ETIOLOGY AND EPIDEMIOLOGY OF COLLAR ROT COMPLEX OF COWPEA

THESIS

By

SIDDHARTH ANAND
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Submitted to

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Dr. D.K.Banyal
Professor
(Plant Pathology)

Department of Plant Pathology, CSK
Himachal Pradesh Krishi Vishvavidyalaya,
Palampur (H.P.)
India - 176062

CERTIFICATE – I

This is to certify that the thesis entitled **"Etiology and epidemiology of collar rot complex of cowpea"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mr. Siddharth Anand** son of **Sh. R. N. Chaurasia** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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

Place : Palampur
Dated : 30th September, 2019

Dr. D. K. Banyal
Major Advisor

CERTIFICATE- II

This is to certify that the thesis entitled **"Etiology and epidemiology of collar rot complex of cowpea"** submitted by **Mr. Siddharth Anand (Admission No. A-2017-30-074)** son of Sh. R. N. Chaurasia to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

Dr. D. K. Banyal
Chairperson
Advisory Committee

( )
External Examiner

Dr. Amar Singh
Member

Dr. P. N. Sharma
Member

Dr. V.K. Sood
Member

Dr. Naveen Kumar
Dean's nominee

Head of the Department

Dean, Postgraduate Studies

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Needless to say, all omissions and errors are mine.

Place: Palampur
Dated: 30th September, 2019 ***(Siddharth Anand)***

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LIST OF ABBREVIATIONS USED

Abbreviation	**Full form**
%	Per cent
&	And
/	Per
@	At the rate
°C	Degree Celsius
µm	Micro meter
cv.	Cultivar
cvs.	Cultivars
et al.	Et alii (and others)
viz.,	vi delicet (namely)
p.	Page
i.e.	Id est (that is)
Fig.	Figure
g	Gram
mm	Millimeter
sp./spp.	Species
w.r.t.	With respect to
cm	Centimeter
etc	Et cetera (and other similar things)
min.	Minute

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Department of Plant Pathology
COA, CSK Himachal Pradesh Krishi Vishvavidyalaya Palampur – 176 062 (HP)

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ABSTRACT

The present investigation entitled "Etiology and epidemiology of collar rot complex of cowpea" was undertaken to identify the associated pathogen(s), factors affecting the pathogen & disease development and effect of weather variables on the diseases development. Collar rot of cowpea is known to be caused by number of pathogens and is one of the most destructive disease in Himachal Pradesh. Isolation was taken from the diseased samples and three fungal pathogen were isolated among which pathogenecity was established only with *Sclerotium* sp. On the basis of symptological and morpho-cultural characteristics of test pathogen, the pathogen was ascertained as *Sclerotium rolfsii*. Maximum mycelial growth and sclerotial production was observed on potato dextrose agar and oat meal agar at 30°C to 25°C, respectively. Incubation and latent period of *S. rolfsii* was observed to be 7 and 13 days respectively. Inoculum load of pathogen was tested between 1g-5g/kg of soil and 2g/kg of soil was found optimum, which gave 86.66 percent disease incidence. Young and early generation of cultures gave maximum disease incidence of collar rot. Significant decrease in the disease incidence was observed with increase in age and sub-culturing of pathogen. The disease incidence was observed maximum at low soil moisture and disease incidence was decreased with increase in soil moisture. Sandy clay loam soil gave the maximum per cent incidence of disease and minimum was observed with silty clay loam. The disease incidence was observed minimum in early sown crop as compared to normal and late sown crop. The minimum disease incidence was also observed at wider spacing (60 cm), as compared to normal (45 cm) and closer (30 cm) spacings. Maximum disease incidence 76.53 per cent was observed on late sown and narrow spaced crop (30 cm) as compared to timely and normal sowing and wider spacing. Disease incidence was highly positively correlated with temperature (minimum, maximum and average) and relative humidity (minimum, maximum and average) on all the dates and sowing. The coefficient of determination revealed that the temperature and relative humidity contributed 97.8, 90.06 and 97.1 per cent towards incidence of collar rot on crop sown at 7th June, 22nd June and 8th July, respectively. AUDPC and infection rate (r) followed the similar trend of disease incidence with respect to 7th June, 22nd June and 8th July date of sowing.

Siddharth Anand
Student
Date:

Dr. D. K. Banyal
Major Advisor
Date:

Head of the Department

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is an important Dicotyledon belonging to family fabacae. This annual legume is perhaps the oldest source of human food grain around the world (Iqbal et al. 2005). Cowpea seed is a nutritious component in the human diet and livestock feed and its green pods can also be used as vegetable. It is highly nutritious and reported to contain 24 per cent of crude protein, 53 per cent carbohydrate and 2 per cent fat (FAO 2012). Its seed contain 25 per cent protein and 64 per cent carbohydrate (Magloire 2011; Singh et al. 2012). Cowpea vine and leaves is often used as fodder and provides a high quality protein rich forage (Anele et al. 2011). Leaves and shoot of cowpea usually contain more than 20 per cent protein depending on the stage of maturity and seasonal climatic variation (Mullen 1999).

Cowpea has capacity to manure the soil and enriching the microbial population which gives immense stress on the utility of this legume in the present era. When the crop is fully nodulated it can fix upto 20 to 140 kg residual N/ha into the soil. This gives a significant bonus to later crops in the rotation (Mullen 1999). Broad and drooping leaves of this crop help in conserving soil moisture. This crop is successfully grown in most regions because of its adaptability in low fertile (Elowad et al. 1982) and even in alkaline soils (West and Francois 1982). Even though acquisition with varied range of soils and rainfall patterns, it is confined to arid and semi-arid regions of the world. Cowpea is mainly grown in North and South America, Africa, Europe and Asia primarily in the region of semi-arid humid tropical regions. In Asia it is mainly grown in India, Srilanka, Bangladesh, Myanmar, China, Nepal, Malaysia and Philippines.

In world the cowpea is grown over 1.25 million ha with production of 7.4 million tons (FAO 2017) however, its 75 percent production is obtained only from Africa (Singh et al. 2002). Among all cowpea growing countries Africa is leading continent sharing 68 per cent, followed by Brazil (17%), Asia (3%) and USA (2%), whereas, the remaining (10%) production is contributed by rest of the world (Gomez 2004). In India area under cowpea is 3.9 million hectares with a production of 2.21 millon tons with the national productivity of 6.83 q/ha (Singh et al.2012).

In India, it is mainly cultivated in arid and semiarid parts of Rajasthan, Karnataka, Tamilnadu, Gujrat and parts of Punjab, Haryana and Himachal Pradesh. In Himachal

Pradesh, cowpea is either taken as sole crop or inter crop with maize in lower and mid hill of Kangra, Hamirpur, Mandi, Kullu, Chamba, Bilaspur and Una dictricts. Cowpea is affected by many fungal disease *viz.* pod blight (*Macrophomina phaseolina* and *Rhizoctonia bataticola*), cecospora leaf spot (*Cerecospora curenta* and *C. canescens*), powdery mildew (*Erysiphe polygoni*), rust (*Uromyces appendiculatus*) and stem rot (*Sclerotium rolfsii*). Wet and humid environmental conditions predispose the crop to the attack of many diseases. Among these, collar rot caused by complex of pathogens like *Rizoctonia solani* Khun (Lakshmanan et al. 1979), *Fsarium solani* Mart and *Sclerotium rolfsii* Sacc. (Singh et al. 1997) and is the most devastating disease of cowpea which causes severe losses in Himachal Pradesh.

Aigbe et al. (1972) first time reported collar rot of cowpea from Nigeria and the causal agent was identified as *Fusarium equiseti.* Lakshmanan et al. (1979) reported *Rhizoctonia solani* as causal agent of cowpea collar rot from India. *Sclerotium rolfsii* was reported as causal agent of collar rot of cowpea from Benin by Adandonon and Aveling (2004). *Sclerotium rolfsii* had been also reported to cause collar rot in other crops like tomato (Rolfs 1892), chickpea (Mathur and Shinha 1968) and soybean (Debbarma et al. 2017).

Cowpea collar rot is most severe at seedling stage and causes 54.3 per cent losses of dry seed yield (Frey and Dukes 2002). Yield losses due to collar rot of cowpea upto 27 per cent also been reported from India by Chohan (1974). Collar rot of cowpea caused by *S. rolfsii* caused seedling mortality of 20 per cent in 5-10 days old seedling (Tanimu et al. 2018). In Himachal Pradesh, collar rot of cowpea is one of the major threats with an incidence of 10-45 per cent in each cropping season (Billah et al. 2017).

Symptoms of collar rot of cowpea is characterized by appearance of oval to spindle shaped, brown to black lesions having length ranging from 0.2-8 cm at soil level near collar region, grinding of basal portion affect the stem, growth of white mycelial often studded with small sclerotia also appear on the infected stem (Vavilapalli and Celine 2014). Symptoms of this disease also described as initial appearance of small circular brown spot and often show concentric banding and become surrounded by water soaked lesion, under humid condition the lesion develop rapidly and coalesce eleading to extensive blighting and defoliation (Allen and Lenne 1998).

In Himachal Pradesh, cowpea is grown by marginal farmers as pulse as well as fodder crop and the cropping season overlapped with moderate to heavy rainfall which limits its cultivation and yield. Though the several aspects of disease of cowpea have been studied in details but, due to its complex nature the cause is still not established in Himachal Pradesh. So, it is necessary to ascertain the causal agent, etiology and epidemiology of cowpea collar rot complex, particularly under Himalayan conditions. Thus, keeping in view the economic importance of disease, crop and virtual lack of information with respect to collar rot pathogens, the present study was conducted to generate information on the following objectives:

I. To study the cause and biology of pathogen(s) associated with cowpea collar rot

II. To study the factors affecting the disease development

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is an important Dicotyledon belonging to family fabacae. This annual legume is perhaps the oldest source of human food grain around the world (Iqbal et al. 2005). Cowpea seed is a nutritious component in the human diet and livestock feed and its green pods can also be used as vegetable. It is highly nutritious and reported to contain 24 per cent of crude protein, 53 per cent carbohydrate and 2 per cent fat (FAO 2012). Its seed contain 25 per cent protein and 64 per cent carbohydrate (Magloire 2011; Singh et al. 2012). Cowpea vine and leaves is often used as fodder and provides a high quality protein rich forage (Anele et al. 2011). Leaves and shoot of cowpea usually contain more than 20 per cent protein depending on the stage of maturity and seasonal climatic variation (Mullen 1999).

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I. To study the cause and biology of pathogen(s) associated with cowpea collar rot

II. To study the factors affecting the disease development

3. MATERIALS AND METHODS

In the present investigation, various experiments concerning the etiology of disease, factors affecting the pathogen and disease development and environmental factors affecting development of collar rot were conducted in the laboratory, net house and field of the Department of Plant Pathology, CSK HPKV Palampur and the material and methods used to carry out these experiments are described as below:

3.1 Collection of diseased sample

3.2 Isolation, purification, maintenance and multiplication of pathogen

3.3 Symptomatology

3.4 Sterilization

3.4.1 Sterilization of glass wares

3.4.2 Sterilization of inoculation needle and cork borer

3.4.3 Sterilization of pots and soil

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3.8.2 Effect of soil texture

3.9 Effect of date of sowing and spacing on the development of disease

3.10 Effect of weather variables on the development of disease

3.11 Statistical analysis

3.1 Collection of diseased sample

Field visits of different cowpea growing areas of Himachal Pradesh *i.e.* Mandi, Hamirpur and Kangra districts were undertaken during 2018 in the month of June – August for collection of diseased samples. Plants showing the typical symptoms of collar rot were collected, placed in paper bags and brought to laboratory for further studies. Isolation from the infected samples was taken in order to ascertain the associated pathogen with the disease.

3.2 Isolation, purification, maintenance and multiplication of pathogen

Pathogen was isolated from the infected root and stem of the plant and maintained on potato dextrose agar medium. Under laminar air flow, small 5 mm bits were cut from the intermittent zone of healthy and diseased tissue and were surface sterilized with 0.1 per cent of mercuric chloride for 15-20 seconds and subsequently washed in sterilized distilled water for 3-4 times. Excessive moisture was removed by using sterilized blotting discs and bits were then transferred to PDA slants using sterilized inoculation needle. These test tubes were incubated at 28±1°C for one week. Precautions were taken to avoid contamination. The pure culture of the isolate was obtained by using hyphal tip isolation method. For this, diluted mycelial suspension was spread uniformly on 2 per cent water agar plates. Single mycelial hyphae were marked and allowed to grow. The hyphal tip was then cut and transferred to the PDA slants with the help of inoculation needle under aseptic conditions and incubated at temperature of 28±1°C for 4 days, later the mycelial

bits of the fungus were placed in the center of Petri plates containing potato dextrose agar medium and incubated at 28±1°C.

3.3 Symptomatology

Different nearby cowpea growing fields were visited during the cropping season and the samples showing the typical symptoms of collar rot were collected and kept in paper bag and brought to laboratory for studying the typical symptoms of the collar rot.

3.4 Sterilization

3.4.1 Sterilization of glass wares

To conduct various laboratory related experiments, glass wares like beakers, conical flasks, measuring cylinders, Petri plates and test tubes were dipped overnight in potassium dichromate ($K_2Cr_2O_7$) and HCl solution. Then, these were rinsed thrice in tap water and sterilized in hot air oven at 180^oC for 2 hours. Media were sterilized in autoclave at 1.05 kg/cm^3 pressure at temperature of 121±1°C for 20 minutes. Inoculation chamber, working platform and hands were surface sterilized with rectified spirit.

3.4.2 Sterilization of inoculation needle and cork borer

Clean inoculation needle and cork borer were sterilized by dipping in spirit and heating over the flame until red hot. It was repeated 2-3 times. Inoculating needle was used for transferring inoculum from one culture tube to another and also in Petri plates. Cork borer was used for making 5 mm fungal disc (measured amount) for cultural studies, purification of isolates and other laboratory studies.

3.4.3 Sterilization of pots and soil

Pots were surface sterilized by swapping with sodium hypochlorite (2%) and ethanol (5%). Soil required in bulk was sterilized by using 5 per cent formalin solution.

3.5 Pathogenicity assay

3.5.1 Preparation of inoculum

To conduct experiments for the effect of inoculum load, age of pathogen, sub culturing, soil moisture and texture on the development of disease, the inoculum of pathogen was mass multiplied on oat meal medium. The 100 g oat seeds were soaked overnight and kept in 250 ml of conical flask and were sterilized in autoclave at 1.05 kg/cm^3. After sterilization, the flasks were inoculated with small bits of pathogen culture cut with the help of cork borer under aseptic conditions and incubated at 28±1°C. The flasks were shaken on alternate days to get uniform growth.

3.5.2 Preparation of sick soil

The mixture of fine clay soil, sand and FYM (2:1:1) was prepared and sterilized. Sterilized soil was made sick by mixing pathogen inoculum. The sick soil was filled in surface sterilized plastic pots (6" diameter) and thoroughly moistened with water.

3.5.3 Method of inoculation

Pots filled with sterilized soil were inoculated at depth of 2 cm with 7 days old mass multiplied pathogen culture @ of 2g/kg soil. Pots were watered and kept for incubation before sowing of cowpea seeds upto 24 hrs which were further utilized for various experiments.

3.5.4 Sowing of seeds

Cowpea seeds of variety 'C-475' were sown in pots filled with sterilized soil for different experiments one day after the inoculation of pathogen culture.

3.5.5 Pathogenicity test

Pots filled with sterilized soil were inoculated with 7 days old mass multiplied pathogen @ 2g/kg soil at depth of 2 cm in soil and these pots were watered and kept for 24 hrs of incubation. On next day, seeds of cowpea were sown in the inoculated pots and watered daily. These pots were regularly observed for the development of disease. Suitable controls without any application of inoculum were also maintained. The pathogen was re-isolated from the infected seedling and pathogenicity of the test pathogen was confirmed by identifying the pathogen through microscopic examination.

3.6 Identification of pathogen and disease

3.6.1 On the basis of symptomatology

The symptoms of collar rot of cowpea were induced in pots by inoculating with pathogen @ 2g/kg soil at depth of 2 cm, developed symptoms were observed from first appearance till the death of seedlings.

3.6.2 On the basis of morpho-cultural characteristic

Pathogen which was found pathogenic on causing collar rot of cowpea was confirmed by pathogenicity test and identified by studying the morphological and cultural characteristics of pathogen on PDA dispensed into 9 cm diameter Petri plates. Mycelial bits of 5 mm diameter were cut with the help of cork borer from the margin of an actively growing colony and placed in the center of media plates. Culture was incubated at 28±1°C in BOD incubator. The culture of the pathogen was replicated thrice having three plates each. Observations were recorded on colony diameter, colony colour and type of growth, colour and number of sclerotia.

(i) Colony diameter

Colony diameter of the isolated pathogen was observed after 5 days of incubation with the help of scale. Diameter of the colony was recorded from two side of the Petri plate and average obtained was taken as final value.

(ii) Colony colour

Mycelium colour (white or dull white) of the isolated pathogen was observed visually from front side of the Petri plate.

(iii) Colony growth

Growth pattern (cottony, fluffy, sparse or dense) of the isolated pathogen were recorded by observing the mycelium minutely.

(iv) Colour and number of sclerotia

Colour and number of sclerotia were observed after 7^{th} day of incubation at temperature28±1°C temperature. Sclerotial characters i.e. colour and number of sclerotia was recorded by observing them very minutely.

3.7 Factors effecting development of pathogen

3.7.1 Effect of media

Five solid media viz. Potato dextrose agar (PDA), Oat meal agar (OMA), Corn meal agar (CMA), Richard's agar and Malt extract agar (MEA) were used (Appendix-1) to study the best medium for mycelial growth and sclerotial production of *S. rolfsii*. Media were prepared and sterilized by autoclaving at pressure of 1.05 kg/cm^3 (121.6^{0}C) for 20 min. Each medium was poured in Petri plates (9 cm) containing equal quantity (20 ml) followed by inoculation with 5 mm culture bits taken from the 4 days old fungus culture. Three replications were kept and incubated at 28±1°C in BOD (Biological Oxygen Demand) incubator. Radial growth of the colonies after 48, 72, 96 and 120 hrs on each culture medium was recorded and analysed statistically for finding out the most suitable medium for optimal fungal growth. The sclerotia formation was recorded up to 7 days and observations on colour and number of sclerotia were recorded for finding out the most suitable medium for sclerotial formation.

3.7.2 Effect of temperature

Effect of five different temperatures viz. 15, 20, 25, 30 and 35^{0}C were studied for the mycelial growth and sporulation of pathogen. The culture of *S. rolfsii* was grown on PDA for 7 days and mycelial bit of 5 mm diameter from actively growing colony margin were cut with sterilized cork borer. Each mycelial bit was transferred on to 9 cm Petri plates each containing 20 ml of PDA medium followed by incubation at 15, 20, 25, 30

and 35^0C, respectively. Three replications were kept for each temperature. To find out the best temperature for mycelial growth, the colony diameter was measured at regular interval of 24 hrs till 120 hrs of incubation. Formation of sclerotia was also recorded at 24 hrs interval upto 7 days for finding out the best temperature for sclerotial production.

3.7.3 Incubation and latent period

To study the incubation and latent period the sterilized soil in pots were inoculated with culture of *S. rolfsii* at the rate of 2g/kg soil of inoculum load at depth of 2 cm. Cowpea seeds were sown after 24 hrs of incubation. These pots were watered daily and observed regularly for the appearance of first symptom and formation of sclerotia. Each treatment was replicated thrice along with un-inoculated pot for each treatment which serves as control

3.7.4 Effect of inoculum load

Different inoculum loads i.e. 1, 2, 3, 4 and 5g/kg soil were studied for their effect on the development of disease of collar rot. The inoculum was taken from mass multiplied culture of *S. rolfsii* and the pots were inoculated upto the depth of 2 cm. These pots were kept for 24 hrs of incubation before seeding. Next day cowpea seeds (15 seed/pots) were sown and observed for the development of the disease. Each treatment was replicated thrice along with un-inoculated pot for each treatment which served as control.

Per cent disease incidence was calculated by formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plant}}{\text{Total number of plants}} \times 100$$

3.7.5 Effect of pathogen sub culturing

To study the effect of sub culturing of pathogen on the development of collar rot, the first isolated and purified culture of *S. rolfsii* was considered as first generation culture which was allowed to grow for 4 days. From this first generation culture, subsequent sub culturing of pathogen was done up to ninth generation and incubated at 28±1°C. From all these sub cultured generation 1^{st}, 3^{rd} 5^{th}, 7^{th} and 9^{th} generation were mass multiplied and incubated for 7 days and inoculum load of 2g/kg soil were inoculated in pots filled with sterilized soil at depth of 2 cm and kept for 24 hrs of incubation. Next day, cowpea seeds were sown and observed for the development of the disease. Each treatment was replicated thrice along with un-inoculated pot for each treatment which serves as control. Per cent disease incidence was calculated by formula as given in 3.7.4.

3.7.6 Effect of age of culture

Pathogen culture of different age i.e. 7, 14, 21 and 28 days old was inoculated in pots filled with sterilized soil with inoculum load of 2g/kg soil at depth of 2 cm in soil. Cowpea seeds were sown after 24 hrs of incubation and these pots were watered daily. Observations on the incidence of collar rot were recorded. Each treatment was replicated thrice along with un-inoculated pot for each treatment which served as control. Per cent disease incidence was calculated by formula as given in 3.7.4.

3.8 Soil factors affecting disease development

3.8.1 Effect of soil moisture

Soil samples were sieved using a 2 mm mesh and its moisture holding capacity, available water content and maximum water holding capacity were calculated. Five soil moisture levels *viz*. 15, 20, 25, 30 and 35 per cent were maintained on weight basis throughout the experiment. These desired moisture levels were obtained by automizing and mixing required amount of soil with calculated quantity of water. Different moisture levels were maintained throughout the experiment by adding required amount of sterilized water after every 24 hrs by taking weight of each pot to assess the water loss. Per cent disease incidence was calculated by formula as given in 3.7.4.

3.8.2 Effect of soil texture

Soil of different texture *viz.* clay loam, sandy clay loam, silty loam, sandy silty loam and sandy loam were collected from their designated areas of Himachal Pradesh. To find the soil texture, 20 g of soil sample was taken and the texture was determined by the international pipette method (Anonymous 1997). Pots were filled with sterilized soil of different texture and inoculated with pathogen culture followed by overnight incubation. Cowpea seeds were sown on the next day and regular watering was done. Observation on the disease incidence was recorded and per cent disease incidence was calculated as given at 3.7.4.

3.9 Effect of date of sowing and spacing on the development of disease

The effect of date of sowing and spacing (row to row) on the development of collar rot was studied by conducting an experiment under field conditions in Split Plot Randomized Block Design (SPRBD) comprising 3 main plots (date of sowing) and 3 sub-plots (row to row spacing). Cowpea seed variety ‘C-475’ were sown during *Kharif* 2018 and 2019 in the fields of Department of Plant Pathology, CSK HPKV Palampur at three different spacing (row to row) *viz.* 30, 45 and 60 cm on three different dates, i.e. 7th June,

22nd June and 8th July and their effect on disease development was studied by recording per cent disease incidence at weekly intervals.

3.10 Effect of weather variables on the development of disease

To study the effect of weather variables i.e. temperature (maximum, minimum and average) and relative humidity (maximum, minimum and average) on the development of disease, an experiment was conducted in the field of Department of Plant Pathology, CSK HPKV, Palampur during *Kharif*, 2019. Crop was sown at three different dates i.e. 7th June, 22nd June and 8th July and data on disease incidence were recorded weekly. Data on weather variables (temperature and relative humidity) were collected from the Department of Agronomy, Fodder and Forage production, CSK HPKV, Palampur. Correlation between disease incidence (%) and weather variables were calculated and regression equations were derived. The relationship between disease incidence and various weather variables for disease development was determined by studying simple, partial and multiple correlations. Regression coefficients were calculated and regression equations were formed. From the data on disease incidence, further AUDPC and infection rate (r) were calculated by using respective formulas.

The AUDPC was calculated using the formula given by Shaner and Finney (1977).

$$AUDPC = \sum (y_i + y_{i+1}) / 2 \times (t_{i+1} - t_i)$$

where, y_i = Disease incidence at time t_i

y_{i+1} = Disease incidence at time t_{i+1}

The infection rate (r) was calculated by using the equation given by Vander Plank (1963).

$$r = \frac{2.3}{t_2 - t_1} \times \text{Log}_{10} \frac{X_2 (1 - X_1)}{X_1 (1 - X_2)}$$

where, X_1 = Proportion of infected tissues at time t_1

X_2 = Proportion of infected tissues at time t_2

Expected disease incidence was also calculated from regression equation developed and compared with observed disease incidence for testing the fitness of the calculated regression equation.

3.11 Statistical analysis

The data recorded were subjected to statistical analysis wherever required. The differences exhibited by the treatment in subsequent experiments for their significance by employing SPRBD. Each data was analyzed by using CPCS-1, MS and other applicable softwares.

4. RESULTS AND DISCUSSION

Collar rot of cowpea caused by *Sclerotium rolfsii* Sacc. is one of the most destructive diseases of cowpea. Results obtained in the current investigations are presented and discussed here in the light of the documented literature under the following headings:

4.1 Symptomatology

4.2 Pathogen associated with disease

4.3 Identification of pathogen and disease

4.3.1 On the basis of symptomatology

4.3.2 On the basis of morpho-cultural characteristic

4.4 Factors affecting development of pathogen

4.4.1 Effect of media

4.4.2 Effect of temperature

4.4.3 Incubation and latent period

4.4.4 Effect of inoculum load

4.4.5 Effect of sub-culturing

4.4.6 Effect of age of culture

4.5 Soil factors affecting the development of disease

4.5.1 Effect of soil moisture

4.5.2 Effect of soil texture

4.6 Effect of date of sowing and spacing on the development of disease

4.7 Effect of weather variables on the development of disease

4.1 Symptomatology

The diseased samples at different stages of seedling were collected from near by places and brought to laboratory for the observation on collar rot symptoms. Yellowing of leaves and wilting of whole plant and severe rotting at collar region was observed in all the samples. Dark brown to black coloured lesions appeared on the affected part of the stem,

white mycelial growth was also appeared near collar region of affected stem. Infected plants did not show proper root development and in severe case, plants were collapsed and died (Plate 4.1).

All these symptoms were in the conformity with Asghari and Mayee (1991). They observed typical symptoms of cowpea collar rot caused by *S. rolfsii* as yellowing and wilting of branches and presence of white mycelial growth at collar region. Chupp and Sherf (1960) also observed the first visible symptoms of cowpea collar rot as progressive yellowing or whitening of foliage starting from lower leaves and stem stand upright and become defoliated, white lesions on stem were covered with a white weft of coarse fungal mycelia. Nene et al. (1989) observed symptoms of seedling affected by collar rot and described it as appearance of rotting at collar region. Later on the plants found to turn yellow without drooping of leaves and terminal buds. The rotten portion was covered with distinct whitish mycelial strands.

4.2 Pathogen associated with disease

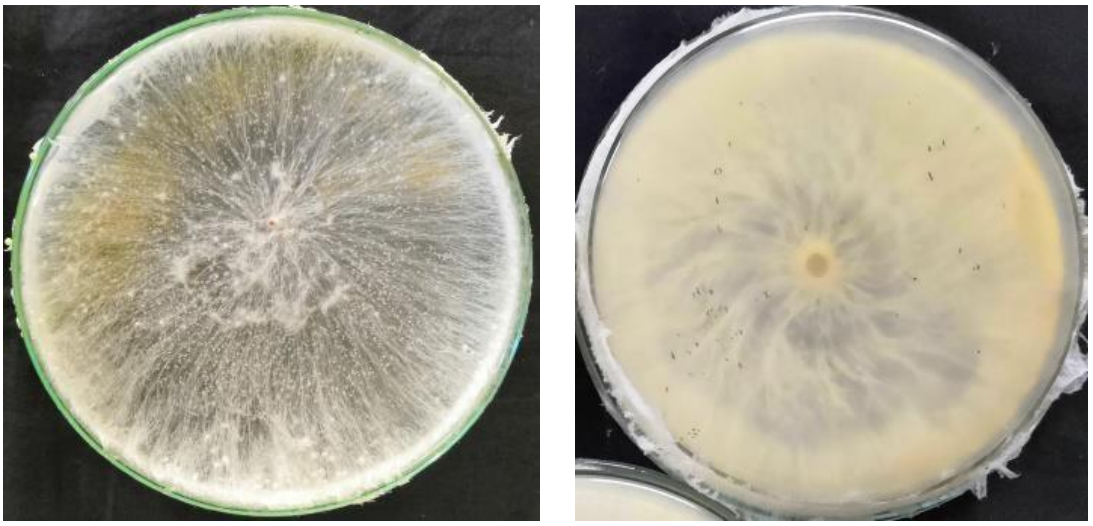
Collar rot of cowpea is a complex disease and reported to be caused by many pathogens. Isolations were taken from the collected diseased sample and three fungal pathogens were isolated and primarily identified on the morpho-cultural characteristics (mycelial growth, colour of colony and pigmentation) as *Fusarium* sp., *Sclerotium* sp. and *Collectotrichum* sp. (Plate 4.2). The pathogenicity tests with these three pathogens were conducted under net house condition, but the pathogenicity was proved only with *Sclerotium* sp, which gave typical symptoms of collar rot. Further, all the studies were conducted with the *Sclerotium*, with which pathogenicity was proved.

4.3 Identification of pathogen and disease

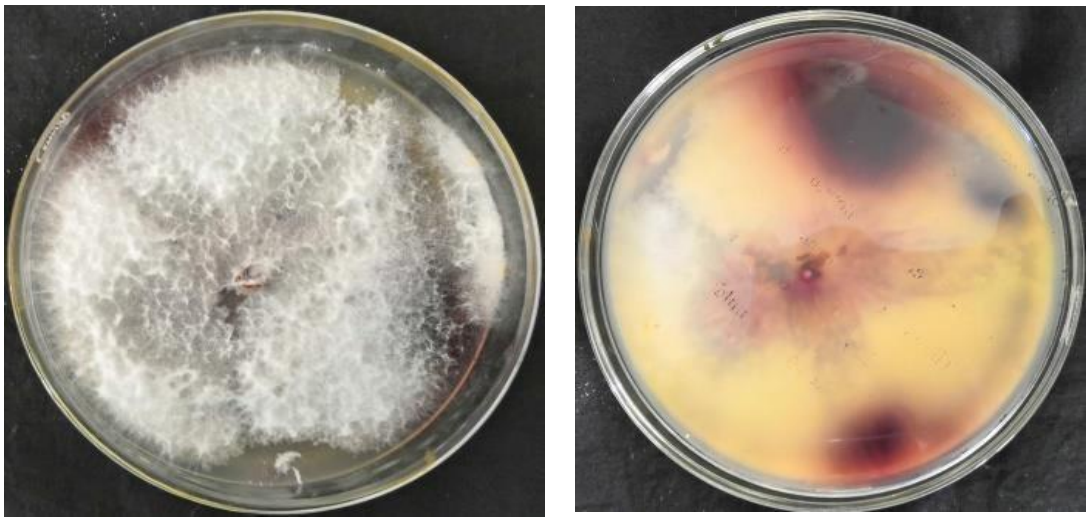
4.3.1 On the basis of symptomatology

The symptoms of collar rot of cowpea were observed under *in vivo* in net house of Department of Plant Pathology CSKHPKV Palampur. The characteristic symptoms of collar rot were observed as wilting of young seedling and yellowing of leaves, oval shaped necrotic lesion also appeared on the wilted seedling at collar region near soil surface. As the disease progressed, white cottony fungal mycelia appeared on affected part of the stem. Tan to brown sclerotia also appeared in the soil near collar region of affected stem after 14 days of inoculation. Infected seedling did not show any growth further, ultimately seedling withered and died.

Plate 4.1 Symptoms of cowpea collar rot caused by *Sclerotium rolfsii* in field and under net-house



Sclerotium sp.



Fusarium sp.

Colletotrichum sp.

Plate 4.2 Isolated fungal species from the collected samples cowpea collar rot

Similar types of symptoms of collar rot of cowpea caused by *S. rolfsii* had been described by Vavilappalli and Celine (2014), who reported that collar rot of cowpea was most sever at seedling stages and described the symptoms of disease as appearance of oval to spindle shaped, brown to black lesions at collar region near soil surface, white mycelial growth often studded with small sclerotia was also appeared on the basal part of the affected stem, which later on turned brown and looked like mustard seeds. Affected plant showed poor root development. Gupta and Shyam (1998) described the symptoms of collar rot of cowpea caused by *S. rolfsii* as yellowing of lower leaves and appearance of water-soaked spots near stem at collar region. As the disease progressed, water-soaked lesions also appeared on upper part of stem and leaves shed prematurely. White mycelium intermixed with a large number of sclerotia was produced at the base of stem. Karat et al. (1985) also observed the symptoms of cowpea collar rot caused by *S. rolfsii* as wilting and yellowing of plants, which was soon followed by drying of foliage and death of affected plants. As the disease progressed, tan to brown coloured sclerotia and white mycelial growth appeared on the stem epidermis near soil surface.

The symptoms produced by the test pathogen were observed similar to that of collar rot caused by *S. rolfsii* as reported in literature. Hence on the basis of symptomatology, the disease was identified as collar rot of cowpea caused by *S. rolfsii.*

4.3.2 On the basis of morpho-cultural characteristics

To identify pathogen causing collar rot, the morphological and cultural characteristics of test pathogen were observed on PDA at 28±1°C and described in table 4.1and plate 4.3. Isolated pathogen produced cottony growth pattern with highly dense mycelium which gave fan like and colour of mycelium was milky white to dull white. Mycelium was hyaline with sparse cross-walls (septation) and profusely branched and had clamp connections when observed under microscope. Sclerotia were produced at soil surface over mycelium, which were initially irregular in shape and white in colour later turned tan to dark brown. The sclerotia were observed spherical at the maturity.

All these morpho-cultural characteristics of test pathogen do match with *S. rolfsii* as described in the literature. Tanimu et al. (2018) identified the fungus causing collar rot of cowpea as *S. rolfsii* on the basis of morphological characteristics i.e. white radial growth of mycelia, hyphae was hyaline, thin & septate and represented scattered

branching. On the mycelia, the fungus also formed small, white globose sclerotia which subsequently turned from light brown to dark brown.

Table 4.1 Identification of associated pathogen with collar rot of cowpea on the basis of morpho-cultural characteristics

Character	Morphological and cultural characteristics	
	Test pathogen	*Sclerotium rolfsii* Sacc. (Kumar et al. 2014)
Hyphae	Hyaline, with sparse cross wall and profusely branched	Hyaline, thin walled septate and profusely branched
Mycelial growth pattern	Cottony-fluffy growth pattern with highly dense mycelium, giving fan like appearance, having clamp connections and prolific growth of mycelia	Mycelium was first milky white, later turned to dull white with radial spreading giving fan like appearance and have clamp connection
Colony colour	Milky white to dull white	Milky white
Sclerotia	Tan to dark brown, small spherical in shape resembling with mustard seeds	Deep brown or brownish black coloured, spherical to irregular in shape

Kumar et al. (2014) studied the cultural and morphological characteristics of *S. rolfsii* infecting groundnut and described the morphological characters as milky white mycelium, which later turned to dull white giving fan like appearance, hyphae was hyaline, thin walled, septate and profusely branched. When attained maturity, deep brown or brownish black coloured spherical to irregular shaped sclerotia appeared. Mordue (1974) studied the disease collar rot caused by *S. rolfsii* and described the fungal mycelium as septate, hyaline and branched bearing clamp connection. Subramanian (1964) and Mahen et al. (1995) described sclerotia of *S. rolfsii* as white coloured fungal structures which later on turned light to dark brown at maturity. The shape of the sclerotia varied from spherical to sub spherical, whose surface was finely wrinkled and sometime flattened. Barnett and Hunter (1972) also observed spherical to irregular sclerotia which on maturity resembled with mustard seeds.

The mopho-cultural and sclerotial characteristics of the test pathogen were observed similar to that of standard descriptions of *S. rolfsii* reported in the literature. Hence, the test pathogen was identified as *S. rolfsii*.

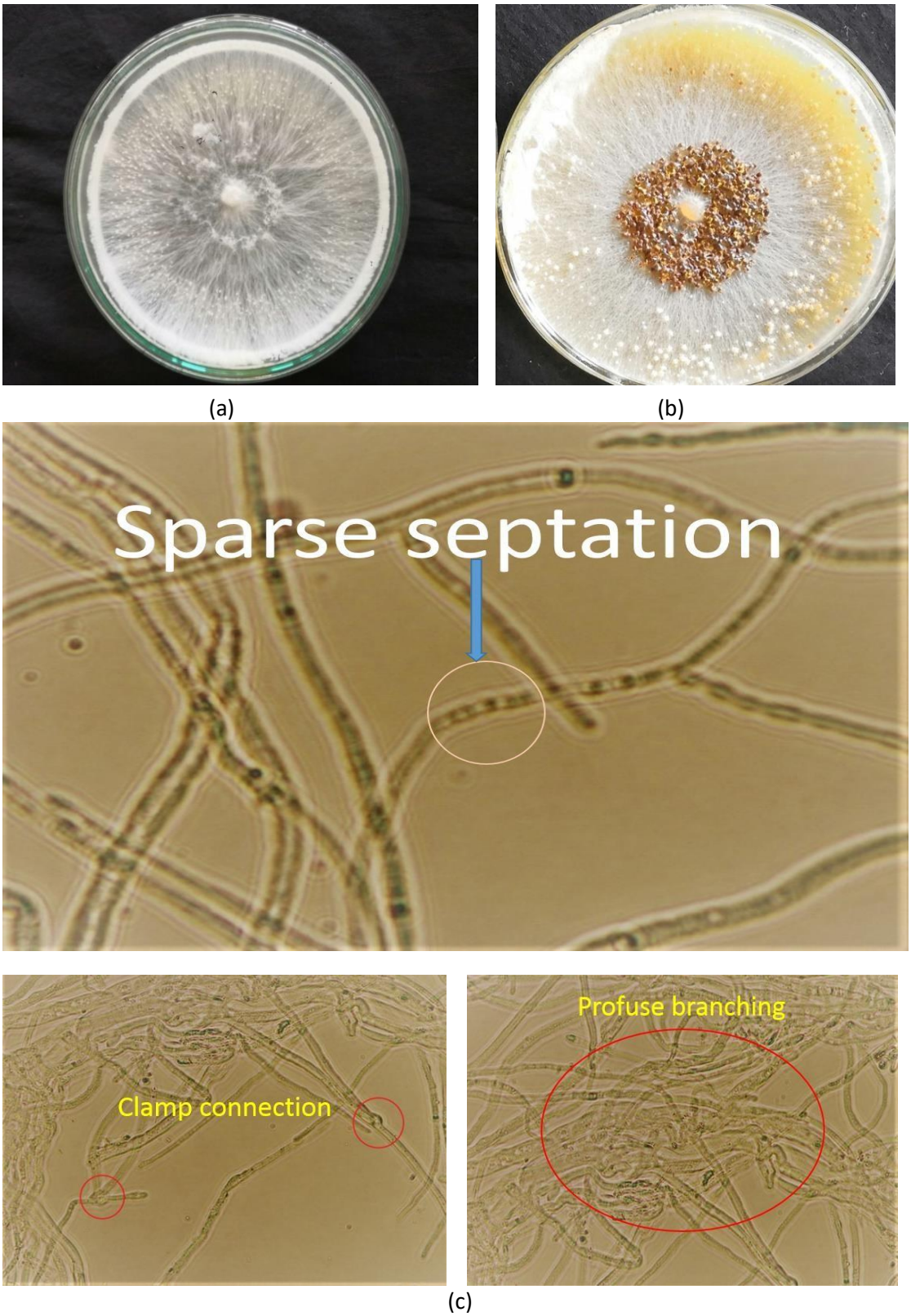


Plate 4.3 Morpho-cultural characteristic of *Sclerotium rolfsii* causing collar rot of cowpea (a) Mycelial, (b) Sclerotia and (c) Microscopic observations

4.4 Factors affecting development of pathogen

4.4.1 Effect of media

To study the best medium for the mycelial growth, cultural characteristic and sclerotial production of *S. rolfsii*, five solid media i.e. potato dextrose agar, oat meal agar, corn meal agar, Richard's agar and malt extract agar were tested and data on mycelial growth, cultural characteristics and sclerotial production were recorded at 28±1°C and presented in table 4.2 and 4.3.

a. Effect on mycelia growth

The data on mycelia growth in each media were recorded at 24 hrs of interval from 48 to 120 hrs of incubation and tabulated in table 4.2 and plate 4.4. Maximum mycelia growth of *S. rolfsii* was observed on PDA (3.56 cm) followed by OMA (2.70 cm), CMA (2.50 cm) and Richard's agar (1.23 cm) whereas, minimum mycelia growth was observed on MEA (0.50 cm) after 48 hrs of incubation. Similar trend of mycelia growth after 72 hrs of incubation was also observed and maximum mycelia growth was found on PDA (6.20 cm) and minimum on MEA (1.50 cm). The full mycelia growth i.e. 9 cm was observed on PDA and OMA whereas, it was minimum on MEA (2.90 cm) after 96 hrs of incubation. The mycelia growth was only 5.60 and 4.63 cm on CMA and Richards's agar respectively, after 96 hrs of incubation. Full growth i.e. 9 cm could not obtained on CMA, Richard's agar and MEA even after 120 hrs of incubation and it was 8.93, 7.63 and 5.40 cm, respectively.

Table 4.2. Effect of different media on mycelial growth of *Sclerotium rolfsii*

Media	**Mycelial growth (cm) after hrs of incubation**			
	48	72	96	120
Potato dextrose agar (PDA)	3.56	6.20	9.00	9.00
Oat meal agar (OMA)	2.70	5.80	9.00	9.00
Corn meal agar (CMA)	2.50	3.10	5.60	8.93
Richard's agar	1.23	2.80	4.63	7.63
Malt extract agar (MEA)	0.50	1.50	2.90	5.40
CD (p=0.05)	**0.16**	**0.20**	**0.15**	**0.81**

b. Effect on sclerotial production

The cultural characteristics of pathogen and sclerotial production were also recorded on different media up to 4 and 7 days of incubation, respectively and the result

were presented in table 4.3. The mycelial growth of *S. rolfsii* was found dense and thick on potato dextrose agar as compared to other medium on which mycelial growth was sparse, fluffy and thin. The mycelial growth was sparse on Richard's agar whereas, it was very sparse in malt extract agar and corn meal agar. The mycelial colour was observed milky white on PDA whereas, it was yellowish white, dull white, light white and much light white on oat meal agar, corn meal agar, Richard's agar and malt extract agar, respectively. The sclerotial production was very high on OMA and high on PDA whereas, moderate and less on CMA and MEA, respectively. However, no sclerotia were observed on Richard's agar medium.

Therefore, PDA was used as standard medium for further studies, as it gave best mycelial growth at 28±1°C. However, for the preparation of inoculum for *in vivo* experiments, oat meal was used as it gave best sclerotial production among all tested media.

Table 4.3. Effect of media on cultural characteristic and sclerotial production of *Sclerotium rolfsii*

Media	Cultural characters		Sclerotial characters	
	Mycelial growth	Mycelial colour	Colour	Number
Potato dextrose agar (PDA)	Dense and thick	Milky white	Light brown	High
Oat meal agar (OMA)	Sparse and Fluffy	Yellowish white	Brown	Very high
Corn meal agar (CMA)	Sparse and thin	Dull white	White	Moderate
Richard's agar	Sparse and fluffy	Light white	Sclerotia not observed	
Malt extract agar (MEA)	Very much spare	Much light white	White	Less

Potato dextrose agar was also reported earlier as best media for mycelial growth by many workers. Zape et al. (2013) reported potato dextrose agar as most suitable media for the mycelial growth of *S. rolfsii*. Bhagat (2011) also evaluated different media for the mycelial growth of *S. rolfsii* and observed maximum mycelial growth on potato dextrose agar followed by yeast dextrose agar whereas, minimum mycelial growth was observed on corn meal dextrose agar. Basamma (2008) studied the factor affecting the physiology of *S. rolfsii* and concluded that radial growth of fungus and sclerotial production was maximum on oat meal agar, potato dextrose agar and Sobouraud's agar followed by carrot dextrose agar. Sharma and Kaushal (1979) evaluated different solid media for mycelial growth and sclerotial production of *S. rolfsii* and reported that potato dextrose agar and potato maltose agar supported good growth of fungus and highest number of

sclerotia of bigger size were formed on potato dextrose agar. Perez et al. (1986) studied the effect of culture media on growth behavior of *S. rolfsii* and found that maize meal agar and malt extract agar were best for the mycelial growth as well as for sclerotial formation. Mridha and Alamgir (1987) studied the growth behavior and sclerotia production of *S. rolfsii* on different media and observed PDA as best media for growth and sclerotial production.

4.4.2 Effect of temperature

To study the best temperature for mycelial growth, cultural characteristics and sclerotial production of *S. rolfsii*, five temperatures i.e. 15, 20, 25, 30, 35°C were evaluated on PDA and data on mycelial growth, cultural characteristics and sclerotia production were recorded and presented in table 4.4 and 4.5.

a. Effect on mycelial growth

The data on mycelial growth on each temperature were recorded at 24 hrs of intervals from 48 to 120 hrs of incubation and tabulated in table 4.4 and plate 4.5. Maximum mycelial growth of *S. rolfsii* was observed at 30°C (4.80 cm) followed by 25°C (3.60 cm), 35°C (1.70 cm) and 20°C (0.70 cm) whereas, minimum mycelial growth was observed at 15°C (0.60 cm) after 48 hrs of incubation. The similar trend of mycelial growth was observed after 72 hrs of incubation and maximum mycelial growth was found at 30°C (7.00 cm) and minimum at 15°C (2.60 cm). The full growth i.e. 9 cm was observed at 30 and 25°C whereas, it was minimum at 15°C (4.50 cm) after 96 hrs of incubation.

Table 4.4. Effect of temperatures on mycelial growth of *Sclerotium rolfsii*

Temperature (°C)	**Mycelial growth (cm) after hrs of incubation**			
	48	72	96	120
35	1.70	3.20	6.40	9.00
30	4.80	7.00	9.00	9.00
25	3.60	6.20	9.00	9.00
20	0.70	2.80	5.50	7.10
15	0.60	2.60	4.50	5.80
CD (p=0.05)	**0.18**	**0.21**	**0.17**	**0.82**

The mycelial growth was observed as 5.5 cm and 6.4 cm at 20°C and 35°C after 96 hrs of incubation, respectively. Full mycelial growth i.e. 9 cm was not observed at 20°C and 15°C even after 120 hrs of incubation and growth was found restricted to 7.1 cm and 5.8 cm, respectively.

b. Effect on sclerotial production

The sclerotial characteristics and sclerotial production were also recorded at different temperatures up to 4 and 7 days of incubation, respectively and results were presented in table 4.5. The mycelial growth of *S. rolfsii* was fluffy, thick, dense at 25°C as compared to other temperatures. It was observed as cottony fluffy, dense fluffy and fluffy sparse at 35°C, 30°C and 20°C, respectively. The mycelial growth was observed much sparse at 15°C. Mycelial colour was observed as milky white at 30°C whereas, it was white, dull white, light white and much lighter white at 35°C, 25°C, 20°C and 15°C, respectively. The sclerotial production was very high and high at 25°C and 30°C, respectively. However, it was moderate at 35°C, less at 20°C and very less at 15°C, even up to 7 days of incubation.

So, 30°C temperature was selected as standard temperature for the further experiments as it gave best mycelial growth. However, for sclerotial production temperature of 25°C was found best as it gave highest number of sclerotia.

Table 4.5. Effect of temperature on cultural characteristic and sclerotia production of *Sclerotium rolfsii*

Temperature (°C)	Cultural characters		Sclerotial characters	
	Mycelial growth	Mycelial colour	Colour	Number
35	Cottony and fluffy	White	White	Moderate
30	Dense and fluffy	Milky white	Brown	High
25	Fluffy, thick and dense	Dull white	Brown	Very high
20	Fluffy and sparse	Light white	Dull white	Less
15	Sparse	Much light white	Light white	Very less

The result on the effect of temperature on mycelial growth and sclerotial production of *S. rolfsii* was also observed by many workers and Zape et al. (2013) observed maximum mycelial growth of *S. rolfsii* at temperature of 30°C whereas, maximum sclerotial production observed at 25°C. Prasad et al. (2012) also observed maximum radial growth of mycelia and excellent sclerotial formation of *S. rolfsii* at

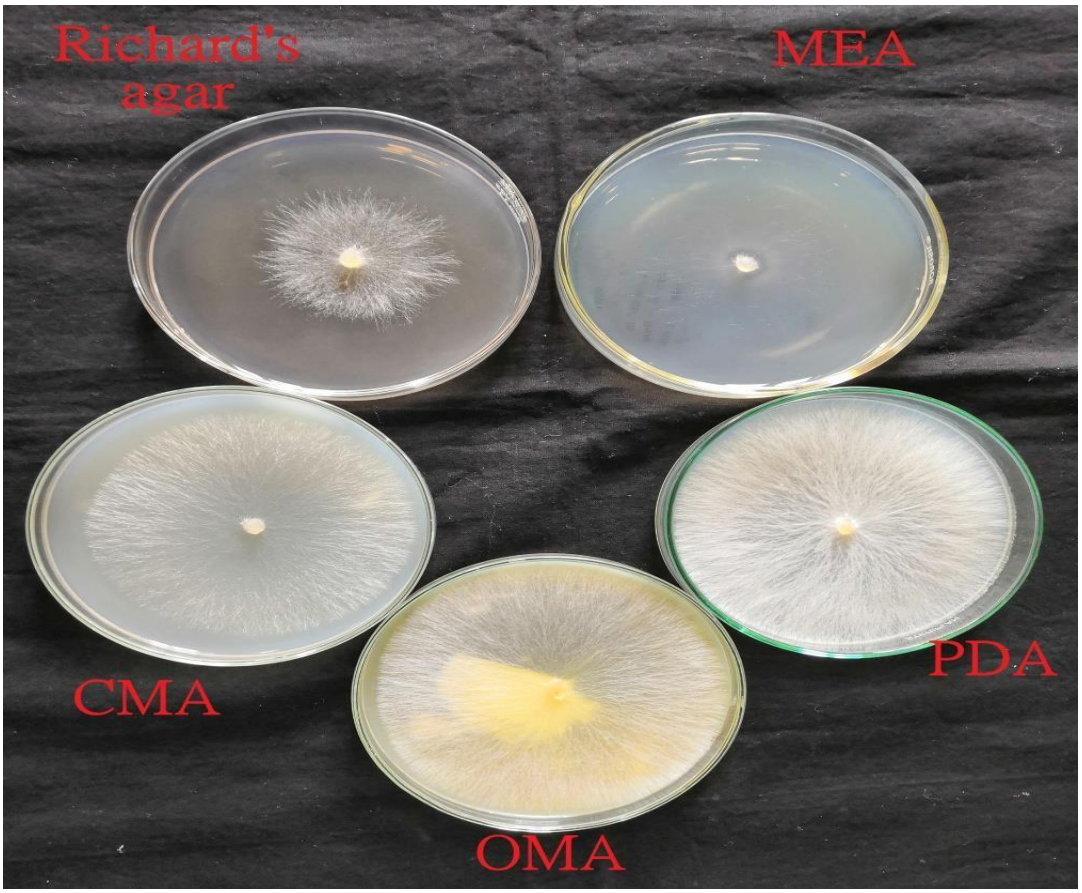


Plate 4.4 Mycelial growth of *Sclerotium rolfsii* on different solid media

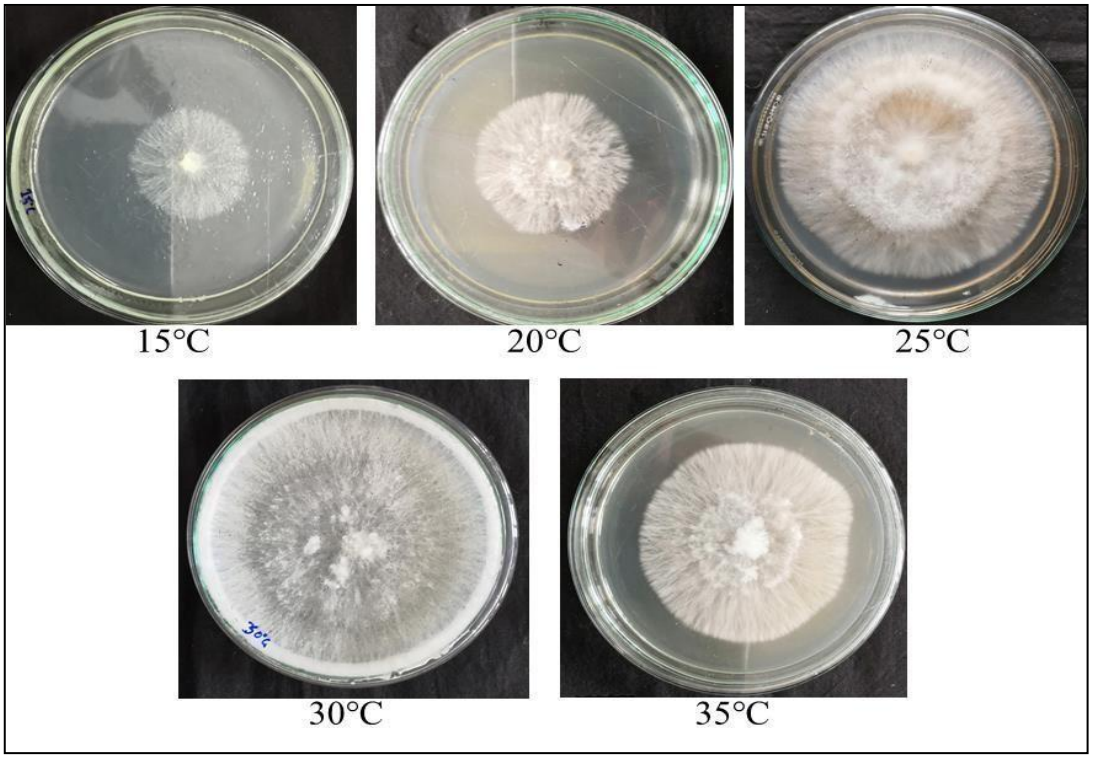


Plate 4.5 Mycelial growth of *Sclerotium rolfsii* at different temperature

temperature of 30°C and 25°C, respectively. Jadon and Tiwari (2011) observed maximum mycelial growth and highest sclerotial production of *S. rolfsii* at 30°C and 25°C, respectively. Daami-Remadi et al. (2010) studied the effect of temperature on mycelial growth rate of *S. rolfsii* and observed that temperature range between 30°C-35°C was optimum for the mycelial growth whereas, no mycelial growth was observed at 5°C, 10°C and 40°C. Kanzaria and Patel (1994) also observed maximum mycelial growth and sclerotial formation at 30°C.

4.4.3 Incubation and latent period

To study the incubation and latent period of *S. rolfsii*, appearance of symptoms and sclerotial production on the infected seedling were observed after inoculation. The first symptom (incubation period) of collar rot was observed after 7 days of incubation whereas, sclerotial formation (latent period) occurred after 13 days of incubation. Hence, incubation and latent period of *S. rolfsii* causing collar rot of cowpea was observed as 7 and 13 days, respectively.

These results on incubation and latent period were in conformity with Shew (1984) who reported incubation and latent period of cowpea collar rot caused by *S. rolfsii* as 7 and 14 days, respectively. Punja (1985) studied the biology, ecology and control of *S. rolfsii* and reported first symptom (incubation period) of collar rot and sclerotia formation (latent period) after 7 and 14 days of incubation, respectively. Aycock (1966) studied stem rot and other diseases caused by *S. rolfsii* and observed that incubation and latent period of pathogen was 6 and 14 days, respectively. Taubenhaus (1919) also observed incubation period of 6 days in *S. rolfsii*.

4.4.4 Effect of inoculum load

To study the effect of inoculum load on the incidence of collar rot, an experiment was conducted with inoculum load of 1, 2, 3, 4 and 5g/kg soil and data on disease incidence were recorded and presented in table 4.6 and plate 4.6. Data indicates that with the increase in inoculum load, per cent disease incidence was also increased. Maximum 100 per cent disease incidence of was observed with the inoculum load of 5g/kg soil whereas, minimum 11.10 per cent incidence was observed with inoculum load of 1 g/kg soil. Inoculum load of 2g/kg gave 88.88 per cent which was statistically at par with inoculum load of 3 g/kg of soil which gave 91.10 per cent disease incidence. Inoculum load of 4 g/kg of soil gave 97.77 per cent of disease incidence which was also statistically

at par with load of 3 g/kg of soil. The per cent disease incidence was very low at inoculum load of 1 g/kg soil (11.10%) but, it increased to 88.88 per cent with 2g/kg soil. From these results, it was concluded that inoculum load of 2 g/kg soil provide sufficient disease incidence and used further as standard inoculum load in all the experiments.

Table 4.6. Effect of inoculum loads on the development of collar rot caused by *Sclerotium rolfsii*

Inoculum load (g/kg soil)	Disease incidence (%)
1	11.10 (3.44)
2	88.88 (9.47)
3	91.10 (9.59)
4	97.77 (9.93)
5	100.00 (10.05)
CD (p=0.05)	0.63

*Figures within parentheses are square root transformed values

The increase in the disease incidence with the increase in the inoculum load of *S. rolfsii* was also reported by Palakshappa et al. (1987) who observed high foot rot infection when betelvine were inoculated with 2 and 3 per cent of inoculum load of *S. rolfsii* and 100 per cent infection was observed with 4 per cent and above inoculum levels. Harlapur (1998) studied the effect of inoculum load on the incidence of foot rot of wheat caused by *S. rolfsii* and reported that for successful infection 2 per cent inoculum was essential. However, 4 per cent and above gave 100 per cent incidence of disease. Singh and Thapliyal (1998) tested inoculum load from 2.5g to 10g per kg of soil on the incidence of seedling rot caused by *S. rolfsii* and found that incidence was significantly increased with the increase of inoculum level.

4.4.5 Effect of sub culturing

An experiment was conducted *in vivo* to study the effect of sub culturing of the pathogen on the incidence of collar rot caused by S. *rolfsii* and data on disease incidence were recorded and presented in table 4.7 and plate 4.7.

1g 2g 3g

4g 5g

Plate 4.6 Effect of inoculum loads of *Sclerotium rolfsii* (1-5 g/kg soil) on the incidence of collar rot of cowpea

1st 3rd 5th

7th 9th

Plate 4.7 Effect of sub-culturing on the development of cowpea collar rot caused by *Sclerotium rolfsii*

Table 4.7. Effect of sub-culturing of *Sclerotium rolfsii* on the development of cowpea collar rot

Subculture	Disease incidence (%)
1st	95.33 (9.86)*
3rd	91.10 (9.59)
5th	73.33 (8.61)
7th	42.22 (6.57)
9th	0.00 (1.00)
CD (p=0.05)	0.33

*Figures within parentheses are square root transformed values

Disease incidence with first sub culturing was maximum i.e. 95.33 per cent followed by third generation sub culture which gave 91.10 per cent with non-significant difference. However, 73.33 and 42.22 per cent disease incidence was observed with fifth and seventh sub culturing of pathogen, respectively. No disease incidence was observed with ninth generation of sub culturing. Data revealed that, with successive sub culturing of pathogen the disease incidence decreased significantly. From these results, it was concluded that pathogen loses its pathogenic ability with sub culturing. Hence, pathogen cultures upto third sub culturing was used for inoculation in all the other experiments for inducing a proper disease incidence.

The result of effect of sub culturing on disease incidence were in conformity with Sennoi et al. (2013) who studied the effect of growth stage and re-isolation on the incidence of stem rot caused by *S. rolfsii* and observed that inoculum derived from sub culture loses its pathogenicity significantly and causes lower incidences of disease than inoculum derived from re-isolated cultures.

4.4.5 Effect of age of culture

To study the effect of age of culture of pathogen on the disease development, an experiment was conducted in pots with pathogen culture of 7, 14, 21 and 28 days old and data on incidence were recorded and presented in table 4.8. The data on disease incidence revealed that 7 days old culture was most virulent causing 86.66 per cent disease incidence of collar rot followed by 14 days old which gave 57.77 per cent of incidence of collar rot. Minimum disease incidence i.e. 11.10 per cent was observed with 28 days old culture; however, 21 days old culture provided only 28.88 per cent of disease incidence.

Table 4.8. Effect of age of culture of *Sclerotium rolfsii* on the development of collar rot

Age of pathogen culture (Days)	Disease incidence (%)
7	86.66 (68.87)
14	57.77 (49.49)
21	28.88 (32.38)
28	11.10 (19.30)
CD (p=0.05)	7.09

*Figures within parentheses are arc sine transformed values

From the results, it was clear that young culture cause more disease incidence as compared to old culture. The disease incidence was significantly reduced with the increase of age of culture. Thus, young culture of pathogen was used for further studies as it was highly virulent in causing collar rot disease as compared to old culture.

Similar trend on the virulence of young culture was also reported by many workers. Suriachandraselvan and Seetharaman (2003) studied the effect of culture age on the incidence of collar rot of chickpea caused by *S. rolfsii* and observed that culture age of one week old cause more incidence of disease as compared to culture of 3-4 weeks old. Sharma et al. (2015) studied the factor affecting the incidence of dry root rot of chickpea and reported that younger culture of pathogen was found to be more aggressive in causing infection than the older culture, while the susceptibility of the crop increased with increase in plant age. Monga and Raj (1994) studied the effect of age of culture on the incidence of root rot caused by *R. bataticola* and reported maximum incidence of disease with young culture of 7-10 days old whereas, minimum incidence was observed with 28-30 days old culture. Rana and Tripathi (1984) studied the factors affecting incidence of dry rot of mung bean and reported that maximum incidence of 82 per cent was observed against inoculation with culture age of 10-15 days old whereas, it was minimum i.e. 22 per cent with inoculation of culture age of 20-30 days old.

4.5 Soil factors affecting the development of disease

4.5.1 Effect of soil moisture

The effect of soil moisture on the incidence of collar rot of cowpea caused by *S. rolfsii* was studied with five moisture levels i.e. 15, 20, 25, 30 and 35 per cent *in vivo* and the data on per cent disease incidence are presented in table 4.9.

Table 4.9. Effect of soil moisture on the incidence of collar rot caused by *Sclerotium rolfsii*

Soil moisture (%)	Disease incidence (%)
15	91.10 (72.85)
20	82.22 (65.12)
25	66.66 (54.78)
30	46.66 (43.05)
35	26.66 (30.95)
CD (p=0.05)	6.94

*Figures within parentheses are arc sine transformed values

Data showed that maximum disease incidence i.e. 91.10 per cent was observed at 15 per cent of soil moisture followed by 20 per cent which gave 82.22 per cent of disease incidence. Disease incidence i.e. 66.66 and 46.66 per cent were observed at 25 and 30 per cent soil moisture, respectively. Minimum disease incidence i.e. 26.66 per cent was observed at 35 per cent soil moisture. From the data on disease incidence at different soil moisture, it was concluded that with the increase in soil moisture there was significant reduction in corresponding disease incidence of collar rot.

The reduction in disease incidence with increase of soil moisture was also reported in literature. Mahato et al. (2017) studied the effect of soil edaphic component on the incidence of tomato collar rot caused by *S. rolfsii* and concluded that disease incidence decreased with increase of soil moisture and maximum disease incidence was recorded at 15 per cent and minimum at 35 per cent soil moisture. Banyal et al. (2008) also reported maximum collar rot incidence in tomato at 15 per cent of soil moisture level whereas, minimum at 35 per cent soil moisture. Harlapur (1998) studied the effect of soil moisture and reported that the *S. rolfsii* survived better at low soil moisture levels as compared to high level. Devi et al. (1999) also reported that fungus survived better on low soil moisture ranging between 15-25 per cent as compared to high soil moisture ranging

between 35-40 per cent. Reddy et al. (1972) conducted a series of laboratory experiments with *S. rolfsii* isolated from wheat and recorded the highest seedling mortality at 25 per cent moisture holding capacity of soil and minimum at 40 per cent of soil moisture.

4.5.2 Effect of soil texture

Five soil texture classes i.e. sandy clay loam, sandy loam, silty clay loam, clay loam and silty loam were evaluated for their effect on the development of disease and data on disease incidence were presented in table 4.10. Data revealed that maximum incidence of collar rot was observed in sandy clay loam (88.88%) followed by sandy loam (82.22%) which was statistically at par with each other, whereas, minimum disease incidence i.e. 26.66 per cent was observed in silty loam soil. The disease incidence i.e. 60.66 and 55.55 per cent with non-significant difference were observed in silty clay loam and clay loam soil, respectively.

Table 4.10. Evaluation of soil textural classes on the incidence of collar rot caused by *Sclerotium rolfsii*

Soil texture	Disease incidence (%)
Silty loam	26.66 (30.95)*
Sandy loam	82.22 (65.12)
Sandy clay loam	88.88 (70.70)
Clay loam	55.55 (48.17)
Silty clay loam	60.66 (54.78)
CD (p=0.05)	6.45

*Figures within parentheses are arc sine transformed values

Maximum disease incidence on sandy clay loam was also reported by Banyal et al. (2008) who observed maximum incidence of collar rot of tomato caused by *S. rolfsii* in sandy clay loam followed by sandy loam, silty clay loam, clay loam whereas, minimum in silt loam soil. Hussain et al. (2006) evaluated different types of soil texture for the incidence of collar rot of chickpea and found maximum (94%) seedling mortality in clay soil followed by 82, 78 and 60 per cent in clay loam, sandy loam and sandy soil, respectively.

4.6 Effect of date of sowing and spacing on the development of disease

The effect of date of sowing and row to row spacing for the development of collar rot was studied by sowing the seeds of cowpea on three dates (7th June, 22nd June and 8th

July) at three row to row spacing (30, 45 and 60 cm) during *Kharif* season in 2018 and 2019 under field conditions and the results are presented in table 4.11.

The data revealed that disease incidence was more during 2018 in crop sown on 8th July (72.65%) followed by 7th June (61.81%) and less on 22nd June (59.53%) as compared to 2019 where the disease incidence was 69.77, 66.35 and 37.68 per cent, respectively. Data also revealed that the average disease incidence of two years on all three spacing was maximum in the crop sown on 8th July (71.21%) as compared to 22nd June (62.94%) and 7th June (49.75%) sowing.

Among all the three spacings evaluated, average maximum disease incidence i.e. 76.53, 67.40 and 54.94 per cent was observed at 60 cm followed by 72.05, 63.21 and 50.34 per cent at 45 cm and 65.06, 58.23 and 43.97 at 60 cm on 8th July, 22nd June and 7th June sown crop, respectively. Data showed that maximum disease incidence was observed when crop was sown at 30 cm as compared to 45 and 60 cm of row to row spacing on all the three dates (7th June, 22nd June and 8th July) of sowing. The effect of spacing on date of sowing on the development of disease showed that in all the three spacings, maximum average disease incidence i.e. 76.53, 72.05 and 65.06 per cent was observed on 8th July sown crop and minimum average disease incidence i.e. 59.94, 50.34 and 43.97 per cent on 7th June sown crop at 30, 45 and 60 cm spacing (row to row), respectively. However, the average disease incidence was observed as 60.40, 63.21 and 58.23 per cent on 22nd June sown crop at 30, 45 and 60 cm spacing (row to row), respectively.

Among all three dates of sowing, average maximum disease incidence i.e. 76.53, 72.05 and 65.06 per cent was observed on 8th July followed by 67.40, 63.21 and 58.23 on 22nd June and 54.94, 50.34 and 43.97 on 7th June at 30, 40 and 60 cm spacing, respectively. Data showed that maximum disease incidence was observed when crop was sown at 8th July as compared to 22nd June and 7th June of sowing on all the three (30, 45 and 60 cm) row to row spacing. Effect of three dates of sowing at three spacing on the development of disease showed that in all three dates of sowing, maximum average disease incidence i.e. 54.94, 67.40 and 76.53 per cent was observed at 30 cm row to row spacing and minimum average disease incidence of 43.97, 58.23 and 65.06 per cent was observed at 60 cm row to row spacing on 7th June, 22nd June and 8th July sowing, respectively. However, average disease incidence of50.34, 63.21 and 72.05 per cent was observed at 45 cm spacing on 7th June, 22nd June and 8th July of sowing, respectively.

Table 4.11. Effect of date of sowing and spacing on the development of collar rot caused by *Sclerotium rolfsii*

Date of sowing	Spacing Row x Row (cm)	Per cent disease incidence		
		2018	2019	Mean
7th June	30	66.27	43.60	54.94
	45	61.24	39.44	50.34
	60	57.94	30.00	43.97
	Average	61.81	37.68	49.75
22nd June	30	64.79	70.00	67.40
	45	58.81	67.61	63.21
	60	55.01	61.44	58.23
	Average	59.53	66.35	62.94
8th July	30	78.73	74.33	76.53
	45	74.66	69.44	72.05
	60	64.57	65.55	65.06
	Average	72.65	69.77	71.21
CD (p=0.05)	A	3.56	1.98	-
	B	3.19	1.69	-
	A x B	6.17	3.43	-

The combination of date of sowing and spacing showed that average maximum disease incidence, i.e. 76.53 per cent was observed in late sown crop (8th July) with narrow row to row spacing (30 cm) whereas, average minimum disease incidence of. 43.97 per cent was observed in early sown crop (7th June) with wider row to row spacing (60 cm). Present study, indicated that high disease incidence occurred in late sown crop at narrow spacing as compared to normal and early sown crop and wider spacing.

Lower incidence of disease in early sown crop may be due to availability of low moisture because of dry climate in the early part of June. The secondary spread of disease may also be restricted at wider spacing. Hence, the disease incidence was less on early sown crop and wider spacing.

Higher disease incidence on late sown crop as compared to early sown and with wide spacing as compared to narrow spacing were also observed by many workers. Deka et al. (2015) evaluated date of sowing and spacing for the incidence of collar rot of cluster bean caused by *S. rolfsii* and reported minimum incidence of disease in early sown crop

i.e. 1st July at wider spacing 60×30 cm (row to plant) whereas, maximum incidence was observed on late sown crop i.e. 1st August with narrow spacing 45×30 (row to plant). Young and Morris (1927) observed that closer plant spacing in collar rot of sunflower caused by *S. rolfsii* was more efficient in the spread of pathogen creating more potential infection loci promoting higher incidence of disease. Whereas, in wider spacing lower incidence of disease was observed because of presence of fewer potential infection loci with less spread of pathogen. Dubey and Dwivedi (1989) observed maximum disease incidence of ground nut collar rot caused by *S. rolfsii* on late sown crop i.e. 15th October whereas, minimum on early sown crop i.e. 1st October.

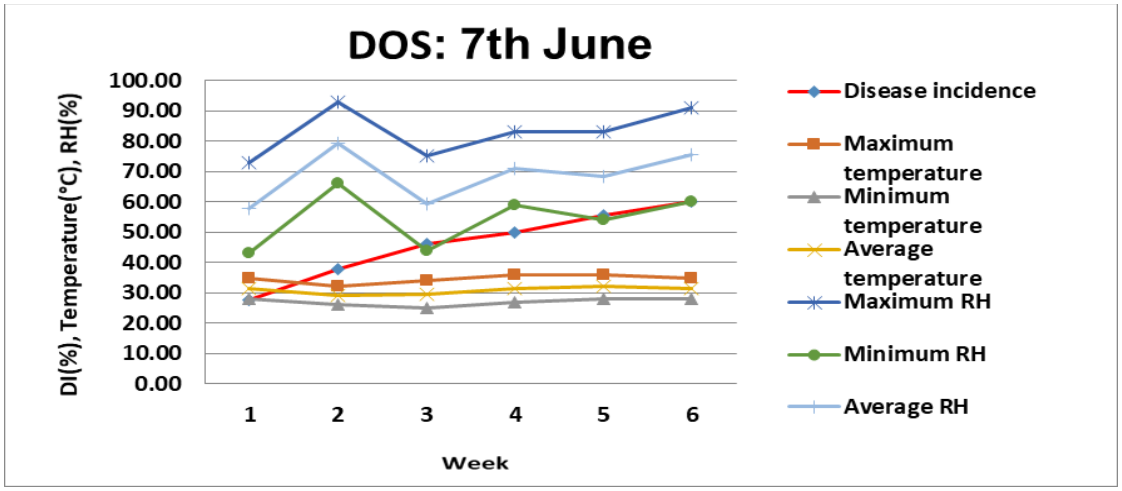
4.7 Effect of weather variables on the development of disease

To study the effect of the weather variables (temperature and relative humidity) on the development of collar rot, an experiment was conducted by sowing the crop on 7thJune, 22nd June and 8th July during *Kharif* 2018 under field conditions. The data on disease incidence (%), temperature (°C) and relative humidity (%) were recorded at weekly interval and presented in table 4.12 and figure 4.1.The data revealed that disease incidence was more on 8th July (65.22%) sown crop in comparison to 22nd June (52.16%) and 7th June (46.16%) sown crop.

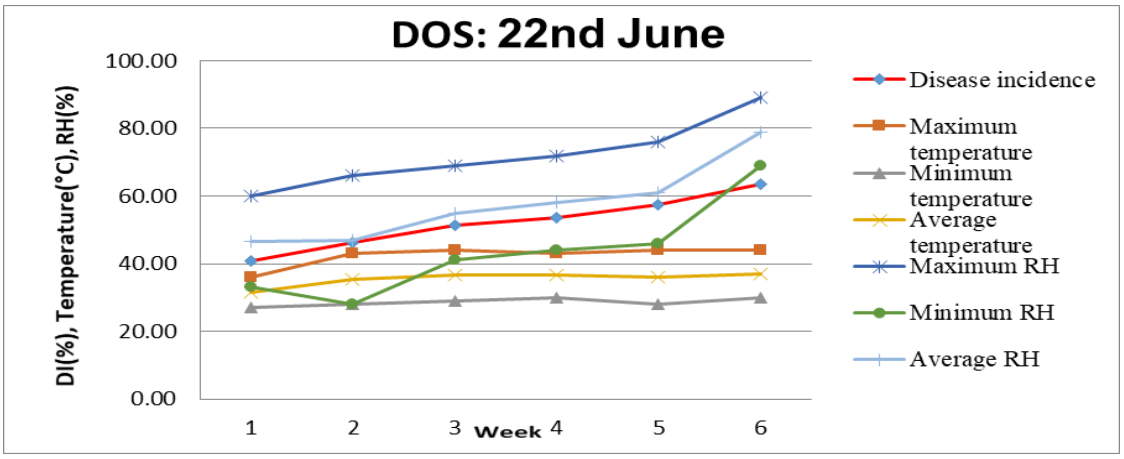
Data on disease incidence of three dates of sowing were correlated with temperature (maximum, minimum and average) and relative humidity (maximum, minimum and average) and the correlation coefficient and regression equations were calculated and presented in table 4.13.

Table 4.12. Disease incidence and weather variable on different dates of sowing

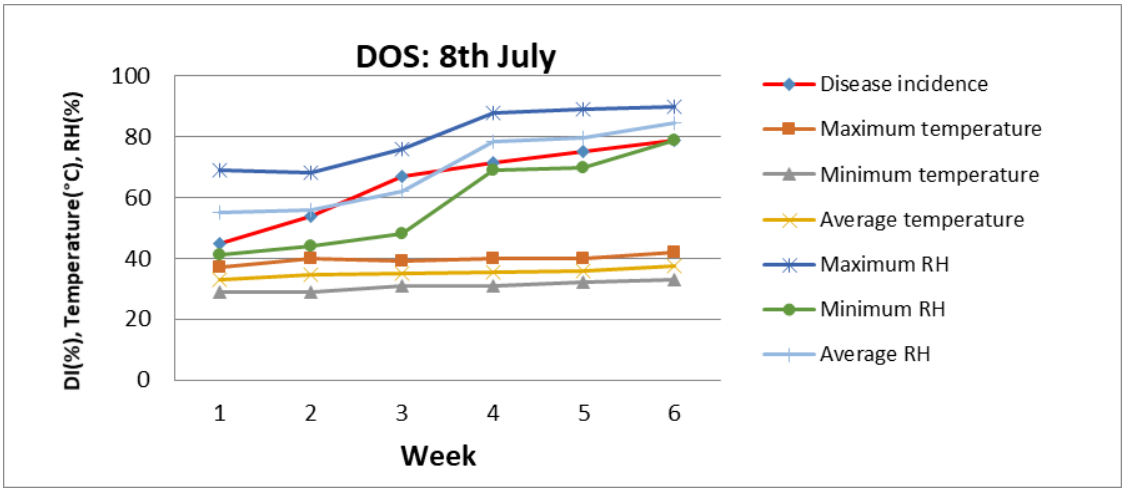
Disease incidence (%)	Temperature (°C)			Relative humidity (%)		
	Maximum	**Minimum**	**Average**	**Maximum**	**Minimum**	**Average**
a. 1st date of sowing (7th June, 2018)						
27.67	35	28	31.5	73	43	58.0
37.67	32	26	29.0	93	66	79.5
46.00	34	25	29.5	75	44	59.5
50.00	36	27	31.5	83	59	71.0
55.67	36	28	32.0	83	54	68.5
60.00	35	28	31.5	91	60	75.5
(Av.) 46.16						
b. 2nd date of sowing (22nd June, 2018)						
40.67	36	27	31.5	60	33	46.5
46.33	43	28	35.5	66	28	47.0
51.33	44	29	36.5	69	41	55.0
53.67	43	30	36.5	72	44	58.0
57.33	44	28	36.0	76	46	61.0
63.67	44	30	37.0	89	69	79.0
(Av.) 52.16						
c. 2nd date of sowing (22nd June, 2018)						
44.67	37	29	33.0	69	41	55.0
54.00	40	29	34.5	68	44	56.0
67.00	39	31	35.0	76	48	62.0
71.33	40	31	35.5	88	69	78.5
75.33	40	32	36.0	89	70	79.5
79.00	42	33	37.5	90	79	84.5
(Av.) 65.22						



(a) 1st date of sowing



(b) 2nd date of sowing



(c) 3rd date of sowing

Fig 4.1 Disease incidence, temperature and relative humidity on different dates of sowing i.e. (a) 7th June, (b) 22nd June and (c) 8th July

Correlation coefficients of simple correlation table (4.13 a) showed positive correlation of disease incidence with maximum temperature (0.435, 0.758 and 0.808), minimum temperature (0.182, 0.745 and 0.959), average temperature (0.359, 0.816 and 0.923), maximum relative humidity (0.430, 0.978 and 0.909), minimum relative humidity (0.544, 0.901 and 0.909) and average relative humidity (0.544, 0.901 and 0.909) on all the dates of sowing i.e. 7thJune, 22ndJune and 8th July, respectively. Correlation of disease incidence was highly and positively correlated with temperature and relative humidity in 3rd (8th July) followed by 2nd (22ndJune) and 1st (7th June) date of sowing.

Correlation coefficients of partial correlation (table 4.13b) also showed positive correlation of disease incidence with maximum temperature (0.992, 0.541 and 0.905), minimum temperature (0.981, 0.604 and 0.948), average temperature (0.825, 0.582 and 0.912), maximum relative humidity (0.986, 0.127 and 0.996), minimum relative humidity (0.973, 0.278 and 0.997) and average relative humidity (0.64, 0.212 and 0.989) on all dates of sowing i.e. 7th June, 22ndJune and 8th July, respectively. The partial correlation of disease incidence was highly and positively correlated with temperature in the 3rd date of sowing (8th July) followed by 2nd (22nd June) and 1st (7th June) date of sowing.

Hence, positive correlation coefficients of simple and partial correlation revealed that increase in temperature and relative humidity will also increase the disease incidence.

Regression coefficients (table 4.13c) was observed positive with maximum temperature, average temperature and average relative humidity and negative with minimum temperature and maximum relative humidity on all the three dates of sowing (8th July, 22nd June and 7th July) except maximum relative humidity which was positive during 8th July sowing.

The coefficient of determination (R^2) revealed that selected weather variables i.e. temperature and relative humidity contributed 97.8, 98.0 and 97.1 per cent towards incidence of collar rot on crop sown on7th June, 22nd June and 8th July, respectively. Consequently, the temperature and relative humidity were observed as very important weather variable for the development of collar rot of cowpea. To study the relationship of disease incidence with AUDPC and infection rate (r), the data on incidence of three date of sowing (7th June, 22nd June and 8th July) were utilized to calculate AUDPC and infection rate (r) and presented in table 4.14. The AUDPC value was maximum in 8thJuly (329.8) followed by 22nd June (260.33) and 7thJune (266.32) date

Table 4.13. Effect of weather variables on the development of collar rot caused by *Sclerotium rolfsii*

a. Simple correlation coefficients between disease incidence and weather variables

Date of sowing	Per cent disease incidence (Mean)	Simple Correlation Coefficients					
		DS x maxT	DS x minT	DS x avgT	DS x maxRH	DS x minRH	DS x avgRH
7th June	46.16	0.435	0.182	0.359	0.430	0.544	0.408
22nd June	52.16	0.758	0.745	0.816	0.978*	0.901*	0.943*
8th July	65.22	0.808	0.959*	0.923*	0.909*	0.909*	0.929*

b. Partial correlation coefficients between disease incidence and weather variables

Date of sowing	Per cent disease incidence (Mean)	Partial Correlation Coefficients					
		DS x maxT	DS x minT	DS x avgT	DS x maxRH	DS x minRH	DS x avgRH
7th June	46.16	0.992*	0.981*	0.825	0.986*	0.973*	0.964*
22nd June	52.16	0.541	0.604	0.582	0.127	0.278	0.212
8th July	65.22	0.905	0.948	0.912	0.996*	0.997*	0.989*

c. Regression equation between disease incidence and weather variables

Date of sowing	Per cent disease incidence (Mean)	Regression equation	Multiple correlation coefficient (R)	Coefficient of determination (R^2)
7th June	46.16	Y = -337.276 + 11.231 (X1) – 7.848 (X2) + 0 X(3) -3.960 (X4) - 2.250 (X5) + 0 (X6)	0.989	0.978
22nd June	52.16	Y = -56.670 + 0.50 (X1) – 0.951 (X2) + 1 (X3) – 0.5 (X4) - 1 (X5) + 0 (X6)	0.990	0.980
8th July	65.22	Y = -385.998 + 0.719(X)1 – 1.435(X)2 + 8.717 (X3) + 0 (X4) – 2 (X5) + 4 (X6	0.997	0.971

*values = significantly correlated
DI (Y) = Per cent disease incidence (%)
maxT (X1) = Maximum temperature (°C)
minT (X2) = Minimum temperature (°C)
avgT (X3) = Average temperature (°C)
maxR (X4) = Maximum Relative humidity (%)
minR (X5) = Minimum Relative humidity (%)
avgR (X6) = Average Relative humidity (%)

of sowing. Similar trends of infection rates were also observed with per cent disease incidence. Infection rate (r) was observed maximum i.e. 0.38/week on 8th July followed by 0.23/ week on 22nd June and 0.22/ week on 7th June sown crop. The values of AUDPC and infection rate followed the same increasing trend of disease incidence i.e. 65.26 per cent followed by 52.16 per cent and 46.16 per cent on 8th July, 22nd June and 7th June date of sowing, respectively.

Expected disease incidence was also calculated through the derived regression equation and it was observed that calculated value of disease incidence were almost similar to that of observed disease incidence on all the three date of sowing (table 4.14). The calculated disease incidence was 41.56, 52.24 and 66.14 per cent whereas, observed disease incidence was 46.16, 52.16 and 65.22 per cent on 7th June, 22nd June and 8th July date of sowing, respectively.

Table 4.14. Relationship of collar rot incidence with infection rate (r), AUDPC and expected disease incidence

Date of sowing	AUDPC	Rate of infection (r/ week)	Disease incidence (Observed)	Disease incidence (Expected)
7th June	266.32	0.22	46.16	41.56
22nd June	260.53	0.23	52.16	52.24
8th July	329.81	0.38	65.22	66.14

The data clearly shows that the weather variables taken for calculation of regression equation were very important and regression equation calculated fitness was good for forecasting of collar rot.

Positive correlation of weather factors with disease incidence and high influence of relative humidity and temperature on the development of disease were also reported by many workers. Kumar and Kudada (2018) studied the effect of weather conditions on incidence of root rot of french bean and observed that pre and post root rot incidence was highly significantly positively correlated with minimum soil temperature whereas, it was non significantly positively correlated with maximum, minimum temperature and maximum soil temperature. Also average relative humidity and sunshine showed non-significant correlation with disease incidence and green pod yield. Pinheiro et al. (2010) observed optimum development of *S. rolfsii* at 70 per cent of soil moisture level combined with temperature range of 25°C-30°C. Sharma and Tripathi (2001) reported

that higher aerial temperature (28°C-30°C), relative humidity (> 80%) and soil temperature (28°C-30°C) favoured high disease severity of web blight caused by *S. rolfsii*. High soil moisture, relative humidity (> 80%) and temperature (28°C) were also observed best for the root rot development of french bean caused by *R. solani* by Upmanyu (2002). Dubey and Dwivedi (1989) observed that 26°C–28°C temperature and 90 per cent of relative humidity favours higher infection and rapid incidence of disease. Emechebe and Florini (1997) observed that collar rot of cowpea caused complete destruction of leaf canopy during periods of heavy rain with long periods of overcast skies. They further observed aggravation of disease in portion of field that contains stagnant water for than 24 hrs or more. Epps et al. (1951) observed that the *S. rolfsii* was most active in soil at temperature of 30°C- 35°C and activity gradually decreases as temperature declined at 15°C or below.

5. SUMMARY AND CONCLUSIONS

Studies on etiology and epidemiology of collar rot complex of cowpea was undertaken to identify the cause, factors affecting pathogen & disease development and effect of date of sowing, spacing & weather variables on the development of collar rot of cowpea. The experiments were conducted in nethouse, laboratory and field of Department of Plant Pathology, CSKHPKV Palampur.

Isolations were taken from the collected samples and three fungal pathogen i.e. *Sclerotium* sp., *Colletotrichum* sp. and *Fusarium* sp. were isolated and identified primarily on the basis of morpho-cultural characteristics. However, the pathogenicity was proved only with *Sclerotium* sp. and all further studies were only conducted with this pathogen.

The pathogen produced typical symptoms of collar rot under nethouse and was described as appearance of brown coloured oval shaped lesion near collar region on the seedling. Wilting and collapsing of affected seedling, appearance of white coloured fungal mycelial growth over the affected part of stem, dark brown coloured sclerotia were produced at collar region near soil level. Diseased seedling do not grow further and ultimately died.

The morphological and cultural characteristics of test pathogen were observed as, hyphae were hyaline, sparsely septate and profusely branched and mycelia had clamp connection, colour of colony was milky white to dull white and colour of sclerotia was initially white which later on turned brown and sclerotia at maturity looks like mustard seeds.

The test pathogen was identified on the basis of symptomatology and morpho-cultural characteristic as *Sclerotium rolfsii* Sacc. as causal agent of collar rot of cowpea.

Among five solid media tested, potato dextrose agar medium was found best for the mycelial growth of *S. rolfsii* as compared to oat meal agar, corn meal agar, Richard agar and Malt extract agar. Sclerotial production was observed best on oatmeal agar at 28±1°C.

Effect of different temperatures *viz.*15, 20, 25, 30 and 35°C on mycelial growth was studied on PDA and 30°C temperature was found best for mycelial growth whereas, 25°C best for sclerotial production.

Incubation period of *S. rolfsii* causing collar rot of cowpea was observed 7 days whereas, latent period was observed 13 days under *in vivo* conditions.

Inoculum loads i.e.1, 2, 3, 4 and 5 g/kg soil were tested for the development of disease and 100 per cent disease incidence was observed with 5g/kg soil of inoculum load whereas, minimum was observed with 1g/kg soil. However, optimum disease incidence was observed with inoculum load of 2g/kg soil.

The cultures of 7, 14, 21 and 28 days old of *S. rolfsii* were tested for development of disease and maximum incidence of collar rot was observed with 7 days old culture whereas, minimum with 28 days old culture. Significant decrease in disease incidence was observed with the increase in the age of culture of pathogen.

Sub-culture of generations i.e. 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 9^{th} were studied for development of collar rot of cowpea and it was observed that maximum per cent disease incidence was observed with 1^{st} generation sub culture and it was reduced significantly with the increase in sub culturing. No disease was observed with 9^{th} generation sub culture of the pathogen.

Five soil moisture levels i.e. 15, 20, 25, 30 and 35 per cent were tested for the development of disease and it was found that maximum incidence of collar rot was observed at 15 per cent of soil moisture whereas, it was minimum at 35 per cent of soil moisture. However, the disease incidence at 20, 25 and 30 per cent soil moisture was moderate and ranged between 46.66 to 82.22 per cent.

Effect of soil textures i.e. sandy clay loam, clay loam, sandy loam, silty loam and silty clay loam were studied for the development of collar rot of cowpea and maximum disease incidence of collar rot (88.22%) was observed in sandy clay loam whereas, minimum was observed i.e. 26.66 per cent in silty loam soil. In other soil it was ranged between 55.55 to 82.22 per cent.

The effect of date of sowing and row to row spacing for the development of collar rot was studied by sowing the seeds of cowpea on three dates (7^{th} June, 22^{nd} June and 8^{th} July) at three row to row spacing (30, 45 and 60 cm) and it was observed that the average disease incidence of two years on all three spacing was maximum in the crop sown on 8^{th}

July (71.21%) as compared to 22nd June (62.94%) and 7th June (49.75%) sowing. Among all the three spacing average maximum disease incidence i.e. 76.53, 67.40 and 54.94 per cent was observed at 60 cm followed by 72.05, 63.21 and 50.34 per cent at 45 cm and 65.06, 58.23 and 43.97 at 60 cm on 8th July, 22nd June and 7th June sown crop, respectively. Among all the three dates of sowing average maximum disease incidence i.e. 76.53, 72.05 and 65.06 per cent was observed on 8th July followed by 67.40, 63.21 and 58.23 on 22nd June and 54.94, 50.34 and 43.97 on 7th June at 30, 40 and 60 cm spacing, respectively.

The combination of date of sowing and spacing showed that average maximum disease incidence, i.e. 76.53 per cent was observed in late sown crop (8th July) with narrow row to row spacing (30 cm) whereas, average minimum disease incidence, i.e. 43.97 per cent was observed in early sown crop (7th June) with wider row to row spacing (60 cm).

The correlation and regression analysis of weather variable (temperature and relative humidity) with per cent incidence of collar rot showed that simple and partial correlation coefficients were positively correlated with maximum temperature, minimum temperature, average temperature, maximum relative humidity, minimum relative humidity and average relative humidity on all the dates of sowing i.e. 7th June, 22nd June and 8th July, respectively. Simple and partial correlations of disease incidence were highly positively correlated with temperature and relative humidity in 3rd date of sowing (8th July) followed by 2nd (22ndJune) and 1st (7th June) date of sowing. The coefficient of determination (R^2) revealed that selected weather variable i.e. temperature and relative humidity contributed 97.8, 98.0 and 97.1 per cent toward incidence of collar rot on crop sown on 7th June, 22nd June and 8th July, respectively.

The AUDPC value and infection rate (r/week) was maximum in 8thJuly (329.8 and 0.38) followed by 22ndJune (260.33 and 0.23) and 7thJune (266.32 and 0.22) date of sowing, respectively. The values of AUDPC and infection rate follow the similar trend as of disease incidence. The regression equations fits well as calculated disease incidence i.e. 41.56, 52.24 and 66.14 per cent fall very near to the values of observed disease incidence were 46.16, 52.16 and 65.22 per cent on 7th June, 22nd June and 8th July date of sowing, respectively.

CONCLUSIONS

- Causal agent of collar rot of cowpea was identified as *Sclerotium rolfsii* Sacc.
- For mycelial growth and sclerotial production best solid media was potato dextrose agar and oat meal agar, respectively.
- For the mycelial growth and sclerotial production of *S. rolfsii* best temperature was 30ºC and 25ºC, respectively.
- Incubation and latent period of *S. rolfsii* causing collar rot of cowpea was 7 and 13 days, respectively.
- Pathogenic ability of *S. rolfsii* decreases with increase in culture age and sub culturing.
- Low soil moisture supported best development of collar rot as compared to high soil moisture.
- Sandy clay loam and sandy loam soil supported high disease incidence as compared to clay and silty loam.
- Late sowing and narrow spacing leads to higher incidence of collar rot as compared to early and timely sowing with wider spacing.
- Temperature and relative humidity have significant and positively correlated with the incidence of collar rot of cowpea.

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APPENDIX - 1

Potato dextrose agar

Peeled and sliced potatoes	200 g
Dextrose	20 g
Agar-Agar	20 g
Distilled water	1000 ml

Malt extract Agar

Malt extract	25 g
Agar-Agar	20 g
Distilled water	1000 ml

Oat meal Agar-Agar

Oat flakes	30 g
Agar - Agar	20 g
Distilled water	100 ml

Corn meal Agar

Corn meal	30 g
Agar-Agar	20 g
Distilled water	1000 ml

Richard's agar

Sucrose	50 g
Potassium dihydrogen phosphate	5 g
Potassium nitrate	10 g
Magnesium sulphate	2.5 g
Ferric chloride	0.02 g
Agar-Agar	20 g
Distilled water	1000 ml

Brief Biodata of student

Name	Mr.Siddharth Anand
Father's Name	Sh. R.N. Chaurasia
Mother's Name	Smt. Sampat Devi
Date of Birth	23.01.1994
Permanent Address	Railway colony, Sadar , Nagpur (MH)

Academic Qualifications

Qualification	Month	Year	School/Board/University	Marks(%)	Division
10^{th}	March	2009	CBSE Board	65%	First
10+2	March	2011	CBSE Board	73%	First
B.Sc. Agriculture	July	2016	Dr. PDKV, Akola	82.2%	First
M.Sc. Plant Pathology	July	2019	CSKHPKV Palampur	71.4%	Second