Management of Stem Rot and Foliar Blight of Cowpea (*Vigna unguiculata* (L.) Walp.)

by

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(2018-11-023)

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PADANNAKKAD, KASARAGOD - 671314

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THESIS

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DEPARTMENT OF PLANT PATHOLOGY
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PADANNAKKAD, KASARAGOD - 671314
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2020

DECLARATION

I, hereby declare that the thesis entitled **"Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other university or society.

Padannakkad **Nayana Sunil. M. V**

Date 2018-11-023

CERTIFICATE

Certified that this thesis entitled **"Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)"** is a bonafide record of research work done independently by Ms. Nayana Sunil. M. V under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Padannakkad
Date 21/08/2020

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We, the undersigned members of the Advisory Committee of Ms. Nayana Sunil. M.V., a candidate for the degree of **Master of Science in Agriculture** with major field in Plant Pathology, agree that the thesis entitled **"Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)"** may be submitted by Ms. Nayana Sunil. M. V, in partial fulfilment of the requirement for the degree.

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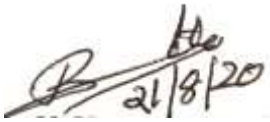

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Nayana Sunil M V

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LIST OF ABBREVIATIONS AND SYMBOLS USED

et al.	And other co workers
%	Per cent
^{0}C	Degree celsius
pm	picometer
cm	centimeter
mm	millimeter
m	meter
ha	hectares
dia	diameter
s	second
min	minutes
h	hours
mg	milligram
g	gram
kg	kilogram
t	tonne
µl	microlitre
ml	millilitre
l	litre
DAI	Days after inoculation
CD	Critical difference
SE	Standard error
sp. or spp.	Species (singular and plural)
viz	Namely
Fig.	Figure
No.	Number

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Introduction

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the versatile and indispensable vegetable crops which can provide food, feed, fodder and green manure. Since it is highly nutritive and common choice for human diet, farmers are highly motivated to go for cowpea cultivation especially in Kerala. Cowpea production has been distributed East and Central Africa, South and Central America and Asia, recording largest production in Africa (Gomez, 2004). India is also one among the countries, contributing largest percentage of cowpea production all over the world.

Nutritional value of cowpea is highly remarkable, since it possesses plenty of proteins, carbohydrates and minimum amount of fat when compared to cereals. High amount of lysine, dietary fiber, phenolic compounds and minerals can be supplemented by cowpea through food and provide health benefits like anti-diabetic and anti-cancer properties which make it a highly valuable crop (Jayathilake *et al.*, 2018).

Apart from nutritional benefit to human, cowpea has significant role in soil fertility by fixing nitrogen to the soil which can be used by succeeding crop. It points out the scope of crop rotation, intercropping and mixed cropping which are widely practiced in Kerala (KAU, 2016). Incorporation of cowpea into the soil at pre flowering stage can accumulate 68 kg N/ha in the soil, specifying green manuring effect of this crop (John *et al.*, 1992).

Since cowpea is included under the most demanding vegetables for farmers in Kerala, especially in Kasargod district, cultivation is promoted throughout the year allocating all over the district. It creates a condition which can conserve the inoculum of plant pathogenic microorganism and make the plants vulnerable to various diseases which result in severe yield reduction and economic loss for farmers. Cowpea plants are highly susceptible to bacteria, fungus and viral diseases which were reported to cause severe crop loss (Williams, 1975).

Recently stem rot and foliar blight disease caused by *Sclerotium rolfsii* were noticed in farmer's fields and the severity of more than 40 % was found during rainy season. Wilting and yellowing of the plants, rotting of the stem, white fungal mycelial growth and sclerotial formation on the infected portion are the common symptoms noticed with stem rot disease whereas water soaked foliar lesion having concentric rings are observed in foliar blight disease. Since the pathogen is soil borne and can survive by sclerotia in debris and weeds and has wide host range, make it very difficult to manage (Kator *et al.*, 2015). Hence a proper management package has to be recommended for the suppression of disease.

Thus present study entitled 'Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)' was conducted to identify and characterize fungal pathogen causing stem rot and foliar blight in cowpea and to evolve a management strategy for these diseases.

The objectives of the study were:

- To identify and characterize fungal pathogen causing stem rot and foliar blight in cowpea
- To evaluate various biocontrol agents and chemical fungicides against the pathogen
- To evolve a management strategy against the disease

The following programmes were experimented for the study

- Collection of samples and isolation of pathogen
- Evaluation of pathogenicity
- Identification of pathogen based on cultural, morphological and molecular characterization
- Study of symptomatology of diseases
- *In vitro* evaluation of biocontrol agents and chemical fungicides
- Study of compatibility of fungicides with antagonists
- *In vivo* evaluation of biocontrol agents and chemical fungicides

Review of Literature

2. REVIEW OF LITERATURE

Cowpea (*Vigna unguiculata* (L.) Walp.) is a versatile crop suitable for tropical and subtropical regions including Asia, Europe, The United States and Central and South America with a purpose of consumption as food and animal feed (Carvalho *et al*., 2017). It is also a main vegetable crop cultivated in Kerala throughout the year and found highly desirable in homestead gardening and intercropping (KAU, 2019).

Diseases incited by various microorganisms such as fungi, bacteria and nematode were found to be threatening towards cowpea production and among which basal stem rot caused by *Sclerotium rolfsii* Sacc. is responsible for severe yield loss (Emechebe and Lagoke, 2002). Basal stem rot, southern blight, damping off and foliar blight are the other major fungal diseases reported in cowpea through different studies.

2.1. COLLECTION OF SAMPLES

Siddaramaiaha *et al.* (1979) recorded 7.8 % incidence of *S. rolfsii* in groundnut plants growing in Dharwad district and complete death of infected plants were observed 70 to 90 days after infection. Anahosur and Kulkarni (1997) studied wilt disease in potato caused by *S. rolfsii* and gave detailed description on disease incidence across different fields of Belganvi and Dharwad districts. Highest incidence was reported at Puddalakatti (40 %) followed by Tadakod (20 %). Sarma *et al*. (2002) made collection of 26 isolates of *S. rolfsii* out of which 24 isolates exhibited collar region infection and 4 exhibited foliar infection. Isolation was done from different host plants located at different geographical regions.

Adandonon *et al*. (2004) conducted survey in cowpea fields of five agro-ecological zones in Benin during 2001 and 2002 to assess the occurrence of cowpea diseases and revealed various fungi associated with each disease. *S. rolfsii* isolated from the diseased samples was found prevalent in all the zones which mentioned as the main causal agent of cowpea damping off and stem rot disease in Benin. Ahohuendo *et al.* (2005) reported *S. rolfsii* as causal organism of damping off of

cowpea which was a serious problem for cowpea production in Ouémé valley of Bénin.

Le *et al*. (2012) collected groundnut samples infected with *S. rolfsii* from eight locations in Central Vietnam and percent disease incidence exhibited a range of 5 to 25 %. Stem rot caused by *S. rolfsii* on cluster bean was reported for the first time in Gujarat by Ronakkkumar and Sumanbhai (2014). As per them, Banaskantha district recorded a disease incidence of 4.8 to 9.8 % for which maximum was recorded at Dantiwada tehsil and least was at Vav.

Poornima (2015) reported that disease incidence of pod rot and stem rot caused by *S. rolfsii* was higher during *kharif* season and recorded highest value of stem rot incidence (73.3 %) and pod rot incidence (100 %) at Yarballi village in Chitradurga district. Groundnut fields in Anantapur and Chittoor districts of Andhra Pradesh were surveyed by Rani *et al*. (2016). Maximum disease incidence was observed at Chittoor (10.06 %) district and minimum in Mahaboobnagar (7.75 %).

2.2. ISOLATION OF THE PATHOGEN

According to Fery and Dukes (2002), southern blight of cowpea was caused by *S. rolfsii* and for isolation dry sclerotia produced on the infected stem were placed in acid PDA plates after surface sterilized with ethanol (95 %) followed by keeping plates for incubation at 30^0C till new sclerotia were formed. Maintenance of isolate was done by keeping dry sclerotia in glass vials.

Incidence of southern blight disease in soybean caused by *S. rolfsii* was reported in Nigeria for the first time by Akem and Dashiell (1991) and they isolated the pathogen in acidified potato dextrose agar (PDA) at 25^0C. Sennoi *et al.* (2010) isolated this fungus from sclerotia found on the rotted portion of Jerusalem artichoke after surface sterilization with alcohol (70 %) followed by washing in sterilized water each for one minute. These were placed in petridishes containing PDA medium for incubation at room temperature.

Tortoe and Clerk (2012) reported that different isolates of *S. rolfsii* were obtained from American taro and African oil palm in Ghana and matured brown sclerotia were used for the isolation. The collected sclerotia were taken for surface sterilization with sodium hypochlorite (1%) followed by three successive washing with sterile water and then placed on the solidified PDA in petri plates. For snake bean infected with basal stem rot pathogen, isolation was done by surface sterilizing the infected stem with 70 % alcohol followed by placing thin sections of stem in carnation leaf-piece agar (CLA) and potato carrot agar (PCA) (Songvilay *et al*., 2013).

Kumar *et al.* (2014) conducted survey in fields of Southern Andhra Pradesh during September 2012 where stem rot of groundnut caused by *S. rolfsii* was severe and isolated the pathogen from the diseased plants by tissue segment method in PDA. 0.5 to 1 cm sized tissues containing both healthy and diseased portion were cut from the collar region followed by surface sterilization, washing and placing the PDA for incubation.

Sclerotium rot caused by *S. rolfsii* on ground apple was reported in South Korea for the first time by Kwon *et al.* (2014). Surface sterilization of sclerotia was done for 1 min and placed in PDA after successive washing with sterile water. Sclerotia were formed after 18 days of incubation. Sudina *et al*. (2014) isolated *S. rolfsii* from infected leaves of cowpea showing foliar blight symptom from Kerala and it was the first report of *S. rolfsii* infection on cowpea leaves.

S. rolfsii was isolated in PDA medium causing fruit rot of pumpkin in many fields of Southern Karnataka with a characteristic symptom of water soaked regions with white mycelial growth on the fruit surface (Mahadevakumar *et al.*, 2016). Fifteen locations of Cuddalore district in Tamil Nadu under both irrigated and rainfed conditions were surveyed by Sivakumar *et al.* (2016) for disease incidence of *S. rolfsii* in groundnut causing stem rot disease and isolated the pathogen from infected stem pieces in PDA after proper surface sterilization and washing. Pumpkin fruits infected with *S. rolfsii* were cut into pieces (0.5 cm^2) and isolated after strict surface sterilization with 1.5 % sodium hypochlorite for about 2 minute and wash with sterile

distilled water (Najera *et al.,* 2018). All pieces were placed on PDA in petridishes and incubated at room temperature for 5 days.

According to Tanimu *et al.* (2018), sclerotia produced by *S. rolfsii* on *Echinochloa* spp. a grass host found in cowpea farm used for the isolation in which sclerotia were dried and stored at 30^0C in petridish. Santosh *et al.* (2020) isolated *S. rolfsii* in PDA from tissue fragments of cowpea infected with the pathogen after strict surface sterilization with 70 % alcohol (30 s) and sodium hypochlorite (2 min) followed by successful washing with sterile water.

2.3. PATHOGENICITY TEST

S. rolfsii is pathogenic to more than 500 species of plants of both monocots and dicots in the tropics and subtropics. (Aycock, 1966; Punja, 1985). Among these legumes, crucifers, cucurbits, maize, alfalfa, bean, carrot, onion, radish, cotton and garlic were reported as the common hosts.

According to Epps *et al.* (1951), pathogenicity screening of *S. rolfsii* done in eight hosts concluded that the tomato, cotton, cowpea, soybean, broad bean, potato and sugar beet were highly infected at seedling stage whereas woody plants imparted the resistance. Fleshiness of roots were more prone to infection throughout the season.

Pathogenicity of *S. rolfsii* was evaluated by Infantino *et al.* (1997) in sunflower by growing seedlings of 20 day old in sterilized peat/ soil mixture filled in pots under green house and five sclerotia were used to place near the crown of each plant. Pathogenicity of *S. rolfsii* which incited seed and seedling rot in soybean was done by seed and soil inoculation methods for which sorghum grains were soaked in sucrose (2 %) solution for 12 h. and after sterilization these were inoculated by pathogen followed by incorporation in soil taken in plastic pots (Singh and Thapliyal, 1998).

Adandonon *et al.* (2004) evaluated the pathogenicity of isolates of *S. rolfsii* on cowpea by millet seed inoculum technique in which pathogen multiplied in millet seeds were used as soil inoculum. Seedling damping off and stem rot in mature

cowpea were observed in the plants grown in the inoculated soil. Pathogenicity of *S. rolfsii* was tested on different hosts like sunflower, sugar beet, mungbean, tomato, sweet pumpkin, cabbage, lentil and cauliflower by Yaqub and Shahzad (2005). In the experiment 2-3 cm long water soaked wheat straw were taken in a conical flask and undergone sterilization. All flasks were inoculated with *S. rolfsii* and developed sclerotia were used for soil inoculation.

Flores-Moctezuma (2006) studied diversity of 20 isolates of *S. rolfsii* obtained from Mexico by inoculating into 51 species of plants with recording disease severity level and could observe variation of susceptibility and resistance among the species tested. Five plant species exhibited high susceptibility for all isolates whereas rest of them showed isolate specific response for resistance and susceptibility.

Pathogenicity of *S. rolfsii* on saffron was tested by planting corms in pot containing 1 kg sterilized soil mixed with 50 ml mycelial suspension of the fungus, and soil without inoculum served as control (Kalha *et al.*, 2007). ILC-263, C-727, Dasht, CM-2000 and Pb-1 were the chickpea cultivars used by Azhar *et al.* (2009) for finding variability in pathogenicity shown by all isolates of *S. rolfsii* from Pakistan and proved that all isolates were capable of producing disease intensity at same level and caused pre-emergence mortality except AT-3. Increase in phenolic production was reported by Saraswathi and Reddy (2012) in groundnut plants infected with *S. rolfsii* compared to healthy plants which indicated the defence reactions occurred during pathogenesis. *S. rolfsii* mass multiplied in sand maize media (50 g) were used for inoculation of plot of 1 m^2 area, 10 days before transplanting of tomato seedling and symptoms of blight, wilting and damping off were recorded (Singh *et al.*, 2013).

Pusa falguni, a variety of cowpea which was highly susceptible to stem rot disease was selected for the evaluation of pathogenicity and inoculum was introduced into the plant grown in pots in net house by soil, seed, soil-seed and mycelium bit inoculation (Kachhadia, 2013). Among these soil and mycelial bit inoculation showed greatest potential in pathogenicity. Pathogenicity test of eight isolates of *S. rolfsii*

obtained from eight districts of Tamil Nadu was conducted by Muthukumar and Venkatesh (2013) and pathogen isolated from Coimbatore showed most virulence and caused 93.66 % collar rot incidence in peppermint where 5 % inoculum load of *S. rolfsii* in 1 kg of soil caused 92.66 % collar rot incidence.

Chaurasia *et al.* (2014) tested pathogenicity of the *S. rolfsii* on brinjal plants grown in earthen pots containing un-inoculated sterile soil and inoculated soil through different media such as wheat grains, sand oat meal, potato dextrose broth and PDA. Among this oat meal and infested wheat grain resulted 100 % disease incidence whereas mycelia in PDB could not infect even a single plant.

Pathogenicity test of *S. rolfsii* isolated from brinjal, tomato, elephant foot yam, groundnut, potato and mesta was conducted by cross inoculation technique for which 100 numbers of sclerotia were inoculated into soil before crop was planted and all isolates showed pathogenicity without any host specificity and observed a range 0.12-6.14 % disease incidence with highest for elephant foot yam (Mahato and Mondal 2014). Pathogenicity of *S. rolfsii* isolated from infected chilli on GVC 111 variety was tested by different methods and found that soil inoculation and mycelial bit inoculation could provide 100 percent infection followed by seed inoculation (80 %) and seed-cum-soil inoculation (60 %) (Mehta, 2014).

Eslami *et al.* (2015) studied resistance of peanut plants towards *S. rolfsii* during virulence evaluation. Pathogen mass multiplied in sterilized barley seeds was used for inoculation of pots containing 500 g sterile soil and disease severity was calculated by observing wilting of the plant, yellowing or death, sclerotial production on stem/soil and lesion length.

According to Khan and Javaid (2015), 500 g bajra seeds were boiled after one hour soaking in water followed by sterilization in 500 ml conical flasks. 15 g grains inoculated with *S. rolfsii* were used to mix the soil for inoculum development and

chickpea seeds were sown in the respective pots for the study of mortality of plants.

One of the species of *Sclerotium* was mass multiplied in sorghum grains for the evaluation of its pathogenicity in cotton plant (Mukherjee *et al.*, 2015). Sorghum grains after sterilization and infection with pathogen were used for inoculation in the seedling. Seedling rot and sclerotial production were noted after three days of inoculation.

Infection of *S. rolfsii* in *Physalis minima* was confirmed by Nandi *et al.* (2017) by pathogenicity testing where parafilm-wrapped PDA plug containing both sclerotia and mycelium were placed at the collar region of the same plant. Yellowing of basal leaves followed by drooping and wilting were the symptoms noticed in inoculated plants while plants kept as control did not show any symptom.

Pathogenicity of *S. rolfsii* on potato was studied by soil infestation method in pots where 1.0 kg sterilized soil was taken and added *S. rolfsii* mass multiplied wheat grains at 20 g/kg soil followed by transplantation of potato seed tubers (Rubayet *et al.*, 2017). Southern blight of *Vigna radiata* caused by *S. rolfsii* was reported for the first time in China by Sun *et al.* (2020). Pathogenicity trials conducted in green gram, soybean, common bean, cowpea, pea, adzuki bean and peanut recorded wilting after 4 days of inoculation followed by death of plants except in maize which showed reduction in growth only.

2.4. IDENTIFICATION OF THE PATHOGEN

2.4.1. Cultural Characterization

S. rolfsii is a soil born fungus reported by Rolfs (1892) for the first time as a causal organism of tomato blight in Florida. The fungus was named as *S. rolfsii* by Saccardo (1911). Abeygunawardena and Wood (1957) studied two isolates of *S. rolfsii* on different media such as potato extract, carrot extract, maize meal, malt extract and weindling's medium and observed luxurious growth of mycelium in carrot extract and weindling's medium.

According to Sulladmath *et al.* (1977) among the crops tested, isolate of *S. rolfsii* obtained from pigeon pea produced higher number of sclerotial bodies followed by sunflower, wheat and groundnut. Three species of *Sclerotium* were studied by Punja and Damiani (1996) and results showed that best media for growth was V8 agar media for *S. coffeicola* and *S. rolfsii* whereas PDA for *S. delphinii*. All species could produce clamp connections and average nuclei for each cell was two.

Sarma *et al.* (2002) collected isolates of *S. rolfsii* from various hosts at different geographical regions which conserved cultural and morphological diversity including morphology of colony, mycelial growth rate, sclerotial formation and basidiocarp formation, observing that isolates with fluffy mycelium were more in number compared to isolates having compact mycelium.

According to Kokub *et al.* (2007), *Sclerotium* strains isolated from infected parts of chickpea in Pakistan exhibited variation in growth rate and sclerotial production in PDA where growth rate was ranged from 0.86-1.35 mm/ h. Okereke and Wokocha (2007) isolated *S. rolfsii* from cowpea which produced silky white mycelium in PDA and later turned to dull in appearance. Sclerotia were produced at the periphery of the plates after 11 days of inoculation with 1.2 mm diameter.

S. rolfsii isolated from chickpea at 12 locations of Punjab showed variability in mycelial growth rate, size and colour of sclerotial bodies. The study of mycelial incompatibility helped in identification of vegetative compatibility groups among the isolates (Akram *et al.*, 2008).

Bagwan (2011) performed a study on 59 isolates of *S. rolfsii* obtained from groundnut causing stem rot disease and out of which 35 isolates were seemed to be fluffy and 24 were compact. More than 500 sclerotia of small size were produced by some isolates with variability in colour such as dark brown, reddish brown and light brown. Prasad *et al.* (2012) explained that 22 isolates of *S. rolfsii* obtained from sunflower growing areas showed variability in morphological features. Sclerotial bodies were internally divided into three regions such as rind, cortex and medulla and

produced at the edges of petri plates after 11-25 days of inoculation. Sclerotial production showed variability among different isolates such as some isolates could produce more than 200 per plate.

21 isolates of *Sclerotium* collected from different hosts in Ranchi showed variability in their radial growth, colour of mycelium, morphology and number of sclerotial bodies such as extra white to off white colony, light brown to dark brown sclerotium of round, spherical and irregular shape with number varied from 38-485 (Lal, 2013). According to Muthukumar and Venkatesh (2013), maximum growth of *S. rolfsii* was occurred at 30^0C under *in vitro* conditions whereas growth was declined below 20^0C and above 35^0C.

El-Nagar *et al*. (2013) examined initiation of sclerotial production in PDA as white aggregates which changed into black colour after 14 days and the diameter measured was 0.3 to 3.0 mm. All isolates showed differences in the growth rate as well as diameter of sclerotia. Banakar *et al*. (2017) observed that growth rate of *S. rolfsii* was different in different media in which maximum colony diameter occurred in PDA followed by oat meal agar medium and slowest was observed in Sabouraud's Dextrose Agar (SDA) followed by Nutrient agar and color of mycelium showed variation such as dull white, pure white and cottony white.

Nandi *et al*. (2017) conducted morphological studies of *S. rolfsii* and concluded that the fungus produced white coloured branches from hypha of 4.37 µm diameter and clamp connections. Sclerotia were smooth, spherical or ellipsoidal, dark brown in colour with 159.48 µm (134.86 - 200.46 µm) in diameter and were produced after 3 days of inoculation in PDA (groundnut isolate). Pandi *et al*. (2017) made observation on colony characters like pigmentation, diameter, radial growth and concentric rings along with colony morphology, growth rate of mycelium, sclerotial number, size and colour. Three isolates of *S. rolfsii* had fluffy, dull white colonies but rest of the isolates exhibited dull white and compact colonies.

Variation shown by *S. rolfsii* isolates from different crops in Karnataka were studied by Manu *et al.* (2018). As per the study the colony diameter was observed in a range of 4.1 to 8.0 cm with 261.7 to 1048.7 sclerotia per plate. According to Ayed *et al.* (2018), initiation of sclerotia were observed within three days of incubation and complete maturation after 15-16 days at 30^0C and 35^0C. Identification of pathogen causing fruit rot in *Cucurbita argyrosperma* was confirmed as *S. rolfsii* by cottony aerial mycelium, fast growth of mycelium, septate, hyaline, branched hyphae with clamp connections (in some) and light brown sclerotia (Najera *et al.*, 2018). Position and shape of sclerotial bodies were different with respect to the media used. On basal agar medium sclerotial bodies were seen as irregular, uniform and at near edges of the petri plates and same pattern was noticed in carrot agar medium also (Shete *et al.*, 2018).

White, extra white, light white colonies and cottony, fluffy, thin pattern of mycelia were observed in 20 isolates of *S. rolfsii* isolated from chickpea along with difference in the arrangement of sclerotium in PDA and Czapex Dok Agar medium (Srividya *et al.*, 2018). Out of the seven media tested for the growth of *S. rolfsii*, PDA was found best followed by Asthana and Hawker's agar medium (58.16 mm). In PDA 453.33 mg dry weight of *S. rolfsii* was noted which was the maximum followed by Richard's broth medium (Kushwaha *et al.*, 2019).

2.4.2. Morphological Characterization

Higgins (1922) explained that feeding branches of *S. rolfsii* which entered into the medium, host tissue and those involved in sclerotium formation were slender in which branches were formed at any point, whereas clamp connection was the characteristic feature of broad hyphae and branches were producing at its back. Initially sclerotium was tufts of intertwined branches with region of active growth at periphery which was stained well and later turned to dark brown, shiny and smooth sclerotium.

Fan like fungal mycelia produced on onion agar medium and its hosts showed straight, broad and elongated cells having clamp connections at each septa which

produced branches occasionally and basidial stage of *S. rolfsii*, was identified as *Corticium centrifugum* (Goto, 1930). *S. rolfsii* isolated from plantain produced basidiospores on four sterigmata eruct on clavate basidia in onion-asparagin agar within 40 days with subsequent development of dense white hymenium at 17.9^0C to 29.1^0C in test tube and petridishes (Venkatakrishnaiya, 1946). West (1947) changed the name of genus from *Corticium* to *Pellicularia*, since this fungus showed morphological characteristics such as aerolate hymenia, short celled, stout hyphae and mycelia with right angle branching. Currently followed name of perfect stage of *S. rolfsii* is *Athelia rolfsii* (Punja and Grogan, 1983).

Perfect stage of *S. rolfsii* was produced in Brown's agar medium after 33-39 days of incubation at room temperature (Misra and Haque, 1960) and this medium was poor in nutrients and basidiospores produced in this medium when cultured in PDA could develop into mycelial mat and sclerotia. Chet *et al*. (1969) conducted study of sclerotium by electron microscopy and explained the presence of differentiated cells of thick walled rind, thin walled cortex and medulla cells filled with reserve materials. Plenty of melanine in the rind, structure and organization of cell wall provided more resistant to biological degradation.

Melanized rind surrounding hyphal aggregates were the typical characters of true sclerotium and variability of the hyphal cells in medulla region was observed in different species (Willets, 1972). Kwon and Park (2004) isolated *S. rolfsii* from Tawny Daylily in water agar and observed that aerial mycelium produced in PDA had narrow mycelial strands having 4.2- 10.4 µm width and microscopic observation revealed the presence of clamp connections in it.

Sporulation of *Sclerotium* sp. often requires specific environmental conditions in the laboratory and production in natural conditions is very rare. Since production of basidiospores were reported in some species, they are included under *Basidiomycetes* (Agrios, 2005). Pandey *et al.* (2005) pointed out sexual stage of *S. rolfsii* having clavate shaped basidia with four hyaline subglobose to ellipsoid basidiospores

erect on sterigmata in *Cyperus rotundus* rhizome meal agar medium.

According to Kirk *et al.* (2008), this fungus is considered as anamorphic fungus and placed under "Mycelia Sterilia" group since asexual spores are not produced. Sclerotia on okra infected with southern blight disease when cultured in PDA could produce hyphae at the edge of colonies which had large straight cells with clamp connections at every septum and absent in secondary and tertiary hyphae. Sclerotia were produced on the culture plate after 5 to 7 days of inoculation (Kone *et al.*, 2010).

2.4.3. Molecular Characterization

Punja and Li-Juan (2001) conducted a study on phylogenetic relationships between 132 isolates of *S. rolfsii* obtained from 36 separate host species grown in 13 countries during 1967-1997 by RAPD analysis and mycelial compatibility groupings. rDNA ITS sequences based phylogenetic relationship for *S. rolfsii* and *S. delphinii* were studied by Okabe and Matsumoto (2003) where cloning and sequencing of ITS regions were used for the identification of r-1, r-2, and r-3 ITS types.

Adandonon *et al.* (2005) did analysis of ITS rDNA sequences and mycelial compatibility to evaluate the variability among the *S. rolfsii* isolated at different locations of Benin. Durgaprasad *et al.* (2008) followed RAPD, ITS-PCR and ITS-RFLP methods for the characterization of *S. rolfsii* isolates infecting groundnut. ITS region of rDNA when amplified could produce around 650–700 bp specific to *S. rolfsii*.

Rasu *et al.* (2013) differentiated isolates of *S. rolfsii* obtained from different host plants in Tamil Nadu by morphological and molecular characterization technique in which RAPD-PCR analysis revealed that all the isolates had 54 % similarity coefficient. Strains of *S. rolfsii* caused stem rot of groundnut were obtained from different locations of Tamil Nadu by Jebaraj *et al.* (2017) and genetic diversity was studied by nuclear markers such as RAPD and ISSR. All the 22 isolates

were confirmed as *Sclerotium* and proved that ISSR markers were good at genetic diversity study than RAPD.

2.5. SYMPTOMATOLOGY

Paintin (1928) explained process of infection of *S. rolfii* and mycelium was present in the stele of the crown region of cowpea and hyphae started to move in to the stem and roots which led to the complete disintegration of the central cylinder. According to Karat *et al.* (1985), wilting, yellowing and drying of foliage and death were the sequences of events associated with collar rot in cowpea caused by *S. rolfsii*. As disease progressed, sclerotial bodies and white mycelia were clearly visible on the stem near soil surface.

Jenkins and Averre (1986) detected tomato fruits infected with *S. rolfsii* had white fungal mycelium and mustard like sclerotial bodies on it and infection on stem produced decayed cortex with white mycelial growth whereas roots of potato and sweet potato after infection produced shallow corky lesions of circular spot. *S. rolfsii* also causes damping off in seedlings, stem canker, crown blight, crown rot, root rot, bulb rot, tuber rot and fruit rot diseases in plants with brown lesions on the stem (for stem rot) followed by wilting of the plant and production of white mycelium and sclerotial bodies (Gobayashi *et al.*, 1992).

Anahosur (2001) narrated symptoms associated with *S. rolfsii* infected potato tubers which are sunken, yellow to tan spots on the tuber surface along with white fan like spreading mycelia and sclerotial bodies and later infected portion converted into watery and rotten portion. Water soaked lesions of small size at the collar regions are the first symptom of stem rot disease caused by *S. rolfsii* in pepper, peanut and tomato plants (Mullen, 2001). As the lesion size increases, girdling occur which make the plant to wilt and produce mustard like sclerotial bodies along with white mycelium.

Tomato plants infected by *S. rolfsii* showed rotting in stem and crown regions followed by complete blighting on the plants along with white mycelia and sclerotial bodies (Kwon and Park, 2002). Kwon *et al.* (2009) reported that water soaked lesion,

white mycelial mat, globoid sclerotia and rotting of fruit were the typical symptoms and sign on the melon infected with *S. rolfsii*.

Incidence of *S. rolfsii* in pepper was reported for the first time in Spain by Remesal *et al.* (2010). Water soaked lesions were observed in the plant parts especially crown and stem region which was in contact with the soil followed by yellowing, wilting and collapsing of the entire plant. White coloured mycelial mat with spherical brown sclerotial bodies were formed at the infected parts.

Sudina *et al.* (2014) reported foliar blight of yard long bean caused by *S. rolfsii* for the first time in Kerala. Lesions with water soaked appearance were present on the leaves which later turned to concentric rings of dark brown colour with pale brown halo around it on both leaf surfaces. Round small sclerotial bodies were also found under side of the leaves.

Southern blight and leaf spot disease on *Phaseolus vulgaris* caused by *S. rolfsii* was reported in India for the first time by Mahadevakumar *et al.* (2015) and in leaf spot disease water soaked lesions having concentric rings of necrotic spots were observed on the leaves of 30 to 45 days old plants. Fan like characteristic growth of fungal mycelium on the stem near soil line, vascular discolouration inside the stem and mustard like sclerotial bodies were also typical symptoms of the disease.

Paul *et al.* (2017) studied symptomatology of blight disease in sweet potato caused by *S. rolfsii* and observed sudden wilting of plants, initiation of infections at the collar region of the sweet potato with necrotic lesions and cottony white mycelia which spread over newly grown roots. Sclerotia were also found on the roots in association with mycelium. Bhuiyan *et al.* (2019) explained symptoms of sugarcane plants attacked by *S. rolfsii* as failure of germination, water soaked light brown lesions, sclerotial and mycelial production on stem base and death of the plants.

2.6. *IN VITRO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES AGAINST PATHOGEN

2.6.1. *In Vitro* Evaluation of Biocontrol Agents

Sclerotia of *S. rolfsii* were put in cell suspension of *Pseudomonas fluorescens* for 1 h and 1 week and observed germination losses of 10-20 % and 50-60 % respectively. (Ganesan and Gnanamanickam, 1987). Parasitisation of sclerotium by *Trichoderma harzianum* was understood by SEM, TEM and light microscopy. Multiplication of antagonist on sclerotial surface and penetration into the rind was clearly explained by light microscopy (Benhamou and Chet, 1996).

Desai and Schlosser (1999) clearly stated that *Trichoderma* isolates showed variability in the ability to infect and kill the sclerotia. Among the 44 isolates of *Trichoderma,* 14 could infect all the inoculated sclerotia, 13 could make infection on some and 17 did not alter the sclerotial viability. Role of endo-chitinase enzyme produced by *T. harzianum* in mycoparasitism of *S. rolfsii* was studied under *in vitro* conditions by Carsolio *et al.* (1999) in which transgenic *Trichoderma* strain having gene of Ech42 could produce more chitinase enzyme and showed antifungal activity.

Antifungal activities of *T. harzianum* was proved by El-Katatny *et al.* (2001) and showed that enzymes produced by *T. harzianum* such as Chitinase and β-1,3-glucanase could break the polysaccharides of *S. rolfsii*. *Bacillus subtilis* was proved to have inhibitory effect on *S. rolfsii* about 40.0 % to 57.8 % (Muhammad and Amusa, 2003). According to Bhatia *et al.* (2005), PSI and PSII were the isolates of *P. fluorescens* tested against *S. rolfsii* on TSM plate and 73 % and 70 % inhibition were observed *in vitro*.

Nalisha *et al*. (2006) reported that *Bacillus subtilis* exhibited higher antifungal activity against *S. rolfsii* because of the production of bioactive compounds by the antagonist. Vinod Babu (2006) conducted study on management of stem rot in groundnut and found that one of the isolates of *T. harzianum* showed mycoparasitism on *S. rolfsii* by coiling the host hyphae. The antagonist could penetrate the hyphae of pathogen through haustoria like structures.

Phenazine antibiotics produced by *Pseudomonas* sp. showed antifungal effect on *S. rolfsii* (Rane *et al*., 2007). According to Mundhe *et al.* (2009) *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtilis* showed maximum inhibition of *S. rolfsii* which was more than 70 % over control. Amin and Razdan (2010) studied antifungal activity of *T. harzianum* and *T. viride* against *S. rolfsii* and revealed that sclerotial production was highly inhibited by *T. viride* (80.18 %) with 67.91 % mycelial inhibition on dual culture plate. *In vitro* evaluation of antagonism by *Trichoderma* sp. on *S. rolfsii* showed 80.29 to 81.36 percent inhibition which revealed that *Trichoderma* sp. was better than *Aspergillus* sp. and *Penicillium* sp. as biocontrol agent (Bosah *et al.,* 2010).

According to Madhavi and Bhattiprolu (2011) 57.5 % mycelial inhibition was shown by *T. harzianum* against *S. rolfsii* isolated from infected chilly which was followed by *T. viride* I (55.8 %) and *T. viride* II (53.63 %). *P. fluorescens* expressed less potential as biocontrol agent under *in vitro* assay with 40.7 percent inhibition. Isolation of 11 *Pseudomonas* sp. from rhizosphere soil was carried out to evaluate the performance of antagonism against *S. rolfsii* (Rakh *et al*., 2011) and found that *Pseudomonas* cf. *monteilii* was highly efficient, since it could inhibit 94 per cent dry weight of *S. rolfsii* in dual culture plate.

Khan (2012) observed a band at the point of contact between *S. rolfsii* and *Trichoderma* in dual culture plate and recorded 54 to 73 % mycelial inhibition of *S. rolfsii* by all isolates of *Trichoderma*. Khirood and Jite (2012) tested efficacy of *T. viride* as biocontrol agent against *S. rolfsii* under *in vitro* conditions by dual culture assay and 75 % inhibition in radial growth of pathogen was observed. Faster growth rate of *T. viride* compared to *S. rolfsii* in dual culture plate implied the competitive ability of the antagonist for space and nutrients. According to Mohamed *et al.* (2012), linear growth of *S. rolfsii* in culture medium was inhibited by culture filtrate of *T. harzianum* by 75.28 % followed by *T. viride* (69.62 %) and *Bacillus subtilis* (54.36 %). *B. subtilis*, *T. viride* and *T. harzianum* were tested to have more antifungal ability on *S. rolfsii* isolated from cowpea whereas *P. fluorescens*, *Trichoderma fasciculatum* and *Trichoderma virens* were less effective in inhibiting the pathogen (Kachhadia, 2013).

Gomashe *et al*. (2014) explained the antifungal activity of *Bacillus subtilis* on *S. rolfsii* by pointing out the ability of *B. subtilis* to produce chitinase which led to the formation of zone of hydrolysis in the dual culture plate. Since major part of cell wall of *S. rolfsii* was chitin, chitinase produced by the antagonist could effectively inhibit the pathogen. High antagonism of *T. harzianum* under *in vitro* assay was mentioned with 70.82 % inhibition on *S. rolfsii* at which *Penicillium notatum* and *Aspergillus niger* failed to exhibit potential of antagonism (Javeria *et al*., 2014). *T. harzianum* and *T. virens* checked the mycelial growth of *S. rolfsii* in dual culture method. Five species of *Bacillus* and *Pseudomonas* exhibited similar inhibition on mycelia of the same fungi (Ramzan *et al*., 2014).

Fifteen isolates of *Trichoderma viride* were examined for their antagonistic potential against *S. rolfsii*, causal organism of jasmine wilt disease and *T. viride* 2 (isolate) showed 75.38 percentage of inhibition followed by *T. viride* 1 with 72.72 % (Jeyaprabha and Raja, 2015). Antagonistic effect of nine microorganisms were tested against *S. rolfsii* isolated from diseased groundnut plant by dual culture method and the results indicated that *Trichoderma asperullum* showed maximum inhibition with 75.00 % and *T. viride* (NBAII) and *T. harzianum* (Th6) were least effective with 51.67 % inhibition (Nagamma *et al*., 2015). Shifa *et al*. (2015) determined antifungal activity of *B. subtilis*-1 through the production of antibiotics by agar well diffusion assay. The results revealed that fraction with Rf 0.48 suppressed the growth of *S. rolfsii* with 10 mm inhibition zone. Gas chromatography-mass spectrometry analysis could find about 22 different antibiotics from the bioactive compounds of *B. subtilis*.

According to Javaid and Ali (2016), inhibition showed by *Trichoderma viride* on *S. rolfsii* in dual culture plate was about 68 % which confirmed more antagonistic potential than *T. harzianum* (57 %). Out of the 11 isolates of *Trichoderma* tested against *S. rolfsii*, Tvs12 strain could show more mycoparasitism and found that chitinase and β-1,3-glucanase produced by the antagonist were responsible for antifungal activity (Hirpara *et al*., 2017). Most of the endophytes isolated from tomato growing region inhibit the mycelial development of *S. rolfsii* by less than 50 % (Sahu *et al*., 2019).

2.6.2. *In Vitro* Evaluation of Chemical Fungicides

Management of collar rot of cotton caused by *S. rolfsii* was evaluated by systemic and non-systemic fungicides and among these propiconazole, hexaconazole, tridemorph, thiram and mancozeb showed better inhibition of fungi under *in vitro* conditions (Prabhu and Hiremath, 2003). Sancozeb at 100 ppm caused reduction in growth of *S. rolfsii* about 60 % and almost same results were observed with dithane m-45 at 100 ppm (Yaqub and Shahzad, 2006). Benomyl and carbendazim were also found better against *S. rolfsii* with inhibition percentage of >50 % Haralpatil and Raut (2008) conducted *in vitro* testing of difenoconazole (0.05 %), metalaxyl + mancozeb (0.1 %), propiconazole (0.05 %), metiram (0.1 %) and hexaconazole (0.05 %) against *S. rolfsii* causing collar rot in betelvine plantations and fungicidal effect was proved to be successful.

Results of poisoned food technique conducted with ridomil, rovral, bavistin, provax 200, dithane M45 and tilt at three different concentrations revealed 100% inhibition of S. *rolfsii* by provax at all the tested concentrations. Tilt was on par with rovral in mycelial inhibition (Bhuiyan *et al.*, 2012). Effectiveness of chlorothalonil was found the least among the fungicides tested *in vitro* and had only 11.18 % inhibition at 1000 ppm on *S. rolfsii*. Among the non-systemic fungicides evaluated, mancozeb recorded maximum inhibition of 83 % mycelial growth (Manu *et al.*, 2012).

Bhagat and Chakraborty (2013) tested systemic fungicides (thiodan, calixin, captan, carbendazim and indofil M-45) *in vitro* and found superiority over control on the inhibition of growth of *S. rolfsii*. Thiodan and calixin showed 100 per cent inhibition on growth of fungus even at 0.0125 %. Carbendazim (Bavistin), captan and indofil M-45 did not perform well even at 0.1 % concentration. Calixin inhibited sclerotial germination when compared to captan, carbendazim and thiodan.

Das *et al.* (2014) presented data on evaluation of fungicides on growth of mycelium of *S. rolfsii* and they could confirm highest fungicidal potential of hexaconazole and tebuconazole at four different concentration ranged from 1 to 100 ppm. Mahato and Mondal (2014) conducted poison food technique and observed 95

% restriction in fungal growth in vitavax followed by kavach with inhibition percentage of 93.55. Negligible effect was shown by blitox against *S. rolfsii*.

The systemic fungicides carboxin, propiconazole and hexaconazole showed 100 % inhibition on the growth of *S. rolfsii*. Carbendazim was found the least effective in controlling the pathogen with 11.85 % inhibition. Mancozeb showed 100 % management at all concentrations tested whereas copper oxychloride could not show effectiveness even at high concentration (Rakholiya, 2015).

Propiconazole and tricyclazole exhibited 100% inhibition of *S. rolfsii* at all concentrations tested whereas thiophanate methyl was less effective at 500 ppm with having <50 % inhibition. Carbendazim did not perform well at all concentrations. Mancozeb and thiram were found best among the non-systemic fungicides tested and combination product of carbendazim and mancozeb had 47.40 % and 87.03 % inhibition at 500 and 1000 ppm respectively (Rangarani *et al.,* 2017).

According to Venkatarao and Manjula (2017) mancozeb and hexaconazole with their combination with insecticides were proved to have greater potential in the inhibition of pathogen causing stem rot of groundnut. Liquid bioassay method in potato dextrose broth was done for the evaluation of three fungicides (thiram, carbendazim, and metalaxyl) and carbendazim showed complete inhibition of the pathogen. Metalaxyl incorporated PDB had 202.57 mg *S. rolfsii* which specified least inhibition among the fungicides tested (Wavare *et al.,* 2017).

Both systemic and non-systemic fungicides were tested *in vitro* by poisoned-food technique in PDA against *S. rolfsii* and observed that hexaconazole and difenaconazole at 125 ppm had 100 % inhibition on fungal growth followed by mancozeb which was superior to chlorothalonil (Sharma and Dhruj, 2018).

2.7. *IN VITRO* STUDIES ON COMPATIBILITY OF EFFECTIVE FUNGICIDES AND ANTAGONISTS

According to Malathi *et al.* (2002), *in vitro* evaluation of carbendazim and thiophanate methyl showed compatibility with *Pseudomonas fluorescens* upto 500

ppm and *Trichoderma* did not grow in the medium amended with 1 ppm of carbendazim and 10 ppm of thiophanate methyl.

Manjula *et al*. (2004) found that, 13 isolates of *Trichoderma* sp. and 57 bacterial isolates showed antifungal activity and 4 isolates of *Pseudomonas* and *T. viride* pq 1 were found to show high antagonistic potential against *S. rolfsii*. Moreover, two isolates of *P. fluorescens* proved compatibility with thiram and *T. viride* pq 1, integrated approach could be suggested for the management of sclerotium stem rot in groundnut.

Archana *et al.* (2012) reported that *B. subtilis* and *P. fluorescens* could grow in the medium poisoned with higher concentration of 300 ppm of azoxystrobin 23 SC. Chennakesavulu *et al*. (2013) evaluated compatibility of six fungicides including carbendazim, hexaconazole, propiconazole, tebuconazole, mancozeb and cheshunt compound with *P. fluorescens* isolate CPF4 and indicated that carbendazim was more compatible followed by mancozeb.

Poisoned food technique and turbidity method were used to test the compatibility of *P. fluorescens* and *B. subtilis* with azoxystrobin 25 SC and they were found compatible at 5, 10, 50, 100 and 250 ppm concentration (Devi and Prakasam, 2013). *P. fluorescens* showed compatibility with chlorothalonil and carbendazim at 0.05-0.1 % under *in vitro* conditions but found sensitive at higher concentrations (Telangre *et al*., 2013).

According to Valarmathi *et al*. (2013), *P. fluorescens* and *B. subtilis* showed compatibility with copper hydroxide (Kocide 3000) at 300 ppm and *T. viride* was found incompatible with copper hydroxide above 2500 ppm. Lab experiments of mancozeb at 3000 ppm showed compatible reaction with *T. viride* having 7 % inhibition (Vasundara *et al*., 2015). It was reported that *P. fluorescens* was compatible with propiconazole, tebuconazole, azoxystrobin, carbendazim at every concentration tested from 0.05 %- 0.3 % (Louis *et al*., 2016).

Basamma and Kulkarni (2017) tested compatibility of *Bacillus subtilis* against different fungicides viz., carbendazim, wettable sulphur, mancozeb, difenoconazole, hexaconazole, tebuconazole and kresoxim methyl at their seven concentrations from

50 to 5000 mg/lit and µg/lit with respect to solid and liquid formulations. It was found that, *B. subtilis* showed compatibility with carbendazim among the solid formulation fungicides and hexaconazole and kresoxim methyl exhibited maximum compatibility at 3000 µl/l.

Results of compatibility test of fungicides and fertilizers with *T. viride* and *P. fluorescens* showed that *T. viride* was compatible with potassium phosphonate and fosetyl aluminium and incompatible with carbendazim and hexaconazole. For *P. Fluorescens*, 100 % compatibility was observed with hexaconazole and potassium phosphonate + hexaconazole mixture (Dhanya *et al*., 2017).

Compatibility test for contact, systemic and combination fungicides with *T. harzianum* were examined and contact fungicides were found more compatible than the systemic fungicides *viz.,* hexaconazole, propiconazole and carbendazim (Sonavane and Venkataravanappa, 2017). Growth of *T. viride* was inhibited by carbendazim (94.2 %) and carbendazim+ mancozeb (93.8 %) which revealed that these fungicides were not compatible with the given biocontrol agent (Kumar *et al*., 2018). Six fungicides were tested for compatibility with *B. subtilis* and among these carbendazim showed maximum compatibility at 0.3 % which was followed by carbendazim 12 %+ mancozeb 63 %WP whereas, tricyclazole 18 % + mancozeb 62 % WP was found incompatible with *B. subtilis* at 0.3 % (Rajkumar *et al.,* 2018).

Mancozeb 75 %WP showed compatibility with *T. viride* at concentrations less than 50 ppm. In addition, thiram also exhibited 30.74 % compatibility and inhibition percentage of chorothalonil was 75.29 % at 1000 ppm (Kumar, 2019). Among the systemic fungicides tested *in vitro*, *T. viride* and *T. harzianum* were highly compatible with 0.05 to 0.15 % of azoxystrobin followed by metalaxyl while carbendazim showed incompatibility (Shashikumar *et al.,* 2019).

2.8. *IN VIVO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES

Dutta and Das (2002) conducted field experiment for the management of collar rot in tomato caused by *S. rolfsii* and they observed that treatment with *T. harzianum* resulted only 39.7 % disease incidence and yield of 497.4 g pods per plant. *T. viride*

treated plants showed 40.3 % disease incidence and 482.7 g pods per plant. Wijetilaka (2003) isolated *Pseudomonas* sp. from Sri Lanka and observed significant reduction in collar rot disease after seeds were treated with this antagonist which was highly effective than soil drenching under field condition. Reduction in seedling mortality was estimated as 30-40 % in plants were seed treatment was employed.

Chandrasehar *et al.* (2005) pointed out overgrowth of *T. viride* and *T. harzianum* on *S. rolfsii* under *in vitro* conditions whereas in pot culture experiment 89.5 % survival was observed by seed treated with *T. viride* which was higher than soil application of the given antagonist. FDP-15, a strain of *P. fluorescens* isolated from roots of groundnut was found more efficient against collar rot disease and also caused significant increase in seed germination, nodulation and pod yield (Ganeshan and Manoj, 2005). Yaqub and Shahzad (2008) reported that pelleting of seeds with *T. harzianum* reduced damping off and root colonisation of *S. rolfsii* in cowpea and sunflower. Local isolates of *Trichoderma* sp. were screened to assess its ability to suppress *S. rolfsii* under pot culture and field trials on plants. *Trichoderma* mass multiplied in paddy and barley seeds when applied in field could reduce the incidence of disease by 75.54 % (Jegathambigai *et al.*, 2010).

Jadon (2011) evaluated eco-friendly management methods against collar rot disease where soil and seedling dip treatment with *Trichoderma viride* was responsible for only 1.58 % disease incidence which was recorded 205 days of transplanting whereas in plants undergone treatments with *Pseudomonas fluorescens* showed 6.34 % disease incidence. The isolates, BCPF8 and BCPF7 of *P. fluorescens* obtained from rhizosphere of chilly and rice were tested against *S. rolfsii* infection in cowpea and seeds treated with BCPF 8 plants showed highest germination per cent (86 %), higher shoot length, root length and fresh weight (Nandi *et al.*, 2013).

Various bioagents such as *T. viride*, *T. harzianum*, *P. fluorescens* and organic amendments like neem, ocimum, and datura were evaluated against collar rot of sunflower caused by *S. rolfsii* in farmer's field in Sundarbens. The results showed that, *T. viride* and *T. harzianum* were good at disease suppression followed by cow's urine (Maji and Nath, 2016). Rajendraprasad *et al.* (2017) reported that application of *T. harzianum* -1 and *Pseudomonas fluorescence* could increase germination and

reduce collar rot in tomato plants where the pots were inoculated with *S. rolfsii*. These were also found effective in increasing the weight of shoots and roots of tomato plants.

Experiments conducted under glass house with fungicides showed that soil drenching with hexaconazole was highly effective for the management of stem rot of bell pepper caused by *S. rolfsii* (Chowdary *et al.*, 1998). Grichar *et al.*, (2000) carried out field studies at 11 locations for studying the efficacy of azoxystrobin against *S. rolfsii* causing stem rot in peanut. Results indicated that azoxystrobin applied at 0.22 to 0.45 kg/ha was good at inhibiting fungal growth and increase peanut yield over untreated plants. Application of carboxin (0.05 %) and hexaconazole (0.1 %) resulted in 20 % wilt disease incidence caused by *S. rolfsii* in potato (Baswaraj, 2005). Groundnut seeds treated with mancozeb could overcome the groundnut root rot disease caused by *S. rolfsii* and resulted in higher seedling emergence, lower mortality of plants, high shelling percentage as well as higher productivity (Tarekegn *et al.*, 2007).

Field evaluation conducted with fungicides for the suppression of disease caused by *S. rolfsii* in groundnut indicated that propiconazole 25 EC showed 68.9 % efficacy in disease control and 36.0 % increase in pod yield compared to control plants (Gour and Pankaj, 2010). Khan and Javaid (2015) conducted *in vivo* experiment with fungicides for the management of collar rot disease in chickpea. Plants treated with thiophanate methyl and mancozeb showed 95 % and 50 % reduction in death rate respectively which was estimated 30 days after sowing.

Shahiduzzaman (2015) conducted studies on management of foot and root rot of lentil caused by *S. rolfsii* and found that mortality rate in lentil plants treated with bavistin 50WP was less (9.33 %) compared to untreated plants (12.9 %). Yield increase of 34.23 % over control was also observed in the case of treatment with bavistin. According to Suryawanshi *et al.* (2015), Seed treatment with combination of vitavax (1.5 g/kg), thiram (1.5 g/kg) and *P. fluorescens* resulted in 80 % of seed germination, 69.96 % reduction in pre-emergence as well as 55.43 % post-emergence mortality in brinjal seeds which suggested good management recommendation for brinjal collar rot disease. Significant suppression in the mycelial growth and

sclerotium production were noticed under *in vitro* conditions when the medium was amended with bavistin 50WP. Moreover, under field conditions bavistin and topgan treated brinjal plants showed lower disease severity as well as higher yield compared to other fungicide treated plants (Siddique *et al.*, 2016). Zero per cent mortality in chickpea plants were recorded when seeds were treated with propiconazole and azoxystrobin, whereas treatment with carbendazim only resulted in 69.84 % mortality and 14.97 % decrease in disease over control (Shirsole *et al.*, 2019).

Materials & Methods

3. MATERIALS AND METHODS

The study entitled 'Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)' was conducted in the Department of Plant Pathology, College of Agriculture, Padannakkad during the period of 2018-2020. The detailed descriptions of materials and methods performed for the experiments are given below.

3.1. COLLECTION OF SAMPLES

Cowpea plants with characteristic symptoms of stem rot and foliar blight were collected from instructional farm of College of Agriculture, Padannakkad and different cowpea growing tracts of Kasargod district during south west monsoon season of 2019. Collected samples were taken to the laboratory for isolation of the pathogen.

3.1.1. Assessment of Percent Disease Incidence and Percent Disease Index

Percent disease incidence and disease index of different fields were recorded during the sample collection by using score charts (Table 1, Plate 1) and other procedures. As per the given formula assessment of percent disease incidence was performed (Wheeler, 1969).

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total no. of plants observed}} \times 100$$

The Percent disease index (PDI) was calculated using the following formula (Mc Kinney, 1923)

$$\text{Percent disease index} = \frac{\text{Sum of grades of each leaf}}{\text{No. of leaves assessed} \times \text{Maximum disease grade used}} \times 100$$

Table 1. Score chart of foliar blight disease of cowpea

Sl. No	Scale	Per cent of leaf area affected
1	0	No disease
2	1	1-10% leaf area with lesions
3	2	11-25% leaf area with lesions
4	3	26-50% leaf area with lesions and yellowing
5	4	Over 50% or more of the leaf area with lesions and yellowing
6	5	Drying of leaf and defoliation

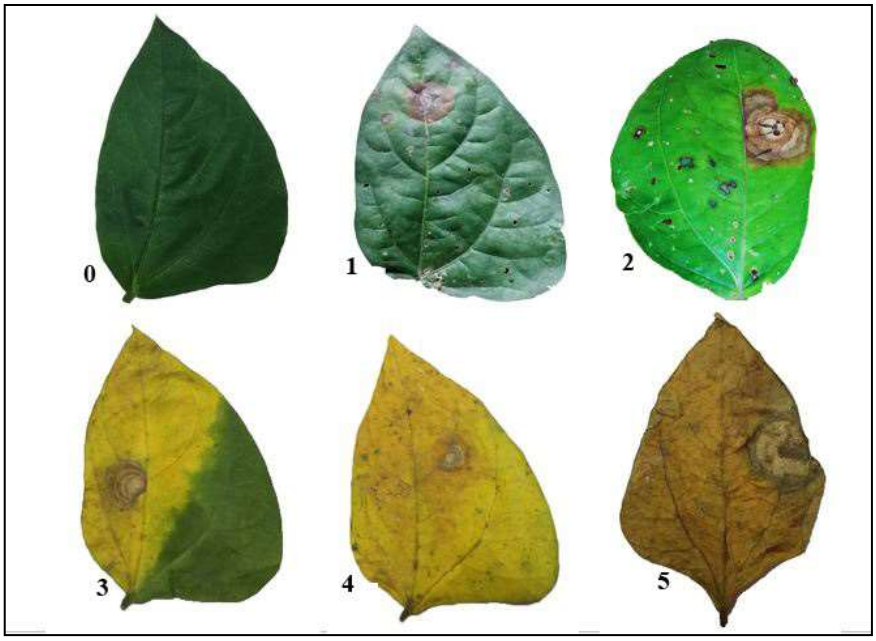


Plate 1. Disease scoring of foliar blight disease in cowpea

3.2. ISOLATION OF THE PATHOGEN

Infected cowpea plant samples collected from different locations were isolated separately. The plant parts showing the typical symptoms of stem rot and foliar blight were washed thoroughly and then cut into small pieces. The isolation procedure was performed under aseptic condition as per Pandi *et al.* (2017). Surface sterilization of the cut bits was done using 0.1 % $HgCl_2$ followed by three consecutive sterile distilled water washing. Washed bits were allowed to dry on sterilized filter paper and then placed into the sterile petridish containing 15 ml solidified sterile potato dextrose agar (PDA) medium. After proper sealing petridishes were kept for incubation at $28+2^0$C for 3 days. Further purification of fungus in PDA was done by hyphal tip method ensuring strict aseptic conditions (Rangaswamy, 1972). Pure cultured samples of different isolates were stored in refrigerator at 15^0C for further studies.

3.3. PATHOGENICITY TEST

Pathogenicity of the fungal isolates obtained from different locations were proved on cowpea (Variety: Kanakamani) by Koch's postulates. Cowpea plants were maintained in grow bags. The pathogen multiplied in paddy grains and fresh PDA culture plates were used as inoculum for the soil and foliar inoculation respectively. After the characteristic symptom development upon artificial inoculation observations such as number of days taken for the appearance of disease symptom, number of lesions and size of lesions were recorded. Followed by this reisolation of the pathogen from the artificially inoculated plant parts was performed and compared with previously isolated pathogen to prove Koch's postulates.

3.3.1. Multiplication of Fungal Inoculum

100 g disease free paddy grains were soaked in water for 2 hours and washed properly to remove the unwanted materials and dirt. Grains were boiled in water for 20 min and excess water was decanted. Boiled grains (3 g) were taken in test tubes and plugged with non-absorbent cotton and sterilized at 121^0C and 1.05 kg/cm^2 for

one hour. Sterilized grains in the test tubes were allowed to cool at room temperature. Each test tube with paddy grains were inoculated with 2-3 culture discs (5mm dia.) from actively growing tips of 3 day old cultures of isolate grown in PDA medium. Enough moisture in the paddy grains was ensured at the time of inoculation. Inoculated test tubes were then incubated at room temperature at $28+2^0$C for 15 days. Infected grains with profuse mycelial growth and sclerotial production, were taken for the soil inoculation in order to screen the pathogenicity of different isolates (Chaurasia *et al.*, 2014).

3.3.2. Inoculation of the Pathogen in the Soil

Sterilization of the potting mixture (equal quantity of sand, soil and dried powdered cow dung) required for the sowing was done by autoclaving at 121^0C and 1.05 kg/cm^2 for 20 min. Disease free cowpea seeds, surface sterilized with 0.1 % $HgCl_2$ were then sown in grow bags (3 seeds per bag) filled with sterilized potting mixture. Plants were thinned out at five leaf stage by keeping one vigorous plant only per grow bag. Separate plants were maintained in grow bags for each isolate. Five to ten paddy grains with sufficient fungal mycelium and sclerotia were incorporated in the soil near the collar region of the plant (Chaurasia *et al.*, 2014). Cowpea plant grown in uninoculated soil served as control. Humid microclimate for the growing plants was ensured by covering with a polythene bag. Apart from this moisture maintained around the plant by proper irrigation and keeping moist cotton inside the polythene bag. Three replications were maintained for each isolate.

3.3.3. Inoculation of the Pathogen on the Leaf

Artificial inoculation of each isolate was done on the healthy leaf of cowpea plant. One healthy and green matured leaf was selected and wiped with 70 % alcohol. Pin pricks were made on the leaf with sterilized needle. 5 mm culture disc of pathogen with one sclerotium was placed on the pin pricked area and covered with a thin layer of cotton moistened with sterilized water. One set of plant was kept as control by placing five mm solidified agar disc on pin pricked leaf and a thin layer of wetted

cotton above it. Inoculated plant parts were then covered by polythene cover to provide sufficient humidity.

3.3.4. Virulence Rating of Pathogen

Virulence rating of the different fungal isolates were carried out in terms of growth rate of the fungus in the petridish and disease development during pathogenicity test. 15 ml of molten PDA was poured in sterile petridishes and allowed for solidification. 5 mm disc of actively growing fungal culture was taken using a sterile cork borer and placed at the centre of petridishes in aseptic conditions. These petri dishes were kept for incubation at room temperature of $28+2^0$C and daily observations were recorded on the colony diameter of the pathogen on PDA till it covered the petridish completely.

Based on the above mentioned parameters used for virulence rating, most virulent fungal isolate was selected and further studies were performed with the most virulent pathogen.

3.3.5. Maintenance of Culture

The most virulent fungal isolate selected through virulence was subcultured in PDA medium and actively growing region of the mycelium was cut aseptically and placed in PDA slants. After obtaining sufficient fungal growth in the PDA slants, these were kept in refrigerator at 15^0C. Virulence of the pathogen was maintained by inoculating the fungus and reisolating the same from the host plant in three months interval.

3.4. IDENTIFICATION OF THE PATHOGEN

Identification of the pathogen was done based on cultural, morphological and molecular characterization. Cultural, morphological and molecular characterization were done for the most virulent fungal isolate along with cultural and morphological variability studies among the isolates.

3.4.1. Cultural Characterization

Cultural and morphological characteristics of fungal isolate was recorded by

observing its growth in PDA medium. 5 mm mycelial disc from three day old culture was inoculated aseptically at the centre of solidified PDA medium in petridish. Three replications were maintained for each isolate. After the incubation at room temperature $28+2^0$C, daily observations were recorded till 25 days of inoculation. The following characters were considered for identification (Pandi *et al.*, 2017).

- Rate of growth of mycelium
- Colour of mycelium
- Pattern of mycelia growth
- Days taken to produce sclerotia
- Sclerotial arrangement
- Size of sclerotia
- Colour of sclerotia
- Shape of sclerotia

3.4.2. Morphological Characterization

Microscopic characteristics of fungal hyphae such as septation, branching pattern, clamp connections and cellular arrangement of rind and medullary region of sclerotia were visualized using Carl Zeiss binocular microscope at 400X magnification. Sclerotia were cut into two equal halves. Thin layer of cross section was made ensuring that both rind and medullary portion were included in the section. This section was stained with lacto phenol cotton blue and observed under Carl Zeiss binocular microscope 400 X magnification.

3.4.2.1. Slide Culture Technique (Riddell, 1950)

Slide culture unit was prepared by placing filter paper, glass slide, cover slips, and two glass rods inside a petridish. Entire unit and water agar (2 %) were sterilized in autoclave at 121^0C & 1.05 kg/cm^2 for 20 min. These were taken into the laminar airflow chamber. Water agar was poured in the sterile petridish. Slide culture unit was rearranged by keeping two glass rods on the filter paper to hold the glass slide above it. 5 mm square agar block was cut and placed on the glass slide inside the slide culture unit. Actively growing mycelium was placed at the four corners of the agar block and covered with cover slip provided that all conditions were strictly aseptic.

Moistened the filter paper with sterile water to provide humid condition required for the fungus and prevent drying of agar. Slide culture unit was kept for incubation and observations were taken on mycelial growth of the fungus. When sufficient mycelial growth was seen, coverslip was removed from the glass slide and stained with lacto phenol cotton blue. Slide was observed through compound microscope at 400X magnification.

3.4.3. Molecular Characterization

Most virulent isolate causing stem rot and foliar blight disease in cowpea was selected for molecular characterization and the sample was done at UniBiosys Biotech Research Labs at South Kalamassery, Cochin. Identification of microbial culture was done by using D1/D2 region of LSU (Large Sub Unit: 28S rDNA) based molecular technique.

3.4.3.1. DNA Isolation

0.01 g of fungal mycelium was ground in 75 µl of STE extraction buffer (320 mM Sucrose, 10 mM Tris-Cl, 20 mM EDTA, 75 mM NaCl and 2.5 ml of 20 % SDS) along with 5 mg of Polyvinylpyrrolidone and 0.1 g of silica powder. After incubation at 65°C for 10 min, centrifugation of sample was done 13,000 rpm for 10 min. To the supernatant, equal volume of chloroform: isoamyl alcohol was added and process of centrifugation was repeated. 2/3 volume of isopropanol was added to the aqueous layer, and centrifuged at 13,000 rpm (10 min). It was followed by washing the pellet with 70 % ethanol by centrifuging and the pellet was dried, dissolved in 50 µl TE buffer.

3.4.3.2. Analysis of DNA Purity & Quality

Quantification of DNA stock samples was done using UV spectrophotometer at 260 and 280 nm noted that one absorbance unit at 260 nm wavelength equals 50 µg DNA per ml. The Ultra violet (UV) absorbance was checked at 260 and 280 nm for determining concentration and purity of DNA. Optical density ratio at 260:280 nm was used for the Purity analysis of DNA. The DNA having ratio within 1.8 to 2.0 was considered to be of good purity. The given formula was used for the estimation of concentration of DNA

Concentration of DNA (mg/ml) = OD 260 x 50 x Dilution factor

Quality of DNA was again checked by agarose gel electrophoresis. The 0.8 % agarose was prepared (0.8 g agarose power / 100 ml 1 X TBE), and was melted. After pouring 30 ml agarose into the casting tray, the gel was allowed to solidify and the comb and tape was removed. 1 X TBE (Tris-Borate- EDTA; electrophoresis buffer) was added to the chamber until the buffer just covers the top of the gel. The samples were loaded with Bromophenol blue loading dye, without puncture the well bottoms and the power pack was run at 100 V. The gel was viewed on a UV transilluminator after electrophoresis. The DNA was used further for PCR.

3.4.3.3. Polymerase Chain Reaction

D1/D2 region was amplified by PCR from fungal genomic DNA using PCR universal primers:

Details of primers used for PCR

DR - 5'-GGTCCGTGTTTCAAGACGG-3'

DF- 5'-ACCCGCTGAACTTAAGC-3'

PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube.

PCR tubes containing the mixture were tapped gently and spin briefly at 10,000 rpm. The PCR tubes with all the components were transferred to thermal cycler. The PCR protocol designed for 30 cycles for the primers and Components and volume per reaction are given below

Deionized water	-	18.7 µl
Taq buffer with MgCl2 (10 X)	-	2.5 µl
Forward Primer (10 pM/µl)	-	0.5 µl
Reverse Primer (10 pM/µl)	-	0.5 µl
dNTPs mix(10 mM each)	-	0.5 µl
Taq DNA Polymerase (5U/µl)	-	0.3 µl
Template DNA (25 ng/µl)	-	2.0 µl
Final Volume	-	25.0 µl

PCR conditions are

A. Initial denaturation - 95°C- 5 min
B. Denaturation - 94°C- 30 s
C. Annealing -48°C- 30 s
D. Extension -72°C- 45 s

Go to step B -D for 29 times

E. Final Elongation - 72°C- 10 min -End

3.4.3.4. Analysis of DNA Amplification by AGE

a. Standard DNA Markers.

Commercially available 100 bp ladder was used as standard molecular weight DNA (Thermo Scientific USA).

b. PCR-Product Electrophoresis

Loaded 3 µl of PCR product with 4 µl bromophenol blue (Loading Dye) in 1.5 % agarose gel. Gel was run at constant voltage of 100V and current of 45A for a period of 30 min till the bromophenol blue has travelled 6 cm from the wells. Gels were viewed on UV transilluminator and photograph of the gel was taken using a gel documentation system, Alphaimager, USA.

3.4.3.5. Purification of DNA Samples

PCR product obtained after amplification was purified using column purification according to manufacturer's guidelines (Thermo Scientific USA), and further used for sequencing reaction.

3.4.3.6. Sequencing of Purified D1/D2 of LSU 28SrDNA Gene Segment

The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI3730xl Genetic Analyzer (Applied Biosystems, USA).

a. Sequence Analysis of D1/D2 region

- Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequence at the 3 'and 5' ends (considering peak and Quality Values for each base) using the sequence analysis tools.
- The edited sequences were then used for similarity searches using

BLAST (Basic Local Alignment Search Tool) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) DNA database for identifying the fungal strains.

3.5. SYMPTOMATOLOGY OF DISEASE

Symptomatology of both stem rot and foliar blight disease under natural conditions were studied while collecting the infected samples from different locations. In artificial conditions symptomatology was studied by both soil inoculation and leaf inoculation methods. For stem rot disease the culture was mass multiplied in paddy grains (mentioned in 3.3.1.) and inoculated in the soil near the stem base of cowpea grown in polythene bags, ensuring that the pathogen would easily access the plant. Initial symptoms observed as water soaked lesions on the stem upon progression lead to the complete wilting and death of the entire plant. Symptomatology of foliar blight disease was studied by inoculating the leaf with the isolates. It was done by placing 5mm disc of pathogen grown in PDA medium with one sclerotium on the pin pricked area of leaves and covering it with a thin layer of moist cotton. The entire plant was covered with polythene cover to provide humidity which is essential for the development of symptom.

3.6. *IN VITRO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES AGAINST THE PATHOGEN

Present study was undertaken with an aim to evaluate the efficacy of biocontrol agents and chemical fungicides against the stem rot and foliar blight causing pathogen.

3.6.1. *In Vitro* Evaluation of Biocontrol Agents

KAU released fungal and bacterial biocontrol agents such as *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* (Table 2) were evaluated against the most virulent fungal isolate causing stem rot and foliar blight disease in cowpea by dual culture technique. The experimental design selected for statistical analysis was Completely Randomized Design (CRD) with five treatments and four replications.

Table 2. Biocontrol agents selected for *in vitro* evaluation against pathogen

Sl. No	Treatment	Biocontrol agents
1	T1	*Trichoderma viride*
2	T2	*Trichoderma harzianum*
3	T3	*Pseudomonas fluorescens*
4	T4	*Bacillus subtilis*
5	T5	Control

3.6.1.1. In Vitro Evaluation of Fungal Antagonist

T. viride and *T. harzianum* were tested for its antagonism by dual culture technique. 7 mm culture disc of pathogen and antagonist were cut out from the actively growing margin of their four day old culture in the petridishes (Manjula *et al.*, 2004). These were placed on the PDA medium at opposite edges of the petridish (2 cm away from the edge) having 9 cm diameter. Each treatment was replicated four times. Control plates were kept by inoculating pathogen at the centre of the petridish without antagonist fungi. Both dual culture and control plates were incubated at room temperature 28 ± 2^0C. Observations were taken at the interval of 24 h. Radial growth of the pathogen was recorded when complete growth was observed in control plates. Percent inhibition of the pathogen by antagonist fungi was calculated by using the following formula given by Vincent (1927).

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

Where

I: Percent inhibition

C: Mycelial growth in control (mm)

T: Mycelial growth in treatment (mm)

3.6.1.2. In Vitro Evaluation of Bacterial Antagonists

KAU released bacterial biocontrol agents such as *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against isolated fungal pathogen by dual culture method (Nandi *et al.*, 2013). It was performed by inoculating the pathogen at the centre of the sterile petri plate with nutrient agar (NA) medium. Bacterial cultures were streaked as parallel lines at both side of the pathogen keeping 2 cm distance from the periphery of the plate. Pathogen inoculated at the centre in NA media without bacterial antagonists were kept as control. Four replications were maintained for each treatment. All the inoculated plates were incubated at room temperature

28 ± 2^0C. Measurements from treatment plates were taken when the pathogen completed its growth in control plate. Per cent inhibition on mycelial growth of pathogen was calculated based on the equations given at 3.6.1.1.

3.6.2. *In Vitro* Evaluation of Chemical Fungicides

Evaluation of efficacy of seven fungicides (Table 3) including four systemic and three contact fungicides at three different concentrations were done by poisoned food technique (Nene and Thapliyal, 1993). CRD with 22 treatments and three replications were used for statistical analysis.

Initially 50 ml double strength potato dextrose agar (DPDA) medium was prepared in 250 ml conical flask. 50 ml distilled water, DPDA and petridishes (9.0 cm diameter) were kept for sterilization in autoclave at 121^0C and 1.05 kg/cm^2 for 20 min. After sterilization, required concentration of the fungicide was added to 50 ml sterilized water and shaken well for uniform mixing. Well mixed fungicidal suspension then transferred to 50 ml molten DPDA ensuring that required concentration was obtained. 15 ml poisoned media was poured into the petridishes, after solidification 5 mm mycelial disc of four days old culture of pathogen was inoculated at the centre of the petridish. Each treatment was replicated thrice. Control plates were maintained by inoculating pathogen in the poison less PDA medium. All procedures mentioned above were strictly followed aseptic conditions. Treatment plates were kept for incubation at room temperature 28 ± 2^0C. Growth of pathogen in the poisoned media were recorded and compared with control plate showed full growth of the fungus. Percentage inhibition was estimated by using the equation depicted in 3.6.1.1.

3.7. *IN VITRO* STUDIES ON COMPATIBILITY OF EFFECTIVE FUNGICIDES AND ANTAGONISTS

Compatibility of effective biocontrol agents and the effective chemical fungicides were evaluated by poisoned food technique under *in vitro* condition. 15 ml poisoned PDA media was poured into the sterilized petridishes and allowed for

Table 3. Chemical fungicides selected for *in vitro* evaluation against pathogen

Sl. No	Treatment	Fungicide	Concentration (%)
1	T1	Thiram (75WS)	0.1
2	T2	Thiram (75WS)	0.2
3	T3	Thiram (75WS)	0.3
4	T4	Mancozeb (75WP)	0.1
5	T5	Mancozeb (75WP)	0.2
6	T6	Mancozeb (75WP)	0.3
7	T7	Chorothalonil (75WP)	0.1
8	T8	Chorothalonil (75WP)	0.2
9	T9	Chorothalonil (75WP)	0.3
10	T10	Copper oxychloride (50WP)	0.1
11	T11	Copper oxychloride (50WP)	0.2
12	T12	Copper oxychloride (50WP)	0.3
13	T13	Carbendazim (50WP)	0.05
14	T14	Carbendazim (50WP)	0.1
15	T15	Carbendazim (50WP)	0.2
16	T16	Propiconazole (25EC)	0.05
17	T17	Propiconazole (25EC)	0.1
18	T18	Propiconazole (25EC)	0.2
19	T19	Azoxistrobin (23SC)	0.05
21	T20	Azoxistrobin (23SC)	0.1
21	T21	Azoxistrobin (23SC)	0.2
22	T22	Control	-

solidification. 5 mm dia. culture disc from four day old cultures of biocontrol agents were inoculated at the centre of petridishes. All the petri plates were kept for incubation at room temperature 28 ± 2^0C. Observations were taken when biocontrol agents inoculated in the control plate reached its full growth and the growth of the biocontrol agent was compared with the growth in petri plates without fungicides. Percent of inhibition was calculated by using the formula given in 3.6.1.1.

3.8. *IN VIVO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES

Most effective fungicides, biocontrol agents and their compatible combinations were short listed based on their efficacy under *in vitro* evaluation. The effectiveness of selected treatments against the disease was assessed by pot culture experiment. Fumigation of the potting mixture was carried out to disinfect the soil before the evaluation.

Design	: CRD
Treatments	: 15
Replications	: 3
Number of plants per replication	: 4
Crop	: Cowpea
Variety	: Kanakamani (PTB- 1)

3.8.1 Fumigation (Godfrey and Young, 1943)

Potting mixture containing sand, soil and cow dung (in equal proportions) required to fill grow bags were taken and prepared raised bed of 4 m^2 area with 6-8 inches height. Small pits were made on the bed at a rate of 6 pits per m^2 with 12 inches depth. Formaldehyde solution (40 %) was poured in all holes at 25 ml/m^2. Aeration was allowed in the pits by filling them with sand. Polythene sheet of 150-200 gauge thickness were used to cover the entire bed after it was moistened with water. Soil was used to seal the sides of the bed and kept as such for two weeks. Immediately after the sheet was removed, soil was raked and moistened with water for five days. This fumigated soil was used for filling 90 grow bags.

3.8.2. Inoculation of the pathogen

Most virulent pathogen isolate was multiplied in paddy grains and were used for soil inoculation as mentioned in 3.4.1. to induce stem rot disease. Ten paddy grains fully covered with white fungal mycelia along with brown sclerotia were incorporated in the soil near surface level

3.8.3. Sowing of Seeds

Cowpea variety used for the pot culture experiment was Kanakamani (PTB-1), a high yielding semi trailing variety released by Regional Agricultural Research Station, Patambi. Three seeds were sown per grow bag and one healthy plant was retained for the further study. The plants were sustained as per Package of Practice Recommendations of KAU (KAU, 2016) for proper growth of the plant.

3.8.4. Application of Treatments

Seven pre-sowing treatments were applied after one week of inoculation and 7 days before sowing. The treatments were repeated 20, 40 and 60 days after sowing the seeds. Pre-sowing treatments including fungicides, biocontrol agents and their effective combinations were applied by soil drenching. Seven treatments such as fungicides, biocontrol agents and their effective combinations were given 20, 40 and 60 days after sowing the seeds as foliar sprays and soil drenching. The plants grown in soil inoculated with pathogen and without any treatment application was considered as control. Each treatment was replicated three times. Spray solution of fungal biocontrol agents was confirmed to have spore content of 4×10^9 cfu/ml by Neubauer haemocytometer (Barbedo, 2013).

3.8.5. Main Items of Observations

Disease incidence, lesion size, lesion number, occurrence of other pests and diseases and yield of the plant were examined in all the treatments and control plants. Data obtained by the above observations were subjected to statistical analysis.

3.9. STATISTICAL ANALYSIS

Data obtained from *in vitro* and *in vivo* studies were undergone analysis of variance (ANOVA) by using Wasp 2 and OPSTAT software after appropriate transformations wherever needed. Critical difference (CD) values were calculated for each observation in significant treatments using t' values at 5 per cent level of significance and the significance of the treatments was compared with CD values.

Results

RESULTS

Cowpea (*Vigna unguiculata* (L.) Walp) is an important protein rich vegetable legume crop which can be grown through the year under Kerala condition. Stem rot and foliar blight disease has been recently found severe in Kerala fields especially during rainy season. Hence, a study on "Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)" was conducted during 2018-2020 with an aim of identifying and characterizing fungal pathogen causing stem rot and foliar blight of cowpea followed by evaluation of various biocontrol agents and chemical fungicides against the pathogen. Results of experiments carried out under *in vitro* and *in vivo* conditions are described below.

4.1. COLLECTION OF SAMPLES

Plants showing symptoms of stem rot and foliar blight of cowpea were collected from six different locations of Kasargod district such as Cheemeni, Pallikkara, Nileswar (College of Agriculture, Padannakkad), Trikaripur, Udinur and Periya during south west monsoon period of 2019. Stem infection was found prevalent in Cheemeni and Trikaripur, however foliar blight was more common in rest of the locations. Incidence of both stem rot and foliar blight disease were noticed at Nileswar.

All stages of the crop were susceptible to the disease. Yellowing and wilting of aerial parts were the common symptoms found on cowpea plants infected with stem rot disease. Water soaked lesion, formation of white mycelial strands of pathogen, mustard like brown sclerotial bodies and rotting of collar region were the characteristic symptoms noticed at the basal region of stem portion. Leaf blight symptoms included water soaked lesions having concentric rings with white mycelial growth of pathogen. Details regarding isolates of pathogen, location of collection and the part infected are included in Table 4.

Table 4. Isolates of pathogen collected from different locations in Kasargod district

Sl. No	Isolate Code	Location	Latitude & Longitude	Symptom
1	Sr1	Cheemeni	12° 14' 33.5112"N 75° 14' 1.7916"E	Wilting and yellowing of leaves, rotting of stem, white mycelia and sclerotial bodies at stem base
2	Sr2	Pallikkara	12° 14' 50.028"N 75° 8' 14.6544"E	Water soaked foliar lesions with concentric rings along with white mycelia of pathogen
3	Sr3	Nileswar (COA Padannakkad)	12° 15' 24.012"N 75° 6' 59.8176"E	Rotting of the stem along with leaf spots having concentric rings.
4	Sr4	Trikaripur	12° 8' 23.7264"N 75° 10' 34.6476"E	Wilting and yellowing of leaves, necrosis and girdling at the base of the stem, white mycelia and sclerotial bodies at stem base
5	Sr5	Udinur	12° 9' 41.9616"N 75° 10' 10.9596"E	Light brown large spots having concentric rings and shot holes on leaves
6	Sr6	Periya	12° 24' 35.1108"N 75° 5' 30.4908"E	Water soaked lesion with 3-4 circular rings

4.1.1. Assessment of Percent Disease Incidence and Percent Disease Index

Disease incidence and disease index in infected fields were assessed as per 3.1.1. Score chart for determining diseases index was prepared based on the area of leaf affected by the disease. Disease index was recorded on the scale of 0-5.

Percent disease incidence (PDI) was calculated for all plants with stem rot and foliar blight symptoms while percent disease index (PDI) was noted only for plants with foliar blight symptoms (Table 5). Among the six locations, percent disease incidence and percent disease index showed a range of 12 to 80 per cent and 8 to 51 per cent respectively (Plate 2). Highest incidence of disease was noticed at Nileswar (80 %) followed by Pallikkara (58 %) whereas lowest was recorded at Periya (12 %) followed by Trikaripur (20 %). Cheemeni and Udinur had 22 % and 44 % disease incidence respectively. Pallikkara (51 %) was found superior than Nileswar (48 %) with respect to disease index. Least percentage of disease index was recorded from Periya (8 %) followed by Udinur (12 %). Details regarding disease incidence and disease index are given in the Table 5.

4.2. ISOLATION OF THE PATHOGEN

Pathogen associated with stem rot and foliar blight diseases was isolated in PDA medium from infected samples collected from six locations in Kasargod district. Isolation was carried out with standard procedures depicted under 3.2. White mycelial growth of fungus in PDA was observed from the second day of incubation at room temperature. Hyphal disc cut from actively growing tip of fungal colony was transferred to another PDA plate and then to PDA slant for pure culturing. Six different isolates were obtained from the survey locations and were named with prefix ‘Sr’ such as Sr1, Sr2, Sr3, Sr4, Sr5 and Sr6. Slants of each isolates were maintained in refrigerator at 15^0C and used for further studies.

Table 5. Disease incidence and Disease index of stem rot and foliar blight disease in cowpea at different locations

Sl. No	Location	Isolate code	Disease incidence (%)*	Disease index (%)
1	Cheemeni	Sr1	22 [d]	-
2	Pallikkara	Sr2	58 [b]	51
3	Nileswar	Sr3	80 [a]	48
4	Trikaripur	Sr4	20 [e]	-
5	Udinur	Sr5	44 [c]	12
6	Periya	Sr6	12 [f]	8
		CD (0.05)	1.79	
		SE (m)	0.58	

*Disease incidence and disease index were calculated based on 10 plants
Figures with same letter do not have significant difference according to one way ANOVA at P=0.05

Cheemeni

Pallikkara

Nileswar

Trikaripur

Periya

Udinur

Plate 2. Cowpea fields with stem rot and foliar blight incidence

4.3. PATHOGENICITY TEST

Evaluation of pathogenicity of the isolates was done by following Koch's postulates. Soil and leaf inoculation methods were used for pathogenicity testing on cowpea grown in polythene bags.

4.3.1. Multiplication of Fungal Inoculum

Each fungal isolate was multiplied in paddy grains and used for soil inoculation (Plate 3). White fungal mycelia covered entire paddy grains in the test tube within three days of inoculation and sclerotial production started after five days of incubation.

4.3.2. Inoculation of Pathogen in the Soil

The isolates of pathogen showed difference in number of days taken for infection and lesion size (Table 6, Plate 4). White thread like mycelial mat was formed on the soil near the collar region within two to four days of inoculation and produced mustard like sclerotial bodies. Irregular, brown, water soaked lesions were formed at the infected portion on the stem especially in the collar region followed by formation of sclerotium, wilting, yellowing and death of the plants which were the typical characters of stem rot disease. The affected plants showed complete wilting and died within two weeks of fungal inoculation. Plants treated as control did not show any symptom of disease and retained as healthy plant.

The isolate Sr3 produced water soaked lesion at the basal region of stem within two days of soil inoculation whereas Sr2, Sr5 and Sr6 took three days for the symptom appearance. The isolates Sr1 and Sr4 showed delayed infection and they took four days for initiation of infection. Complete wilting of Sr3 inoculated plants were observed within five days of inoculation which was earliest compared to other isolates.

All the isolates produced single lesion on the stem, but variation in lesion size was recorded till five days of infection. At fifth day of infection the plants showed

complete wilting. Largest lesion area was produced as a result of the infection by Sr3 (6.4 cm^2) followed by Sr1 (4.5 cm^2) and Sr4 (4.1 cm^2). Smallest lesion area of 1.3 cm^2 was recorded in Sr6 infection followed by 2.8 cm^2 in Sr5 on 7 days of pathogen inoculation on soil.

4.3.3. Inoculation of the Pathogen on the Leaf

All isolates produced disease symptoms on the leaves however they showed difference in number of days for the appearance of symptom and lesion size (Table 6). Light brown and small water soaked lesions were formed on both sides of the leaves at the first day of infection. Size of the lesion started to increase covering the whole area of leaf in the next three days along with white mycelial growth of pathogen on the lesion. White sclerotial initials and mustard like sclerotia were formed on the lesion. Yellowing, drying and shedding of leaves were noticed within 5-7 days of inoculation. Leaves of control plants remained healthy without having any symptoms of foliar blight.

Leaves inoculated with Sr3 showed initiation of water soaked lesion within 24 h and they also showed abscission earlier than other inoculated plants (Plate 5). The isolates Sr5 and Sr6 were also found as more virulent as they took only two days for infection. Among the six isolates, Sr4 caused delay in infection taking four days for initiating infection. Only one lesion was produced as a result of inoculation of all the six isolates on cowpea leaves. Lesion size was recorded maximum in Sr3 covering 9.6 cm^2 area of leaf followed by Sr5 (4.9 cm^2). Smallest lesion was produced by Sr1 (0.9 cm^2) followed by Sr4 (1.3 cm^2) on five days of leaf inoculation.

4.3.4. Virulence Rating of Pathogen

Virulence of all fungal isolates was rated based on their growth rate in petri dish and pathogenicity in cowpea plant. All isolates were found virulent having the ability to produce stem rot and foliar blight disease in cowpea. Sr3 showed infection on the stem within two days of inoculation while it could produce foliar infection within 24 h

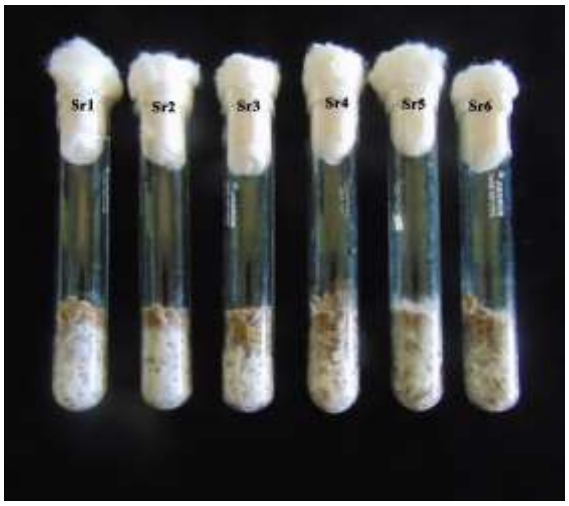
Table 6. Symptom development by different isolates in cowpea

Sl. No.	Isolate	Stem rot symptom		Foliar blight	
		No. of days taken to produce symptom	Lesion size on stem (cm^2)*	No. of days taken to produce symptom	Lesion size on leaf (cm^2)*
1	Sr1	4	4.5 [b]	3	0.9 [f]
2	Sr2	3	3.8 [d]	3	3.1 [d]
3	Sr3	2	6.4 [a]	1	9.6 [a]
4	Sr4	4	4.1 [c]	4	1.3 [e]
5	Sr5	3	2.8 [e]	2	7.1 [b]
6	Sr6	3	1.3 [f]	2	4.9 [c]
		CD(0.05)	0.37		0.96
		SE (m)	0.12		0.31

*Lesion size on stem was taken after 7 days of inoculation

*Lesion size on leaf was taken after 5 days of inoculation

Figures with same letter do not have significant difference according to one way ANOVA at P=0.05



A.

B.

C.

Plate 3. Pathogenicity test for stem rot of cowpea A) Pathogen multiplied in paddy grains B) Incorporation of inoculated grains in soil near basal part of stem C) Inoculated plant is covered with polyethylene cover

Plate 4. Stem rot symptom developed by different isolates in cowpea during pathogenicity test. (Observation taken 7 days after soli inoculation)

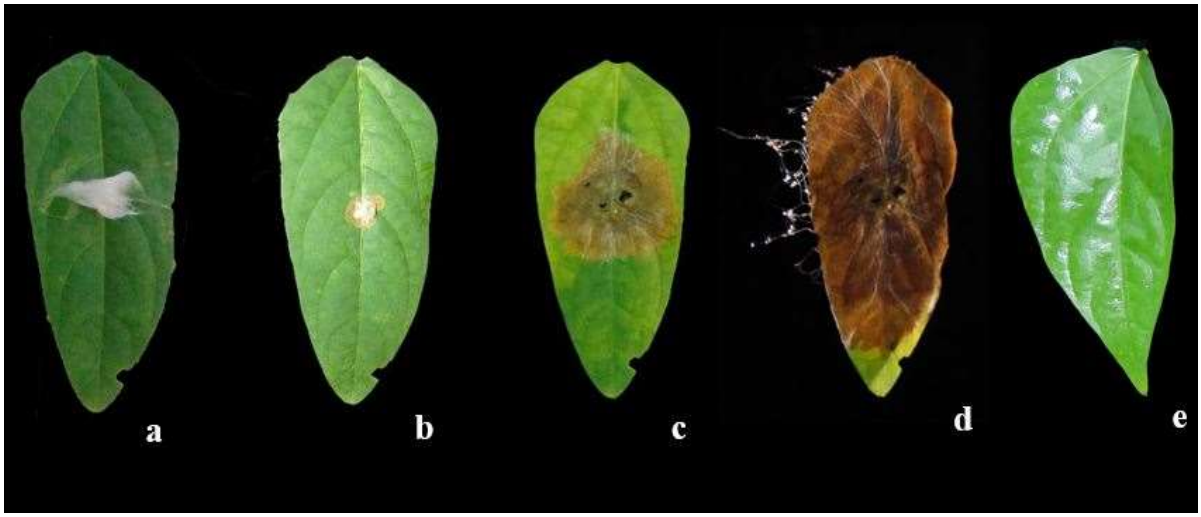


Plate 5. Stages of leaf blight by most virulent isolate Sr3. a) Inoculation of pathogen on the leaf. B- 1 DAI, c- 3 DAI and d- 5 DAI are the symptom development in inoculated leaf. e) Uninoculated leaf (control)

of inoculation. Sr3 was found more virulent as it could infect plant within 24 h and produced the largest lesion area on both stem and leaf.

Growth rate of six isolates were observed in PDA as a part of virulence rating (Plate 6,7). Significant difference in the radial growth of the fungus was observed at third day of inoculation (72 h after inoculation) ranges from 3.8 – 8.8 cm and maximum radial growth of 8.8 cm was showed by Sr3 and considered it as very fast grower (Table 7). Sr1 (7.4 cm) and Sr4 (7.3 cm) were found on par and superior than Sr5 and Sr6 which attained radial growth of 6.8 cm and 6.1 cm respectively at 3rd day of inoculation. Hence, Sr1, Sr4, Sr5 and Sr6 were considered as fast growers. Sr2 was showing slower growth among the six isolates which could attain only 3.8 cm of radial growth, which was below half of the radial growth of Sr3 at 3rd day of inoculation. So Sr2 was categorised as slow grower.

From the results of pathogenicity test (Table 6) and growth rate in PDA (Table 7), Sr3 isolate was found as most virulent isolate and taken for further study.

4.4. IDENTIFICATION OF THE PATHOGEN

Most virulent isolate Sr3 was identified with its cultural, morphological and molecular characteristics.

4.4.1. Cultural Characteristics

Colony characters of Sr3 such as rate of growth of mycelium, colour of mycelium, pattern of mycelial growth, days taken to produce sclerotia, sclerotial arrangement, size and shape of sclerotia were studied in PDA (Plate 8, 9, 10, 11). The pathogen showed rapid growth and covered Petridish of 90 mm diameter within 4 days of inoculation (Table 7). It produced cottony, fluffy and thick mycelium of pure white colony with slight zonations in PDA (Table 8). White tufts of loosely intertwined small branches of fungal hypha called sclerotial initials having round shape were produced in the fungal colony after eight days of inoculation (Plate 12A, 12B). Maturation of sclerotial initials resulted in round, smooth and light brown

Table 7. Radial growth of six isolates on PDA

Sl. No.	Isolate	Colony diameter of isolate in PDA in petridish (cm)			
		24 h	**48 h**	**72 h***	**96 h**
1	Sr1	1.1	5.0	7.4 [b]	9.0
2	Sr2	1.0	2.2	3.8 [e]	7.0
3	Sr3	1.2	5.6	8.8 [a]	9.0
4	Sr4	1.0	5.5	7.3 [b]	9.0
5	Sr5	1.1	3.1	6.8 [c]	9.0
6	Sr6	1.0	3.1	6.1 [d]	8.8
			CD (0.05)	0.18	
			SE (m)	0.05	

*Colony diameter of isolates at 72 h of inoculation was taken for statistical analysis. Figures with same letter do not have significant difference according to one way ANOVA at P=0.05

Plate 6. Radial growth of six isolates at 3 days after inoculation

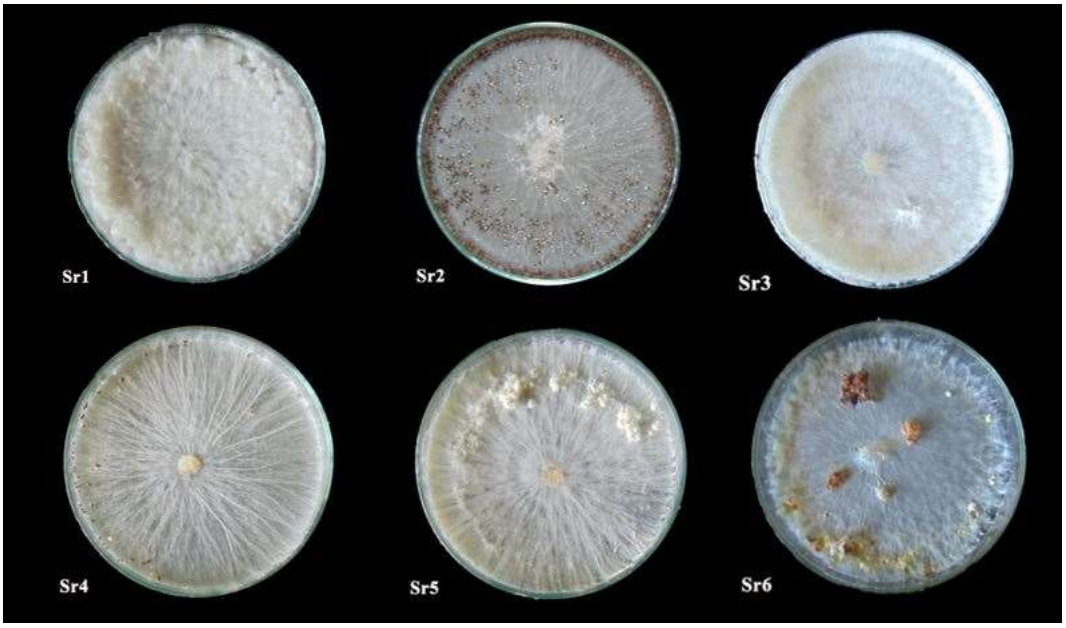


Plate 7. Colony characters of six isolates on PDA

Table 8: Morphological characters of isolate Sr3

Sl.	Parameters	Observations
1	Culture colour	Pure white with dense cottony mycelia
2	Reverse plate colour	Dull white
3	Culture margin	Regular
4	Topography	Flat with fluffy mycelia
5	Zonation	Concentric ring like zone
6	Substrate colour	White
7	Colony diameter (cm)	Completes petri plate (9 cm diameter) on 4^{th} day of inoculation
8	Mycelia	Septate with clamp connections
9	Colour of mycelia	White
10	Sporulation	Rare, sexual spores are produced at particular environmental conditions only.

Plate 8. Variability in shape and texture of sclerotia of different isolates.

a) Irregular shaped clustered sclerotia with dark brown spots b) Spherical shaped clustered sclerotia c) Spherical shaped scattered sclerotia d) Large sized yellowish white sclerotia e) Irregular shaped sclerotia with dark brown spots and cavities

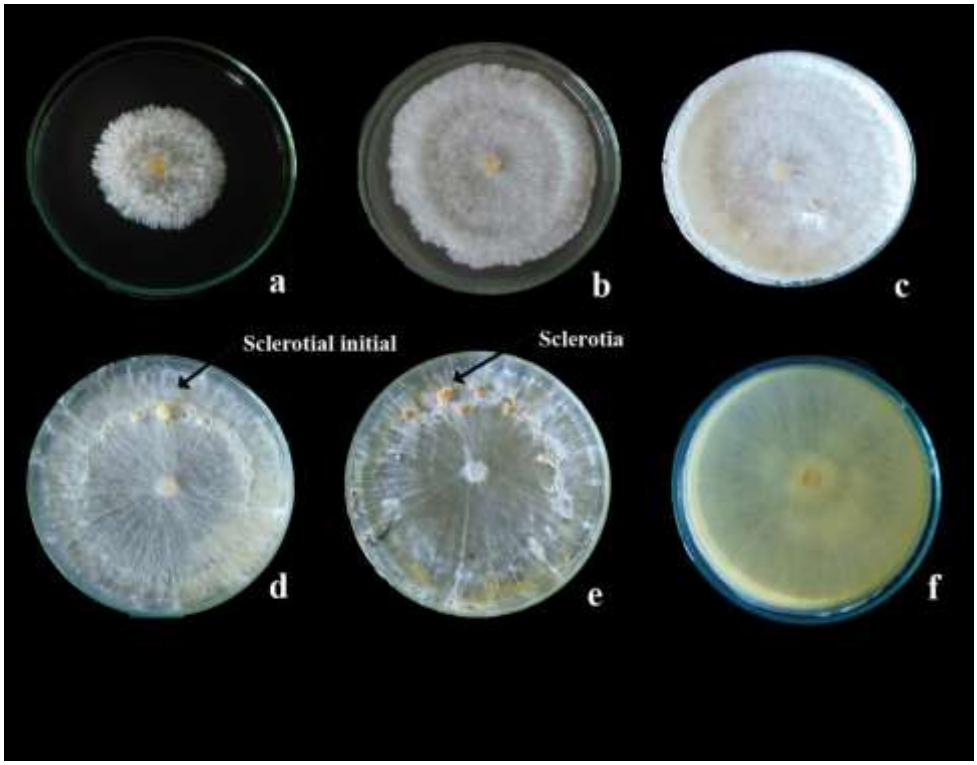
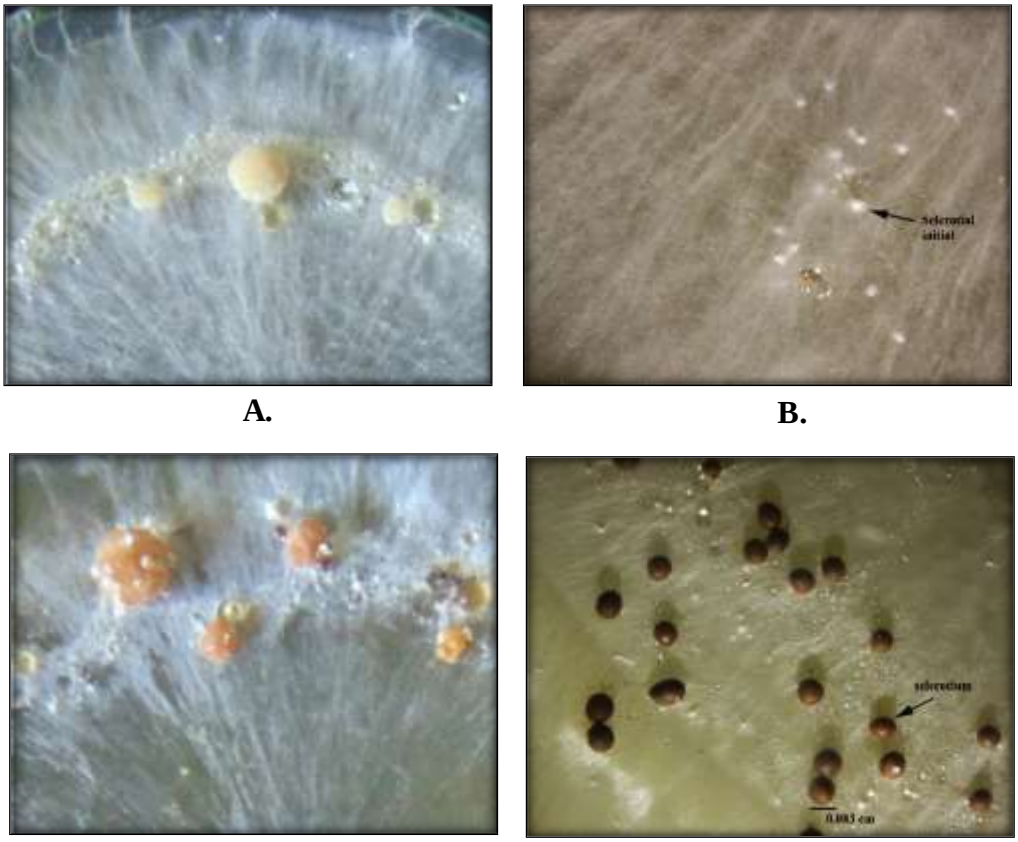


Plate 9. Colony morphology of Sr3 at its different growth stages in PDA a) 2 DAI b) 3 DAI c) 6 DAI d) 8 DAI e) 14 DAI f) Reverse side view

Plate 10. Growth of Sr3 in PDB

Plate 11. Growth of Sr3 in PDA slant



A. **B.**

C. **D.**

Plate 12. Stages of sclerotial formation of Sr3 in PDA. A & B are the white sclerotial initials and C & D are the mature light brown and brown sclerotial bodies

sclerotial bodies in the fungal colony after 14 days of inoculation. Total number of sclerotia formed in the fungal colony ranged from 25 to 30 in which four were large sized (Plate 12C) and 25 were small sized (Plate 12D). Small sized sclerotia of 1 mm dia. were arranged at the periphery whereas large sized sclerotia of 6 mm dia. were observed in semicircle manner at 3 cm away from the centre (Table 10).

Cultural variability was also observed among the six isolates (Table 9, 10). Sr1 and Sr3 produced fluffy colony whereas Sr2, Sr4 and Sr6 produced flat colony with thin mycelial strands. Presence of concentric zones in the colony were the characteristics of Sr3, Sr5 and Sr6. Slight variations in colour of mycelium such as off white and pure white were also noticed among isolates. Earliest production of sclerotial bodies was noted in Sr2 (8 days) and late production in Sr5 (17 days). Sr1 showed peripheral arrangement of sclerotia while scattered arrangement was seen in Sr2. Sclerotia were produced in groups by the isolates Sr3, Sr5 and Sr6 (Plate 8a) and among these, Sr3 and Sr5 produced sclerotia in semi-circle manner at 3 cm away from the centre.

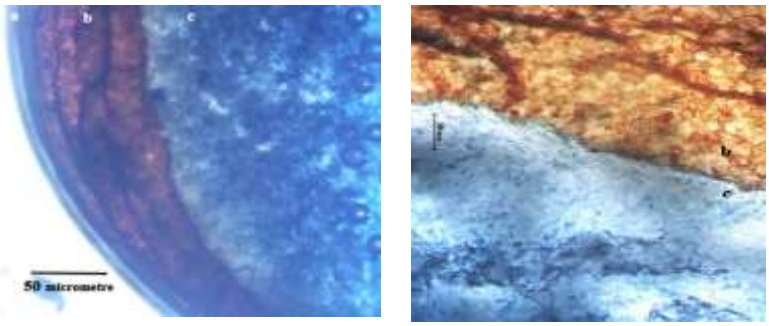
Maximum number of sclerotia was produced by the isolate Sr2 (310 per plate) and minimum by Sr1 (12 per plate). Size of sclerotia showed a range of 1-6 mm with light brown, brown and dark brown colour having characteristic spots and cavities on some (Plate 8e). Irregular shaped sclerotial bodies were observed in Sr5 and Sr6 while others produced only spherical ones.

4.4.2. Morphological Characteristics

Morphological characteristics of fungal hyphae were examined through Carl Zeiss binocular microscope under 400X magnification (Plate 13). Two types of hyphae were observed with one having coarse, straight and large cells (150-250 µm diameter) with clamp connection at septa. Some produced branching at the place of clamp connection. Another type of hypha was slender forming branches irregularly, but clamp connections were absent (Plate 13c). Cross section of sclerotium revealed that it had soft cream coloured inner portion (Plate 14 A).

Plate 13. Morphology of fungal hypha a) Clamp connections b) Coarse hypha c) Slender hyphae with no clamp connection

A.



B.

Plate 14. Morphology of sclerotium. A. Cross section of sclerotium B. Different layers of Sclerotium under 400X magnification a) Rind, b) Cortex and c) Medullary cells

Table 9. Colony characters of six isolates of pathogen

Sl. No	Isolates	Rate of growth of mycelium	Colour of mycelium	Pattern of mycelia growth
1	Sr1	Very fast	White	Cottony, fluffy, dense at periphery, upright growth, thick strands
2	Sr2	Slow	White	Flat, thin mycelium, dense at center
3	Sr3	Very fast	Pure white	Cottony, fluffy, thick mycelium with light zonation
4	Sr4	Very fast	White	Root like, flat with thin strands
5	Sr5	Very fast	Pure white	Fan like appearance at the periphery, light zonation near center
6	Sr6	Fast	White	Flat, suppressed, dense at margin, zonation present at initial stage.

Table 10. Characters of sclerotia of six isolates

Isolate	No. of days for sclerotial production		Sclerotial arrangement	No. per plate	Diameter (mm)	colour	Shape
	SI*	SM*					
Sr1	8	13	Periphery	12	1.37	Brown	Spherical
Sr2	5	8	Scattered, more number at periphery	310	1.49	Brown	Spherical
Sr3	8	14	3 cm from the centre in semi-circle manner and periphery	25-30	Small – 1.0 Large- 6.0	Light brown.	Spherical
Sr4	7	11	Periphery	124	1.2	Light brown	Spherical
Sr5	10	17	Aggregated, half circle arrangement, 3.5 cm away from the centre	28	2.0	Light brown with dark brown spots	Spherical
Sr6	8	13	Aggregated irregularly	32	2.0	Dark brown and light brown	Irregular

*SI- Sclerotial Initiation

*SM- Sclerotial Maturation

Sclerotium was internally differentiated into four different layers having an outer thick skin followed by rind with thick cells, cortex with thin walled cells and medulla region of loosely filamentous hyphae (Plate 14B). Based on the results of cultural and morphological studies, most virulent isolate Sr3 was identified as *Sclerotium rolfsii* Sacc.

4.4.3. Molecular Characteristics

Molecular characterization of most virulent isolate was carried out for the identification of fungus at molecular level. Identification of microbial culture was done by using D1/D2 region of LSU (Large Sub Unit: 28SrDNA) based molecular technique. Consensus Sequence are given as follows (560bp).

TTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGG
ATTCCCCTATGGAGAAGTGTTTTCTGTGCTGGGCCGTGTACAAGT
CTCTTGGAATAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGA
CACGGACATCCAGTGCTCTGTGATGCACTCTCAAAGAGTCGAGT
TGTTTGGGAATGCAGCTCAAAATGGGTGGTAAATTCCATCTAAA
GCTAAATATTGGCAAGAGACCGATAGCGAACAAGTACCGTGAGG
GAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTG
AAATTGTTGAAAGGGAAACACTTGAAGTCAGTCGCGTCTAGCAA
GGATCAGCCTTTCTCGGAAGGTGTATCTCTTGCTTGACGGGTCAA
CATCAATTTTGACTACTGGAAAAAGGCCAGGGGAAGGTGGCACC
TTCGGGTGTGTTATAGCCTTTGGTCATATACAGTAGTTGGGATTG
AGGAATTCAGCATGCCTTCATGGCTGGGGTTCGCCCACATTCAT
GCTTAGGATGTTGGCATAATGGCTTTAAGCGAC

Based on the D1/D2 Region- PCR analysis, the fungal culture Sr3 showed 99.61 % similarity with *Athelia rolfsii* (Accession No:JN811675.1), which was the perfect stage of *Sclerotium rolfsii* (Table 11).

Table 11. Nucleotide identity of Sr3 isolate with reported isolates of *Athelia rolfsii*

Sl. No	Description	Max. Core	Query Coverage	E value	Identity (%)	Accession
1	*Athelia rolfsii* isolate S-78	931	91%	0.0	99.61	JN811675.1
2	*Athelia rolfsii* isolate IGFRI 1	926	100	0.0	99.41	MT225781.1
3	*Athelia rolfsii* isolate BSR19	926	91	0.0	99.41	MN078808.1
4	*Sclerotium delphini* strain CBS 672.71	926	98	0.0	99.41	MH872052.1
5	*Athelia rolfsii* isolate DGADY11	926	100	0.0	99.41	MG195622.1
6	*Athelia rolfsii* isolate DGADY09	926	100	0.0	99.41	MG195621.1
7	*Athelia rolfsii* strain SPL15005	926	98	0.0	99.41	KY446371.1
8	*Athelia rolfsii* strain SPL15001	926	98	0.0	99.41	KY446368.1
9	*Sclerotium delphinii* isolate IVT 17	926	91	0.0	99.41	KY172990.1
10	*Athelia rolfsii* strain ATCC 201126	926	99	0.0	99.41	AF499019.1

Based on the cultural, morphological and molecular characterization, isolate Sr3 responsible for stem rot and foliar blight disease was confirmed as *Sclerotium rolfsii.*

4.5. SYMPTOMATOLOGY OF DISEASE

Symptomatology of stem rot and foliar blight disease in cowpea caused by *S. rolfsii* was studied under natural and artificial conditions (Plate 15 & Plate 16). It was observed that similar symptoms were noticed in both conditions.

Infection was initiated with water soaked, brown lesions at the base of the stem which expanded its size thereby progressing upwards (Plate 16 a1). As a result, lower leaves started to droop followed by wilting and yellowing of aerial parts (Plate 15b2, 16a2). White thread like mycelial strands were formed on the infected parts on the stem at the soil line (Plate 15a1, a2 & a3). White sclerotial initials were formed on the mycelial mat of *S. rolfsii* followed by maturation of these into brown, mustard like sclerotial bodies (Plate 15b1,16b1).Water soaked lesions then turned to necrotic and became rotten (Plate 15b4). Girdling on the stem was also visible at the base of the stem (Plate 15b3). Infected plants died under extreme disease severity without producing pods but plants with mild infection produced pods with comparatively less yield. White mycelium with sclerotial bodies started to spread on the soil and infected nearby plants at the collar region.

Foliar blight symptoms under natural and artificial conditions were similar except for concentric ring formation on lesion. Initially light brown circular water soaked lesions were produced in the infected leaves. Lesion expanded with three to four concentric rings on both side of the leaf and turned to dark brown in colour with irregular shape (Plate 17). White mycelia of pathogen were clearly visible on the lesions which later produced brown mustard like sclerotial bodies. Entire leaf turned to yellow and dry but under dry climate, only lesion became dry without showing complete yellowing of leaves and fall down resulting shot hole symptom.

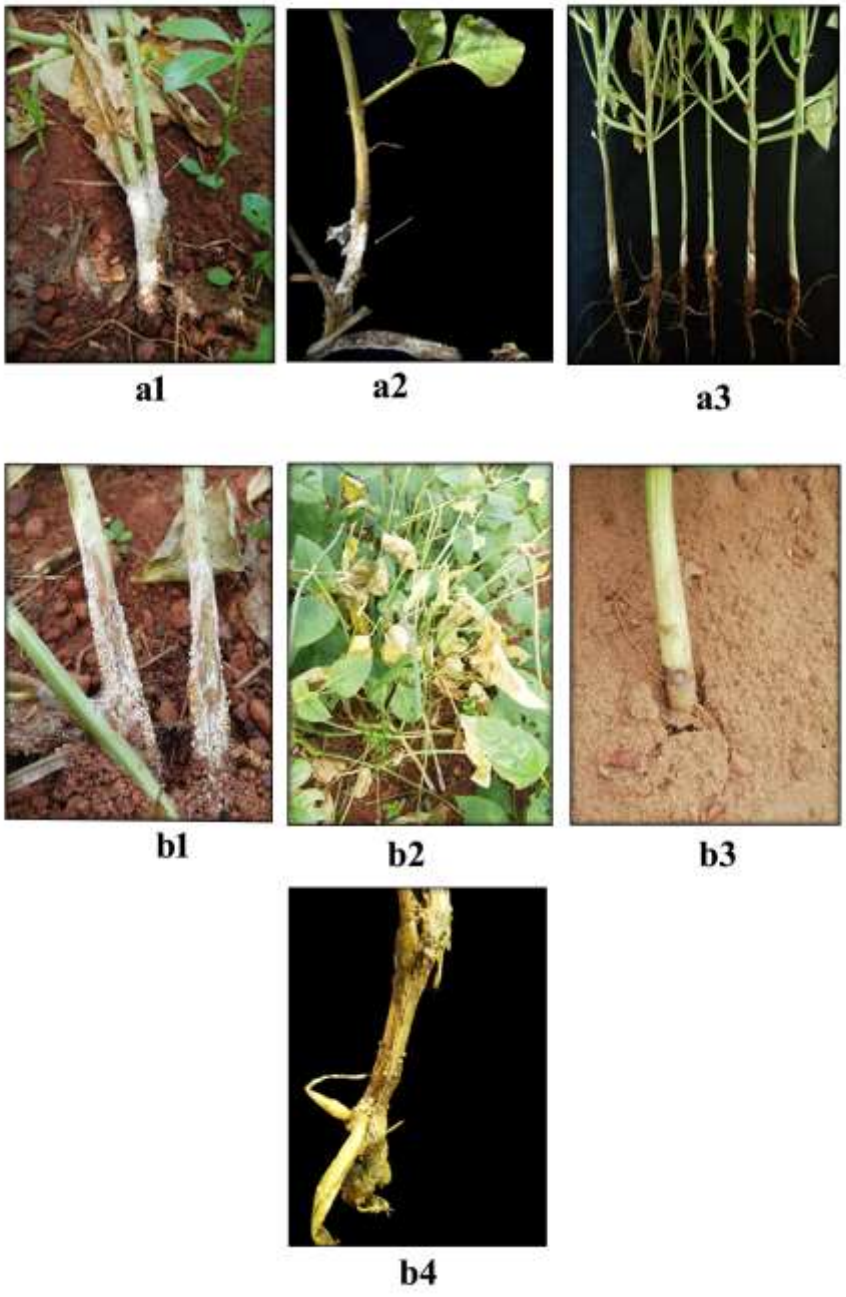


Plate 15. Symptomatology of stem rot of cowpea at natural conditions. a1, a2, a3 – White fungal mycelial growth at basal region of stem. b1) Formation of mustard like sclerotial bodies at the stem base b2) Wilting and yellowing of leaves b3) Girdling of stem b4) Rotted stem

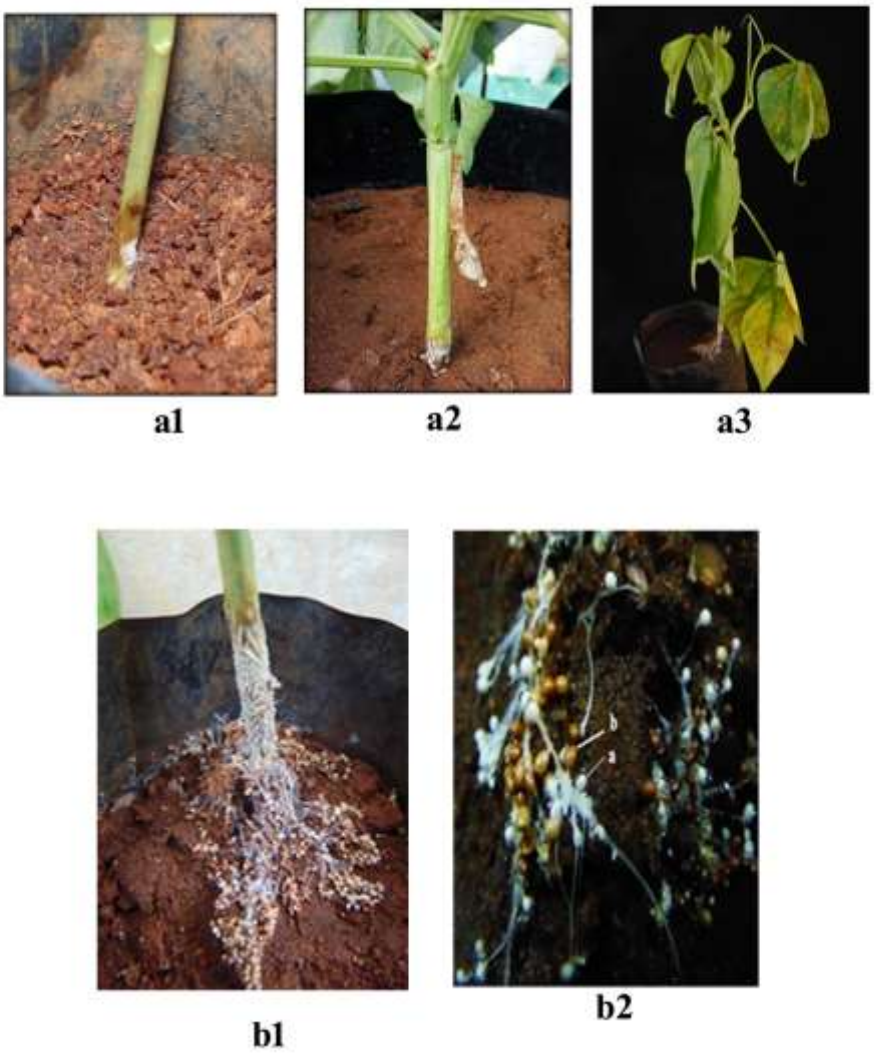
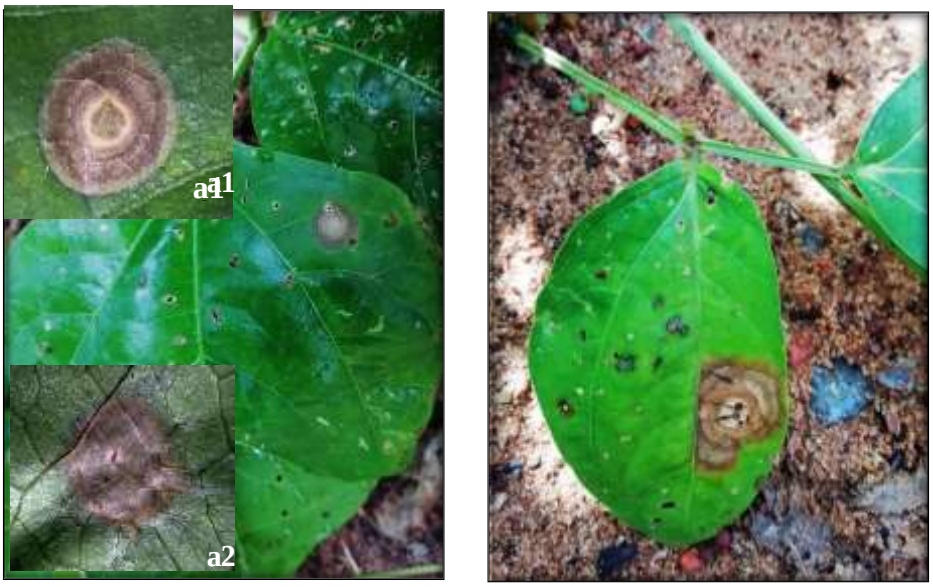


Plate 16. Symptomatology of stem rot disease in artificial conditions

a1) Water soaked brown lesion at the basal region of stem. a2) White mycelial growth on the infected portion a3) Yellowing and wilting of the plant. b1) Extensive white mycelium, white sclerotial initials and brown matured sclerotia formation at the infected region b2) a- Sclerotial initial, b- Sclerotial bodies



A. B.

C. D.

Plate 17. Symptomatology of foliar blight of cowpea in natural conditions A. Round water soaked brown spots with concentric rings (a1 upper surface, a2 lower surface) B. Big irregular brown spots with concentric rings C. Round spots with yellowing of leaf D. Dried leaf

Leaves artificially inoculated with pathogen could produce all symptoms observed under natural conditions except concentric rings formation (Plate 5).

4.6. *IN VITRO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES AGAINST PATHOGEN

In vitro evaluation of four biocontrol agents released by KAU and seven fungicides available in the market were conducted against most virulent isolate of *S. rolfsii*, Sr3 by dual culture and poisoned food technique respectively. Results obtained from this experiment are presented below.

4.6.1. *In Vitro* Evaluation of Biocontrol Agents

KAU released biocontrol agents such as *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were used for testing their antagonism against *S. rolfsii* under *in vitro* condition. Dual culture method was followed for both these fungal and bacterial antagonists (Plate 18).

Fungal biocontrol agents were found to be superior over bacterial agents against *S. rolfsii* for which highest inhibition percentage was recorded with *T. harzianum* (84.44 %) followed by *T. viride* (71.11 %). Bacterial biocontrol agents showed less inhibition of pathogen under *in vitro* assay where *Bacillus subtilis* checked radial growth of fungus about 38.89 %. *P. fluorescens* showed no inhibition in the mycelial growth of the pathogen (Table 12).

4.6.2. *In Vitro* Evaluation of Chemical Fungicides

In vitro evaluation of seven fungicides was done at three concentrations against *S. rolfsii* by poisoned food technique in double strength PDA. All fungicides were tested at three different concentrations including recommended dose and its lower and higher dose (Table 13).

Among the fungicides, mancozeb (75 WP) and propiconazole (25EC) were found superior with 100 % inhibition at all the three concentration (0.1 %,0.2 % and 0.3 %) followed by thiram (75WS) with 99.63 % inhibition at its highest concentration (0.3 %) (Plate 19). Chlorothalonil (75 WP) was also tested effective

Table 12. Effect of biocontrol agents against *S. rolfsii in vitro*

Treatment	Biocontrol agents	Radial growth of pathogen in petridish (cm)*	Percentage of mycelial inhibition of *S. rolfsii* *
T1	*Trichoderma viride*	1.30	71.11 (57.47) [b]
T2	*Trichoderma harzianum*	0.70	84.44 (66.75) [a]
T3	*Pseudomonas fluorescens*	4.50	0.00 (0.00) [d]
T4	*Bacillus subtilis*	2.75	38.89 (38.56) [c]
T5	Control	4.50	0.00 (0.00) [d]
		CD (0.05)	1.63
		SE (m)	0.54

*mean of four replications

Values in parenthesis are arcsine transformed

Observations were taken after 4 days of inoculation

Figures with same letter do not have significant difference according to one way ANOVA at P=0.05

T1 *Trichoderma viride*

T2 *Trichoderma harzianum*

T3 *Pseudomonas fluorescens*

T4 *Bacillus subtilis*

T5 Control

Plate 18. Effect of biocontrol agents against *Sclerotium rolfsii* (Sr3) *in vitro*

Table 13. Effect of different fungicides against *S. rolfsii in vitro*

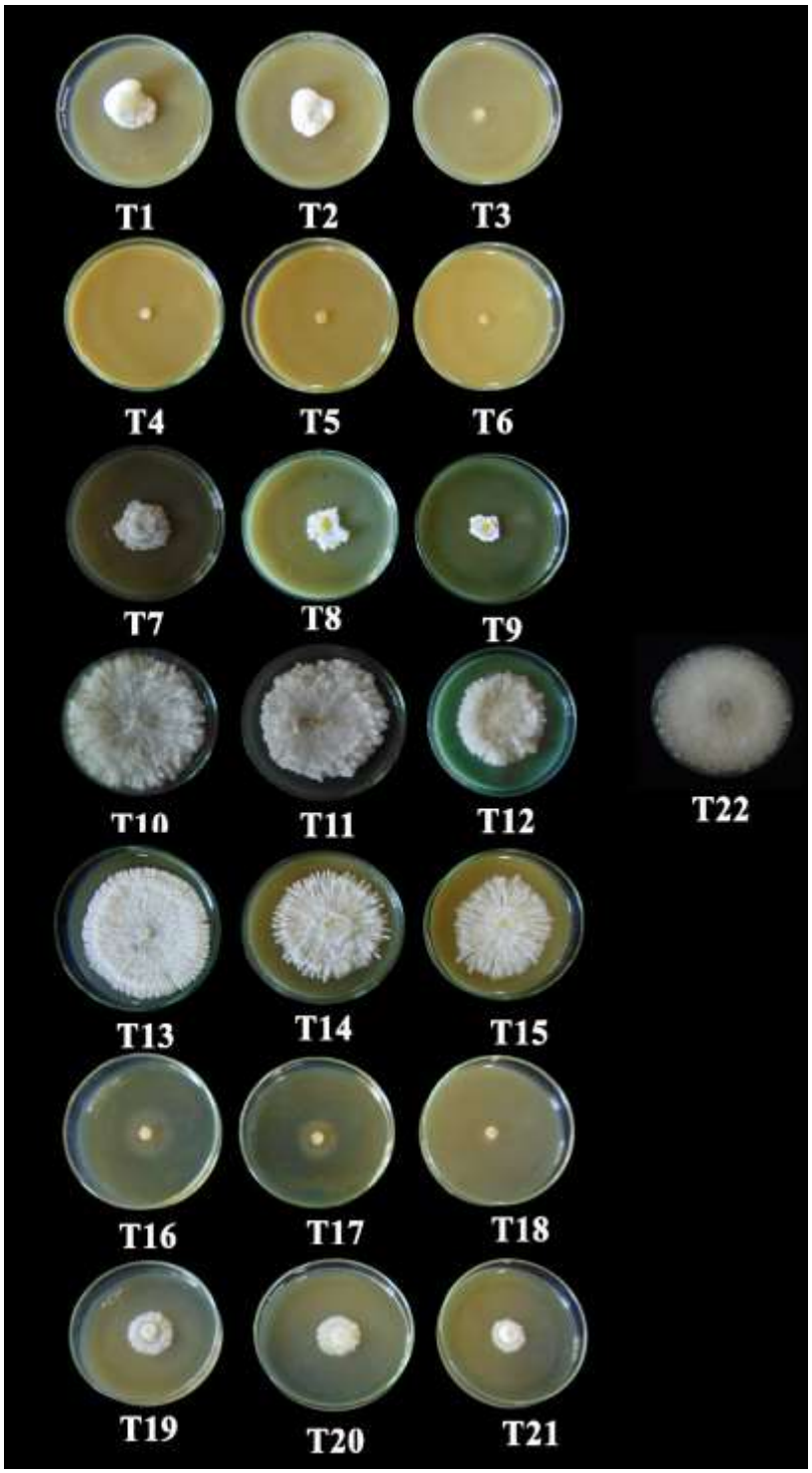
Treatment	Fungicide- concentration in per cent	Radial growth of pathogen in petridish (cm)*	Percentage mycelial inhibition of *S. rolfsii* (%)*
T1	Thiram (75WS) – 0.1 %	3.10	65.60 (54.00)^{g}
T2	Thiram (75WS) – 0.2 %	2.63	70.70 (57.20)^{e}
T3	Thiram (75WS) – 0.3 %	0.03	99.60 (88.00)^{a}
T4	Mancozeb (75 WP) – 0.1 %	0.00	100.00 (90.00)^{a}
T5	Mancozeb (75 WP) – 0.2 %	0.00	100.00 (90.00)^{a}
T6	Mancozeb (75 WP) – 0.3 %	0.00	100.00 (90.00)^{a}
T7	Chorothalonil (75 WP) – 0.1 %	3.56	60.40 (51.00)^{h}
T8	Chorothalonil (75 WP) – 0.2 %	2.50	72.20 (58.20)^{d}
T9	Chorothalonil (75 WP) – 0.3 %	1.83	79.60 (63.20)^{b}
T10	Copper oxychloride (50WP) – 0.1%	8.53	5.20 (13.10)^{n}
T11	Copper oxychloride (50WP) – 0.2%	7.60	15.60 (23.20)^{m}
T12	Copper oxychloride (50WP) – 0.3%	6.26	30.70 (33.70)^{j}
T13	Carbendazim (50WP) – 0.05 %	7.83	12.80 (21.00)^{m}
T14	Carbendazim (50WP) – 0.1 %	7.06	21.50 (27.60)^{k}
T15	Carbendazim (50WP) – 0.2 %	6.10	32.20 (34.57)^{i}
T16	Propiconazole (25EC) – 0.05 %	0.00	100.00 (90.00)^{a}
T17	Propiconazole (25EC) – 0.1 %	0.00	100.00 (90.00)^{a}
T18	Propiconazole (25EC) – 0.2 %	0.00	100.00 (90.00)^{a}
T19	Azoxistrobin (23SC) – 0.05 %	2.83	68.50 (55.90)^{f}
T20	Azoxistrobin (23SC) – 0.1 %	2.51	72.18 (58.20)^{d}
T21	Azoxistrobin (23SC) – 0.2 %	2.10	76.70 (61.10)^{c}
T22	Control	9.00	0.00 (0.00)^{op}
		CD (0.05)	1.03
		SE (m)	0.36

*mean of three replications

Values in parenthesis are arcsine transformed

Observation was taken after 4 days of inoculation

Figures with same letter do not have significant difference according to one way ANOVA at P=0.05



T1 Thiram (75WS) – 0.1%
T2 Thiram (75WS) – 0.2%
T3 Thiram (75WS) – 0.3%
T4 Mancozeb (75 WP) – 0.1%
T5 Mancozeb (75 WP) – 0.2%
T6 Mancozeb (75 WP) – 0.3%
T7 Chorothalonil (75 WP) – 0.1%
T8 Chorothalonil (75 WP) – 0.2%
T9 Chorothalonil (75 WP) – 0.3%
T10 Copper oxychloride (50WP) – 0.1%
T11 Copper oxychloride (50WP) – 0.2%
T12 Copper oxychloride (50WP) – 0.3%
T13 Carbendazim (50WP) – 0.05%
T14 Carbendazim (50WP) – 0.1%
T15 Carbendazim (50WP) – 0.2%
T16 Propiconazole (25EC) – 0.05%
T17 Propiconazole (25EC) – 0.1%
T18 Propiconazole (25EC) – 0.2%
T19 Azoxistrobin (23SC) – 0.05%
T20 Azoxistrobin (23SC) – 0.1%
T21 Azoxistrobin (23SC) – 0.2%
T22 Control

Plate 19. Effect of fungicides against *Sclerotium rolfsii* (Sr3) *in vitro*

at all the tested concentrations (0.1 %, 0.2 % & 0.3 %) having inhibition percentage of 79.6 %, 72.22 % and 60.37 %. Chlorothalonil 75WP at its recommended concentration (0.2%) was on par with recommended concentration of azoxystrobin 23 SC (0.1 %) in suppressing mycelial growth of pathogen. Among all the treatments, 16 treatments showed more than 60 % inhibition on fungus. Least inhibition percentage was recorded with copper oxychloride (50WP) followed by carbendazim (50WP) having inhibition of 5.18 and 12.8 per cent at 0.1 % and 0.05 % respectively. They were not good at inhibiting the fungi even at their highest concentration tested such as 0.3% for copper oxychloride (50WP) and 0.2 % for carbendazim (50WP) where inhibition percentage was reduced below 35 % .

4.7. *IN VITRO* STUDIES ON COMPATIBILITY OF EFFECTIVE FUNGICIDES AND ANTAGONISTS

Biocontrol agents such as *T. viride* and *T. harzianum* which showed high potential of antagonism in dual culture experiment were selected for testing compatibility with most effective chemical fungicides such as mancozeb (75 WP), propiconazole (25EC) and chorothalonil (75 WP) which were found superior in poisoned food method. Compatibility of these biocontrol agents and fungicides were studied under *in vitro* condition by poisoned food technique (Plate 20). From this experiment it was observed that mancozeb 75 WP was more compatible to both *T. viride* and *T. harzianum* at 0.2 % concentration and among these 100 per cent compatibility was shown with *T. harzianum*. Only 7.61 % mycelial inhibition was recorded with *T. viride*. Propiconazole (0.1 %) and chlorothalonil (0.2 %) were highly incompatible with both species of *Trichoderma* where 100 % inhibition was shown by propiconazole over two fungi. Chlorothalonil also showed incompatibility with *T. viride* and *T. harzianum* having inhibition percentage of 77.14 and 74.11 respectively (Table 14).

Table 14. Effect of fungicides on antagonists *in vitro*

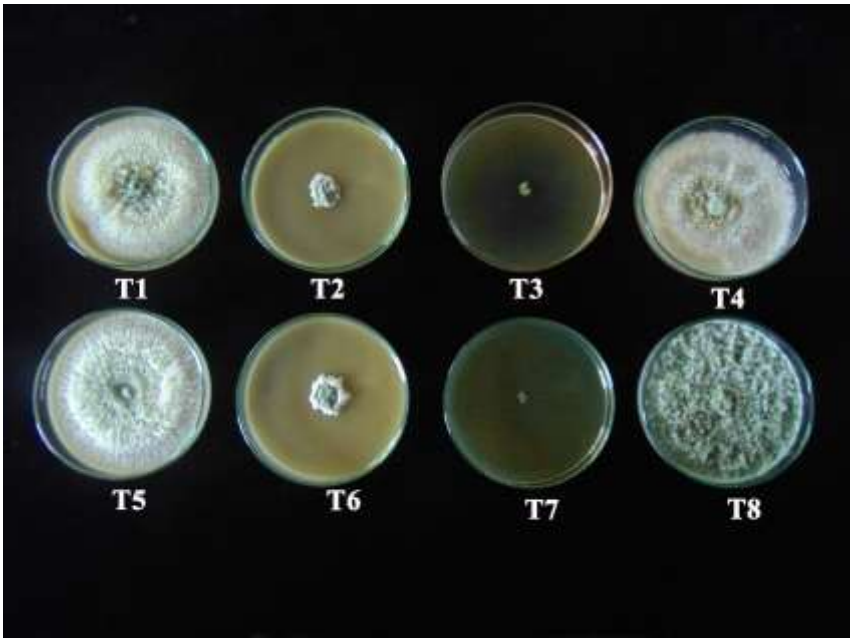
Treatment	Fungicide and biocontrol agent (Concentration in per cent)	Radial growth of biocontrol agent in petridish (cm)*	Percentage mycelial inhibition of biocontrol agent (%)*
T1	*T. viride* X Mancozeb (0.2 %)	8.32	7.61(16.00)[d]
T2	*T. viride* X Chorothalonil (0.2 %)	2.05	77.14 (61.40)[b]
T3	*T. viride* X Propiconazole (0.1 %)	0.00	100.00(90.00)[a]
T4	*T. viride* only	9.00	0.00 (0.00)[e]
T5	*T. harzianum* X Mancozeb (0.2 %)	9.00	0.00 (0.00)[e]
T6	*T. harzianum* X Chorothalonil (0.2 %)	2.33	74.11(59.40)[c]
T7	*T. harzianum* X Propiconazole (0.1 %)	0.00	100.00(90.00)[a]
T8	*T. harzianum* only	9.00	0.00 (0.00)[e]
		CD (0.05)	0.34
		SE (±)	0.12

*mean of four replications

Values in parenthesis are arcsine transformed

Observation taken after 4 days of inoculation

Figures with same letter do not have significant difference according to one way ANOVA at P=0.05



T1 *T. viride* X Mancozeb (0.2 %)
T2 *T. viride* X Chorothalonil (0.2 %)
T3 *T. viride* X Propiconazole (0.1 %)
T4 *T. viride* only
T5 *T. harzianum* X Mancozeb (0.2 %)
T6 *T. harzianum* X Chorothalonil (0.2 %)
T7 *T. harzianum* X Propiconazole (0.1 %)
T8 *T. harzianum* only

Plate 20. Effect of fungicides on antagonists *in vitro*

4.8. *IN VIVO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES

Best treatments from *in vitro* evaluation of biocontrol agents and chemical fungicides along with their compatible combinations were selected for *in vivo* studies. *T. harzianum*, *T. viride*, mancozeb 75 WP, propiconazole 25 EC, chlorothalonil 75 WP and combinations of mancozeb with *T. viride* and *T. harzianum* were considered as the treatments for field level evaluation (Table 15).

All plants were maintained in grow bags and arranged in one and half cent area. Potting mixture was fumigated with formaldehyde (40 %) before sowing the seeds (Plate 21). Among the 15 treatments, fungicides and bioagents were applied as soil drenching (seven treatments) and foliar spraying after 20, 40 and 60 days of sowing (Plate 22). Rest of the treatments fungicides and bioagents were applied as post sowing soil drenching and foliar spraying after 20, 40 and 60 days after sowing and inoculated control plants were maintained without any fungicides and bioagents application.

Seeds were germinated in all the treatments within five days of sowing except in grow bags drenched with propiconazole where more than ten days were taken and phytotoxicity symptoms were observed in the seedlings.

4.8.1. Percent Disease Incidence

Among the 15 treatments least and zero per cent incidence of stem rot disease was observed with mancozeb at 0.1 % (T1) which was given as pre-sowing and post sowing treatment. All treatments could reduce disease incidence below 50 % whereas in control plants 100 per cent disease incidence was observed (Table 16). Those treatments which included pre-sowing soil drenching showed superiority in disease reduction over the remaining treatments. Among the pre-sowing treatments, propiconazole (T2) and combination of mancozeb with *T. harzianum* (T6) and *T. viride* (T7) showed lesser percentage of disease incidence (8.33 %) which was on par with mancozeb (T8) given as post treatment only. 25 per cent disease incidence was recorded with cholorothalonil and among these treatments *T. viride* was found

Table 15. List of treatments used in field level management of *S. rolfsii*

Sl. No	Treatments (Concentration in percentage)	Method and time of application
T1	Mancozeb (75 WP) 0.1 %	Soil drenching 7 days before sowing + soil drenching and foliar spraying 20, 40 & 60 days after sowing
T2	Propiconazole (25EC) 0.05 %	-do-
T3	Chorothalonil (75 WP) 0.2 %	-do-
T4	*Trichoderma harzianum* 2 %	-do-
T5	*Trichoderma viride* 2 %	-do-
T6	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%	-do-
T7	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%	-do-
T8	Mancozeb (75 WP) 0.1 %	Soil drenching and foliar spraying 20, 40 & 60 days after sowing
T9	Propiconazole (25EC) 0.05 %	-do-
T10	Chorothalonil (75 WP) 0.2 %	-do-
T11	*Trichoderma harzianum* 2 %	-do-
T12	*Trichoderma viride* 2 %	-do-
T13	*Trichoderma harzianum* 2 % + Mancozeb (75 WP) 0.1 %	-do-
T14	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1 %	-do-
T15	Control	-

Plate 21. Fumigation of potting mixture

Plate 22. Field view of experimental plot

least effective since 41.67 % plants were infected by *S. rolfsii*. Foliar blight incidence was not observed in any of the treatments.

Same trend of disease incidence was recorded in treatments which applied only after sowing of the seeds. Among these, disease incidence was least when mancozeb was applied at 0.1% (8.33 %) followed by propiconazole at 0.05 % (25 %). Among the biocontrol agents applied, *T. harzianum* (2 %) (T11) was found superior having less incidence of disease (33.33%), which was on par with its combination with mancozeb (T13). *T. viride* (2 %) was least effective since 50 % plants were infected by *S. rolfsii* followed by chlorothalonil (0.2 %) with 41.67 % disease incidence.

4.8.2. Lesion Number

Number and size of lesions formed on the stem associated with Stem rot disease caused by *S. rolfsii* was recorded in all treatments (Table 16). Water soaked lesion was produced around the stem near soil line and it enlarged as it proceeds upwards (Plate 24). Lesion was continuous in almost all treatments so that number of lesion was noted as one. Lesion was absent in plants where soil was drenched with mancozeb 75 WP before sowing (T1). Plants subjected to propiconazole treatments produced four irregular shaped brown lesions on the stem which was restricted on the surface only. In most of the treatments water soaked lesion was confined to stem surface and it became dry and caused girdling of the stem. Though stem rot lesion was produced on the stem, in majority of the plants interior portion of the stem was free of pathogen and retained as green and healthy. Plants maintained as control produced water soaked lesion at the base of the stem followed by necrosis and complete disintegration which resulted in rapid yellowing and wilting of the plant. White mycelial growth and brown mature sclerotial bodies were found abundant on the infected part of these plants.

4.8.3. Lesion Size

Lesion size on the stem was found significantly differ (Table 16). Initially all lesions were found at stem base near soil line. Later it gradually expanded its size

towards the crown (Plate 24). Pre-sowing drenching of Mancozeb (75WP) (0.1 %) made the plants free of stem rot lesion. In all other treatments, no considerable increase in lesion size was observed after first two applications of treatments were over. Remarkable difference was noticed among treatments with respect to the time of application such as pre-sowing drenching produced small size of lesion as compared to treatments which applied only after sowing. Lesion area was highest for control plants which was on par with combination of *T. harzianum* and mancozeb (T6) and superior to all other treatments. Combination of mancozeb and *T. viride* (T14) was found on par with chorothalonil (0.2 %), *T. viride* (T12) and propiconazole (T9) at 0.05 %. Lesion area was least for mancozeb (T1) at 0.1 %.

4.8.4. Yield

Periodical harvest was made from cowpea plants after 45 days of planting. Effect of different treatments on yield of cowpea was clear from the results obtained during the *in vivo* evaluation (Table 17, Plate 23). Yield was measured in terms of average weight of pods per plant. Yield per hectare was extrapolated from the results obtained. Average number of pods per plant ranged from 5- 34 and weight from 20 to 135 g. Minimum yield was recorded from the control plants where average number and weight of pods were 4.7 and 20.7 g respectively. Treatments which included prophylactic soil drenching were found superior than the corresponding treatments without pre-sowing treatments.

Among the treatments pre-sowing and post sowing treatment with mancozeb 75WP (T1) at 0.1 % concentration were found superior with respect to yield (134.7g/plant) of cowpea which was on par with treatments (T2 & T9) including propiconazole 25EC at 0.05 % having yield of 130.7 & 133.7 g/plant. Post sowing treatment of mancozeb (T8) produced significantly higher yield (125.3 g/plant) at 0.1 % than biocontrol agents and its combinations. Combination of mancozeb (0.1 %) with *T. harzianum* (2 %) and *T. viride* (2 %) was found superior than the individual application of biocontrol agents. Among the biocontrol agents pre-sowing and post sowing treatment with *T. harzianum* at 2 % concentration (T4) produced

Table 16. Effect of different treatments on *S. rolfsii* in cowpea

Sl. No.	Treatments	Per cent disease incidence* (%)	No. of lesion on stem	Lesion area on stem surface (cm^2)
	Pre-sowing + post sowing treatments			
T1	Mancozeb (75 WP) 0.1 %	0.00 (0.00) [d]	0	0 [J]
T2	Propiconazole (25EC)0.05 %	8.33 (10.00) [cd]	4	5.1 [def]
T3	Chorothalonil (75 WP) 0.2 %	25.00 (25.00) [bcd]	1	3.1 [ghi]
T4	*Trichoderma harzianum* 2 %	25.00 (25.00) [bcd]	1	2.5 [hi]
T5	*Trichoderma viride* 2 %	41.67 (40.00) [b]	3	4.5 [efg]
T6	*Trichoderma harzianum* 2 % + Mancozeb (75 WP) 0.1 %	8.33 (10.00) [cd]	1	9.4 [a]
T7	*Trichoderma viride* 2 % + Mancozeb (75 WP) 0.1 %	8.33 (10.00) [cd]	1	1.57 [ij]
	Post sowing treatments only			
T8	Mancozeb (75 WP) 0.1 %	8.33 (10.00) [cd]	1	3.8 [fgh]
T9	Propiconazole (25EC) 0.05 %	25.00 (30.00) [bcd]	4	6.2 [bcd]
T10	Chorothalonil (75 WP) 0.2 %	41.67 (40.00) [b]	1	6.9 [bc]
T11	*Trichoderma harzianum* 2 %	33.33 (30.00) [bc]	1	4.1 [fg]
T12	*Trichoderma viride* 2%	50.00(45.00) [b]	1	6.6 [bcd]
T13	*Trichoderma harzianum* 2 % + Mancozeb (75 WP) 0.1 %	33.33 (35.00) [bc]	1	5.7 [cde]
T14	*Trichoderma viride* 2 % + Mancozeb (75 WP) 0.1 %	33.33 (35.00) [bc]	1	8.5 [b]
T15	Control	100.00 (90.00) [a]	1	10.1 [a]
CD (0.05)		27.08		1.57
SE (±)		9.4		0.53

*mean of three replications, Values in parenthesis are arcsine transformed
Figures with same letter do not have significant difference according to one way ANOVA at P=0.0

Table 17. Economics of field evaluation of different treatments for the management of stem rot and foliar blight of cowpea

Treatment No.	No. of pods/ Plant *	Weight of pods /plant (g)*	Yield (t/ha)	Cost over control (Rs./ha)	Benefit over control (Rs./ha)	B:C ratio
T1	33.7	134.7 ^{a}	9.9	2600	422222	1.94
T2	32.7	130.7 ^{ab}	9.7	5400	407407	1.86
T3	22.7	90.7 ^{f}	6.7	2760	259259	1.31
T4	24.7	100.7 ^{ef}	7.5	4200	296296	1.44
T5	18.0	72.3 ^{g}	5.4	4200	191358	1.04
T6	30.0	120.3 ^{bc}	8.9	3400	369135	1.73
T7	26.0	104.7 ^{de}	7.8	3400	311111	1.50
T8	31.3	125.3 ^{abc}	9.3	1950	387654	1.81
T9	32.7	133.7 ^{a}	9.9	4050	418518	1.92
T10	23.3	89.3 ^{f}	6.6	2070	254321	1.29
T11	24.0	95.7 ^{ef}	7.1	3150	277778	1.38
T12	17.0	70.3 ^{g}	5.2	3150	183950	1.01
T13	28.7	114.7 ^{cd}	8.5	2550	348148	1.65
T14	23.3	101.7 ^{ef}	7.5	2550	300000	1.47
T15	4.7	20.7 ^{h}	1.5	0	0	0.30
CD(0.05)	2.8	12.54				
SE (m)	0.9	4.3				

*mean of three replications

Figures with same letter do not have significant difference according to one way ANOVA at P=0.05

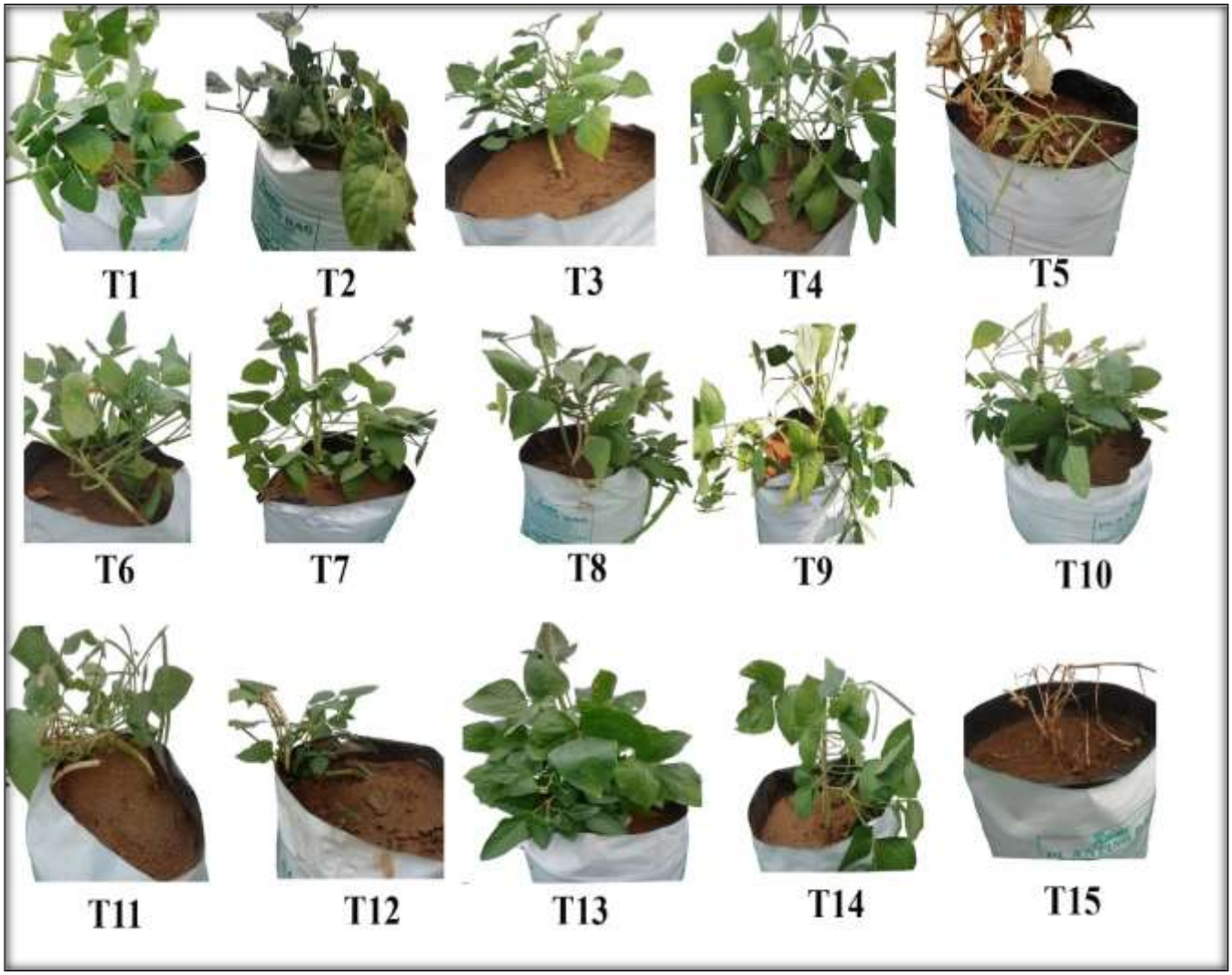


Plate 23. Effect of different treatments on cowpea during the management study of stem rot and foliar blight of cowpea.

Plate 24. Effect of different treatments on lesion size and lesion number on cowpea stem during the management study of stem rot and foliar blight of cowpea

significantly higher yield (100.7 g/plant) which was on par with post sowing treatment with *T. harzianum* (T11). Both these treatments were found superior than chlorothalonil 75WP (0.2 %). Among the fungicides and biocontrol agents least production of cowpea was recorded with *T. viride* alone (T12) at 2 % concentration having yield of 70.4g/plant.

4.8.5. Economics of Treatments

Data obtained from field evaluation on cowpea was subjected to economic analysis and benefit cost ratio (BC ratio) of each treatment was calculated. All treatments were found having BC ratio greater than one (Table 17). Highest BC ratio of 1.94 and highest benefit of 4,22,222/- per hectare over control were observed in T1 (Mancozeb 75WP) followed by T9 (propiconazole 25EC – 1.92). Combinations of Mancozeb and *Trichoderma* had higher BC ratio (1.73 & 1.65). Among the biocontrol agents, *T. harzianum* showed highest BC ratio of 1.44 which was higher than the BC ratio of chlorothalonil. Among the fungicides and biocontrol agents lowest BC ratio was recorded with *T. viride* (1.01) whereas in control plants, BC ratio was very low (0.3).

4.8.6. Incidence of Other Pests and Diseases

Apart from stem rot disease caused by *S. rolfsii*, other pest and disease incidence were noted in cowpea during field level examination and proper management at early stages could reduce the extent of damage (Table 18).

4.8.6.a. Aphid Infestation

Aphid (*Aphis craccivora*) infestation was noticed in two cowpea plants at its initial stages (10 leaf stage). Dense colonies of aphid were observed at base of the stem and growing parts. Since it was the early stage of infestation, no characteristic symptoms were produced on the plant (Plate 25A). Application of pongamia oil emulsion (2 %) reduced the infestation completely. Symptoms of cowpea bud necrosis virus disease were observed in some cowpea plants at its earlier stages.

Table 18. Incidence of other pests and diseases in cowpea

Pest and disease	Symptom	Management adopted
Aphid infestation	Initial stages (10 leaf stage of cowpea). Dense colonies of aphis at growing tip of plants.	Application of pongamia oil (2%)- reduction in infestation
Intumescence (Oedema) on plant	Greenish-white galls on the leaf Rusty coloured minute spots	Optimum amount of water was maintained by avoiding over irrigation
Bud Necrosis Virus Disease	Initial stages of cowpea Typical chlorotic spots	Application of neem oil garlic emulsion (2%) reduced spread of the disease.

4.8.6.b. Intumescence (Oedema) on Plant

Oedema appeared on the leaf since two leaf stage of plants numerous small bumps on the leaf. First observed symptom was greenish-white galls on the leaf followed by rusty colored minute spots (Plate 25 B).

4.8.6.c. Bud Necrosis Virus Disease

Typical chlorotic spots were observed on the leaves of some young cowpea plants. Later it turned to necrotic spots. Plants showed symptoms of this virus disease were removed from the field and neem oil garlic emulsion (2 %) were sprayed which reduced spread of the disease (Plate 25 C).

A. Aphid infestation

B. Intumescence (Oedema) on plant

C. Bud Necrosis Virus Disease

Plate 25. A, B & C -Incidence of other pests and diseases

Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp)) is one of the most demanding vegetable and legume crops in Kerala. Since it is nutritionally rich, its cultivation is highly promoted by farmers throughout the year. Nowadays stem rot and foliar blight disease has emerged as a severe problem in Kerala fields. Disease incidence is more common during monsoon season. This disease is a threat in cowpea cultivation since the pathogen survive by sclerotia and is primarily soil borne, its survival in debris, weeds and wide host range make it very difficult to manage. Hence reliant on any single method for control may not be effective for suppression of disease. Study was conducted on 'Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)' during 2018-2020 with an aim of identifying and characterizing fungal pathogen causing stem rot and foliar blight of cowpea and developing a management strategy against the pathogen using various biocontrol agents and chemical fungicides.

5.1. COLLECTION OF SAMPLES

Cowpea is one of the most cultivated vegetable crops in Kerala especially in Kasargod district. Since cowpea is considered as most priority choice for farmers, cultivation is not restricted to a particular place and found throughout the district. Hence collection of diseased samples from different locations gave a detailed description on distribution of disease and its prevalence. Disease incidence and disease index of stem rot and foliar blight disease at Cheemeni, Pallikkara, Nileswar, Trikaripur, Udinur and Periya showed variability with higher percent disease incidence (80 %) and disease index (48 %) at Nileswar. A range of 12 to 80 % and 8 to 51 % were observed for disease incidence and disease index respectively. From this data it is clearly understood that stem rot and foliar blight disease is prevalent in Kasargod. Distribution of *S. rolfsii* in the tropical and subtropical regions was reported by Aycock (1966).

Variety of host plants, relative humidity, temperature, soil parameters like moisture, pH and genetic potential of the pathogen may be the reason for the variability in disease incidence observed across the fields. This is in line with

Poornima (2015) who conducted survey during *Kharif* season and reported different value of percent incidence of stem rot and pod rot of groundnut across the fields of Karnataka.

One of the main observations during sample collection was that high humidity caused by rainfall created favourable conditions for the sclerotium to germinate and cause infection in the samples. Edmund *et al.*, 2003 already observed that high moisture in the soil enhanced infection of *S. rolfsii*. It was observed that, rain water is the main source of spread of sclerotium in the soil which led to severe incidence in Nileswar areas. Pande and Rao (2000) also observed high percentage of *Sclerotium* infection in Andhra Pradesh during rainy season. Lack of recommended spacing between cow pea plants created high population density which further led to high humidity condition as described by Punja (1985). In Pallikkara area, weed attack was high and weeds were showing the same symptoms of Sclerotium infection. This might be the reason for stem rot symptom of cowpea in this area. It indicated that weed plants can also act as the host of *S. rolfsii* and pathogen can survive on it. Farmers in Udinur followed monocropping of cowpea continuously in the same field without following crop rotation and any management measures. *S. rolfsii* is a soilborne fungus and sclerotial bodies can remain in soil for long period (Smolińska and Kowalska, 2018). Therefore this might be the reason for inoculum development in the soil over the seasons and cause epidemic of stem rot disease (Chaube and Singh, 2011). The soil acidity, which favours infection of *S. rolfsii* might be one of the reasons for prevalence of stem rot disease in Kasargod (Kator *et al.*, 2015).

5.2. ISOLATION OF THE PATHOGEN

Six isolates of pathogen were obtained from infected tissues of the collected samples. Isolation from both stem portion and leaf portion were equally successful and produced white fungal mycelium in the PDA within 24 h. Fery and Dukes (2002) used dry sclerotia for isolation of *S. rolfsii* instead of infected stem bits. Sudina *et al.* (2014) isolated *S. rolfsii* from leaf tissues of cowpea. Isolation of *S. rolfsii* from different host plants was reported by several scientists which pointed out wide host

range of this pathogen (Sennoi *et al.*, 2010; Tortoe and Clerk, 2012; Songvilay *et al.*, 2013: Kwon *et al.*, 2014).

5.3. PATHOGENICITY TEST

Present study included pathogenicity testing of six isolates in cowpea by following Koch's postulates. *S. rolfsii* was pathogenic to many species of plants which included legumes also (Aycock, 1966; Punja, 1985). Both Soil and leaf inoculation methods have the ability for faster infection on cowpea. Multiplication of pathogen in paddy grains could produce abundant fungal inoculum for the study and also production of white mycelia and sclerotial bodies were earlier and faster compared to culture production in petridish. Similar to paddy grains, other cereal and millet grains such as sorghum, wheat, millets and maize were also reported suitable for mass multiplication of *S. rolfsii* (Singh and Thapliyal, 1998; Adandonon *et al.*, 2004; Yaqub and Shahzad, 2005; Eslami *et al.*, 2015).

All isolates showed infection in cowpea with variation in days taken for infection. Infection process was same in all plants, ie plants underwent same stages of disease development such as water soaked lesion at the base of the stem, formation of white fungal mycelia and mustard like sclerotial bodies, wilting and yellowing of leaves and death of the plants. Same symptoms were noticed by Karat *et al.* (1985). Since high percentage of humidity was maintained around the plants, disease progression was faster. Foliar infection also started with water soaked lesion and white mycelial growth over the lesion. Later it led to browning and drying of the entire leaf area. Small sclerotial bodies were also noticed on the developing lesion. Foliar lesion was continuous and resulted in defoliation.

Lesion number on the stem and leaves were same for all isolates, but lesion size were different. Lesion was initiated from the collar region and then increased its area upwards. Mycelium was present in the stele of the crown region of cowpea and hyphae penetrate in to the stem and roots which led to the complete disintegration of the central cylinder (Paintin, 1928). Lesion formed on the stem surface was continuous and brown discolouration was observed at the inner parts of stem. Among

these largest area of lesion was produced by Sr3 isolate (6.4 cm^2) followed by Sr1 (4.5 cm^2) and Sr4 (4.1 cm^2).

Mechanism of infection by *S. rolfsii* can be predicted with respect to enzymes involved during the process. Production of enzymes by *S. rolfsii* during infection can cause changes in plant cells and that will lead penetration of the pathogen into the cells and tissue disintegration. Pectin methylesterase, cutinase, phosphatidase, arabanase, galactanase, mannanase, xylanase and J3-g1ucosidases are the extracellular enzymes produced by *S. rolfsii* which might have role in pathogenesis. Cellulase enzyme produced by the pathogen during infection could also be one of the reasons for tissue destruction during infection (Punja, 1985). Production of pectic enzymes by *S.rolfsii* was reported as the reason for tissue maceration in the plant (Bateman (1972). These enzymes can degrade pectin in the cellwall result in liquefaction of the pectic substances and weakening of cell wall. All these process can lead to tissue maceration. Oxalic acid production of *S. rolfsii* was reported by Kritzman *et al.* (1977) during the process of infection which also considered as the reason for pathogenesis.

Disease development in the plant depends on pathogen factors, host factors and environmental factors (Keane and Kerr, 1997). Since uniform environmental conditions were provided for all plants and cowpea plants selected for pathogenicity test were of same variety and same age, hence virulence of the pathogen determined the intensity of disease. From these observations, it was revealed that Sr3 inoculated plants showed early infection and wilting symptom. Lesion area produced on the stem (6.4 cm^2) and leaf (9.6 cm^2) also were highest compared to other isolates. Field from where it collected also had higher percentage of disease incidence and index. Same isolate also showed very fast growth rate in PDA. All these data pointed out that Sr3 as the most virulent isolate. Eslami *et al.* (2015) used criteria like wilting of the plant, yellowing or death, sclerotia produced on the stem/soil and lesion length for virulence rating.

Pandey *et al.* (2005) reported that among the eight isolates of *S. rolfsii*, most virulent one possessed highest mycelial growth rate in petridish. Since all isolates were obtained from different localities, role of environmental factors in virulence of the pathogen is important along with genetic factors of the fungus (Narayanaswamy, 2011).

5.4. IDENTIFICATION OF THE PATHOGEN

Cultural, morphological and molecular characterization of the virulent isolate Sr3 was done to identify the pathogen.

5.4.1. Cultural Characterisation

Cultural characterization of fungus has important role in identification. White mycelia, sclerotial initials and sclerotial bodies are the main identification keys of *S. rolfsii* (Manu *et al.*, 2018). Same colony characters were observed in *Rhizoctonia solani* having difference in shape and size of sclerotia and pattern of mycelia growth (Desvani *et al.*, 2018).

Sr3 was a very fast growing fungi which cover the petridish of 90 mm diameter within 4 days of inoculation and started to grow over the lids after that. Cottony, fluffy and thick mycelium of pure white colour with slight zonations were the characteristics of this fungus.

White tufts of loosely intertwined small branches of fungal hypha called sclerotial initials having round shape were produced in the fungal colony after eight days of inoculation. This observation was in line with reports of Higgins (1922) and El-Nagar *et al.* (2013). Maturation of sclerotial initials resulted in round and smooth sclerotial bodies in the fungal colony after 14 days of inoculation. Total number of sclerotia formed in the fungal colony ranged from 25 to 30. Size of sclerotia ranged from 1 to 6 mm. Peripheral and central arrangement of sclerotia were also noticed. These are in line with observation made by Nandi *et al.* (2017) and Shete *et al.* (2018).

Cultural variability of fungal isolates was observed with respect to colony colour, pattern of mycelial growth, growth rate, number and size of sclerotium.

Variability may be due to differences in soil type, variety of cowpea and other environmental factors across the geographical regions from which isolates were obtained. Same observation was made by Sulladmath *et al.* (1977) and Sarma *et al.* (2002). Mutation (Marquardt, 1972), hybridization (Stukenbrock, 2016), heterokaryosis (Parmeter *et al.*, 1963) and other genetical factors can also induce variations in fungal pathogen. Cultural variability was also studied in different isolates obtained from the same and different host plant (Pandi *et al.*, 2017 and Srividya *et al.*, 2018).

5.4.2. Morphological Characterisation

Present study revealed that the pathogen had two types of hypha with one having coarse, straight and large cells (width of 150-250 µm) with clamp connection at septa. Branching at the place of clamp connection was also noted on some of the hyphae. Second type of hypha was slender which produced branches irregularly without any clamp connections. Same characters were also noted by Higgins (1922) and he explained clamp connections as the property of broad hyphae. Kwon and Park (2004) observed that aerial mycelium produced in PDA had narrow mycelial strands having 4.2- 10.4 µm width and presence of clamp connections. Koné *et al.* (2010) also confirmed presence of clamp connections at every septum of fungal hypha with large straight cells.

Cross section of sclerotium revealed that it had soft cream coloured inner portion and internal differentiation into different layers such as outer thick skin followed by rind with thick cells, cortex with thin walled cells and medulla region of loosely filamentous hyphae. Same result was recorded by Chet *et al.* (1969) and presence of differentiated cells of thick walled rind, cortex and medulla cells filled with reserve materials were explained by him. Willets (1972) also made explanation on melanized rind surrounding hyphal aggregates as typical characters of true sclerotium and variability in the hyphal cells of medulla region among different species. Internal differentiation into different layers was absent in sclerotium of *Rhizoctonia solani* which helps in identification of both fungi (Agrios, 2005).

Based on the results of cultural and morphological studies, it was understood that most virulent isolate Sr3 met all characters of *S. rolfsii* described by Chet *et al.* (1969). Therefore, the pathogen causing stem rot and foliar blight of cowpea was identified as *Sclerotium rolfsii* Sacc.

5.4.3. Molecular Characteristics

Identification of microbial culture at molecular level was done by using D1/D2 region of LSU (Large Sub Unit: 28S rDNA) based molecular technique.

Based on the D1/D2 Region- PCR analysis, the fungal culture Sr3 showed 99.61 % similarity with *Athelia rolfsii* (Accession No:JN811675.1), perfect stage of *S. rolfsii*. According to Punja and Li-Juan (2001), RAPD analysis and mycelial compatibility groupings was used for finding phylogenetic relationships between 132 isolates of *S. rolfsii* from 36 host species of 13 countries. rDNA ITS sequencing protocol was used by Okabe and Matsumoto (2003) for getting the phylogenetic relationship for *S. rolfsii* and *S. delphinii*. Rasu *et al.* (2013) also used RAPD-PCA analysis for the differentiation of *S. rolfsii* isolated from different host plants in Tamil Nadu and revealed that all the isolates had 54 % similarity coefficient.

Based on the cultural, morphological and molecular characterization, isolate Sr3 responsible for stem rot and foliar blight disease was confirmed as *S. rolfsii*.

5.5. SYMPTOMATOLOGY OF DISEASE

Symptomatology of stem rot and foliar blight disease in cowpea under natural conditions were compared with symptoms produced in artificially inoculated plants. Post infectional changes occurred in artificially inoculated plants were exactly same as that of natural infections with respect to stem rot disease in cowpea.

Since it was a soil borne fungus, infection started with water soaked brown lesions at the base of the stem which expanded its area with disease progress. As a result lower leaves started to droop followed by wilting and yellowing of all leaves. This was according to the observation made by Karat *et al.* (1985). The characteristic feature of sclerotium stem rot disease was white thread like mycelial strands along

with sclerotial initials and mustard like sclerotial bodies on the infected region. This characteristic symptom distinguish stem rot disease by *S. rolfsii* from stem rot caused by other fungi. Lesion became necrotic and rotted along with girdling of stem. Girdling of the stem was the main reason for wilting of the plant infected with *S. rolfsii*. In some plants, even though water soaked lesion was produced at the stem base, it did not show wilting symptom. Surface lesions on the stem will not cause wilting, since it doesn't interrupt the translocation of water and minerals and internal tissues remain green and healthy. Under favourable conditions like high humidity (> 80 %), high moisture and high temperature (21^0C to 30^0C), disease development became more fast (Borah and Saikia, 2019). Water saturated soil was found more favourable for sclerotial germination and fungal mycelial growth (Mullen, 2001). Infection with *S. rolfsii* in all plants followed same sequence of morphological changes in the plants which were clearly explained by Jenkins and Averre (1986) in tomato, Anahosur (2001) in potato and Remesal *et al.* (2010) in pepper also.

Foliar symptoms produced by pathogen in artificially inoculated plants showed characteristic difference in concentric ring formation when compared to plants had natural infections. In both natural and artificial infections light brown and circular water soaked lesions were produced in the infected leaves. But in natural conditions lesion expanded with three to four concentric rings on both side of the leaf and turned to dark brown in colour with irregular shape. White mycelia of pathogen were clearly visible on the lesions which later produced brown mustard like sclerotial bodies. Same observations were made by Sudina *et al.* (2014) and Mahadevakumar *et al.* (2015). High humidity and high inoculum density at the place of inoculation might be the reason for lack of concentric ring formation under artificial conditions. In the present study some infections turned entire leaf into yellow but under dry climate, lesion became dry without showing complete yellowing of leaves and fell down producing shot hole symptom. It clearly explained role of environmental factors on symptomatology of disease.

5.6. *IN VITRO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES AGAINST PATHOGEN

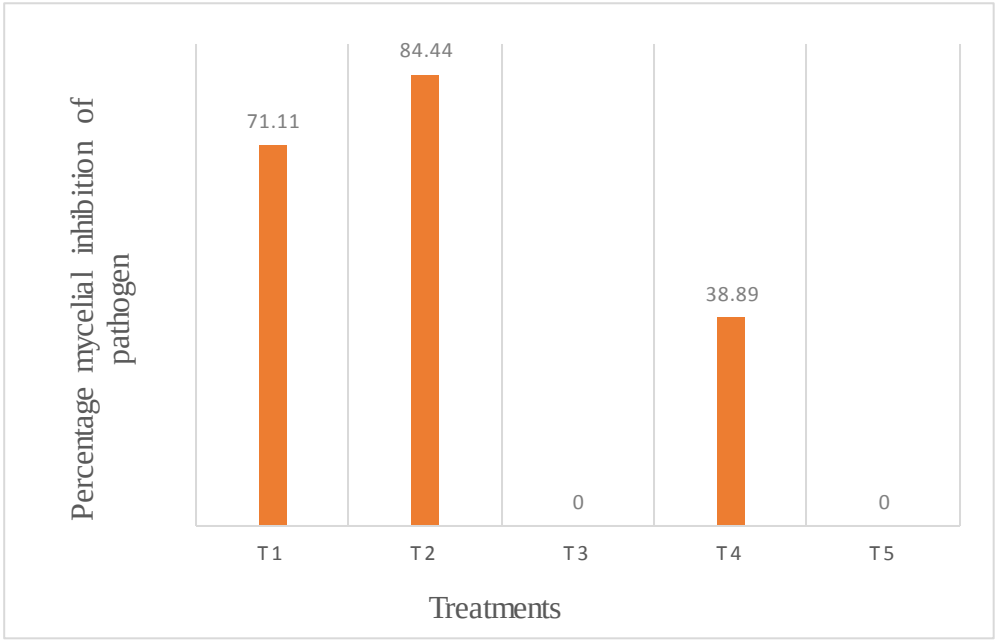
Four biocontrol agents released by KAU and seven fungicides available in the market were evaluated against most virulent isolate of *S. rolfsii*, Sr3 by dual culture and poisoned food technique respectively.

5.6.1. *In Vitro* Evaluation of Biocontrol Agents

Since present agriculture gives more focus on eco-friendly strategies in plant disease management, KAU released biocontrol agents such as *T. viride*, *T. harzianum*, *P. fluorescens* and *B. subtilis* were evaluated to find out their potential against *S. rolfsii* by dual culture method (Fig.1).

Hyphal coils, appressoria and hooks produced by *Trichoderma* on the pathogen and production of β (1-3) glucanase and chitinase enzymes might be the reason for mycoparasitism (Elad *et al*., 1983). Chitinase enzyme can degrade chitin of *S. rolfsii* which is the component of fungal cell wall. Same observation was made by El-Katatny *et al.* (2001) and Hirpara *et al*. (2017). Coiling of hypha of *S. rolfsii* by *T. harzianum* and penetration into the host hyphae by haustoria like structure were in mycoparasitism (Vinod Babu, 2006).

In the present study bacterial biocontrol agents were not effectively inhibiting *S. rolfsii* under *in vitro* assay where as B. *subtilis* checked radial growth of fungus about 38.89%. *P. fluorescens* showed zero percentage inhibition over the pathogen. These results are in accordance with observations made by Madhavi and Bhattiprolu (2011). Kachhadia (2013) also revealed that *P. fluorescens* was not much effective in inhibiting *S. rolfsii*, but according to him *B. subtilis* was tested to have more antifungal activity. Findings of Mundhe *et al*. (2009) also not in line with present results, since they could report high antifungal activity for *P. fluorescens* and *B. subtilis* with more than 70% inhibition over control.



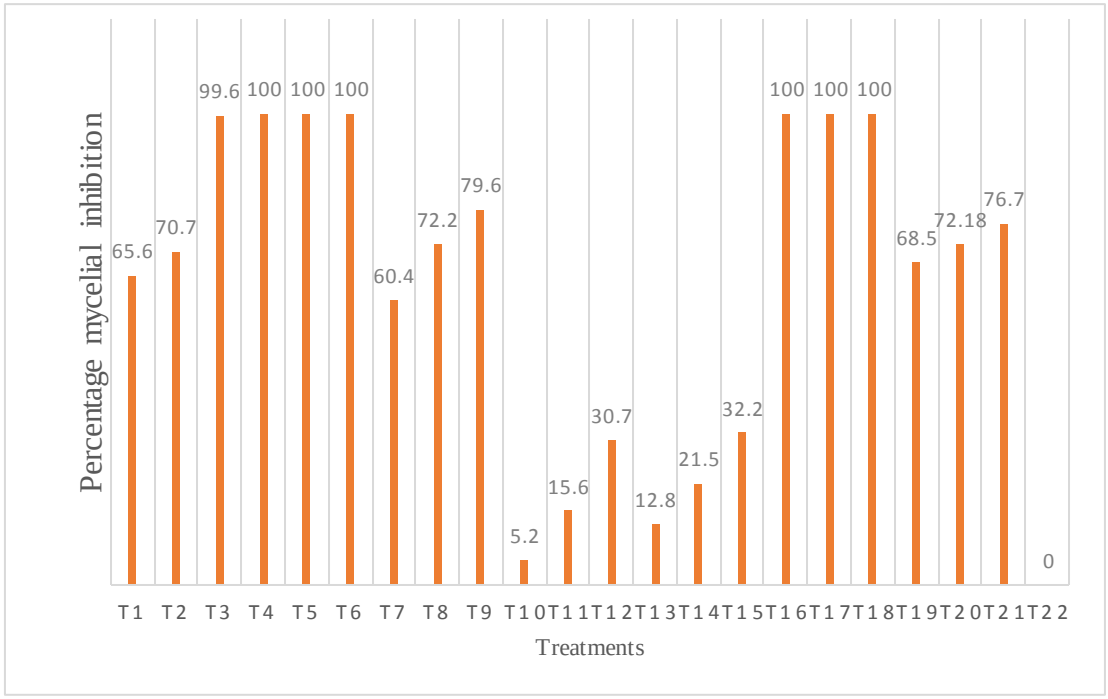
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Pseudomonas fluorescens*
T4	*Bacillus subtilis*
T5	Control

Fig.1. Effect of biocontrol agents against *Sclerotium rolfsii in vitro*

5.6.2. *In Vitro* Evaluation of Chemical Fungicides

Seven fungicides were evaluated against *S. rolfsii* (Sr3) at three different concentrations by poisoned food technique. Among the fungicides tested, mancozeb 75 WP (0.1 %, 0.2 % & 0.3 %) and propiconazole 25EC (0.05 %. 0.1 % & 0.2 %) were found best having 100 % inhibition followed by thiram75WS having 99.63 % inhibition at its highest concentration (0.3 %) (Fig. 2). Prabhu and Hiremath (2003) made similar conclusions in which propiconazole, thiram and mancozeb showed more efficacy in inhibition of fungi under *in vitro* conditions. 83% inhibition of *S. rolfsii* by mancozeb was reported by Manu *et al.* (2012). Inhibition of sclerotial production by sulphur containing compounds was described by Trevethick and Cook (1971) which pointed out the fungicidal action of mancozeb which contains thiol group. Effect of sulphur containing aminoacid like methionine in suppression of sclerotial formation was well explained by Paster and Chet (2011).

Chlorothalonil (75 WP) was found highly effective against *S. rolfsii* at all the tested concentrations (0.1 %, 0.2 % & 0.3 %) with inhibition percentage of 79.6 %, 72.22 % and 60.37 %. Chlorothalonil 75WP at 0.2% and azoxistrobin 23 SC at 0.1% were having similar effect on the pathogen. Among the fungicides, the performance of copper oxychloride (50WP) and carbendazim (50WP) were not effective, since they showed least inhibition of *S. rolfsii* of 5.18% for copper oxychloride (50WP) (0.1 %) and 12.8 % for carbendazim (50WP) (0.05 %). Effectiveness of both these chemicals were low on inhibition of the fungi even at their highest concentration tested such as 0.3 % for copper oxychloride (50WP) and 0.2 % for carbendazim (50WP). Rakholiya (2015) also reported low effectiveness of copper oxychloride and 100% inhibition by mancozeb. Superiority of mancozeb over chlorothalonil was reported by Sharma and Dhruj (2018). According to Rangarani *et al.* (2017), 100 % inhibition was recorded with propiconazole and poor performance was noticed with carbendazim. Bhagat and Chakraborty (2013) also observed low efficiency of carbendazim at 0.1 % against *S. rolfsii*. Higher efficiency of azoxystrobin was reported by Grichar *et al.* (2000).



T1	Thiram (75WS) – 0.1%
T2	Thiram (75WS) – 0.2%
T3	Thiram (75WS) – 0.3%
T4	Mancozeb (75 WP) – 0.1%
T5	Mancozeb (75 WP) – 0.2%
T6	Mancozeb (75 WP) – 0.3%
T7	Chorothalonil (75 WP) – 0.1%
T8	Chorothalonil (75 WP) – 0.2%
T9	Chorothalonil (75 WP) – 0.3%
T10	Copper oxychloride (50WP) – 0.1%
T11	Copper oxychloride (50WP) – 0.2%
T12	Copper oxychloride (50WP) – 0.3%
T13	Carbendazim (50WP) – 0.05%
T14	Carbendazim (50WP) – 0.1%
T15	Carbendazim (50WP) – 0.2%
T16	Propiconazole (25EC) – 0.05%
T17	Propiconazole (25EC) – 0.1%
T18	Propiconazole (25EC) – 0.2%
T19	Azoxistrobin (23SC) – 0.05%
T20	Azoxistrobin (23SC) – 0.1%
T21	Azoxistrobin (23SC) – 0.2%
T22	Control

Fig.2. Effect of fungicides against Sclerotium rolfsii *in vitro*

5.7. *IN VITRO* STUDIES ON COMPATIBILITY OF EFFECTIVE FUNGICIDES AND ANTAGONISTS

Integration of biocontrol agents and fungicides are highly recommended in the present day agriculture. Since majority of the highly efficient fungicides exhibit antifungal effect on beneficial microbes like *Trichoderma*, their application in the field are not being promoted.

In this context compatibility test of *T. viride* and *T. harzianum* with mancozeb (75WP), propiconazole (25EC) and chorothalonil (75WP) was essential. It was observed that mancozeb 75WP was highly compatible to both *T. viride* and *T. harzianum* at 0.2 % concentration. Among these 100 % compatibility was shown with *T. harzianum* and 7.61 % mycelial inhibition with *T. viride*. So combination of mancozeb and *Trichoderma* can be recommended. Similar result was reported by Vasundara *et al.* (2015) where 7 % inhibition was noted in *T. viride* at 3000 ppm of mancozeb. Kumar (2019) also found compatibility of *T. viride* with mancozeb 75WP. Shashikumar *et al.* (2019) explained that both *T. viride* and *T. harzianum* were highly compatible with mancozeb.

100 per cent inhibition was shown by propiconazole over two fungi at 0.05 % concentration. Chlorothalonil also showed incompatibility with *T. viride* and *T. harzianum* having inhibition per cent of 77.14 and 74.11 respectively. Tomer *et al.* (2018) reported that propiconazole was toxic to *T. harzianum* which resulted complete incompatibility with each other, but negligible toxic effect was expressed by mancozeb at 25, 50, 75 and 100 ppm concentration towards this fungus. Kumar *et al.* (2019) explained 75.29 % incompatibility of chlorothalonil with *T. viride* at 1000 ppm concentration which is in line with the result obtained from the present experiment.

5.8. *IN VIVO* EVALUATION OF BIOCONTROL AGENTS AND CHEMICAL FUNGICIDES

Highly effective fungicides and biocontrol agents which produced good result in the *in vitro* evaluation were brought into the field. All the results obtained under *in vivo* evaluation was in line with the results of *in vitro* evaluation.

Cowpea seeds sown in soil which was undergone pre-sowing drenching with propiconazole 25EC at 0.05 % showed late germination, phytotoxic action and some of the seeds failed to germinate (Plate 26). Germinated seeds also showed abnormality in growth. Under *in vitro* studies growing in paper towel dipped in propiconazole (0.05 %), some seeds showed drying at the tip of radicle (Plate 27). It might be the reason for abnormality and failure of germination under field conditions.

Among the 15 treatments lower percentage of disease incidence was noticed in those treatments which included prophylactic drenching (Fig.3). Plants undergone only post sowing treatments were found to have comparatively higher disease incidence which were less than 50 %. Control showed 100 percent disease incidence. With respect to disease incidence, mancozeb (T1) (0.1 %) was found best having zero per cent incidence of stem rot disease. Those treatments which included pre-sowing soil drenching mancozeb was followed by propiconazole (0.05 %) and combinations of mancozeb with *T. harzianum* and *T. viride*. All of these showed 8.33 % disease incidence. Charde *et al.* (2002) reported greater potential of propiconazole in suppressing the stem rot disease in groundnut. Shirsole *et al.* (2019) reported that treatment with propiconazole and mancozeb significantly reduced collar rot disease in chickpea and percent disease incidence was zero and 16.66 % respectively. Among the biocontrol agents *T. harzianum* (25 %) was best compared to *T. viride* (41.67 %) and also on par with chlorothalonil at 0.2%. According to Mohamed *et al.* (2012), lower incidence of *S. rolfsii* infection was reported in cowpea treated with *T. harzianum* and explained that increased production of peroxidase and polyphenol oxidase was the reason for this.

Plate 26. Phytotoxicity symptoms on cowpea grown in soil drenched with propiconazole (0.05%) at seven days before sowing

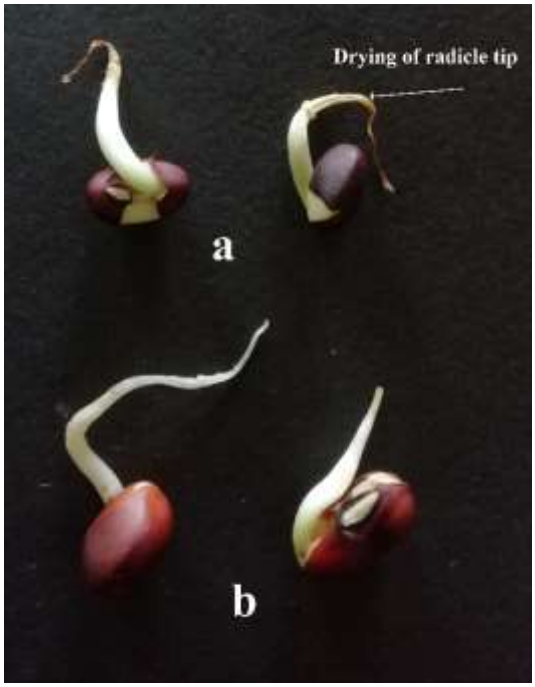


Plate 27. Effect of propiconazole (0.05%) on cowpea seed germination a. seeds germinated in propiconazole (0.05%) showed drying of tip of radicle. b. seeds germinated in water showed normal growth of radicle

Jegathambigai *et al.* (2010) revealed that *Trichoderma* mass multiplied in paddy and barley seeds when applied in field could reduce the incidence of disease by 75.54 %. Same order of superiority was seen among the fungicides and biocontrol agents which were applied as post sowing only. Among this mancozeb (8.33 %) was followed by propiconazole (25 %). Disease incidence of combinations of mancozeb and *Trichoderma* was same as that of individual application of *T. harzianum* (33.33 %) which was superior to chlorothalonil 25EC. 50 % plants showed stem rot incidence in plants undergone treatment with *T. viride.*

Water soaked lesions were formed at the basal region of cowpea plants which was clearly explained in many plants infected with *S. rolfsii* (Kwon *et al.*, 2009; Remesal *et al.*, 2010; Paul *et al.*, 2017; Bhuiyan *et al.*, 2019). In majority of the plants lesion was continuous and numbered as one. But prophylactic drenching of mancozeb at 0.1% made the plants free from infection and lesion formation. Plants undergone treatments with propiconazole 25EC could produce four irregular shaped brown lesions on the stem.

From the study it was observed that there was no relationship with lesion area and wilting of the plant, since it was seen outer surface of the stem only. Same observation was reported by Mullen (2001) where he noticed decay of stem cortex in tomato and pepper, but stem cylinder did not decay and plants did not show wilting symptom. Paintin (1928) also reported that complete disintegration of central cylinder was the reason for wilting of the plant. Even though stem rot lesion was produced on the stem, in majority of the plants interior portion of the stem was free of pathogen and retained as green and healthy, but in inoculated control plants complete rotting of the stem base was happened and which led to wilting and drying of the plant. Observable variations in lesion size between treatments were found. Initially all lesions were found at stem base near soil line. Then it gradually expanded upwards. No considerable increase in lesion size was observed when first two applications of treatments were over.

Treatments with prophylactic soil drenching produced comparatively higher yield than the corresponding treatments having only post sowing treatments since the pre sowing treatments can protect the younger stage of crop and reduce the early infection. Chauhan *et al*. (1988) reported that pre-sowing drenching was most effective than post sowing drenching. Mancozeb applied at 0.1 % was the best and most effective treatment followed by propiconazole 25EC (130.7 g) at 0.05 % (Fig.4). According to Gour and Pankaj (2010) field application of propiconazole 25EC for the suppression of *S. rolfsii* infection in groundnut resulted 36.0 % increase in pod yield when compared to control plants. Maji and Nath (2016) found out high ability of propiconazole and mancozeb for the reduction of collar rot in sunflower and recorded 14.5 % and 4.46 % increase yield respectively over control.

Performance of mancozeb and *Trichoderma* combinations were remarkable, since they could produce comparatively higher yield and benefit over control (Fig.5) than applications of *Trichoderma* alone. Among the biocontrol agents *T. harzianum* (2 %) was best, which was higher than chlorothalonil 75WP also. Dutta and Das (2002) also explained efficiency of *T. harzianum* for suppressing collar rot of tomato and producing high yield which was greater than *T. viride* and less than mancozeb. Among the treatments least production of cowpea was observed with *T. viride*. But Jadon (2011) observed that brinjal seedlings dipped in *T. viride* @ 4g/Kg was found best for collar rot disease.

Benefit cost ratio explains economic viability of a treatment from the farmer's point of view. Therefore BC ratio is an important parameter to be considered while analysing the treatments. Highest BC ratio was obtained for mancozeb (1.94) followed by propiconazole (1.92) (Fig.6). Combinations of biocontrol agents and mancozeb produced higher benefit (1.47 to 1.73) followed by *T. harzianum* (1.38 to 1.44). Lowest value was estimated with *T. viride* (1.01), whereas control plants showed BC ratio of 0.30.

From the present study, it was clearly understood that, *S. rolfsii* was the causal organism of stem rot and foliar blight disease which could cause severe yield

reduction in cowpea. Prophylactic drenching of mancozeb (0.1 %) or propiconazole (0.05 %) or combinations of mancozeb and *T. harzianum* or *T. viride* at 7 days before sowing and post sowing drenching and spraying at 20, 40 and 60 days after sowing can be recommended for the management of stem rot and foliar blight disease in cowpea. Since the efficiency of *T. harzianum* is highly notable, it pointed out the scope of ecofriendly management of disease. When we approach for integrated management of stem rot and foliar blight of cowpea, fungicides, biocontrol agents and their combinations mentioned here can be well utilized.

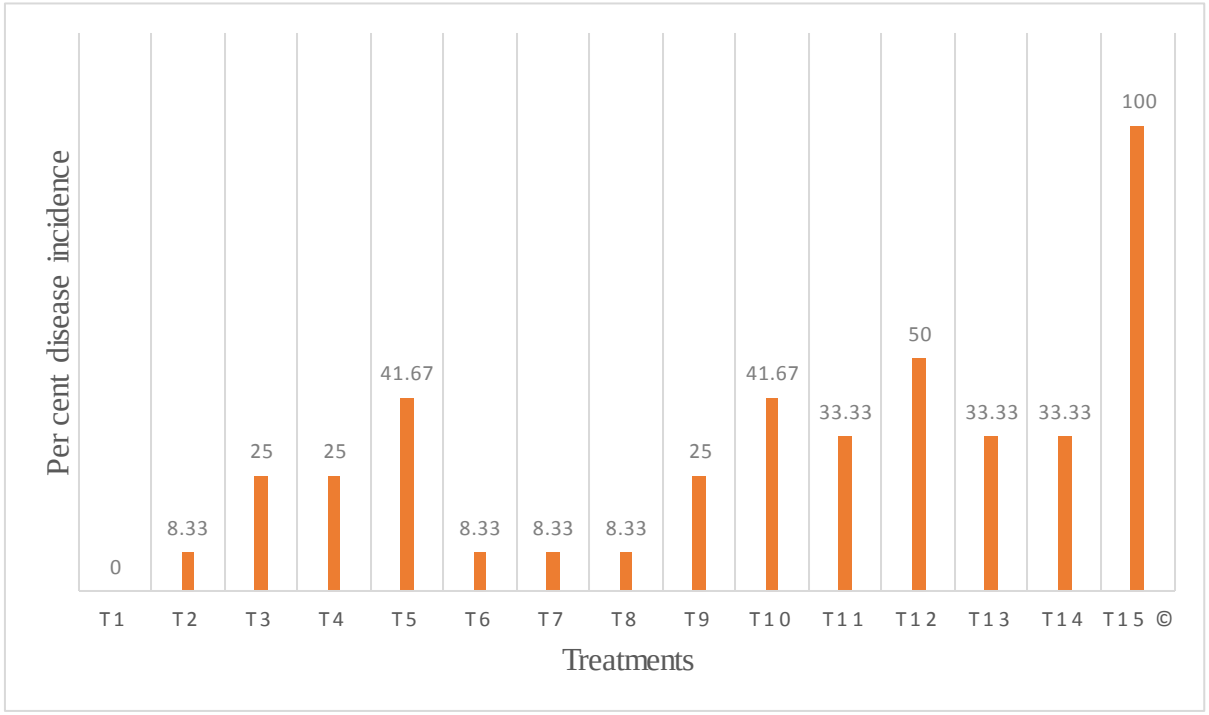


Fig.3. Effect of different treatments on per cent disease incidence in cowpea

Pre-sowing + post sowing treatments	
T1	Mancozeb (75 WP) 0.1%
T2	Propiconazole (25EC)0.05%
T3	Chorothalonil (75 WP) 0.2%
T4	*Trichoderma harzianum* 2%
T5	*Trichoderma viride* 2%
T6	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T7	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
Post sowing treatments only	
T8	Mancozeb (75 WP) 0.1%
T9	Propiconazole (25EC) 0.05%
T10	Chorothalonil (75 WP) 0.2%
T11	*Trichoderma harzianum* 2%
T12	*Trichoderma viride* 2%
T13	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T14	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
T15	Control

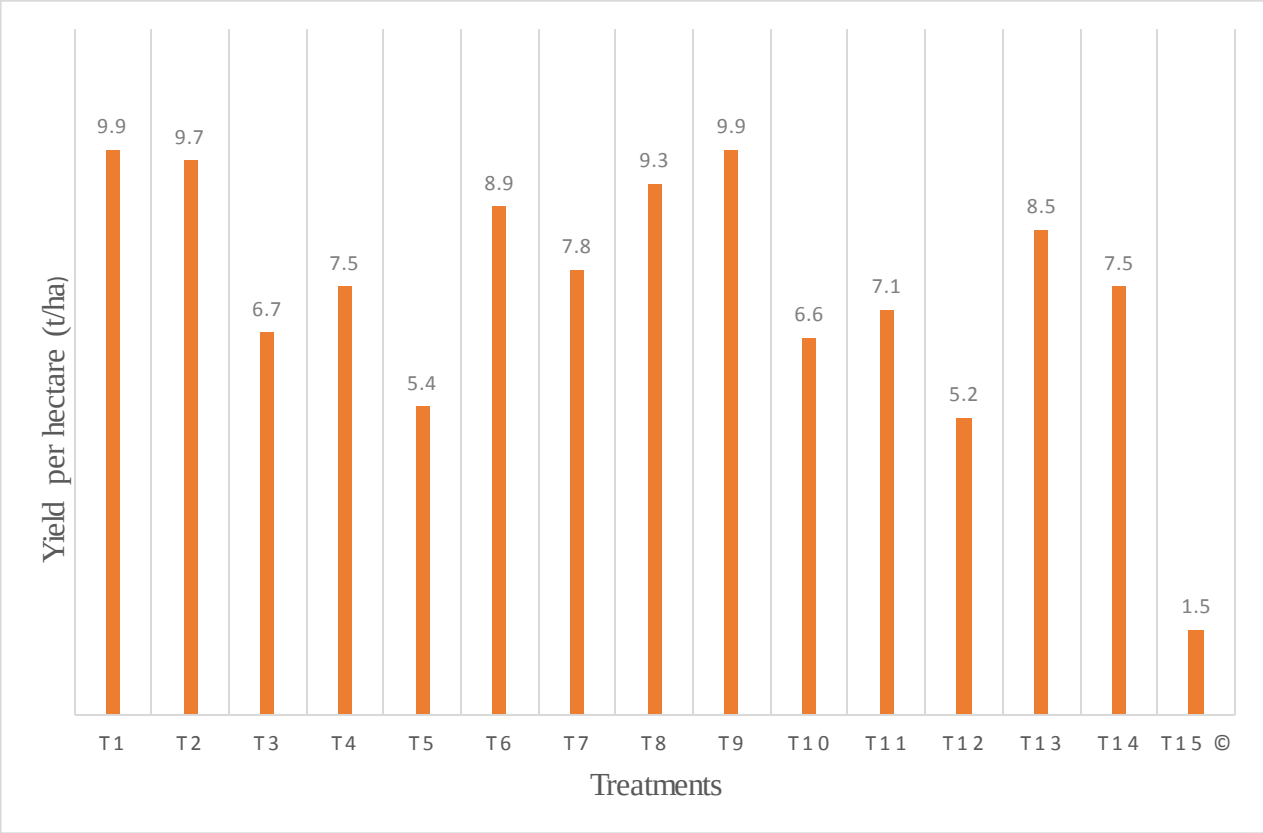


Fig.4. Effect of different treatments on cowpea yield (t/ha)

Pre-sowing + post sowing treatments	
T1	Mancozeb (75 WP) 0.1%
T2	Propiconazole (25EC)0.05%
T3	Chorothalonil (75 WP) 0.2%
T4	*Trichoderma harzianum* 2%
T5	*Trichoderma viride* 2%
T6	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T7	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
Post sowing treatments only	
T8	Mancozeb (75 WP) 0.1%
T9	Propiconazole (25EC) 0.05%
T10	Chorothalonil (75 WP) 0.2%
T11	*Trichoderma harzianum* 2%
T12	*Trichoderma viride* 2%
T13	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T14	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
T15	Control

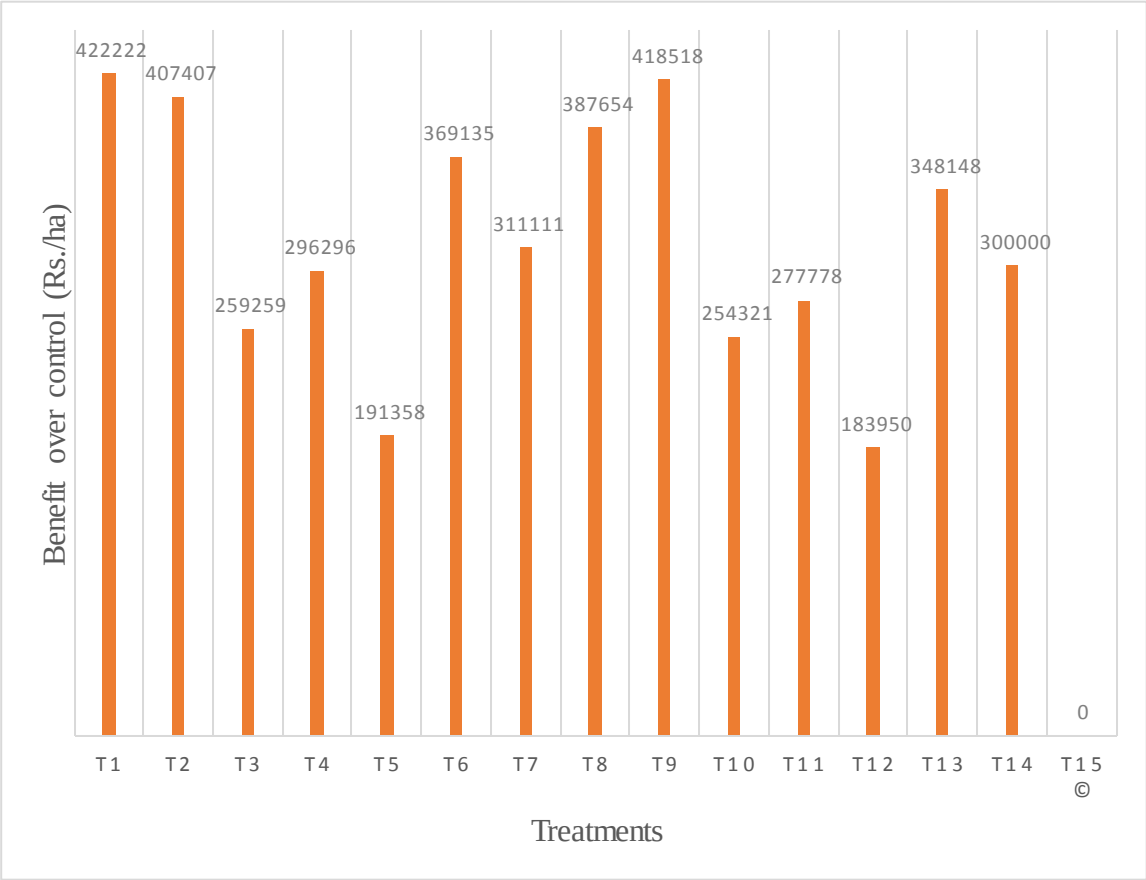


Fig.5. Benefit over control (Rs. /ha) obtained from different treatments during the management study of stem rot and foliar blight of cowpea.

Pre-sowing + post sowing treatments	
T1	Mancozeb (75 WP) 0.1%
T2	Propiconazole (25EC)0.05%
T3	Chorothalonil (75 WP) 0.2%
T4	*Trichoderma harzianum* 2%
T5	*Trichoderma viride* 2%
T6	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T7	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
Post sowing treatments only	
T8	Mancozeb (75 WP) 0.1%
T9	Propiconazole (25EC) 0.05%
T10	Chorothalonil (75 WP) 0.2%
T11	*Trichoderma harzianum* 2%
T12	*Trichoderma viride* 2%
T13	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T14	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
T15	Control

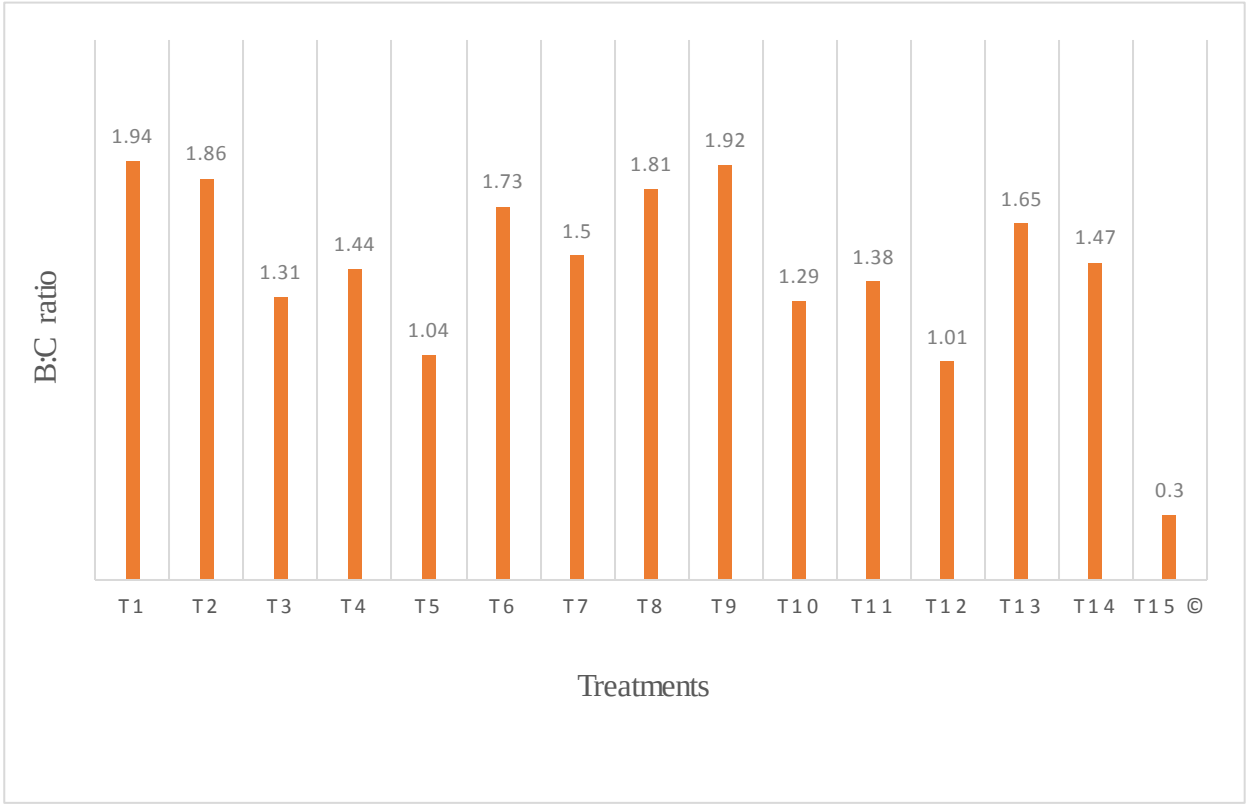


Fig.6. Benefit cost ratio of different treatments during the management study of stem rot and foliar blight of cowpea

Pre-sowing + post sowing treatments	
T1	Mancozeb (75 WP) 0.1%
T2	Propiconazole (25EC)0.05%
T3	Chorothalonil (75 WP) 0.2%
T4	*Trichoderma harzianum* 2%
T5	*Trichoderma viride* 2%
T6	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T7	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
Post sowing treatments only	
T8	Mancozeb (75 WP) 0.1%
T9	Propiconazole (25EC) 0.05%
T10	Chorothalonil (75 WP) 0.2%
T11	*Trichoderma harzianum* 2%
T12	*Trichoderma viride* 2%
T13	*Trichoderma harzianum* 2% + Mancozeb (75 WP) 0.1%
T14	*Trichoderma viride* 2% + Mancozeb (75 WP) 0.1%
T15	Control

Summary

6. SUMMARY

Cowpea (*Vigna unguiculata* (L.) Walp) is highly nutritious and multipurpose crop which can be benefited as vegetable, legume and green manure and found common in farmers' fields in Kerala. Cultivation of cowpea is highly encouraged by people in Kasargod district and also they witnessed severe yield reduction due to various diseases associated with this crop.

Stem rot and foliar blight caused by *Sclerotium rolfsii* has become serious problem in cowpea especially during rainy period. Since it was not a serious disease earlier, not much work has been done so far regarding the etiology of the disease, characterization of pathogen and management strategies. In this background the present study emphasized to identify and characterize fungal pathogen associated with stem rot and foliar blight in cowpea and to evolve a management strategy for the disease.

Cowpea plants showed symptoms of stem rot and foliar blight disease were collected from different cowpea growing areas of Kasargod like Cheemeni, Pallikkara, Nileswar, Trikaripur, Udinur and Periya during rainy season of 2019. Stem rot infection was found severe at cheemeni and Trikaripur whereas foliar blight incidence was noted at Pallikkara, Udinur and Periya. Incidence of both stem rot and foliar blight disease was occurred at Nileswar.

High percent of disease incidence (80 %) was at Nileswar followed by Pallikkara (58 %) and least percentage was recorded at Periya (12 %) which showed lowest value for disease index (8 %) also. Highest index of foliar blight disease was recorded at Pallikkara (51 %). Since all these locations showed significant variation in percent disease incidence and index, it can be correlated with difference in variety of host, relative humidity, temperature, soil parameters like moisture, pH and genetic potential of the pathogen for disease development.

Six isolates of pathogen was isolated from infected plant samples collected which was indicated with prefix 'Sr' such as Sr1, Sr2, Sr3, Sr4, Sr5 & Sr6. All isolates could infect cowpea variety called Kanakamani and produced water soaked

lesion on stem followed by wilting and yellowing of the plant after soil inoculation with pathogen multiplied in paddy grains.

Leaf inoculation with fresh PDA culture plates also produced water soaked lesion on both side of the leaves which covered entire leaf area having white mycelium and mustard like sclerotial bodies on it. Mechanism of infection by *S. rolfsii* can be explained with respect to enzyme s involved in the process like pectin methylesterase and cutinase during infection and can cause plant cell alteration that will lead penetration of the pathogen into the cells and tissue disintegration.

Symptom development by different isolates during pathogenicity test was studied and observed difference in number of days taken by each isolate for symptom appearance. Sr3 made stem infection at 2^{nd} day of inoculation and foliar infection at 24 h of inoculation which were found earlier than other isolates. Lesion number was same for all isolates having significant variation in lesion area for both stem and leaf. Sr3 produced highest lesion area such as 6.4 cm^2 on stem at 7^{th} day of inoculation and 9.6 cm^2 at 5^{th} day of leaf inoculation which confirmed superiority of this isolate over other isolates in disease development.

Radial growth of six isolates in PDA showed critical difference at 3^{rd} of inoculation and observed largest value of colony diameter (8.8 cm) for Sr3 which was followed by Sr1 (7.4 cm), Sr4 (7.3 cm), Sr5 (6.8 cm) and Sr6 (6.1 cm). Sr2 was found slow grower since it attained only 3.8 cm at 3^{rd} of inoculation.

Based on the disease development during pathogenicity test and radial growth in PDA, Sr3 was considered as most virulent isolate. Environmental factors and genetic factors can be the reasons for variability observed among the isolates with respect to virulence.

Cultural, morphological and molecular identification were studied for most virulent isolate (Sr3). Sr3 was very fast growing fungus which completed 9 cm of colony diameter at 4th day of inoculation. Cottony, fluffy and thick mycelium of pure white colour with slight zonation were the colony characters produced by this fungus. White tufts of fungal hyphae called sclerotial initials were produced at 8^{th} day of inoculation which matured into light brown sclerotial bodies at 14^{th} day of

inoculation. Two types of sclerotia like large (6 mm) and small (1 mm) were also produced. Cultural variability with respect to radial growth, colour of mycelium, sclerotial production was observed among the isolates.

Morphological characterization revealed presence of two types of hyphae with septation and clamp connection. Internal region of sclerotium was differentiated into Outer thick skin, rind with thick cells, cortex with thin walled cells and medullary region. Based on the results of cultural and morphological studies, most virulent isolate Sr3 possessed all characters of *S. rolfsii* described by Chet *et al.* (1969).

Molecular identification was done based on D1/D2 region of LSU (Large Sub Unit: 28S rDNA) and noticed 99.61% similarity with *Athelia rolfsii* (Accession No: JN811675.1), which was the perfect stage of *S. rolfsii.* Hence fungal isolate responsible for stem rot and foliar blight disease was confirmed as *S. rolfsii.*

Symptomatology of stem rot disease under natural and artificial conditions initiated with water soaked brown lesion at the stem base followed by expansion of lesion area progressing upwards, drooping of lower leaves, wilting and yellowing of aerial parts. White thread like fungal mycelia were formed at the infected portion which produced brown mustard like sclerotial bodies when the disease became severe. Water soaked lesion then became necrotic and cause girdling on the stem. At the extreme severity of disease, stem rotting along with complete wilting and death of the plants occurred.

Foliar blight disease under natural and artificial conditions started with light brown, circular water soaked lesion on the leaves which expanded its size with three to four concentric rings (absent under artificial conditions) and became dark brown and irregular shape. White mycelia of fungus was visible on the lesion which later produced small mustard like sclerotial bodies. Entire leaf turned to yellow and under dry climate short hole symptom was produced on the lesion.

In vitro evaluation of biocontrol agents released by KAU such as *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluorescens* were evaluated against *S. rolfsii* by dual culture method. Highest potential of antagonism was exhibited by *T. harzianum* with inhibition percentage of 84.44 % followed by *T.*

viride (71.11 %). Efficacy of bacterial biocontrol agents were not good against pathogen. Among these *B. subtilis* inhibited 38.89% fungal mycelia whereas least and zero inhibition was shown by *P. fluorescens*.

Effect of seven fungicides were tested at recommended, its lower and higher dose on mycelial inhibition of *S. rolfsii* by poisoned food method. Mancozeb 75WP (0.1 %, 0.2 % & 0.3 %) and propiconazole 25EC (0.05 %, 0.1 % & 0.2 %) were tested as best fungicide with 100% mycelial inhibition at all the three concentration tested followed by thiram75WS (0.3 %), chlorothalonil 75WP (0.3 %) and azoxistrobin 23SC (0.2 %).

Performance of copper oxychloride and carbendazim were very poor against the pathogen. Least inhibition was shown by copper oxychloride 50WP at 0.1 % having mycelial inhibition of 5.2 % followed by carbendazim 50WP (0.05 %) having 12.8 % inhibition. They did not inhibit the fungus even at their highest concentration tested.

Effect of fungicides on antagonists was evaluated by poisoned food method and results showed 100 % compatibility of mancozeb (0.2 %) with *T. harzianum* and 92.39 % compatibility with *T. viride*. Propiconazole (0.1 %) showed 100 % incompatibility with both fungal biocontrol agents. Chlorothalonil (0.2 %) also expressed incompatible reaction with *T. viride* (77.14 %) and *T. harzianum* (74.11 %). From these results, combinations of mancozeb and *Trichoderma* sp. can be recommended for field level application.

In vivo evaluation of most efficient fungicides such as mancozeb (0.1 %), propiconazole (0.05 %) and chlorothalonil (0.2 %), biocontrol agents like *T. viride* (2 %) and *T. harzianum* (2 %) and the compatible combinations of both mancozeb and *Trichoderma* were undertaken on cowpea with pot culture experiment.

Among the 15 treatments seven were given as soil drenching at 7 days before sowing and soil drenching and foliar spraying after 20, 40 and 60 days of sowing. Plants which were taken prophylactic soil drenching were superior in yield compared to plants without prophylactic treatment. Among the treatments fungicides were found best followed by combinations of fungicides and biocontrol agents and biocontrol agents alone.

Percent disease incidence was zero for mancozeb (0.1 %) followed by propiconazole (0.05 %) and combinations of fungicide and biocontrol agents which were applied as both pre-sowing and post sowing treatments (8.33 %). It was on par with mancozeb taken as post sowing treatment only. 100 % incidence of stem rot disease was found in plants grown as inoculated control. Foliar blight incidence was not observed in any of the plants which pointed out better management of treatments applied.

Single lesion was produced in all plants except those plants, which underwent treatment with propiconazole and *T. viride* which had multiple number of lesions on plant stem. Lesion area on stem was significantly differ with highest value of 10.1 cm^2 for inoculated control plants and lowest (zero) for pre-sowing and post sowing treatment of mancozeb (0.1 %). Lesion area and yield of the plants were not strictly correlated since most of the lesions were seen at the stem surface only.

Highest yield (134.7 g/plant, 9.9 t/ha) of cowpea, benefit over control (4,22,222/ ha) and BC ratio (1.94) were observed for pre sowing and post sowing treatment with mancozeb 75WP (0.1 %), followed by propiconazole 25EC (0.05 %). Mancozeb and *Trichoderma* combinations were also significantly superior than chlorothalonil. Among the fungal biocontrol agents *T. harzianum* best with BC ratio of 1.44. Least efficiency for stem rot management and cowpea production was noted with *T. viride* (2 %).

Prophylactic soil drenching at 7 days before sowing along with post sowing drenching and foliar spraying at 20, 40 and 60 days of sowing with fungicides like mancozeb 75WP (0.1 %), propiconazole 25EC (0.05 %), integrated application of mancozeb with *T. harzianum* and *T. viride* and *T. harzianum* alone can be recommended for stem rot and foliar blight disease of cowpea caused by *S. rolfsii*. High potential of biocontrol agents towards stem rot and foliar blight disease in cowpea emphasized scope for eco-friendly approach towards disease management.

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Appendices

APPENDIX -I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes -200 g

Dextrose - 20 g

Agar-Agar - 20 g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar –agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in the mixture. The volume made up to 1000 ml with distilled water and sterilized at 15 psi and 121°C for 15 min.

2. Potato dextrose broth

Peeled and sliced potatoes -200 g

Dextrose - 20 g

Distilled water - 1000 ml

3. Double strength potato dextrose agar

Peeled and sliced potatoes -400 g

Dextrose - 40 g

Agar-Agar - 40 g

Distilled water - 1000 ml

4. Water agar

Agar - 2 g

Distilled water - 100 g

5. Nutrient agar

Peptone - 5 g

NaCl - 5 g

Beef extracts - 3 g

Agar - 20 g

Distilled water - 1000 ml

APPENDIX- II

COMPOSITION OF STAIN USED

1. Lactophenol- cotton blue

Anhydrous lactophenol - 67.0 ml

Distilled water - 20.0 ml

Cotton blue - 0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid and 3 ml glycerol.

Abstract

Management of Stem Rot and Foliar Blight of Cowpea (*Vigna unguiculata* (L.) Walp.)

by
NAYANA SUNIL. M. V
(2018-11-023)

ABSTRACT OF THE THESIS

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ABSTRACT

Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.)

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the highly demanding, nutritionally rich vegetable and legume crops cultivated in Kerala throughout the year. Stem rot and foliar blight disease in cowpea was emerged as a serious disease in farmer's fields especially during monsoon period and resulted in severe yield reduction and economic loss. Hence present study was undertaken during 2018-2020 with an aim of identifying and characterizing fungal pathogen causing stem rot and foliar blight disease in cowpea and to evaluate biocontrol agents and chemical fungicides against it.

Cowpea plants showing symptoms of stem rot and foliar blight disease were collected from six locations of Kasargod district such as Cheemeni, Pallikkara, Nileswar, Trikaripur, Udinur and Periya showing disease incidence ranging from 12 to 80 % and percent disease index of 8 to 51 %. Highest disease incidence recorded at Nileswar and lowest at Periya. Six isolates obtained were named with index 'Sr' such as Sr1, Sr2, Sr3, Sr4, Sr5 and Sr6.

Pathogenicity test of six isolates were done in cowpea by soil inoculation and leaf inoculation methods. Isolate Sr3 produced symptom on stem at 2^{nd} day and on leaf at 24 h of inoculation which was earlier than other isolates. Area of water soaked lesion on stem (6.4 cm^2) and leaf (9.6 cm^2) was also highest for Sr3 and observed fastest radial growth compared to others and covering 90 mm petridish at third day of inoculation. Based on disease development and growth rate on PDA, Sr3 was considered as most virulent isolate.

Pathogen was identified based on cultural, morphological and molecular characterestics. Cottony and fluffy pure white colony with light zonation and presence of two types of fungal hyphae with clamp connection were observed. Two types of sclerotial bodies were detected and cross section of sclerotium revealed the presence of different layers such as outer thick skin, rind, cortex and medullary cells under

compound microscope. Molecular identification based on D1/D2 region of LSU revealed 99.61 % similarity with *Athelia rolfsii*. Based on all these characteristics isolate Sr3 was identified as *Sclerotium rolfsii*.

Symptoms associated with stem rot disease were water soaked lesion at the basal region of stem followed by wilting, yellowing of the aerial parts, necrosis, girdling and rotting of the stem. White mycelia and sclerotial bodies were formed at the infected portion. Foliar blight disease showed water soaked lesion on the leaf with concentric ring formation. Both mycelia and sclerotial bodies were also produced over the lesion.

Effect of biocontrol agents on *S. rolfsii* tested by dual culture method explained higher inhibition per cent of *Trichoderma harzianum* followed by *Trichoderma viride*. Bacterial biocontrol agents showed least potential of antagonism in which *Pseudomonas fluorescens* exhibited zero inhibition on pathogen.

Among the fungicides tested against *S. rolfsii* by poisoned food method, mancozeb 75WP (0.1 %, 0.2 % & 0.3 %) and propiconazole 25EC (0.05 %, 0.1 % & 0.2 %) were found best having 100 % inhibition over the pathogen whereas copper oxychloride 50 WP (0.1 %, 0.2 % & 0.3 %) and carbendazim 50WP (0.05 %, 0.1% & 0.2 %) were not effective. Compatibility of mancozeb, propiconazole and chlorothalonil were tested with *T. harzianum* and *T. viride*. Mancozeb (0.2 %) exhibited 100 % compatibility with *T. harzianum* and 92.39 % with *T. viride*. Propiconazole (0.1 %) was highly (100 %) incompatible with both of these followed by chlorothalonil (0.2 %).

Field evaluation with most efficient fungicides, biocontrol agents and combination of both were experimented on cowpea (variety: Kanakamani) in pot culture method. Pre-sowing drenching with post sowing drenching and spraying of mancozeb (0.1 %) and propiconazole (0.05 %) at 20, 40, 60 days of sowing were found best for the management of stem rot and foliar blight disease having BC ratio

of 1.9 followed by combinations of mancozeb and *Trichoderma* sp. Among the biocontrol agents *T. harzianum* were found superior than *T. viride*.

Hence soil drenching at 7 days before sowing and soil drenching and aerial spraying at 20, 40, 60 days after sowing with mancozeb (0.1 %) or propiconazole (0.05 %) or mancozeb-*Trichoderma* combinations or *T. harzianum* (2 %) can be recommended for management of stem rot and foliar blight disease in cowpea. future line of work should be focused on field level study of stem rot and foliar blight disease in other districts of Kerala and to evolve local specific management with native isolates of biocontrol agents and their metabolites.