibition of growth with 2, 8 and 10% respectively (Falade, 2017).

Furthermore, botanicals were evaluated against *C. lindemuthianum in vitro.* and it was found that Datura leaf extract at 5 % concentration least per cent inhibition of 10.22 %, followed by Neem leaf extract at 5 % (20.84 %) (Sushmita and Zacharia, 2021).

In the field experiment, four best botanicals observed in lab experiment were tested on field, in which maximum percent disease control was recorded in *Tagetus erecta* followed by *Aloe barbadensis, Alium sativum* and *Alium cepa.* Which was found in agreement with the work of Mishra *et al.* (2011), who tested six plant extracts *viz; A. indica*, *P. juliflora*, A. sativum, *Ocimum sanctum*, *Datura metel* and *Tagetes erecta* against *C. capsici*, causing anthracnose of urdbean *in vivo* (foliar sprays) and found that leaf extracts of *A. indica* and *P. juliflora* recorded higher (59.9-63.0%) disease control efficacy compared to the control. The garlic extract also showed good (52.1%) efficacy in controlling disease severity.

Correspondingly, evaluation was done to evaluate botanicals against *C. lindemuthianum in vivo*. It was found that foliar application of Neem leaf extract at 5 % on plant, moderately inhibit the pathogen with disease intensity of (33.90 %), while Datura leaf extract treated plant at 5 % concentration showed maximum disease intensity (35.97 %) (Sushmita and Zacharia, 2021).

Like wise, a experiment was conducted to evaluate efficacy of powdered leaf extracts of *Aloe vera* (Linn) and *Aloe schweinfurthii* (Baker) using cold water, hot water and ethanol against four fungal spp., *viz*, *Alternaria solani, Colletotrichum lindemuthianum, Sclerotium rolfsii* and *Trichophyton rubrum*. Ethanolic extract of *A. vera* at 50 mg/ml and 100 mg/ml had the greatest impact on *A. solani* and *C. lindemuthianum* respectively. Like wise, cold water extract of *A. schweinfurthii* at 100 mg/ml was the most effective against S.

rolfsii and T. rubrum. (Alejo, A.O. *et. al.* 2019).

In the present investigation, *in vitro* experiment was conducted to evaluate the inhibitory action of six organic farm products against *Colletotrichum lindemuthianum* and it was found that maximum inhibition of the test pathogen was done by Neemastra and found to be the most prominent among other organic farm products, which is followed by Jivamrut, Bijamrut, Fermented butter milk, Vermicompost and FYM as compared to untreated control. The present investigation is in corroboration with, Ashlesha and Paul (2014), who tested five organic and natural farming inputs viz; cow urine, panchgavya, vermiwash, biosol and buttermilk against *C. capsici* causing anthracnose in bell pepper under *in vitro* at different concentration, It was observed that under *in vitro* cow urine showed 100% mycelial growth inhibition @ 10 per cent conc., followed by fermented butter milk (98.22%), panchgavya (97.91%), vermiwash (97.40%) and biosol (93.91%).

Equivalently, it was observed that maximum per cent inhibition of spore germination was observed with cow urine (75.57 %), followed by fermented butter milk (64.59%), panchgavya (62.70%) and vermiwash (59.73%). While least inhibition was recorded in cow milk (56.10%) (Kulkarni, 2009).

Similarly, Sharma (2020) studied five bioformulation *viz*; Jeevamrit, Bijamrit, Fermented butter milk (Tamerlassi), Vermiwash and Cow urine against *C. lindemuthianum* at different concentration (2,4.6,8 and 10%). It was observed that Jeevamrit completely inhibited the pathogen at 4 and above concentrations, while at 2 per cent, pathogen growth inhibition was 40.56% followed by Cow urine and Tamerlassi (100%) at 6 and above concentrations, however at 2 and 4% concentrations, Cow urine inhibited 63.81 and 75.81% mycelial growth respectively. Bijamrit was next best product (70 - 77.78%) at all concentration followed by Tamerlassi (29.37 and 57.89%) at 2 and 4 concentrations respectively.

Futhermore, an experiment was carried to test organic and natural farming inputs viz; panchgavya, jeevamrit, beejamrit, tamarlassi and eupatorium ark at 10, 20 and 30 per cent concentration against *Colletotrichum truncatum* under *in vitro* and as foliar spray *in vivo.* Under *in vitro* results showed that, beejamrit and jeevamrit were highly effective and showed complete mycelial inhibition even at 10 per cent concentration whereas, tamarlassi with 22.80 per

cent mycelial inhibition even at 30 per cent concentration was found least effective. Under *in vivo* among all treatments natural farming panchgavya was found most effective with disease severity of 51.37 % (Chatak, 2020).

In the field experiment, three best organic farm products found in the lab experiment were tested on field to evaluate their efficacy, among them Neemastra significantly reduced the disease severity among other treatments and found to be the best over other treatments, which is followed by Jivamrut and Bijamrut. The result is in conformity with the earlier reports made by Ashlesha and Paul (2014), who tested five organic and natural farming inputs viz; cow urine, panchgavya, vermiwash, biosol and buttermilk against *C. capsici* causing anthracnose in bell pepper under *in vivo* at different concentration, They observed that cow urine was the most effective against anthracnose with 74.59 per cent disease control followed by panchgavya (70.46%), biosol (68.94%), buttermilk (64.39%) and vermiwash (63.45%) under field conditions.

Similar experiment was carried to test organic and natural farming inputs viz; panchgavya, jeevamrit, beejamrit, tamarlassi and eupatorium ark at 10, 20 and 30 per cent concentration against *Colletotrichum truncatum* as foliar spray under *in vivo.* It was observed that among all treatments natural farming panchgavya was found most effective with disease severity of 51.37 % (Chatak, 2020).

Correspondingly, Pandia *et. al.* (2019) studied efficacy of Jivamrit and its components against leaf spot of mungbean caused by Alternaria alternata in field condition and observed that Percent disease index PDI and PDC of 17.34 and 75.22% respectively in Jiwamrit-2 treated plants, which significantly inhibited the pathogen and found superior over other treatments, followed by Jiwamrit-1 PDI (22.0) and PDC (68.57) and Jiwamrit-3 PDI (24.67) and PDC (64.75).

Furthermore, Yadav (2021) evaluated six natural farm products for their effectivity against anthracnose of urdbean caused by *C. lindemuthianum* and reported that Jiwamrit significantly inhibited the pathogen and found superior over other treatment (PDI -20.89%), followed by Cow urine (31.56%), Compost tea (44.30%), vermiwash (48.74%), Compost sat (50.22%) and Cow milk (54.07%).

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

Chapter-VI

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

Blackgram (*Vigna mungo* L. Hepper) also known as urd, udad, udid, mash in india. Blackgram is originated from India (De Candolle, 1986). It is one of the important grain legume crop, belonging to the sub-genus *Ceratotropis*, domesticated from *V. mungo var. silvestris .* It is extensively cultivated in the Indian sub-continent throughout the country in all the three seasons such as in Kharif, Rabi, and Summer. Most of the urdbean production comes from Madhya Pradesh, Rajasthan, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Maharashtra, Jharkhand, Gujrat, Karnataka and West Bengal. India is the largest producer as well as consumer of urdbean. Sustainable production of urdbean is affected by varying degree of biotic stress i.e. pests and diseases, Disease is one of the major constraints to yield throughout most of the part of Asia. Most serious fungal diseases of Urdbean is Anthracnose (*Colletotrichum lindemuthianum*), which infest the crop particularly under cool and humid environmental conditions and cause 80 to 100 per cent yield losses.

Summary:

The present study entitled as **"Studies on Anthracnose of Urdbean caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) and it's eco-friendly management"** was conducted to study the morphological and cultural characteristics of the pathogen, evaluation of urdbean genotypes against anthracnose, laboratory and field evaluation of botanicals and organic farm products for eco-friendly management of the disease which would be helpful in the identification and selection of cost effective eco-friendly inputs for controlling the disease, so that can be used for the improvement of yield in urdbean. The finding has been summarized as under.

Initially the symptoms appeared as minute water soaked necrotic spots around 2-4 mm on the margins of the leaflets, which were dark brown in colour. Later spots coalesced together giving papery appearance to the leaves which caused shot hole and defoliation of the plant. Symptoms appeared on stem as light brown, irregular shaped necrotic spots followed by drying of the stem. Later on large number of acervuli was found scattered all over the affected twigs.

While infection appeared on pods as reddish, dark brown circular blotches. Infected pods developed less number of seeds as compared to the healthy pods.

Colletotrichum produced branched, septate and hyaline mycelium. The acervuli was rounded and black in colour and measures across 60 x 270 um. The conidia was produced from conidiospores and appear as unicellular and measures across 10.5-15.5 x 3.5-4.5 um in size.

Out of 20 genotypes of urdbean evaluated, none of the them were found to be highly resistant to *C. lindemuthianum*. Two cultivars (PU 1617 and PU 1514) were found moderately resistant. Three cultivars (VBG 12-034, KPU 520-69 and KU 96-3) were found moderately susceptible. Thirteen cultivars (Azad - 3, PU 31, LBG 752, PU 14-19, TBG 12, NGU 24, IPU 72, KU 17-08, TPU 4, Uttara, TU 94-2 and T-9) were found to be susceptible, while two cultivars (AKU 1608 and SBC 50) were found to be highly susceptible.

An *in vitro* laboratory experiment was conducted to test the inhibition property of organic farm products against *C. lindemuthianum*. A total of six organic farm product were evaluated at 20% concentration in CRD in three replications. The mean colony diameter of the pathogen at 7 days after inoculation varied from 22.23 mm to 76.50 mm. Neemastra was found was significantly superior to other organic farm products with mean colony diameter of 22.23 mm and highest per cent inhibition of 70.94%, while minimum per cent inhibition was observed in FYM (5.92%).

An *in vitro* experiment was carried out to evaluate the efficacy of botanical extracts in regulating *C. lindemuthianum*. A total of six botanical were tested at 20% concentration in CRD with 3 replications. The radial growth of the test pathogen at 7 days after inoculation varied from 35.53 mm to 86.63 mm. The maximum radial growth of *C. lindemuthianum* was inhibited by *Tagetes erecta* (35.53 mm) and found to be significantly superior over other treatments with maximum per cent inhibition (58.99%), while minimum per cent inhibition was observed in Parthenium (6.04%).

A field experiment was conducted to evaluate the selected botanicals and organic farm products after *in vitro* screening viz; *Tagetus erecta, Aloe barbadensis, Alium cepa, Alium sativum*, Jivamrut, Bijamrut and Neemastra, against anthracnose of urdbean caused by *C. lindemuthianum* at 20%

concentration in RBD with 3 replications. The percent disease severity varied from 25.14 % to 60.62 %. Neemastra significantly reduce the anthracnose severity with maximum percent disease control (58.53) and found to be superior over other treatments, while minimum percent disease control was recorded in *Alium cepa* (7.77%).

Conclusion:

- After screening of all the 20 urdbean genotypes maximum disease severity was recorded in AKU 1608 (57.65 %) and found to be highly susceptible, while minimum diease severity was recorded in PU 1617 (5.09 %) and found to be moderately resistant.
- Among all the six botanical products evaluated *in vitro Tagetus erecta* was found significantly superior to other botanical products with maximum percent inhibition (67.60 %), while minimum percent inhibition was found in *Parthenium hysterophorus* (5.87).
- Evaluation of six organic farm products under *in vitro* condition showed that Neemastra was found significantly superior to other farm products with maximum percent inhibition of (71.20 %), while minimum percent inhibition was found in FYM (5.92 %).
- Field evaluation of seven eco-friendly treatments depicted, percent disease control was recorded significantly in Neemastra (58.53%), while minimum percent disease control was recorded in *Alium cepa* (8.97%).

Suggestions for further work:

- Identification of the fungus based on morphology, symptoms on host surface and pathogenicity is time-consuming and needs a specialist in taxonomy, therefore an accurate, sensitive and effective diagnostic tool is necessary.
- Detailed study of the pathogen needs to be done in order to know more about its habit and characters for implementing its management.
- To know source and wide base of resistance in urdbean genotypes, screening can be done under different locations for calibrating their resistant.

- Eco-friendly disease management has a vast scope in itself, so more amount of work is needed to test different organic farm products and botanicals in combination and in different concentrations.

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APPENDICES

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Anova table for table-8

SV	DF	SS	MSS	Fcal	Sig
Replications	1	6.04	6.04	7.912	0.01
Treatment	19	2,509.01	132.05	173.13	0.00
Error	19	14.49	0.76		
Total	39	2,529.54	138.85	181.04	0.01

Anova table for table-9 (3 Days After Inoculation)

SV	DF	SS	MSS	Fcal	Sig
Farm products	6	943.73	157.28	88.66	0.00
Error	14	24.84	1.77		
Total	20	968.56	159.05		

Anova table for table-9 (5 Days After Inoculation)

SV	DF	SS	MSS	Fcal	Sig
Farm products	6	2,591.01	431.84	178.83	0.00
Error	14	33.81	2.42		
Total	20	2,624.82	434.26		

Anova table for table-9 (7 Days After Inoculation)

SV	DF	SS	MSS	Fcal	Sig
Farm products	6	5,845.22	974.20	579.36	0.00
Error	14	23.54	1.68		
Total	20	5,868.77	975.88		

Anova table for table-10 (3 Days After Inoculation)

SV	DF	SS	MSS	Fcal	Sig.
Botanicals	6	906.16	151.03	66.77	0.00
Error	14	31.67	2.26		
Total	20	937.83	153.29		

Anova table for table-10 (5 Days After Inoculation)

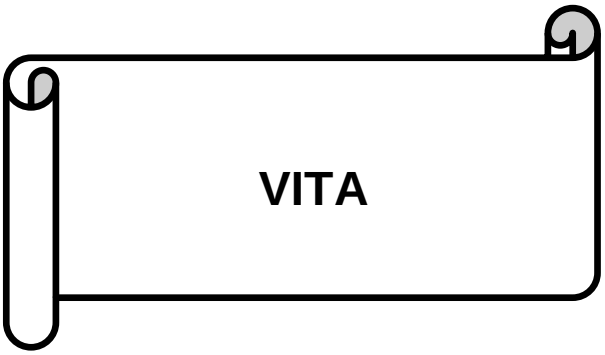
SV	DF	SS	MSS	Fcal	Sig.
Botanicals	6	3030.05	505.01	360.84	0.00
Error	14	19.59	1.40		
Total	20	3049.65	506.41		

Anova table for table-10 (7 Days After Inoculation)

SV	DF	SS	MSS	Fcal	Sig
Botanicals	6	5,129.27	854.88	391.96	0.00
Error	14	30.535	2.18		
Total	20	5,159.80	857.06		

Anova table for table-11

SV	DF	SS	MSS	Fcal	Sig
Replications	2	44.17			
Treatment	7	1,094.13	156.31	39.216	0.00
Error	14	55.80	3.99		
Total	23	1,194.11	160.30		


VITA

VITA

The author of this thesis **Mr. Aashish Deshmukh** S/o Shri Babu Rao Deshmukh, was born in Indore, (M.P.) on December 4, 1996. He has completed his high school education with 8.0 c.g.p.a, in the year 2013 and higher secondary in 2015 with 76.2 per cent marks from Delhi Public Academy, Gwalior, (M.P.), which is affiliated to C.B.S.E. Board.

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After completion of his graduation, he appeared for PG entrance exam (JEE) and got selected in Department of Plant Pathology, College of Agriculture, RVSKVV, Gwalior, (M.P.) for the post-graduation degree programme. He has passed all the academic subjects of M.Sc. (Ag.) Plant Pathology degree in 2022 with 81.4 per cent marks and submitting his thesis of the research work entitled **"Studies on Anthracnose of Urdbean caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) and it's eco-friendly management"** in the partial fulfilment of the requirements for the degree of **Master of Science (Ag.)** in **Plant Pathology**.

Place : Gwalior

Date: **Aashish Deshmukh**