STUDIES ON PIGEONPEA STEM BLIGHT CAUSED BY *Phytophthora drechsleri* f. sp. *cajani*

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B.Sc. (Agriculture)

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IN
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PARBHANI-431 402 [M.S.] INDIA

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BY

PATIL TEJAS SUNIL

B.Sc. (Agriculture)

A thesis submitted to
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
in partial fulfillment of the requirement for the Degree of

MASTER OF SCIENCE
IN
AGRICULTURE
(PLANT PATHOLOGY)

DEPARTMENT OF PLANT PATHOLOGY
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VASANTRAO NAIK MARATHWADA KRISHI VIDYAPEETH
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2022

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled, **"Studies on Pigeonpea Stem Blight Caused by *Phytophthora drechsleri* f. sp. *cajani* "**, submitted by me is based on the actual work carried out by me under the guidance and supervision of **Dr. Sunita Janardhanrao Magar.** The extent of information derived from the existing literature have been duly cited and referenced. The existing researchwork or its any part is not submitted anywhere else for the award of any degree or diploma.

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THESIS ABSTRACT 1. Title of the thesis : Studies on Pigeonpea Stem Blight Caused By Phytophthora drechsleri f. sp. cajani 2. Full name of condidate : Patil Tejas Sunil 3. Full name of Research Guide : Sunita Janardhanrao Magar 4. Department : Plant Pathology 5. College / University : College of Agriculture , Latur (VNMKV, Parbhani) 6. Degree to be awarded : M. Sc. (Agriculture) ABSTRACT Pigeonpea (Cajanus cajan) stem blight caused by Phytophthora drechsleri f. sp. cajani are most widely distributed and destructive disease of pigeonpea, causing about 70-80 per cent yield losses. Therefore, present studies were undertaken with the objectives viz ., collection of disease samples, symptomatology, isolation, pathogenicity test and different media, morpho-cultural characteristics and efficacy of various fungicides, bioagents and phytoextracts against the Phytophthora drechsleri f. sp. cajani . The pigeonpea plants exhibiting typical stem blight symptoms were collected from farmer's field. The P. drechsleri f. sp. cajani was isolated by applying tissue isolation technique on PDA plates, these was purified and maintained on PDA slant tubes for further studies. Therefore, pathogenicity of these P. drechsleri f. sp. cajani was proved by applying soil drenching method. However, soil drenching method could reveal pathogenic association of the P. drechsleri f. sp. cajani with pigeonpea stem blight. Based on symptomatology, pathogenicity test, morpho-cultural characteristics and microscopic observations of the P. drechsleri f. sp. cajani identified and further confirmed by comparing with their authentic description. The cultural and morphological characteristics on different culture media and various physiological parameters i.e ., effect of temperature, effect of p H and effect of nitrogen sources on growth of P. drechsleri f. sp. cajani causing stem blight of pigeonpea. All of the fungicides, bioagents and phytoextracts evaluated in vitro were proved to be potent antimicrobials with significant mycelial growth inhibition of the P.
drechsleri f. sp. cajani , over untreated control. However, the most effecient fungicides found were Metalaxyl MZ 35% WS, Fosetyl AL 80% (each @ 500 and 1000 ppm), Mancozeb 75% WP, Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8% + Mancozeb 64% WP, Thiophanate methyl 45% + Pyraclostrobin 5% FS (each @ 2000 and 2500 ppm). Among the test bioagents, Trichoderma hamatum , T. harzianum and T. asperellum . whereas among the test phytoextracts, Allium sativum , Zingiber officinale , Lawsonia inermis and Eucalyptus globulus were found effective against the P. drechsleri f. sp. cajani causing stem blight of pigeonpea. (Key Words :, Cajanus cajan , Stem blight, Phytophthora drechsleri f. sp. cajani, Fungicides, Bioagents, Phytoextracts, Inhibition)

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"Success is not fine, failure is not fatal: it is the courage to continue that counts."

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(Patil T. S.)

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ABBREVIATIONS

/	-	Per
@	-	At the rate
&	-	And
%	-	Per cent
µm		Micro meter
<	-	Less than
>	-	More than
Avg.	-	Average
C.D.	-	Critical difference
mm	-	milimeter (s)
CRD	-	Completely Randomized design
Conc.	-	Concentration (s)
Col.	-	Colony
Dia.	-	Diameter
^{0}C	-	Degree Celsius
e.g.	-	Exampli Gratia (For example)
et al.	-	and other
etc.	-	Etcetera
Fig	-	Figure (s)
g	-	Gram
ha	-	Hectare (s)
Kg	-	Kilogram (s)
m	-	Meter (s)
Max.	-	Maximum
NA	-	Nutrient agar
No.	-	Number (s)
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
pH	-	Log H^+ ion treatment
S.E.	-	Standard error
spp.	-	Species
T	-	Treatment
t/ha	-	Tonns per hectare
viz.,	-	videlicet (Namely)

THESIS ABSTRACT

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2. Full name of candidate	: Patil Tejas Sunil
3. Full name of Research Guide	: Sunita Janardhanrao Magar
4. **Department**	: Plant Pathology
5. College / University	: College of Agriculture , Latur (VNMKV, Parbhani)
6. Degree to be awarded	: M. Sc. (Agriculture)

ABSTRACT

Pigeonpea (*Cajanus cajan*.) stem blight caused by *Phytophthora drechsleri* f. sp. *cajani* are most widely distributed and destructive disease of pigeonpea, causing about 70-80 per cent yield losses. Therefore, present studies were undertaken with the objectives *viz*., isolation, identification and pathogenicity test, effect different cultural media, different nitrogen sources, pH and temperature on growth of *Phytophthora drechsleri* f. sp. *cajani* and efficacy of various fungicides, bioagents and phytoextracts on it.

The pigeonpea plants showing stem blight symptoms were isolated by applying tissue isolation technique on PDA plates and pure culture was maintained on PDA slant tubes for further studies. Pathogenicity of these *P. drechsleri* f. sp. *cajani* was proved by applying soil drenching method. This method reveal pathogenic association of the *P. drechsleri* f. sp. *cajani* with pigeonpea stem blight.

Based on symptomatology, pathogenicity test, morpho-cultural characteristics and microscopic observations of the *P. drechsleri* f. sp. *cajani* identified and further confirmed by comparing with their authentic description. *P. drechsleri* f. sp. *cajani* on different cultural media exhibited wide range of variation in morphological and cultural characters. At 30 ºC temperature and 6.5 pH *P. drechsleri* f. sp. *cajani* produce higher mycelial growth. Among different nitrogen sources Ammonium Ferrous Sulphate $(NH_4)_2Fe(SO_4)_2.6H_2O$ was found most effective with no mycelial growth.

All of the fungicides, bioagents and phytoextracts evaluated *in vitro* were proved to be potent antimicrobials with significant mycelial growth inhibition of the *P. drechsleri* f. sp. *cajani*, over untreated control. However, the most efficient fungicides found were Metalaxyl MZ 35% WS, Fosetyl AL 80% (each @ 500 and 1000 ppm), Mancozeb 75% WP, Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8% + Mancozeb 64% WP, Thiophanate methyl 45% + Pyraclostrobin 5% FS (each @ 2000 and 2500 ppm). Among the test bioagents, *Trichoderma hamatum*, *T. harzianum* and *T. asperellum*. whereas among the test phytoextracts, *Allium sativum*, *Zingiber officinale*, *Lawsonia inermis* and *Eucalyptus globulus* were found efficient against the *P. drechsleri* f. sp. *cajani* causing stem blight of pigeonpea.

(Key Words:, *Cajanus cajan*, Stem blight, *Phytophthora drechsleri* f. sp. *cajani*, Fungicides, Bioagents, Phytoextracts, Inhibition)

CHAPTER – I

INTRODUCTION

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INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) *Millisp*.) is a cross-pollinated crop belonging to Fabaceae family, commonly known as Adhaki, Arhar, Pigeon pea and Tur in Sanskrit, Hindi, English and Bengali respectively (Pal *et al.* 2011). It is diploid, perennial grain legume having India, the primary center of origin. Pigeonpea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. It is an economically important grain legume of the small and marginal farmers in India. Pigeonpea is cultivated as a sole crop and intercrop with rainfed cereals, millets, oilseeds and other pulses; thereby, it enhances the productivity and net income to the small and marginal farmers.

It is the most important legume in the World. Globally, pigeonpea production is around 4 million metric tonnes. In Maharashtra, during year 2020-2021 area under pigeonpea was about 1236.1 thousand ha with production is about 1251.8 thousand tonnes and productivity 1012.7 Kg/ha, respectively (Anonymous, 2021). The Indian sub-continent, eastern Africa and Central America are the World's three main pigeonpea producing regions. Pigeonpea is cultivated in more than 25 tropical and subtropical countries, either as a sole crop or as an intercrop with cereals and other legumes (Mallikarjun *et al*. 2011).

Pigeonpea is one of the major and inseparable dietary protein sources to the large mass of the Indian population (Varshney *et al*. 2010). Pigeonpea is an important source of proteins, carbohydrates, vitamins B-complex and certain minerals, iron, iodine and essential amino acids like lysine, cystine, methionine, tryptophan and arginine. Pigeonpea is drought tolerant crop, but is highly sensitive to water logging condition. The crop has many uses like grain is cooked and eaten as dal, fresh pigeonpea is eaten as a vegetable. The wood is used as fuel and the leaves and husks provide feed to livestock.

Cajanus cajan being a forage crop has been utilized as an important remedy for various ailments. The Garo tribal community of Bangladesh utilizes it for the treatment of diabetes and as an energy stimulant. In Trinidad and Tobago, the leaves of *C. cajan* are used in food poisoning as colic and in constipation. In Chinese folk medicine, pigeon pea leaves are used to staunch blood, as an analgesic and to kill parasites. In some parts of Tamil Nadu in India, the leaf, seeds and young stems are used to cure gingivitis, stomatitis and as a toothbrush. It is also an important folk medicine in eastern Rajasthan as fresh juice/boiled leaves are given orally to nullify the effect of intoxication and as a laxative. Leaf paste is applied in oral ulcers and inflammations. Leaves and seeds are applied as poultice over the breast to induce lactation (Pal *et al.* 2011).

Pigeonpea is susceptible to many diseases such as wilt *(Fusarium udum*), sterility mosaic (Pigeonpea Sterility Mosaic Virus), *Alternaria* blight (*Alternaria alternata*), anthracnose (*Colletotrichum spp.*), stem blight (*Phytophthora drechsleri*) and *Cercospora* leaf spot (*Cercospora cajani*). Among all the diseases, only a few of them are of economically important (Nene *et al*. 1996 and Dhar *et al*. 2004).

After wilt (*Fusarium udum*) and sterility mosaic (Pigeonpea Sterility Mosaic Virus), *Phytophthora* blight (PB) caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* is the third potentially important disease of pigeonpea in India (Kannaiyan *et al*. 1984) causing complete crop loss upon its infection. *Phytophthora* stem blight (PSB) has also been reported as the most important production in constraint north eastern states of India (Mishra and Shukla 1987 and Chauhan *et al*. 2002).

Phytophthora drechsleri Tucker f. sp. *cajani* belongs to Kingdom Chromista, Phylum Oomycota, Order Peronosporales, Family Peronosporaceae, Genus *Phytophthora*, species *P. cajani* (Amin *et al*. 1978).

The *Phytophthora drechsleri* Tucker f. sp. *cajani* survives in soil and infected plant parts as chlamydospores, oospores and dormant mycelium. Chlamydospore is thick-walled long-term survival spores, as they are produced through asexual means of reproduction. Whereas, oospores are sexual spores and these are produced from fertilization of the oogonium by an antheridium. Mycelium of *Phytophthora* is coenocytic, hyaline and profusely branched mainly of monopodial branches. The septa are formed at the time of reproduction (Naik *et al*. 2020).

Though the disease is sporadic in nature, occasionally it assumes epidemic proportions in places of heavy and frequent rainfall leading to mortality of young plants (Kannaiyan *et al*. 1984). The disease caused heavy plant mortality at seedling and vegetative stages, resulting in poor plant stand and lower yield (Mishra and Shukla, 1987). Characteristic symptoms of the disease are water-soaked lesions on the leaves and slightly sunken lesions on stems and petioles. Lesions girdle the stem and the foliage dries up (Dhar *et al*. 2005).

Phytophthora blight may appear at any growth stage; however, the seedling stage is most vulnerable. *Phytophthora* blight incidence data from last decade clearly determined the most favorable conditions for an outbreak were ≥ 300 mm rainfall in 1 week with maximum temperature of 28–35 °C, minimum of 12–24 °C and relative humidity >75%. Recently, *Phytophthora* blight become endemic and a potential treats to pigeonpea production, especially during excessive rain within a short time that creates temporary flooding coupled with hot and humid weather (Sharma *etal*. 2006 and Pande *et al*. 2011).

Phytophthora drechsleri, attacks on young (1-7 week-old) plants of pigeonpea, which in turn kills the young plants at the early stage of crop. Crop stand to leave large gaps in overall plant stand. Yield losses are generally higher in early maturing pigeonpea in comparison to medium and long-duration varieties, because of favorable disease triangle components in early pigeonpea (Naik *et al*. 2020). Soil moisture status is an important factor in biology of *Phytophthora* spp. Poor soil aeration and prolonged soil saturation enhances reproduction and dissemination of *Phytophthora* (Mamta *et al*. 2019).

Information on economic losses caused by PB is meager, but in last few years 100% yield losses under favorable environment was recorded (Sharma *et al*. 2015).

Considering the economic importance of the disease, present study was undertaken with following objectives.

OBJECTIVES

1. To isolate, identify and prove pathogenicity of *Phytophthora drechsleri* f. sp. *cajani*
2. To study the effect of different culture media on morphological and cultural characteristics of test pathogen
3. To study the effect of various pH, Temperature and Nitrogen sources on growth of *P. drechsleri* f. sp. *cajani*
4. To evaluate *in vitro* efficacy of various fungicides, biocontrol agents and phytoextracts against *P. drechsleri* f. sp. *cajani.*

CHAPTER - II

REVIEW OF LITERATURE

CHAPTER- II

REVIEW OF LITERATURE

The relevant literature available on different aspects of stem blight of pigeonpea (*Phytophthora drechsleri*) and other crop hosts and different species of organism have been reviewed herein under following sub-heads.

2.1 Occurrence, distribution of *Phytophthora drechsleri* f. sp. *cajani*

A serious stem blight of pigeon pea [*Cajanus cajan* (L.) Millsp.] was first reported in India in 1966 (Williams *et al*. 1968).

The disease caused by *Phytophthora drechsleri* has spread to most pigeonpea growing countries like Asia(Pal *et at.* 1970 and Williams *et al*. 1975), Africa (Birch, 1988), Dominican Republic, Kenya, Panama and Puerto Rico (Nene *et al*. 1996).

Kannaiyan and Nene (1985) evaluated that, *Atylosia scarabaeiodes* and *A. platyacarpa* as an alternative host for *Phytophthora drechsleri* pathogen to survive and perpetuate from season to season. The disease has been reported from most pigeonpea growing areas inAsia, Africa, die Americas, Australia, Dominican Republic, Kenya, Panama and PuertoRico.

Mishra and Shukla (1987) observed that, incidence of *P. drechsleri* f. sp. *cajani* was highest in Kanpur (22.3%). No infection was recorded in 10 of the 40 districts surveyed. Incidence was lower in western districts, where the crop is grown in a rotation, than in central and eastern districts of Uttar Pradesh where, pigeon pea is the main pulse crop and grown continuously in the same field.

The stem blight was first observed during Kharif, 1982 at Amgaon in Bhandara district, Vidarbha, India. Symptoms included brown lesions on stems of 1-3-month-old plants at or just above ground level. The lesions increased in size, encircling the stem and causing it to swell. Plants gradually died. The symptoms were thought similar to those reported for *P. drechsleri* f.sp. *cajani* from Andhra Pradesh. None of the 650 cultivars examined during the 1982-83 and 1983-84 seasons was resistant, although 38-2, AKT8, T2, ICP7701 and ICP7958 were moderately resistant (less than 10% incidence) (Raut and Somani, 1988).

A pigeon pea plot at the ICRISAT Centre, Patancheru, India was observed daily during the 1987 and 1988 rainy seasons for blight infection (caused by *P. drechsleri* f. sp. *cajani*). Disease onset occurred on July 10 and on Aug. 4 in 1987 and 1988, respectively. In both the years, infection and subsequent disease development occurred when day temperature were < 28°C. Rain was recorded on 7 consecutive days prior to disease onset in 1987 and on 5 of 7 days in 1988. An increase in soil inoculum levels was also associated with a decrease in day temperature with higher rainfall and cloudy weather (Reddy *et al*. 1992).

The intermittents of *Phytophthora* blight in the recent past was a major risk to pigeonpea production and productivity in the Deccan Plateau of India and was reported irrespective of cropping system, soil types and genotypes (Sharma *et al*. 2006 and Pande *et al*. 2006).

Stem blight disease in the pigeonpea was observed in growing areas of Sehore, Bhopal and Rajgarh district of Gwalior (Madya Pradesh). Disease incidence ranged from 3.50 to 37.90 %. Maximum per cent disease incidence was recorded in Rajgarh tehsil followed by Icchawar, Ashta and Huzur, while minimum disease incidence was recorded in Narsullaganj followed by Jawar, Rehti and Sehore (Patel, 2016).

2.2 Yield losses caused by *Phytophthora drechsleri* f. sp. *cajani*

Since from 1966, the disease has spread to most pigeon-pea-growing areas in India, resulting in heavy economic losses (Pal *et al*. 1970 and Williams, 1975).

Pal *et al.* (1970) estimated yield losses due to *Phytophthora* blight to be 98%, since the affected plants dry up rapidly**.**

In South India, total yield loss was observed in some short-duration pigeonpea crops (Reddy and Sheila, 1994).

The loss of more than 84.5% of the plant population was recorded in short duration cultivar (Singh, 1996).

Information on economic losses caused by PB is meager, but in last few years 100% yield losses under favorable environment was recorded (Sharma *et al*. 2015).

2.3 Symptomology

Phytophthora drechsleri present symptomless in the rhizosphere of pigeonpea, and the infection was only evident, when the favorable disease triangle exists (Stanier *et al*. 1971).

The symptoms of the *Phytophthora* blight disease on pigeonpea have been described in detail by Pal *et al*. (1970) as stem rot, by Williams *et al*. (1975) as stem blight and by Kaiser and Melendez (1978) as a stem canker.

Agrawal and Khare (1987) reported that, per centage of rainy days had a significant positive correlation with infection index and increase in the lesion size of *Phytophthora drechsleri* f. sp. *cajani*. Plant mortality due to the disease was favored by max. rain and rainy days, high RH and small differences between max. and min. temperature.

In India, the fungus affects the collar region and all above-ground parts of the plant. In Australia, *Phytophthora drechsleri* has been reported to cause a serious root rot in addition to chlorosis and lesions on stems of pigeonpea (Wearing and Birch, 1988).

Symptoms included brown lesions on stems of 1-3-month-old plants at or just above ground level. The lesions increased in size, encircling the stem and causing it to swell. Plants gradually died. (Raut and Somani, 1988).

Characteristic symptoms of the *Phytophthora* blight were water-soaked lesions on the leaves and slightly sunken lesions on stems and petioles. Lesions girdle the stem and the foliage dries up (Dhar *et al*. 2005).

In seedling stage, young seedlings were killed within 3 to10 days. On foliage, water-soaked lesions were seen on the leaves and whole foliage gives desiccated appearance. Brown to dark brown lesions, distinctly different from the healthy green portions. These lesions increase rapidly and usually girdle the stem; infected stems break easily at lesion site. Phloem is smoky gray colored and the xylem remains clear. It is also common to find stem and branches swollen at base or else turning into a cankerous hyper tropical structure. The roots of the PB infected plants are healthy and cannot be uprooted easily (Mamta *et al*. 2011).

Phytohpthora blight symptoms occurs, when cloudy weather accompanied by intermittent rains and moderate temperatures of 25 ± 1°C during seedling stage. *Phytophthora* blight symptoms observed as circular or irregular water-soaked lesions on the leaves and slightly sunken lesions on stem and petioles. The stem girdles by lesion and the foliage dries up, stems swollen into cankerous structures. Phloem vessels show smoky gray colored discoloration and xylem vessels remain healthy (Asmita 2015).

PB symptoms resemble damping-off disease that causes seedling to die suddenly. Infection in seedlings was visible as water-soaked lesions in the trifoliate leaves, which subsequently become necrotic. The leaflet lesions were circular to irregular and up to 1 cm in diameter. The whole foliage can exhibit a blighted appearance under condition of high humidity (>80). On stems, brown to dark brown lesions distinctly marked from the healthy green portion were formed near ground level. The lesions on the stem and branches increase rapidly, causing the portion of the plant above the lesion to dry out but remain attached to the plant. Wind easily break up stems at the infected point. Some infected pigeonpea plants often produce large galls on their stems. As the disease progresses, patches of diseased plants become conspicuous in the field from a distance (Mamta and Ghosh, 2018).

Phytophthora stem blight resembles damping off disease at the early stage of infection that causes young seedlings to die after infection. Further infected plants have water-soaked lesions on their leaves and brown to black spots, slightly sunken lesions on their stems and petioles. Infected plant parts lose turgidity and become desiccated. Lesions strap the affected main stem or a branch, which leads to break at that infected point, causing the foliage above the lesion to dry up and lodging. Pigeonpea plants that were infected by blight, but not killed, often produce large galls on their stems especially at the edges of the lesions (Naik *et al*. 2020).

2.4 Isolation of the *Phytophthora drechsleri* f. sp. *cajani*

Williams *et al*. (1968) isolated *Phytophthora* blight causing pathogen from pigeonpea plant with stem canker symptoms at New Delhi, India.

Kannaiyan *et al*. (1980) isolated different *Phytophthora* spp. from blighted pigeonpea (*Cajanus cajan*) plants from different locations in India.

Shen (2011) isolated different *Phytophthora* spp. from plants with blight and wilt symptoms, which were observed in commercial vegetable farms in Changhua, Taiwan.

Mamta and Ghosh (2016) isolated *Phytophthora drechsleri* f. sp. *cajani* from the infected pigeonpea plants by tissue isolation method.

Mamta and Ghosh (2018) isolated *Phytophthora drechsleri* f. sp. *cajani* from pigeonpea plants by stem tissue isolation method.

2.5 Identification of the *Phytophthora drechsleri* f. sp. *cajani*

Pande *et al*. (2011) identified of *Phytophthora drechsleri* Tucker f. sp. *cajani* based on morphological characters. Sporangia of *Phytophthora drechsleri* Tucker f. sp. *cajani* were proliferating type with size ranging from 42-43 × 28-48 µm. Oogonium and oospore size showed ranging from 19-29 to 34-44 µm.

Jung *et al*. (2011) identified on basis of morphological characters of sporangia *viz*., obpyriform, ovoid or elongated, non-papillate often with tapering base and having size 52 × 28 µm.

Mamta and Ghosh (2016) identified of *Phytophthora* on the basis of morphological characters like mycelial type and sporangia shape as well as molecular level using ITS region sequencing.

Patel (2016) identified stem blight as *P. drechsleri* f. sp. *cajani* of pigeonpea. On tomato juice agar, at 25±2ºC, the culture of *P. drechsleri* f. sp. *cajani* was dull white colour with fluffy growth. Mycelium was hyaline, branched, filamentous to slender, coenocytic when young but later septate with thick plugs and abundant hyphal swelling of mycelium was hyaline 11-14 µ in diameter with tube like projections.

Mamta and Ghosh (2018) observed that, hyphae were coenocytic and branched, some isolates formed hyphal swelling. The sporangium varied in different isolates from broadly ovoid, obpyriform to elongate and non-pappilate.

Abad *et al*. (2019) observed that, sporangia papillation and caducity were important characters for identification, as the sporangiophore shape and the presence/absence and shape of hyphal chlamydospores.

Jadesha *et al.* (2020) observed that, the pathogen was homothallic with amphigynous antheridium and oogonium and able to produce oospore *in vitro*. Sporangium was non-papillate, non-caducous, oviod-obpyriform shape. The colony characteristics were dull white, flat and rosette pattern.

Naik *et al*. (2020) identified *Phytophthora drechsleri* Tucker f. sp. *cajani* as hyphae lack cross wall septa and diploid phase. The *Phytophthora drechsleri* Tucker f. sp. *cajani* has terminal papillate hyphae which in turn produced the spores. The sizes of sporangia of *Phytophthora drechsleri* var. *cajani* ranging from 42 to 83 × 29 to 48 µm (average 61.8 × 37.3 µm) and the sporangial stalks was either narrowly tapered or widened some what at the base of the sporangium.

2.6 Pathogenicity of the *Phytophthora drechsleri* f. sp. *cajani*

Nene *et al*. (1981) proved pathogenicity by soil drenching method and spray inoculation method on seedlings of HY-3C variety of pigeonpea. They used V8 juice broth for mass multiplication of *Phytophthora drechsleri*.

Mamta and ghosh (2016) proved pathogenicity by spore suspension method using sporangia and zoospore suspension containing 1.5 x 10 zoospores/ml.

Patel (2016) proved pathogenicity of *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea by three different methods *viz*., stem inoculation method, leaf attach method and spray method applied to prove the Koch's postulates. He reported that, symptoms of *Phytophthora* stem blight developed start after five days in infected leaf attach method, symptoms were initially expressed brown to dark brown lesions distinctly marked from the healthy green portion, were formed near ground level after three days of inoculation in stem inoculation method. While, first symptom on the seedling appeared as water soaked lesion on primary and trifoliate leaves within 4 days after inoculation in mycelium mat spray method.

Chand *et al*. (2017) proved pathogenicity by node inoculation method. In this method, the petiole on pigeonpea was removed for easy, precise inoculation of node with *Phytophthora drechsleri* f. sp. *cajani*.

Jadesha (2020) proved pathogenicity of *P. drechsleri* f. sp. *cajani* with susceptible variety ICP 7119 by soil drenching method. He reported that, first symptom on the seedling appeared as water-soaked lesion on primary and trifoliate leaves within 48 hours after inoculation. The seedlings showed characteristics symptoms of crown rot which completely collapsed and died within 7 days.

2.7 Effect of different media on growth of *Phytophthora drechsleri* f. sp. *cajani*

Medina and Platt (1999) reported that, effect of nine media were compared to rye agar (RA) which was frequently used to culture *Phytophthora infestans*. Poor fungal growth across most isolates was observed on soybean agar, carrot agar and carrot agar with sterols relative to RA.

Asmita (2015) studied ten culture media and reported that, nine culture media exhibited better mycelial growth of *P. drechsleri* f. sp. *cajani* of pigeonpea crop. She reported that, most suitable media were Oat meal agar with maximum radial mycelia growth (88.66mm), followed by V8 juice agar (86.33 mm), Pigeonpea seed meal agar

(84.00 mm), Yeast mannitol agar (82.33 mm) and Corn meal agar (82.00 mm). The colonies produced were mostly white, aerial and fluffy dense.

Patel (2016) studied six different culture media *viz*., Potato dextrose agar, Pigeonpea seed extract agar, Bean seed extract agar, V-8, Tomato juice agar and Pea seed extract agar which were evaluated to find out the appropriate growth of *P. drechsleri* f. sp. *cajani* of pigeonpea. He reported that, among the culture medium, significantly maximum mycelial growth was recorded in Pea seed extract agar (90.00 mm) followed by Bean seed extract agar (77.78 mm), V-8 (77.45 mm) and Tomato juice agar (76.67 mm). However, minimum mycelial growth was recorded in Pigeonpea seed extract agar (48.33 mm) followed by Potato dextrose agar (69.44 mm).

Mohsan *et al*. (2017) studied different growth media like PARP (Pimaricin + Ampicilin + Rifampicin + PCNB), Carrot Agar, Rye Agar and V8 Juice Agar were evaluated against mycelial growth of *Phytophthora capsici*. During isolation, PARP was found the best medium as mean colony diameter of 2.2 cm was observed while, no growth of *P. capsici* was found on any other media.

Thomas and Naik (2017) reported that, eight different culture media *viz*., V8 Juice Agar, Richard's Synthetic Agar, Sabouraud Dextrose Agar, Oat Meal Agar, Corn M6l Agar, Carrot Dextrose Agar, Potato Dextrose Agar and Rye Agar A used for growth of *Phytophthora capsici* and proved that, Oat meal agar, V8 Juice agar, Potato dextroseagar and Rye agar A supported maximum colony diameter (90 mm).

Saykar *et al*. (2018) reported that, eight artificial media, Oat meal agar medium (OMA), Potato dextrose agar medium (PDA), Host leaf extract agar medium (HLEA), Carrot agar medium (CAM), Tomato agar medium (TAM), Soybean agar medium (SAM), Water agar medium (WAM) and V8 agar medium (V8) were assessed *in vitro* for growth analysis of *Phytophthora colocasiae* Racib causal agent of leaf blight disease of Colocasia. They proved that OMA was the most effective medium, as it recorded maximum (90mm) mycelial growth of the pathogen, after 7 days of inoculation. It was followedby HLEA (87.67mm), CAM (87.33mm), PDA (84.67mm), TAM (54.67mm), WAM (41mm), SAM (38mm) and V8 (28.33mm) was the least effective medium, respectively.

Kumar *et al*. (2019) studied different growth media, such as Potato dextrose agar, V8 juice agar, Maltose agar, Corn meal agar, Potato sucrose agar, Tomato juice agar, Czapek's sucrose agar and carrot agar were evaluated against mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea under *in vitro* condition. The maximum mycelial growth (90.0 mm) was recorded on Potato dextrose agar medium ± 28°C after seven days of incubation followed by V-8 juice agar medium (89.8mm). The lowest mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* (15.0 mm) were observed in corn meal agar medium.

Jadesha *et al*. (2020) studied different media like Oat meal agar, Corn meal agar, V8 juice agar, Pigeonpea seed meal agar, Chickpea seed agar, Potato dextrose agar, Czapek dox agar, Carrot agar, Tomato extract agar, Potato dextrose agar and Potato glucose agar under *in vitro* condition. After growing at 30°C for six days, *P. cajani* of pigeonpea reported the fastest growth on tomato extract agar medium (90 mm) and next best was V8 juice agar medium (89.0 mm) and least growth of 40.67 mm was observed on carrot agar medium.

2.8 Effect of different nitrogen sources on growth of *Phytophthora drechsleri* f.sp. *cajani*

Singh (1973) studied nutrition of two isolates of *Phytophthora palmivora* causes pod rot of cocoa in pure culture. Several amino acids supported good growth of both isolates but casein hydrolysate was the best nitrogen source for one isolate. Ammonium sulphate, urea and potassium nitrate were poor nitrogen sources for both isolates.

Riga *et al*. (2000) studied on toxic effects of solutions of inorganic nitrogen compounds HNO_2 and NH_3 on mycelium growth of *P. capsici* isolated from *Capsicum annuum* grown in the greenhouse. Experiments were carried out in *in vitro* conditions at different concentrations (from 0 to 106 µM HNO_2 and from 0 to 471 µM NH_3) and treatment duration (6, 15 and 24 h). Cumulative growth recorded for 3 days after HNO_3 treatment showed a lethal effect at 96 µM for 6 h of treatment, 42 µM for 15 h and 21-42 µM for 24-h treatment. NH_3 solutions from 0 to 471 µM had no lethal effect and at 235 µM had inhibitory effect.

Faisal *et al*. (2005) studied the effect of different nitrogen sources on the growth of *Phytophthora cactorum* causing root rot of apricot on basal agar medium. Ammonium nitrate was the best for mycelial growth of the fungus.

Nakova (2011) found that, most of the amino acids stimulated the mycelial growth of *Phytophthora cactorum* with the exception of L-hydroxyproline, L-phenylalanine, L- triptophane and L-leucine. NH_4NO_3 added to the medium showed higher rate of the mycelial growth compared to the control (no nitrogen). An opposite tendency- a slowermycelial growth was found in *Phytophthora citrophthora*, when amino acids were added as a nitrogen source. That was indicated by the suppressed development of the fungi. The colony size was similar to the control (no nitrogen) when L-tyrosine, L- treonine, KNO_3, NH_4NO_3 or $NaNO_3$ were added to the medium.

Asmita (2015) studied that, nitrogen sources exhibited varied radial mycelial growth of the *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea. She reported that, highest radial mycelial growth (82.66 mm) was recorded on Potassium nitrate, followed by Ammonium nitrate (75.00 mm), Cysteine (70.66) and Leucine (62.00 mm). Least radial mycelial growth (35.00 mm) was recorded on Magnesium nitrate.

Morales *et al*. (2019) reported that, effect of different N sources on the mycelial growth of *Phytophthora cinnamoni* in Walnut and proved that, Ammonium nitrate (NH_4NO_3) and Ammonium sulphate [$(NH_4)_2SO_4$] allowed for greater control of *Phytophthora cinnamoni* mycelia in comparison with Calcium nitrate [Ca $(NO_3)_2$] and Potassium nitrate (KNO_3) when used with 1000 ppm nitrogen.

2.9 Effect of temperature on growth of *Phytophthora drechsleri* f. sp. *cajani*

Brasier (1969) reported that, the optimum temperature for sexual reproduction of *Phytophthora palmivora* (Butl.) Bud., *in vitro* and in the dark, lay close to 20°C, whereas, that for growth and sporangial formation was 27-30°C.

Kannaiyan *et al*. (1980) studied the morphology and growth rates of different isolates of *P. drechsleri* f. sp. *cajani* were studied on CV8A, at the following temperatures: 5, 9, 15, 21, 24, 27, 30, 33, 36 and 39°C. The optimum temperature for growth of all isolates was 27 to 33°C, minimum 9°C and maximum 36°C.

Purwantara *et al*. (1997) evaluated that, effect of temperature on the growth of three races of *Phytophthora clandestine* and observed that, temperature for growth in culture, infection and race identification was approximately 20°C in clover cultivars.

Simpfendorfer *et al*. (2001) reported that, mycelial growth occurred at temperature from 10 to 30°C. Sporangial production was greatest between 20°C and 25°C of *Phytophthora clandestine* in subterranean clover (*Trifolium subterraneum* L.) causes tap root rot.

Singh *et al*. (2010) proved that, the average growth (1.69 cm) at 30°C temperature was recorded with all the isolates followed by average growth (1.25 cm) at 35°C temperature.

Varsha *et al*. (2010) reported maximum vegetative growth of *Phytophthora drechsleri* f. sp. *cajani* was recorded on oat meal agar medium at 30°C temperature.

Sporangial germination of the pathogen started at 15°C after 180 minutes and at 20-25°C after 120 minutes.

Asmita (2015) studied *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea exhibited differential response to various temperature regimes. She reported that, the test fungus could grow in a wide temperature range of 5 - 40°C; however, it could proliferate better with significantly highest mycelial growth of 86.33 and 84.00 mm at the temperatures of 30 and 25°C, respectively. However, the temperatures below 10°C and above 40°C were found to inhibit the growth of the test fungus.

Singh *et al.* (2017) reported average growth of *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea on potato dextrose agar medium was 1.69 cm^2 at 30°C temperature recorded with all the isolates followed by average growth (1.25 cm^2) at 35°C temperature in pigeonpea.

Jadesha *et al*. (2020) studied growth of *P. cajani* on tomato extract agar medium at different temperatures of 5, 10, 15, 20, 25, 30, 35 and 40°C. They reported fastest growth which was observed at 30°C (90.0 mm) followed by 25°C (87.4 mm). Pathogen did not showed any growth when, plates were incubated at temperature of 5, 10 and 40°C whereas, 35°C showed the growth of 23.5 mm. Hence, *P. cajani* can tolerate high temperature of 35°C.

2.10 Effect of pH on growth of *Phytophthora drechsleri* f. sp. *cajani*

Duncan (1985) reported that, spores germinated between pH 4-8 and the optimum for germ tube formation was pH 5.5 to 7.

Kim *et al*. (1989) studied influence of various *in vitro* conditions on growth of *Phytophthora capsici* pathogen of pepper crown and root rot. The rates of 11 zoospore release and germination of encysted zoospore was found highest at pH 7 with 73.4% andfollowed by 62.1% at pH 6 and 34.0% and pH 5, respectively. However, no zoospore was released atpH 4.

Sahu *et al*. (2000) studied *in vitro* effect of pH on the growth of *Phytophthora cajani*. The pH of 6.5 was the optimum condition for the pathogen growth.

Simpfendorfer *et al*. (2001) reported that, mycelial growth occurred at pH 3.5 to 9.0 and sporangial production was greatest between pH 4.0 to 9.0 of *Phytophthora clandestine* in subterranean clover (*Trifolium subterraneum* L.) causes tap root rot.

Gul *et al*. (2005) reported about physiological studies on *Phytophthora syringae* causing root rot of almond. The best pH level for the colony growth of the fungus was found 6.

Varsha *et al*. (2010) reported that, maximum vegetative growth of *Phytophthora drechsleri* f. sp. *cajani* was recorded on oat meal agar medium at 6.5 pH. Maximum sporangial germination was observed at 6.5 pH in complete dark condition.

Asmita (2015) studied on different pH of culture media from 4 to 8, tested and encouraged variable growth and sporulation of *P. drechsleri* f. sp. *cajani* of pigeonpea crop. She recorded mean colony growth with all the pH levels was ranged from 30.33 mm at pH 4.0 to 87.33 mm at pH 6.5. However, significantly maximum mean mycelial growth (87.33 mm) was recorded at pH 6.5. The second and third best pH levels found was pH 6 (84.33 mm) and pH 7 (84.00 mm). This was followed by pH 7.5 (81.66 mm), pH 8.0 (79.66 mm). The pH 4.0 and 4.5 was found least suitable and which recorded minimum mycelial growth 30.33 mm and 39.00 mm, respectively of the test pathogen.

2.11 Disease management strategies

2.11.1 Efficacy of fungicides

Pal and Grewal (1983) reported that, the best protection against *Phytophthora drechsleri var. cajani* was given by Brestan 60 % WP [fentin acetate] in trials with 7 fungicides applied to potted plants before inoculation.

Kanniyan and Nene (1984) proved that, significant control of the blight was achieved with Metalaxyl 35 % WS (1.75 g a. i./kg seed) in greenhouse trial.

Agrawal (1987) tested efficacy of 13 fungicides *in vitro*, mycelial growth was completely inhibited by 250 ppm. of zineb 75 % WP, copper oxychloride 50 % WP, metalaxyl 35 % WS, thiram 75 % WP and captan 75 % WP and by 1000 ppm. Mancozeb 75 % WP. When 7 selected fungicides were tested in the field, applications of metalaxyl 35 % WP at 30 and 45 days after sowing gave max. reduction in stem blight incidence of pigeonpea.

Bhisht *et al*. (1988) studied on application of metalaxyl 4 % + mancozeb 64 % WP (Ridomil) as a seed dressing, a soil treatment prior to sowing and sprayed at 40, 55 and 70 days after sowing were evaluated for control of *P. drechsleri* f. sp. *cajani* on 2 pigeon pea lines, HY 3C (susceptible to the predominant P_2 isolate) and ICP 1 (tolerant of P_2). Disease incidence was much higher on HY 3C than on ICP 1. Seed dressing alone was ineffective, but spray treatment alone or in combination with seed dressing gave the most effective control of P. blight. Spraying was most effective on the tolerant line, resulting in 23% disease incidence as compared with 86% in the untreated control.

Jadeja *et al*. (2000) reported efficacy of various chemical treatments for the control of gummosis (*Phytophthora nicotianae* var. *parasitica*) of citrus sp. Six-year-old acid lime (Kagzi lime) trees were treated with Bordeaux paste [Bordeaux mixture], Ridomil MZ 72 % WP [metalaxyl 8 % + mancozeb 64 %], streptomycin sulphate + copper oxychloride, fosetyl Al 80 % WP [fosetyl] and aureofungin solution, alone and in various combinations. The results revealed that, Bordeaux paste application and soil drenching of metalaxyl 8 % + mancozeb 64 % as well as fosetyl Al 80 % WP either alone or in combination with foliar application of streptomycin sulphate + copper oxychloride significantly reduced gummosis incidence.

Perry *et al*. (2006) reported that, fungicide fosetyl Al 80 % WP (Aliette) may be used on a number of ornamental plant species to help prevent *Phytophthora* infections. When applied as a foliar spray, it is absorbed by foliage and moves into roots. However, do not rely on fungicide applications alone to control root and crown rot diseases.

Jagtap *et al.* (2012) studied nine fungicides tested *in vitro* against *Phytophthora nicotianae*, *Phytophthora citrophthora* and *Phytophthora palmivora* of citrus. He reported that, Cymoxynil 8 % + Mancozeb 64 % WP (Curzate M-8) recorded minimum mean colony diameter (16.12 mm) and maximum mean inhibition (82.09%) of mycelial growth of the test pathogen over untreated control (mean colony diameter 90.00 mm and mean inhibition 0.00) followed by the fungicide Metalaxyl-M 4% + Mancozeb 64% (Ridomil), Metiram 70 % WG (Polyram), which recorded mean colony diameter of 20.16 mm, 24.16 mm and mean mycelial inhibition of 77.59 %, 73.14 %, respectively.

Jadehsa (2014) studied two fungicides *viz*., metalaxyl and mefenoxam against *phytophthora drechsleri* f. sp. *cajani* under *in vitro* condiion. He reported that, mefenoxam was statistically superior over metalaxyl in reducing the growth and inhibition of zoospore. A concentration of 2.0 µg/ml inhibited 100 per cent of mycelial growth in both the fungicides, whereas 100 per cent inhibition in induction of zoospores was achieved at 2.0µg/ml and 5.0 µg/ml concentration of mefenoxam and metalaxyl, respectively.

Asmita (2015) studied four systemic and four non-systemic tested were found effective against *P. drechsleri* f. sp. *cajani* of pigeonpea. However, systemic fungicides *viz*., Metalaxyl 4 % + Mancozeb 64 % WP (97.42 %), Cymoxanil 8 % + Mancozeb 64

% WP (93.70 %) and Propineb 70 % WP (81.41 %) recorded significantly highest average mycelial inhibition of the test pathogen. This was followed by non-systemic fungicide Mancozeb (78.14%).

Patel (2016) reported that, complete mycelium inhibition was recorded in fungicides *viz*., Trifloxystrobin 25 % WG, Chlorothalonil 75 % WP, Pyrachlostrobin 20 % WG, Fosetyl Al 80 % WP, Mancozeb 64 % + Mefenoxam 4 % WP and Copper Oxychloride 50 % WP (0.00mm) at 200, 1500, 2000, 1000, 2500 and 2500 ppm concentraction, respectively. While, the growth reduction was less in Azoxystrobin (79.33 mm) followed by Metalaxyl 4 % + Mancozeb 64 % WP (27.67 mm) against *P. drechsleri* f. sp. *cajani* of pigeonpea.

Rolando *et al*. (2016) proved that, phosphite, Copper oxychloride 50 % WP and Metalaxyl-M 35 % have potential to protect commercially planted *Phytophthora radiata* from *Phytophthora kernoviae* and *Phytophthora pluvialis* these two *Phytophthora* species.

Thomas and Naik (2017) reported that, out of the ten fungicides tested *in vitro*, the systemic fungicide Azoxystrobin 23 % SC at all three concentrations (250 ppm, 500 ppm and1000 ppm), the combi products Fenamidone 10 % + Mancozeb 50 % WG (Sectin) and Famaxodone 22.1 % + Cymoxanil 16.6 % SC (Equation pro) at all the three concentrations (1000 ppm, 2000 ppm and 3000 ppm) were found highly inhibitory against *Phytophthora capsici*. Bordeaux mixture (1%) at 2000 ppm and 3000 ppm gave 100% mycelial inhibition of the fungus.Potassium phosphonate at 1000 ppm were also inhibitory to the pathogen. The least effective was dimethomorph followed by Cymoxanil 8 % + Mancozeb 64 % WP combination (Curzate M8).

Muhammad and Syed (2018) studied at Potato Research Station, Sialkot during 2014-15 and 2015-16 crop season found out suitable fungicides to combat the late blight disease of potato. Six fungicides *viz*., Ridomil Gold (Metalaxyl 4 % + Mancozeb 64 % WP), Defeater Plus, Puslsor (Thifluzamide 24 % SC), Success 72 % WP, Aleitte (Fosetyl Al 80 % WP) and Dithane M-45 75 % WP were tested against the disease in two consecutive seasons. They reported that, all fungicides reduced the late blight disease incidence of potato over control. Among the fungicides, alternate spray of Ridomil Gold (Metalaxyl 4 % + Mancozeb 64 % WP) and Dithane M-45 75 % WP was found highly effective to minimize the late blight and to increase the yield of potato.

Jadesha *et al*. (2019) proved that, commercial formulations of Mefenoxam 4 % + Mancozeb 64 % (Ridomil Gold MZ 68 WG) and Metalaxyl 35 % WS (Apron 35 SD) were highly inhibitory to mycelial growth of *Phytophthora cajani* in *in vitro*.

Kumar *et al*. (2020) evaluated different six fungicides namely Potassium paramagnate, Bavistin 50 % WP, Metalaxyl 50 % WP, Tebuconazole 50 % WP, Mancozeb 75 % WP and Tricyclozole 75 % WP on mycelial growth of *Phytophthora infestans* (Mont.) de Bary and proved that, minimum radial growth with 0.75 mm in diameter was recorded at 300 ppm concentration, which was followed by 200 ppm and 100ppm and 50 ppm. However, no mycelial growth was recorded at 400 ppm. As per concern of per cent inhibition, the highest with 100 % inhibition was recorded from 400 PPM concentration of Metalaxyl 35 % WS.

2.11.2 Efficacy of bioagents

Shahidi *et al*. (2006) studied on 130 Actinomycetes isolates against *Phytophthora drechsleri* Tucker causes gummosis and root rot of pistachio and proved that, 12 Actinomycetes inhibited growth of the pathogen of pistachio gummosis in culture plates and four of the most active isolates exhibited biological control of the pathogen under greenhouse condition.

Singh and Dubey (2010) reported that, *Pseudomonas fluorescens* produced maximum inhibition zone and lysed the mycelium of *P. drechsleri* f. sp. *cajani of* pigeonpea crop. The inhibition zone formed by *B. subtilis* was significantly lower than the *Pseudomonads*, whereas no zone of inhibition was observed in the case of *Trichoderma. viride* and *T. hamatum*. *Trichoderma* grew faster than *P. drechsleri* f. sp. c*ajani* and the bacterial bioagents and overgrew on the colony of the pathogen. A clearly visible yellow band was formed near the zone of contact. After seven days of incubation, the entire Petri dish was covered with a greenish white growth of *T. viride* and *T. hamatum*.

Tabarraei *et al*. (2011) studied *in vitro* screening of 210 bacterial isolates of *Pseudomonas* spp. Against *Phytophthora drechsleri* of cantaloupe plants (*Cucumis mello* L.) and reported that, 16 isolates of *Pseudomonas* spp., which were the most effective antagonists, which inhibited the mycelial growth of *Phytophthora drechsleri* by production of antibiotics and extra cellular compounds. Two isolates of *P. putida* (SCh) and *P. aeruginosa* (Zp) produced cyanide hydrogen. Seven of the 16 isolates, including *P. aeruginosa* (SG1 and Zh2), *P. putida* (SG2 and Zh1) and *P. fluorescens*

(SSh, ZK1 and ZK2) produced siderophore and inhibited spores of *Geothricum candidum*. Isolates *P*. *fluorescens* (ZA, SSh and SU1) *P*. *putida* (SCh and SG2) and *P*. *aeruginosa* (SG1) produced Protease. Only one isolate of *P*. *putida* (SK1) produced cellulose.

Jagtap *et al.* (2012) studied six bioagents tested *in vitro* against *Phytophthora nicotianae*, *P*. *citrophthora* and *P*. *palmivora* of citrus. They evaluated that, *Trichoderma harzianum* recorded minimum mean colony diameter (7.73 cm^2) and highest inhibition (87.85%) of mycelial growth of *P*. *nicotianae* over untreated control followed by the bioagents *T. viride*, *T. koningii* which recorded mean colony diameter of 9.95 cm^2, 14.15 cm^2 and mean mycelial inhibition of 84.36 %, 77.76%, respectively.

Jadesha (2014) studied the five isolates of *Trichoderma* and four isolates of *Pseudomonas* against the growth of *P*. *drechsleri* f. sp. *cajani*. He reported that, *Trichoderma* isolate-3 (80.52 %) and *Pseudomonas* isolate-1 (71.90 %) gave highest growth inhibition followed by *Trichoderma* isolate-4 (75.48 %) and *Pseudomonas* isolate-4 (63.90 %). The least growth inhibition was in *Trichoderma* isolate-1 (60.85 %) and *Pseudomonas* isolate-2 (54.30 %).

Asmita (2015) studied the five fungal and two bacterial antagonists tested, exhibited significant mycelial growth inhibition of *P*. *drechsleri* f. sp. *cajani*. However, *T. harzianum* recorded significantly highest mycelial growth inhibition (80.74%), followed by *T. viride* (70.74%) and *T. hamatum* (67.03%) and *Pseudomonas fluorescens* (65.55%). Rest of the bioagents tested also caused significant mycelial inhibition of the test pathogen. *B. subtilis* was found comparatively less effective with minimum of 38.51 per cent mycelial inhibition.

Rashmi *et al*. (2015) evaluated *in vitro* efficacy of two bioagents against *Phytophthora parasitica* causing root rot in citrus. They reported that, maximum mycelial growth inhibition with *Trichoderma viride* (75.33%) followed by *Pseudomonas fluorescens* (70.22%).

Bae *et al*. (2016) evaluated various *Trichoderma* species which are well-known biological control agents and reported that, *Trichoderma atroviride/petersenii* (KACC, Korea Agricultural Culture Collection, 40557) and *Trichoderma virens* (KACC 40929) showed the strongest inhibitory activities against *Phytophthora* isolates.

Thomas and Naik (2017) reported that, *Pseudomonas fluorescens* was found most effective ininhibiting the growth of *Phytophthora capsici* in black paper.

Fariba *et al*. (2018) proved that, *Phytophthora drechsleri* can be controlled in pistachios by using PGPR bacteria. *Pseudomonas fluorescens* VUPF5 and *B. subtilis* Bs96 demonstrated highest efficacy in the suppression of diseases by increasing plant defense enzymes.

Muhammad *et al*. (2019) evaluated that, *Trichoderma viz*., *T. asperellum* TH, *T. harzianum* HM, *T. harzianum* HK and two morphologicall characterized isolates of *Bacillus subtilis* against *Phytophthora capsici* (Leonian), a threating pathogen of fruit rot of chili and reported that, in dual culture assay, *T. asperellum* showed maximum (61.6%) mycelial growth inhibition followed by *Bacillus subtilis* A (54.3%), *T. harzianum* HK (51.4%), *T. harzianum* HM (47.2%) and *B. subtilis* B (41.5%), respectively.

Muhammad *et al*. (2019) studied on root rot of muskmelon caused by *Phytophthora drechsleri* (Tucker) considered as the most important disease limiting muskmelon production in Pakistan. In this study, three molecularly characterized isolates of *Trichoderma* (*T. harzianum* HK, *T. harzianum* HM, *T. asperellum* TH) and phenotypically characterized isolate of *Bacillus subtilis* were evaluated against *P. drechsleri* under laboratory conditions. The different antagonism assays such as, dual culturing, non-volatile, volatile metabolites and field conditions were tested. The results showed that, all tested isolates of *Trichoderma* and *Bacillus subtilis* significantly ($p < 0.001$) inhibited the mycelial growth of the pathogen. In all dual cultures, *T. asperellum* TH showed maximum mycelial inhibited followed by *T. harzianum* HM and *Bacillus subtilis*. Non-volatile metabolites were more effective against pathogens than volatile metabolites.

Jadesha *et al*. (2019) evaluated efficacy of *Trichoderma asperellum* against *Phytophthora cajani* tested and proved that, *T. asperellum* inhibited the growth by 80.52 % in *in vitro.*

Pour *et al*. (2019) proved that, pistachio gummosis caused by *Phytophthora drechsleri* could be controlled by *Bacillus subtillis* bacteria. In addition, *B. subtilis* VRU1 and BS-VRU showed high efficacy in the suppression of diseases through several biocontrol mechanisms.

2.11.3 Efficacy of phytoextracts

Four species of *Brassicaceae viz*., *Sinapis alba* L., *Brassica carinata*, *B.* nigra and *B. oleracea* were tested against *Phytophthora nicotianae* and proved that *B. nigra* and *S. alba* were found the most effective (53.6 and 52.5% inhibition, respectively) (Rodriguez *et al*. (2012).

Bahraminejad *et al.* (2012) studied crude aqueous and methanol extracts of 121 plant species from 41 families, collected from the west of Iran, screened for antifungal activity against mycelial growth of *Phytophthora drechsleri*. They reported that, bioassay used was based on the paper disc diffusion method with four replicates. Extracts of 38 of 121 (about 31 %) plant species had inhibitory activity against this phytopathogenic fungus, among which 23 species measurably inhibited the growth of *P*. *drechsleri*. A methanol extract of *Xanthium strumarium* had the strongest inhibitory activity (17.79±1.35 mm) against *P. drechsleri* followed by extracts of *Glycyrrhiza glabra*, *Verbascum sp.*, *Hypericum perforatum*, *Centaura depressa*, *Centaura sp*., *Lamium amplexicaule* and *Haplophyllum perforatum*, respectively.

Jagtap *et al.* (2012) tested different botanicals against *Phytophthora nicotianae*, *P*. *citrophthora* and *P*. *palmivora* of citrus. Plant extract (@ 5, 10 and 15%) of garlic, recorded lowest mean colony diameter (47.45 mm) and highest mean mycelial growth inhibition (47.26%) followed by Neem and Onion which recorded the mycelial growth of 55.20 mm 60.85 mm, and the mean mycelial growth inhibition of 38.65%, 32.38%, respectively.

Asmita (2015) studied aqueous extracts of the 9 botanicals evaluated *in vitro* (each @ 10, 15 and 20%) were found antifungal to *P. drechsleri* f. sp. *cajani* of pigeonpea. However, on the basis of highest average mycelial growth inhibition recorded in *A. sativum* (62.96 %), followed by *Z. officinale* (50.73 %), *A. indica* (49.25 %), *A. cepa* (43.70 mm) and *L. innermis* (41.55 mm). The botanicals *M. citrifolia* (19.62 mm) and *C. longa* (17.03 mm) were found comparatively less effective.

Rashmi *et al*. (2015) evaluated that, five botanicals viz., Neem, Tulsi, Onion, Chrysanthemum and Garlic, which were used @ 5% concentration *in vitro* against *Phytophthora* parasitica by applying poisoned food technique and reported that the highest reduction in the mycelial growth of *P*. *parasitica* was recorded by Garlic (0.00 mm) followed by Neem (27.33 mm), Onion (31.33 mm), Chrysanthemum (33.33mm), respectively, while, Tulsi found less effective and recorded minimum inhibition of mycelial growth of *P*. *parasitica* (41.00 mm), respectively.

Mohsan *et al*. (2017) evaluated *in vitro* efficacy of phytoextracts *viz.*, *Calotropis gigantean* (Akk), *Cassia fistula* (Amaltas), *Nerium oleander* (Kaner), *Oscimum basilicum* (Niazbo), *Parthenium hysterophorus* (*Parthenium*) and *Azadirachta indica*

(Neem) against *Phytophthora capsica* of pepper. They reported that, *Parthenium hysterophorus* was found most effective with cent per cent (100%) mycelial inhibition, followed by *Nerium oleander* and *Oscimum basilicum*, respectively.

Anju *et al*. (2018) evaluated acetone, methanol and hexane extracts of 10 plant species *viz.*, *Azadirachta indica* (seed), *Cassia augustifoli* (leaf), *Terminalia bellerica* (seed), *Psoraleaco rylifolia* (seed), *Tamarindus indica* (leaf), *Annona squamosa* (leaf), *Ricinus communis* (seed), *Pongamia pinnata* (leaf), *Ricinus communis* (seed) and *Phyllanthus asperlatus* (seed) were tested against the mycelial growth of *Phytophthora infestans* at 500 ppm. Acetone, methanol and hexane extracts of *Terminalia bellerica* completely inhibited the mycelial growth. They reported that, acetone extracts of *Psoralea corylifolia* also showed complete inhibition and were significantly superior to others but acetone, methanol and hexane extracts of *Cassia tora* showed no inhibition.

Ndala et al. (2019) evaluated crude extracts from *Plectranthus barbatus*, *Tephrosia vogelii*, *Sphaeranthus suaveolens* and *Lantana camara*. The results showed significant differences ($P \leq 0.001$) among the extracting solvents on per centage inhibition of *Phytophthora infestans*. Methanol was superior in inhibiting the of mycelial growth of *P. infestans* as compared with ethyl acetate. Furthermore, all plants tested showed antifungal activity against *P. infestans*. The *P. barbatus*, *L. camara* and *S. suaveolens* were compatible with the commercial fungicide in inhibiting the growth of *P. infestans*. In this study, *T. vogelii* extract showed poor results in inhibiting the mycelial growth of *P. infestans* as compared with other plant extracts, respectively.

CHAPTER - III

MATERIALS AND METHODS

CHAPTER- III

MATERIALS AND METHODS

During the present investigations on *Phytophthora drechsleri* f. sp. *cajani* of pigeonpea (*Cajanus cajan* (L.) *Millisp*.), various experiments were conducted at the Department of Plant Pathology, College of Agriculture, Latur during *Kharif-Rabi,* 2021-22, to fulfill the objectives defined. The details of the materials used and methods adopted for various experiments are being described here in following various sub-heads.

3.1 Materials

The various kinds of materials *viz*., seeds, fertilizers, fungicides, chemicals, glassware's, culture media, bioagents and other miscellaneous items required for conducting present studies were obtained from the Department of Plant Pathology, College of Agriculture, Latur.

3.1.1 Glassware's

The common glasswares *viz*., Petri dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rod, beakers, funnel, pipette and autoclavable storage bottles etc. were obtained from the Department of Plant Pathology, College of Agriculture, Latur.

3.1.3 Chemicals

Standard chemicals, reagents, pesticides and fungicides required for various experiments were obtained from the Department of Plant Pathology, College of Agriculture, Latur.

3.1.4 Nutritional sources

The various nitrogen sources *viz*., Urea (CH_4N_2O), Ammonium Chloride, Ammonium Sulphate ((NH4)2 SO4), Ammonium Nitrate ($(NH_4)_2$ NO_3), Ammonium Ferrous Sulphate $(NH_4)_2Fe(SO_4)_2.6H_2O$, Potassium Nitrate (KNO3), Ammonium Oxalate $(NH_4)2C_2O_4$) and Sodium Nitrate ($NaNO_3$) were obtained from the Department of Plant Pathology, College of Agriculture, Latur.

3.1.5 Equipments and instruments

The laboratory equipments *viz.*, Autoclave, Hot air oven, Laminar airflow Cabinet, BOD Incubator, Refrigerator, Binocular Research Microscope, Electronic balance and pH meter etc. available at the Department of Plant Pathology, College of Agriculture, Latur were used as per requirement.

3.1.6 Culture media

Potato Dextrose Agar (PDA) was used as basal medium for isolation, purification, multiplication and maintenance of the pure culture of *Phytophthora drechsleri* f. sp. *cajani*. For studying cultural characteristics of *Phytophthora drechsleri* f. sp. *cajani*, ready-made (make Hi media) synthetic media *viz.*, Czapek's dox agar (CDA), V8 juice agar and non-synthetic media *viz.*, potato dextrose agar, host leaf extract agar, corn meal agar, oat meal agar,tomato juice agar and carrot dextrose agar were used. The biocontrol agents were maintained and multiplied on respective selective synthetic culture media.

3.1.7 Fungicides

Following 15 fungicides (systemic, non-systemic and combi product fungicides) were used for *in vitro* experiments conducted during present studies.

Table 3.1 List of the fungicides used

Sr. No.	Common/Technical name	Trade name	Active ingredient	Company/ Manufacturer
Systemic fungicides (each @ 500 and 1000 ppm)				
1.	Azoxystrobin	Amistar	23% SC	Syngenta IndiaLtd., Pune
2.	Dimethomorph	Ditaf	50% WP	Tata Phaltan, Satara, Maharashtra
3.	Metalaxyl MZ	Tagron 35WS	35% WS	Tropical Agrosystem (India) Pvt. Ltd.
4.	Fosetyl AL	Aliette	80% WP	Syngenta IndiaLtd., Pune
5.	Difenoconazole	Score	25% EC	Syngenta IndiaLtd., Pune
6.	Thiophanate methyl	Roksin	70% WP	Shivalik CropSci. Ltd. Chandigarh
7.	Hexaconazole	Counter Plus	5% EC	GCS Pvr. Ltd.Jaipur, Rajasthan

Contact and combi-product fungicides (each @ 2000 and 2500 ppm)				
1.	Mancozeb	Dithane M-45	75% WP	Shivalik CropSci. Ltd. Chandigarh
2.	Chlorothalonil	Kavach	75% WP	Syngenta IndiaLtd., Pune
3.	Copper oxychloride	Blue copper	50% WDG	Syngenta IndiaLtd., Pune
4.	Copper hydroxide	Dupont kocide 2000	77% WP	Dupont IndiaPvt. Ltd.
5.	Tebuconazole + Trifloxystrobin	Nativo	50% + 25% WG	Bayer, Vapi, Gujrat
6.	Mancozeb + Cymoxanil	Curzate	64 % + 8 % WP	Corteva Agriscience
7.	Metalaxyl + Mancozeb	Ridomil Gold	8% + 64% WP	Syngenta IndiaLtd., Pune
8.	Thiophanate methyl + Pyraclostrobin	Xerola	45% + 5% FS	BASF Group Company, Navi Mumbai
9.	Iprovalicarb + Propineb	Melody Duo	5.5% + 61.25% WP	Bayer, Vapi, Gujarat

3.1.8 Bioagents

Pure culture of the biocontrol agents *viz*., *Trichoderma asperellum*, *T. harzianum*, *T. hamatum*, *T. koningii*, *Verticillium leccani*, *Metarhizium anisopliae*, *Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from the Spawn Production-cum-biocontrol Laboratory, Department of Plant Pathology, VNMKV, Parbhani and College of Agriculture, Latur; multiplied and maintained on appropriate culture media and used in present studies.

3.1.9 Phytoextracts

Following plant species reported to exhibit antifungal and antibacterial properties against phytopathogens were collected from the farms of College of Agriculture, Latur and adjoining fields and their acetone solvent extracts were used in present studies.

Table 3.2: List of phytoextracts used

Sr. No.	Common Name	Scientific Name	Plant parts used
1.	Nilgiri	*Eucalyptus globulus*	Leaves
2.	Nirgudi	*Vitex negundo*	Leaves
3.	Mehandi	*Lawsonia inermis*	Leaves
4.	Neem	*Azadirachta indica*	Leaves
5.	Ghaneri	*Lantana camera L.*	Leaves
6.	Garlic	*Allium sativum L.*	Clove
7.	Ginger	*Zingiber officinale*	Rhizome
8.	Onion	*Allium cepa*	Bulb

3.1.10 Miscellaneous material

Miscellaneous experimental material *viz.,* inoculation needle, forceps, blotter paper, paper bags, polythene bags, spirit lamp, sodium hypochlorite, earthen pots, labels and scales *etc.* were obtained from the Department of Plant Pathology, College of Agriculture, Latur.

3.2 Methodology

Details of the methodologies adopted for various experiments under present study were being described here in under following subheads.

3.2.1 Collection of disease specimens

The stem blight diseased specimens of pigeonpea were collected from the farmers fields and brought to the laboratory. Visual symptoms were recorded and subjected to tissue isolation on potato dextrose agar (PDA).

3.2.2 Symptomatology

Visual observations were recorded of the stem blight symptoms on the diseased specimens of pigeonpea stems collected from the farmers fields. Temporary mounts of these pigeonpea diseased specimens were prepared in Lactophenol Cotton Blue stain, on clean glass slides and observed under research microscope, for presence of pathogens structure (mycelium, spores and fruiting bodies etc.). Based on symptomatology and microscopic observations, the stem blight specimens were preliminarily ascertained.

3.2.3 Isolation of the pathogen

The stem blight diseased pigeonpea stem specimens collected from the fields were subjected to tissue isolation on PDA plates. For the purpose, diseased pigeonpea stem was washed thoroughly under running tap water to remove inert materials, cut into small pieces (5mm) with a sterilized blade in such a way that the piece contains half healthy and half infected portion. Then, it was surface sterilized by dipping into 1 per cent Sodium hypochlorite solution for 30-60 seconds, rinsed by three sequential changes in distilled water, blot dried and inoculated on autoclaved and solidified, cooled PDA medium in Petri plates and were incubated at (27 ± 2^{o} C) for a week. After a week of incubation, the typical fungus growth developed will be transferred onto fresh PDA plates and incubated further. By applying hyphal tip isolation technique, the test fungus isolates were sub-cultured, purified and their pure cultures were maintained separately on PDA slant tubes in refrigerator.

3.2.4 Pathogenicity test

Seeds of pigeonpea ICP-7119 susceptible to *Phytophthora* blight were surface sterilized with 0.1% sodium hypochlorite and sown (@ 5 seeds/pot) in the earthen pots filled with sterilized potting mixture of soil: sand: FYM (2: 1: 1). Two healthy growing pigeonpea seedlings per pot were maintained, watered regularly and kept in the screen house forfurther development. The pathogenicity test was proved by soil drenching method.

The pathogen *P. drechsleri* f. sp. *cajani* was mass multiplied on potato dextrose broth (100 ml) in flasks and incubated at 28°C for 15 days. This fungus inoculum (mycelial mat + broth) was macerated in a blender for 1-2 min, diluted this suspension with glass rod and tap water was added to get final volume as 200 ml. Then 7-10 days old seedlings were inoculated by pouring 100 ml inoculum around the base of the seedlings in a pot. The seedlings were irrigated as and when required. Observations on disease development were recorded.

3.2.5 Identification of the pathogen

Based on symptomatology, cultural and morphological characteristics and pathogenicity test, the test pathogen was identified and further confirmed by comparing its authentic description / characters.

3.2.6 Morpho-cultural characteristics

Cultural characteristics of the pathogen associated with stem blight of pigeonpea were studied, using PDA as basal culture medium. For the purpose, autoclaved and cooled PDA medium was filled (20 ml/plate) in sterilized glass Petri plates (90mm) and allowed to solidify at room temperature. On solidification of PDA, these plates were inoculated aseptically by putting 5mm fungal culture disc in the center was obtained from actively growing 10 days old pure culture of the test pathogens and incubated at 28 ± 2°C. After 10 days incubation, radial growth was measured.

Observations on cultural characteristics (at an interval of 24/48 hrs) *viz*., growth rate, colony characters such as colony type, colony colour, colony margin, colony elevation and topography etc. were recorded at 10 days after incubation and sporulation. For studying morphological characteristics, the temporary mounts on glass slide in a drop of lactophenol cotton blue stain of the pure culture (10-12 days old) of the isolated of test pathogen, covered with cover slip was observed under research microscope. Observations on hyphae, mycelium, spores, oospores and sporangia etc. in respect of their size and shape were recorded.

3.2.7 Effect of culture media on *Phytophthora drechsleri* f. sp. *cajani*

To study the effect of different solid culture media on characteristics of *Phytophthora drechsleri* f. sp. *cajani*, eight culture media *viz*., potato dextrose agar, oat meal agar, Czapek's dox agar, corn meal agar, host leaf extract, V8 juice agar, tomato juice agar and carrot dextrose agar were used.

The media was sterilized in autoclave at 15 lbs pressure for 15 minutes. Autoclaved and cooled media was poured (@ 20 ml/plate) in sterilized Petri plates (90 mm diameter) and allowed to solidify at room temperature. On solidification of the media, Petri plates of each culture medium were inoculated by placing in the center a 5 mm mycelial disc of actively growing 7 days old pure culture of *P. drechsleri* f. sp. *cajani*. Each culture medium was replicated thrice.

Experimental details

Design: Completely Randomized Design (C.R.D.)

Replications : Three

Treatments : Eight

Treatment details:

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Potato dextrose agar	T_5	Host leaf extract
T_2	Oat meal agar	T_6	V8 juice agar
T_3	Czapek's dox agar	T_7	Tomato juice agar
T_4	Corn meal agar	T_8	Carrot dextrose agar

Plates were incubated at temperature 28 ± 2°C. The observations on mean colony diameter, colony growth and cultural characteristics were recorded after 7 days of inoculation.

3.2.8 Physiological studies

3.2.8.1 Effect of temperature

For studying the temperature effects, different levels of temperature were maintained either in refrigerator, incubator at 10, 15, 20, 25, 30, 35, 40 and 45°C as per the adjustability of the instruments. Experiment was conducted on Potato dextrose agar (PDA) in Petri plates. PDA was sterilized and poured in sterile Petri plates. For each treatment, a 5 mm inoculum disc used. Plates were incubated at respective treatment of temperature and observations on colony diameter was recorded after seven days of inoculation.

Experimental details

Design **:** Completely Randomized Design (C.R.D.)

Replications **:** Three

Treatments **:** Eight

Treatment details: Temperature °C

Tr. No.	Treatments (Temperature °C)	Tr. No.	Treatments (Temperature °C)
T_1	10°C	T_5	30°C
T_2	15°C	T_6	35°C
T_3	20°C	T_7	40°C
T_4	25°C	T_8	45°C

3.2.8.2 Effect of pH

The pH plays a key role in the maintenance of metabolic rate of the pathogen *Phytophthora drechsleri* f. sp. *cajani*. Potato dextrose agar was used as basal medium for this experiment.

The pH level of PDA will be adjusted by using 0.1 N HC1 or 0.1 N NaOH. After adjustment of pH, basal medium PDA was sterilized in autoclave. The cooled (40^0C) medium was poured in Petri plates (@ 20 ml/plate) and allowed to solidify at room temperature. On solidification of the media, Petri plates were inoculated with a 5 mm disc of actively growing culture organism and incubated at temperature 28 ± 2°C for seven days. Three replications of each treatment were maintained and mean colony diameter were recorded.

Experimental details

Design **:** Completely Randomized Design (C.R.D.)

Replications **:** Three

Treatments **:** Nine

Treatment details : pH

Tr. No.	Treatments (pH)	Tr. No.	Treatments (pH)
T_1	4.0	**T_6**	6.5
T_2	4.5	**T_7**	7.0
T_3	5.0	**T_8**	7.5
T_4	5.5	**T_9**	8.0
T_5	6.0		

3.2.9 Nutritional studies

3.2.9.1 Effect of nitrogen sources

Different fungi are known to have different requirements of nitrogen content for their optimum growth and sporulation. Potato dextrose agar (PDA) was used as basal medium for this experiment.

Nitrogen content in the PDA changes by using different nitrogen sources *viz*., Urea (CH_4N_2O), Ammonium Chloride (NH_4Cl), Ammonium Sulphate ((NH4)2 SO4), Ammonium Nitrate ($(NH_4)_2$ NO_3), Ammonium Ferrous Sulphate $(NH_4)_2Fe(SO_4)_2$. $6H_2O$, Potassium Nitrate (KNO3), Ammonium Oxalate ($(NH_4)2C_2O_4$) and Sodium Nitrate ($NaNO_3$) were used. For evaluation of nitrogen sources, basal mediumPDA was enriched separately with the test nitrogen compounds. Quantity of nitrogen sources were measured on the basis of total nitrogen in source.

Basal medium PDA was sterilized in autoclave. The cooled (40^0C) medium poured in Petri plates (@ 20 ml/plate) and allowed to solidify at room temperature. On solidification of the media, Petri plates were inoculated with 5 mm disc of actively growing culture organism. Untreated PDA plates (without nitrogen sources) inoculated separately with pure culture disc of the test fungus per treatment per replication were maintained and incubated at temperature 28 ± 2°C for seven days. Three replications of each treatment were maintained and mean colony diameter was recorded.

Experimental details

Design : Completely Randomized Design (C.R.D.)

Replications : Three

Treatments : Nine

Treatment details: **Nitrogen sources**

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Ammonium Chloride (NH_4Cl)	T_6	Urea (CH_4 N_2O)
T_2	Ammonium Sulphate $(NH_4)_2$ SO_4)	T_7	Potassium Nitrate (KNO_3)
T_3	Ammonium Nitrate $(NH_4)_2$ NO_3)	T_8	Ammonium Oxylate $(NH_4)2C_2O_4$)
T_4	Sodium Nitrate ($NaNO_3$)	T_9	Control (Untreated)
T_5	Ammonium Ferrous Sulphate $(NH_4)_2Fe(SO_4)_2.6H_2O$		

3.2.10 Disease management strategies

3.2.10.1 *In vitro* evaluation of systemic, non-systemic and combi product fungicides

The conventional and newer systemic (each @ 500 and 1000 ppm), non-systemic and combi - product (each @ 2000 and 2500 ppm) fungicides were evaluated against *P. drechsleri* f. sp. *cajani*, causal organism of stem blight of pigeonpea by applying Poisoned food technique (Nene and Thapliyal, 1993) and using PDA as basal culture medium. Requisite quantity of the test fungicides were calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (40^0C) PDA medium in glass conical flasks (250 ml capacity) to obtain their desired concentrations.

This PDA medium separately amended with the test fungicides were then poured (20 ml/plate) aseptically in sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its test concentration,two plates per treatment per replication were maintained. After solidification of the PDA medium, these plates were inoculated aseptically by placing in the centre 5 mm culture disc of the test fungus obtained from actively growing 7 days old pure culture of test pathogen. Untreated PDA plates (without fungicide) inoculated separately with pure culture disc of the test fungus per treatment per replication were maintained. Both treated and untreated PDA plates were incubated in an inverted position at 28±2^{0}C in BOD incubator, for a week.

Experimental details: Systemic fungicides (each @ 500 and 1000 ppm)

Design: Completely Randomized Design (C.R.D.)

Replications : Three

Treatments : Eight

Treatment details : Systemic fungicides (each @ 500 and 1000 ppm)

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Azoxystrobin 23% SC	T_5	Difenconazole 25%EC
T_2	Dimethomorph 50% WP	T_6	Thiophanate methyl 70% WP
T_3	Metalaxyl MZ 35% WS	T_7	Hexaconazole 5% EC
T_4	Fosetyl AL 80% WP	T_8	Control (Untreated)

Experimental details: Contact and combi-product fungicides (each @ 2000 and 2500 ppm)

Design : Completely Randomized Design (C.R.D.)

Replications : Three

Treatments : Ten

Treatment details : Contact and combi-product fungicides (each @ 2000 and 2500 ppm)

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Mancozeb 64% + Cymoxanil 8% WP
T_2	Chlorothalonil 75%WP	T_7	Metalaxyl 8% + Mancozeb 64% WP
T_3	Copper Oxychloride 50% WDG	T_8	Thiophanate methyl 45% + Pyraclostrobin 5% FS
T_4	Copper hydroxide 77% WP	T_9	Iprovalicarb 5.5% + Propineb 61.25%
T_5	Tebuconazole 50% + Trifloroxystrobin 25% WG	T_{10}	Control (Untreated)

Observations on radial mycelial growth colony diameter (mm) of the test fungi at an interval of 24 hrs of incubation were recorded and continued upto seven days or till the untreated PDA plates covered fully with mycelial growth of the test fungus. Based on cumulative data, per cent mycelial growth inhibition of the test fungi with the test fungicides, over untreated control will be calculated by applying following formula (Vincent, 1927).

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of test fungus in untreated control plate

T = Growth (mm) of test fungus in treated plates

3.2.10.2 *In vitro* evaluation of bioagents

Most potential fungal and bacterial biocontrol agents were evaluated against the test fungi, by applying ‘Dual Culture Technique’ (Dennis and Webter, 1971) and using PDA as basal culture medium. Seven days old cultures of the test bioagents and test fungi grown on respective culture media were used for the study. One each 5 mm culture disc of the test fungus and the test fungal bioagents cut out with sterilized cork borer was placed at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates. For bacterial biocontrol agents, a culture disc (5mm) for the test fungus was placed along periphery of the PDA plate and exactly opposite to it, pure culture suspension of the test bacterial biocontrol agent was streaked with wire inoculation needle loop. The PDA plate inoculated (in the centre) only with pure culture disc of the test fungus was maintained as untreated control.

Experimental details :

Design :Completely Randomized Design (CRD)

Replications : Three

Treatments : Nine

Treatment details :

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperellum*	T_6	*Metarhizium anisopliae*
T_2	*T. harzianum*	T_7	*Bacillus subtilis*
T_3	*T. hamatum*	T_8	*Pseudomonas fluorescens*
T_4	*T. koningii*	T_9	Control (Untreated)
T_5	*Verticillium leccani*		

Observations on colony diameter (mm) of the test fungus and the test bioagents were recorded at an interval of 24 hrs of incubation and continued upto seven days or till the untreated control plates get fully covered with mycelial growth of the test fungus. Based on cumulative data, per cent mycelial growth inhibition of the test fungus with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhay,1978).

$$\text{Growth Inhibition (\%)} = \frac{\text{Colony growth of the fungus in control plate} - \text{Colony growth of the fungus in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

3.2.10.3 *In vitro* evaluation of phytoextracts

Solvent (acetone) extracts of locally available higher plant species were evaluated (each @ 10 and 20 %) separately against the test fungi, by applying Poisoned food technique (Nene and Thapliyal, 1993) and using PDA as a basal culture medium. Plant parts (leaves, bulbs, cloves and rhizomes etc.) of the selected plant species were washed thoroughly with distilled water, chopped into small bits with sterilized sharp knife, separately crushed and homogenized in pestle and mortar in equal quantity of distilled water (1:1 W/V). The homogenate obtained were then strained through double layered muslin cloth and the extract / filtrate obtained, were further passed through Whatman's filter paper No. 1 using glass volumetric flask (100 ml capacity) and funnel. The clear supernatent obtained was the plant extract of 100% concentration.

An appropriate quantity of each plant extract (100%) were separately mixed thoroughly with autoclaved and cooled (40^0 C) PDA medium in conical flasks (250 ml) to make the PDA medium of 10 and 20 per cent concentration of the test phytoextracts.

These PDA medium amended separately with plant extract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. Two PDA plates per test phytoextract per replication were replicated thrice. Upon solidification of the PDA, all these treatment plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc, obtained from a week old actively growing pure culture of the fungi. Plain PDA plates without any plant extract and inoculated with mycelial disc of the test fungus were maintained untreated as control. All these plates were incubated at 28 ± 2^0C temperature for a week or until the untreated control plates fully covered with mycelial growth of the test fungus.

Experimental details :

Design : C.R.D (Completely Randomized Design)

Replication : Three

Treatments : Nine

Treatment details : P**hytoextracts each @ 10 and 20%**

Tr. No.	Treatments	Plant Parts used	Tr. No.	Treatments	Plant Parts used
T_1	*Eucalyptus globulus* (Nilgiri)	Leaves	T_6	*Zingiber officinale* (Ginger)	Rhizome
T_2	*Allium cepa* (Onion)	Bulb	T_7	*Vitex negundo* (Nirgudi)	Leaves
T_3	*Allium sativum L.* (Garlic)	Clove	T_8	*Lawsonia inermis* (mehandi)	Leaves
T_4	*Azadirachta indica* (Neem)	Leaves	T_9	Control (Untreated)	
T_5	*Lantana camera L.* (Ghaneri)	Leaves			

Observations on radial mycelial growth / colony diameter (mm) were recorded at regular interval and continued upto seven days after incubation or till the untreated PDA plates covered fully with mycelial growth of the test fungus. Based on cumulative data per cent mycelial growth inhibition of the test fungi with the test phytoextracts over untreated control was calculated by applying following formula (Vincent, 1927).

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of test fungus in untreated control plate

T = Growth (mm) of test fungus in treated plates

3.2.11 Statistical analysis

The data obtained in all the experiments (*in vitro*) were subjected to statistical analysis. The standard error (S.E.) and critical difference (C.D.) @1 % level of significance were worked out (Panse and Sukhatme, 1978). Per cent data was transformed into arc sine values.

CHAPTER - IV

RESULTS AND DISCUSSION

CHAPTER- IV

RESULTS AND DISCUSSION

Present investigation entitled studies on pigeonpea stem blight caused by *Phytophthora drechsleri* f. sp. *cajani* were conducted on the aspects *viz*., collection of disease samples, symptomatology, isolation, pathogenicity test and different media, morpho-cultural characteristics and efficacy of various fungicides, bioagents and phytoextracts against *P. drechsleri* f. sp. *cajani*. The results obtained on all these aspects are being narrated and discussed here in this chapter under following sub-heads.

4.1 Symptomatology

Phytophthora stem blight of pigeonpea disease specimens collected (Plate 4.1) which was manifested with typical symptoms as: In older plants that have age 1 to 3 month old, they showed brown, dark brown to black lesions above the ground level on main stem and on branches (Plate 4.1 A). As the disease progressed, lesions enlarged in size and girdle the stem (Plate 4.1 B).

During severe infection, lesions on stem developed converted into cankers or gall (Plate 4.1 C) and infected bark cracked (Plate 4.1 D). Phloem of infected plants was smoky grey and xylem remains clear (Plate 4.1 E).

Similar symptoms of *Phytophthora* stem blight of pigeonpea (*Phytophthora drechsleri* f. sp. *cajani*) were reported earlier by several workers (Williams *et al*. 1975; Raut and Somani, 1988; Dhar *et al*. 2005; Mamata *et al*. 2011; Mamata and Ghosh, 2018 and Naik *et al*. 2020).

4.2 Isolation of the pathogen

The test pathogen (*Phytophthora drechsleri* f. sp. *cajani*) was isolated from stem blight diseased specimens collected from fields, by tissue isolation technique on Potato dextrose agar medium. Test pathogen *P. drechsleri* f. sp. *cajani* produced mycelium which was greyish white growth, slightly elevated with rough and irregular margin (Plate 4.2).

Applying hyphal-tip isolation technique, these test pathogens were aseptically sub-cultured, purified and maintained separately on agar slant tubes and preserved in BOD incubator for further studies. Based on their cultural and morphological characteristics, which were earlier reported by several workers (Willians *et al.* 1968;

Kannaiyan *et al*. 1980; Mamta and Ghosh, 2016 and 2018), the test fungi were preliminarily identified.

4.3 Pathogenicity of the test fungi

The pathogenicity of *Phytophthora. drechsleri* f. sp. *cajani* was studied in pot culture by soil drenching method (Plate 4.3 A).

For pathogenicity ten days old seedlings of susceptible variety ICP-7119 were artificially inoculated with *P. drechsleri* f. sp. *cajani* by soil drenching method. The first symptom on the seedlings appeared as: water-soaked lesion on primary and trifoliate leaves within 4 days after inoculation (Plate 4.3 B). The seedlings showed characteristics symptoms of crown rot (Plate 4.3 C). Some plants showed oozing symptoms at nodal region (Plate 4.3 D). Un-inoculated seedlings remained healthy throughout the experiment. Re-isolation was done from infected plants and the morphological and cultural characters of the fungus were found similar to the original isolates.

Similar studies of *Phytophthora* stem blight of pigeonpea (*Phytophthora drechsleri* f. sp. *cajani*) were reported earlier by several workers (Nene *et al*. 1981 and Jadesha, 2014).

4.4 Cultural characters and morphological characters

4.4.1 Cultural characters

Test pathogen *P. drechsleri* f. sp. *cajani* produced greyish white mycelium growth which was slightly elevated with rough and irregular margin (Plate 4.2).

Similar cultural characters of *Phytophthora* stem blight of pigeonpea (*Phytophthora drechsleri* f. sp. *cajani*) were reported earlier by several workers (Jung *et al*. 2011and Jadesha, 2014).

4.4.2 Morphological characters

Test pathogen *P. drechsleri* f. sp. *cajani* produced mycelium hyaline, branched, filamentous to cylindrical, coenocytic when young but later septation was noticed with thick plugs and abundant hyphal swelling (Plate 4.4 A and B). Hyphal swelling was common and intercalary.

A. Stem blight (initial stage)

B. Stem blight (Advanced stage)

C. Stem gall

D. Splitting of stem

E. Smoky grey coloured phloem vessel

Plate 4.1: Symptoms of *Phytophthora* blight disease of pigeonpea (A to E)

Front view

Back view

Plate 4.2: Cultural characteristics of *Phytophthora drechsleri* f. sp. *cajani*

A. Pathogenicity experiment

B. water-soaked lesions

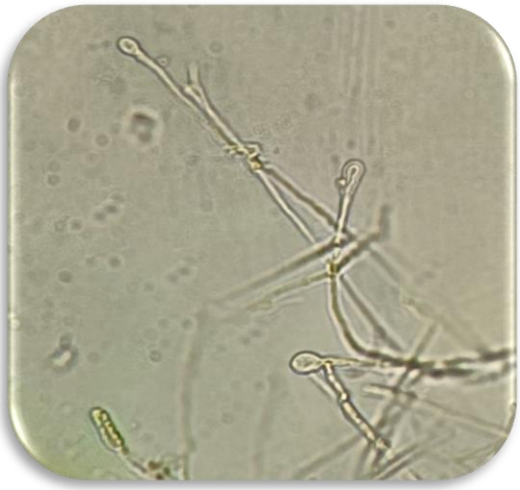
C. Crown rot symptoms

D. Oozing symptoms

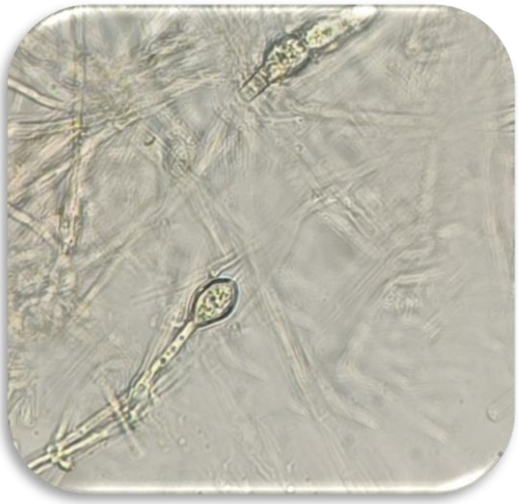
Plate 4.3: Pathogenicity of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight (A to D)

A. Mycelium

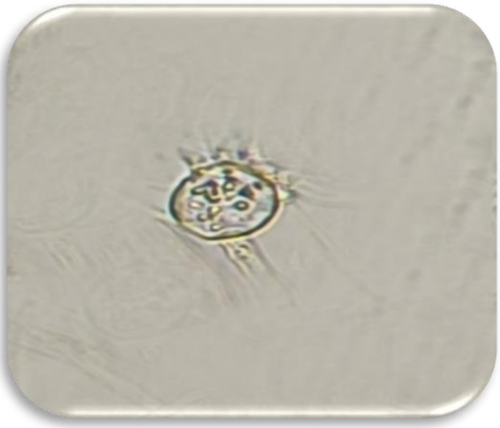
B. Hyphal swelling



C. Sporangiaphore



D. Sporangia



E. Encysted zoospore

F. Oospore

Plate 4.4: Morphological characters of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight (A to F)

The sporangia were proliferating type and sporangiaphore was simple sympodial (Plate 4.4 C). Sporangia *viz*., obpyriform, elongated, non-papillate often with tapering base and having size 40-42 × 27-30 µm (Plate 4.4 D).

Released zoospores were encysted with diameter 11 µm (Plate 4.4 E). Average diameter of oospores is about 18 µm (Plate 4.4 F).

Similar morphological characters of *Phytophthora* stem blight of pigeonpea (*Phytophthora drechsleri* f. sp. *cajani*) were reported earlier by several workers (Pal *et al*. 1970; Williams *et al*. 1968; Kannaiyan *et al*. 1980 and Singh *et al*. 1992; Pande *et al*. 2011; Jung *et al*. 2011 Jadesha, 2014, Jadesha 2020 and Naik *et al*. 2020).

4.5 Effect of different culture media on morpho-cultural characters of *Phytophthora drechsleri* f. sp. *cajani*

The cultural and morphological characteristics on different culture media of *P. drechsleri* f. sp. *cajani*, causing stem blight of pigeonpea was studied and there results are presented in the Table 4.1 and 4.2 and depicted in the Plates 4.5a to 4.5d and 4.6.

4.5.1 Morphological characteristics

On eight different media, *P. drechsleri* f. sp. *cajani* test pathogen was evaluated for morphological characteristics in respect of 7th day microscopic images and the results are presented in Table 4.1 and Plate 4.6, fig. 4.1.

Table 4.1 Morphological characteristics of *P. drechsleri* f. sp. *cajani* on different culture media

Tr. No.	Different media	Morphological Characteristics
T_1	Potato dextrose agar	Aseptate mycelium
T_2	Oat meal agar	Septate mycelium
T_3	Czapek’s dox agar	Aseptate mycelium
T_4	Corn meal agar	Aseptate mycelium
T_5	Host leaf extract	Aseptate mycelium
T_6	V8 juice agar	Aseptate mycelium
T_7	Tomato juice agar	Septate mycelium, starting of hyphal swelling
T_8	Carrot dextrose agar	Aseptate mycelium

The results (Table 4.1, Plate 4.5 and Fig.4.1) revealed that, morphological characteristics of *P. drechsleri* f. sp. *cajani* varied on different cultural media. Potato dextrose agar, Czapek's dox agar, Corn meal agar, Host leaf extract, V8 juice agar and Carrot dextrose agar showed aseptate mycelium. Oat meal agar showed septate mycelium. Tomato juice agar was showed septate mycelium and hyphal swelling was started.

4.5.2 Cultural characteristics

On eight different culture media, *P. drechsleri* f. sp. *cajani* test pathogen was evaluated for cultural characteristics in respect of growth rate, colony colour, colony shape, colony margin, elevation and topography, respectively. Observations were recorded on 7th day and the results are depicted in Table 4.2, Plate 4.6a to 4.6d and Fig.4.1.

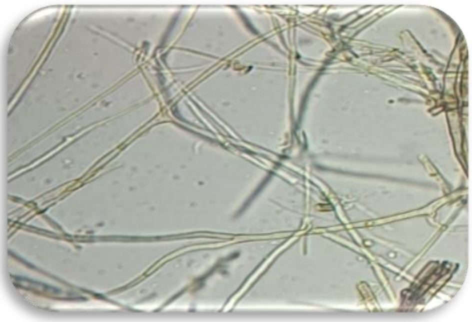
4.5.2.1 Growth rate and colony diameter

The results (Table 4.2, Plate 4.6a to 4.6d and Fig.4.1) revealed that, growth rate of *P. drechsleri* f. sp. *cajani* varied from slow to fast. However, it was fast on Oat meal agar, Corn meal agar, V8 juice agar and Tomato juice agar; moderate on Potato dextrose agar, Carrot dextrose agar and slow on Czapek's dox agar and Host leaf extract.

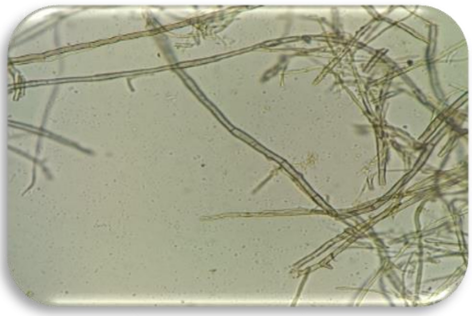
Colony diameter of test pathogen varied from 22.50 to 89.50 mm. However it was highest on Oat meal agar (89.50 mm), followed by the cultural media *viz*., Corn meal agar (88.50 mm), Tomato juice agar (87.50 mm), V8 juice agar (81.50 mm), Carrot dextrose agar (69.00 mm), Potato dextrose agar (50.00 mm), Host leaf extract (32.50 mm) and Czapek's dox agar (22.50 mm), respectively.

4.5.2.2 Colony colour (front and rear)

The colony of Potato dextrose agar, Oat meal agar, Tomato juice agar, Carrot dextrose agar was greyish white, whereas whitish in colour of Host leaf extract and V8 juice agar, dull white on Corn meal agar and cottony white on Czapek's dox agar. Whereas, that of rear colony colour varied from black at centre and white at margin in Potato dextrose agar, Oat meal agar, Czapek's dox agar, and Tomato juice agar. Greyish at centre and white at margin in Carrot dextrose agar, whitish grey in Host leaf extract and V8 juice agar. Dark grey in Corn meal agar.



A. PDA

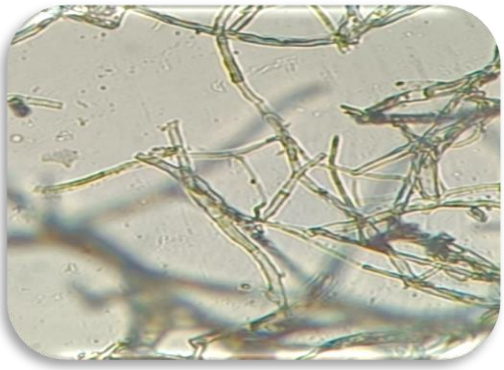


B. Oat meal agar

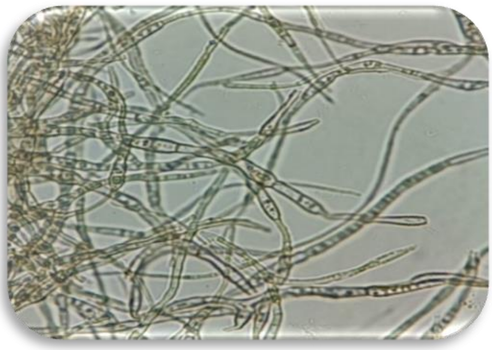
C. Czapek's dox agar

D. Corn meal agar

E. Host leaf extract



F. V8 juice agar



G. Tomato juice agar

H. Carrot dextrose agar

Plate 4.5: Effect of different media on morphological character *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

Front view

Back view

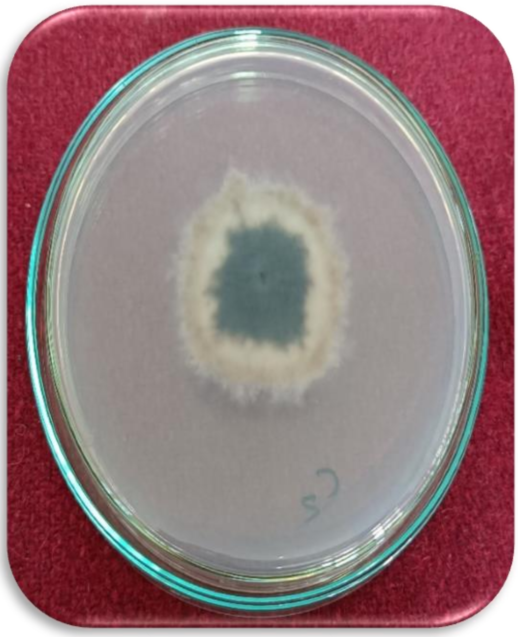
T$_1$: PDA

Front view

Back view

T$_2$: Oat meal agar

Plate 4.6a : Effect of different media on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight



Front view **Back view**

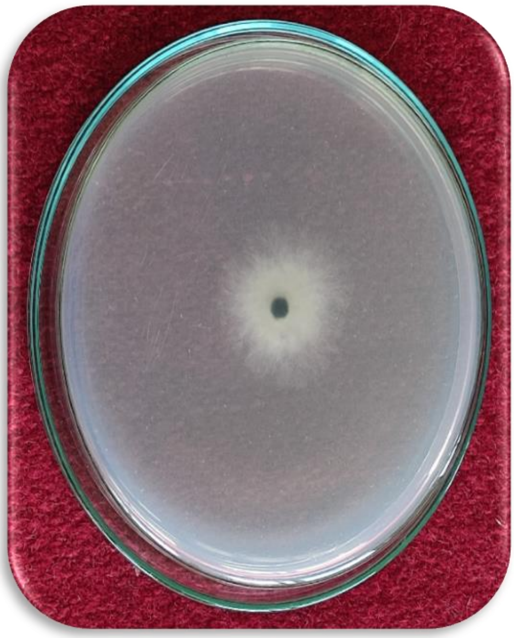
T_3: Czapek's dox agar

Front view **Back view**

T_4: Corn meal agar

Plate 4.6b : Effect of different media on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

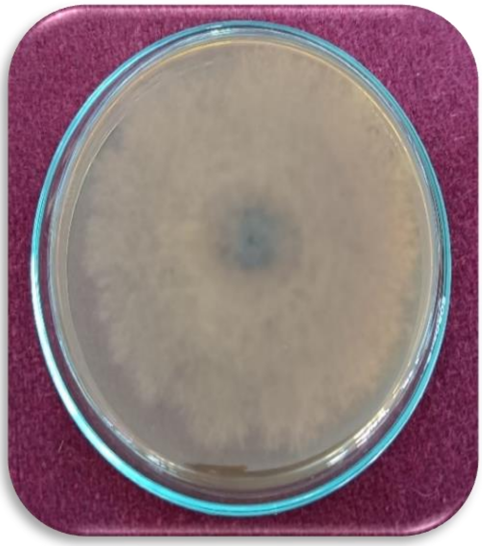
Front view



Back view

T_5: Host leaf extract

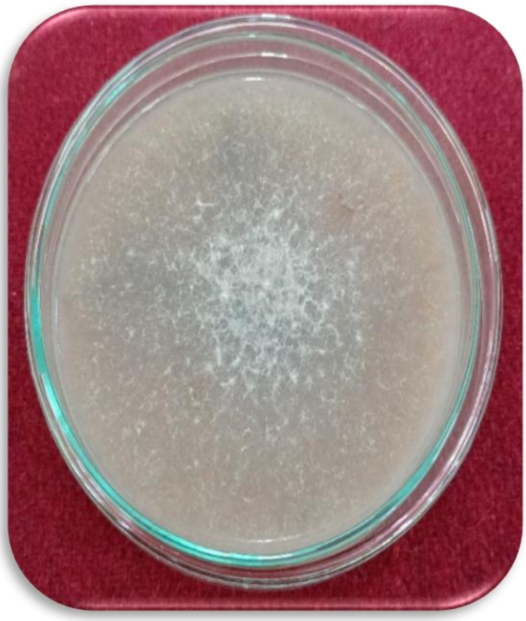
Front view



Back view

T_6: V8 juice agar

Plate 4.6c : Effect of different media on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight



Front view

Back view

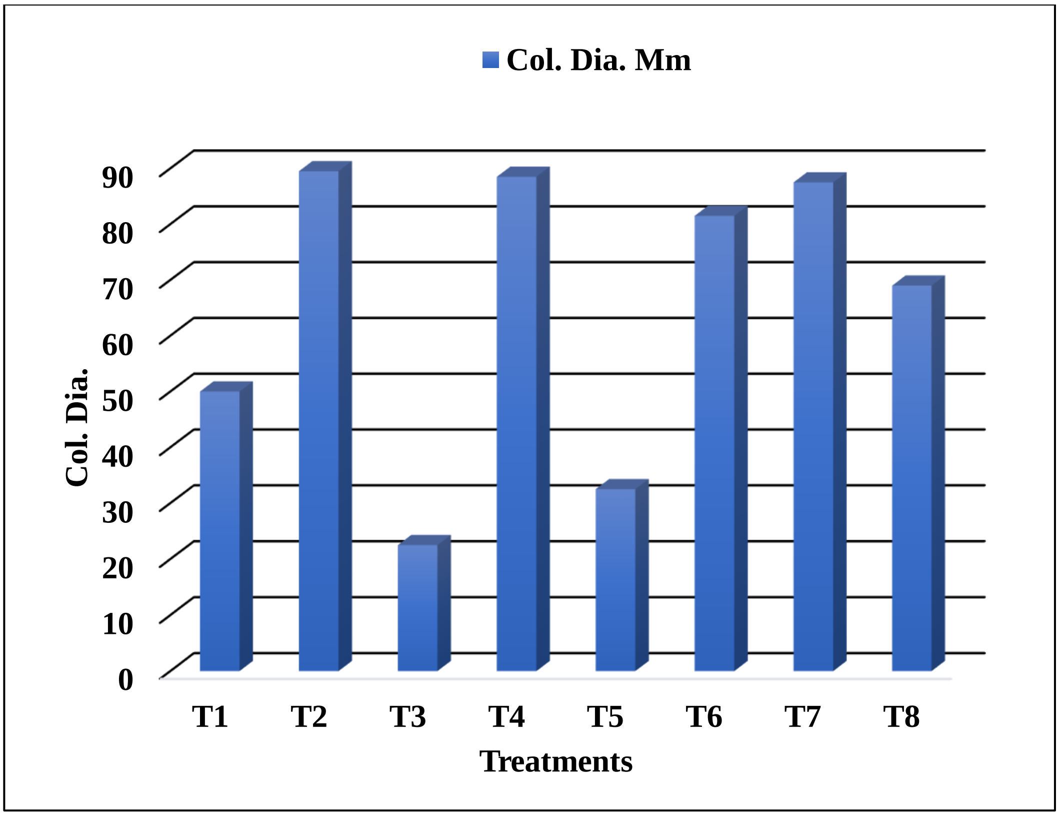
T_7: Tomato juice agar

Front view

Back view

T_8: Carrot dextrose agar

Plate 4.6d : Effect of different media on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight



Tr. No.	Treatments	Tr. No.	Treatments
T_1	Potato dextrose agar	T_5	Host leaf extract
T_2	Oat meal agar	T_6	V8 juice agar
T_3	Czapek's dox agar	T_7	Tomato juice agar
T_4	Corn meal agar	T_8	Carrot dextrose agar

Fig 4.1: Effect of different media on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

4.5.2.3 Colony appearance and shape

Colony appearance was mostly feathery on Oat meal agar, Corn meal agar, Tomato juice agar, V8 juice agar, Carrot dextrose agar, Potato dextrose agar and cottony on Host leaf extract, Czapek's dox agar. Colony shape of test pathogen on all media were circular except Host leaf extract was irregular.

4.5.2.4 Colony topography, elevation and margin

Colony topography of test pathogen on Oat meal agar, Tomato juice agar, V8 juice agar, Carrot dextrose agar, Potato dextrose agar and Czapek's dox agar was rough. On Corn meal agar and Host leaf extract was rough and raised. Colony elevation of *P. drechsleri* f. sp. *cajani* on all eight media was slightly raised.

Colony margin on Potato dextrose agar, Czapek's dox agar, Corn meal agar, V8 juice agar, Tomato juice agar and Carrot dextrose agar showed regular margin. Oat meal agar and Host leaf extract showed irregular margin.

Different cultural media showed different growth characters of *P. drechsleri* f. sp. *cajani*. Similar reports regarding morpho-cultural characters were given by earliest workers (Asmita, 2015; Patel, 2016; Thomas and Naik, 2017; Saykar *et al*. 2018; Kumar *et al*. 2019 and Jadesha *et al*. 2020).

Table 4.2 Cultural characteristics of *P. drechsleri* f. sp. *cajani* on different culture media

Tr. No.	Different media	Cultural Characteristics								
		Growth rate	Col. Dia.* (mm)	Colony Colour		Appearance	Shape	Topography	Elevation	Margin
				Front	Rear					
T_1	Potato dextrose agar	Moderate	50.00	Greyish white	Black at centre and white at margin	Feathery	circular	Rough	Slightly raised	regular
T_2	Oat meal agar	Fast	89.50	Greyish white	Black at centre and white at margin	Feathery	circular	Rough	Slightly raised	irregular
T_3	Czapek's dox agar	Slow	22.50	Cottony white	Black at centre and white at margin	Cottony	circular	Rough	Slightly raised	regular
T_4	Corn meal agar	Fast	88.50	Dull white	Dark grey	Feathery	circular	Rough and raised	Slightly raised	regular
T_5	Host leaf extract	Slow	32.50	Whitish	Whitish grey	Cottony	irregular	Rough and raised	Slightly raised	irregular
T_6	V8 juice agar	Fast	81.50	Whitish	Whitish grey	Feathery	circular	Rough	Slightly raised	regular
T_7	Tomato juice agar	Fast	87.50	Greyish white	Black at centre and white at margin	Feathery	circular	Rough	Slightly raised	regular
T_8	Carrot dextrose agar	Moderate	69.00	Greyish white	Greyish at centre and white at margin	Feathery	circular	Rough	Slightly raised	regular
	S.E.	**-**	**0.586**	-	-	-	-	-	-	-
	C.D.(P=0.01)	**-**	**1.771**	-	-	-	-	-	-	-

* Col. Dia. : Colony diameter

4.6 Effect of Temperature pH and Nitrogen sources on growth of *P. drechsleri* f. sp. *cajani*

4.6.1 Effect of temperature on growth of *P. drechsleri* f. sp. *cajani*

Mycelial growth

The results revealed that, *P. drechsleri* f. sp. *cajani* could grow on different temperature varied from 10°C to 45°C. Mean mycelial growth recorded with different temperature regimes, which ranged from 17.50 to 87.00 mm and presented in Table 4.3, Plate 4.7a and 4.7b, Fig. 4.2.

Table 4.3 Effect of temperature on growth of *P. drechsleri* f. sp. *cajani*

Tr. No.	Treatments (Temperature)	Mean Col. Dia. *(mm)
T_1	10°C	17.50
T_2	15°C	39.50
T_3	20°C	60.50
T_4	25°C	80.50
T_5	30°C	87.00
T_6	35°C	84.50
T_7	40°C	55.00
T_8	45°C	38.00
	S.E.	**0.462**
	C.D.(P=0.01)	**1.397**

*Mean of three replications. Dia.: Diameter

The highest mycelial growth of *P. drechsleri* f. sp. *cajani* was observed at temperature 30°C (87.00 mm) followed by 35°C (84.50 mm), 25°C (80.00 mm), 20°C (60.50 mm), 40°C (55.00 mm), 15°C (39.50 mm), 45°C (38.00 mm) and 10°C (17.50 mm), respectively.

On different temperature ranges *P. drechsleri* f. sp. *cajani* showed slightly variation in colony colour and margin on culture media. From 25°C to 35°C colony colour was greyish white with regular margin, as temperature increases and decreases colony colour changed from greyish white to whitish with irregular margin.

Growth of *P. drechsleri* f. sp. *cajani* varies according to different range of temperatures. Similar findings were earlier reported by several scientist (Kannaiyan *et al*. 1980; Singh *et al*. 2010; Varsha *et al*. 2010; Asmita, 2015; Singh *et al*. 2017 and Jadesha *et al*. 2020).

4.6.2 Effect of pH on growth of *P. drechsleri* f. sp. *cajani*

Mycelial growth

The results revealed that, *P. drechsleri* f. sp. *cajani* could grow on different pH levels varied from 4.0 to 8.0. Mean mycelial growth was recorded with different pH ranges which ranged from 34.00 to 88.00 mm and presented in Table 4.4, Plate 4.8 and Fig. 4.3.

Table 4.4 Effect of pH on growth of *P. drechsleri* f. sp. *cajani*

Tr. No.	Treatments (pH level)	Mean Col. Dia. *(mm)
T_1	4.0	34.00
T_2	4.5	47.00
T_3	5.0	61.50
T_4	5.5	69.50
T_5	6.0	83.00
T_6	6.5	88.00
T_7	7.0	81.00
T_8	7.5	79.50
T_9	8.0	73.50
	S.E.	**0.520**
	C.D.(P=0.01)	**1.556**

*Mean of three replications. Dia.: Diameter

The highest mycelial growth of *P. drechsleri* f. sp. *cajani* was observed at pH 6.5 (88.00 mm) followed by 6.0 (83.00 mm), 7.0 (81.00 mm), 7.5 (79.50 mm), 8.0 (73.50 mm), 5.5 (69.50 mm), 5.0 (61.50 mm), 4.5 (47.00 mm) and pH 4.0 (34.00), respectively.

There are different growths of *P. drechsleri* f. sp. *cajani* on different pH levels. Similar growth was reported by several workers (Duncan, 1985; Kim *et al*. 1989; Sahu *et al*. 2000; Simpfendorfer *et al.* 2001; Gul *et al.* 2005; Varsha *et al*. 2010 and Asmita, 2015).

4.6.3 Effect of different nitrogen sources on growth of *P. drechsleri* f. sp. *cajani*

To study the effect of nitrogen sources on *P. drechsleri* f. sp. *cajani*, eight nitrogen sources were used and PDA was used as basal medium.

T1. 10 ºC

T2. 15 ºC

T3. 20 ºC

T4. 25 ºC

Plate 4.7a: Effect of different temperature ranges on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

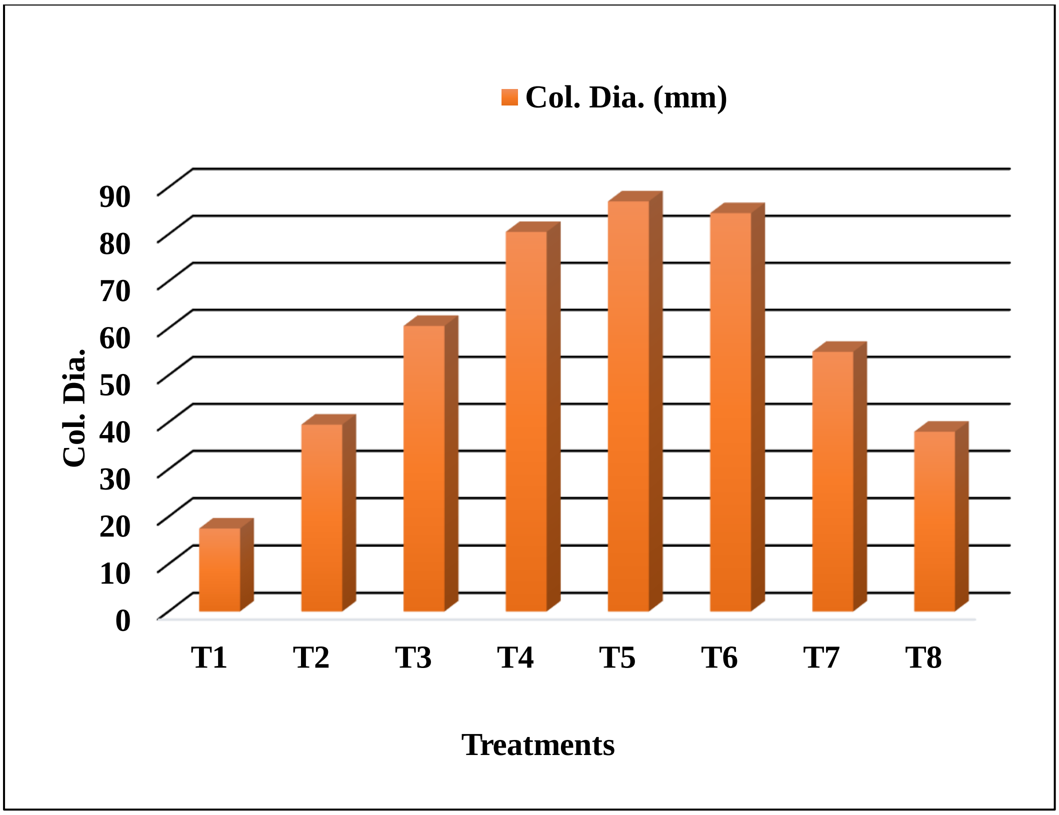
T_5. 30 °C

T_6. 35 °C

T_7. 40 °C

T_8. 45 °C

Plate 4.7b: Effect of different temperature ranges on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

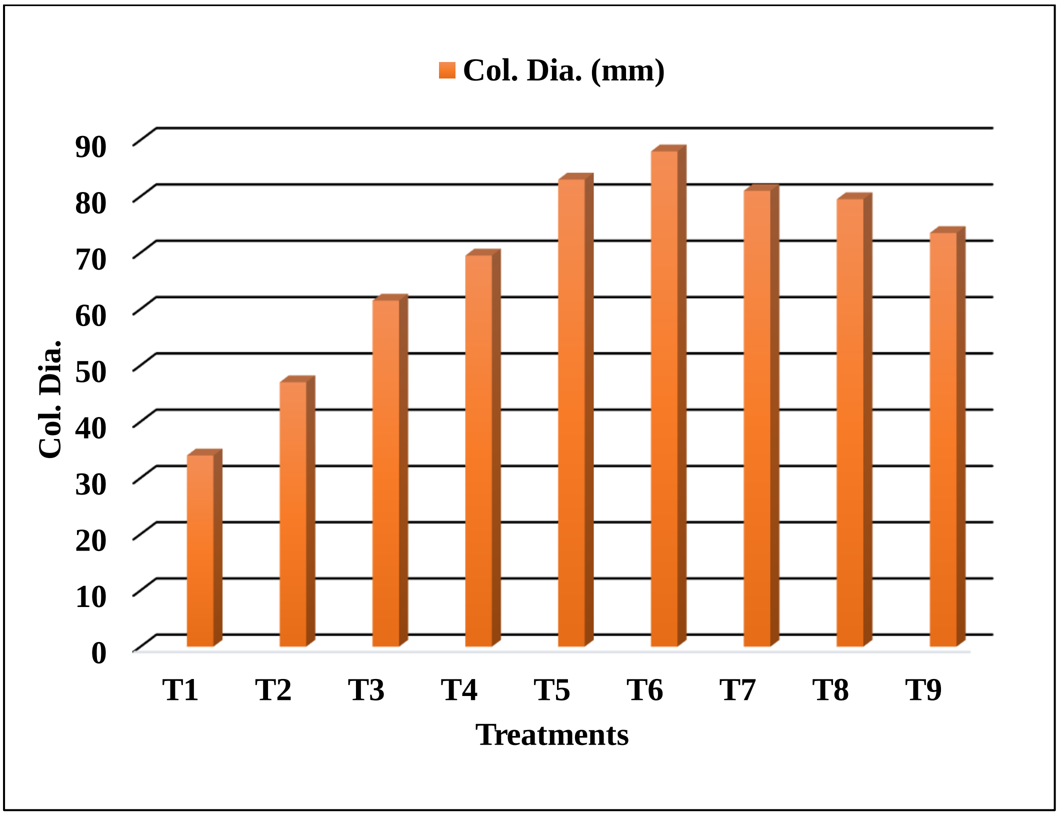


Tr. No.	Treatments	Tr. No.	Treatments
T_1	10°C	T_5	30°C
T_2	15°C	T_6	35°C
T_3	20°C	T_7	40°C
T_4	25°C	T_8	45°C

Fig. 4.2: Effect of different Temperature ranges on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

Tr. No.	Treatments (pH)	Tr. No.	Treatments (pH)
T_1	4.0	**T_6**	6.5
T_2	4.5	**T_7**	7.0
T_3	5.0	**T_8**	7.5
T_4	5.5	**T_9**	8.0
T_5	6.0		

Plate 4.8: Effect of different pH levels on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight



Tr. No.	Treatments (pH)	Tr. No.	Treatments (pH)
T_1	4.0	T_6	6.5
T_2	4.5	T_7	7.0
T_3	5.0	T_8	7.5
T_4	5.5	T_9	8.0
T_5	6.0		

Fig. 4.3: Effect of different pH levels on mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

The results revealed that all the nitrogen evaluated *in vitro* exhibited antifungal activity against *P. drechsleri* f. sp. *cajani* and significantly inhibited its growth, over untreated control and presented in Table 4.5, Plate 4.9 and Fig. 4.4.

Table 4.5 Effect of different nitrogen sources on growth of *P. drechsleri* f. sp. *cajani*

Tr. No.	Treatments	Col. Dia. *(mm)	Inhibition %
T_1	Ammonium Chloride (NH_4Cl)	12.50	86.11 **(68.11)****
T_2	Ammonium Sulphate ($(NH_4)_2\ SO_4$)	34.00	62.22 **(52.07)**
T_3	Ammonium Nitrate ($(NH_4)_2\ NO_3$)	29.00	67.77 **(55.40)**
T_4	Sodium Nitrate ($NaNO_3$)	42.00	53.33 **(46.90)**
T_5	Ammonium Ferrous Sulphate $(NH_4)_2Fe\ (SO_4)_2.6H_2O$	00.00	100.00 **(90.00)**
T_6	Urea ($CH_4.N_2O$)	12.00	86.66 **(68.57)**
T_7	Potassium Nitrate (KNO_3)	38.00	57.77 **(49.47)**
T_8	Ammonium Oxalate ($(NH_4)_2C_2O_4$)	15.50	82.77 **(65.47)**
T_9	Control (Untreated)	90.00	00.00 **(00.00)**
	S.E.	**0.462**	**0.635**
	C.D.(P=0.01)	**1.383**	**1.901**

*: Mean of three replications. Dia.: Diameter,

**Figures in parentheses are arc sine transformed values.

The results revealed that, Ammonium Ferrous Sulphate $(NH_4)_2Fe\ (SO_4)_2.6H_2O$ was found most effective nitrogen source with significantly smallest mycelial growth (00.00 mm) and significantly uppermost mycelial growth inhibition (100 %), followed by Urea (12.00 mm and 86.66 %), Ammonium Chloride (NH_4Cl) (12.50 mm and 86.11%), Ammonium Oxalate ($((NH_4)_2C_2O_4$ (15.50 mm and 82.77%), Ammonium Nitrate ($(NH_4)_2NO_3$) (29.00 mm and 67.77 %), Ammonium Sulphate $(NH_4)_2SO_4$ (34.00 mm and 62.22%), Potassium Nitrate (KNO_3) (38.00 mm and 57.77%) and Sodium Nitrate ($NaNO_3$) (42.00 mm and 53.33%), respectively.

Nitrogen sources used in present study against pigeonpea stem blight causing *P. drechsleri* f. sp. *cajani* and also against other *Phytophthora* spp. infecting various crop hosts were reported as potential antagonists, by several earlier scientist (Singh, 1973; Riga *et al.* 2000; Faisal *et al.* 2005; Nakova, 2011; Asmita, 2015 and Morales 2019).

4.7 Disease management strategies

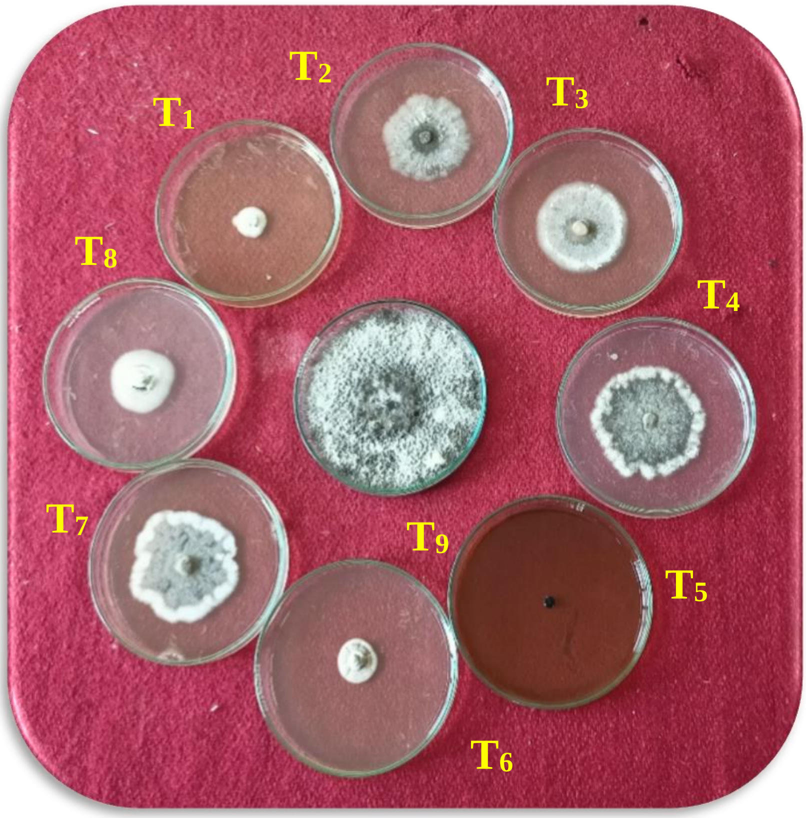
Based on symptomatology and pathogenicity test of *Phytophthora drechsleri* f. sp. *cajani*, causing stem blight of pigeonpea were selected to evaluate *in vitro* efficacy of the fungicides, biocontrol agents and phytoextracts against them and the results thereof are described under following sub-heads.

4.7.1 *In vitro* efficacy of systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani*, causing stem blight of pigeonpea

All of the seven systemic fungicides *viz.*, Azoxystrobin 23 % SC, Dimethomorph 50 % WP, Metalaxyl MZ 35 % WS, Fosetyl AL 80 % WP, Difenoconazole 25 % EC, Thiophanate methyl 70 % WP and Hexaconazole 70 % WP evaluated *in vitro* by poisoned food technique (each @ 500, 1000 ppm) showed antifungal activity against *Phytophthora drechsleri* f. sp. *cajani*, which numerically affected mycelial growth and its corresponding inhibition, over untreated control and presented in Table 4.6, Plate 4.10 and Fig. 4.5.

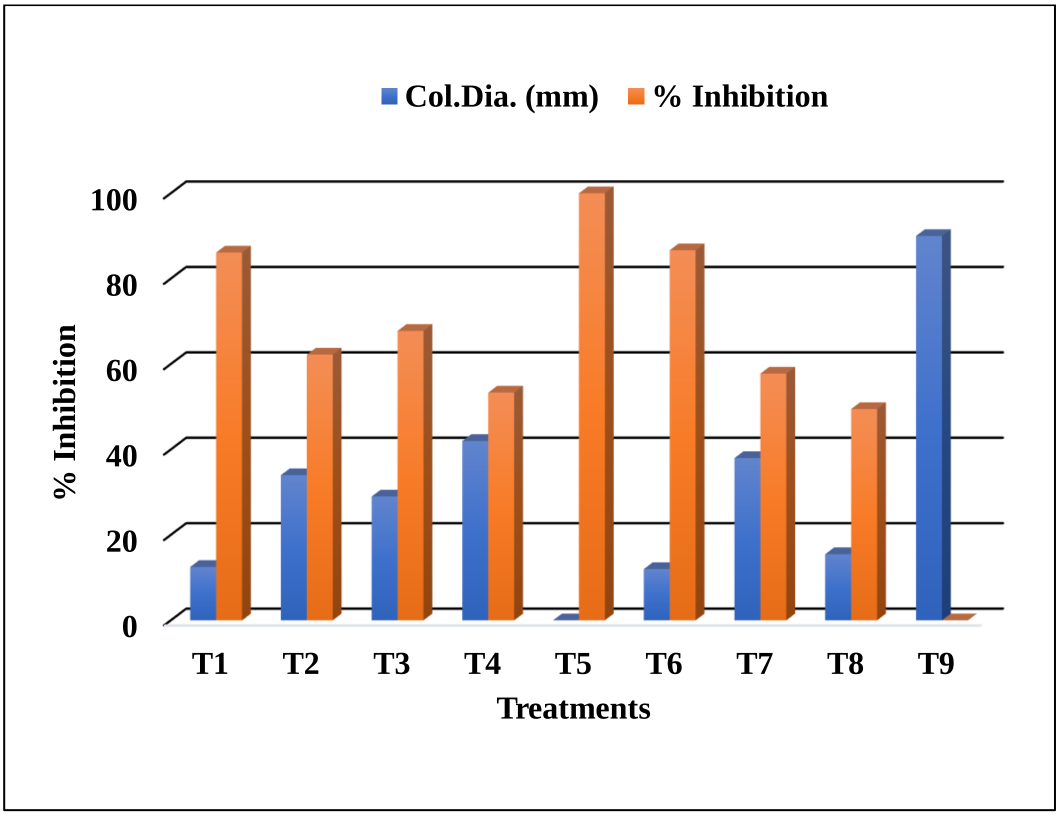
4.7.1.1 Effect on mycelial growth

The results revealed that, the test systemic fungicides exhibited a wide range mycelial growth of *P. drechsleri* f. sp. *cajani*, which was found to be decreased with increase in the concentration of systemic fungicides. However, Metalaxyl MZ 35% WS, Fosetyl AL 80% WP (each @ 500 and 1000 ppm) showed no mycelial growth of the test pathogen. These were followed by Hexaconazole 70%WP (13.50 and 6.50 mm), Dimethomorph 50% WP (15.00 and 8.50 mm), Difenconazole 25% EC (20.00 and 14.00 mm), Azoxystrobin 23% EC (20 and 16.50 mm) and Thiophanate methyl 70% WP (25.00 and 12.50 mm), respectively @ 500 and 1000 ppm, against maximum mycelial growth (90 mm) in untreated control.



Tr. No.	Treatments	Tr. No.	Treatments
T_1	Ammonium Chloride (NH_4Cl)	T_6	Urea
T_2	Ammonium Sulphate ($(NH_4)_2 SO_4$)	T_7	Potassium Nitrate (KNO_3)
T_3	Ammonium Nitrate ($(NH_4)_2 NO_3$)	T_8	Ammonium Oxalate ($(NH_4)2C_2O_4$)
T_4	Sodium Nitrate ($NaNO_3$)	T_9	Control (Untreated)
T_5	Ammonium Ferrous Sulphate $(NH_4)_2Fe(SO_4)_2.6H_2O$		

Plate 4.9: Effect of different nitrogen sources against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea



Tr. No.	Treatments	Tr. No.	Treatments
T_1	Ammonium Chloride (NH_4Cl)	T_6	Urea (CH_4N_2O)
T_2	Ammonium Sulphate ($(NH_4)_2$ SO_4)	T_7	Potassium Nitrate (KNO_3)
T_3	Ammonium Nitrate ($(NH_4)_2$ NO_3)	T_8	Ammonium Oxylate ($(NH_4)2C_2O_4$)
T_4	Sodium Nitrate ($NaNO_3$)	T_9	Control (Untreated)
T_5	Ammonium Ferrous Sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$)		

Fig. 4.4: Effect of different nitrogen sources against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea

Table 4.6 ***In vitro*** **efficacy of systemic fungicides against** ***Phytophthora drechsleri*** **f. sp.** ***cajani***

Tr. No.	Treatments	Col. Dia. *(mm) at ppm		Av. (mm)	% Inhibition *at ppm		Av. Inhibition (%)
		500	1000		500	1000	
T_1	Azoxystrobin 23% SC	20.00	16.50	18.25	77.77 **(61.86)****	81.66 **(64.64)**	79.71 **(63.22)**
T_2	Dimethomorph 50% WP	15.00	8.50	11.75	83.33 **(65.90)**	90.55 **(72.09)**	86.94 **(68.81)**
T_3	Metalaxyl MZ 35% WS	00.00	00.00	00.00	100.00 **(90.00)**	100.00 **(90.00)**	100.00 **(90.00)**
T_4	Fosetyl AL 80% WP	00.00	00.00	00.00	100.00 **(90.00)**	100.00 **(90.00)**	100.00 **(90.00)**
T_5	Difenoconazole 25%EC	20.00	14.00	17.00	77.77 **(61.86)**	84.44 **(66.76)**	81.10 **(64.23)**
T_6	Thiophanate methyl 70%WP	25.00	12.50	18.75	72.22 **(58.19)**	86.11 **(68.11)**	79.16 **(62.83)**
T_7	Hexaconazole 70% WP	13.50	6.50	10.00	85.00 **(67.21)**	92.77 **(74.40)**	88.88 **(70.52)**
T_8	Control	90.00	90.00	90.00	00.00 **(00.00)**	00.00 **(00.00)**	00.00 **(00.00)**
	S.E.	**0.500**	**0.574**	**-**	**0.456**	**0.462**	**-**
	C.D.(P=0.01)	**1.512**	**1.737**	**-**	**1.380**	**1.397**	**-**

*: Mean of three replications. Dia.: Diameter, Av.: Average

**Figures in parentheses are arc sine transformed values.

Average radial mycelial growth recorded with the systemic fungicides, which ranged from 00 mm to 18.75 mm. However, least average mycelial growth was showed by Hexaconazole 70%WP (10.00 mm), Dimethomorph 50% WP (11.75 mm), Difenconazole 25% EC (17.00 mm), Azoxystrobin 23% EC (18.25 mm) and Thiophanate methyl 70% WP (18.75 mm), respectively.

4.7.1.2. Effect on mycelial growth inhibition

The result revealed that, all systemic fungicides tested (each @ 500 and 1000 ppm), were inhibited mycelial growth of *P. drechsleri* f. sp. *cajani*, over control and it was directly proportional to the concentration of the test fungicide.

Among systemic fungicides tested, Metalaxyl MZ 35% WS, Fosetyl AL 80% WP (each @ 500 and 1000 ppm) resulted for cent per cent mycelial growth inhibition (100%). These were followed by Hexaconazole 70%WP (85.00% and 92.77%), Dimethomorph 50% WP (83.33% and 90.55%), Difenconazole 25% EC (77.77% and

84.44%), Azoxystrobin 23% EC (77.77 and 81.66 %) and Thiophanate methyl 70% WP (72.22% and 86.11%), respectively each @ 500 and 1000 ppm and these fungicides were significantly with each other.

Average mycelial growth inhibition recorded with test fungicides, which ranged from 79.71 to 100 %. However, it was maximum and cent per cent with Metalaxyl MZ 35% WS, Fosetyl AL 80% WP. These were followed by Hexaconazole 70%WP (88.88%), Dimethomorph 50% WP (86.94%), Difenconazole 25% EC (81.10%), Azoxystrobin 23% EC (79.71%) and Thiophanate methyl 70% WP (79.16%), respectively.

These results were confirmed with the findings of several earlier workers. They reported regarding effectiveness of systemic fungicides *viz*., Metalaxyl MZ 35 % WS, Fosetyl AL 80 % WP, Azoxystrobin 23 % EC against *P. drechsleri* f. sp. *cajani* and also against other *Phytophthora* spp. infecting various crop hosts (Kanniyan and Nene, 1984; Agrawal, 1987; Bisht *et al.* 1988; Perry *et al*. 2006; Jadesha 2014; Rolando *et al.* 2016; Patel, 2016; Thomas and Naik, 2017 and Kumar *et al*. 2020).

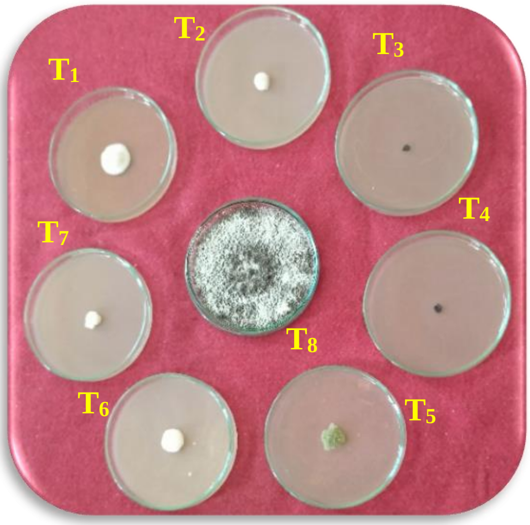
4.7.2 *In vitro* efficacy of non-systemic and combi product fungicides against *Phytophthora drechsleri* f. sp. c*ajani*, causing stem blight of pigeonpea

All of the nine non-systemic and combi product fungicides *Viz.*, Mancozeb 75 % WP, Chlorothalonil 75 % WP, Copper Oxychloride 50 % WDG, Copper hydroxide 77 % WP, Tebuconazole 50% + Trifloxystrobin 25%WG, Mancozeb 64% + Cymoxanil 8% WP, Metalaxyl 8% + Mancozeb 64% WP, Thiophanate methyl 45% + Pyraclostrobin 5% FS and Iprovalicarb 5.5% + Propineb 61.25% WP evaluated *in vitro* by poisoned food technique (each @ 2000, 2500 ppm) showed antifungal activity against *Phytophthora drechsleri* f. sp. *cajani*, which numerically affected mycelial growth and its corresponding inhibition, over untreated control presented in Table 4.7, Plate 4.11 and Fig 4.6.

4.7.2.1 Effect on mycelial growth

The results revealed that, the test non-systemic and combi product fungicides exhibited a wide range of mycelial growth of *P. drechsleri* f. sp. *cajani,* which was found to be decreased with increase in the concentration of systemic fungicides. However, Mancozeb 75% WP, Mancozeb 64% + Cymoxanil 8% WP, Metalaxyl 8% +

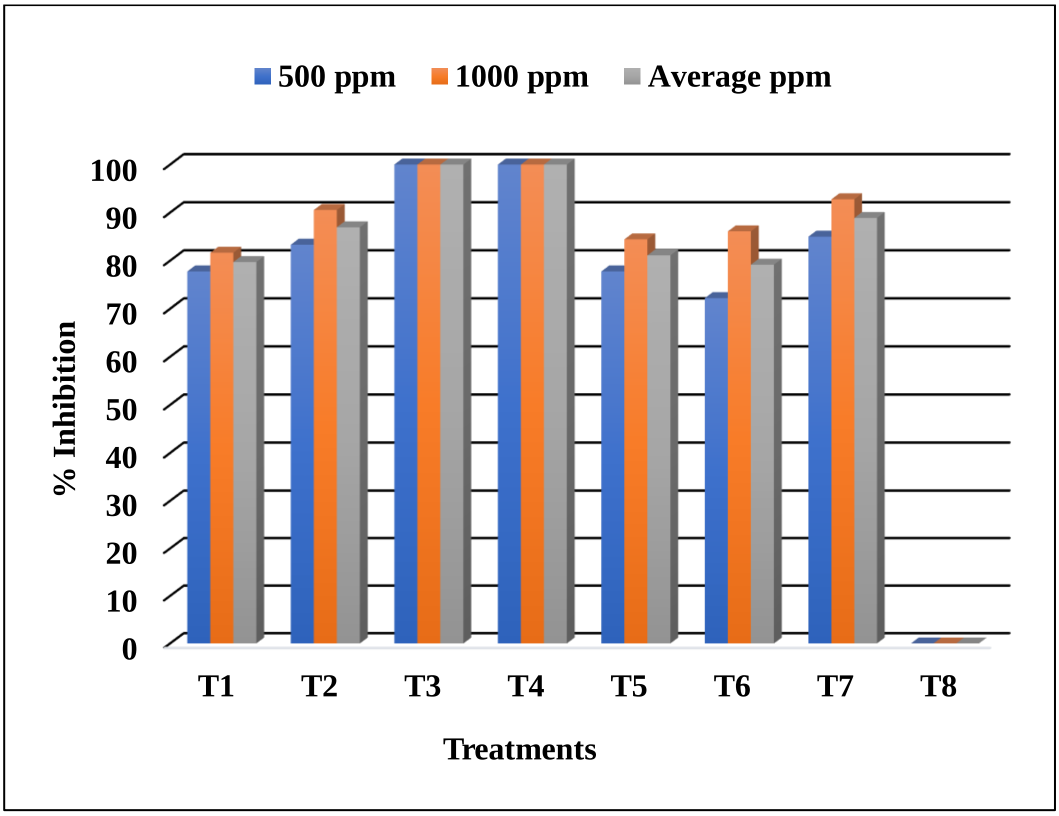
A. @ 500 ppm



A. @ 10 00 ppm

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Azoxystrobin 23% SC	T_5	Difenconazole 25%EC
T_2	Dimethomorph 50% WP	T_6	Thiophanate methyl 70% WP
T_3	Metalaxyl MZ 35% WS	T_7	Hexaconazole 5% EC
T_4	Fosetyl AL 80% WP	T_8	Control (Untreated)

Plate 4.10: *In vitro* efficacy of systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea



Tr. No.	Treatments	Tr. No.	Treatments
T_1	Azoxystrobin 23% SC	T_5	Difenconazole 25%EC
T_2	Dimethomorph 50% WP	T_6	Thiophenate methyl 70% WP
T_3	Metalaxyl MZ 35% WS	T_7	Hexaconazole 5% EC
T_4	Fosetyl AL 80% WP	T_8	Control (Untreated)

Fig. 4.5: *In vitro* efficacy of systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of Pigeonpea

Mancozeb 64% WP, Thiophanate methyl 45% + Pyraclostrobin 5% FS (each @ 2000 and 2500 ppm) showed no mycelial growth of the test pathogen. These were followed by Iprovalicarb 5.5% + Propineb 61.25% WP (18.50 and 16.50 mm), Tebuconazole 50% + Trifloxystrobin 25%WG (20.00 and 15.00 mm), Chlorothalonil 75%WP (22.50 and 18.50 mm), Copper hydroxide 77% WP (27.50 and 13.50 mm) and Copper Oxychloride 50% WDG (36.50 and 15.50 mm), respectively @ 2000 and 2500 ppm, against maximum mycelial growth (90 mm) in untreated control.

Table 4.7 *In vitro* efficacy of non-systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani*

Tr. No.	**Treatments**	**Col. Dia. *(mm) at ppm**		**Av. (mm)**	**% Inhibition *at ppm**		**Av. Inhibition (%)**
		2000	**2500**		**2000**	**2500**	
T_1	Mancozeb 75% WP	00.00	00.00	00.00	100.00 **(90.00)****	100.00 **(90.00)**	100.00 **(90.00)**
T_2	Chlorothalonil 75%WP	22.50	18.50	20.50	75.00 **(60.00)**	79.44 **(63.03)**	77.22 **(61.49)**
T_3	Copper Oxychloride 50% WDG	36.50	15.50	26.00	59.44 **(50.44)**	82.77 **(65.47)**	71.10 **(57.48)**
T_4	Copper hydroxide 77% WP	27.50	13.50	20.35	69.44 **(56.43)**	85.00 **(67.21)**	77.22 **(61.49)**
T_5	Tebuconazole 50% + Trifloxystrobin 25 % WG	20.00	15.00	27.50	77.77 **(61.86)**	83.33 **(65.90)**	80.55 **(63.83)**
T_6	Mancozeb 64% + Cymoxanil 8% WP	00.00	00.00	00.00	100.00 **(90.00)**	100.00 **(90.00)**	100.00 **(90.00)**
T_7	Metalaxyl 8% + Mancozeb 64% WP	00.00	00.00	00.00	100.00 **(90.00)**	100.00 **(90.00)**	100.00 **(90.00)**
T_8	Thiophanate methyl 45% + Pyraclostrobin 5% FS	00.00	00.00	00.00	100.00 **(90.00)**	100.00 **(90.00)**	100.00 **(90.00)**
T_9	Iprovalicarb 5.5% + Propineb 61.25% WP	18.50	16.50	17.50	79.44 **(63.03)**	81.16 **(64.27)**	80.30 **(63.65)**
T_{10}	Control	90.00	90.00	90.00	00.00 **(00.00)**	00.00 **(00.00)**	00.00 **(00.00)**
	S.E.	**0.577**	**0.520**	**-**	**0.462**	**0.548**	**-**
	C.D.(P=0.01)	**1.715**	**1.544**	**-**	**1.372**	**1.629**	**-**

* Mean of three replications. Dia.: Diameter, Av.: Average

**Figures in parentheses are arc sine transformed values.

Average radial mycelial growth recorded with the systemic fungicides which ranged from 00 mm to 27.50 mm. However, least average mycelial growth showed by Iprovalicarb 5.5% + Propineb 61.25% WP (17.50 mm) followed by Copper hydroxide 77% WP (20.35 mm), Chlorothalonil 75%WP (20.50 mm), Copper Oxychloride 50% WDG (26.00 mm) and Tebuconazole 50% + Trifloxystrobin 25%WG (27.50 mm), respectively.

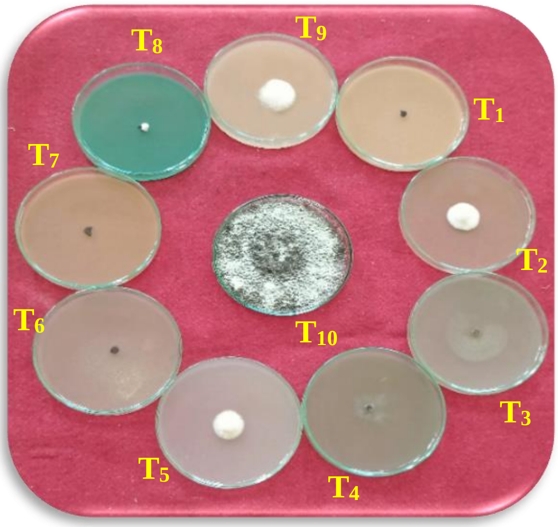
4.7.2.2. Effect on mycelial growth inhibition

The result revealed that, all non-systemic and combi product fungicides tested (each @ 2000 and 2500 ppm) inhibited mycelial growth of *P. drechsleri* f. sp. *cajani*, over control and it was directly proportional to the concentration of the test fungicide.

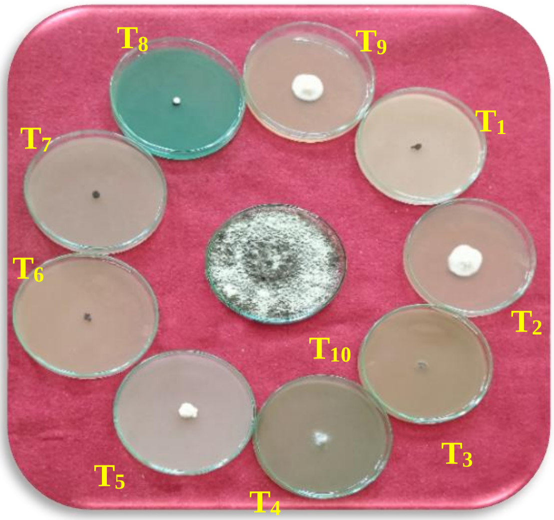
Among non-systemic and combi product fungicides tested, Mancozeb 75% WP, Mancozeb 64% + Cymoxanil 8% WP, Metalaxyl 8% + Mancozeb 64% WP, Thiophanate methyl 45% + Pyraclostrobin 5% FS (each @ 2000 and 2500 ppm) resulted cent per cent mycelial growth inhibition (100%). These were followed by Iprovalicarb 5.5% + Propineb 61.25% WP (79.44 and 81.16 %), Tebuconazole 50% + Trifloxystrobin 25%WG (77.77 and 83.33 %), Chlorothalonil 75%WP (75.00 and 79.44 %), Copper hydroxide 77% WP (69.44 and 85.00 %) and Copper Oxychloride 50% WDG (59.44 and 82.77 %), respectively each @ 2000 and 2500 ppm and these fungicides were significantly on par with each other.

Average mycelial growth inhibition recorded with test fungicide, which ranged from 71.10 to 100 %. However, it was numerically maximum and cent per cent with Mancozeb 75 % WP, Mancozeb 64% + Cymoxanil 8% WP, Metalaxyl 8 % + Mancozeb 64% WP, Iprovalicarb 5.5 % + Propineb 61.25 % WP. These were followed by Thiophanate methyl 45 % + Pyraclostrobin 5 % FS (80.30 %) Tebuconazole 50 % + Trifloxystrobin 25 % WG (80.55 %), Chlorothalonil 75 % WP and Copper hydroxide 77 % WP (77.22 %) and Copper Oxychloride 50 % WDG (71.10 %), respectively.

These results are confirmity with the finding of several earlier workers. Systemic fungicides *viz*., Mancozeb 75% WP, Mancozeb 64% + Cymoxanil 8% WP, Metalaxyl 8% + Mancozeb 64% WP effective against *P. drechsleri* f. sp. *cajani* and also against other *Phytophthora* spp. infecting various crop hosts (Jagtap *et al*. 2012; Jadesha, 2014; Asmita, 2015; Rolando *et al*. 2016; Patel, 2016; Thomas and Naik, 2017; Muhammad and Syed, 2018).



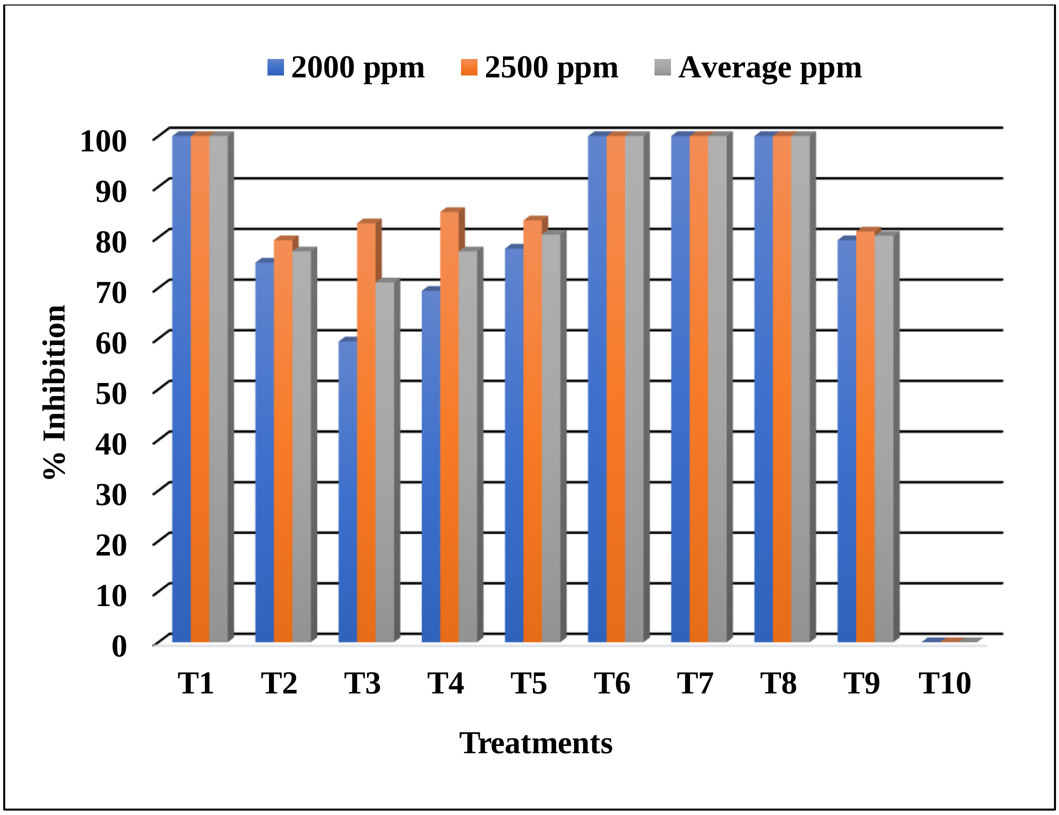
A. @ 2000 ppm



A. @ 2500 ppm

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Mancozeb 64% + Cymoxanil 8% WP
T_2	Chlorothalonil 75%WP	T_7	Metalaxyl 8% + Mancozeb 64% WP
T_3	Copper Oxychloride 50% WDG	T_8	Thiophanate methyl 45% + Pyraclostrobin 5% FS
T_4	Copper hydroxide 77% WP	T_9	Iprovalicarb 5.5% + Propineb 61.25%
T_5	Tebuconazole 50% + Trifloroxystrobin 25% WG	T_{10}	Control (Untreated)

Plate 4.11: *In vitro* efficacy of non- systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea



Tr. No.	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Cymoxanil 8% + Mancozeb 64% WP
T_2	Chlorothalonil 75%WP	T_7	Metalaxyl 8% + Mancozeb 64% WP
T_3	Copper Oxychloride 50% WDG	T_8	Thiophanate methyl 45% + Pyraclostrobin 5% FS
T_4	Copper hydroxide 77% WP	T_9	Iprovalicarb 5.5% + Propineb 61.25%
T_5	Tebuconazole 50% + Trifloroxystrobin 25% WG	T_{10}	Control (Untreated)

Fig. 4.6: *In vitro* efficacy of non- systemic fungicides against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea

Antimicrobial or antifungal activity of fungicides against test pathogen, Metalaxyl and Mancozeb inhibits the RNA synthesis. Metalaxyl highly inhibits sporangium formation and reduces chlamydospore and oospore formation. It prevents colonization of leaf tissue by mycelium since it inhibits the growth of hyphae (Jadesha, 2014).

4.7.3 *In vitro* efficacy of bioagents against *P. drechsleri* f. sp. *cajani* causing stem blight of pigeonpea.

To study the *in vitro* effect of bioagents *viz., Trichoderma asperellum, T. harzianum, T. hamatum, T. koningii, Verticillium lecani, Metarhizium anisopliae, Bacillus subtilis* and *Pseudomonas fluorescens* against *P. drechsleri* f. sp. *cajani*. The results revealed that, all the bioagents evaluated *in vitro*, exhibited antifungal activity against *P. drechsleri* f. sp. *cajani* and significantly inhibited its growth, over untreated control and presented in Table 4.8, Plate 4.12, and Fig. 4.7.

Table 4.8 *In vitro* efficacy of bioagents against *Phytophthora drechsleri* f. sp. *cajani*

Tr. No.	Treatments	Col. Dia. *(mm)	Inhibition %
T_1	*Trichoderma asperellum*	12.50	86.11 **(68.11)****
T_2	*T. harzianum*	10.50	88.33 **(70.02)**
T_3	*T. hamatum*	6.00	93.33 **(75.03)**
T_4	*T. koningii*	23.50	73.88 **(59.26)**
T_5	*Verticillium lecani*	46.00	45.68 **(42.52)**
T_6	*Metarhizium anisopliae*	21.00	76.66 **(61.11)**
T_7	*Bacillus subtilis*	70.00	22.22 **(28.12)**
T_8	*Pseudomonas fluorescens*	67.50	25.00 **(30.00)**
T_9	Control (Untreated)	90.00	00.00 **(00.00)**
	S.E.	**0.520**	**0.589**
	C.D.(P=0.01)	**1.556**	**1.763**

*: Mean of three replications, Dia.: Diameter,

**Figures in parentheses are arcsine transformed values

The results revealed *Trichoderma hamatum* was found as most effective bioagent with significantly least mycelial growth (6.00 mm) and significantly highest mycelial growth inhibition (93.33 %) followed by *Trichoderma harzianum* (10.55 mm and 88.33%), *T. asperellum* (12.50 mm and 86.11%), *Metarhizium anisopliae* (21.00 mm and 76.66 %), *T. koningii* (23.50 mm and 73.88%), *Verticillium lecani* (46.00 mm and 45.68%), *Pseudomonas fluorescens* (67.50 mm and 25%) and *Bacillus subtilis* (70.0 mm and 22.22%), respectively.

The test bioagents found most effective in present study against pigeonpea stem blight causing *P. drechsleri* f. sp. *cajani* similar finding regarding effectiveness of bioagent and also *Trichoderma* spp. as potential antagonist against *P. drechsleri* f. sp. *cajani* and *Phytophthora* spp. infecting various crop hosts by several earlier workers (Jagtap *et al*. 2012; jadesha 2014; Rashmi *et al*. 2015; Asmita, 2015; Muhammad, 2019 and Jadesha *et al*. 2019).

Antimicrobial or antifungal activity of bioagents against test pathogen due to *Trichoderma* spp. and bacterial bioagents produce mycolytic enzymes which play an important role in the degradation of target pathogens (Jagtap *et al*. 2012; Jadesha, 2014 and Rashmi *et al*. 2015).

4.7.4 *In vitro* efficacy of phytoextracts against *P. drechsleri* f. sp. *cajani* causing stem blight of pigeonpea

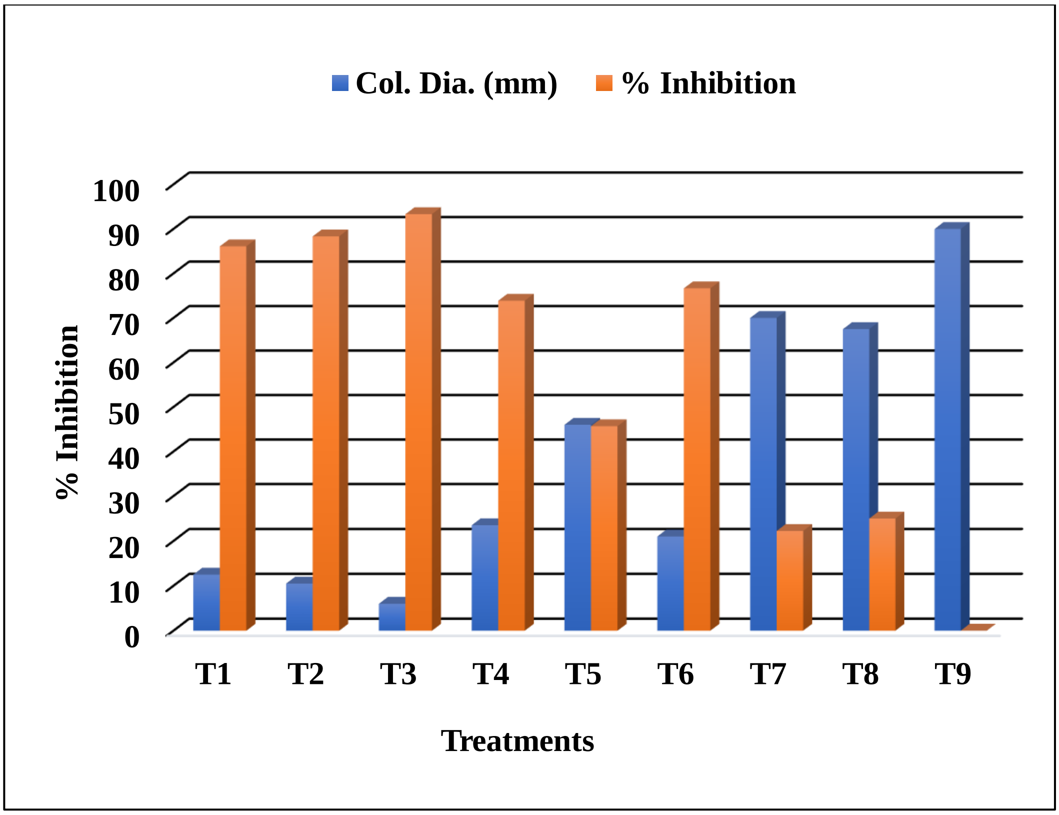
Solvent (acetone) extracts (leaf / rhizome / bulb) of eight plant species *viz*., *Eucalyptus globulus* (Nilgiri), *Allium cepa* (Onion), *Allium sativum* L. (Garlic), *Azardirachta indica* (Neem), *Lantana camera* L. (Ghaneri), *Zingiber officinal* (Ginger), *Vitex negundo* (Nirgudi), *Lawsonia innermis* (Mehandi) were evaluated *in vitro* (each @ 10 and 20 %) against *P. drechsleri* f. sp. *cajani* and the results obtained on mycelial growth and its inhibition are presented in Table 4.9, Plate 4.13 and Fig. 4.8.

4.7.4.1 Effect on mycelial growth

At 10 per cent concentration, radial mycelial growth of *P. drechsleri* f. sp. *cajani* ranged from 8.50 to 75.50 mm. However, it was significantly least with *A sativum L.* (8.50 mm), followed by *Z. officinale* (12.50 mm), *L. inermis* (14.50 mm), *E. globulus* (32.50 mm), *L. camara* (40.50mm) *A. cepa* (42.50 mm), *A. indica* (72.00 mm) and *V. negundo* (75.50 mm), respectively.

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperellum*	T_6	*Metarhizium anisopliae*
T_2	*T. harzianum*	T_7	*Bacillus subtilis*
T_3	*T. hamatum*	T_8	*Pseudomonas fluorescens*
T_4	*T. koningii*	T_9	Control (Untreated)
T_5	*Verticillium leccani*		

Plate 4.12: *In vitro* efficacy of various bioagents against *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight



Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperellum*	T_6	*Metarhizium anisopliae*
T_2	*T. harzianum*	T_7	*Bacillus subtilis*
T_3	*T. hamatum*	T_8	*Pseudomonas fluorescens*
T_4	*T. koningii*	T_9	Control (Untreated)
T_5	*Verticillium leccani*		

Fig. 4.7: *In vitro* efficacy of various bioagents against *Phytophthora drechsleri* f. sp. *cajani* causing pigeonpea stem blight

Table 4.9 *In vitro* efficacy of phytoextracts against *Phytophthora drechsleri* f. sp. *cajani*

Tr. No.	Treatments	Col. Dia. *(mm) at ppm		Av. (mm)	% Inhibition *at		Av. Inhibition (%)
		10%	20%		10%	20%	
T_1	*Eucalyptus globulus* (Nilgiri)	32.50	30.00	31.25	63.88 **(53.05)****	66.66 **(54.73)**	65.27 **(53.89)**
T_2	*Allium cepa* (Onion)	42.50	40.00	41.25	52.77 **(46.58)**	55.55 **(48.18)**	54.16 **(47.38)**
T_3	*Allium sativum L.* (Garlic)	8.50	5.50	7.00	90.55 **(72.09)**	93.88 **(75.67)**	92.21 **(73.79)**
T_4	*Azadirachta indica* (Neem)	72.00	66.50	69.25	20.00 **(26.56)**	26.11 **(30.72)**	23.05 **(28.69)**
T_5	*Lantana camara L.* (Ghaneri)	40.50	38.00	39.25	55.00 **(47.86)**	57.77 **(49.47)**	56.38 **(48.66)**
T_6	*Zingiber officinale* (Ginger)	12.50	10.00	11.25	86.11 **(68.11)**	88.88 **(70.52)**	87.49 **(69.28)**
T_7	*Vitex negundo* (Nirgudi)	75.50	72.50	74.00	16.11 **(23.66)**	19.44 **(26.16)**	17.77 **(24.93)**
T_8	*Lawsonia inermis* (Mehandi)	14.50	10.50	12.50	83.88 **(66.32)**	88.33 **(70.02)**	86.10 **(68.10)**
T_9	Control	90.00	90.00	90.00	00.00 **(00.00)**	00.00 **(00.00)**	00.00 **(00.00)**
	S.E.	**0.577**	**0.520**	**-**	**0.635**	**0.520**	**-**
	C.D.(P=0.01)	**1.729**	**1.556**	**-**	**1.901**	**1.556**	**-**

*Mean of three replications. Diam.: Diameter, Av.: Average

**Figures in parentheses are arcsine transformed values

At 20 per cent concentration, radial mycelial growth of *P. drechsleri* f. sp. *cajani* ranged from 5.50 to 72.50 mm. However, it was significantly least with *A. sativum* (5.50 mm), followed by *Z. officinale* (10.00 mm), *L. innermis* (10.50 mm), *E. globulus* (30.00

mm), *L. camara* (38.00 mm), *A. cepa* (40.00 mm), *A. indica* (66.50 mm) and *V. negundo* (72.50 mm), respectively.

Average radial mycelial growth of *P. drechsleri* f. sp. *cajani* ranged from 7.00 to 74.00 mm. However, it was least with *A. sativum* (7.00 mm), followed by *Z. officinale* (11.25 mm), *L. innermis* (12.50 mm), *E. globulus* (31.25 mm), *L. camara* (39.25 mm), *A. cepa* (41.25 mm), *A. indica* (69.25 mm) and *V. negundo* (74.00 mm), respectively.

4.7.4.2 Effect on mycelial growth inhibition

The results revealed that, the phytoextracts tested were significantly inhibited the mycelial growth of *P. drechsleri* f. sp. *cajani*, over untreated control and it was increased with the increase in concentration of the phytoextracts tested.

At 10 per cent concentration, mycelial growth inhibition of *P. drechsleri* f. sp. *cajani* ranged from 16.11 to 90.55 per cent. However, it was significantly maximum with *A. sativum* (90.55 %), followed by *Z. officinale* (86.11%), *L. innermis* (83.88%), *E. globulus* (63.88%), *L. camara* (55.00%) and *A. cepa* (52.77%), *A. indica* (20.00%) and *V. negundo* (16.11%), respectively.

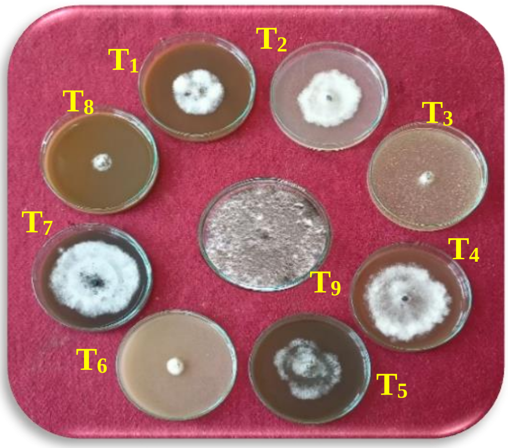
At 20 per cent concentration, mycelial growth inhibition of *P. drechsleri* f. sp. *cajani* ranged from 19.44 to 93.21 per cent. However, it was significantly maximum with *A. sativum* (93.21%), followed by *Z. officinale* (88.88%), *L. innermis* (88.83%), *E. globulus* (66.66%), *L. camara* (55.57%) and *A. cepa* (55.55%), *A. indica* (26.11%) and *V. negundo* (19.44 %), respectively.

Average mycelial growth inhibition of *P. drechsleri* f. sp. *cajani* ranged from 17.77 to 92.21 per cent. However, it was highest with *A. sativum* (92.21%), followed by *Z. officinale* (87.49%), *L. inermis* (86.10%), *E. globulus* (65.27%), *L. camara* (56.38%) and *A. cepa* (54.16%), *A. indica* (23.05%) and *V. negundo* (17.70%).

Thus, based on average mycelial growth inhibition, the most potential antifungal phytoextracts found in their order of merit were *A. sativum* > *Z. officinale* > *L. innermis* > *E. globulus* > *L. camara*, respectively.

These results of the present study on fungicidal / fungistatic potential of the test phytoextracts are in agreement with the findings of several earlier workers. The effectiveness of phytoextracts *viz.*, *A. sativum* and *Z. officinale* were earlier reported by several workers (Jagtap *et al*. 2012; Asmita, 2015 and Rashmi *et al*. 2015).

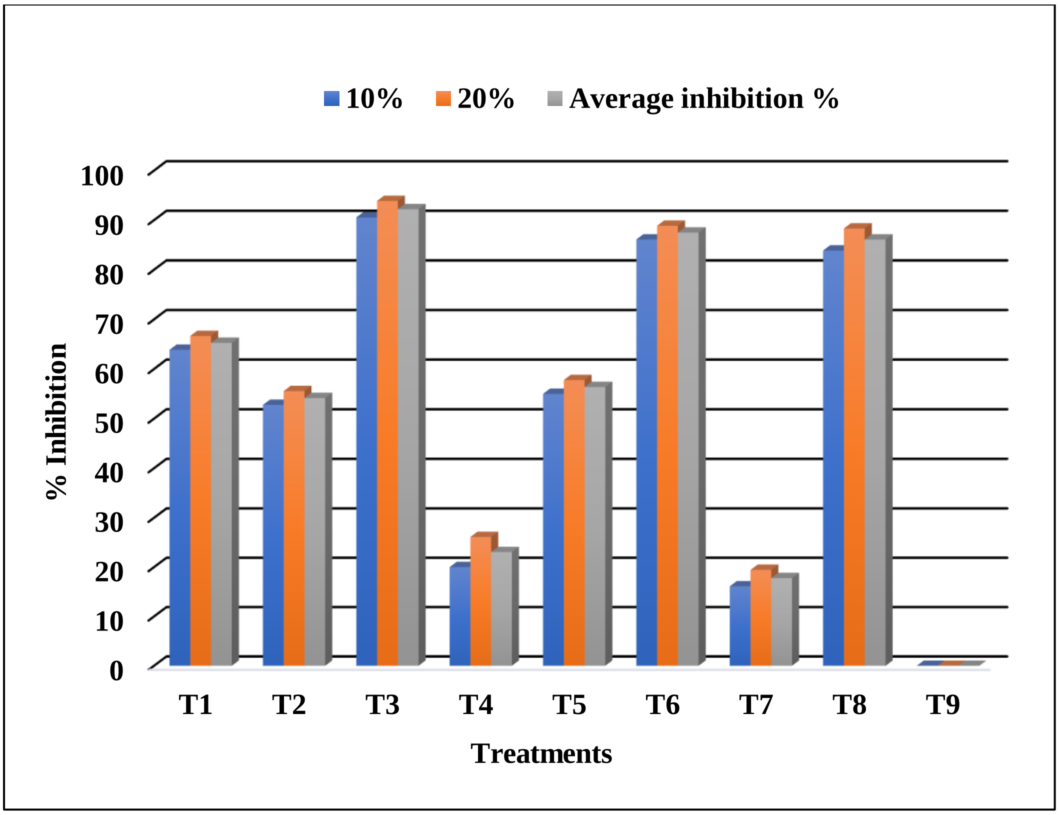
A. @ 10 %



A. @ 20 %

Tr. No.	Treatments	Plant parts used	Tr. No.	Treatments	Plant parts used
T_1	*Eucalyptus globulus* (Nilgiri)	Leaves	T_6	*Zingiber officinale* (Ginger)	Rhizome
T_2	*Allium cepa* (Onion)	Bulb	T_7	*Vitex negundo* (Nirgudi)	Leaves
T_3	*Allium sativum L.* (Garlic)	Clove	T_8	*Lawsonia innermis* (mehandi)	Leaves
T_4	*Azadirachta indica* (Neem)	Leaves	T_9	Control (Untreated)	
T_5	*Lantana camera L.* (Ghaneri)	Leaves			

Plate 4.13: *In vitro* efficacy of phytoextracts against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of pigeonpea



Tr. No.	Treatments	Plant parts used	Tr. No.	Treatments	Plant parts used
T_1	*Eucalyptus globulus* (Nilgiri)	Leaves	T_6	*Zingiber officinale* (Ginger)	Rhizome
T_2	*Allium cepa* (Onion)	Bulb	T_7	*Vitex negundo* (Nirgudi)	Leaves
T_3	*Allium sativum L.* (Garlic)	Clove	T_8	*Lawsonia innermis* (mehandi)	Leaves
T_4	*Azadirachta indica* (Neem)	Leaves	T_9	Control (Untreated)	
T_5	*Lantana camera L.* (Ghaneri)	Leaves			

Fig. 4.8: *In vitro* efficacy of phytoextracts against *Phytophthora drechsleri* f. sp. *cajani* causing stem blight of Pigeonpea

Antimicrobial or antifungal activity of phytoextracts against test pathogen due to garlic contains diallyl disulphide, diallyl sulphide, diallyl trisulphide, allyl propyl disulphide, allyl alcohol, dimethyl trisulphide, allyl methyl trisulphide, and allycin (diallyl trisulphonate) in its aromatic oils and these compounds have antimicrobial effects. The inhibitory action of neem due to azadirachtin present in seed kernels which retards the growth and activation of the pathogen. Microbial activity of onion bulb extract due to presence of antifungal compounds such as cycloallin and carbohydrate propenyl sulphuric acid (Rashmi *et al*. 2015; Jagtap *et al*. 2012).

CHAPTER - V

SUMMARY AND CONCLUSIONS

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Pigeonpea (*Cajanus cajan* (L.) *Millisp*.) is a cross-pollinated crop belonging to Fabaceae family, commonly known as Adhaki, Arhar, Pigeon pea and Tur in Sanskrit, Hindi, English and Bengali respectively. Pigeonpea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. It is an economically important grain legume of the small and marginal farmers in India. It being a forage crop has been utilized as an important remedy for various ailments. Pigeonpea is one of the major and inseparable dietary protein sources to the large mass of the Indian population.

However, the crop is susceptible to many diseases such as wilt *(Fusarium udum*), sterility mosaic (Pigeonpea Sterility Mosaic Virus), *Alternaria* blight (*Alternaria alternata*), anthracnose (*Colletotrichum* spp.), stem blight (*Phytophthora drechsleri*) and *Cercospora* leaf spot (*Cercospora cajani*) amongst which, the major one is stem blight causes quantitative as well as qualitative losses.

Considering these issues, the present studies on "Studies on pigeonpea stem blight caused by *Phytophthora drechsleri* f. sp. *cajani*" were planned and conducted with well-defined objectives at the Department of Plant Pathology, College of Agriculture, Latur, during *Rabi*, 2020-21 and the findings thereof are being summarized in following paragraphs.

The pigeonpea plants exhibiting typical stem blight symptoms were collected from farmer's field. The *P. drechsleri* f. sp. *cajani* was isolated by applying tissue isolation technique on PDA plates revealed the typical cultural growth of the fungi Therefore, pathogenicity of these *P. drechsleri* f. sp. *cajani* was proved by applying soil drenching method. However, soil drenching method could reveal pathogenic association of the *P. drechsleri* f. sp. *cajani* with pigeonpea stem blight.

The cultural characterization of test pathogen *P. drechsleri* f. sp. *cajani* produced mycelium greyish white growth which was slightly elevated with rough and irregular margin and morphological characters were produced mycelium hyaline, branched, filamentous to cylindrical, coenocytic when young but later septation was noticed with thick plugs and abundant hyphal swelling. Hyphal swelling was common and intercalary and the sporangia were proliferating type and sporangiophore was simple sympodial. Sporangia *viz*., obpyriform, elongated, non-papillate often with tapering base and having size 40-42 × 27-30 μm.

Effect of different culture media on morpho-cultural characters of *Phytophthora drechsleri* f. sp. *cajani* revealed that, Potato dextrose agar, Czapek's dox agar, Corn meal agar, Host leaf extract, V8 juice agar and Carrot dextrose agar shows aseptate mycelium. Oat meal agar and Tomato juice agar shows septate mycelium in morphological characters. Cultural characteristics on Potato dextrose agar, Oat meal agar, Tomato juice agar and Carrot dextrose agar produced greyish white colony colour with rough topography. Host leaf extract and V8 juice agar produce white colony colour with rough, raised and rough respectively, Czapek's dox agar and Corn meal agar produced cottony white and dull white colony colour, respectively with rough topography.

Effect of temperature on growth of *P. drechsleri* f. sp. *cajani* revealed that highest mycelial growth of *P. drechsleri* f. sp. *cajani* was observed at temperature 30°C (87.00 mm) followed by 35°C (84.50 mm), 25°C (80.00 mm), 20°C (60.50 mm), 40°C (55.00 mm), 15°C (39.50 mm), 45°C (38.00 mm) and 10°C (17.50 mm), respectively.

Effect of pH on growth of *P. drechsleri* f. sp. *cajani* revealed that highest mycelial growth of *P. drechsleri* f. sp. *cajani* was observed at pH 6.5 (88.00 mm) followed by 6 (83.00 mm), 7 (81.00 mm), 7.5 (79.50 mm), 8 (73.50 mm), 5.5 (69.50 mm), 5 (61.50 mm), 4.5 (47.00 mm) and pH 4 (34.00), respectively.

Effect of nitrogen sources on growth of *P. drechsleri* f. sp. *cajani* resulted that Ammonium Ferrous Sulphate $(NH_4)_2Fe\ (SO_4)_2.6H_2O$ was found most effective nitrogen source with significantly smallest mycelial growth (00.00 mm) and significantly uppermost mycelial growth inhibition (100 %), followed by Urea (12.00 mm and 86.66 %), Ammonium Chloride (NH_4Cl) (12.50 mm and 86.11%), Ammonium Oxalate ($(NH_4)_2C_2O_4$ (15.50 mm and 82.77%), Ammonium Nitrate $NH_4)_2NO_3$) (29.00 mm and 67.77 %), Ammonium Sulphate $(NH_4)_2SO_4$ (34.00 mm and 62.22%), Potassium Nitrate (KNO_3) (38.00 mm and 57.77%) and Sodium Nitrate ($NaNO_3$) (42.00 mm and 53.33%), respectively.

Seven systemic fungicides evaluated *in vitro* (each @ 500 and 1000 ppm), Metalaxyl MZ 35% WS, Fosetyl AL 80% WP resulted with cent per cent mycelial growth inhibition (100%) in *Phytophthora drechsleri* f. sp. *cajani*, followed by Hexaconazole 70% WP (88.88%), Dimethomorph 50% WP (86.94%), Difenconazole 25% EC (81.10%), Azoxystrobin 23% EC (79.71%) and Thiophanate methyl 70% WP (79.16%), respectively.

Similarly, all of the non-systemic / combiproduct fungicides (each @ 2000 and 2500 ppm) evaluated *in vitro* were found most effective with significantly highest mean mycelial growth inhibition of *Phytophthora drechsleri* f. sp. *cajani,* over untreated control. However, it was maximum and cent per cent with Mancozeb 75% WP, Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8% + Mancozeb 64% WP, Iprovalicarb 5.5% + Propineb 61.25% WP. These were followed by Thiophanate methyl 45% + Pyraclostrobin 5% FS (80.30 %) Tebuconazole 50% + Trifloroxystrobin 25%WG (80.55 %), Chlorothalonil 75%WP and Copper hydroxide 77% WP (77.22 %) and Copper Oxychloride 50% WDG (71.10%), respectively.

All eight test bioagents evaluated *in vitro* significantly inhibited mycelial growth over untreated control, of the test pathogenic fungi *Phytophthora drechsleri* f. sp. *cajani,* causing stem blight of pigeonpea. However, *Trichoderma hamatum* was found as most effective bioagent with significantly smallest mycelial growth (6.00 mm) and significantly uppermost mycelial growth inhibition (93.33 %), followed by *Trichoderma harzianum* (10.55 mm and 88.33%), *T. asperellum* (12.50 mm and 86.11%), *Metarhizium anisopliae* (21.00 mm and 76.66 %), *T. koningii* (23.50 mm and 73.88%) and *Verticillium lecani* (46.00 mm and 45.68%), respectively.

All of the nine phytoextracts evaluated (each @ 10 and 20%) *in vitro* significantly inhibited mycelial growth of *Phytophthora drechsleri* f. sp. *cajani* over untreated control. But, there were no remarkable differences between the test phytoextracts concentrations of 10 and 20 per cent, in respect of per cent mycelial growth inhibition of *Phytophthora drechsleri* f. sp. *cajani*. However, Average mycelial growth inhibition, the most fungistatic / fungicidal phytoextracts found were *A. sativum* (92.21%), followed by *Z. officinale* (87.49%), *L. inermis* (86.10%), *E. globulus* (65.27%), *L. camara* (56.38%) and *A. cepa* (54.16%), respectively.

Conclusions

Thus, from the results obtained on various aspects during present investigations on "Studies on pigeonpea stem blight caused by *Phytophthora drechsleri* f. sp. *cajani*" following conclusions are being drawn:

- *P. drechsleri* f. sp. *cajani* was identified and confirmed, based on symptomatology, morpho-cultural characteristics and pathogenicity test.
- *P. drechsleri* f. sp. *cajani* on different media exhibited a wide range of variations in their morpho-cultural characteristics.

- Effect of temperature and pH on growth of *P. drechsleri* f. sp. *cajani* at temperature 30 °C produced highest mycelial growth pH 6.5 produces highest mycelial growth.
- Among the nitrogen sources evaluated *in vitro* Ammonium Ferrous Sulphate $(NH_4)_2Fe\ (SO_4)_2.6H_2O$ was found most effective nitrogen source with no mycelial growth and highest mycelial growth in Sodium Nitrate (NaNO3).
- Among the fungicides evaluated *in vitro*, Metalaxyl MZ 35% WS, Fosetyl AL 80% WP and Hexaconazole 70%WP (systemic); Mancozeb 75% WP, Cymoxanil 8% + Mancozeb 64% WP, Metalaxyl 8% + Mancozeb 64% WP, Iprovalicarb 5.5% + Propineb 61.25% WP (non-systemic and combi-product) were found most prominent in significantly inhibiting maximum mycelial growth of the *P. drechsleri* f. sp. *cajani* with pigeonpea stem blight.
- Among the bioagents tested *in vitro*, *Trichoderma hamatum* was found as most potent antagonists and among phytoextracts tested *A. sativum* and *Z. officinale* were found efficient with significantly highest mycelial growth inhibition of the *P. drechsleri* f. sp. *cajani* with pigeonpea stem blight.

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LITERATURE CITED

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APPENDICES

APPENDICES

List of contents of media

1) Potato Dextrose Agar (PDA)

i)	Potato	200 g
ii)	Dextrose	20 g
iii)	Agar – Agar	20 g
iv)	Distilled water	1000 ml
v)	pH	6.9 – 7.1

2) Nutrient Agar (NA)

i)	Beef Extract	3 g
ii)	Peptone	5 g
iii)	Agar – Agar	15 g
iv)	Distilled water	1000 ml
v)	pH	6.9 – 7.1

3) Oat meal agar

i)	Rolled oats	40 g
ii)	Agar	20 g
iii)	Distilled water	1000 ml

4) Czapek's dox agar

i)	Sodium nitrate ($NaNO_3$)	2 g
ii)	Potassium dihydrogen phosphate (K_2HPO_4)	1 g
iii)	Magnesium sulphate (MgSO4. 7H2O)	0.5 g
iv)	Ferrous sulphate (FeSO4. 7H2O)	0.01 g
v)	Sucrose (C12H22O11)	20 g
vi)	Agar	20 g
vii)	Distilled water	1000 ml

5) Corn meal agar

i)	Corn meal	20 g
ii)	Peptone	20 g
iii)	Glucose	20 g
iv)	Agar	15 g
v)	Distilled water	1000 ml

6) Host leaf extract

i)	Pigeonpea leaf	200 g
ii)	Dextrose	20 g
iii)	Agar	20 g
iv)	Distilled water	1000 ml

7) V8 juice agar

i)	V-8 Juice (100 ml)	8.3 g
ii)	L-Asparagine	10 g
iii)	Yeast extract	2 g
iv)	Calcium carbonate	2 g
v)	Glucose	2 g
vi)	Agar	20 g
vii)	Distilled water	1000 ml

8) Tomato juice agar

i)	Tomato juice	200 ml
ii)	Calcium carbonate (CaCO3)	2 g
iii)	Agar	20 g
iv)	Distilled water	1000 ml

9) Carrot dextrose agar

i)	Carrot	200 g
ii)	Dextrose	20 g
iii)	Agar	20 g
iv)	Distilled water	1000 ml

10) Potato dextrose broth

i)	Potato	200 g
ii)	Dextrose	20 g
iii)	Distilled water	1000 ml

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Patil

Signature of Student

(Tejas S. Patil)

Place : Latur

Date : 30 / 11 /2022