ASSESSMENT AND MANAGEMENT OF RICE SHEATH BLIGHT DISEASE IN KUTTANAD

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(2022-11-081)

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by

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THESIS

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2025

DECLARATION

I, hereby declare that this thesis entitled **"Assessment and management of rice sheath blight disease in Kuttanad"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Assessment and management of rice sheath blight disease in Kuttanad"** is a record of research work done independently by **Ms. Archana Gilbert (2022-11-081)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Archana Gilbert**, a candidate for the degree of **Master of Science in Agriculture**, with major in Plant Pathology, agree that the thesis entitled **"Assessment and management of rice sheath blight disease in Kuttanad"** may be submitted by **Ms. Archana Gilbert (2022-11-081)**, in partial fulfilment of the requirement for the degree.

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Archana Gilbert

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LIST OF ABBREVIATIONS USED

ºC	Degree Celsius
&	And
%	Per cent
µm	Micrometre
µl	Microlitre
AEU	Agroecological Unit
AG	Anastomosis Group
bp	Base pairs
CD	Critical Difference
cm	Centimetre
CRD	Completely Randomised Design
DAP	Days After Planting
DI	Disease Incidence
DNA	Deoxyribo nucleic acid
DS	Disease severity
et al.	And coworkers
Fig.	Figure
g	Gram
gL^{-1}	Gram per litre
i.e.,	That is
IRRI-SES	International Rice Research Institite – Standard Evaluation System
ITS	Internal transcribed spacer
KAU	Kerala Agricultural University
kb	Kilobase pair

kg	Kilogram
kg ha^{-1}	Kilogram per hectare
L^{-1}	Per litre
m	Metre
mg	Milligram
min.	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
No.	Number
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RDF	Recommended Dose of Fertilizers
rpm	Rotations per minute
s	Second
SE (m)	Standard error of mean
Sl.	Serial
sp.	Species
SPD	Split Plot Design
TBE	Tris - Borate - EDTA
TE	Tris - EDTA
V	Volt
viz.	Namely

Introduction

INTRODUCTION

Rice (*Oryza sativa L.*), one of the three primary cereals, serves as the primary food source for more than 60% of the world population (Singh *et al*., 2024). Since it is the staple food for more than two-thirds of the Indian population, it holds the key to food security and plays a significant role in the national economy (Ashwini *et al*., 2024). Even though technological advancements have been successfully adapted for the production of the crop, various pathogen groups, including bacteria, fungus, nematodes and viruses, cause diseases leading to economic losses. Hence, protective methods should be available to reduce the loss in both quantitative and qualitative levels (Pal and Mandal, 2023).

Among rice diseases, sheath blight caused by *Rhizoctonia solani* (*Thanatephorus cucumeris*) is considered as the second most important disease of rice after blast (Manibhusanrao, 1995). It is a destructive disease reported in almost all rice growing regions (Zheng *et al*., 2021) causing 10-30% yield loss which increases upto 50% during advanced stages (Qingzhong *et al*., 2001).

The disease was first reported in Japan by Miyake (1910). In India, it was first reported in Gurdaspur in Punjab (Paracer and Chahal, 1963). Currently, the disease is prevalent in major rice growing tracts of the country causing significant reduction in grain yield (Laha *et al*., 2016). In Kerala, sheath blight incidence was first observed in Regional Agricultural Research Station, Pattambi, Palakkad by Prabhat (1969). It is a location specific disease in Kuttanad causing 30 to 37 per cent reduction in grain yield in rice (Surendran *et al*., 2021). Krishnan *et al*. (2024) reported that rice production in Kuttanad wetland ecosystem is significantly affected by the disease leading to decline in grain yield extending upto 50% under favourable conditions.

Sheath blight disease of rice, also named as snake skin disease, banded blight of rice is caused by *R. solani* and is one of the major fungal diseases affecting rice (Roy,

1993). The disease symptoms are visible during the tillering and flowering stages. Spots appear on leaf sheaths at or above the water level. Coalescing of the spots leads to the formation of lesions appearing as brown bands alternating with green colour (Surendran *et al*., 2019). The lesions enlarge with pale-green to grey centre surrounded by an irregular purple border (Webster and Gunnel, 1992). Severely infected plants produce poorly filled grains and may cause death of immature panicles (Dasgupta, 1992).

The pathogen, *R. solani*, survives in soil and spreads through contact between plant parts like tillers (Tsiboe *et al.*, 2017). The primary inoculum is soil borne which forms lesions on the sheaths, while the secondary inoculum is the mycelial strands formed by the primary lesions that run on the surface of the leaves to establish new lesions (Savary *et al*., 1995).

The rDNA-ITS region analysis is an established molecular technique for understanding the genetic linkage of *R. solani* isolates (Lakshman *et al*., 2016). Lack of resistant rice varieties and absence of single resistance genes for use in breeding are major constrains in sheath blight disease management (Singh *et al*., 2019).

Agricultural chemicals have been significant in the management of rice diseases including sheath blight. Prophylactic and therapeutic sprays of fungicides along with seed and soil application have effectively controlled sheath blight disease (Viswanathan and Mariappan, 1980). As the pathogen races continue to evolve due to various genetic mechanisms, it is crucial to identify new combination of fungicides to target currently evolving strains and races of pathogen.

However, the development of pesticide resistance and potential environmental issues associated with prolonged use of chemicals led to the search for safer and more effective alternative means against rice sheath blight disease (Willocquet *et al*., 2000). Biological control is considered as both safe and reliable solution for the above-mentioned concerns. Biocontrol agents such as *Bacillus* sp., *Pseudomonas* sp. and

Trichoderma sp. being naturally derived pesticides, have their own complex mechanisms to reduce the disease incidence along with preventing the development of resistance by the pathogen. Moreover, biocontrol agents are observed to improve the plant attributes and thereby promote their growth.

The study entitled "Assessment and management of rice sheath blight disease in Kuttanad" is undertaken with the aim of assessment of extent of rice sheath blight disease incidence caused by *R. solani* in Kuttanad region, screening for host plant resistance and evaluation of different management strategies.

The major objectives of the research work include:

- Assessment of extent of sheath blight disease incidence in Kuttanad tract
- Isolation of the pathogen involved and its characterization
- *In vitro* evaluation of bacterial biocontrol agents and fungicides against sheath blight pathogen
- Screening for host plant resistance
- *In vivo* testing of the efficacy of bacterial biocontrol agents and commercial fungicides against sheath blight pathogen

Review of Literature

REVIEW OF LITERATURE

Rice (*Oryza sativa*) is an important food crop that provides nutritional security to half of the world population. 90 per cent of the rice is cultivated and consumed by Asian countries like China, India, Japan and Bangladesh. The crop is cultivated worldwide in around 165 million hectares with a yield of 758.8 million tons per annum. In India, rice is cultivated over an area of 46.4 million hectares with annual production of 196.2 million tons (FAO, 2022) thereby ensuring its importance in global food security. However, the crop is affected by various biotic and abiotic stresses resulting in the reduction in yield (Richa *et al*., 2016).

Among the biotic stresses affecting the crop, diseases are the major constraints which can lead to the reduction in quantity and quality of grains (Yellareddygari *et al*., 2014). Rice production is affected by numerous diseases leading to 30 to 40 per cent loss in grain yield. Among the diseases, sheath blight is one of the most severe diseases infecting rice leading to more than 50 per cent reduction in grain yield (Bhukal *et al*., 2015; Yu *et al*., 2017). In severe conditions the disease may affect the whole plant leading to 100 per cent yield loss (Majumdar *et al*., 2017).

2.1 ASSESSMENT OF EXTENT OF SHEATH BLIGHT DISEASE INCIDENCE IN KUTTANAD TRACT

Rice sheath blight is one of the major diseases in rice affecting the crop worldwide leading to 10 to 30 per cent grain yield (Skamnioti and Gurr, 2009). According to Chahal *et al*. (2003), the yield loss has been estimated to be upto 54.3 per cent in India.

The disease was first reported in Japan by Miyake (1910). Subsequently its occurrence was reported throughout major rice growing countries of Asia, Africa and

America (Kozaka, 1975; Gangopadhyay and Chakrabarty, 1982; Shahjahan *et al*., 1986). In India, the disease was first reported in Gurdaspur in Punjab (Paracer and Chahal, 1963). Later the disease was reported in Uttar Pradesh (Singh and Pavgi, 1969) and West Bengal (Amin *et al*., 1974). The disease is prevalent in Andhra Pradesh, Kerala, Orissa, Bihar Tamil Nadu, West Bengal, U.P. and Uttarakhand causing significant reduction in grain yield (Laha *et al*., 2016; Yadav *et al.*, 2019).

In Kerala, sheath blight incidence was first observed in Regional Agricultural Research Station, Pattambi, Palakkad by Prabhat (1969). The disease causes 30 to 37 per cent reduction in grain yield of rice in Kerala. It is a location specific disease in Kuttanad, a major rice cultivating tract in Kerala (Surendran *et al*., 2021). Rice production in Kuttanad wetland ecosystem is significantly affected by sheath blight disease leading to decline in grain quality along with 50% loss in grain yield (Krishnan *et al*., 2024).

Thera *et al*. (2021) conducted a survey in major rice growing areas of Eastern Uttar Pradesh. A roving survey was carried out in five major rice-growing districts *viz.,* Varanasi, Mirzapur, Prayagraj, Chandauli, and Jaunpur. Highest disease incidence was observed at paddy field of Sadalpura (80%) in Chandauli region followed by Cholapur (70%) in Varanasi and the least disease incidence was observed in Narayanpur location of Mirzapur (20%).

Sandhya *et al*. (2021) conducted survey in five major rice growing districts of Andhra Pradesh. Rice plants showing typical sheath blight disease symptoms were collected from the fields of different mandals in West Godavari, East Godavari, Nellore, Y.S.R. Kadappa and Chittoor districts. The data indicated that among all the locations the per cent disease incidence ranged from 11.31 to 45.66 per cent and highest per cent disease incidence was observed at Penumanchili village (45.66%) of West Godavari followed by Alamuru (44.14%) in East Godavari. Lowest disease incidence was recorded at Koppolu (11.31%) in YSR Kadappa district.

An intensive roving survey was conducted by Prasad *et al*. (2011) in major rice growing regions of Karnataka to determine the incidence of sheath blight of rice in the farmer's fields. The highest incidence of sheath blight was recorded at Gangavati (37.79%) of Koppal district. This was followed by Sindhanur (34.92%) of Raichur district and Siruguppa (31.14%) of Bellary district. Least incidence was recorded in Manvi (23.74%) of Raichur district.

Pal *et al*. (2015) conducted studies from early tillering to booting stages of the crop to observe the prevalence of sheath blight disease in three major rice growing districts of Odisha. The disease incidence was recorded highest in Bargarh district (30.19%), followed by Sambalpur (25.22%). Least disease incidence was recorded in Jharsuguda district (21.98%).

Yaduman *et al*. (2018) conducted a roving survey in five blocks of Allahabad district to record the incidence of sheath blight disease in rice. Ten to fifteen villages were randomly selected. The disease incidence ranged between 15 to 42 per cent causing significant reduction in grain yield. Highest disease incidence was recorded in Bahadurpur block followed by Chaka block. Among the villages, highest disease incidence was recorded in Leelapur Kalan (42%) followed by Andawa (38%) in Bahadurpur and least disease incidence (15%) was recorded in Tatarganj village of Jasra block and Pandila village of Soraon block.

Ahmad *et al*. (2023) conducted a survey in 21 rice growing districts in Uttar Pradesh to record the frequency of occurrence and disease incidence of sheath blight. The incidence of sheath blight in the rice fields ranged from 7.20 to 38.90 per cent. The highest rate of disease occurrence was recorded in Muzaffarnagar district whereas highest disease incidence (38.9%) was recorded in Saharanpur followed by Mathura district.

Neha *et al*. (2016) conducted a fixed plot survey in major rice growing areas of Cuddalore district in Tamil Nadu to assess the per cent disease index (PDI) of rice

sheath blight disease. Highest disease severity (36.5%) was recorded in Naduthittu region followed by Vadakkumangudi (32.4%) whereas moderate incidence was noticed in Shivapuri (19.5%) and Periya kannadi (19.1%) locations. Least level of incidence was recorded in Ramapuram region (10.5%).

A survey was conducted in 50 villages in two major rice growing districts of Uttarakhand i.e. Udham Singh Nagar and Nainital by Kabdwal *et al*. (2018) to record the disease incidence and severity of rice sheath blight disease. The disease incidence varied from 11.33 to 34.00 per cent whereas disease severity ranged from 24.00 to 57.33 per cent. Highest disease incidence was recorded in Dhamola village (34%) followed by Bachipur (31.33%). The maximum disease severity (57.33%) was recorded in Sunderpur village followed by Jaipur Kheema (53.33%).

A survey was conducted in major rice growing regions of Chhattisgarh by Parshuram *et al*. (2017) to assess the occurrence and incidence of sheath blight disease in rice. The disease incidence in survey locations varied from 20 to 90 per cent. Highest disease incidence was recorded in fields of Kokdi region (90%) followed by Gariyaband (80%). Among 41 locations, 26.8% (11) locations showed very high incidence (>50%), 29.2% (12) locations showed high incidence (31% - 50%) and 43.9% (18) locations exhibited moderate incidence (20% - 30%).

Reddy *et al*. (2018) conducted a survey in major rice growing regions of Telangana to assess the incidence of sheath blight disease. The disease incidence ranged from 20 to 80 per cent in the survey locations. Highest disease incidence was observed in Miryalaguda (80%) followed by Huzarnagar district (75%) whereas, lowest disease incidence (20%) was recorded in Buddipally, Rudrur, Vangapally and Wyra districts.

Survey conducted by Mary *et al*. (2022) in Tamil Nadu revealed that Chitrakudi village of Thanjavur district recorded highest disease incidence (19.97%) followed by

Keelvellore village (16.66%) of Thiruvarur district. The least disease incidence of 3.34% was recorded in Tenkarai village of Nagapattinam district.

Survey was conducted by Prasad *et al*. (2014) in ten rice growing regions of Thrissur district of Kerala to assess the occurrence of sheath blight disease. The per cent disease incidence in the surveyed locations varied from 24.53 to 51.95 per cent whereas, disease severity ranged from 44.76 to 85.53 per cent in the survey locations. The highest disease incidence was recorded in fields of Adat region (51.95%) followed by Irinjalakuda (49.18%) and Pudurkara (48.10%). Least disease incidence was recorded in Ollukkara region (24.53%). Similarly, highest per cent disease severity was recorded in fields of Adat (85.53%) and Chalakudy (84.30%) followed by Pudurkara (81.76%), Iranjalakuda (78.93%) and Mannuthy (73.31%) while least per cent disease severity was recorded in fields of Madakathara region (44.76%).

Sheath blight symptoms usually appear on leaf sheaths during tillering stage. In the initial stages, the spots will be 1 – 3 cm in length which in advanced stages lead to coalescing of spots giving the appearance of snake skin (Hollier *et al*., 2009).

Oval to irregular spots are formed on the base of leaf sheath with grey centre and dark brown margins (Lee, 1983). The spots will coalesce and eventually causes infection in panicles resulting in formation of unfilled or partially filled grains with brownish black spots or patches leading to reduction in grain yield (Acharya *et al*., 2004).

Greenish grey spots with brown margins are formed near the water level and under favourable conditions, it will spread to upper plant parts leading to the death of the plants. The infected plants appear in circular patterns, referred to as ‘bird’s nest’ (Hollier *et al*., 2009). The symptoms appear as water soaked lesions on stalks and leaf sheaths (Zhang *et al*., 2021).

The disease emerges during late tillering to joint elongation stages. Later becomes aggressive during panicle differentiation stage. The typical symptom of

sheath blight is formation of spots with irregular grey to dark brown margins on sheaths and leaf blades leading to softness and lodging of sheath and causing the inhibition in grain filling (Wu *et al*., 2012).

Neha *et al*. (2017) reported that along with reducing the vigour and yield of the crops, the disease also causes discolouration of grains. Srinivas *et al*. (2013) reported that a total crop loss is observed when the aerial parts of the crop is affected.

The characteristic symptom of sheath blight disease is the presence of greenish grey water soaked lesions on the rice leaf sheath. The lesions will get coalesced and become grey to light brown in advanced stages (Shahbazi *et al*., 2023).

The pathogen survives in soil and spreads through contact between plant parts like tillers (Tsiboe *et al.*, 2017). The primary inoculum is soil borne which forms lesions on the sheaths, while the secondary inoculum is the mycelial strands formed by the primary lesions that run on the surface of the leaves to establish new lesions (Savary *et al*., 1995). The pathogen penetrates the sheath through infection pegs formed from infection cushion and lobate appressoria and leads to the formation of lesions (Marshall and Rush, 1980).

The pathogen produces two types of mycelia i.e., straight and branched, and lobate. Among this only the latter one is infectious. The primary lesion is formed by the lobate mycelium whereas the straight type mycelium extends the lesion leading to further spread of the lesion within the leaf sheath. The presence of larger spots on the leaf sheaths causes the death of the leaves and in severe stages, the death of the whole plant (Ou, 1973).

The disease develops significantly at flowering stage when the canopy is most dense, creating a microclimate conducive for fungal growth and proliferation (Pan *et al.*, 1999). The pathogen survives in water and soil in the form of sclerotia and can remain viable upto three years. The sclerotia on contact with plants produces mycelia and causes infection in the plants (Kumar *et al*., 2009). In the initial stages, the hyphae

produced from the sclerotia form a network around the base of the plant and penetrates at or near the water level (Ou, 1985).

The spots formed on the leaf sheath are greenish grey colour with a length of about ten mm in the initial stages. Later the spots enlarge and reach 2 – 3 cm length with irregular margins and brown to grey centre. The spots coalesce to form a distinctive banded appearance. Under favourable conditions, the infection will spread to the upper regions, killing the entire plant. Brown coloured silky mycelium are present on the surface of the lesions. In advanced stages, dark brown coloured sclerotia will be formed which eventually fall off the plant and will remain in the soil leading to further infection (Singh, 2005).

2.2 ISOLATION OF THE PATHOGEN INVOLVED AND ITS CHARACTERIZATION

2.2.1 Isolation of the pathogen and its pathogenicity studies

Jayaprakashvel and Mathivanan (2012) isolated pathogen from sheath blight disease samples of rice. Samples with prominent symptoms were cut into small bits of 5 cm length. The samples were then subjected to sterilization processes such as treatment with two per cent sodium hypochlorite for two min, treatment with 70 per cent ethanol for 15 s and washing with sterile water. The samples were then blot dried and placed on Potato Dextrose Agar (PDA) media and kept for incubation at room temperature.

Guleria *et al*. (2007) isolated 19 isolates of *R. solani* from diseased rice plants. The infected samples were cut into small bits containing both healthy and infected tissues, then sterilized with one per cent sodium hypochlorite and rinsed with sterile water. The bits were then placed on PDA medium containing plates and incubated in room temperature to observe the fungal growth.

Sheath blight infected rice samples were isolated in PDA media. Pure culture was obtained by reisolation of hyphal tips and maintained at (27± 2)°C throughout the studies (Mishra *et al*., 2014). Rangaswami and Mahadevan (2004) isolated *R. solani* from diseased rice samples using PDA media and purified the culture by hyphal tip method/ single sclerotial method.

Sheath and leaf samples of rice plants with sheath blight symptoms were surface sterilized with two per cent sodium hypochlorite solution and then placed on PDA medium. The hyphal tip from mycelia were then subcultured on PDA media (Banniza *et al*., 1999).

Singh *et al*. (2002) tested the pathogenicity of *R. solani* by artificially inoculating the pathogen to healthy rice plants. 0.2 mg of pathogen inoculum was placed in the leaf sheath of healthy plants. The pathogenicity of the isolates of *R. solani* was tested by placing the mycelial discs of the pathogen in the base of the leaf sheath of healthy rice seedlings (Jia *et al*., 2007 and Neeraja *et al*., 2003).

Willocquet *et al*. (2011) tested pathogenicity by inoculating sclerotia to the base of leaf sheaths of rice seedlings. Adhipathi *et al*. (2013) tested pathogenicity of *R. solani* using sclerotia. Four day old sclerotia were inoculated into sheath and moistened with sterile water. The inoculated plants produced symptoms of sheath blight such as elliptical lesions with greyish white centre.

Brooks (2007) tested pathogenicity by placing mycelial bits of *R. solani* in inner regions of the sheaths of healthy rice seedlings leading to the formation of sheath blight symptoms. Taheri *et al*. (2007) tested the pathogenicity of *R. solani* and observed the symptoms of ellipsoidal to oval shaped lesions with 1.5 – 6 cm lesion length which initially appeared green in colour and later turned to spots with greyish centre and brown margins. Similar findings were reported by Kumar *et al*. (2008).

2.2.2 Cultural and morphological characterization of isolates

Goswami *et al*. (2010) observed that *R. solani* exhibited cream to dark brown coloured cultures. Palo (1926) observed that colony colour appeared to be various shades of brown. The younger colonies appeared to be white in colour, while older ones were brown in colour. Similar findings were reported by Ahuja and Payak (1985); Singh and Singh (1994); Susheela *et al*. (2004).

Mughal *et al*. (2017) observed variation in colour of mycelia ranged from cream to dark brown in *R. solani* isolates. Mishra *et al*. (2014) identified isolates with light brown to dark brown colours in *R. solani*. Similarly, Yaduman *et al*. (2019) reported that colony colour varied from light yellow to dark brown.

Susheela and Reddy (2013) grouped isolates as fast, medium and slow growers based on the rate of mycelial growth. Isolates with fast and slow growth rates were identified in studies conducted by Parmeter and Whitney (1970). Similar reports were given by Matsumoto (1921); Matz (1921); Nabi *et al.* (2024).

Gopireddy *et al*. (2017) reported that *R solani* isolates took 4 – 10 days for sclerotia formation. Singh *et al*. (2014) observed the time required for initiation of sclerotia to be 3 – 25 days. A positive correlation existed between growth rate and virulence of the isolate (Akai *et al*., 1960; Basu *et al*., 2004). Yang *et al*. (1996) reported that diameter of sclerotia ranged from 0.5 – 2.0 mm. Lal *et al*. (2014) reported sclerotial diameter to be 1.13 – 1.50 mm.

Srinivas *et al*. (2007) reported sclerotia of light brown, dark brown and reddish brown colour in isolates of *R. solani*. Singh *et al*. (2014) observed that the sclerotia formed were light brown to dark brown in colour with rough or smooth surface. Similar findings were observed by Gopireddy *et al*. (2017). Singh *et al*. (1999) recorded that colour of *R. solani* sclerotia varies from white to dark brown.

The pattern of sclerotia formation was observed as sub-central, peripheral and irregular (Singh *et al*., 2014). Srinivas *et al*. (2007) reported that pattern of sclerotia formation varied among isolates. Isolates were categorized into two based on the pattern *i.e*., peripheral and scattered. Similar findings were observed by Rajput and Harlapur, (2016). Variation in sclerotial distribution pattern was reported by Moni *et al*. (2016); Yugander *et al*. (2015).

2.2.3 Molecular characterization of virulent isolate

Lakshman *et al*. (2016) reported that higher taxonomic levels such as genera and family can be distinguished using 18S and 28S, while organisms can be characterized at species level using the ITS. The rDNA-ITS region analysis is an established molecular technique for understanding the genetic linkage of *R. solani* isolates.

Kuninaga (1996) evaluated the ITS sequences of rDNA of 45 isolates of *R. solani* from different host and observed above 96% similarity in the sequences in isolates belonging to same anastomosis groups. Amaradasa *et al.* (2013) reported that DNA fragments ranging from 550 to 700 bp were obtained from the amplified ITS region of the isolates of *R. solani*. To determine the phylogenetic links between the isolates, a neighbor joining tree was built.

Al-Fadhal *et al.* (2019) conducted molecular characterization of isolates using ITS1/ITS4 and observed 97% similarity with *R. solani*. Similar findings were reported by Al-Abedy *et al*. (2018); Lübeck (2004).

Sayler and Yang (2007) reported 99% sequence identity with *R. solani* sequences by molecular characterization of *R. solani* isolates using ITS primers. Similarly, Toda *et al*. (2004) observed ITS sequences of the isolates and reported 99 – 100 per cent similarity with *R. solani*.

Nadarajah *et al*. (2014) reported that molecular characterization of different isolates obtained DNA fragments of 550 bp and upon examination of the ITS sequences, it was observed to have a similarity of 98 – 99 per cent with *R. solani.* While Bintang *et al*. (2017) reported 100% similarity of the obtained isolates with *R. solani*.

2.3 *IN VITRO* EVALUATION OF BACTERIAL BIOCONTROL AGENTS AND FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

2.3.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture technique

Maslennikova *et al*. (2023) conducted *in vitro* studies of *Bacillus amyloliquefaciens* against *R. solani* using dual culture assay and observed 81.00% inhibition in mycelial growth of the pathogen. Solanki *et al*. (2015) reported that *B. amyloliquefaciens* inhibited the mycelial growth of *R. solani* by 62.00% under *in vitro* conditions.

Karimi *et al.* (2016) observed that biocontrol agent *B. amyloliquefaciens* inhibited *R. solani* by 74.30%. Imran *et al*. (2022) conducted dual culture assays and reported an inhibition of 42.60% by *B. amyloliquefaciens* against *R. solani*. Tuyong *et al*. (2000) screened endophytic bacteria including *B. amyloliquefaciens* against *R. solani* and observed inhibition of mycelial growth. *B. amyloliquefaciens* exhibited 74.50% against *R. solani* under *in vitro* conditions (Kumar *et al*., 2011).

Srivastava *et al*. (2016) tested biocontrol efficacy of *B. amyloliquefaciens* strains against *R. solani* by dual culture technique and observed a reduction in more than 50% in fungal dry mass of *R. solani.* Jamal *et al*. (2015) conducted *in vitro* studies using biocontrol agents against *R. solani* and observed that *B. amyloliquefaciens* caused mycelial growth inhibition of 70.00% in *R. solani.*

Reddy *et al*. (2010) obtained isolates of *Pseudomonas fluorescens* strains from rhizosphere of rice seedlings and conducted dual culture assay to assess the antagonistic activity against *R. solani* and reported 78.00% mycelial growth inhibition. Maurya *et al*. (2014) isolated different strains of *P. fluorescens* from various agroecological zones and recorded mycelial growth inhibiton of 68.23% against *R. solani*.

Sandhya *et al*. (2018) conducted dual culture assay using *P. fluorescens* isolates against soil borne pathogens and observed mycelial growth inhibition of 66.76%, 63.32% and 57.30% using various isolates against *R. solani*. Bautista *et al.* (2007) observed 77.80% mycelial growth inhibition while conducting dual culture assays with *P. fluorescens* strains against *R. solani.* Pande and Chaube (2003) conducted dual culture assays using six different isolates of *P. fluorescens* which resulted in the mycelial growth inhibition of *R. solani* and formation of inhibition zones of 3.3 – 12.0 mm in different isolates.

Afsharmanesh *et al.* (2010) reported a growth inhibition of 88.00% on dual culture assay conducted against *R. solani* using *P. fluorescens* isolate. Similarly, Swati *et al*. (2015) analyzed the antagonistic activity of *P. fluorescens* against *R. solani* and observed mycelial inhibition of 47.67 – 55.47% on using different isolates.

Gupta *et al*. (2020) isolated sixteen fluorescent Pseudomonads from different crop rhizosphere soils and tested against soil borne fungal plant pathogens and observed mycelial growth inhibition of 55.66% against *R. solani*. Devi *et al*. (1989) conducted *in vitro* studies using various isolates of *P. fluorescens* against *R. solani* and observed 50 – 100 per cent inhibition of mycelial growth. Similar findings were observed by Islam (2003); Bashar *et al*. (2010); Sharma *et al*. (2004).

2.3.2 *In vitro* evaluation of fungicides against sheath blight pathogen by poisoned food technique

Kumari (2017) conducted studies on *in vitro* efficacy of fungicides on *R. solani* and observed that kresoxim methyl 40% + hexaconazole 8% WG showed 91.46% inhibition of mycelial growth in *R. solani.* Kumar *et al*. (2014) reported that fungicide kresoxim methyl 40% + hexaconazole 8% WG, was found to be effective in inhibiting the mycelial growth of *R. solani*.

Gauda *et al*. (2021) reported complete inhibition of *R. solani* mycelial growth against fungicides kresoxim methyl, trifloxystrobin + tebuconazole and hexaconazole. Pawar *et al*. (2015) conducted *in vitro* studies and reported 100% inhibition of *R. solani* mycelial growth with fungicides such as hexaconazole, propiconazole, difenoconazole and azoxystrobin. Similarly, Sharma *et al*. (2024) evaluated effect of fungicides on mycelial growth of *R. solani* under *in vitro* conditions and observed that among different fungicides tested, azoxystrobin + difenoconazole inhibited the mycelial growth by 72.70%.

Yadav *et al*. (2021) conducted *in vitro* studies to assess the efficacy of fungicides against *R. solani* and reported complete inhibition of fungal mycelial growth in treatments hexaconazole and propiconazole while the treatment with kresoxim methyl exhibited 94.16% inhibition and treatment trifloxystrobin + tebuconazole showed 96.50% inhibition.

Dhami and Maharjan (2023) conducted *in vitro* studies and reported that trifloxystrobin + tebuconazole completely inhibited the mycelial growth of *R. solani*. Similar results were observed by Bag (2009); Persuad *et al*. (2013).

Mohanty *et al*. (2020) studied *in vitro* efficacy of fungicides against *R. solani* and observed 100% mycelial growth inhibition with trifloxystrobin + tebuconazole and hexaconazole followed by azoxystrobin with 90.60% inhibition. Nagaraju and Manjunath (2017) reported significant mycelial growth inhibition by fungicide

hexaconazole. Similarly, Agrawal and Sunder (2013) reported that hexaconazole exhibited complete inhibition of *R. solani* mycelial growth under *in vitro* conditions.

Swamy *et al*. (2009) evaluated the efficacy of fungicides under *in vitro* conditions and reported that trifloxystrobin + tebuconazole and hexaconazole significantly inhibited the mycelial growth of *R. solani*. Prakash *et al*. (2013) reported that hexaconazole inhibited the mycelial growth by 71.22%. Similar findings were observed by Ali and Archer (2003).

2.4 SCREENING FOR HOST PLANT RESISTANCE

The most popular rice variety in Kerala is Uma (MO 16) released by M. S. Swaminathan Rice Research Station, Moncompu followed by Jyothi (PTB 39), released by Regional Agricultural Research Station, Pattambi. Other varieties popular in Kerala in their preference order are Aiswarya, Kanchana, Bhadra, Krishnanjana, Makom, Gouri etc. KAU varieties Kanchana and Aiswarya exhibit resistance to sheath blight. (KAU, 2024).

Adhipathi *et al*. (2013) evaluated ten rice varieties and reported that variety Sarju – 52 was observed to be highly resistant, while varieties Jaya, UPR – 2005 – 38 and IET – 15182 showed moderate resistance and variety, Pusa Basmati-1 was observed to be susceptible to sheath blight disease.

Prasad *et al*. (2020) evaluated 31 rice varieties against sheath blight disease. The plants were artificially inoculated and it was observed that no varieties were found to be immune to the disease. 21 varieties were resistant and nine varieties were moderately resistant with IRRI - SES score 3 and 5 respectively. One variety was observed to be susceptible to sheath blight disease.

Bhandarkar *et al*. (2018) evaluated 68 rice varieties for resistance against sheath blight disease and reported that three varieties were highly resistant, 33

varieties were resistant and 26 varieties were moderately resistant with IRRI – SES score of 1, 3 and 5 respectively. Among the varieties screened, 16 varieties were moderately susceptible and two varieties were susceptible to the disease with score 7 and 9 respectively.

Shamim *et al*. (2014) evaluated rice varieties against sheath blight disease and reported that two varieties, IR 42 and TKM 9 exhibited moderate resistance against the disease. Lakshmanan (1991) evaluated 87 lines and observed five resistant lines against sheath blight disease.

Pavani *et al*. (2018) conducted screening of rice germplasm for resistance to sheath blight and observed that among 196 germplasms, none was found to be immune or resistant while, 57 were observed to be moderately resistant to the disease. Kumar *et al*. (2019) evaluated 307 rice genotypes and reported that only one genotype exhibited resistance against sheath blight while five show moderate resistance.

Azharudheen *et al*. (2018) evaluated nineteen rice varieties and observed that three varieties exhibited moderate resistance to sheath blight disease. Dubey *et al*. (2014) conducted evaluation of 32 varieties and observed that seven varieties showed tolerance to sheath blight disease while, none was observed to be resistant.

Arshad *et al*. (2020) screened 85 rice varieties against sheath blight disease and reported none of them exhibited immune response against the disease while two varieties showed resistance to the pathogen. Eight varieties were reported to be moderately resistant and 26 varieties were found to be moderately susceptible to the disease.

Mansi (2022) reported that among 512 genotypes screened against sheath blight disease, twenty-nine genotypes were observed to be resistant. Turaidar *et al*. (2017) conducted evaluation of thirty varieties and observed that only one variety exhibited moderate resistance while 15 varieties were susceptible and 11 were highly susceptible to sheath blight disease.

Ashwini *et al*. (2024) screened 100 rice genotypes against sheath blight disease and reported two varieties exhibited resistance to the disease while 38 were moderately resistant and 33 were moderately susceptible. Among the screened varieties, 25 were identified as susceptible and two were identified as highly susceptible.

Nagaraju (2013) screened 139 rice genotypes against sheath blight disease and observed that five genotypes were resistant while none were identified to be immune to the disease. Goswami *et al*. (2019) evaluated 261 genotype and observed that 57 genotypes were resistant while, 169 were identified to be moderately resistant. Xiaoping (2004) conducted screening of nine cultivars and identified one cultivar exhibiting stable resistance to sheath blight throughout two growing seasons.

Timsina *et al*. (2022) evaluated 122 rice genotypes and identified 24 genotypes to be moderately resistant and 38 genotypes to be moderately susceptible. 40 genotypes were susceptible and 20 were identified to be highly susceptible to sheath blight disease. Naveenkumar *et al.* (2022) evaluated 63 rice genotypes against sheath blight disease and identified 23 genotypes to be moderately resistant, 38 to be moderately susceptible and two genotypes to be highly susceptible to the disease.

Chandra *et al*. (2016) screened 108 rice germplasm and reported that none of them were observed to be immune or resistant, while 45 were moderately resistant, 37 moderately susceptible and 14 were identified to be susceptible. Kumar *et al*. (2008) identified two varieties NDR – 359 and Ajaya to be highly resistant to sheath blight disease.

2.5 *IN VIVO* TESTING OF THE EFFICACY OF BACTERIAL BIOCONTROL AGENTS AND COMMERCIAL FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

Nagendran *et al*. (2014) conducted *in vivo* studies using *B. amyloliquefaciens* against sheath blight disease and reported that application of *B. amyloliquefaciens* as seed treatment (4 gkg^{-1}) along with soil application (500 gha^{-1}) and foliar application was observed to have lowest disease severity of 33.00% with a disease reduction of 55.00% over control plants.

Margani *et al*. (2018) evaluated efficacy of *Bacillus* sp. in reducing rice sheath blight disease incidence under *in vivo* conditions and observed that *B. amyloliquefaciens* was effective in inhibiting the infection of the pathogen with disease reduction of 30.43% over control.

Khan *et al*. (2024) evaluated various endophytic bacteria in controlling rice sheath blight disease and observed that *B. amyloliquefaciens* expressed lowest disease incidence (7.20%) and highest disease suppression (78.80%) compared to control.

Kakar *et al*. (2018) conducted field evaluation of rice associated bacteria against sheath blight disease and reported that *B. amyloliquefaciens* exhibited significant reduction in disease incidence compared to control plants. Kumar *et al*. (2012) conducted field studies and reported that application of *B. amyloliquefaciens* as seed treatment (10 gkg^{-1} seed), soil treatment (1 kg $acre^{-1}$) and foliar spray (20 gL^{-1}) recorded minimum disease incidence and severity.

Murugavel and Kannan (2020) conducted field study on the efficacy of *P. fluorescens* on disease reduction of sheath blight and observed that the application of *P. fluorescens* as seed treatment at 10gkg^{-1} of seeds and foliar spraying at 0.2% recorded the minimum disease incidence (12.12%).

Singh and Sinha (2012) reported that application of *P. fluorescens* resulted in 59.60 – 64.40% reduction in sheath blight severity and 36.70 – 40.40% reduction in disease incidence along with 30.60 – 32.30% increase in grain yield and 27.20 – 29.50% increase in thousand grain weight compared to control plants. Vidhyasekaran and Muthamilan (1999) reported that application of *P. fluorescens* as seed, soil and foliar treatments exhibited least per cent disease index compared to other treatments. Similar results were observed by Mawaddah *et al*. (2023).

Mandal *et al*. (2024) evaluated the efficacy of fungicides under field conditions against sheath blight disease and reported that kresoxim methyl 40% + hexaconazole 8% was highly effective in with highest reduction in disease severity (72.6%) followed by azoxystrobin18.2% + difenoconazole 11.4% SC and trifloxystrobin 25% + tebuconazole 50% WG with per cent reduction of 69.00% and 57.55% in disease severity over control plots. The analysis of grain yield data reported that highest grain yield was observed using kresoxim methyl 40% + hexaconazole 8% WG followed by azoxystrobin 18.2% + difenoconazole 11.4% SC and trifloxystrobin 25% + tebuconazole 50% WG.

Kumar and Veerabhadraswamy (2014) reported that kresoxim methyl + hexaconazole was highly effective against sheath blight disease. Lore *et al*. (2012) conducted *in vivo* studies using fungicides against sheath blight disease and observed that kresoxim methyl + hexaconazole exhibited highest reduction in disease incidence compared to other treatments. Chandra (2016) reported hexaconazole significantly reduced per cent disease incidence and disease severity compared to control plots.

Kumar and Chethana (2022) reported kresoxim methyl 40% + hexaconazole 8% was effective against sheath blight disease recording the lowest disease incidence of 11.85% compared to other treatments.

Singh *et al*. (2015) evaluated the efficacy of fungicides and reported that the plots treated with kresoxim methyl was observed with lowest per cent disease index (12.00) along with increased grain yield.

Pal and Mandel (2023) conducted field evaluation of fungicides against sheath blight disease and reported that spraying azoxystrobin + difenoconazole at 1 mlL^{-1} was most effective. It was observed with 82.00% reduction over control and with highest grain yield. Sundaravadana *et al*. (2007) reported that azoxystrobin suppressed disease development by 64.00% under field conditions compared to control plots.

Similarly, Bhuvaneswari and Raju (2012) evaluated the efficacy of fungicides under *in vivo* conditions and observed that azoxystrobin + difenoconazole was effective with least disease incidence of 16.43% and disease severity of 21.37%. It was observed that hexaconazole was also effective against the disease by exhibiting disease incidence and severity of 23.09% and 31.06% respectively whereas, azoxystrobin alone recorded disease incidence and disease severity of 27.06% and 32.89%.

Gouda *et al*. (2021) conducted field studies on efficacy of fungicides on sheath blight disease and observed that trifloxystrobin + tebuconazole was effective in reduction of disease severity (83.30%) followed by hexaconazole (74.02%). Swamy *et al*. (2009) evaluated fungicides against sheath blight under *in vivo* conditions and observed that trifloxystrobin + tebuconazole reduced disease incidence to 25.00% compared to control plots.

Pramesh *et al*. (2017) reported that trifloxystrobin 25% + tebuconazole 50% was highly effective against sheath blight disease by recording least per cent disease index of 24.70 along with highest grain yield compared to other treatments. Kumar *et al*. (2018) reported that tebuconazole 50% + trifloxystrobin 25% WG was observed to be effective against sheath blight disease in reducing the disease incidence by 79.12% compared to the control.

Surendran *et al*. (2019) reported that tebuconazole was found to be effective against sheath blight disease with lowest disease incidence of 9.56% whereas hexaconazole and kresoxim methyl exhibited disease incidence of 14.6% and 15.5% respectively. The yield assessment revealed that highest yield was obtained in treatment using tebuconazole (5214 $kgha^{-1}$) followed by hexaconazole (4988 $kgha^{-1}$) and kresoxim methyl (4816 $kgha^{-1}$).

Wu *et al*. (2014) reported that crop management practices cause variation in disease incidence and severity. Cu *et al*. (1996) reported that application of nitrogen fertilizer at rates above those need to maximize grain yield caused increase in sheath blight disease incidence and severity. Wu *et al*. (2012) reported a close relationship between rate of application of nitrogen fertilizers and disease intensity. Peng *et al*. (2010) reported that yield reduction is often associated with excess nitrogen application due to higher chances of lodging and incidence of disease and pest.

Savary *et al*. (1995) reported that intensity of infection increased significantly with increased nitrogen application. The increased rate of nitrogen fertilizers increases the vertical spread of the pathogen and thereby facilitating the disease development (Savary *et al*., 1997; Castilla *et al*., 1996).

Similarly, Slaton *et al*. (2003) observed higher disease incidence in treatments with higher nitrogen fertilizer rates. Wu *et al*. (2015) observed significant increase in lesion length in treatment with nitrogen at rate of 240 $kgha^{-1}$ compared to 120 $kgha^{-1}$. Studies conducted by Shahjahan *et al.* (1990) recorded increased disease incidence in treatments with higher application of nitrogen fertilizers compared to treatments with low nitrogen application.

Rice varieties cultivated with higher dose of nitrogen fertilizers creates a denser canopy and provides a microclimatic condition conducive for the development of sheath blight disease (Basu and Gupta, 1996; Norman *et al*., 2001; Hu *et al*., 2004; Zhong *et al*., 2006; Tang *et al*., 2007).

Li *et al*. (2012) conducted field evaluation to analyze the relation between nitrogen application and sheath blight disease incidence. It was reported that sheath blight incidence was less than 25.00% in treatment plots with low nitrogen application while it was more than 46.00% in treatments with higher nitrogen dose. Similarly, grain yield was observed to be higher in treatments with low nitrogen application due to reduced disease incidence.

Prasad *et al*. (2020) conducted field trials with different doses of potassium fertilizers and reported that excess dose of potassium caused an increase in incubation period and lead to 67.57% increase in phenols. This led to 32.31% reduction in disease severity compared to control plots. Whereas, application at recommended dose and lower doses produced 27.99% and 18.45% increased phenols respectively with 19.73% and 12.58% reduction in disease severity compared to control plots.

Sweeny *et al*. (2000) reported increased application of potassium fertilizers reduce sheath blight disease incidence along with increasing grain yield. Bhaskar *et al*. (2001) reported that disease incidence decreased with increase in potassium application from 0 - 140 $kgha^{-1}$ whereas, phenol content in leaf and leaf sheath increased. Bhaskar *et al*. (2002) reported higher grain yield (3909 $kgha^{-1}$) at higher potassium rates (140 $kgha^{-1}$) and lower grain yield (2900 $kgha^{-1}$) at lower rates of potassium.

Materials and Methods

MATERIALS AND METHODS

The present research work, entitled "Assessment and management of rice sheath blight disease in Kuttanad" was undertaken between 2022 and 2024 at the Department of Plant Pathology, College of Agriculture, Vellayani and Division of Plant Pathology, M. S. Swaminathan Rice Research Station, Moncompu. The details of materials used and the experimental methods employed in this study are described below.

3.1 ASSESSMENT OF EXTENT OF SHEATH BLIGHT DISEASE INCIDENCE IN KUTTANAD TRACT

A purposive sampling survey was conducted in three major rice growing districts *viz.,* Alappuzha, Kottayam and Pathanamthitta of Kerala which come under agroecological unit (AEU) – 04 (Kuttanad). The survey was conducted in 22, 12 and 6 locations in Alappuzha, Kottayam and Pathanamthitta districts respectively during 2023 - 2024 to assess the disease incidence and severity of sheath blight disease of rice in farmer's field. Rice fields infected with the disease were identified from each survey location (Table 1). One square meter area was selected randomly in each field to assess the disease.

The nature of symptoms, number of infected plants, number of infected tillers, number of infected panicles, disease severity and major pest associated were recorded from each survey location.

Disease incidence was recorded on the basis of number of healthy and diseased plants in the locations. The disease severity was calculated using IRRI - SES disease score chart, 2013 (Table 2). The diseased samples per square meter was scored and disease severity was calculated using the formulas.

Disease incidence was calculated using the following formula (Thera *et al*., 2021).

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ tillers}{Total\ number\ of\ tillers} \times 100$$

Disease severity was calculated rating the individual specimens and by using the following formula (Neha *et al*., 2017).

$$Disease\ severity\ (\%) = \frac{Sum\ of\ individual\ rating}{Number\ of\ tillers\ observed\ \times Maximum\ disease\ score} \times 100$$

3.2 ISOLATION OF THE PATHOGEN INVOLVED AND ITS CHARACTERIZATION

3.2.1 Isolation of pathogen and its pathogenicity studies

Diseased specimens were collected from the survey locations for isolation of the pathogen. A bit of sheath portion containing diseased tissues along with healthy part was used. This was thoroughly washed in running tap water to remove soil and other external contaminants adhering to the surface. The bits were surface sterilized using one per cent sodium hypochlorite for 30 s. This was then followed by two subsequent washes with sterile water. The bits were placed on sterile tissue paper to remove excess moisture and then placed on sterile PDA medium in sterile Petri plates. Four leaf bits were placed in each Petri plates and these were then incubated at room temperature (28 ± 2°C) for three days (Jayaprakashvel and Mathivanan, 2012). Fungal mycelial strands were formed and these were then subcultured in sterile Petri plates containing PDA medium. The fungal culture obtained were purified using single hyphal tip method (Lore *et al*., 2015).

These cultures were maintained in PDA slants for further use. Regular subculturing of the isolates were done in sterile PDA slants in order to maintain the

viability of the pathogen. The cultured slants were incubated at room temperature for seven days and then stored at 4°C in refrigerator (Vidhyasekharan *et al*., 1992).

The pathogenicity studies of the fungal isolates were conducted in popular susceptible rice variety, Uma. Pots were filled with sterilized soil, sand and dry cow dung in a proportion of 2:1:1. Healthy seeds of the rice variety Uma were sown in these pots. Crop management practices including cultural operations and fertilizer applications were followed according to Package of practices of Kerala Agricultural University (KAU, 2024).

Artificial inoculation was done to test the pathogenicity of the fungal isolates. The plants were artificially inoculated at tillering stage of the crop. Seven day old cultures of the fungal isolates were used for artificial inoculation. Pinpricking was done using sterile needle to make injury on the sheaths. Mycelial bits of 5mm size were placed on the pinpricked areas. Over these bits, a thin layer of moistened cotton was placed and covered with polythene cover to maintain humidity. The untreated control plants were also maintained. Pinpricked sheath with PDA disc without inoculum and covered with moistened cotton were used as control.

Inoculated plants were labelled and observed for symptom development. Periodic observations were taken including the time taken for symptom development for each isolate. The virulent isolate among the obtained forty isolates was identified by observing the time taken for symptom development, sclerotia formation and disease severity.

After the symptom establishment, the reisolation of the pathogen from artificially inoculated plants were done in PDA medium. The morphological characters of the reisolated pathogens were studied and compared with the original cultures in order to prove Koch’s postulates.

3.2.2 Cultural and morphological characterization of isolates

The cultural and morphological characteristics of all the isolates obtained from different locations were studied to record the morphological variations among the isolates. The colour and appearance of colony, colour and septation of hypha, number of days taken for the formation of sclerotia, colour, texture and size of sclerotia of all the isolates were studied.

The fungal isolates were subcultured to sterile Petri plates containing PDA medium. 5mm mycelial disc of seven day old culture was used. These were incubated at room temperature (28 ± 2°C). Observations on the mycelial growth were recorded. The cultural characters *viz.,* colony colour, texture, number of days taken for full growth in Petri plates, number of days for the formation of sclerotia were recorded.

The morphological characters of the pathogen were studied using compound light microscope (LEICA DM750) at 400X magnification.

Microscopic studies were conducted using the slide culture technique (Bhat, 2017). A slide culture unit was prepared by placing blotter papers on both top and bottom plates of a Petri plate within which glass rods, microscopic glass slide and coverslips were arranged. This unit was then sterilized. Under aseptic condition, the blotter paper was moistened with sterile water and the microscopic glass slide was placed over the glass rods. 2% agar was prepared and sterilized from which agar block was cut using sterilized needle. This was placed on the glass slide and fungi was inoculated on the corners of the agar block. After inoculation, coverslip was placed carefully on the agar block. The Petri plate was then incubated in room temperature and observations were taken within 48 - 72 h for the morphological studies.

3.2.3 Molecular characterization of virulent isolate

3.2.3.1 DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

The virulent isolate among the obtained isolates was identified by molecular characterization. DNA was isolated from the virulent isolate using NucleoSpin® Plant II Kit (Macherey-Nagel). The culture was given to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram for DNA sequencing using ITS primers. The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.2.3.2 PCR amplification using ITS primers

The PCR amplification of the DNA template was carried out using ITS1 and ITS4 primers in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The details of PCR reaction mixture, primer sequence and reaction conditions are provided below.

Components of reaction mix	Concentration	Volume for one reaction
Phire Master Mix	2X	5µL
Distilled water	-	4µL
Forward Primer	10 pM	0.25µL
Reverse Primer	10 pM	0.25µL
DNA	50 ng	1µL

Sequence of ITS primers used in the study

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS 1	Forward	TCCGTAGGTGAACCTGCGG
	ITS 4	Reverse	TCCTCCGCTTATTGATATGC

Reaction conditions

98ºC - 30 s - Denaturation

98ºC - 5 s
54ºC - 10 s } 40 cycles - Annealing
72ºC - 15 s

72ºC - 60 s - Extension

4ºC - ∞

3.2.3.3 Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 4 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 h, until the bromophenol blue front had migrated to almost the bottom of the gel. DNA ladder was also added to the gel to verify the length of the amplicon. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

Purification of the PCR product by ExoSAP IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. Five µl of PCR product is mixed with 0.5µl of ExoSAP-IT and incubated at 37ºC for 15 min followed by enzyme inactivation at 85ºC for 5 min. Sequencing reaction was done in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The sequencing PCR mix consisted of the following components:

3.2.3.3.1 Components of sequencing reaction

Components of reaction mix	Concentration	Volume for one reaction
D/W	-	6.6µL
5X Sequencing Buffer	5X	1.9µL
Forward Primer/ Reverse Primer	10 pM	0.3µL
Sequencing Mix	1X	0.2µL
Exosap treated PCR product	50 ng	1µL

3.2.3.3.2 Thermal profile of sequencing PCR

96ºC	-	2 min	
96ºC	-	30 s	} 30 cycles
50ºC	-	40 s	
60ºC	-	4 min	
4ºC	-	∞	

3.2.3.4 Post sequencing clean up reaction

The sequencing product was then subjected to clean up to remove unused primers, ddNTPs, dNTPs and salt content.

3.2.3.4.1 Components of post sequencing clean up reaction

Components of reaction mix	Concentration	Volume
Distilled water	-	5 µl
Sodium Acetate	3 M	1 µl
EDTA	125 Mm	0.1 µl
Ethanol	100%	44 µl

1. A clean up mix of 125mM EDTA, 3M sodium acetate pH 4.6, 100 % ethanol and nuclease free water was prepared and mixed well.
2. 50 µl of clean up mix was added to each well in the sequencing plate containing sequencing PCR product and subjected to vortexing.
3. The mixture was then incubated at room temperature for 30 min
4. After incubation it was centrifuged at 3700 rpm for 30 min
5. The supernatant was decanted and added 50 µl of 70% ethanol was added
6. It was again subjected to centrifugation at 3700 rpm for 20 min.
7. The supernatant was decanted and was washed with 70% ethanol.
8. The pellet was finally air dried.
9. The cleaned-up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.2.3.4 Sequence analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). The obtained sequences were aligned to the available sequences in the NCBI database with the help of BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the pathogen was identified up to species level. This identified pathogen was used for further studies. Phylogenetic tree of the fungal isolate was constructed based on Neighbor-Joining analysis by genetic matrix calculation based on ClustalW programme using MEGA11 software.

3.3 *IN VITRO* EVALUATION OF BACTERIAL BIOCONTROL AGENTS AND FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

3.3.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture technique

Bacterial biocontrol agents *B. amyloliquefaciens* (B15) and *P. fluorescens* (PN026) from M. S. Swaminathan Rice Research Station, Moncompu were tested against the virulent isolate of the fungal pathogen to assess the antagonistic effect. The evaluation was performed using dual culture technique (Dennis and Webster, 1971). The experiment was conducted in Completely Randomized Design (CRD) with three treatments and seven replications. The treatments are as follows:

Treatments - 3

Replications - 7

T1 – *B. amyloliquefaciens* (B15)

T2 – *P. fluorescens* (PN026)

T3 - Control

Sterilized PDA medium was poured in 90 mm sterile Petri plates. A mycelial disc (5 mm) of *R. solani* was placed on the centre of the Petri plates. Using a sterile inoculation loop, a loopful of bacterial biocontrol agent was streaked on two sides of the pathogen at 1.5 cm away from periphery of the Petri plate. Seven replications were kept for each treatment. A control plate consisting of only pathogen mycelial bit was maintained. The plates were incubated at room temperature. The observations regarding the radial growth of the pathogen were recorded from second day until the pathogen attained full plate growth in the control plate. The per cent inhibition of pathogen over the control was calculated as per the formula given by Vincent (1927).

$$PI = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent Inhibition (%)

C = Growth of the pathogen in control plates (cm)

T = Growth of the pathogen in dual culture (cm)

3.3.2 *In vitro* evaluation of fungicides against sheath blight pathogen by poisoned food technique

In vitro evaluation of the fungicides listed below was carried out using poisoned food technique (Vincent, 1947). The evaluation of these fungicides against the virulent isolate of the pathogen was done at three different concentrations.

Molten sterilized PDA was used as nutrient medium for pathogen and required quantity of each fungicide was added in the 250 ml conical flask separately so as to get a requisite concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 20 ml poisoned medium was poured to each of the 90 mm sterilized Petri plates and allowed for solidification. Mycelial disc of 5 mm diameter was cut

from seven day old culture of fungal pathogen and transferred aseptically to the centre of each Petri plates containing the poisoned solid medium. Three replications were maintained for each of the concentrations of fungicides, while the plates without fungicides served as control. The plates were incubated at room temperature (28± 2°C). The observations were recorded for growth in mm on daily basis. Per cent growth inhibition (PI) of each treatment was calculated as given below.

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

Where,

PI = Per cent inhibition

C = Growth of the pathogen in control plate (cm)

T = Growth of the pathogen in treatment plate (cm)

The experiment was carried out with ten treatments and three replications in Completely Randomized Design (CRD). The treatments are as follows.

Treatments - 10

Replications - 3

T1 - Azoxystrobin 18.2% w/w + Difenoconazole 11.4% ww SC @ 0.25 mlL^{-1}

T2 - Azoxystrobin 18.2% w/w + Difenoconazole 11.4% ww SC @ 0.5 mlL^{-1}

T3 - Azoxystrobin 18.2% w/w + Difenoconazole 11.4% ww SC @ $1mlL^{-1}$

T4 - Kresoxim methyl 40% + Hexaconazole 8% WG @ 0.25 gL^{-1}

T5 - Kresoxim methyl 40% + Hexaconazole 8% WG @ 0.5 gL^{-1}

T6 - Kresoxim methyl 40% + Hexaconazole 8% WG @ 1gL^{-1}

T7 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG @ 0.1 gL^{-1}

T8 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG @ 0.2 gL^{-1}

T9 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG @ 0.4gL^{-1}

T10 - Control

3.4 SCREENING FOR HOST PLANT RESISTANCE

Pot culture experiment was conducted to screen KAU rice varieties (15 number) for resistance to sheath blight disease. The pot culture was performed at M. S. Swaminathan Rice Research Station, Moncompu during 2023 - 2024. KAU varieties screened were Dhanu, Onam (Kayamkulam 3), Bhadra (MO 4), Aruna (MO 8), Kanakom (MO 11), Ranjini (MO 12), Uma (MO 16), Karishma (MO 18), Krishnanjana (MO 19), Pournami (MO 23), Jyothi (PTB 39), Kanchana (PTB 50), Aiswarya (PTB 52), Anaswara (PTB 58) and Manuratna with three replications each. The variety Uma (MO 16) was maintained as susceptible check.

Healthy seeds of the selected rice varieties were sown in micro planter pots filled with paddy field soil. After fifteen days the seedlings were transplanted to plastic planter pots filled with paddy field soil, sand and dry cowdung at the ratio of 2:1:1. The crop management practices like cultural operations and fertilizer application was followed according to Package of Practices of Kerala Agricultural University (KAU, 2024).

The plants were artificially inoculated at tillering stage. Seven days old culture of the virulent isolate of the pathogen were used for artificial inoculation. Using sterile needles, pinpricking was provided to the plants to make injury on the sheaths. Mycelial

bits were placed on the pinpricked areas. A thin layer of moistened cotton was placed over these bits and covered with polythene covers to maintain humidity. The periodic observations for symptom development, lesion size, disease incidence and disease severity were recorded. Disease scoring was also done using IRRI - SES scale, 2013 based on lesion developed on sheath of the inoculated plants to calculate the disease severity by using the following formula (Neha *et al*., 2017).

$$Disease\ severity\ (\%) = \frac{Sum\ of\ individual\ rating}{Number\ of\ tillers\ observed\ \times Maximum\ disease\ score} \times 100$$

3.5 *IN VIVO* TESTING OF THE EFFICACY OF BACTERIAL BIOCONTROL AGENTS AND COMMERCIAL FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

A field experiment was conducted at M. S. Swaminathan Rice Research Station, Moncompu to evaluate the efficacy of bacterial biocontrol agents and commercial fungicides against sheath blight pathogen during *Kharif* 2024 in popular rice variety, Uma (MO 16). The evaluation was conducted in Split Plot Design (SPD) with six main plot treatments and two sub plot treatments with three replications. The treatment details are as follows.

Main plot treatments

M1 - *B. amyloliquefaciens* (B15)

M2 - *P. fluorescens* (PN026)

M3 - Azoxystrobin 18.2% w/w + Difenoconazole 11.4% ww SC @ $1mlL^{-1}$

M4 - Kresoxim methyl 40% + Hexaconazole 8% WG @ $1gL^{-1}$

M5 - Trifloxystrobin 25% + Tebuconazole 50% 75 WG @ $0.4gL^{-1}$

M6 - Untreated control

Sub plot treatments

S1 - 100% RDF (Recommended dose of fertilizers)

S2 - 75% N + 100% P + 125% K of RDF

3.5.1 Methods of application of biocontrol agents

Bacterial biocontrol agents *B. amyloliquefaciens* (B15) and *P. flourescens* (PN026) were applied as seed treatment, soil application and foliar spray. The application methods are as follows.

3.5.1.1 Seed treatment with *B. amyloliquefaciens* and *P. fluorescens*

Seeds of popular rice variety Uma (MO 16) were treated with *P. fluorescens* and *B. amyloliquefaciens* separately. Talc based formulation of these biocontrol agents at the rate 10g kg^{-1} were used for the wet seed treatment. Ten grams talc formulation of *B. amyloliquefaciens* and *P. flourescens* were mixed separately in one litre of water for one kg seed and soaked for twelve hours. It was then drained and transferred to gunny bags for germination. The seeds were soaked in water without biocontrol agents for the control plots. Water was sprinkled over the gunny bags thrice a day. Sprouted seeds were sown in the nursery field after second day. The twenty two day old seedlings were transplanted in the main field at a spacing of 15 cm x 15 cm in each plot of size 5 x 2 m^2. The fertilizer was applied as per the Package of practices of Kerala Agricultural University (KAU, 2024).

3.5.1.2 Soil application of *B. amyloliquefaciens* and *P. fluorescens*

The soil application of *B. amyloliquefaciens* and *P. fluorescens* was done at 35 days after planting (DAP) at the rate of 1 kg $acre^{-1}$. The required quantity of formulation for two main plots (M1 and M2) was taken and mixed with one kg of cow dung and then broadcasted in the field.

3.5.1.3 Foliar spray of *B. amyloliquefaciens* and *P. fluorescens*

The biocontrol agents, *B. amyloliquefaciens* and *P. fluorescens* were applied as foliar spray (20 gL^{-1} of water) at maximum tillering stage.

3.5.1.4 Application of fungicides

Required quantity of fungicidal solutions were prepared in quantities required to spray three main plots (M3, M4 and M5) at maximum tillering stage.

3.5.1.5 Application of fertilizers for subplots

Fertilizers required for subplots were calculated and applied as per Package of practices of Kerala Agricultural University (KAU, 2024).

3.5.1.6 Observations recorded

Disease scoring was done as per IRRI-SES scale, 2013 based on the symptoms. The observations *viz.,* number of infected tillers, per cent infected panicles, infected grains, number of chaffy grains, thousand grain weight, grain yield at 14% moisture content and other major pest and diseases associated were recorded.

Disease incidence was calculated using the following formula (Thera *et al*., 2021).

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ tillers}{Total\ number\ of\ tillers} \times 100$$

Disease severity was calculated rating the individual specimens and by using the following formula (Neha *et al*., 2017).

$$Disease\ severity\ (\%) = \frac{Sum\ of\ individual\ rating}{Number\ of\ tillers\ observed\ \times Maximum\ disease\ score} \times 100$$

3.6 STATISTICAL ANALYSIS

The observed and computed values from pot culture, *in vitro* studies and field experiment was analyzed using standard data analysis software (GRAPES) (Gopinath *et al*., 2021).

Results

RESULTS

The results of study entitled "Assessment and management of rice sheath blight disease in Kuttanad" conducted at M. S. Swaminathan Rice Research Station, Moncompu and Department of Plant Pathology, College of Agriculture, Vellayani during the year 2022 – 2024 are given here.

4.1 ASSESSMENT OF EXTENT OF SHEATH BLIGHT DISEASE INCIDENCE IN KUTTANAD TRACT

A purposive sampling was conducted in agroecological unit – 04 (Kuttanad) comprising Alappuzha, Kottayam and Pathanamthitta districts. The survey was conducted in forty locations *i.e.* 22, 12 and 6 locations from Alappuzha, Kottayam and Pathanamthitta respectively (Table 1).

The symptomatology was studied. The plants expressed symptoms such as presence of water soaked lesions with greenish grey centre and brown margins. The symptoms appeared at the base of the outer leaf sheath at or above the water level. The symptoms of sheath blight disease observed in survey locations are given (Table 2 , Plate 1). The diseased plants were scored according to IRRI-SES scale, 2013 (Table 3). The disease parameters such as disease incidence, disease severity, infected tillers (%) and infected panicles (%) were recorded from these locations (Table 4).

The disease incidence ranged from 15.39 to 72.73 per cent in the survey locations whereas, infected tillers ranged from 28.20 to 75.82 per cent. The panicle infection percentage varied from 10.18 to 56.89 per cent in the survey locations. The disease severity assessed from each location varied from 30.00 to 80.91 per cent.

Neelamperoor region of Alappuzha district exhibited highest disease incidence (72.73%), per cent tiller infection (75.82%), per cent panicle infection (56.89%) as well as disease severity (80.91%) among the forty survey locations. Least disease incidence

(15.39%) and panicle infection (10.18%) were observed in Kallara location of Kottayam district. Lowest per cent tiller infection (28.20%) was observed in Parampuzha location of Kottayam district whereas least disease severity (30.00%) was recorded from Neendoor and Vechoor locations in Kottayam district.

In Alappuzha district, highest disease incidence (72.73%) was observed in Neelamperoor location followed by Kunnumma (69.23%), Kidangara (64.28%) and Pulinkunnu (63.64%) whereas least disease incidence was recorded from Chungam (18.19%) location. In Kottayam district, survey location Kaduthuruthy exhibited highest disease incidence (54.55%) followed by Neendoor (46.15%) and Mannar (41.67%) and least disease incidence (15.39%) was observed in Kallara. Similarly, Niranam location recorded highest disease incidence (50.00%) in Pathanamthitta district followed by Nedumpuram (31.25%) and Kadapra (30.77%) whereas least disease incidence (18.19%) was observed in Peringara location.

Highest tiller infection in Alappuzha district was observed in Neelamperoor (75.82%) followed by Kainakary (70.72%), Edathua (68.00%) and Kidangara (64.22%) locations while least infection (41.42%) was recorded in Kunnumma location. Highest tiller infection in Kottayam (63.30%) district was in Neendoor survey location followed by Kallara (61.63%), Thalayolaparambu (61.54%) and Kumarakom (55.54%) whereas lowest infection (28.20%) was recorded from Parampuzha location. Similarly, in Pathanamthitta district, Thiruvalla exhibited highest tiller infection (67.80%) followed by Kadapra (51.00%) and Nedumpuram (50.09%).

Among the survey locations from Alappuzha district, highest panicle infection (56.89%) was recorded in Neelamperoor followed by Kavalam (44.45%), Edathua (42.36%) and Kidangara (39.67%). Kainady location recorded least panicle infection (10.41%) in Alappuzha district. In Kottayam district, Neendoor recorded highest infection (30.27%) followed by Vaikom (24.27%), Thalayolaparambu (22.75%) and Kaduthuruthy (21.00%) while least infection (10.18%) was recorded in Kallara survey

location of Kottayam district. In Pathanamthitta, survey location Nedumpuram recorded highest infection (24.81%) while least infection (15.18%) was observed in Niranam.

Highest disease severity (80.91%) in Alappuzha district was recorded from Neelamperoor followed by Kainakary (73.64%), Kidangara (72.58%) and Nedumudy (70.12%) and least was recorded in Kunnamkary (41.09%). While, in Kottayam district, highest disease severity (68.18%) was recorded from Thalayolaparambu location followed by Eara (64.54%) and Mannar (62.73%). Lowest disease severity in Kottayam (30.00%) district was observed to be in Neendoor and Vechoor survey locations. Similarly, in Pathanamthitta district, highest disease severity was recorded from Kadapra location (64.54%) followed by Thiruvalla (59.09%). The least disease severity (33.64%) in Pathanamthitta district was recorded from Perumthuruthy survey location. The common insect pests observed in the survey locations were brown plant hopper, rice bug, yellow stem borer, and grass hopper (Table 5).

4.2 ISOLATION OF THE PATHOGEN INVOLVED AND ITS CHARACTERIZATION

The sheath blight infected samples were collected from all forty locations of agroecological unit – 04 (Kuttanad). All the samples were isolated and was tested for pathogenicity on rice variety Uma using pot culture study.

4.2.1 Isolation of the pathogen and its pathogenicity studies

The isolation of the pathogen was done using bits from diseased samples on PDA medium. The obtained pathogen was then subjected to microscopic examination. Then purified subsequently and maintained in refrigerator at 4ºC temperature in PDA slants for further studies. Forty isolates were obtained from survey locations and were used for further studies (Table 6, Plate 2).

Table 1: Locations surveyed in agroecological unit – 04 (Kuttanad)

Sl. No	District	Location	GPS Coordinates
1	Alappuzha	Karumady	9.377752ºN, 76.389006ºE
2		Thakazhy	9.369663ºN, 76.418887ºE
3		Edathua	9.389259ºN, 76.477827ºE
4		Mampuzhakary	9.4271ºN, 76.4785ºE
5		Champakulam	9.415989ºN, 76.407823ºE
6		Kainakary	9.443027ºN, 76.399157ºE
7		edumudy	9.5150ºN,76.3927ºE
8		Kidangara	9.451425ºN, 76.45767ºE
9		Pulinkunnu	9.4596211ºN, 76.433707ºE
10		Kunnumma	9.474017ºN, 76.452083ºE
11		Ramankary	9.4289190ºN, 76.464316ºE
12		Kavalam	9.469117ºN, 76.453152ºE
13		Narakathara	9.46888ºN, 76.46689ºE
14		Vazhappally	9.467871ºN, 76.492456ºE
15		Moncompu	9.4412172ºN, 76.4209105ºE
16		Koilmukku	9.360867ºN, 76.469142ºE
17		Kainady	9.4974203ºN, 76.4757268ºE
18		Chungam	9.495386ºN, 76.355832ºE
19		Kannady	9.459692ºN, 76.431984ºE
20		Puthukary	9.4017501ºN, 76.4729511ºE
21		Neelamperoor	9.4964729ºN, 76.5096732ºE
22		Kunnamkary	9.438036ºN, 76.4836ºE
23		Onamthuruth	9.672177ºN, 76.497664ºE
24		Neendoor	9.680126ºN, 76.507091ºE

25	Kottayam	Parampuzha	9.60965ºN, 76.558226ºE
26		Mannar	9.332125ºN, 76.531977ºE
27		Eara	9.489815ºN, 76.480347ºE
28		Kallara	9.781471ºN, 76.463105ºE
29		Kaduthuruthy	9.6516429ºN, 76.428321ºE
30		Kumarakom	9.781471ºN, 76.463105ºE
31		Kaipuzha	9.791725ºN, 76.457781ºE
32		Vechoor	9.6828ºN, 76.4585ºE
33		Thalayolaparambu	9.590295ºN, 76.426123ºE
34		Vaikom	9.673755ºN, 76.505985ºE
35	Pathanamthitta	Kadapra	9.330969ºN, 76.500685ºE
36		Niranam	9.364012ºN, 76.519303ºE
37		Thiruvalla	9.4018030ºN, 76.55684ºE
38		Nedumpuram	9.3632791ºN, 76.55737ºE
39		Peringara	9.3768265ºN, 76.54426ºE
40		Perumthuruthy	9.409215ºN, 76.550314ºE

Table 2: Sheath blight symptoms observed in survey locations

Location	Symptoms
Karumady	Greyish water soaked lesions with brown margins on leaf sheath at water level. White coloured sclerotia present.
Thakazhy	Brown coloured lesions with grey centers on the base of the leaf sheath.
Edathua	Water soaked spots on the leaf sheath with yellowish brown margins and light brown centre
Mampuzhakary	Grey to dark brown spots and lesions near water level in the leaf sheath
Champakulam	Presence of greyish ellipsoid lesions on sheaths along with brown coloured sclerotia
Kainakary	Necrotic lesions with brown to black margins and light grey to white centres at the base
Nedumudy	Circular to oblong shaped brown spots with grey centre present in sheath near the water level
Kidangara	Large lesions on the base of the sheaths along with the presence of black coloured sclerotia
Pulinkunnu	Elongated water soaked lesions with brown to black margins on base of leaf sheath
Kunnumma	Brown to grey dark lesions near the leaf sheath base with minute black coloured sclerotia
Ramankary	Greenish grey water soaked lesions on leaf sheath
Kavalam	Elongated lesions on sheaths near water level with light grey centres
Narakathara	Circular spots with whitish centre with greyish brown margins on base of sheath
Vazhappally	Brown coloured spots and elongated lesions on the sheath base at water level
Moncompu	Prescence of white coloured lesions with brown margins on the sheaths
Koilmukku	Grey coloured oval to circular spots observed on the sheath
Kainady	Greenish grey water soaked spots near water level
Chungam	Greyish white lesions on the sheath base
Kannady	Expanded brown lesions with pale green to white centres on sheaths near the water line

Puthukary	Small circular spots on the outer leaf sheaths at the base region
Neelamperoor	Coalesced lesions with dark brown to black margins and white centres along with brown sclerotia
Kunnamkary	Elliptical to oval shaped spots with brown margins on leaf sheath
Onamthuruth	Water soaked irregular lesions with brown margin on leaf sheaths on and above the water level
Neendoor	Elongated lesions with white coloured centre and brown to black margins on sheaths
Parampuzha	Circular water soaked spots on the base of leaf sheath
Mannar	Brown coloured irregular spots with white centres
Eara	Greyish white lesions near the water level on the sheaths with white coloured sclerotia
Kallara	Dark brown coloured lesions with white centres bearing black coloured sclerotia
Kaduthuruthy	Pale yellow spots with dark brown coloured margin observed on the base of the plants
Kumarakom	Irregular lesions occurred due to the coalescing of water soaked spots on sheaths near the water level
Kaipuzha	Brown coloured spots on the surface of the sheath
Vechoor	White to yellow spots with white coloured sclerotia
Thalayolaparambu	Greyish spots with brown margins above the water level
Vaikom	Water soaked lesions with light grey coloured margins on leaf bases
Kadapra	Brown to black lesions observed on the base of rice plants along with brown coloured sclerotia
Niranam	Yellow to brown spots near the water level on the outer sheath
Thiruvalla	Greyish spots present on the surface of the sheath near the water level
Nedumpuram	Brown to black water soaked spots on the base of sheath
Peringara	Grey coloured expanded lesions on the leaf sheath with white to light grey coloured centre
Perumthuruthy	Brown water soaked lesions on the leaf sheath above and near the water level

Table 3: IRRI Standard Evaluation System (SES) for rice sheath blight disease

Disease score	Description	Reaction
0	No infection	Immune
1	Lesion limited to the lower 20% of plant height	Resistant
3	Lesion limited to lower 20 – 30% of the plant height	Moderately resistant
5	Lesion limited to the lower 31 – 45% of the plant height	Moderately susceptible
7	Lesion limited to the lower 46 – 65% of the plant height	Susceptible
9	Lesion more than 65% of the plant height	Highly susceptible

Karumady

Thakazhy

Edathua

Mampuzhakary

Champakulam

Kainakary

Nedumudy

Kidangara

Pulinkunnu

Kunnumma

Plate 1: Sheath blight symptoms observed in survey locations

Ramankary

Kavalam

Narakathara

Vazhappally

Moncompu

Koilmukku

Kainady

Chungam

Kannady

Puthukary

Plate 1 (contd.): Sheath blight symptoms observed in survey locations

Plate 1 (contd.): Sheath blight symptoms observed in survey locations

Kaipuzha

Vechoor

Thalayolaparambu

Vaikom

Kadapra

Niranam

Thiruvalla

Nedumpuram

Peringara

Perumthuruthy

Plate 1 (contd.): Sheath blight symptoms observed in survey locations

Table 4: Details of disease parameters assessed in different survey locations

Sl. No.	District	Location	Disease Incidence (%)	Infected tillers (%)	Infected panicles (%)	Disease severity (%)
1.	Alappuzha	Karumady	58.33	50.54	33.00	51.82
		Thakazhy	45.40	56.70	37.00	62.73
		Edathua	31.25	68.00	42.36	64.55
		Mampuzhakary	33.33	47.70	24.82	53.64
		Champakulam	40.00	58.63	29.42	61.82
		Kainakary	41.66	70.72	25.89	73.64
		Nedumudy	50.00	62.63	23.00	70.12
		Kidangara	64.28	64.22	39.67	72.58
		Pulinkunnu	63.64	55.63	27.91	68.25
		Kunnumma	69.23	41.42	30.27	52.55
		Ramankary	50.00	51.55	25.58	54.85
		Kavalam	58.33	47.45	44.45	53.60
		Narakathara	27.27	53.30	12.09	46.36
		Vazhappally	58.33	60.56	24.78	44.54
		Moncompu	36.36	58.64	21.70	55.46
		Koilmukku	35.71	59.80	16.55	46.36
		Kainady	30.00	52.45	10.41	50.00
		Chungam	18.19	58.11	19.45	46.36
		Kannady	36.37	49.83	16.55	48.18
		Puthukary	42.86	52.55	11.34	44.54
		Neelamperoor	72.73	75.82	56.89	80.91

		Kunnamkary	30.00	60.56	35.88	41.09
2.	Kottayam	Onamthuruth	22.23	47.36	13.91	57.27
		Neendoor	46.15	63.30	30.27	30.00
		Parampuzha	27.27	28.20	11.36	39.09
		Mannar	41.67	48.27	17.83	62.73
		Eara	26.67	54.36	15.89	64.54
		Kallara	15.39	61.63	10.18	42.73
		Kaduthuruthy	54.55	47.55	21.00	53.64
		Kumarakom	31.25	55.54	18.58	39.09
		Kaipuzha	33.34	54.36	16.54	31.82
		Vechoor	30.77	48.91	19.45	30.00
		Thalayolaparambu	40.00	61.54	22.75	68.18
		Vaikom	22.22	45.30	24.27	37.27
3.	Pathanamthitta	Kadapra	30.77	51.00	17.00	64.54
		Niranam	50.00	45.25	15.18	42.73
		Thiruvalla	27.28	67.80	16.00	59.09
		Nedumpuram	31.25	50.09	24.81	48.18
		Peringara	18.19	43.27	16.00	35.45
		Perumthuruthy	25.00	44.45	16.54	33.64

Table 5: Details of major pests associated in different locations

Sl.No.	District	Location	Major pest associated
1.	Alappuzha	Karumadi	Rice bug, Grasshopper, Rice leaf folder
		Thakazhi	Yellow stem borer, Grasshopper, Rice bug
		Edathua	Grasshopper, Shield bug, Yellow stem borer
		Mampuzhakari	Green leaf hopper, Rice bug
		Champakulam	Rice bug, Shield bug, Yellow stem borer
		Kainakary	Brown plant hopper, Rice leaf folder, Rice bug
		Nedumudi	Shield bug, Green leaf hopper, Grasshopper
		Kidangara	Yellow stem borer, Rice leaf folder
		Pulinkunnu	Rice bug, Grasshopper
		Kunnumma	Yellow stem borer, Brown plant hopper
		Ramankary	Grasshopper, Yellow stem borer, Rice bug
		Kavalam	Brown plant hopper, Rice bug
		Narakathara	Shield bug, Rice bug, Grasshopper
		Vazhappally	Grasshopper, Rice bug, Yellow stem borer
		Moncompu	Rice leaf folder, Brown plant hopper, Rice bug
		Koilmukku	Grasshopper, Rice bug, Green leaf hopper
		Kainady	Rice leaf folder, Rice bug
		Chungam	Brown plant hopper, Grasshopper, Rice bug
		Kannadi	Brown plant hopper, Grasshopper
		Puthukary	Rice leaf folder, Rice bug

		Neelamperoor	Brown plant hopper, Yellow stem borer, Rice bug
		Kunnamkari	Brown plant hopper, Grasshopper, Rice bug
2.	Kottayam	Onamthuruth	Green leaf hopper, Yellow stem borer
		Neendoor	Grasshopper, Yellow stem borer, Rice bug
		Parampuzha	Rice bug, Brown plant hopper
		Mannar	Grasshopper, Rice leaf folder
		Eara	Yellow stem borer, Grasshopper, Rice bug
		Kallara	Brown plant hopper, Rice leaf folder, Yellow stem borer
		Kaduthuruthy	Rice leaf folder, Grasshopper, Rice bug
		Kumarakom	Brown plant hopper, Rice bug
		Kaipuzha	Green leaf folder, Grasshopper
		Vechoor	Rice bug, Yellow stem borer
		Thalayolaparambu	Brown plant hopper, Shield bug, Rice bug
		Vaikom	Green leaf hopper, Brown plant hopper, Rice bug
3.	Pathanamthitta	Kadapra	Yellow stem folder, Shiled bug
		Niranam	Grasshopper, Yellow stem borer, Rice leaf folder
		Thiruvalla	Rice leaf folder, Rice stem borer, Shield bug
		Nedumpuram	Rice bug, Brown plant hopper
		Peringara	Grasshopper, Yellow stem borer, Rice bug
		Perumthuruthy	Rice bug, Grasshopper, Brown plant hopper

The forty isolates were subjected for pathogenicity test in rice variety, Uma. All the isolates produced symptoms within three to nine days (Plate 3). The symptoms produced by each isolate obtained from different survey locations are described in Table 7. The typical symptoms of sheath blight disease such as presence of water soaked lesions with grey to white centres and brown margins were observed in the plants. The sclerotia was formed on infected leaf sheaths within five to twelve days. The plants were scored using IRRI-SES scale, 2013 and disease severity of the inoculated plants was recorded. The pathogen was re-isolated from the developed symptoms. Upon reisolation the pathogen expressed characters similar to that of the inoculated pathogen.

Plants inoculated with isolate obtained from Neelamperoor in Alappuzha district (I_{21}) took least number of days for symptom development (3 days) and for sclerotia formation (5 days) compared to plants inoculated with other isolates. Similarly, plants inoculated with isolate I_{21} exhibited highest disease severity (88.54%) compared to other plants (Table 8). Hence, the isolate from Neelamperoor region of Alappuzha district (I_{21}) was identified as the virulent isolate.

4.2.2 Cultural and morphological characterization of isolates

The isolates produced mycelia of diverse colours from white to dark brown (Plate 4). It took three to eleven days to complete growth in Petri plates (90 mm). All forty isolates produced sclerotia within three to nine days (Table 9). The mycelial growth nature varied among the isolates. Flat and aerial type growth pattern was observed. Isolates I_2, I_8, I_{10}, I_{11}, I_{16}, $I_{17,}$ I_{18}, I_{21}, I_{22}, I_{26}, I_{33} and I_{38} exhibited aerial growth pattern whereas, all other isolates exhibited flat growth nature. The pattern of sclerotia formation was recorded as scattered or confined to centre or periphery. The pattern of sclerotia formation was observed to be confined to periphery in isolates $I_{3,}$ I_4, I_7, I_{11}, I_{15}, I_{23}, I_{25}, I_{30}, I_{32} and I_{34} and to be confined to centre in isolates I_2, I_8, I_{26}, I_{27}, I_{28} and I_{35} whereas, the pattern was observed to be scattered in all other isolates.

The isolate I_{21} from Neelamperoor location took least number of days to complete growth in Petri plate (3 days) as well as for the formation of sclerotia (3 days) compared to other isolates. Hence, isolate I_{21} was identified as virulent and used in further studies. All the isolates produced hyaline, septate hypha with right angled branching (Table 10). The hyphal width ranged from 1.12 – 1.98 µm. The sclerotia produced were of different colours which varied from white to dark brown (Plate 5). Sclerotia formed by isolates I_4, I_6, I_{14}, I_{18}, I_{19}, I_{21}, I_{31}, I_{33}, I_{36}, I_{37} and I_{38} were initially white in colour and later turned to dark brown whereas, the sclerotia formed by isolates I_5, I_8, I_{15} and I_{27} remained white in colour. The size of sclerotia varied from 1.05 – 1.48 mm (Table 11). The texture of sclerotia was recorded to be two different types *viz.,* smooth and rough textured. Among the isolates, I_1, I_5, I_7, I_8, I_{13}, I_{15}, I_{22}, I_{27}, I_{29}, I_{32} and I_{35} isolates formed sclerotia with smooth surface while others formed sclerotia with rough surface.

4.2.3 Molecular characterization of virulent isolate

The virulent isolate (I_{21}) among the forty isolates obtained was identified by molecular characterization. The culture was given to Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram for DNA sequencing using ITS primers. DNA was isolated from the virulent isolate using NucleoSpin® Plant II Kit (Macherey-Nagel). The DNA was amplified using ITS1 and ITS4 primers and amplicon of 500 bp lenth was obtained. The gel profile of the PCR product is given in Plate 6. The amplicon was purified by Exosap treatments and it was then subjected to Sanger's sequencing by BigDye Terminator method. The DNA sequences obtained are provided in Appendix. The DNA sequence was aligned to the already existing sequences in NCBI database using BLAST software. The isolate showed maximum similarity with *R. solani* isolate with accession code EU591797 (Fig. 3). The sequences obtained was submitted in GenBank and was allotted with accession number PQ658187. The phylogenetic tree showing the evolutionary relationship with other *R. solani* isolates is given in Figure 4.

Table 6: Isolates of pathogen obtained from different survey locations of AEU – 04 (Kuttanad)

Location	Isolates	Location	Isolates
Karumady	I_1	Neelamperoor	I_{21}
Thakazhy	I_2	Kunnamkary	I_{22}
Edathua	I_3	Onamthuruth	I_{23}
Mampuzhakary	I_4	Neendoor	I_{24}
Champakulam	I_5	Parampuzha	I_{25}
Kainakary	I_6	Mannar	I_{26}
Nedumudy	I_7	Eara	I_{27}
Kidangara	I_8	Kallara	I_{28}
Pulinkunnu	I_9	Kaduthuruthy	I_{29}
Kunnumma	I_{10}	Kumarakom	I_{30}
Ramankary	I_{11}	Kaipuzha	I_{31}
Kavalam	I_{12}	Vechoor	I_{32}
Narakathara	I_{13}	Thalayolaparambu	I_{33}
Vazhappally	I_{14}	Vaikom	I_{34}
Moncompu	I_{15}	Kadapra	I_{35}
Koilmukku	I_{16}	Niranam	I_{36}
Kainady	I_{17}	Thiruvalla	I_{37}
Chungam	I_{18}	Nedumpuram	I_{38}
Kannady	I_{19}	Peringara	I_{39}
Puthukary	I_{20}	Perumthuruthy	I_{40}

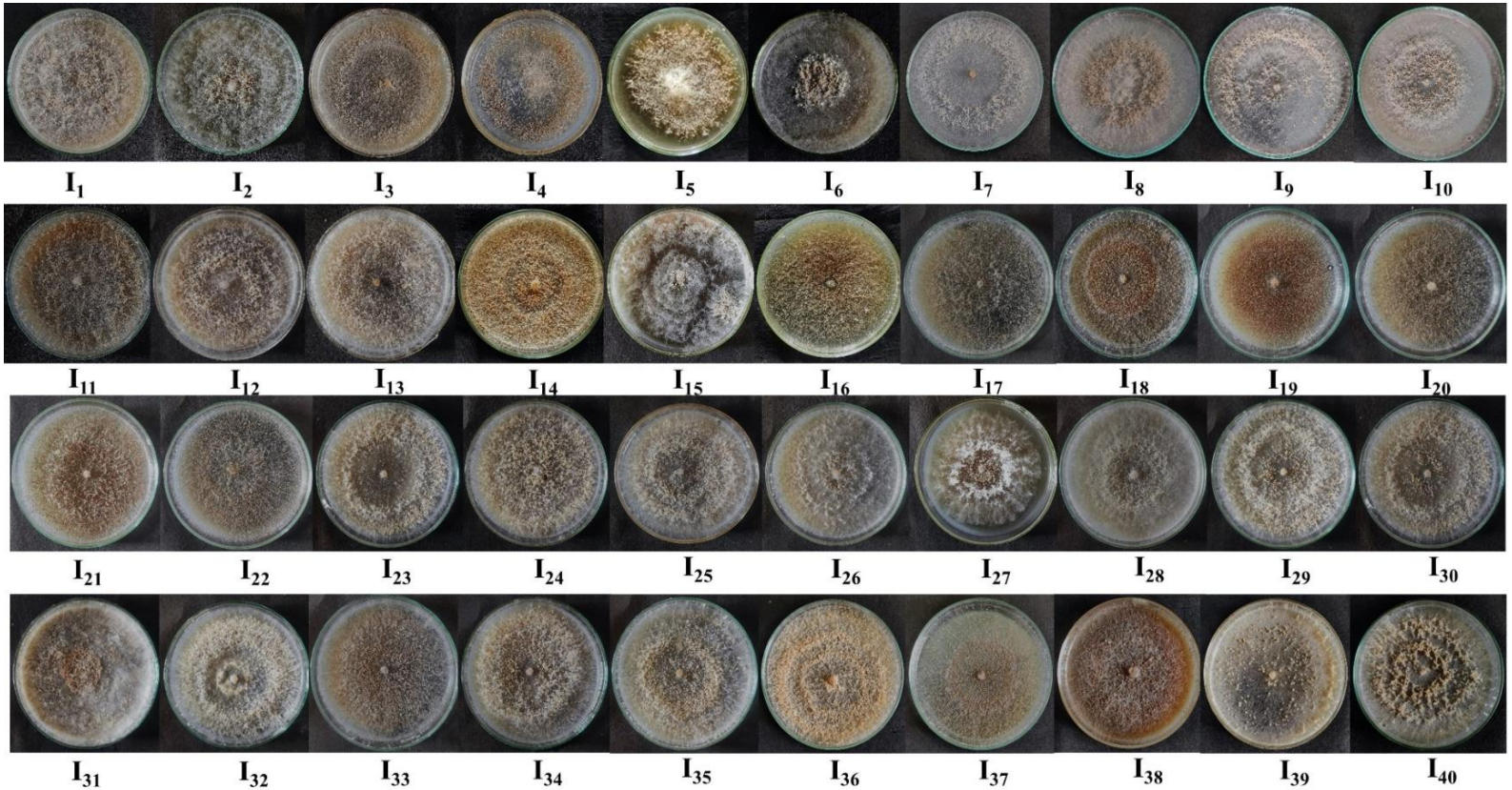


Plate 2: Isolates of pathogen obtained from different survey locations of AEU – 04 (Kuttanad)

Table 7: Symptoms produced by pathogenic isolates upon artificial inoculation

Isolates	Symptoms
I_1	Greenish grey water soaked lesions on the base of the sheath
I_2	Brown coloured spots on the sheath near water level
I_3	Water soaked spots with yellowish brown margins and white centre
I_4	Dark brown spots with whitish grey centre
I_5	Circle to oval shaped lesions with yellow to brown centres
I_6	Grey necrotic lesions with white centres on leaf sheath
I_7	Oblong shaped brown spots with grey centre on the leaf sheath
I_8	Large lesions with whitish grey centre
I_9	Elongated water soaked lesions with brown to black margins
I_{10}	Brown lesions on the leaf sheath near water level
I_{11}	Greenish grey coloured lesions
I_{12}	Elongated lesions with dark grey colours on the sheath
I_{13}	Circular spots with whitish centres
I_{14}	Brown coloured spots on the sheaths near the water level
I_{15}	Whitish grey coloured lesions with brown margins
I_{16}	Grey coloured circular to oval shaped spots and lesions on the sheath
I_{17}	Greenish grey water soaked lesions
I_{18}	Whitish grey lesions on the sheaths
I_{19}	Lesions with pale green to white centres
I_{20}	Circular brown spots on the outer sheaths near the water level

I_{21}	Lesions with dark brown to black margin with white centres
I_{22}	Oval shaped spots with grey centre
I_{23}	Water soaked lesions with irregular margins above the water level
I_{24}	Spots with white coloured centre and black margins
I_{25}	Circular water soaked spots on sheath
I_{26}	Irregular spots with brown coloured margins and grey centres
I_{27}	Greyish green lesions near the water level
I_{28}	Brown coloured lesions with light brown centres
I_{29}	Pale yellow coloured spots with brown margins on the base of the sheath
I_{30}	Irregular water soaked lesions on the sheaths
I_{31}	Brown to black coloured spots on the outer sheaths
I_{32}	White to yellow coloured spots near the water level on the base of leaf sheaths
I_{33}	Greyish green spots on the sheaths
I_{34}	Water soaked lesions with grey centres and brown margins
I_{35}	Brown to black coloured spots
I_{36}	Yellow to light brown coloured spots near water level
I_{37}	Greyish water soaked spots on the leaf sheath
I_{38}	Water soaked spots with brown to black margins
I_{39}	Brown coloured spots with grey centres
I_{40}	Greyish water soaked spots on the leaf sheaths

I_1 I_2 I_3 I_4 I_5

I_6 I_7 I_8 I_9 I_{10}

Plate 3: Symptoms observed upon artificial inoculation of isolates obtained from different survey locations

I_{11}

I_{12}

I_{13}

I_{14}

I_{15}

I_{16}

I_{17}

I_{18}

I_{19}

I_{20}

Plate 3 (contd.): Symptoms observed upon artificial inoculation of isolates obtained from different survey locations

I_{21}

I_{22}

I_{23}

I_{24}

I_{25}

I_{26}

I_{27}

I_{28}

I_{29}

I_{30}

Plate 3 (contd.): Symptoms observed upon artificial inoculation of isolates obtained from different survey locations

I_{31}

I_{32}

I_{33}

I_{34}

I_{35}

I_{36}

I_{37}

I_{38}

I_{39}

I_{40}

Plate 3 (contd.): Symptoms observed upon artificial inoculation of isolates obtained from different survey locations

Table 8: Details of disease parameters upon artificial inoculation of isolates

Isolates	Days for symptom development	Days for sclerotia formation	Disease severity (%)
I_1	$(8 \pm 0.816)^{bc}$	$(11 \pm 0.816)^{ab}$	$27.08\ (31.33)^{klm}$
I_2	$(6 \pm 0.500)^{hi}$	$(8 \pm 0.816)^{gh}$	$35.94\ (36.83)^{hi}$
I_3	$(6 \pm 0.577)^{ij}$	$(9 \pm 0.500)^{defg}$	$56.25\ (48.59)^{d}$
I_4	$(5 \pm 0.011)^{jkl}$	$(6 \pm 0.500)^{kl}$	$64.06\ (53.17)^{c}$
I_5	$(9 \pm 0.577)^{ab}$	$(12 \pm 1.291)^{ab}$	$26.04\ (30.64)^{klm}$
I_6	$(5 \pm 0.500)^{kl}$	$(8 \pm 0.957)^{hi}$	$72.92\ (58.65)^{b}$
I_7	$(8 \pm 0.500)^{cd}$	$(11 \pm 0.957)^{ab}$	$29.17\ (32.67)^{jk}$
I_8	$(6 \pm 0.577)^{ij}$	$(9 \pm 0.500)^{fg}$	$53.65\ (47.09)^{d}$
I_9	$(5 \pm 0.577)^{ij}$	$(8 \pm 0.500)^{gh}$	$54.69\ (47.69)^{d}$
I_{10}	$(6 \pm 0.500)^{fgh}$	$(9 \pm 0.500)^{fg}$	$36.46\ (37.14)^{ghi}$
I_{11}	$(6 \pm 0.957)^{hijk}$	$(9 \pm 0.957)^{defg}$	$35.42\ (36.51)^{i}$
I_{12}	$(7 \pm 0.500)^{def}$	$(10 \pm 0.577)^{abc}$	$34.38\ (35.88)^{i}$
I_{13}	$(9 \pm 0.577)^{a}$	$(11 \pm 1.915)^{abc}$	$16.15\ (23.66)^{n}$
I_{14}	$(9 \pm 0.577)^{a}$	$(12 \pm 2.082)^{ab}$	$15.10\ (22.69)^{n}$
I_{15}	$(7 \pm 0.500)^{cde}$	$(9 \pm 0.957)^{ghij}$	$27.60\ (31.68)^{jklm}$
I_{16}	$(6 \pm 0.500)^{hijk}$	$(8 \pm 1.291)^{ghi}$	$34.38\ (35.89)^{i}$
I_{17}	$(7 \pm 0.011)^{defg}$	$(9 \pm 1.708)^{kl}$	$33.86\ (35.57)^{i}$
I_{18}	$(5 \pm 1.258)^{ijkl}$	$(6 \pm 0.816)^{k}$	$56.25\ (48.59)^{d}$
I_{19}	$(5 \pm 0.577)^{ijkl}$	$(6 \pm 1.414)^{k}$	$35.42\ (36.52)^{i}$
I_{20}	$(5 \pm 1.258)^{ijkl}$	$(7 \pm 0.957)^{hij}$	$60.94\ (51.32)^{c}$
I_{21}	$(3 \pm 0.500)^{m}$	$(5 \pm 0.577)^{l}$	$88.54\ (70.31)^{a}$
I_{22}	$(6 \pm 0.816)^{ghij}$	$(8 \pm 0.816)^{ghij}$	$36.45\ (37.14)^{ghi}$
I_{23}	$(7 \pm 0.816)^{defg}$	$(9 \pm 0.816)^{efgh}$	$39.58\ (38.97)^{fg}$
I_{24}	$(9 \pm 0.957)^{a}$	$(11 \pm 1.708)^{bcde}$	$24.48\ (29.62)^{m}$
I_{25}	$(4 \pm 0.577)^{l}$	$(9 \pm 0.500)^{gfhi}$	$72.39\ (58.32)^{b}$
I_{26}	$(5 \pm 0.577)^{ijkl}$	$(8 \pm 0.500)^{hij}$	$41.67\ (40.19)^{f}$
I_{27}	$(6 \pm 1.291)^{ijkl}$	$(9 \pm 0.816)^{efgh}$	$48.96\ (44.40)^{e}$

I_{28}	$(5 \pm 0.577)^{l}$	$(7 \pm 0.816)^{jk}$	$70.83\ (57.33)^{b}$
I_{29}	$(8 \pm 0.577)^{abc}$	$(10 \pm 0.816)^{cdef}$	$26.56\ (31.01)^{jkl}$
I_{30}	$(7 \pm 0.500)^{def}$	$(9 \pm 1.258)^{defg}$	$29.69\ (33.06)^{j}$
I_{31}	$(5 \pm 0.816)^{jkl}$	$(7 \pm 0.816)^{jk}$	$61.98\ (54.94)^{c}$
I_{32}	$(7 \pm 0.577)^{cde}$	$(9 \pm 0.957)^{fghi}$	$28.13\ (32.02)^{jkl}$
I_{33}	$(4 \pm 0.577)^{l}$	$(7 \pm 0.816)^{jk}$	$73.44\ (58.99)^{b}$
I_{34}	$(9 \pm 0.816)^{ab}$	$(11 \pm 0.957)^{a}$	$24.47\ (29.62)^{m}$
I_{35}	$(9 \pm 0.816)^{ab}$	$(11 \pm 1.291)^{ab}$	$25.52\ (30.31)^{lm}$
I_{36}	$(7 \pm 1.258)^{efgh}$	$(9 \pm 0.957)^{ghij}$	$39.06\ (38.68)^{fgh}$
I_{37}	$(5 \pm 0.957)^{ijkl}$	$(7 \pm 0.577)^{ij}$	$60.94\ (51.34)^{c}$
I_{38}	$(6 \pm 0.500)^{hijk}$	$(8 \pm 0.816)^{ghij}$	$39.06\ (38.67)^{fgh}$
I_{39}	$(8 \pm 0.816)^{bcd}$	$(10 \pm 0.816)^{cd}$	$28.13\ (32.02)^{jkl}$
I_{40}	$(7 \pm 0.500)^{def}$	$(9 \pm 0.500)^{fghi}$	$34.38\ (35.89)^{i}$
SE (m)	0.362	0.505	1.258
CD (0.05)	1.012	1.413	3.522

Values are mean of four replications

*Means of four replication ± Standard Deviation

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 9: Colony characters of isolates obtained from survey locations

Isolate	Colony colour	Days taken for complete growth of mycelia in Petri plate (9 cm)	Days taken for the formation of sclerotia	Growth pattern of mycelia	Sclerotia formation pattern
I_1	Whitish brown	5	8	Flat	Scattered
I_2	White	6	7	Aerial	Central
I_3	Whitish brown	6	8	Flat	Peripheral
I_4	White	8	5	Flat	Peripheral
I_5	White	11	9	Flat	Scattered
I_6	White	8	6	Flat	Scattered
I_7	White	9	8	Flat	Peripheral
I_8	Whitish brown	6	7	Aerial	Central
I_9	White	5	6	Flat	Scattered
I_{10}	White	6	7	Aerial	Concentric circles
I_{11}	Light brown	6	7	Aerial	Peripheral
I_{12}	White	5	8	Flat	Scattered

I_{13}	White	6	8	Flat	Scattered
I_{14}	Light brown	7	4	Flat	Scattered
I_{15}	White	11	9	Flat	Peripheral
I_{16}	Light brown	4	5	Aerial	Scattered
I_{17}	Yellowish brown	5	7	Aerial	Scattered
I_{18}	Centre–dark brown Periphery–Light brown	5	4	Aerial	Scattered
I_{19}	Light brown	6	4	Flat	Scattered
I_{20}	Light brown	6	5	Flat	Scattered
I_{21}	Dark brown	3	3	Aerial	Scattered
I_{22}	Dark brown	4	5	Aerial	Scattered
I_{23}	Light brown	7	8	Flat	Peripheral
I_{24}	Light brown	9	7	Flat	Scattered
I_{25}	Light brown	6	7	Flat	Peripheral
I_{26}	White	5	6	Aerial	Central
I_{27}	White	9	8	Flat	Central

I_{28}	White	6	5	Flat	Central
I_{29}	Light brown	6	6	Flat	Scattered
I_{30}	Whitish brown	11	8	Flat	Peripheral
I_{31}	White	6	5	Flat	Scattered
I_{32}	White	5	6	Flat	Peripheral
I_{33}	Light brown	4	5	Aerial	Scattered
I_{34}	Light brown	8	7	Flat	Peripheral
I_{35}	White	5	7	Flat	Central
I_{36}	Light brown	8	4	Flat	Concentric circles
I_{37}	Centre–dark brown Periphery–Light brown	6	5	Flat	Scattered
I_{38}	Dark brown	4	4	Aerial	Scattered
I_{39}	Light brown	5	7	Flat	Scattered
I_{40}	White	8	7	Flat	Concentric circles

Table 10: Hyphal characters of isolates obtained from survey locations

Sl.No.	Hyphal characters		
	Colour	Septation	Width of hypha (µm)
I_1	Hyaline	Septate	1.14
I_2	Hyaline	Septate	1.32
I_3	Hyaline	Septate	1.15
I_4	Hyaline	Septate	1.32
I_5	Hyaline	Septate	1.45
I_6	Hyaline	Septate	1.15
I_7	Hyaline	Septate	1.25
I_8	Hyaline	Septate	1.41
I_9	Hyaline	Septate	1.36
I_{10}	Hyaline	Septate	1.48
I_{11}	Hyaline	Septate	1.59
I_{12}	Hyaline	Septate	1.56
I_{13}	Hyaline	Septate	1.89
I_{14}	Hyaline	Septate	1.12
I_{15}	Hyaline	Septate	1.15
I_{16}	Hyaline	Septate	1.36
I_{17}	Hyaline	Septate	1.15
I_{18}	Hyaline	Septate	1.21
I_{19}	Hyaline	Septate	1.14
I_{20}	Hyaline	Septate	1.14

I_{21}	Hyaline	Septate	1.14
I_{22}	Hyaline	Septate	1.58
I_{23}	Hyaline	Septate	1.23
I_{24}	Hyaline	Septate	1.56
I_{25}	Hyaline	Septate	1.98
I_{26}	Hyaline	Septate	1.54
I_{27}	Hyaline	Septate	1.89
I_{28}	Hyaline	Septate	1.26
I_{29}	Hyaline	Septate	1.54
I_{30}	Hyaline	Septate	1.25
I_{31}	Hyaline	Septate	1.25
I_{32}	Hyaline	Septate	1.14
I_{33}	Hyaline	Septate	1.18
I_{34}	Hyaline	Septate	1.89
I_{35}	Hyaline	Septate	1.56
I_{36}	Hyaline	Septate	1.25
I_{37}	Hyaline	Septate	1.26
I_{38}	Hyaline	Septate	1.28
I_{39}	Hyaline	Septate	1.27
I_{40}	Hyaline	Septate	1.56

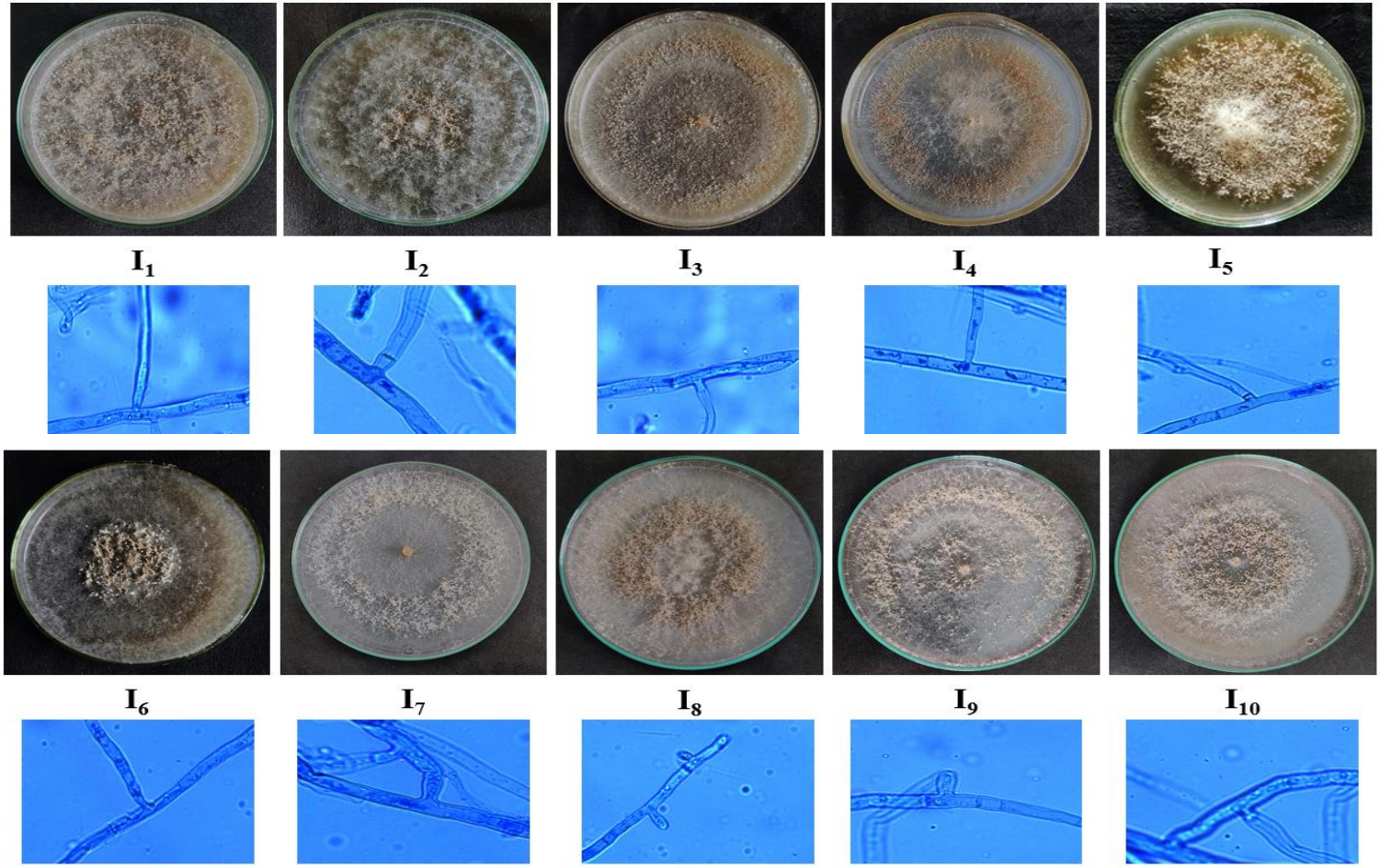


Plate 4: Colony and hyphal characters of isolates obtained from different survey locations

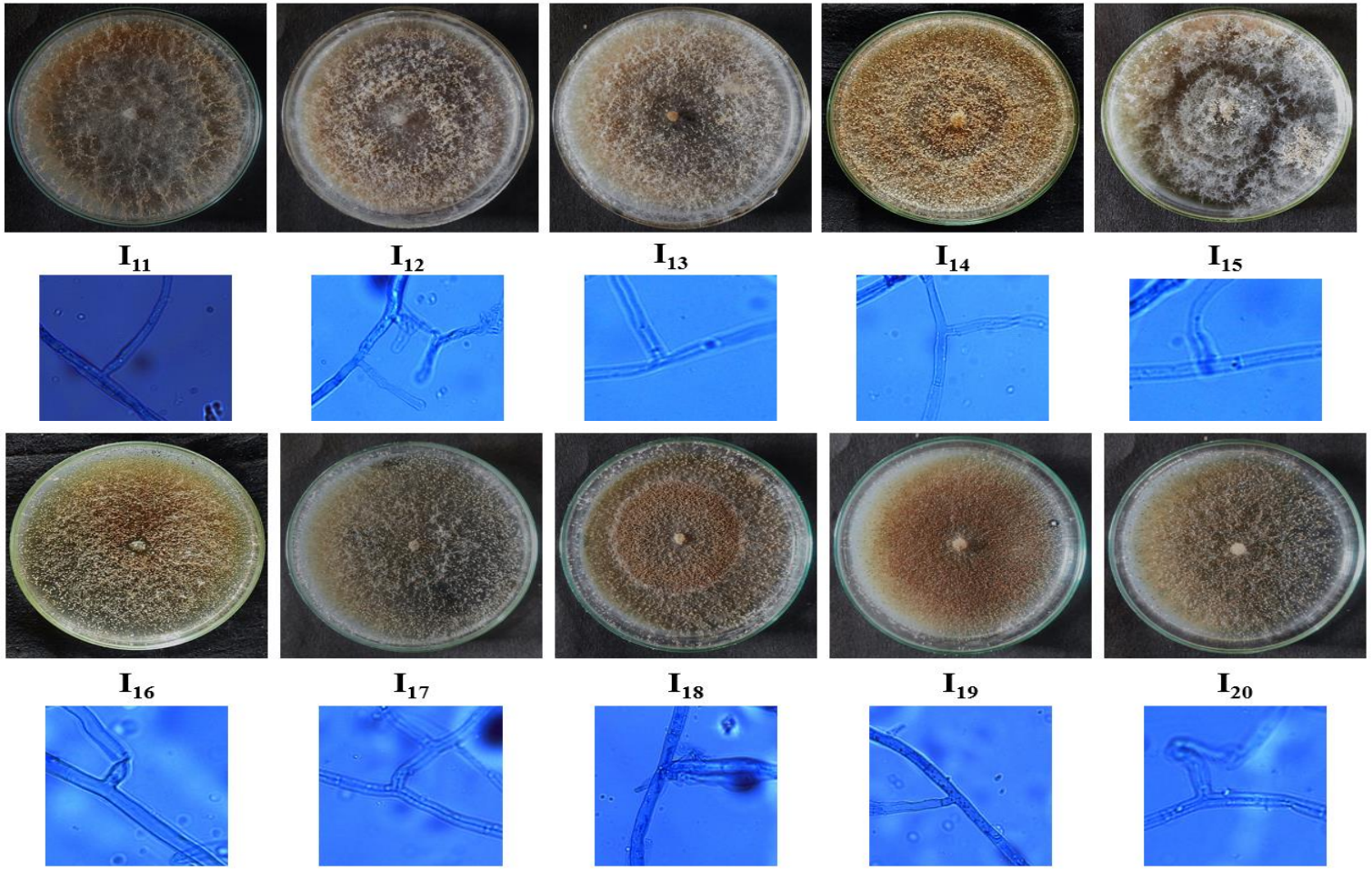


Plate 4 (contd.): Colony and hyphal characters of isolates obtained from different survey locations

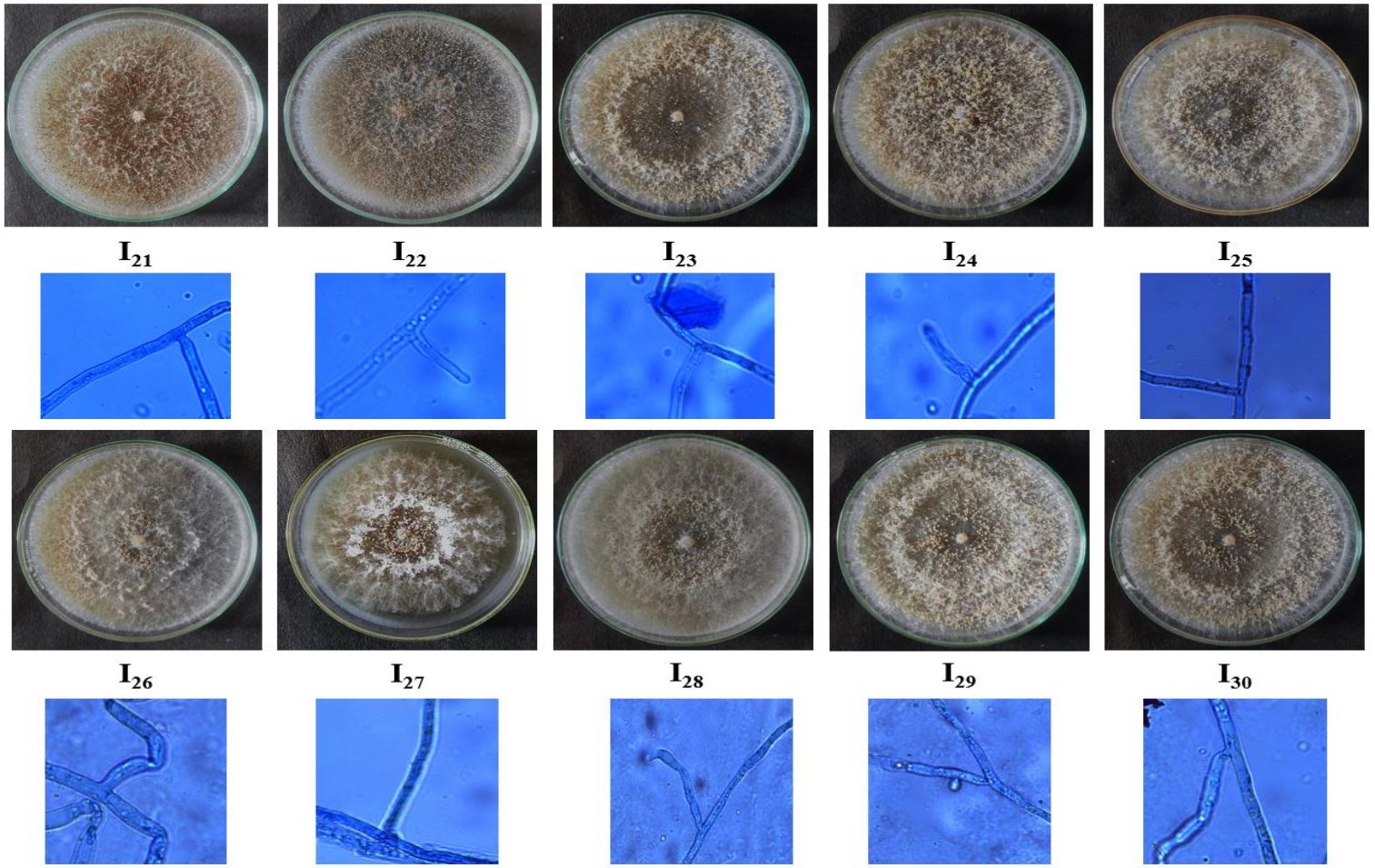


Plate 4 (contd.): Colony and hyphal characters of isolates obtained from different survey locations

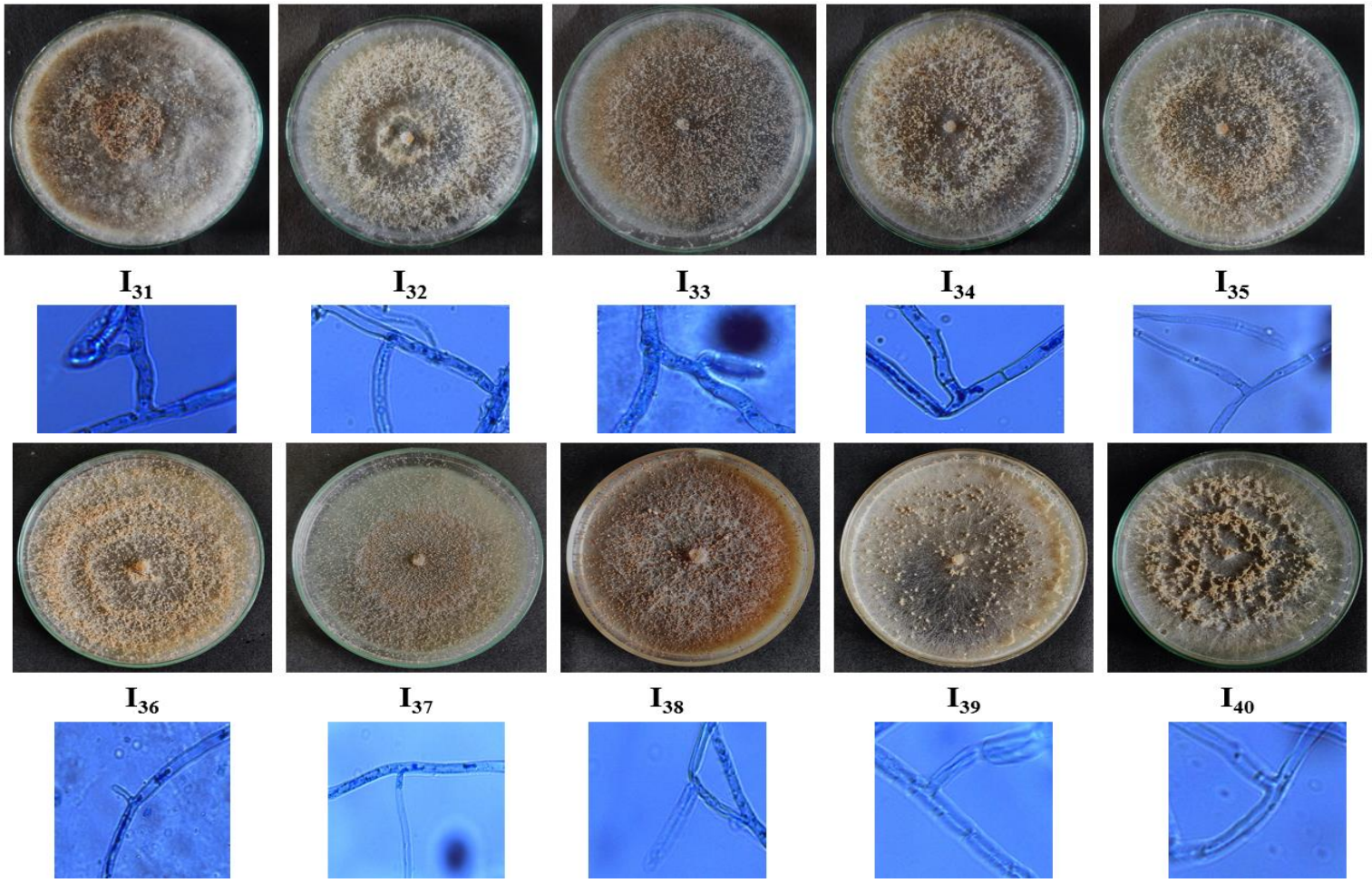


Plate 4 (contd.): Colony and hyphal characters of isolates obtained from different survey locations

Table 11: Sclerotial characters of sclerotia of isolates from survey locations

Sl.No.	Sclerotial characters		
	Colour	Texture	Size (mm)
I_1	Light brown	Smooth	1.12
I_2	Light brown	Rough	1.26
I_3	Light brown	Rough	1.18
I_4	Dark brown	Rough	1.14
I_5	White	Smooth	1.05
I_6	Dark brown	Rough	1.32
I_7	White	Smooth	1.25
I_8	Light brown	Smooth	1.12
I_9	Light brown	Rough	1.15
I_{10}	Light brown	Rough	1.35
I_{11}	Light brown	Rough	1.16
I_{12}	Light brown	Rough	1.21
I_{13}	Light brown	Smooth	1.28
I_{14}	Dark brown	Rough	1.13
I_{15}	White	Smooth	1.08
I_{16}	Dark brown	Rough	1.16
I_{17}	Light brown	Rough	1.12
I_{18}	Dark brown	Rough	1.26
I_{19}	Dark brown	Rough	1.24
I_{20}	Light brown	Rough	1.19

I_{21}	Dark brown	Rough	1.28
I_{22}	Light brown	Smooth	1.12
I_{23}	Light brown	Rough	1.13
I_{24}	Light brown	Rough	1.41
I_{25}	Light brown	Rough	1.21
I_{26}	Light brown	Rough	1.25
I_{27}	White	Smooth	1.48
I_{28}	Light brown	Rough	1.12
I_{29}	Light brown	Smooth	1.25
I_{30}	Whitish brown	Rough	1.18
I_{31}	Dark brown	Rough	1.21
I_{32}	Light yellow	Smooth	1.15
I_{33}	Dark brown	Rough	1.19
I_{34}	Whitish brown	Rough	1.25
I_{35}	Light yellow	Smooth	1.25
I_{36}	Dark brown	Rough	1.31
I_{37}	Dark brown	Rough	1.21
I_{38}	Dark brown	Rough	1.48
I_{39}	Light brown	Rough	1.42
I_{40}	Light brown	Rough	1.38

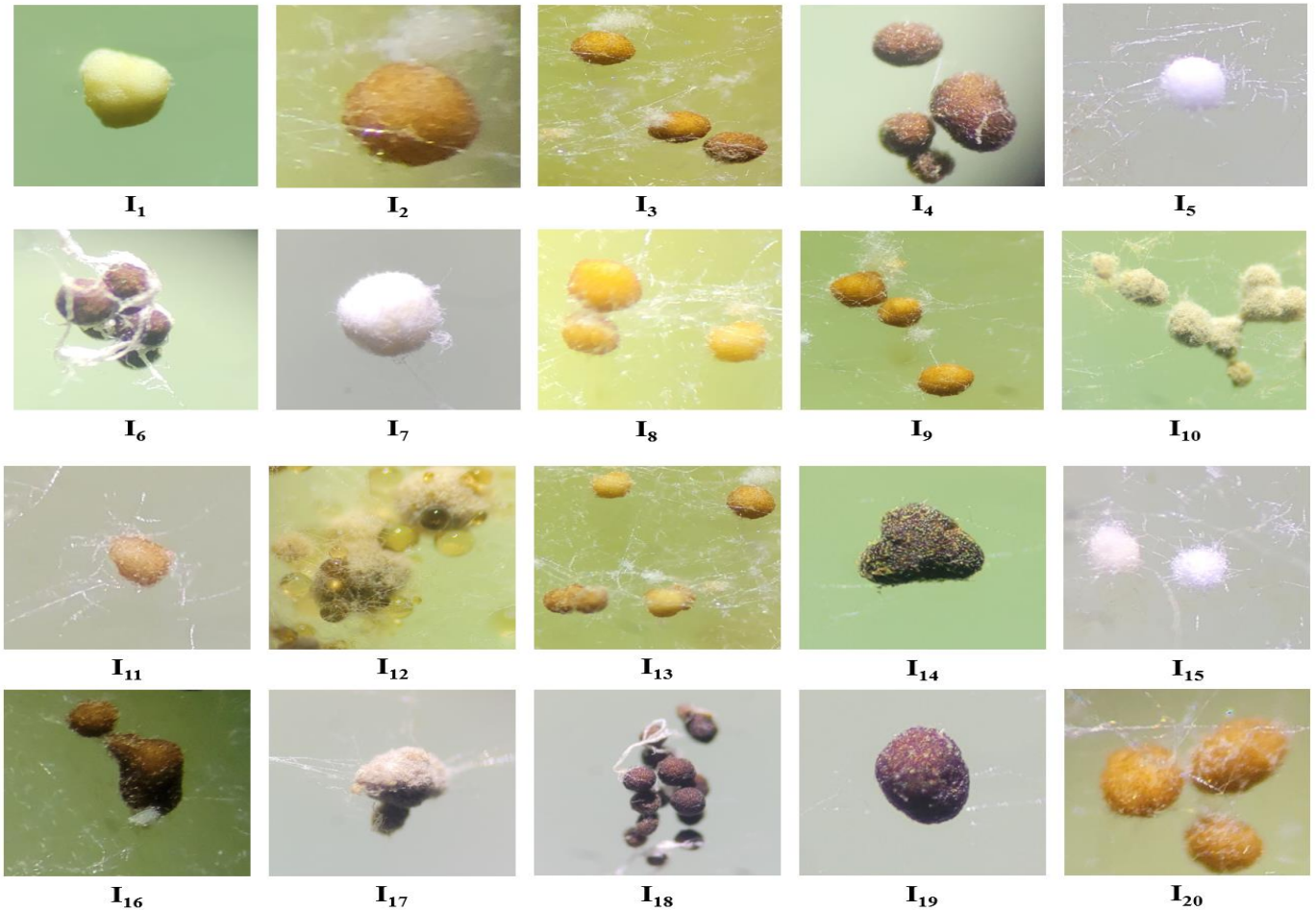


Plate 5: Microscopic view of sclerotia of each isolate

Plate 5 (contd.): Microscopic view of sclerotia of each isolate

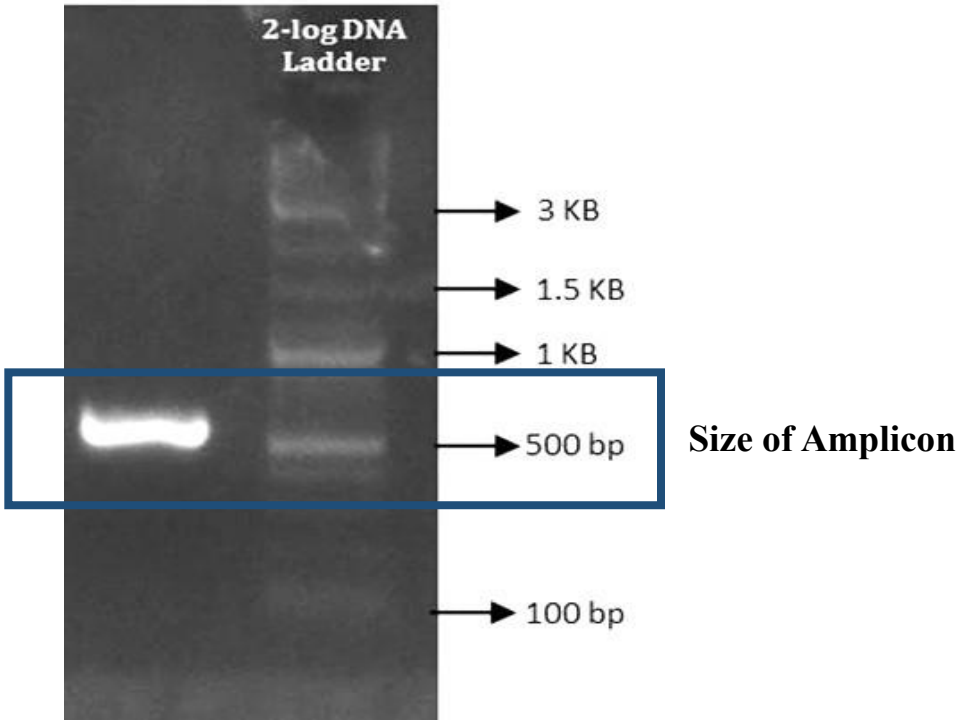


Plate 6: The gel profile of the PCR product

4.3 *IN VITRO* EVALUATION OF BACTERIAL BIOCONTROL AGENTS AND FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

Bacterial biocontrol agents and fungicides were evaluated against virulent pathogen under *in vitro* condition to assess the efficacy prior to field evaluation.

4.3.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture technique

The efficacy of bacterial biocontrol agents *B. amyloliquefaciens* (B15) and *P. fluorescens* (PN026) was evaluated against the virulent isolate of sheath blight pathogen under *in vitro* conditions using Dual culture technique.

The results of *in vitro* evaluation revealed that both *B. amyloliquefaciens* and *P. fluorescens* significantly inhibited the mycelial growth of the pathogen. Highest growth inhibition of the pathogen was observed in treatment with *B. amyloliquefaciens*. *B. amyloliquefaciens* and *P. fluorescens* inhibited the pathogen by 68.64% and 49.78% respectively (Plate 7, Plate 8). The per cent inhibition and radial growth of *R. solani* are given in Table 12.

4.3.2 *In vitro* evaluation of fungicides against sheath blight pathogen by poisoned food technique

Fungicides azoxystrobin 18.2% + difenoconazole 11.4% SC, kresoxim methyl 40% + hexaconazole 8% WG, trifloxystrobin 25% + tebuconazole 50% WG were evaluated at three different concentrations against virulent isolate of sheath blight pathogen *R. solani* under *in vitro* conditions. Azoxystrobin 18.2% + difenoconazole 11.4% SC at 0.25mlL^{-1}, 0.5mlL^{-1}, 1mlL^{-1}; kresoxim methyl 40% + hexaconazole 8% WG at 0.25gL^{-1}, 0.5gL^{-1}, 1gL^{-1} and trifloxystrobin 25% + tebuconazole 50% WG 0.1gL^{-1}, 0.2gL^{-1}, 0.4gL^{-1} concentrations were evaluated against the virulent isolate of *R. solani*.

Among the fungicides evaluated, highest inhibition (100%) was observed with kresoxim methyl 40% + hexaconazole 8% WG at $0.5gL^{-1}$, $1gL^{-1}$ and with azoxystrobin 18.2% + difenoconazole 11.4% SC $1mlL^{-1}$. Kresoxim methyl 40% + hexaconazole 8% WG at $0.25gL^{-1}$ showed an inhibition of 90.16%. Azoxystrobin 18.2% + difenoconazole 11.4% SC at concentrations of $0.5mlL^{-1}$ and $0.25mlL^{-1}$ exhibited 88.19% and 86.07% mycelial growth inhibition respectively. Trifloxystrobin 25% + tebuconazole 50% WG showed 87.63%, 84.26% and 80.71% mycelial inhibition at concentrations $0.1gL^{-1}$, $0.2gL^{-1}$ and $0.4gL^{-1}$ respectively (Plate 9, Plate 10, Plate 11). The mycelial growth of the pathogen and per cent inhibition are given in Table 13.

4.4 SCREENING FOR HOST PLANT RESISTANCE

Rice varieties released by Kerala Agricultural University (KAU) were screened for host plant resistance against sheath blight disease. Fifteen KAU released varieties were used in the experiment. Pot culture was conducted for the study. Artificial inoculation was done at maximum tillering stage. Fifteen KAU released varieties namely, Dhanu, Onam (Kayamkulam 3), Bhadra (MO 4), Aruna (MO 8), Kanakom (MO 11), Ranjini (MO 12), Uma (MO 16), Karishma (MO 18), Krishnanjana (MO 19), Pournami (MO 23), Jyothi (PTB 39), Kanchana (PTB 50), Aiswarya (PTB 52), Anaswara (PTB 58) and Manuratna were screened for resistance.

Upon artificial inoculation the plants produced symptoms of sheath blight disease such as presence of water soaked lesions on sheath near the water level (Table 14, Plate 12). Each plant was scored according to IRRI-SES scale, 2013 and disease severity (%) was assessed for each variety. The disease parameters such as infected tillers (%) and infected panicles (%) were also recorded in each variety (Table 15).

The days taken for symptom development was observed (Table 15). Least number of days (4 days) were taken for the symptom development in variety Uma (MO 16) followed by Jyothi and Karishma which took five days for symptom development.

Table 12: *In vitro* evaluation of bioagents against virulent isolate of *R. solani* using dual culture method

Treatments	Colony diameter (cm)*	Percent inhibition**
***B. amyloliquefaciens* (B15)**	2.823 ± 0.09^{c}	68.64^{a}
***P. fluorescens* (PN026)**	4.520 ± 0.25^{b}	49.78^{b}
Control	9.00 ± 0.00^{a}	-
SE(m)	0.06	0.458
CD (0.05)	0.17	1.411

#Values are means of seven replications

*Means of seven replication ± Standard Deviation

** Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

Plate 7: *In vitro* evaluation of *B. amyloliquefaciens* against *R. solani*

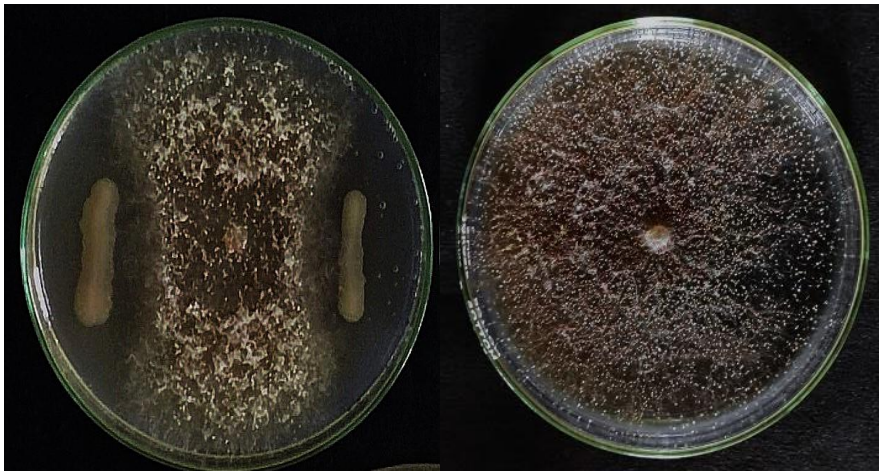


Plate 8: *In vitro* evaluation of *P. fluorescens* against *R. solani*

Table 13: *In vitro* evaluation of fungicides against virulent isolate of *R. solani* using poisoned food technique

Treatments	Colony diameter (cm)*	Percent inhibition**
Azoxystrobin 18.2% + Difenoconazole 11.4% SC 0.25 mlL^{-1}	1.25 (1.324±0.008)^{d}	86.07 (68.089)^{e}
Azoxystrobin 18.2% + Difenoconazole 11.4% SC 0.5 mlL^{-1}	1.06 (1.250±0.005)^{f}	88.19 (69.89)^{c}
Azoxystrobin 18.2% + Difenoconazole 11.4% SC 1 mlL^{-1}	0.00(0.707±0.00)^{h}	100 (90.00)^{a}
Kresoxim methyl 40% + Hexaconazole 8% WG 0.25 gL^{-1}	0.89 (1.178±0.002)^{g}	90.16 (71.71)^{b}
Kresoxim methyl 40% + Hexaconazole 8% WG 0.5 gL^{-1}	0.00(0.707±0.00)^{h}	100 (90.00)^{a}
Kresoxim methyl 40% + Hexaconazole 8% WG 1 gL^{-1}	0.00(0.707±0.00)^{h}	100 (90.00)^{a}
Trifloxystrobin 25% + Tebuconazole 50% 75 WG 0.1 gL^{-1}	2.09 (1.611±0.004)^{b}	80.71 (61.14)^{g}
Trifloxystrobin 25% + Tebuconazole 50% 75 WG 0.2 gL^{-1}	1.78 (1.509±0.002)^{c}	84.26 (63.62)^{f}
Trifloxystrobin 25% + Tebuconazole 50% 75 WG 0.4 gL^{-1}	1.12 (1.271±0.002)^{e}	87.63 (69.41)^{d}
Control	9.00 (3.082 ± 0.00)^{a}	-
SE(m)	0.002	0.053
CD (α = 0.05)	0.06	0.157

#Values are means of three replications *Square root transformed values ± Standard Deviation

** Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

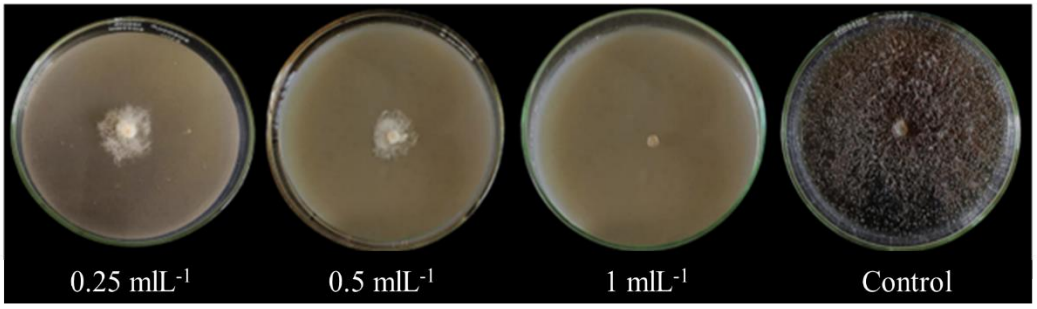


Plate 9: *In vitro* evaluation of Azoxystrobin 18.2% + Difenoconazole 11.4% SC against *R.solani*

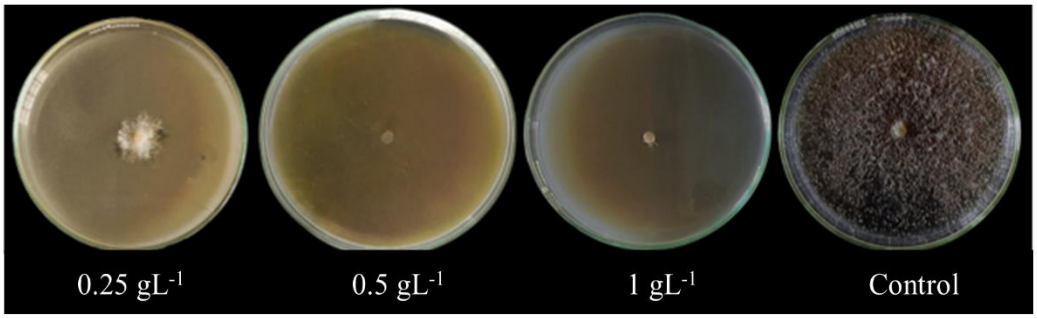


Plate 10: *In vitro* evaluation of Kresoxim methyl 40% + Hexaconazole 8% WG against *R.solani*

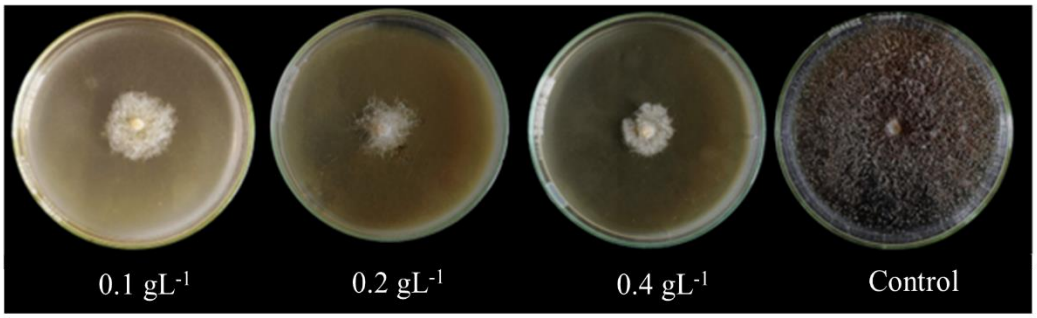


Plate 11: *In vitro* evaluation of Trifloxystrobin 25% + Tebuconazole 50% WG against *R.solani*

Kanchana (PTB 50) and Aiswarya (PTB 52) recorded highest number of days for symptom development *i.e.* 18 and 19 days respectively.

Variety Uma was observed to have highest per cent tiller infection (97.33%) followed by Jyothi (93.03%) and Karishma (86.71%). Similarly, highest panicle infection was recorded in Uma (74.94%) followed by Jyothi (70.21%) and Karishma (67.67%). The disease severity was analyzed and highest (80.11%) was observed in Uma.

Aiswarya was recorded with least per cent tiller infection (29.70%) followed by Kanchana (31.30%). The disease severity was also recorded to be least in Aiswarya (15.55%) and Kanchana (20.01%) whereas, panicle infection was observed to be least in Kanchana (13.64%) followed by Aiswarya (15.36%).

The screened varieties were categorized into immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible based on IRRI-SES scale (Table 16). Among the 15 varieties, no variety was identified to be immune to sheath blight disease. The varieties Kanchana and Aiswarya produced least symptoms and were identified as resistant varieties according to IRRI-SES scale, 2013. Moderate resistance was observed in varieties Dhanu, Onam, Kanakom, Pournami and Anaswara. Varieties Ranjini and Bhadra were assessed as moderately susceptible whereas, varieties Krishnanjana, Manuratna and Aruna were observed to be susceptible to the disease. Uma, Jyothi and Karishma were categorized as highly susceptibile varieties.

Table 14: Symptoms observed in different rice varieties upon artificial inoculation

Variety	Nature of symptoms
Dhanu	Brown spots with greyish brown centres that later coalesce to form lesions on the sheaths
Onam	Small lesions with brown coloured margins and white centres near water level
Bhadra	Brown to grey lesions formed on the sheaths near the water level
Aruna	Dark brown spots appeared near the water level on sheath and coalesce upwards
Kanakom	Water soaked lesions formed in the base of the plant
Ranjini	Yellowish brown lesions on the outer sheaths starting from the base of the plant
Uma	Elongated lesions extending upto the leaves covering the entire plant
Karishma	Black coloured water soaked lesions formed on the sheaths
Krishnanjana	Brown to black coloured elongated lesions on the sheaths
Pournami	Dark brown spots on the sheaths later forming lesions
Jyothi	Greyish irregular spots later coalescing to form lesions on the sheaths covering the whole plant
Kanchana	Brown coloured water soaked spots formed on the base of the plant
Anaswara	Dark brown irregular spots appeared on the sheath and coalesced to form larger lesions
Aiswarya	Brown coloured water soaked spots on the outer sheaths
Manuratna	Water soaked lesions coalesced to form large lesions

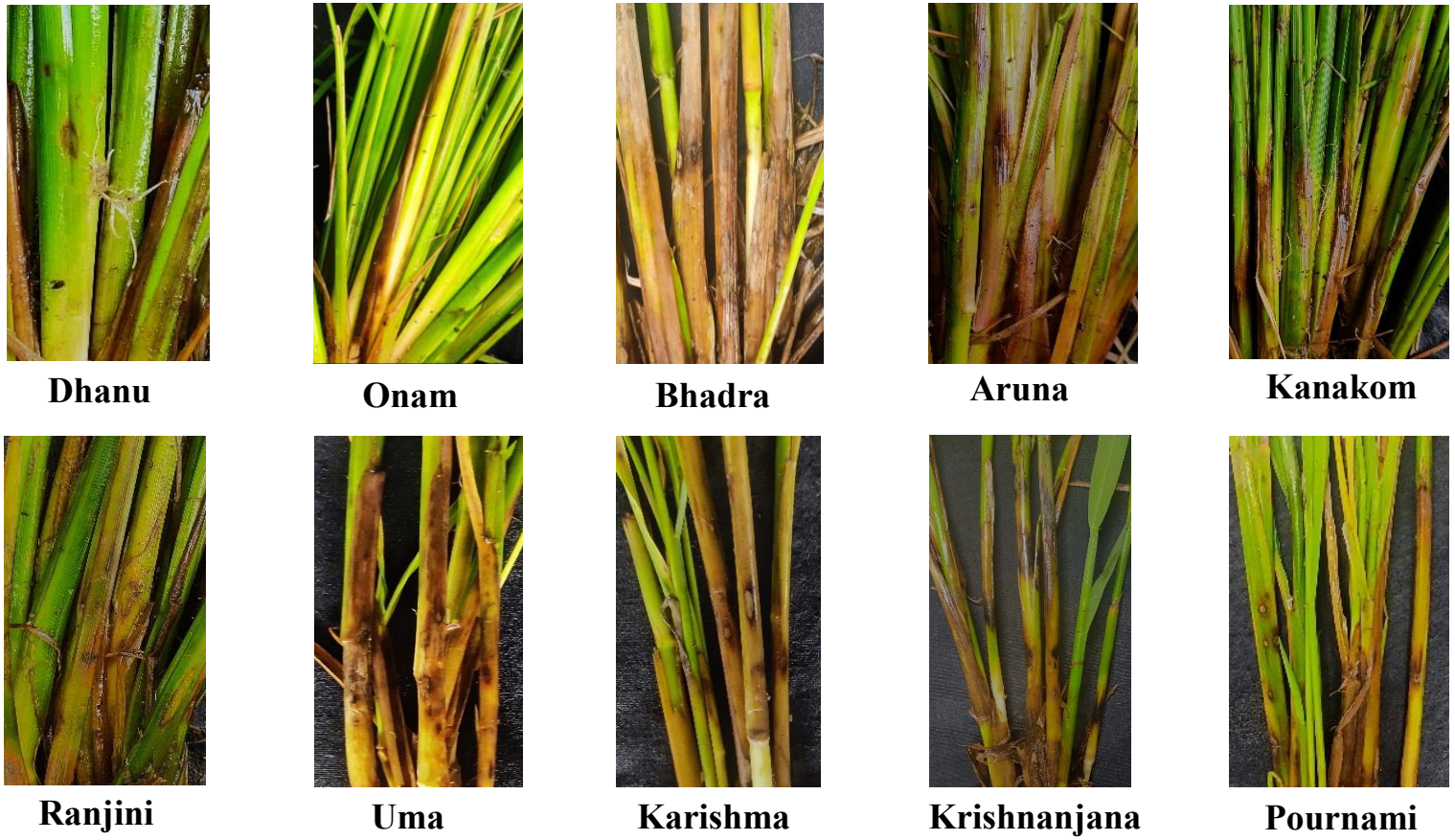


Plate 12: Symptoms observed in different rice varieties upon artificial inoculation

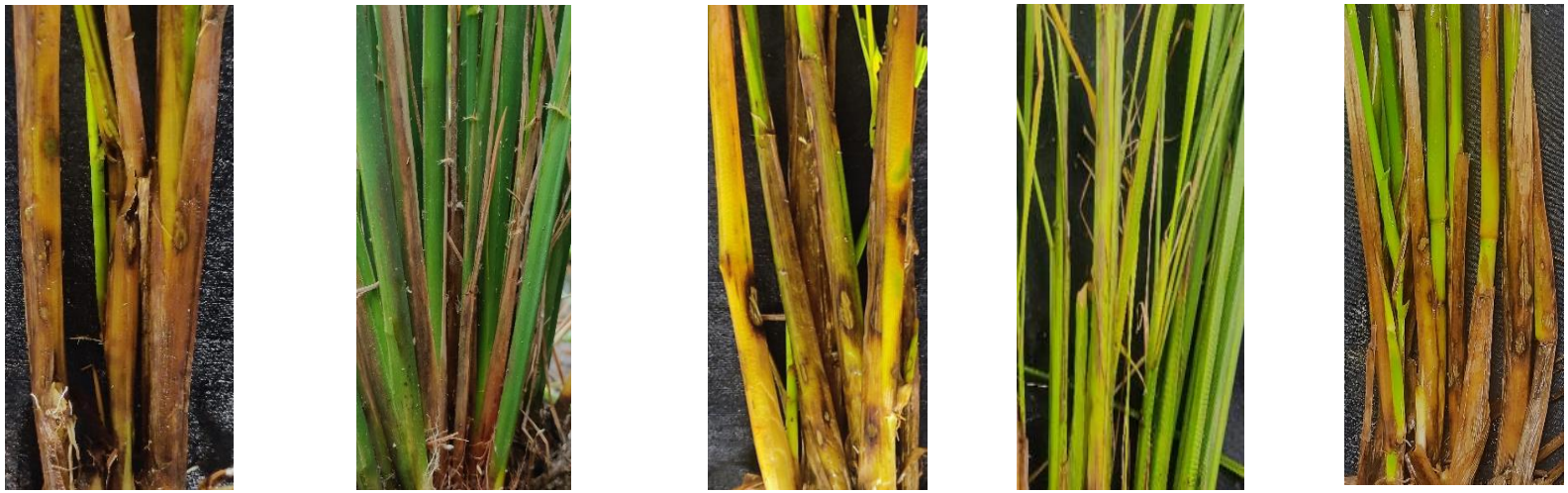


Plate 12 (contd.): Symptoms observed in different rice varieties upon artificial inoculation

Table 15: Details of disease parameters in different rice varieties upon artificial inoculation

Sl.No.	Variety	Infected tillers (%)*	Infected panicles (%)*	Size of lesion (%)	Days Taken For Sclerotia Formation	Disease severity (%)*
V1	Dhanu	54.09 (47.34)^{g}	25.76 (30.48)^{g}	25.02 (29.48)^{g}	13 ± 0.509^{c}	36.67 (37.27)^{ef}
V2	Onam	58.48 (49.88)^{f}	32.91 (35.00)^{f}	26.71(30.12)^{g}	13 ± 0.192^{c}	32.22 (34.58)^{fg}
V3	Bhadra	63.88 (53.06)^{e}	44.24 (41.79)^{e}	36.42 (31.56)^{e}	10 ± 0.385^{d}	53.33 (46.91)^{d}
V4	Aruna	74.24 (59.50)^{d}	56.85 (48.94)^{c}	50.23 (45.23)^{c}	7 ± 0.509^{e}	63.34 (52.75)^{c}
V5	Kanakom	39.39 (38.87)^{h}	32.82 (34.95)^{f}	27.81 (30.42)^{f}	13 ± 0.694^{c}	31.11 (33.86)^{g}
V6	Ranjini	62.97 (52.53)^{e}	45.54 (42.44)^{e}	42.02 (41.22)^{e}	10 ± 0.192^{d}	51.12 (45.64)^{d}
V7	Uma	97.33 (80.66)^{a}	74.94 (59.96)^{a}	79.02 (56.12)^{a}	4 ± 0.333^{g}	80.11 (63.49)^{a}
V8	Karishma	86.71 (68.79)^{c}	67.67 (55.35)^{c}	70.13 (53.11)^{c}	5 ± 0.509^{fg}	72.23 (58.20)^{b}
V9	Krishnanjana	75.09 (60.06)^{d}	56.73 (48.87)^{c}	47.89 (42.13)^{c}	7 ± 0.509^{e}	63.32 (52.74)^{c}
V10	Pournami	60.33 (50.96)^{ef}	31.82 (34.33)^{f}	35.12 (31.28)^{f}	12 ± 0.192^{c}	38.89 (38.58)^{e}

V11	**Jyothi**	93.03 (74.71)^{b}	70.21 (56.95)^{b}	72.15 (52.11)^{b}	5 ± 0.192^{f}	77.78 (61.89)^{a}
V12	**Kanchana**	31.30 (34.01)^{i}	13.64 (21.65)^{h}	15.12 (21.11)^{h}	18 ± 0.694^{b}	20.01 (26.52)^{h}
V13	**Anaswara**	57.87 (49.53)^{fg}	33.82 (34.33)^{f}	35.61 (31.33)^{f}	12± 0.667^{c}	37.77 (37.92)^{e}
V14	**Aiswarya**	29.70 (33.06)^{i}	15.36 (21.65)^{h}	12.13 (20.09)^{h}	19 ± 0.192^{a}	15.55 (23.20)^{h}
V15	**Manuratna**	74.03 (59.36)^{d}	50.39 (45.23)^{d}	46.13 (41.19)^{d}	7 ± 0.509^{e}	63.34 (52.75)^{c}
SE(m)		0.849	1.203	1.019	0.274	1.623
CD (0.05)		2.452	3.475	3.122	0.791	4.687

Values are mean of three replications

**Square root transformed values ± Standard Deviation

*Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 16: Categorization of screened rice varieties based on disease scoring using IRRI-SES scale (2013)

Disease score	Description	Reaction	Varieties
0	No infection	Immune	NIL
1	Lesion limited to the lower 20% of plant height	Resistant	Aiswarya, Kanchana
3	Lesion limited to lower 20 – 30% of the plant height	Moderately resistant	Dhanu, Onam, Kanakom, Pournami, Anaswara
5	Lesion limited to the lower 31 – 45% of the plant height	Moderately susceptible	Ranjini, Bhadra
7	Lesion limited to the lower 46 – 65% of the plant height	Susceptible	Krishnanjana, Manuratna, Aruna
9	Lesion more than 65% of the plant height	Highly susceptible	Uma, Jyothi, Karishma

4.5 *IN VIVO* TESTING OF THE EFFICACY OF BACTERIAL BIOCONTROL AGENTS AND COMMERCIAL FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

Field evaluation of bacterial biocontrol agents and commercial fungicides was conducted at M. S. Swaminathan Rice Research Station, Moncompu during *Kharif* 2024.

Field evaluation was carried out in split plot design comprising of main plot treatments and sub plot treatments. The general view of nursey and field is given in Plate 13. Main plots treatments, sub plot treatments and interaction effects were evaluated separately. Disease parameters such as per cent infected tillers, panicle infection (%), infected grains (%), per cent chaffy grain (%), disease severity (%) along with yield parameters were evaluated in each treatment.

All the treatments were observed to have reduced disease parameters in comparison with the control plots. Significant increase in grain yield, thousand grain weight and B:C ratio was observed in all other plots compared to the control plots. Details of disease parameters assessed in main plot treatments, sub plot treatments and interaction are given in Table 17, Table 19, Table 21. The yield parameters assessed in main plot treatments, sub plot treatments and interaction are given in Table 18, Table 20, Table 22. Per cent reduction of disease parameters and per cent increase in grain yield compared to control plots are given in Table 23.

4.5.1 Main plot treatments

Among the main plot treatments, kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ (M4) was observed to be effective compared to other treatments with least per cent infected tillers (34.15%), panicle infection (31.81%), infected grains (6.07%), per cent chaffy grains (6.94%) and disease severity (21.90%) followed by azoxystrobin 18.2% + difenoconazole 11.4% SC at $1mlL^{-1}$ (M3) in per cent infected tillers (43.06%), panicle infection (40.60%), per cent infected grains (8.23%), per cent chaffy grains

(8.28%) and disease severity (35.24%). The treatment, trifloxystrobin 25% + tebuconazole 50% WG at 0.4 gL^{-1} (M5) recorded higher per cent infected tillers (45.93%), panicle infection (41.21%), per cent infected grains (9.04%), per cent chaffy grains (9.25%) and disease severity (35.71%) compared to other two fungicide treatments.

The yield parameters were assessed and treatment kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ was observed to be significantly higher compared to other treatments. Highest thousand grain weight (26.14 g), grain yield (8183.3 $kgha^{-1}$) and B:C ratio (2.48) was observed in treatment with fungicide kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ followed by azoxystrobin 18.2% + difenoconazole 11.4% SC at $1mlL^{-1}$ in thousand grain weight (25.43 g), grain yield (7300 $kgha^{-1}$) and B:C ratio (2.27). The standard check, trifloxystrobin 25% + tebuconazole 50% WG at 0.4 gL^{-1} recorded lower thousand grain weight (24.99 g), grain yield (7183.34 $kgha^{-1}$) and B:C ratio (2.11) compared to other fungicide treatments.

Among the biocontrol agents, least per cent infected tillers (61.39%), panicle infection (47.27%), per cent infected grains (12.01%), per cent chaffy grains (13.39%) and disease severity (51.43%) were recorded in treatment with *B. amyloliquefaciens* (B15) (M1) followed by *P. fluorescens* (PN026) (M2) in per cent infected tillers (71.69%), panicle infection (53.03%), per cent infected grains (18.40%), per cent chaffy grains (19.94%) and disease severity (67.62%).

In the assessment of yield parameters, *B. amyloliquefaciens* (B15) exhibited higher thousand grain weight (24.82 g), grain yield (6191.67 $kgha^{-1}$) and B:C ratio (1.93) compared to *P. fluorescens* (PN026) in thousand grain weight (22.75 g), grain yield (5750 $kgha^{-1}$) and B:C ratio (1.76).

The highest per cent reduction over control plots for infected tillers (62.99%), panicle infection (65.12%) and disease severity (72.13%) was observed in treatment kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ while azoxystrobin 18.2% +

difenoconazole 11.4% SC at 1mlL^{-1} recorded 53.33, 55.49 and 55.15 per cent reduction in infected tillers, panicle infection and disease severity respectively. The standard check trifloxystrobin 25% + tebuconazole 50% WG at 0.4 gL^{-1} recorded least per cent reduction in infected tillers (50.22%), panicle infection (54.82%) and disease severity (54.55%) compared to other two fungicide treatments. Among biocontrol agents highest per cent reduction over control in infected tillers (33.47%), panicle infection (48.17%) and disease severity (34.54%) were observed in treatment with *B. amyloliquefaciens* (B15) followed by *P. fluorescens* (PN026) in per cent reduction infected tillers (22.30%), panicle infection (41.85%) and disease severity (13.97%) over control plots.

Similarly, the per cent increase in grain yield over control was recorded highest (94.07%) in treatment with kresoxim methyl 40% + hexaconazole 8% WG at 1gL^{-1} followed by azoxystrobin 18.2% + difenoconazole 11.4% SC at 1mlL^{-1} (73.12%) while, trifloxystrobin 25% + tebuconazole 50% WG at 0.4 gL^{-1} recorded 70.35% increase over control. Among biocontrol agents, highest per cent increase was recorded to be 46.85% in treatment with *B. amyloliquefaciens* (B15) whereas treatment with *P. fluorescens* (PN026) recorded 36.37% increase in grain yield over control plots.

4.5.2 Sub plot treatments

Among the sub plot treatments, plots provided with treatment 75% nitrogen, 100% phosphorus and 125% potassium of Recommended Dose of Fertilizers (RDF) (S2) was found to be effective compared to treatment 100% RDF (S1). Treatment 75% nitrogen, 100% phosphorus and 125% potassium of RDF recorded the lowest per cent infected tillers (52.95%), panicle infection (47.98%), per cent infected grains (11.37%), per cent chaffy grains (13.13%) and disease severity (45.87%) while, the treatment 100% RDF exhibited higher per cent infected tillers (63.21%), panicle infection (53.73%), per cent infected grains (13.27%), per cent chaffy grains (15.18%) and disease severity (50.95%).

In the assessment of yield parameters, 75% nitrogen, 100% phosphorus and 125% potassium of RDF exhibited higher thousand grain weight (25.27 g), grain yield (6708.34 $kgha^{-1}$) and B:C ratio (2.03) followed by treatment 100% RDF in thousand grain weight (23.10 g), grain yield (6233.34 $kgha^{-1}$) and B:C ratio (1.98).

Among the sub plots, the treatment 75% nitrogen, 100% phosphorus and 125% potassium of RDF was recorded to have highest per cent reduction in infected tillers (42.61%), panicle infection (47.39%) and disease severity (41.62%) followed by treatment 100% RDF which recorded reduction per cent of 31.49%, 41.09% and 35.15% respectively.

Similarly, in the assessment of yield parameters it was observed that 75% nitrogen, 100% phosphorus and 125% potassium of RDF recorded highest per cent increase in grain yield over control *i.e.* 59.09% while treatment 100% RDF recorded 47.82% increase over control.

4.5.3 Interaction effect

The interaction effect of all main plot treatments and subplot treatments were evaluated. In case of parameters such as infected tillers (%), infected panicles (%), grain yield and B:C ratio, the interaction effect of all main plots and sub plots were observed to be significant while for parameters such as thousand grain weight, disease severity (%), infected grains (%) and chaffy grains (%), the interaction effect was observed to be non-significant.

The interaction effect of main plot treatment, kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ with subplot treatment 75% nitrogen, 100% phosphorus and 125% potassium of RDF (M4S2) was significantly more effective compared to other treatments in terms of infected tillers (%), infected panicles (%) and grain yield.

The treatment combination kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ and 75% nitrogen, 100% phosphorus and 125% potassium of RDF recorded the

lowest per cent infected tillers (31.97%), per cent infected panicles (29.69%), per cent infected grains (5.77%), chaffy grains (5.96%), disease severity (20.48%) and highest grain yield (8316.67 $kgha^{-1}$), thousand grain weight (27.59 g) and B:C ratio (2.54).

This was followed by treatment combination of kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ and 100% RDF (M4S1) in terms of per cent infected tillers (36.32%), per cent infected panicles (33.93%), per cent infected grains (6.36%), chaffy grains (7.93%), disease severity (23.33%), grain yield (8050 $kgha^{-1}$), thousand grain weight (26.68 g) and B:C ratio (2.42).

Among the biocontrol agents, the interaction effect of treatment combination of *B. amyloliquefaciens* (B15) with 75% nitrogen, 100% phosphorus and 125% potassium of RDF (M1S2) recorded lowest per cent infected tillers (53.08%), per cent infected panicles (43.02%), per cent infected grains (10.49%), chaffy grains (11.57%), disease severity (48.09%) and highest grain yield (6416.66 $kgha^{-1}$), thousand grain weight (25.75 g) and B:C ratio (1.90).

The interaction effect of treatment combination of kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ and 75% nitrogen, 100% phosphorus and 125% potassium of RDF (M4S2) recorded highest per cent reduction in infected tillers (65.35%), infected panicles (67.45%) and disease severity (73.93%) over control followed by treatment combination kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ with 100% RDF (M4S1) which recorded 60.63%, 62.80% and 70.31% reduction in infected tillers, infected panicles and disease severity over control respectively.

Similarly, the interaction effect of treatment combination kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ and 75% nitrogen, 100% phosphorus and 125% potassium of RDF recorded highest per cent increase in grain yield (97.23%) followed by treatment combination kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ with 100% RDF which recorded 90.10% increase in grain yield over control.

Among the biocontrol agents, the interaction effect of treatment combination of *B. amyloliquefaciens* (B15) with 75% nitrogen, 100% phosphorus and 125% potassium recorded highest per cent reduction in infected tillers (42.47%), infected panicles (52.83%) and disease severity (38.79%) over control and highest per cent increase in grain yield (52.17%).

4.5.4 Other pest and diseases observed in the field

In case of pest incidence, very few grasshoppers and rice bugs were observed in all the plots. Among diseases, very low incidence of diseases like sheath rot, bacterial leaf blight, grain discolouration, blast and brown leaf spot was observed. The pest and diseases observed are given in Plate 14.

Plate 13: General view of nursery and field

Table 17: Disease parameters assessed among main plot treatments

Treatments	Inf. tillers(%)*	Panicle infection(%)*	Infected grains(%)*	Chaffy grains(%)*	Disease Severity (%)*
***B. amyloliquefaciens* (B15) (M1)**	61.39 (51.68)^{c}	47.27 (43.42)^{c}	12.01 (20.23)^{c}	13.39 (21.42)^{c}	51.43 (36.24)^{c}
***P. fluorescens* (PN026) (M2)**	71.69 (58.11)^{b}	53.03 (46.74)^{b}	18.40 (25.37)^{b}	19.94 (26.49)^{b}	67.62 (46.54)^{b}
Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC @ 1ml L^{-1} (M3)	43.06 (40.99)^{d}	40.60 (39.58)^{d}	8.23 (16.65)^{d}	8.28 (16.72)^{e}	35.24 (31.48)^{d}
Kresoxim methyl 40% + Hexaconazole 8% WG @ 1g L^{-1} (M4)	34.15 (35.73)^{e}	31.81 (34.32)^{e}	6.067 (14.25)^{e}	6.94 (15.23)^{f}	21.90 (27.96)^{e}
Trifloxystrobin 25%+Tebuconazole 50% 75 WG @ 0.4g L^{-1} (M5)	45.93 (42.66)^{d}	41.21 (39.93)^{d}	9.04 (17.48)^{d}	9.25 (17.67)^{d}	35.71 (34.62)^{c}
Untreated control (M6)	92.27 (74.57)^{a}	91.21 (73.13)^{a}	20.17 (26.67)^{a}	27.11 (31.37)^{a}	78.57 (58.29)^{a}
SE(m)	1.611	0.5716	0.4467	0.331	0.948
CD (α = 0.05)	5.077	1.801	1.407	1.042	2.989

#Values are means of three replications

*Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 18: Yield parameters assessed among main plot treatments

Treatments	1000 grain weight (g)**	Grain Yield (kg ha^{-1})	BC Ratio
B. amyloliquefaciens **(B15) (M1)**	24.82 (4.817±0.214)^{b}	6191.67^{c}	1.93^{d}
P. fluorescens **(PN026) (M2)**	22.75 (4.758±0.206)^{b}	5750.0^{d}	1.76^{e}
Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC @ 1ml L^{-1} (M3)	25.43 (5.039±0.215)^{a}	7300.0^{b}	2.27^{b}
Kresoxim methyl 40% + Hexaconazole 8% WG @ 1g L^{-1} (M4)	26.14 (5.168±0.195)^{a}	8183.33^{a}	2.48^{a}
Trifloxystrobin 25%+Tebuconazole 50% 75 WG @ 0.4g L^{-1} (M5)	24.99 (4.877±0.125)^{b}	7183.34^{b}	2.11^{c}
Untreated control (M6)	20.98 (4.578±0.175)^{c}	4216.67^{e}	1.49^{f}
SE(m)	0.051	60.61	0.006
CD (0.05)	0.159	190.987	0.019

#Values are means of three replications

**Values in parenthesis are square root transformed values ± SD

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 19: Disease parameters assessed among sub plot treatments

Treatments	Inf. tillers(%)*	Panicle infection(%)*	Infected grains(%)*	Chaffy grains(%)*	Disease Severity (%)*
100% Recommended dose of Fertilizers (RDF) (S1)	63.21 (54.09)[a]	53.73 (48.12)[a]	13.27 (20.93)[a]	15.18 (22.36)[a]	50.95 (41.12)[a]
75%N, 100%P, 125% K OF RDF (S2)	52.95 (47.16)[b]	47.98 (44.25)[b]	11.37 (19.28)[b]	13.13 (20.60)[b]	45.87 (37.25)[b]
SE (m)	0.682	0.3975	0.2841	0.256	0.538
CD (α = 0.05)	2.107	1.225	0.8753	0.793	1.659

#Values are means of three replications

*Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 20: Yield parameters assessed among sub plot treatments

Treatments	1000 grain weight (g)**	Grain Yield (Kg ha^{-1})	BC Ratio
100% Recommended dose of Fertilizers (RDF) (S1)	23.10 (4.99±0.239)^{b}	6233.34^{b}	1.98^{b}
75%N, 100%P, 125% K OF RDF (S2)	25.27 (4.75±0.231)^{a}	6708.34^{a}	2.03^{a}
SE (m)	0.034	33.101	0.003
CD (α = 0.05)	0.103	101.995	0.009

#Values are means of three replications

**Values in parenthesis are square root transformed values ± SD

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 21: Disease parameters assessed to find the interaction between main plot and sub plot treatments

Treatments	Inf. tillers (%)*	Panicle inf. (%)*	Infected grains (%)*	Chaffy grains (%)*	Disease Severity (%)*
M1S1	69.69 (56.60)	51.51 (45.87)	13.54 (21.56)	15.21 (22.95)	54.76 (38.44)^{e}
M1S2	53.08 (46.77)	43.02 (40.99)	10.49 (18.89)	11.57 (19.88)	48.09 (34.04)^{f}
M2S1	79.13 (62.89)	57.57 (49.36)	19.99 (26.56)	21.82 (27.83)	69.95 (50.02)^{c}
M2S2	64.24 (53.33)	48.49 (44.13)	16.81 (24.18)	18.06 (25.15)	64.28 (43.09)^{d}
M3S1	47.12 (43.35)	42.42 (40.64)	9.63 (18.06)	10.07 (18.49)	36.67 (36.46)^{g}
M3S2	39.00 (38.63)	39.99 (39.23)	8.46 (16.89)	8.43 (16.86)	34.76 (32.79)^{g}
M4S1	36.32 (37.05)	33.93 (35.63)	6.36 (14.60)	7.93 (16.33)	23.33 (28.88)^{i}
M4S2	31.97 (34.42)	29.69 (33.02)	5.77 (13.89)	5.96 (14.13)	20.48 (27.03)^{i}
M5S1	50.65 (45.38)	42.42 (40.64)	8.94 (17.38)	8.46 (16.90)	37.62 (33.18)^{f}
M5S2	41.21 (39.94)	38.79 (38.52)	7.52 (15.91)	8.11 (16.53)	32.85 (29.78)^{h}
M6S1	96.36 (79.25)	94.55 (76.62)	21.17 (27.39)	27.58 (31.68)	82.38 (59.78)^{b}
M6S2	88.18 (69.90)	87.88 (69.64)	19.17 (25.95)	26.64 (31.07)	74.76 (56.79)^{a}
SE(m)	1.671	0.8948	NS	NS	NS
CD (α = 0.05)	6.247	2.783	-	-	-

#Values are means of three replications *Values in parenthesis are arc sine transformed

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 22: Yield parameters assessed to find the interaction between main plot and sub plot treatments

Treatments	1000 grain weight (g)**	Grain Yield (Kg ha^{-1})	BC Ratio
M1S1	23.88 (4.635±0.119)	5966.67^{f}	1.90
M1S2	25.75 (4.999±0.037)	6416.66^{e}	1.95
M2S1	21.65 (4.636±0.130)	5333.34^{g}	1.72
M2S2	23.84 (4.880±0.213)	6166.67^{e}	1.81
M3S1	24.63 (4.962±0.163)	6966.67^{c}	2.21
M3S2	25.82 (4.910±0.177)	7416.67^{c}	2.32
M4S1	26.68 (5.005±0.113)	8050.0^{b}	2.42
M4S2	27.59 (5.330±0.059)	8316.67^{a}	2.54
M5S1	24.18 (4.844±0.067)	6950.0^{d}	2.16
M5S2	26.23 (5.117±0.267)	7633.34^{b}	2.05
M6S1	19.59 (4.427±0.056)	4133.34^{h}	1.48
M6S2	22.36 (4.729±0.068)	4300.0^{h}	1.51
SE(m)	NS	83.431	0.008
CD (α = 0.05)	-	260.147	0.025

#Values are means of three replications **Values in parenthesis are square root transformed values ± SD

#Values followed by similar superscripts are not significantly different at 0.05% level

Table 23: Percent reduction of disease parameters and percent increase of grain yield over control

Treatments	Infected tillers (%)	Infected panicles (%)	Disease severity (%)	Grain yield (%)
M1	33.47	48.17	34.54	46.85
M2	22.30	41.85	13.97	36.37
M3	53.33	55.49	55.15	73.12
M4	62.99	65.12	72.13	94.07
M5	50.22	54.82	54.55	70.35
S1	31.49	41.09	35.15	47.82
S2	42.61	47.39	41.62	59.09
M1S1	24.47	43.52	30.31	41.50
M1S2	42.47	52.83	38.79	52.17
M2S1	14.24	36.88	10.69	26.48
M2S2	30.38	46.83	18.19	46.24
M3S1	48.93	53.49	53.33	65.22
M3S2	57.73	56.16	55.76	75.89
M4S1	60.63	62.80	70.31	90.10
M4S2	65.35	67.45	73.93	97.23
M5S1	45.11	53.49	52.12	64.82
M5S2	55.34	57.27	58.19	81.02

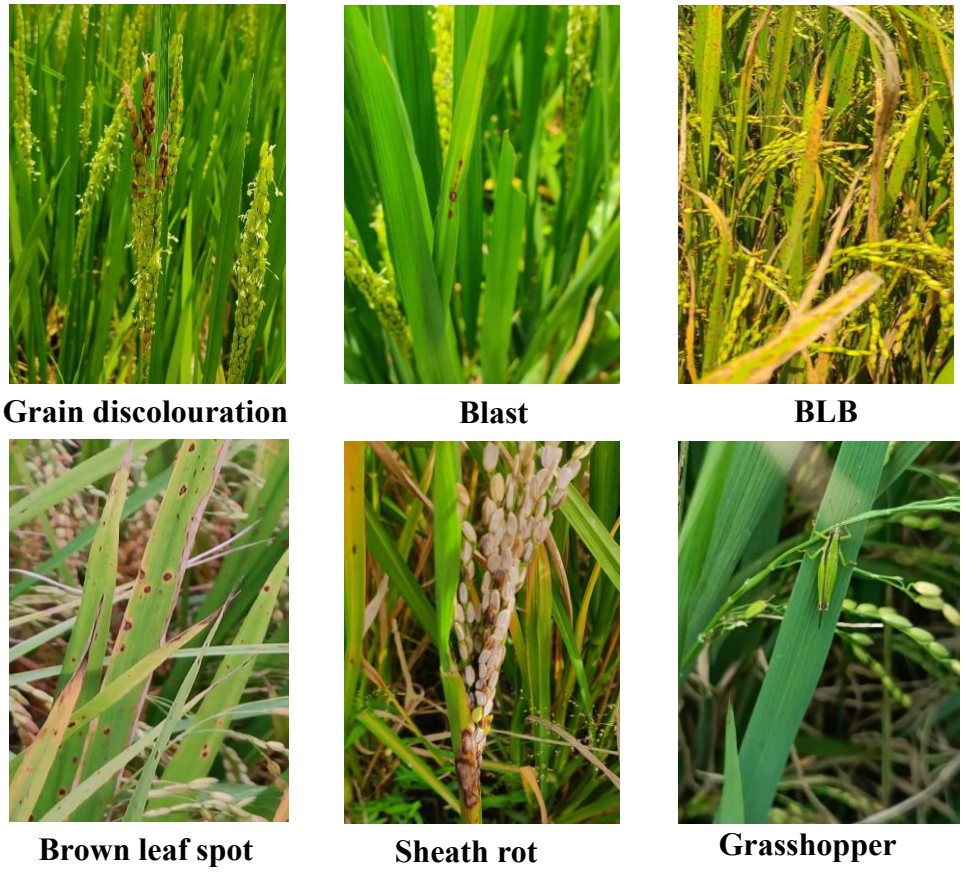


Plate 14: Other pest and diseases observed in the field

Discussion

DISCUSSION

Rice is a cereal crop cultivated and consumed worldwide. It is staple food for majority of the population. However, production of the crop is affected by various biotic and abiotic stresses. Diseases such as blast, sheath blight, bacterial leaf blight and sheath rot reduce the productivity of the crop causing deterioration of quality and quantity. Among the diseases, sheath blight caused by *R. solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*) causes significant reduction in the yield. The incidence and severity of the disease vary among different locations. Management of the disease is difficult because of the absence of resistant genes in the host. Foliar spraying of fungicides is one of the most effective measures for reducing sheath blight infection. The present study "Assessment and management of rice sheath blight disease in Kuttanad" was carried out with the objectives of assessment of extent of rice sheath blight disease incidence caused by *R. solani* in Kuttanad region, screening for host plant resistance and evaluation of different management strategies. The discussion of results obtained is given in this chapter.

5.1 ASSESSMENT OF EXTENT OF SHEATH BLIGHT DISEASE INCIDENCE IN KUTTANAD TRACT

A survey was conducted during 2023-2024 in forty locations in agroecological unit – 04 (Kuttanad) covering Alappuzha, Kottayam and Pathanamthitta districts and sheath blight was observed to be an important disease in AEU – 04. In Kerala, sheath blight incidence was first observed in Regional Agricultural Research Station, Pattambi, Palakkad by Prabhat (1969). It is a location specific disease in Kuttanad, a major rice cultivating tract in Kerala (Surendran *et al*., 2021). Rice production in Kuttanad wetland ecosystem is significantly affected by sheath blight disease leading to decline in grain quality along with 50% loss in grain yield (Krishnan *et al*., 2024).

The survey showed that the incidence of disease in AEU – 04 varied among locations. The per cent disease incidence ranged from 15.39 to 72.73% (Fig. 1). Similarly, the disease severity also varied from 30.00 to 80.91% among the survey locations (Fig. 2). The results were similar to that reported by Prasad *et al*. (2014) while conducting survey in rice growing regions of Thrissur district of Kerala which reported that per cent disease severity ranged from 44.76 to 85.53% in the surveyed locations. The findings are also similar to that reported by Thera *et al*. (2021) which showed the variation of disease incidence from 20.00 to 80.00% in major rice growing areas of Eastern Uttar Pradesh.

The survey revealed that disease incidence and severity was higher in Alappuzha compared to other districts. The rice growing regions of Alappuzha consist of low-lying regions that are about 0.6m – 2.2m below mean sea level (Sheeja *et al*., 2013). The soil humidity of low-lying paddy fields enables the sclerotia to be maintained in hundred per cent survival rate (Feng *et al*., 2017). Similar findings were reported by Kannaiyan and Prasad (1978). The disease was correlated with the climatic and soil conditions prevailing in Kuttanad such as high relative humidity and severely acidic p^H during *Kharif* and *Rabi* seasons (Surendran *et al*., 2021).

The disease symptoms appeared during the tillering and flowering stages. Small circular to oval spots with greenish grey colour were observed in the base of the leaf sheaths. The spots later coalesced and formed large lesions with irregular margins. As the disease progressed, lesions were observed on the aerial parts of the plant. Later the disease affected the panicles leading to discolouration of grains and thereby causing reduction in yield. In severe incidence, white coloured fungal mycelia were observed in the lesions along with brown sclerotia.

Rodrigues *et al*. (2003) reported that the crop was susceptible to sheath blight disease in all life stages with tillering stage being the most susceptible stage which is similar to the results observed in the current study. Rush and Lee (1992) reported that

during tillering stage, the crop canopy leads to the development of high humidity which provides favourable conditions for the development of sheath blight disease.

Singh (2005) reported that greenish grey spots were formed on leaf sheath near the water level. Later the spots enlarged with irregular margins. Under favourable conditions, the lesions spread to the upper regions leading to death of the whole plant. On the surface of lesions, grey to brown mycelia were observed. On further stages, brown coloured sclerotia formed which eventually fall off from the plants. Similar findings were reported by Kumar *et al.* (2016).

5.2 ISOLATION OF THE PATHOGEN INVOLVED AND ITS CHARACTERIZATION

Forty fungal isolates were obtained by the isolation of samples collected from survey locations of AEU – 04 (Kuttanad) *i.e.,* 22, 12 and 6 samples from survey locations of districts *viz.,* Alappuzha, Kottayam and Pathanamthitta respectively

5.2.1 Isolation of the pathogen and its pathogenicity studies

Fungal isolates obtained from samples collected from different survey locations were subjected to pathogenicity studies in rice variety, Uma. The plants exhibited symptoms of disease like presence of grey lesions with brown coloured margins upon artificial inoculation which were similar to that observed in field.The results were similar to the findings reported by Groth and Nowick (1992) which states that the artificially inoculated plants expressed symptoms such as oval or ellipsoid spots with brown or dark brown margins on the leaf sheath near the water level.

The plants on artificial inoculation formed mustard shaped sclerotia with white to dark brown colour which is similar to the results obtained by Singh *et al*. (2003) who reported the presence of brown coloured sclerotia on plants upon artificial inoculation. Kumar *et al.* (2009) conducted artificial inoculation of different isolates of *R. solani*

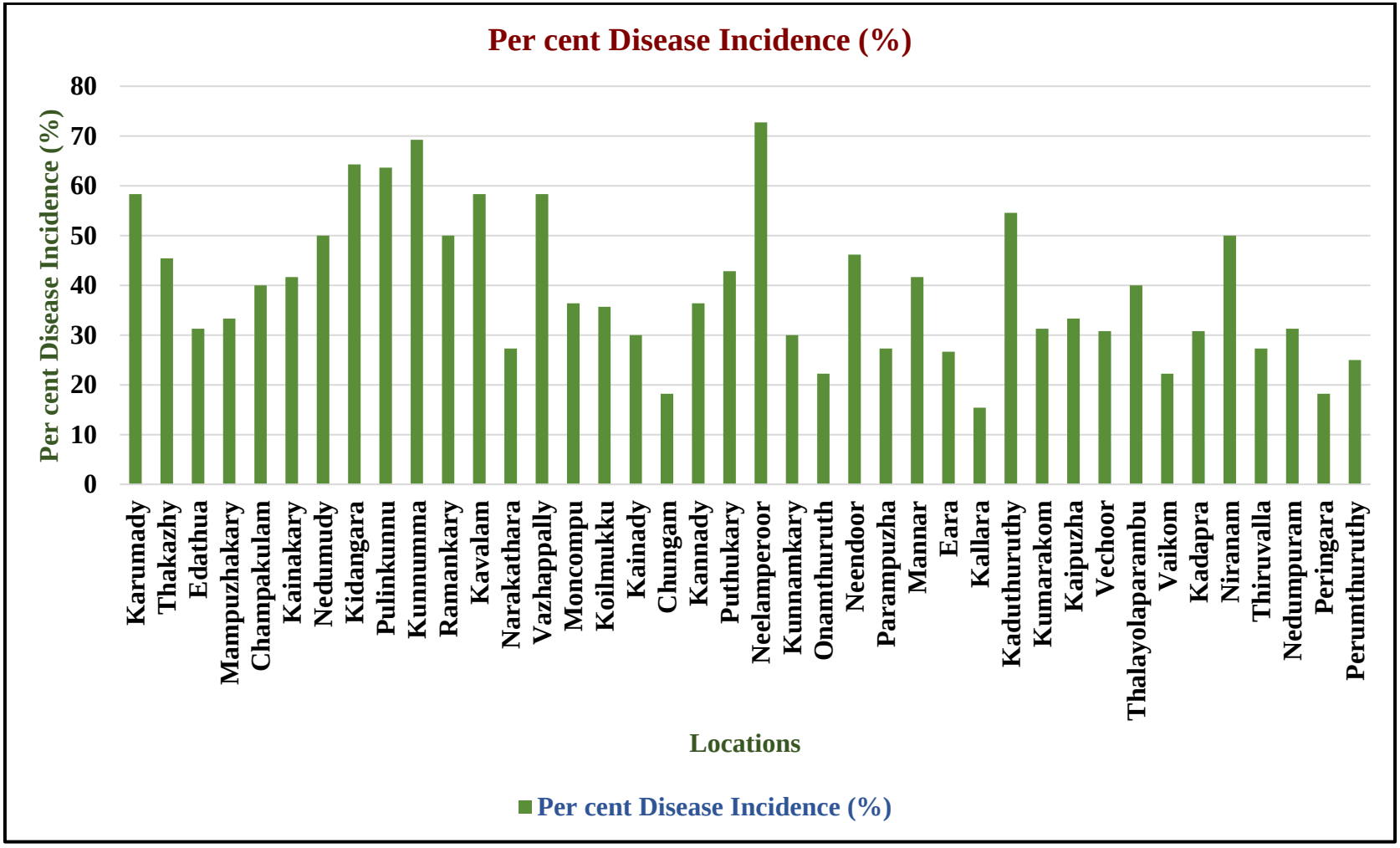


Fig.1: Sheath blight incidence in different locations of Agroecological Unit – 04 (Kuttanad)

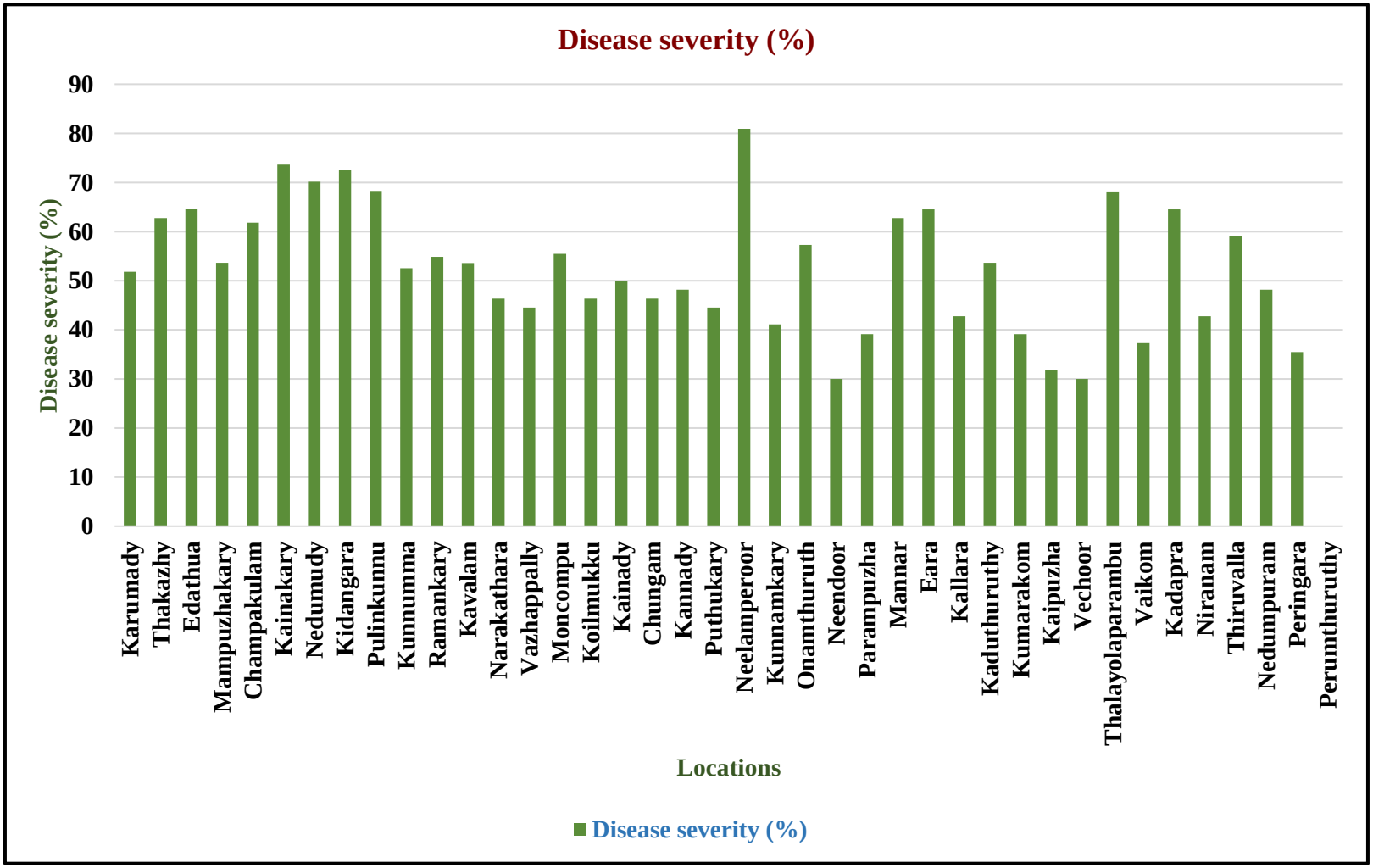


Fig.2: Sheath blight severity in different locations of Agroecological Unit – 04 (Kuttanad)

and observed that water soaked spots appeared within 48 – 96 hours after inoculation. Taheri *et al*., (2007) reported that the isolates on artificial inoculation induced typical symptoms of sheath blight disease such as presence of oval water soaked spots with dark brown margin and grey centre.

The isolate obtained from Neelamperoor region in Alappuzha district formed symptoms on plants in least days (3 days) compared to other isolates and was hence identified as the virulent isolate. Shahjahan *et al*. (1987) reported that growth rate and virulence of the pathogen are positively correlated. Mehi *et al.* (2014) reported that the aerial growth pattern and growth rate of mycelia is linked with the vertical spread of the disease in the plants.

5.2.2 Cultural and morphological characterization of isolates

The cultural and morphological characters of all the isolates obtained were studied. The colour of mycelia and sclerotia, number of days taken for the formation of sclerotia and for full plate (90 mm) growth of mycelia, growth nature of mycelia, pattern of formation of sclerotia, colour, septation and width of hypha and size of scleroia were studied in PDA medium.

Among the forty isolates, the colony colour varied from white, light brown to dark brown. The findings were similar to that reported by Basu and Gupta (1992) which states that colony colour varied from light brown to dark brown. Sunder *et al*. (2003) reported that the discolourations in *R. solani* is due to the pigments produced by the pathogen and the variation in the intensity of the colour is due to the amount of pigments produced.

The isolates took three to eleven days for full plate growth. Mishra *et al*. (2014) recorded that *R. solani* isolates took two to eight days for complete growth in Perti plates (90 mm). Jayaprakashvel and Mathivanan (2012) studied the cultural characters of the isolates of *R. solani* and observed that the growth rate of isolates varied and grouped the isolates as slow growers and fast growers.

The sclerotia formation was observed within three to nine days in every isolate which is similar to findings of Meena *et al.* (2001) which reported that sclerotia formation took place within three to eleven days. Akhtar *et al*. (2009) reported that the sclerotia formation was observed within seven to fifteen days. The colour of sclerotia varied from white to dark brown. Similar findings were observed by Anderson (1982); Sinha and Ghufran (1988); Hoa (1994); Yadav *et al.* (2019).

The size of sclerotia varied from 1.05 – 1.48 mm. This was similar to the observations by Dath (1985) who reported that the size of sclerotia varies from 1 – 3 mm. Lal and Kandari (2009) reported that diameter of sclerotia was observed to vary from 1.13 – 2.03 mm.

The growth pattern of mycelia was studied. Among the forty isolates, twelve isolates exhibited aerial pattern and twenty eight isolates exhibited flat type growth pattern. The findings were similar to the studies by Khodayari *et al*. (2009) which states that the isolates of *R. solani* expressed two types of mycelial growth i.e., aerial and flat. Wamishe *et al*. (2007) reported that the growth nature of the mycelium is correlated to the virulence of the isolate. Mehi *et al*. (2014) reported that the isolates with aerial growth pattern were highly virulent compared to those with flat growth pattern. Tu (1967) reported a positive correlation between aerial mycelial growth of *R. solani* isolates and their virulence.

Microscopic studies revealed that all the isolates exhibited right angled branching, a typical character of *R. solani* with hyaline, septate hypha. Hyphal width varied from 1.12 – 1.98 µm. The microscopic studies are similar to those reported by Sneh *et al.* (1991) and San Aye *et al*. (2008) which states the presence of right angled branching in *R. solani* isolates.

The sclerotia formation pattern was studied and was observed to be scattered or confined to centre or periphery. Similar findings were observed in studies conducted by Singh (1990); Singh *et al*. (2002); Rajput and Harlapur (2016); El-Shafey *et al*.

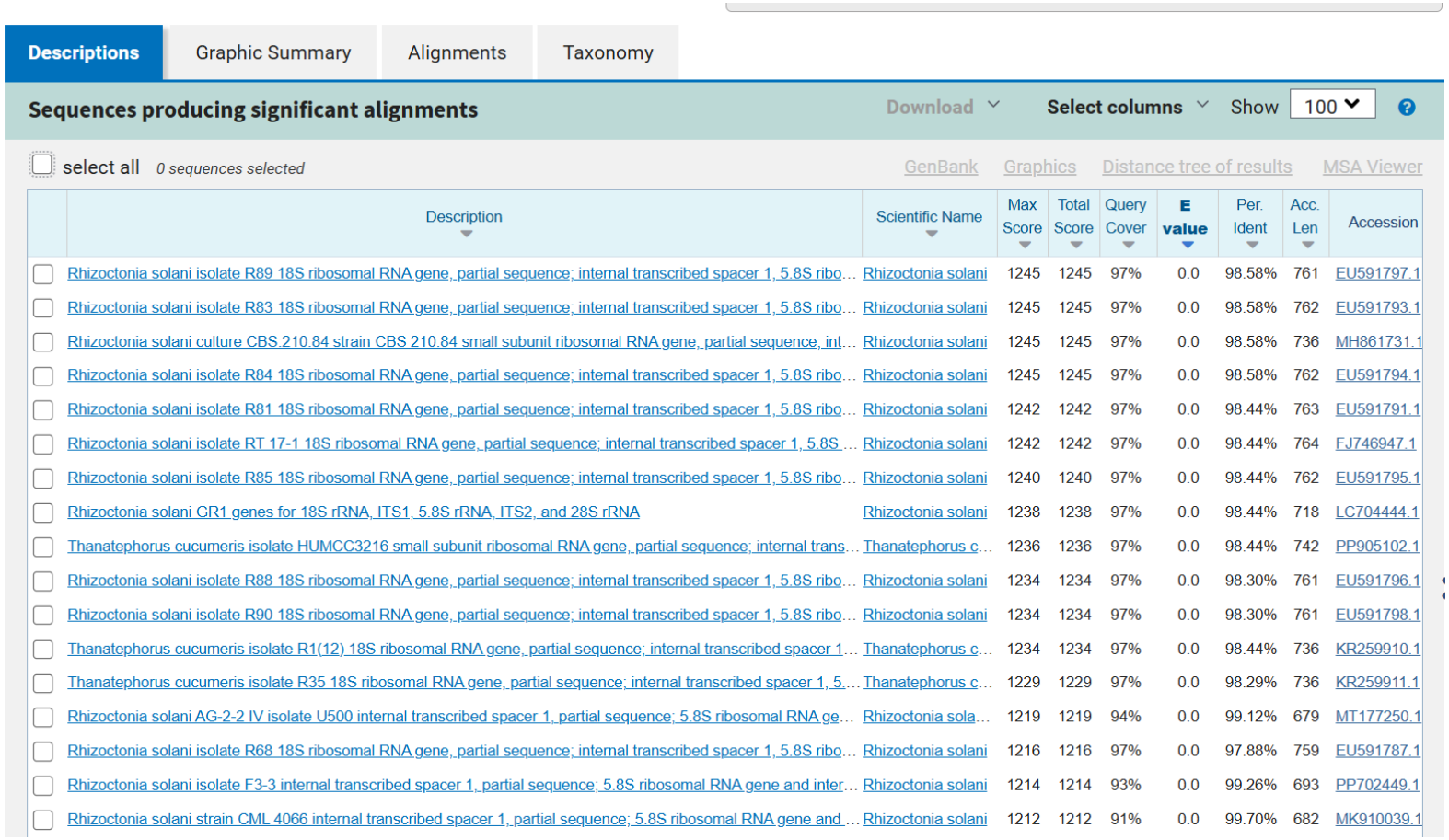
(2019). The texture of sclerotia was observed to be of two different types *i.e.,* smooth and rough textured similar to that reported by Takashi and Tadao (1978); Lal and Kandari (2009); Kumar *et al*. (2014); Kuiry *et al*. (2014).

The isolate from Neelamperoor took least days for complete growth in Petri plate (3 days) and for the formation of sclerotia (3 days). Hence, the culture was confirmed to be the virulent isolate among the obtained isolates. Burpee *et al*. (1980) reported that isolates with fast growth rates were more pathogenic compared to slow growing isolates.

5.2.3 Molecular characterization of virulent isolate

Internal transcribed spacer (ITS) sequences are used in many fungal isolates to identify species (Salazar *et al.,* 2000; Martin *et al*., 2005; Koetschan *et al*., 2010). The primer set ITS1/ITS4 amplifies and sequence ~500 bp rDNA regions, used for the identification of ITS1, 5.8 sRNA and ITS2 of the fungal pathogens (White *et al*., 1990).

The virulent isolate obtained was confirmed by molecular characterization using ITS 1 and ITS 4 primers. The isolate was confirmed as *R. solani* based on the similarity with sequences in GenBank (Fig. 3). The isolate showed 98.58% similarity with *R. solani* isolate with accession code EU591797 with E value 0.0. The phylogenetic tree showing the evolutionary relationship with other *R. solani* isolates is given in Figure 4.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all *0 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhizoctonia solani isolate R89 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1245	1245	97%	0.0	98.58%	761	EU591797.1
Rhizoctonia solani isolate R83 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1245	1245	97%	0.0	98.58%	762	EU591793.1
Rhizoctonia solani culture CBS:210.84 strain CBS 210.84 small subunit ribosomal RNA gene, partial sequence; int…	Rhizoctonia solani	1245	1245	97%	0.0	98.58%	736	MH861731.1
Rhizoctonia solani isolate R84 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1245	1245	97%	0.0	98.58%	762	EU591794.1
Rhizoctonia solani isolate R81 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1242	1242	97%	0.0	98.44%	763	EU591791.1
Rhizoctonia solani isolate RT 17-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S …	Rhizoctonia solani	1242	1242	97%	0.0	98.44%	764	FJ746947.1
Rhizoctonia solani isolate R85 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1240	1240	97%	0.0	98.44%	762	EU591795.1
Rhizoctonia solani GR1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, and 28S rRNA	Rhizoctonia solani	1238	1238	97%	0.0	98.44%	718	LC704444.1
Thanatephorus cucumeris isolate HUMCC3216 small subunit ribosomal RNA gene, partial sequence; internal trans…	Thanatephorus c…	1236	1236	97%	0.0	98.44%	742	PP905102.1
Rhizoctonia solani isolate R88 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1234	1234	97%	0.0	98.30%	761	EU591796.1
Rhizoctonia solani isolate R90 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1234	1234	97%	0.0	98.30%	761	EU591798.1
Thanatephorus cucumeris isolate R1(12) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1…	Thanatephorus c…	1234	1234	97%	0.0	98.44%	736	KR259910.1
Thanatephorus cucumeris isolate R35 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.…	Thanatephorus c…	1229	1229	97%	0.0	98.29%	736	KR259911.1
Rhizoctonia solani AG-2-2 IV isolate U500 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge…	Rhizoctonia sola…	1219	1219	94%	0.0	99.12%	679	MT177250.1
Rhizoctonia solani isolate R68 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Rhizoctonia solani	1216	1216	97%	0.0	97.88%	759	EU591787.1
Rhizoctonia solani isolate F3-3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter…	Rhizoctonia solani	1214	1214	93%	0.0	99.26%	693	PP702449.1
Rhizoctonia solani strain CML 4066 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and …	Rhizoctonia solani	1212	1212	91%	0.0	99.70%	682	MK910039.1

Fig. 3: Sequence comparison of the virulent isolate with other *R. solani* isolates

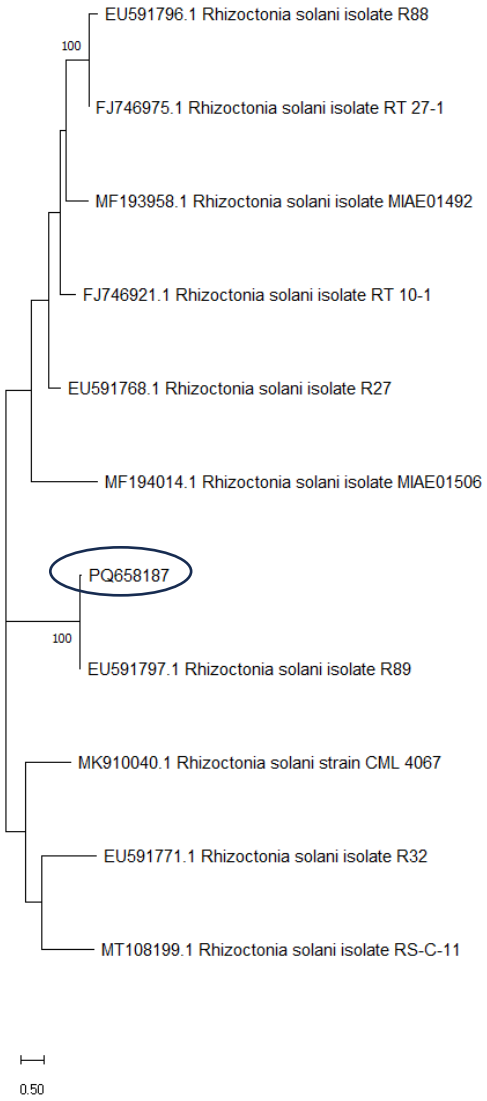


Fig. 4: The phylogenetic tree showing the evolutionary relationship of the virulent isolate with other *R. solani* isolates

5.3 *IN VITRO* EVALUATION OF BACTERIAL BIOCONTROL AGENTS AND FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

In vitro evaluation was conducted to confirm the virulence of the bioagents prior to field evaluation. Biocontrol agents *B. amyloliquefaciens* (B15) and *P. fluorescens* (PN026) were evaluated against virulent isolate of *R. solani* using dual culture technique. Similarly, *in vitro* evaluation of fungicides was conducted using poisoned food technique. Three different fungicides *viz.,* azoxystrobin 18.2% + difenoconazole 11.4% SC, kresoxim methyl 40% + hexaconazole 8% WG and trifloxystrobin 25% + tebuconazole 50% WG were evaluated at different concentrations.

5.3.1 *In vitro* evaluation of bacterial biocontrol agents against sheath blight pathogen by dual culture technique

Both biocontrol agents *i.e., B. amyloliquefaciens* (B15) and *P. fluorescens* (PN026) show inhibition in the mycelial growth of *R. solani* isolate. *B. amyloliquefaciens* (B15) inhibited the pathogen by 68.64% meanwhile, *P. fluorescens* (PN026) show inhibition of 49.78%.

Solanki *et al*. (2015) observed that *B. amyloliquefaciens* caused growth restriction of mycelia of *R. solani* through scanning electron microscope (SEM) and reported that *B. amyloliquefaciens* inhibited the mycelial growth of *R. solani* by 62.00%. Karimi *et al.* (2016) observed that biocontrol agent *B. amyloliquefaciens* inhibited *R. solani* by 74.30%. Solanki *et al*. (2012) reported that *B. amyloliquefaciens* produced chitinase, protease and β - 1,3 - glucanase using *R. solani* cell walls as carbon source. Soliman *et al*. (2022) reported that the antibiosis activity of *B. amyloliquefaciens* was due to the volatile antifungal compounds produced *viz.,* Bis (2-ethylhexyl) phthalate, Bis (2-ethylhexyl) ester, N, N-Dimethyldodecylamine, and Dibutyl phthalate. Yu *et al*. (2002) reported the inhibition of *R. solani* by *B. amyloliquefaciens* is due to the production of iturins by the biocontrol agent. Similar

reports were given by Yoshida *et al*. (2001); Baysal *et al*. (2008). Iturins are antifungal cyclic lipopeptides produced by *Bacillus* sp. (Besson *et al*., 1978; Eshita *et al*., 1995; Ongena and Jacques, 2008).

Maurya *et al*. (2014) evaluated *P. fluorescens* against *R. solani* and observed 55.88% mycelial inhibition of *R. solani.* Nagendran *et al*. (2019) reported that biocontrol agent *P. fluorescens* exhibited 60.33% inhibition of *R. solani.* Nielsen and Sørensen (1999) reported that *P. fluorescens* produced endochitinase and chitobiosidase which inhibited the mycelial growth of *R. solani.* Nagarajkumar *et al*. (2004) observed that production of β - 1,3 - glucanase and HCN are responsible for the inhibition of mycelial growth of *R. solani.* Similar findings were observed in studies conducted by Kumar *et al.* (2012); Paramageetham and Prasada Babu (2012); Akter *et al*. (2016).

5.3.2 *In vitro* evaluation of fungicides against sheath blight pathogen by poisoned food technique

Among the fungicides evaluated, highest inhibition (100%) was observed with kresoxim methyl 40% + hexaconazole 8% WG at $0.5gL^{-1}$, $1gL^{-1}$ and with azoxystrobin 18.2% + difenoconazole 11.4% SC at $1mlL^{-1}$. The per cent inhibition of *R. solani* by the above mentioned fungicides are shown in Fig.5.

Kumari (2017) conducted studies on *in vitro* efficacy of fungicides on *R. solani* and observed that kresoxim methyl 40% + hexaconazole 8% WG showed 91.46% inhibition of mycelial growth in *R. solani.* Lore *et al*. (2011) reported that kresoxim methyl + hexaconazole was highly effective against *R. solani*. Carling *et al.* (1990) reported the efficacy of hexaconazole against *R. solani.*

Birla (2022) reported that hexaconazole inhibited the mycelial growth of *R. solani* by 86.74% at 200 ppm. Yadav *et al*. (2021) reported that kresoxim methyl and hexaconazole inhibited the mycelial growth of *R. solani* by 94.16% and 100% respectively at 200 ppm concentration. Kumar *et al*. (2018) observed that kresoxim

methyl at 5 ppm concentration inhibited the sclerotial development in *R. solani*. Shephard *et al*. (1986) reported that triazole fungicide possess curative, protective, systemic and translaminar properties that enables it to be used excellently against *R. solani*.

Pal and Mandal (2023) conducted *in vitro* assays against sheath blight pathogen and observed that azoxystrobin 18.2% + difenoconazole 11.4% at 200 ppm exhibited 100% inhibition of the pathogen. Similar results were observed in studies conducted by Sundravadana *et al*. (2007); Kumar *et al*. (2018); Sharma *et al*. (2024). Leinhos *et al.* (1997) reported that strobilurin fungicides have been observed to inhibit the germination and pre penetration growth of various plant pathogenic fungi including *R. solani.*

5.4 SCREENING FOR HOST PLANT RESISTANCE

Fifteen KAU released varieties namely, Dhanu, Onam (Kayamkulam 3), Bhadra (MO 4), Aruna (MO 8), Kanakom (MO 11), Ranjini (MO 12), Uma (MO 16), Karishma (MO 18), Krishnanjana (MO 19), Pournami (MO 23), Jyothi (PTB 39), Kanchana (PTB 50), Aiswarya (PTB 52), Anaswara (PTB 58) and Manuratna were screened for resistance against *R. solani*. The pathogen was artificially inoculated and disease parameters were recorded.

Least number of days (4 days) were taken for the symptom development in variety Uma (MO 16). Meanwhile, Kanchana (PTB 50) and Aiswarya (PTB 52) recorded highest number of days for symptom development *i.e.* 18 and 19 days respectively. Disease severity was analyzed and it ranged from 15.55 - 80.11% (Fig.6). Highest disease severity (80.11%) was recorded in Uma (MO 16) whereas, Aiswarya (15.55%) and Kanchana (20.01%) recorded least disease severity. Per cent infected tillers and per cent infected panicles varied from 29.70% - 97.33% and 13.64% - 74.94% respectively among the varieties.

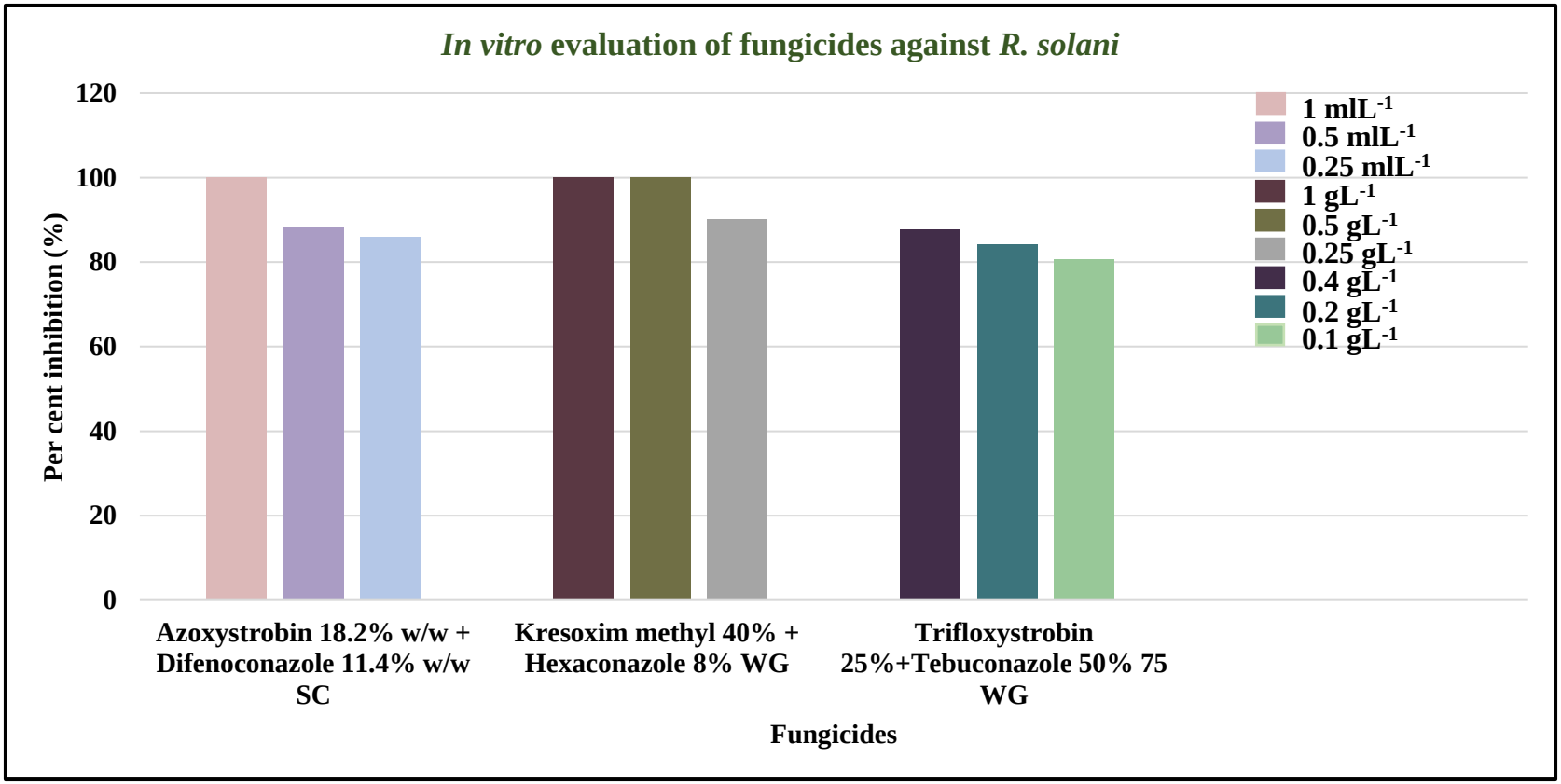


Fig. 5: Per cent growth inhibition of sheath blight pathogen *R. solani* using fungicides

Prasad *et al*. (2010) conducted screening of rice varieties against sheath blight disease and observed that among the 142 varieties screened, one variety was found to be resistant, two were moderately resistant, 73 varieties exhibited moderate susceptibility, 57 varieties were identified as susceptible and eight were found to be highly susceptible to the disease. Reddy *et al*. (1997) evaluated 457 varieties and reported two varieties were resistant to sheath blight.

Similarly, Singh and Borah (2000) reported one resistant variety among sixty local upland rice cultivars. Prasad *et al*. (2020) reported that 21 varieties exhibited resistance to sheath blight disease meanwhile, nine varieties were recorded with moderate resistance. Bhaktavatsalam *et al*. (1978) reported that three varieties were found to be tolerant to sheath blight among the varieties screened.

Rice varieties Kanchana and Aiswarya are reported to be resistant to sheath blight disease (KAU, 2024). Mansi (2022) reported abundant cuticular wax deposition in resistant varieties compared to susceptible varieties which act as barrier and prevent the pathogen establishment on the plant surface. Similarly, biochemical assays revealed higher level of induction of defense related enzymes such as peroxidase, superoxide dismutase, polyphenol oxidase, chitinase and phenylalanine ammonia lyase in resistant varieties.

Marshall and Rush (1980) reported that there was no formation of infection cushions on the resistant cultivars. Even though, the lobate appressoria was formed, further penetration was slowed by cell collapsing on the outer sheaths around the invading hypha. Marshall (1979) reported that resistance exhibited by rice varieties to infection cushion formation by *R. solani* is a dominantly inherited trait. Similarly, failure in invading the culms was also reported in resistant varieties. Flentje *et al.* (1963) reported stimulus formed in susceptible varieties and stimulus – inhibitor complex in resistant varieties upon *R. solani* infection resulted in reduced necrosis in resistant varieties.

According to Rao *et al*. (2020), resistant cultivars showed higher levels of expression of genes encoding polysaccharide lyases (PL), glycoside hydrolases (GH), and glycosyl transferases (GT) than susceptible cultivars following inoculation with *R. solani* resulting in a greater degree of necrosis in susceptible cultivars. The metabolism of phenylpropanoid and phenylalanine also imparts a role in avoiding infection in rice sheath blight resistant strains, according to KEGG enrichment analyses (Kwon *et al*., 2014; Shi *et al*., 2020; Zhang *et al*., 2017).

Oreiro *et al*. (2020) reported the reduced accumulation of reactive oxygen species (ROS) in resistant lines compared to susceptible lines resulting in reduced necrotic lesions in resistant lines. Shi *et al*. (2020) conducted transcriptome analysis of sheath blight resistant lines and susceptible lines and observed the enhanced regulation of trans-cinnamate-4-monooxygenase (C4H), ethylene-insensitive protein 2 (EIN2) and transcriptome factor WRKY33 in resistant lines and reported that these genes contribute to the resistance against sheath blight disease.

5.5 *IN VIVO* TESTING OF THE EFFICACY OF BACTERIAL BIOCONTROL AGENTS AND COMMERCIAL FUNGICIDES AGAINST SHEATH BLIGHT PATHOGEN

Field evaluation of biocontrol agents and fungicides were conducted in split plot design. The results revealed that among the main plot treatments, kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ was observed to be effective compared to other treatments with least per cent infected tillers (34.15%), panicle infection (31.81%), per cent infected grains (6.07%), per cent chaffy grains (6.94%) and disease severity (21.90%) and highest yield parameters such as thousand grain weight (26.14 g), grain yield (8183.33 $kgha^{-1}$) and B:C ratio (2.48).

Among biocontrol agents, *B. amyloliquefaciens* (B15) given as seed treatment (10 gkg^{-1} seed), soil application (1 kg $acre^{-1}$) at 35 days after planting (DAP) and foliar

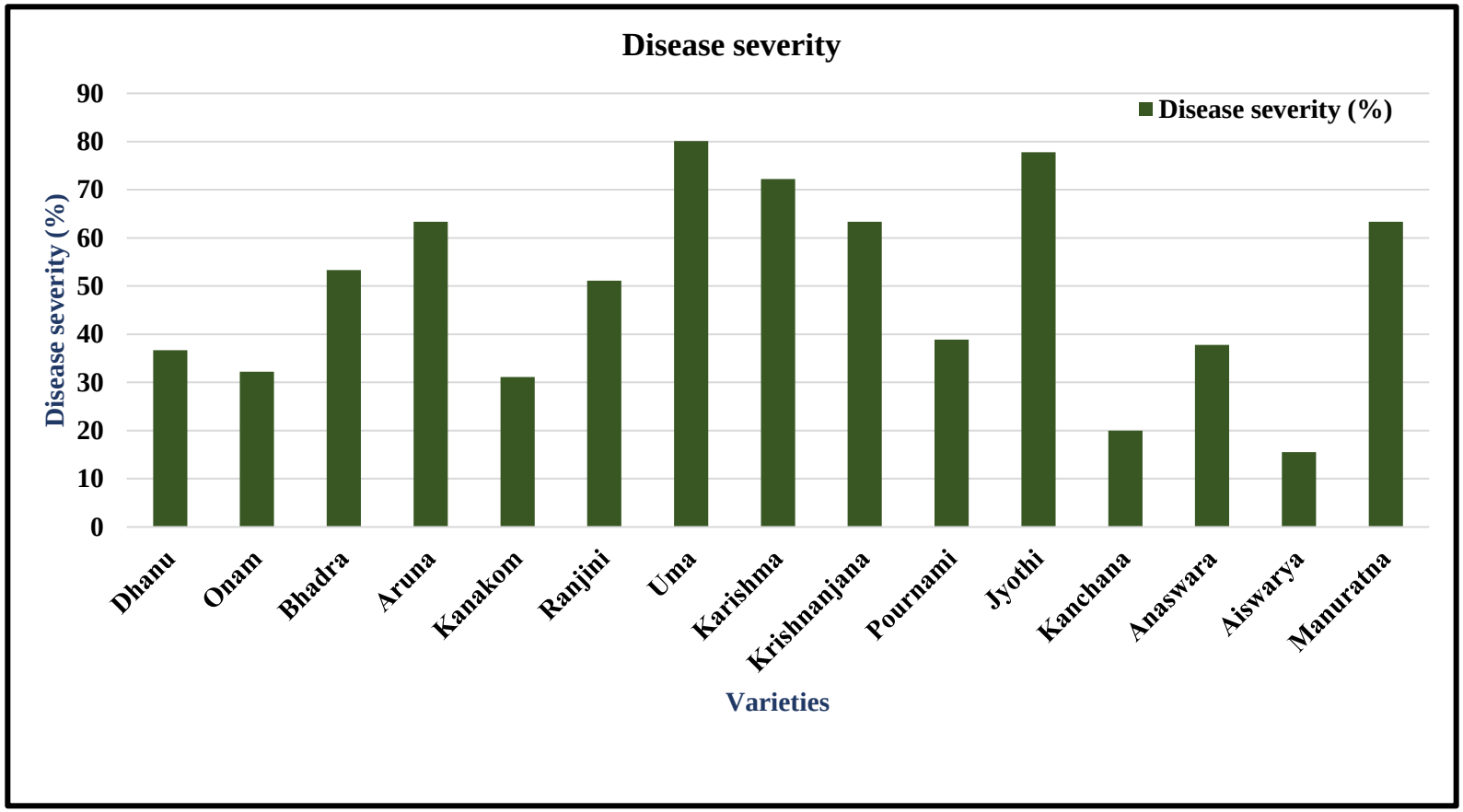


Fig. 6: Variation in disease severity among different KAU rice varieties

spray (20 gL^{-1}) at maximum tillering stage recorded least per cent infected tillers (61.39%), panicle infection (47.27%), per cent infected grains (12.01%), per cent chaffy grains (13.39%) and disease severity (51.43%) with highest yield parameters thousand grain weight (24.82 g), grain yield (6191.67 $kgha^{-1}$) and B:C ratio (1.93). Variation of percent disease index, per cent disease severity and grain yield among the main plot treatments is given in Fig. 7, Fig. 8.

Among the sub plot treatments, 75% nitrogen, 100% phosphorus and 125% potassium of RDF recorded the lowest per cent infected tillers (52.95%), panicle infection (47.98%), per cent infected grains (11.37%), per cent chaffy grains (13.13%) and disease severity (45.87%) with highest thousand grain weight (25.27 g), grain yield (6708.34 $kgha^{-1}$) and B:C ratio (2.03). Variation of disease parameters among sub plot treatments is given in Fig. 9.

The interaction effect of main plot treatment, kresoxim methyl 40% + hexaconazole 8% WG at $1gL^{-1}$ and subplot treatment 75% nitrogen, 100% phosphorus and 125% potassium of RDF (M4S2) was significantly more effective compared to other treatments which recorded the lowest per cent infected tillers (31.97%), per cent infected panicles (29.69%), per cent infected grains (5.77%), chaffy grains (5.96%), disease severity (20.48%) and highest grain yield (8316.67 $kgha^{-1}$), thousand grain weight (27.59 g) and B:C ratio (2.54). Among the biocontrol agents, the interaction effect of treatment combination of *B. amyloliquefaciens* (B15) and 75% nitrogen, 100% phosphorus and 125% potassium of RDF (M1S2) recorded lowest per cent infected tillers (53.08%), per cent infected panicles (43.02%), per cent infected grains (10.49%), chaffy grains (11.57%), disease severity (48.09%) and highest grain yield (6416.66 $kgha^{-1}$), thousand grain weight (25.75 g) and B:C ratio (1.90). Variation of percent disease index, per cent disease severity and grain yield in interaction among the main plot and sub plot treatments is given in Fig. 10, Fig. 11.

Ansari *et al*. (2024) reported that application of *B. amyloliquefaciens* improved the growth parameters of the plants along with reduced disease infection over control. Kumar *et al*. (2012) reported that application of *B. amyloliquefaciens* as seed treatment (10 gkg^{-1} seed), soil treatment (1 $kgacre^{-1}$) and foliar spray (20 gL^{-1}) recorded minimum disease incidence in *in vivo* studies. Khan *et al*. (2024) conducted *in vivo* studies against sheath blight disease in rice and observed that *B. amyloliquefaciens* exhibited highest inhibition of the disease compared to other treatments along with improving growth parameters. In addition to the effectiveness as biocontrol agents, *B. amyloliquefaciens* improved plant growth parameters such as shoot height, root length, fresh and dry weights, number of tillers, and grains per tiller. Flavonoid and indole acetic acid (IAA) concentrations was observed to be increased by 30 to 80%, while plant hormones abscisic acid (ABA) and gibberellic acids (GA3) increased by 35% and 53%, respectively. Similarly, comparing to the control, the levels of proline, carbohydrates, phenolic content, carotenoids, antioxidant enzymatic activity, and chlorophyll (a, b) were also higher.

Buttar *et al*. (2023) recorded that when used as a seedling and foliar spray treatment, the biopesticide formulation of *B. amyloliquefaciens* exhibited disease control of 47.20% and 30.04% in both *Kharif* seasons 2022 and 2023, respectively and therefore, effective in management of rice sheath blight disease. Similar results were obtained in study conducted by Soliman *et al*. (2023).

Nagendran *et al*. (2019) conducted field trials for the evaluation of biocontrol agents against sheath blight disease and observed that application of *P. fluorescens* as seed treatment, soil application and foliar spray recorded least disease incidence. Similar findings were observed in studies conducted by Kazempour (2004); Singh and Sinha (2009); Sivakamasundari and Usharani (2012); Suman *et al*. (2017);

Lore *et al*. (2012) conducted *in vivo* studies using fungicides against sheath blight disease and observed that kresoxim methyl + hexaconazole exhibited highest

reduction in disease incidence compared to other treatments. Similarly, Kumar and Chethana (2022) conducted field evaluation of various fungicides against sheath blight disease and observed that among the fungicides evaluated, highest reduction in disease severity was observed in treatment using kresoxim methyl + hexaconazole. Pal and Mandal (2023) reported that azoxystrobin + difenoconazole exhibited highest reduction in sheath blight infection in field conditions. Similar results were observed in studies conducted by Kumar *et al*. (2018); Thakur *et al*. (2018).

Triazoles are broad spectrum fungicide, inhibiting a large range of fungus by functioning as sterol demethylation inhibitor, which inhibits the growth of the fungus by preventing biosynthesis of ergosterol in their cell membranes. The strobilurin group of fungicides prevent the fungal respiration by binding to Qo site of cytochrome b-c_1 complex causing disruption in electron transport system thereby inhibiting the fungus (Worthington, 2012).

Slaton *et al*. (2003) reported that increased application of nitrogen fertilizers leads to increased sheath blight incidence in field. Studies conducted by Shahjahan *et al.* (1990) recorded increased disease incidence in treatments with higher application of nitrogen fertilizers compared to treatments with low nitrogen application. Similar results were obtained by Cu *et al*. (1996); Savary and Mew (1996).

The establishment and spread of sheath blight disease in rice are facilitated by the application of higher nitrogen fertilizer rates because they directly enhance the density of the foliage, increasing disease contact with the leaves and sheath of rice plants and moisture retention beneath the canopy (Savary *et al*., 1995). Nitrogen supply stimulates crop growth which influences canopy density, leading to increased wetness and frequent contact within the canopy that are conducive for infection of healthy leaves leading to increased incidence of sheath blight (Castilla *et al*., 1996; Wu *et al*., 2012).

Inoue and Uchino (1963) recorded lower disease incidence when potassium was applied at higher dose due to the increase in phenol content in leaf sheaths. Perrenoud (1990) reported the increased application of potassium fertilizer recorded decreased incidence of sheath blight disease and increased grain yield. Haerdter (1997) observed an inverse relationship between rate of potassium fertilizer and disease incidence. Similarly, a negative correlation was observed by Mondal *et al*. (2001) between the application rate of potassium and disease incidence. Similar findings were observed in study conducted by Singh *et al.* (2009).

The results of the study revealed the wide spread occurrence of sheath blight disease in major rice growing districts *viz.,* Alappuzha, Kottayam and Pathanamthitta in AEU – 04 (Kuttanad) of Kerala. The pathogen associated with sheath blight was confirmed as *R. solani*. Screening of KAU released rice varieties revealed Aiswarya and Kanchana to be resistant to sheath blight disease according to IRRI-SES scale, 2013. Biocontrol agent *B. amyloliquefaciens* (B15) given as seed treatment (10 gkg^{-1} seed), soil application (1 kg $acre^{-1}$) at 35 days after planting and foliar spray (20 gL^{-1}) at maximum tillering stage combined with 75%N, 100%P, 125%K of RDF was effective against the disease. Among the fungicides assessed, foliar spraying of commercial fungicide kresoxim methyl 40% + hexaconazole 8% WG at a concentration of 1 gL^{-1} at maximum tillering stage along with 75%N, 100%P, 125%K of RDF significantly reduced disease incidence along with increasing yield.

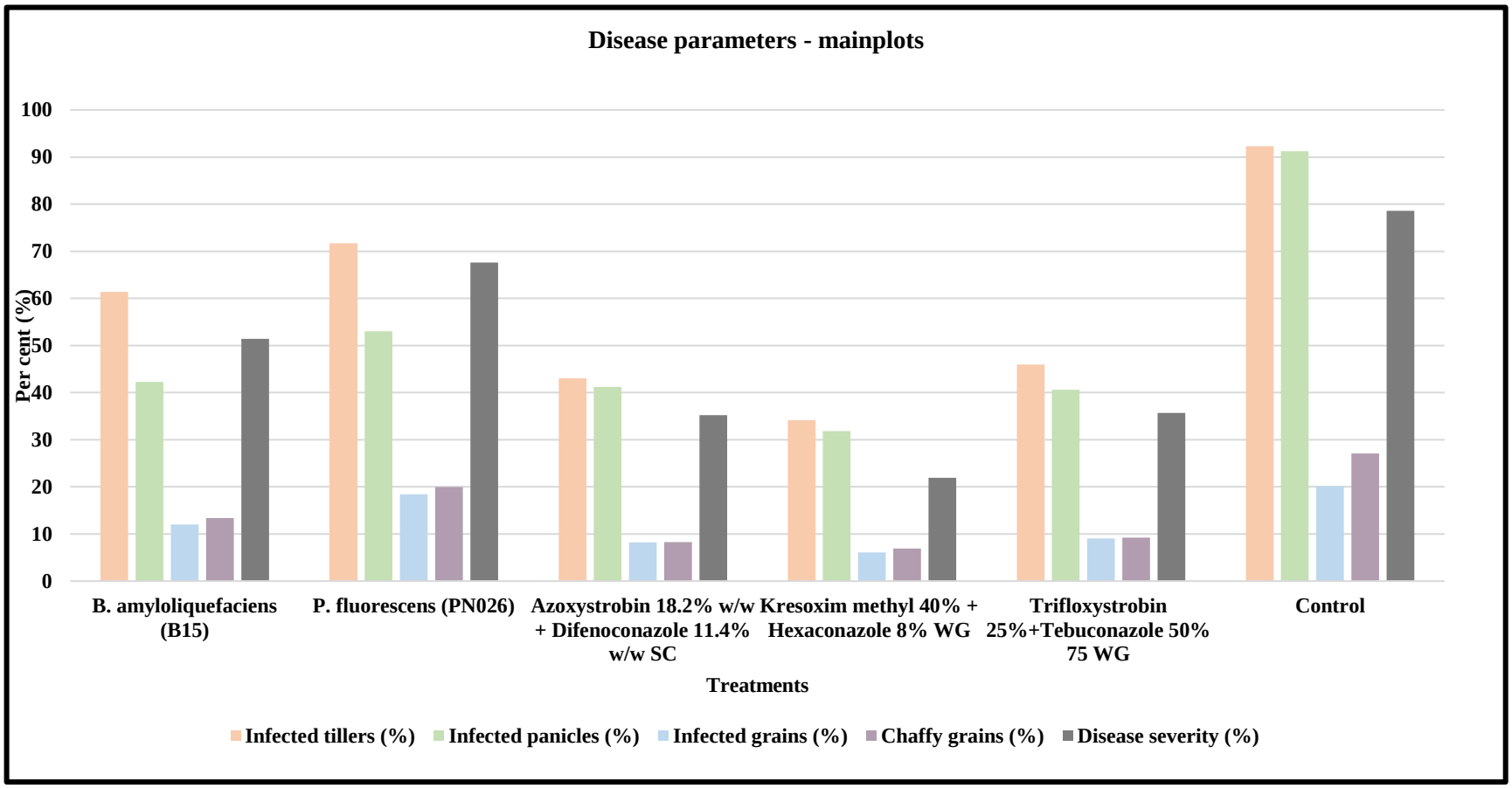


Fig. 7: Variation in disease parameters assessed in main plot

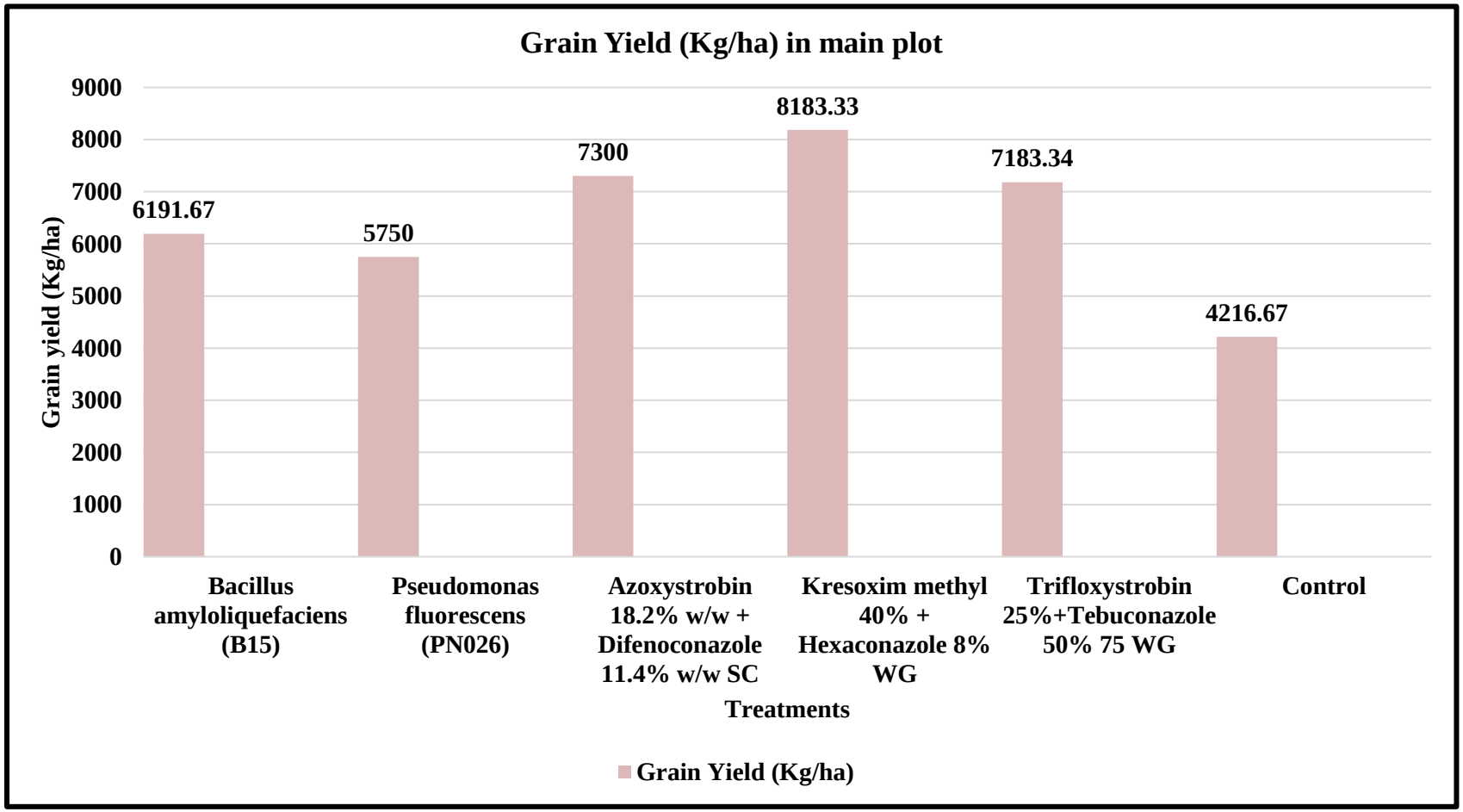


Fig. 8: Variation in grain yield among main plot treatments

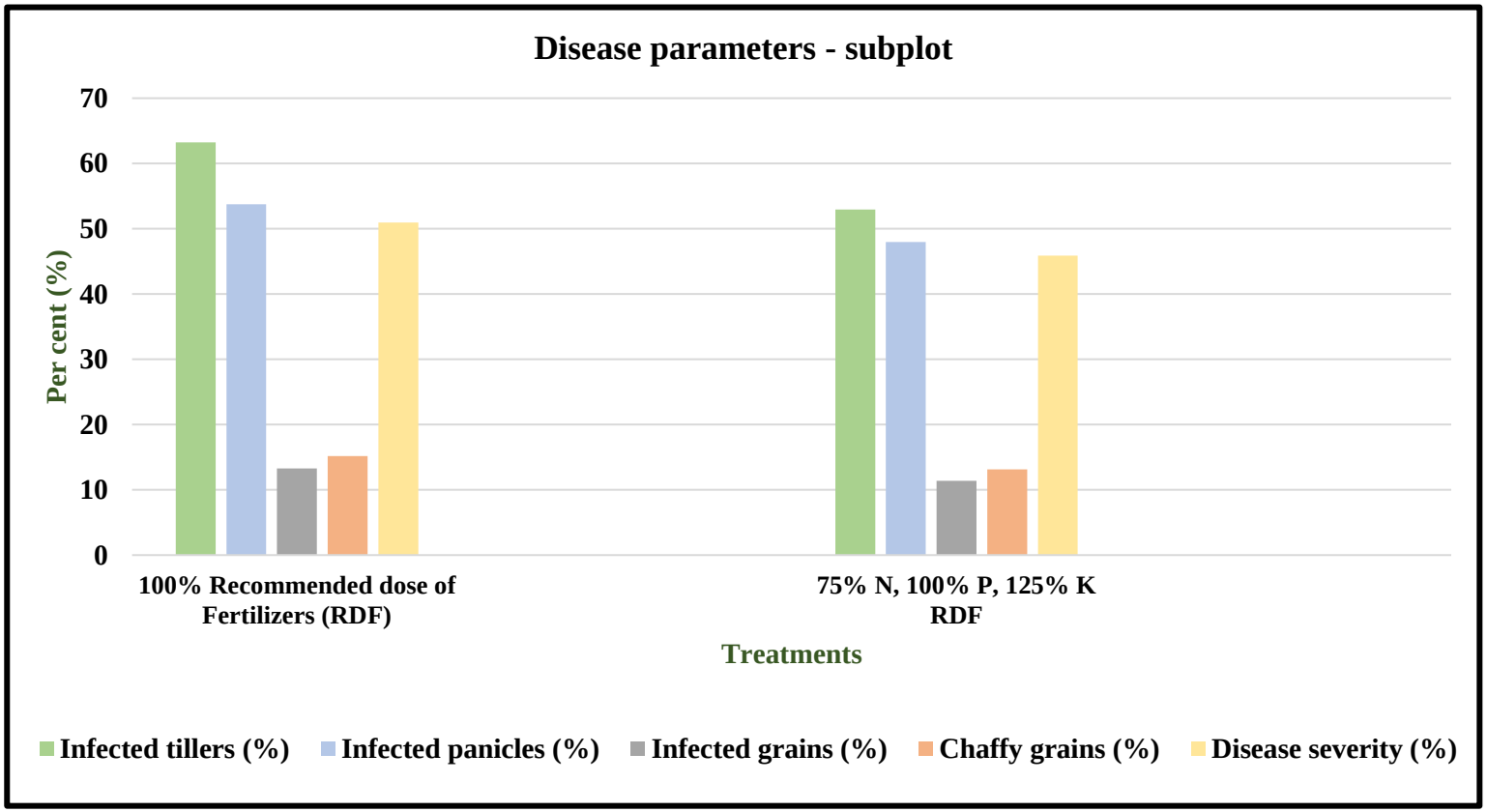


Fig. 9: Variation in disease parameters assessed in sub plot treatments

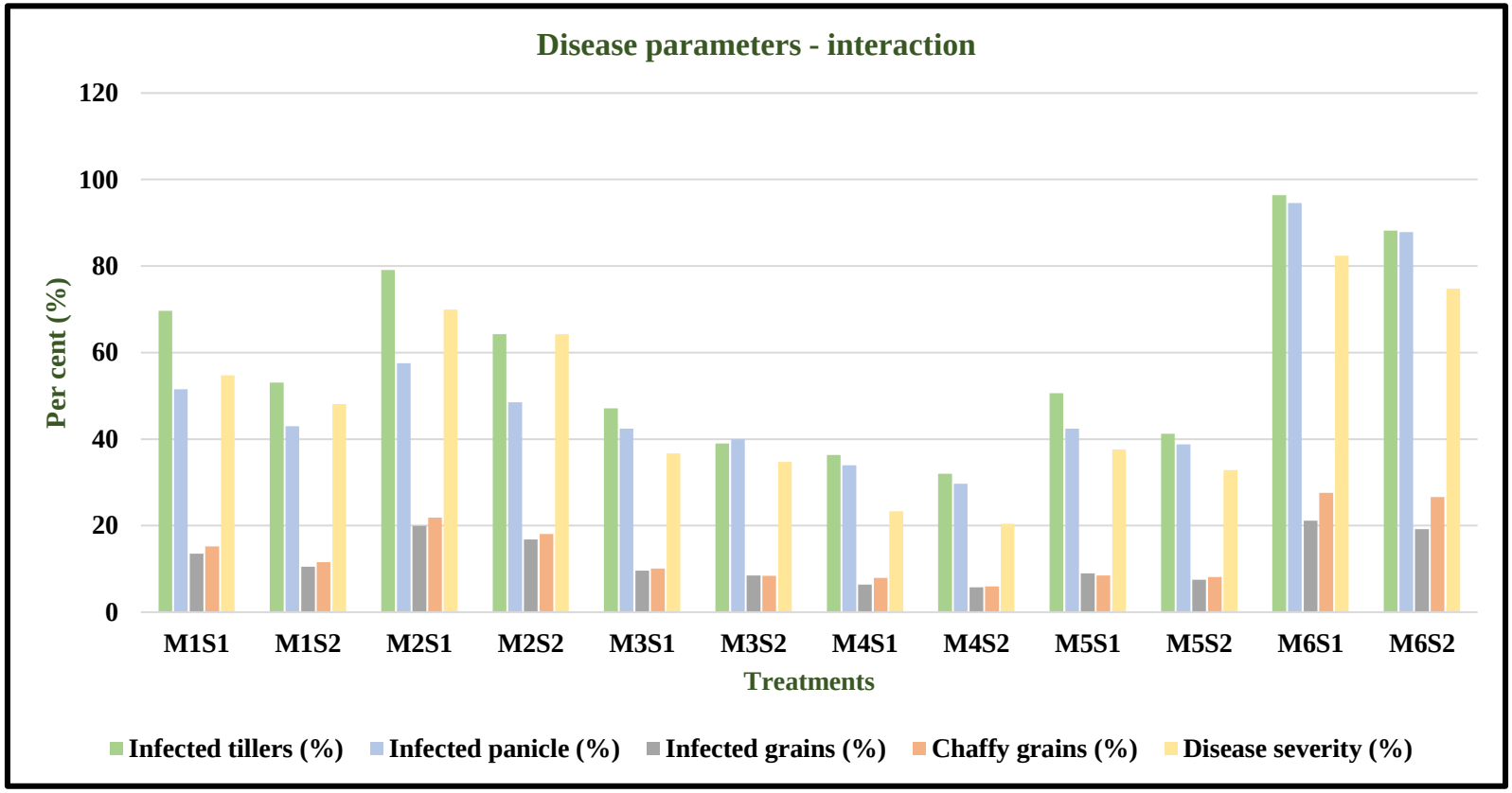


Fig. 10: Variation in disease parameters assessed in interaction between main plot and sub plot treatments

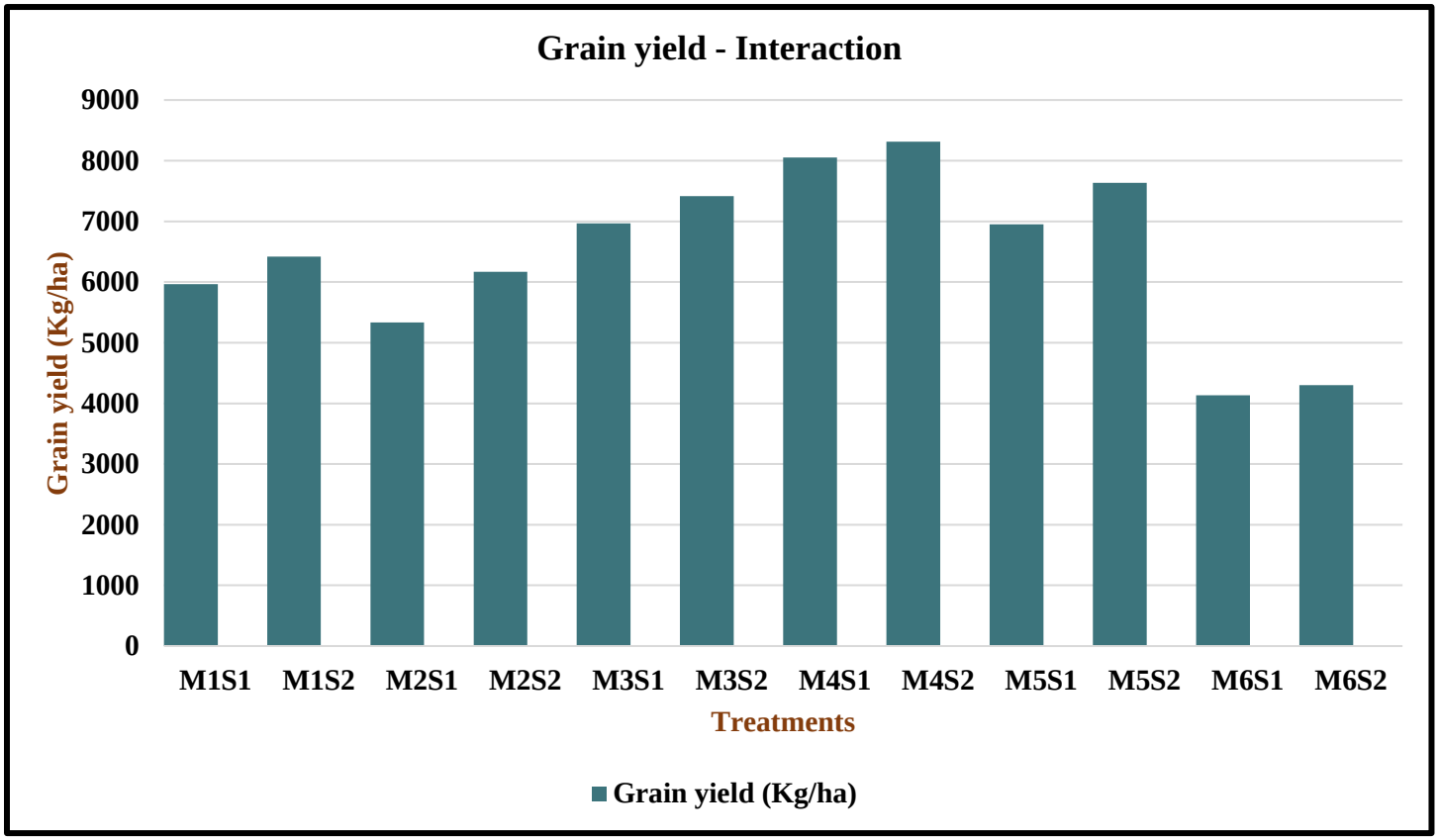


Fig. 11: Variation in grain yield in interaction between main plot and sub plot treatments

Summary

SUMMARY

Rice is a staple food crop consumed worldwide. It has an inevitable role in providing food security to the population. Rice production is influenced by numerous parameters among which disease plays a key role. Among diseases, sheath blight caused by *R. solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*) causes significant reduction in the yield. Management of the disease is difficult because of the absence of resistant genes in the host. The present study entitled "Assessment and management of rice sheath blight disease in Kuttanad" was conducted at Department of Plant Pathology, College of Agriculture, Vellayani and M. S. Swaminathan Rice Research Station, Moncompu during 2022-2024 with the objectives of assessment of extent of rice sheath blight disease incidence caused by *R. solani* in Kuttanad region, screening for host plant resistance and evaluation of different management strategies.

A purposive survey was conducted in forty locations of agroecological unit (AEU) – 04 (Kuttanad) covering Alappuzha, Kottayam and Pathanamthitta districts to assess the extent of sheath blight disease incidence in rice. The symptomatology was studied and plants having symptoms of water soaked lesions with grey centre and brown margins were observed on leaf sheath near the water level. Presence of brown coloured sclerotia were observed in severely infected plants. Disease parameters such as disease incidence, disease severity, infected tillers (%) and infected panicles (%) were recorded from these locations. The disease incidence was assessed and it ranged from 15.39 to 72.73 per cent in the survey locations whereas, and disease severity ranged from 30.00 to 80.91 per cent. Infected tillers (%) varied from 28.20 to 75.82 per cent and panicle infection percentage varied from 10.18 to 56.89 per cent in the survey locations. The highest disease incidence (72.73%), disease severity (80.19%), infected tillers (75.82%) and infected panicles (56.89%) were observed in Neelamperoor region of Alappuzha district.

The diseased samples were collected for the isolation of the pathogen and 40 isolates were obtained. The pathogenicity was confirmed by artificially inoculating the isolates in rice variety, Uma. Days taken for symptom development and sclerotia formation was recorded and isolate from Neelamperoor region (I_{21}) took least number of days for symptom development (3 days) and sclerotia formation (5 days). Similarly, plants inoculated with isolate from Neelamperoor exhibited higher disease severity (88.54%) compared to other plants. Hence, this was identified as the virulent isolate and was used for further studies.

Cultural and morphological studies of all forty isolates were conducted and the colony colour varied from white to dark brown in PDA media. The mycelia formed were aerial and flat type. The number of days for complete growth in Petri plate (90 mm) and for sclerotia formation was 3 to 11 days and 3 to 9 days respectively. The isolate from Neelamperoor took least number of days for complete growth in Petri plate (3 days) and for the formation of sclerotia (3 days). Pattern of sclerotia formation was scattered or confined to centre or periphery. Sclerotia was white to dark brown in colour with smooth and rough surface. Size of sclerotia varied from 1.05 – 1.48 mm. The mycelia were hyaline and septate with right angled branching. Hyphal width ranged from 1.15 – 1.98 µm.

The molecular characterization of the virulent isolate (I_{21}) was done using ITS primers. The DNA sequence was aligned to the already existing sequences in NCBI database using BLAST software. The isolate showed maximum similarity with *R. solani* isolate with accession code EU591797. The sequences obtained was submitted in GenBank and was allotted with accession number PQ658187.

In vitro evaluation of biocontrol agents in growth inhibition of *R. solani* was tested by dual culture technique. Biocontrol agents *B. amyloliquefaciens* (B15) and *P. fluorescens* (PN026) were tested and highest mycelial growth inhibition (68.64%) was observed in *B. amyloliquefaciens* (B15) whereas, *P. fluorescens* (PN026) showed 49.78% inhibition.

Efficacy of fungicides in growth inhibition of *R. solani* at different concentrations was tested under *in vitro* conditions by poisoned food technique. Fungicides, azoxystrobin 18.2% + difenoconazole 11.4% SC, kresoxim methyl 40% + hexaconazole 8% WG and trifloxystrobin 25% + tebuconazole 50% 75 WG were used at three different concentrations. Complete mycelial inhibition was recorded with azoxystrobin 18.2% + difenoconazole 11.4% SC at 1 mlL^{-1} and in kresoxim methyl 40%+ hexaconazole 8% WG at 0.5 gL^{-1} and 1 gL^{-1}. Kresoxim methyl 40% + hexaconazole 8% WG at 0.25gL^{-1} showed an inhibition of 90.16%. Azoxystrobin 18.2% + difenoconazole 11.4% SC at concentrations of 0.5mlL^{-1} and 0.25mlL^{-1} exhibited 88.19% and 86.07% mycelial growth inhibition respectively. Trifloxystrobin 25% + tebuconazole 50% WG showed 80.71%, 84.26% and 87.63% mycelial inhibition at concentrations 0.1gL^{-1}, 0.2gL^{-1} and 0.4gL^{-1} respectively.

Fifteen varieties released by KAU namely, Dhanu, Onam (Kayamkulam 3), Bhadra (MO 4), Aruna (MO 8), Kanakom (MO 11), Ranjini (MO 12), Uma (MO 16), Karishma (MO 18), Krishnanjana (MO 19), Pournami (MO 23), Jyothi (PTB 39), Kanchana (PTB 50), Aiswarya (PTB 52), Anaswara (PTB 58) and Manuratna were subjected to screening for host plant resistance against sheath blight disease. The disease parameters were analyzed and among these, Aiswarya and Kanchana were observed to be resistant with least disease severity (15.55% and 20.01% respectively) whereas varieties Uma, Jyothi and Karishma were found to be susceptible exhibiting disease severity of 80.11%, 77.78% and 72.23% respectively.

In vivo evaluation of efficacy of biocontrol agents and fungicides along with different fertilizer dose for the management of sheath blight disease was conducted in rice variety Uma in split plot design.

Highest per cent reduction in disease over control was observed in treatment combination kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} with 75%N, 100%P, 125%K of recommended dose of fertilizers (RDF) (73.93%) followed by kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} along with 100% RDF

(70.31%). Among biocontrol agents, treatment combination of *B. amyloliquefaciens* (B15) and 75%N, 100%P, 125%K of RDF had better control over other treatments with 38.79% reduction in disease severity.

Highest per cent increase in grain yield was recorded in treatment combination of kresoxim methyl 40% + hexaconazole 8% WG at a concentration of 1 gL^{-1} and 75%N, 100%P, 125%K of RDF (97.23%) followed by kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} with 100% RDF (90.10%).

The present study concludes that sheath blight disease incidence ranged from 15.39 to 72.73% in AEU – 04 (Kuttanad). The varieties Aiswarya and Kanchana exhibited resistance to the disease. Sheath blight disease can be effectively managed by foliar spraying of commercial fungicide kresoxim methyl 40% + hexaconazole 8% WG at a concentration of 1 gL^{-1} at maximum tillering stage along with 75%N, 100%P, 125%K of RDF. Biocontrol agent *B. amyloliquefaciens* (B15) given as seed treatment (10 gkg^{-1} seed), soil treatment (1 kg $acre^{-1}$) at 35 days after planting and foliar spray (20 gL^{-1}) at maximum tillering stage combined with 75%N, 100%P, 125%K of RDF also reduces the disease incidence along with increasing yield parameters.

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Appendices

APPENDIX - I

COMPOSITION OF MEDIA USED

Potato Dextrose Agar (PDA) Medium

Potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml

APPENDIX – II

COMPOSITION OF STAIN USED

Lactophenol - Cotton blue

Anhydrous lactophenol	-	67.0 ml
Distilled water	-	20.0 ml
Cotton blue	-	0.1 g

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

APPENDIX - III

The ITS sequence of *R. solani* isolate (I_{21})

GAGGGGGGGGCTAGCGAGTCTACCTGCATTTGAGATCAGATCATAAAATAA
TAATTTTTATTGTCCAAGTCAATGGACTATTGGAAGCGGTTCATCTGCATTT
ACCTTGGCCACCCTTTTTTACCGGGGTGTCCTCAGCGATAGATAATTTATCA
CGCCGAGTGGAACCAAGCATAACACTGAGATCCAGCTAATGAACGAAGAG
GAGCAGCGTGTGAAGCTGCAAGAACCTCCAATACCAAAGTGAAACCAAAT
TGAGTTAACAAAAAGATTTACTTTGAAGATTTCATGATACTCAAACAGGCA
TGCTCCAAGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGAT
TCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATC
GATGCGAGAGCCAAGAGATCCGTTGTTGAAACTTAGTATTAGATGCGTTAC
ATCCATTACATTCATTTTAAAATAAATTGGGTTTATATTAGAGTTGAGTAGAC
AGAGGGGGGTAGGGGTCCCAATCATTCACCTAAAAGTAAATGAAAGTTTT
CCCATCCATGTCTCTGCCTCACAGGTTCACAGGTGTGTGTGGATTAAAAAA
AAGAGCAAAAGGTGTGCACATGCTCCAAATTAATGGAGCCAGCTACAACC
TAAATACCCTTTGTTTAAATTCAATAATGATCCTTCCGCAGGTCCCCCTACG
GAAA

ASSESSMENT AND MANAGEMENT OF RICE SHEATH BLIGHT DISEASE IN KUTTANAD

by

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(2022-11-081)

ABSTRACT

Submitted in the partial fulfilment of the requirement for the degree of

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Faculty of Agriculture

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ABSTRACT

The study entitled "Assessment and management of rice sheath blight disease in Kuttanad" was conducted at Department of Plant Pathology, College of Agriculture, Vellayani and M. S. Swaminathan Rice Research Station, Moncompu during 2022-2024 with the objectives of assessment of extent of rice sheath blight disease incidence caused by *Rhizoctonia solani* in Kuttanad region, screening for host plant resistance and evaluation of different management strategies.

A survey was conducted in forty locations of agro ecological unit (AEU) – 04 (Kuttanad) in Alappuzha, Kottayam and Pathanamthitta districts to assess the extent of sheath blight disease incidence in rice. The symptomatology was studied and plants with grey water soaked lesions with brown margins were observed on leaf sheath near the water level. Presence of brown coloured sclerotia were observed in severely infected plants. Disease parameters such as disease incidence and disease severity were recorded. The highest disease incidence (72.73%) and disease severity (80.19%) were observed in Neelamperoor region of Alappuzha district.

The diseased samples were collected for the isolation of the pathogen and 40 isolates were obtained. The pathogenicity was confirmed by artificially inoculating the isolates in rice variety, Uma. Days taken for symptom development and sclerotia formation was recorded and isolate from Neelamperoor region took least number of days for symptom development (3 days) and sclerotia formation (5 days).

Cultural and morphological studies of the isolates were conducted and the colony colour varied from white to dark brown in PDA medium. The mycelia formed were aerial and flat type. The number of days for complete growth in Petri plate (90 mm) and for sclerotia formation was 3 to 11 days and 3 to 9 days respectively. The isolate from Neelamperoor took least number of days for complete growth in Petri plate (3 days) and for the formation of sclerotia (3 days). Pattern of sclerotia formation was scattered or confined to centre or periphery. Sclerotia was white to dark brown in colour

with smooth and rough surface. Size of sclerotia varied from 1.05 – 1.48 mm. The mycelia were hyaline and septate with right angled branching. Hyphal width ranged from 1.12 – 1.98 µm.

The molecular characterization of the virulent isolate was done using ITS primers. The DNA sequence comparison showed similarity with *R. solani.* The sequences obtained was submitted in GenBank and was allotted with accession number PQ658187.

In vitro evaluation of biocontrol agents in growth inhibition of *R. solani* was tested by dual culture technique. Biocontrol agents *Bacillus amyloliquefaciens* (B15) and *Pseudomonas fluorescens* (PN026) were tested and highest mycelial growth inhibition (68.64%) was observed in *B. amyloliquefaciens*. Efficacy of fungicides in growth inhibition of *R. solani* at different concentrations was tested under *in vitro* conditions by poisoned food technique. Fungicides, azoxystrobin 18.2% + difenoconazole 11.4% SC, kresoxim methyl 40% + hexaconazole 8% WG and trifloxystrobin 25% + tebuconazole 50% 75 WG were evaluated against virulent isolate of *R. solani*. Complete mycelial inhibition was recorded with azoxystrobin 18.2% + difenoconazole 11.4% SC at 1 mlL^{-1} and in Kresoxim methyl 40%+ Hexaconazole 8% WG at 0.5 gL^{-1} and 1 gL^{-1}.

Fifteen varieties released by KAU were subjected to screening for host plant resistance against sheath blight disease. The disease parameters were analyzed and among these, Aiswarya and Kanchana were observed to be resistant with least disease severity (15.55% and 20.01% respectively) whereas varieties Uma, Jyothi and Karishma were found to be more susceptible exhibiting disease severity of 80.11%, 77.78% and 72.23% respectively.

In vivo evaluation of efficacy of biocontrol agents and fungicides along with different fertilizer dose for the management of sheath blight disease was conducted in rice variety Uma in split plot design. Highest per cent reduction in disease over control

was observed in treatment combination kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} with 75%N, 100%P, 125%K of recommended dose of fertilizers (RDF) (73.93%) followed by treatment combination of kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} with 100% RDF (70.31%). Among biocontrol agents, treatment combination of *B. amyloliquefaciens* (B15) and 75%N, 100%P, 125%K of RDF had better control with 38.79% reduction in disease severity. Highest per cent increase in grain yield was recorded in treatment combination of kresoxim methyl 40% + hexaconazole 8% WG at a concentration of 1 gL^{-1} and 75%N, 100%P, 125%K of RDF (97.23%) followed by kresoxim methyl 40% + hexaconazole 8% WG at 1 gL^{-1} and 100% RDF (90.10%).

The present study concludes that sheath blight disease incidence ranged from 15.39 to 72.73% in AEU – 04 (Kuttanad). The varieties Aiswarya and Kanchana exhibited resistance to the disease. Sheath blight disease can be effectively managed by foliar spraying of commercial fungicide kresoxim methyl 40% + hexaconazole 8% WG at a concentration of 1 gL^{-1} at maximum tillering stage along with 75%N, 100%P, 125%K of RDF. Biocontrol agent *B. amyloliquefaciens* (B15) given as seed treatment (10 gkg^{-1} seed), soil treatment (1 kg $acre^{-1}$) at 35 days after planting and foliar spray (20 gL^{-1}) at maximum tillering stage combined with 75%N, 100%P, 125%K of RDF also reduces the disease incidence along with increasing yield parameters.

സംഗ്രഹം

“കുട്ടനാട്ടിലെ നെല്ലിലെ പോളരോഗത്തിന്റെ നിർണയവും അവയുടെ നിയന്ത്രണവും” എന്ന വിഷയത്തിൽ കോളേജ് ഓഫ് അഗ്രികൾച്ചർ, വെള്ളായണിയിലും എം എസ് സ്വാമിനാഥൻ നെല്ലു ഗവേഷണ കേന്ദ്രം മങ്കൊമ്പിലും ആയി 2022-2024 കാലയളവിൽ പഠനം നടത്തുകയുണ്ടായി. പോളരോഗത്തിന്റെ വ്യാപ്തി നിർണ്ണയിക്കുക, വിവിധ നെല്ലിനങ്ങളിലെ പോളരോഗ പ്രതിരോധശേഷി വിലയിരുത്തുക, നിയന്ത്രണമാർഗങ്ങൾ പരിശോധിക്കുക എന്നിവയായിരുന്നു ലക്ഷ്യങ്ങൾ.

അഗ്രോ ഇക്കോളജിക്കൽ യൂണിറ്റ് (AEU) - 04 (കുട്ടനാട്) - ലെ ആലപ്പുഴ, കോട്ടയം, പത്തനംതിട്ട ജില്ലകളിൽ നാല്പത് സ്ഥലങ്ങളിൽ നെല്ലിലെ പോളരോഗബാധയുടെ വ്യാപ്തി വിലയിരുത്തുന്നതിനായി ഒരു സർവേ നടത്തുകയുണ്ടായി. നെല്ലിലെ രോഗലക്ഷണങ്ങൾ പഠിക്കുകയും, ജലനിരപ്പിന് സമീപമുള്ള ഇലപ്പാളിയിൽ തവിട്ടുനിറത്തിലുള്ള അരികുകളുള്ള ചാരനിറത്തിലെ പാടുകൾ ചെടികളിൽ കാണപ്പെടുകയും ചെയ്തു. ഗുരുതരമായി ബാധിച്ച ചെടികളിൽ തവിട്ട് നിറമുള്ള സ്ക്ലിറോഷ്യയുടെ സാന്നിധ്യം കണ്ടെത്തി. ആലപ്പുഴ ജില്ലയിലെ നീലംപേരൂരിലാണ് ഏറ്റവും കൂടുതൽ രോഗസാധ്യതയും (72.73%) രോഗതീവ്രതയും (80.19%) രേഖപ്പെടുത്തിയത്.

സർവേ നടത്തിയ സ്ഥലങ്ങളിൽ നിന്ന് ശേഖരിച്ച സാമ്പിളുകളിൽ നിന്നും റൈസക്ടോണിയ സോളാനിയെ

വേർതിരിച്ചെടുത്തു. നാല്പത് ഐസൊലേറ്റുകൾ ശേഖരിക്കുകയും അവയെല്ലാം ഉമ എന്ന നെല്ലിനത്തിൽ നടത്തിയ രോഗാരിത്വ പരീക്ഷണത്തിൽ രോഗബാധ ഉണ്ടാക്കുന്നതായ് സ്ഥിരീകരിക്കുകയും ചെയ്തു.

രോഗലക്ഷണ വികസനത്തിനും സ്ക്ലിറോഷ്യ രൂപീകരണത്തിനും എടുത്ത ദിവസങ്ങൾ രേഖപ്പെടുത്തുകയും നീലംപേരൂർ മേഖലയിൽ നിന്നുള്ള ഐസൊലേറ്റ് രോഗലക്ഷണ വികസനത്തിനും (3 ദിവസം) സ്ക്ലിറോഷ്യ രൂപീകരണത്തിനും (5 ദിവസം) ഏറ്റവും കുറവ് ദിവസമെടുക്കുന്നതായി കണ്ടെത്തുകയും ചെയ്തു. അങ്ങനെ ഏറ്റവും തീവ്രമായ ഐസൊലേറ്റായി തിരഞ്ഞെടുത്തു.

ഐസൊലേറ്റുകളുടെ രൂപാന്തരപരമായ പഠനങ്ങൾ നടത്തുകയും കോളനി നിറം പിഡിഎ മീഡിയയിൽ വെള്ള മുതൽ കടും തവിട്ട് വരെ വ്യത്യാസപ്പെടുകയും ചെയ്യുന്നതായി കണ്ടെത്തി. പെട്രി പ്ലേറ്റിലെ (9 സെന്റീമീറ്റർ) പൂർണ്ണ വളർച്ചയ്ക്കും സ്ക്ലിറോഷ്യ രൂപീകരണത്തിനും യഥാക്രമം 3 മുതൽ 11 ദിവസം വരെയും 3 മുതൽ 9 ദിവസം വരെയും എടുക്കുന്നതായി കാണപ്പെട്ടു. നീലംപേരൂരിൽ നിന്നുള്ള ഐസൊലേറ്റിന്റെ പൂർണ്ണ വളർച്ചയ്ക്കും (3 ദിവസം) സ്ക്ലിറോഷ്യ രൂപീകരണത്തിനും (3 ദിവസം) ഏറ്റവും കുറഞ്ഞ ദിവസമെടുത്തു. വെളുത്തതു മുതൽ കടുംതവിട്ടു നിറം വരെയുള്ള സ്ക്ലിറോഷ്യ കാണപ്പെട്ടു. സ്ക്ലിറോഷ്യയുടെ വലിപ്പം 1.05 മുതൽ 1.48 മില്ലിമീറ്റർ വരെ വ്യത്യാസപ്പെട്ടിരുന്നു. മൈസീലിയ ഹൈലിനും സെപ്റ്റേറ്റും

ലംബമായ ശാഖകളുള്ളവയും ആയിരുന്നു. ഹൈഫയുടെ വീതി 1.12 മുതൽ 1.98 മൈക്രോ മീറ്റർ വരെയായിരുന്നു.

രോഗകാരിയിൽ നിന്ന് ഡിഎൻഎ വേർതിരിച്ചെടുക്കുകയും ITS പ്രൈമറുകൾ ഉപയോഗിച്ച് മോളിക്കുലാർ പഠനങ്ങൾ നടത്തുകയും ചെയ്തു. തിരുവനന്തപുരം രാജിവ് ഗാന്ധി സെന്റർ ഫോർ ബയോടെക്നോളജി (RGCB) - യിൽ നിന്ന് മോളിക്കുലാർ ക്യാരക്കൂറൈസേഷനിലൂടെ റൈസോക്ടോണിയ സോളാനിയായി ഈ അനുബന്ധ രോഗകാരിയെ തിരിച്ചറിഞ്ഞു. ഇതിനെ തുടർ പഠനങ്ങൾക്കായി ഉപയോഗിച്ചു. സീകൻസുകൾ GenBank-ൽ സമർപ്പിക്കുകയും PQ658187 എന്ന ആക്സഷൻ നമ്പർ ലഭിക്കുകയും ചെയ്തു.

ബാസില്ലസ് അമിലോലികാഫേഷ്യൻസ് (B15), സ്യൂഡോമോണാസ് ഫ്ലൂറസെൻസ് (PN 026) എന്നിവ ഉപയോഗിച്ച് നടത്തിയ ഡ്യൂവൽ കൾച്ചർ പരീക്ഷണത്തിൽ ഇവ രോഗകാരിയുടെ വളർച്ചയെ ഗണ്യമായി തടയുന്നുവെന്ന് മനസ്സിലാക്കാൻ കഴിഞ്ഞു. ഇതിൽ ബി. അമിലോലികാഫേഷ്യൻസ് (B15) ഉയർന്ന രോഗപ്രതിരോധം (68.64%) കാണിച്ചു. വിവിധ സാന്ദ്രതകളിൽ ആർ. സൊളാനിയുടെ വളർച്ച തടയുന്നതിൽ കുമിൾനാശിനികളുടെ ഫലപ്രാപ്തി ഇൻ വിട്രോയിൽ പരിശോധിച്ചു. അസോക്സിസ്ട്രോബിൻ 18.2% + ഡൈഫെനോക്കോണാസോൾ 11.4% SC, ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG, ട്രൈഫ്ലോക്സിസ്ട്രോബിൻ 25% + ടെബുക്കോണാസോൾ 50% 75 WG എന്നീ കുമിൾനാശിനികൾ ഉപയോഗിച്ചു. ഇതിൽ

അസോക്സിസ്ട്രോബിൻ 18.2%+ ഡൈഫെനോക്കോണാസോൾ 11.4% SC (1 mlL^{-1}), ക്രെസോക്സിം മീഥൈൽ 40%+ ഹെക്സാക്കോണസോൾ 8% WG (0.5 gL^{-1}, 1 gL^{-1}) എന്നിവ രോഗകാരിയുടെ മൈസീലിയൽ വളർച്ചയെ പൂർണ്ണമായും (100%) തടഞ്ഞു.

കെ എ യൂ നെല്ലിനങ്ങളുടെ (15 ഇനങ്ങൾ) പോളരോഗ പ്രതിരോധശേഷി പരിശോധിക്കുന്നതിനായി പോട്ട് കൾച്ചർ പരീക്ഷണം നടത്തി. ഇവയിൽ, ഐശ്വര്യയും കാഞ്ചനയും ഏറ്റവും കുറഞ്ഞ രോഗ തീവ്രത (യഥാക്രമം 15.55%, 20.01%) രേഖപ്പെടുത്തുകയും പ്രതിരോധശേഷിയുള്ളതായി കാണപ്പെടുകയും ചെയ്തു. അതേസമയം ഉമ, ജ്യോതി, കരിഷ്മ എന്നീ ഇനങ്ങളിൽ 80.11% 77.78%, 72.23% യഥാക്രമം രോഗ തീവ്രത രേഖപ്പെടുത്തി.

2022 ഖാരീഫ് സമയത്ത് എം എസ് സ്വാമിനാഥൻ നെല്ല് ഗവേഷണകേന്ദ്രം, മങ്കൊമ്പിൽ ജൈവനിയന്ത്രണമാർഗങ്ങളും കുമിൾനാശിനികളും ഉപയോഗിച്ച് നെല്ലിന്റെ പോളാരോഗത്തിനെതിരെ പരീക്ഷണം നടത്തുകയുണ്ടായി. ഇതിൽ ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG (1 gL^{-1}) + ശുപാർശ ചെയ്യുന്ന രാസവളങ്ങളുടെ (RDF) 75% N, 100%P, 125% K എന്ന ചികിത്സാസംയോജനം രോഗബാധയിൽ ഏറ്റവുമധികം കുറവുണ്ടാക്കുന്നതായി കാണപ്പെട്ടു (73.93%). പിന്നാലെ ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG 1 gL^{-1} ന് ഒപ്പം 100% RDF സംയോജനം 70.31% രോഗനിയന്ത്രണം നൽകുന്നതായും

രേഖപ്പെടുത്തി. ബയോകൺട്രോൾ ഏജന്റുകളിൽ, ബി. അമിലോലികഫേഷ്യൻസ് (B15) + RDF-ന്റെ 75% N, 100%P, 125% K എന്ന സംയോജനം രോഗ തീവ്രതയിൽ 38.79% കുറവുണ്ടാക്കുന്നതായി കാണപ്പെട്ടു. ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG (1 gL^{-1}) + RDF-ന്റെ 75% N, 100%P, 125% K എന്നീ സംയോജനത്തിൽ ധാന്യവിളവിൽ ഏറ്റവും ഉയർന്ന ശതമാനം (97.23%) വർദ്ധനവ് രേഖപ്പെടുത്തി. പിന്നാലെ ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG (1 gL^{-1}) + 100% RDF, 90.10% വർദ്ധനവ് രേഖപ്പെടുത്തി.

ഇപ്പോഴത്തെ പഠനത്തിൽ AEU - 04 (കുട്ടനാട്) - ൽ 15.39% മുതൽ 72.73% വരെ പോളരോഗബാധയുണ്ടായതായി കണ്ടെത്താൻ സാധിച്ചു. ഐശ്വര്യ, കാഞ്ചന എന്നീ നെല്ലിനങ്ങളിൽ രോഗ പ്രതിരോധശേഷി പ്രകടമായിരുന്നു. വാണിജ്യ കുമിൾനാശിനിയായ ക്രെസോക്സിം മീഥൈൽ 40% + ഹെക്സാക്കോണാസോൾ 8% WG, 1 gL^{-1} എന്ന സാന്ദ്രതയിൽ ഇലകളിൽ തളിക്കുന്നതിനോടൊപ്പം RDF - ന്റെ 75% N, 100%P, 125% K നൽകുന്നതും പോളരോഗം ഫലപ്രദമായി നിയന്ത്രിക്കുന്നതായി കണ്ടെത്തി. വിത്ത് പരിചരണത്തിലൂടെയും (10 ഗ്രാം/കിലോ വിത്ത്), മണ്ണു പ്രയോഗത്തിലൂടെയും (ഏക്കറിന് 1 കി.ഗ്രാം), ഫോളിയാർ സ്പ്രേയിലൂടെയും (20 gL^{-1}) ബി. അമിലോലികഫേഷ്യൻസ് (B 15) + RDF ന്റെ 75% N, 100%P, 125% K എന്നിവ നൽകുന്നത് ഒരു പരിധി വരെ രോഗം നിയന്ത്രിക്കാൻ സഹായിക്കുന്നതായും കണ്ടെത്തി.