ORGANIC STRATEGY FOR THE MANAGEMENT OF SHEATH BLIGHT DISEASE OF RICE

by

KARTHIKA S. R.
(2015-11-073)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2017

DECLARATION

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

Vellayani
Date: 16/08/17

Karthika S. R.
(2015 - 11-073)

CERTIFICATE

Certified that this thesis entitled **"Organic strategy for the management of sheath blight disease of rice"** is a record of research work done independently by Ms. Karthika S. R. 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.

Sajeena S 16/08/17

Vellayani
Date: 16/08/17

Dr. Sajeena A.
(Major Advisor, Advisory Committee)
Assistant Professor (Plant Pathology)
IFSRS, Karamana

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Karthika S. R., a candidate for the degree of **Master of Science in Agriculture** with major in Plant Pathology, agree that the thesis entitled **"Organic strategy for the management of sheath blight disease of rice"** may be submitted by Ms. Karthika S. R., in partial fulfilment of the requirement for the degree.

Dr. Sajeena A.
(Chairperson, Advisory Committee)
Assistant Professor (Plant Pathology)
IFSRS, Karamana,
Thiruvananthapuram 695002

16/08/2017

Dr. V. K. Girija
(Member, Advisory Committee)
Professor and Head
Dept. of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram 695522

16/8/17

Dr. Jacob John
(Member, Advisory Committee)
Professor and Head
IFSRS, Karamana,
Thiruvananthapuram 695002

16/8/17

Dr. G. Heera
(Member, Advisory Committee)
Assistant Professor (Plant Pathology)
PRS, Panniyoor
Kannur 670142

16/8/17

External Examiner

Dr. (Mrs.) Veena. S. S.
Principal Scientist (Plant Pathology)
Division of Crop Protection
Central Tuber Crops Research Institute
Sreekariyam, Thiruvananthapuram - 695 017
Kerala, India

Acknowledgement

First and foremost, praises and thanks to the Almighty, for everything that happens to me...

With immense pleasure, I would like to express my sincere gratitude to **Dr. Sajeena. A.** *Assistant Professor, IFSRS, Karamana for the constructive guidance, constant inspiration, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for providing a stress free situation by the open minded approach and for the care and affection bestowed on me throughout the study period.*

I convey my heartfelt thanks to **Dr. V. K. Girija** *Professor and Head, Department of Plant Pathology for the unceasing encouragement, valuable advices and whole hearted approach right from the beginning of the thesis work.*

I extend my sincere gratefulness to **Dr. Jacob John** *Professor and Head IFSRS, Karamana for the valuable suggestions, technical advices and incessant motivation throughout the research work.*

I am extremely thankful to **Dr. G. Heera,** *Asst. professor, PRS,Panniyoor for the unstinting support, suggestions and passionate approach rendered during the period of research work.*

I gratefully acknowledge with thanks **Dr. Umamaheswaran** *Professor, for his constructive comments, creative suggestions and, affectionate approach at all the stages of research work.*

I express my sincere thanks to **Dr. Lulu Das** *Professor, Department of Pathology for the valuable advices, unstinted motivation and for the valuable support.*

I extend my gratefulness to **Dr. Joy M.** *Associate professor, Department of Plant Pathology, for the timely advices and support.*

I am also thankful to **Dr. Susha. S. Thara,** *Assistant professor, Department of Plant Pathology, for her immense support and valuable advices during the research work.*

I express my heartfelt thanks to ***Dr. C. Gokulapalan*** *for his care, affection and support provided during my thesis work.*

I extend my gratitude to ***Dr. Kamala Nayar,*** *for the help and timely advices rendered during the research work.*

I wish to extend my sincere gratitude to ***Dr. Vijayaraghavakumar,*** *Professor and Head, Department of Agricultural Statistics, for the timely advice and statistical interpretation of the experiment data. I thankfully acknowledge* ***Dr. P. Babu Mathew,*** *Professor and Head, Instructional Farm, Vellayani for the critical suggestions and help rendered during my study period.*

I express my sincere thanks to the teaching and non-teaching staff of Department of Plant Pathology for their sincere cooperation and kindly approach and inspiration offered during the study period.

I express my thanks and whole hearted cheers to my batch mates ***'Aparna, Brinda, Chinnu and Ananthu*** *for their help, love, encouragement and support which made my days more colourful. It's my pleasure to express my special thanks to my seniors* ***Radhika mam, Safeer chettan, Parvathy chechi, Theresa chechi, Sumi chechi, Amjada chechi and Gireesh chettan*** *for their valuable advices and support throughout the study period. I express my thanks to my juniors* ***Naveen, Nisha, Geethu, Madhukiran, Anjana, Agnes*** *for their help and support.*

I express my thanks to ***Sreeja chechi*** *and* ***Anju chechi*** *for their help, valuable advices and support.I express my thanks to all the staffs and labourers of IFSRS, Karamana for their help and support throughout the research period.*

Words are inadequate to express my thanks to ***Nyshanth, Revathy, Sajin, Jefy and Krishnaraj*** *for their constant support and care during the thesis work.*

I am beholden beyond words to express my indebtedness to my ***Achan, Amma and Rahul*** *for their unconditional love, sacrifices and support bestowed on me during my hard periods.*

Karthika S. R.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1	Composition of media used	I
2	Composition of stains used	II
3	Molecular identification report	III

LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
^0C	Degree Celsius
%	Per cent
CD	Critical difference
cm	Centimeter
cm^2	Cenitmeter square
CRD	Completely Randomised Design
DAS	Days after sowing
DAT	Days after transplanting
DAI	Days after Inoculation
DNA	Deoxy ribo Nucleic Acid
et al.	And other co-workers
Fig.	Figure
g	Gram
$g\ L^{-1}$	Gram Per litre
g/plant	Gram per plant
h	Hours
ha	Hectare
ha^{-1}	Per hectare
t/ha	Tonnes per hectare
kg/ha	Kilogram per hectare
ITS	Internal Transcribed Space

KAU	Kerala Agricultural University
kg	Kilogram
kg/cm^2	Kilogram per centimetre square
L	Litre
L^{-1}	Per litre
mm	Millimeter
µ	Micro
M	Molar
m*M*	Milli molar
µL	Microlitre
µg/ml	Microgram per millilitre
µg/L	Microgram per litre
mg	Milligram
mg/kg	Milligram per kilogram
mL^{-1}	Per millilitre
ml/L	Millilitre per litre
mm	Millimetre
min	Minutes
NS	Non -significant
No.	Number

Plant^{-1}	Per Plant
ppm	Parts per million
PCR	Polymerase Chain Reaction
RLH	Relative Lesion Height
rDNA	Ribosomal DNA
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely

INTRODUCTION

1. INTRODUCTION

Rice is the staple food of more than 60 per cent of the world's population and occupies 11 per cent of the world's cropped area (Sireesha, 2013). Rice cultivation is often subjected to a variety of biotic and abiotic stresses of which sheath blight disease caused by *Rhizoctonia solani* Kuhn is prominent. Yield loss ranging from 5.5 to 50 per cent is reported due to the disease (Zheng *et al.*, 2013; Bhunkal *et al.*, 2015). In the global scenario, sheath blight is ranked as the second most important disease of rice (Lee and Rush, 1983). In India, the disease was first reported by Paracer and Chahal (1963) from Gurudaspur in Punjab. It is now considered to be a major constraint all over India. In Kerala, rice cultivation is extended over an area of 1.98 lakh ha with a production of 5.6 lakh MT and productivity of 2.8 T/ha (FIB, 2017). The incidence of sheath blight was first reported in Kerala in 1969 at Central Rice Research Station, Pattambi and it was Prabhath (1971) who first recorded the disease in the state (Das, 1986).

R. solani is a competitive saprophyte with a wide host range and worldwide distribution (Sneh *et al.,* 1996). It causes severe damage to field and horticultural crops, worldwide. Thus, the wide host range of the pathogen, high pathogenic diversity and viability of the sclerotia in the soil for several years severely hinder the management of the disease (Taheri and Hofte, 2007). Sheath blight management centers on the use of resistant cultivars, cultural practices, fungicides and bio-control methods. However, no resistant cultivar of rice against sheath blight disease has been developed till date. Fungicides are the most commonly used tool for its management. However, there is considerable pressure to reduce the emphasis on chemical control (Singh *et al.*, 2015), since they result in many adverse effects like residual toxicity, resistance development by pathogens, environmental pollution and human health hazards (Pal *et al.*, 2011).

Attempt for the development of novel plant protectants which interfere with the fungal pathogenicity is relevant from this angle (Srivastava and Singh, 2011). The use of natural products with antifungal potential could serve as an

alternative to chemical fungicides. Great emphasis is being laid on the adoption of safe and ecofriendly methods for crop health and yield. Substitution of synthetic pesticides by indigenous organic preparations or botanicals can greatly affect the host resistance, the balance of pathogens and beneficial microbes in the soil as well as the micronutrient supplies (van Bruggen *et al.*, 2015).

Thus, the present study entitled "Organic strategy for the management of sheath blight disease of rice" was taken up to develop an ecofriendly and safe management strategy for sheath blight disease of rice with the following objectives

- Collection of *R. solani* isolates
- Pathogenicity studies
- Virulence rating
- Characterization of the pathogen
- Identification of the most virulent *R. solani* isolate
- *In vitro* and *in vivo* management studies

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Sheath blight disease of rice caused by the fungus, *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a destructive disease resulting in significant yield loss and quality degradation (Lee and Rush, 1983; Singh *et al*, 2003). Each year, 50 per cent decrease in the crop yield due to the disease has been reported under favourable conditions, worldwide (Zheng *et al*., 2013). Hence the disease and its management have to be addressed. Thus, the present study entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken with an objective to develop an ecofriendly and safe management strategy for sheath blight disease of rice. As part of this strategy development, the information collected regarding the importance of sheath blight disease, yield loss, etiology, symptomatology, characterization of the pathogen as well as its ecofriendly management have been reviewed and detailed in this chapter.

2.1 IMPORTANCE, YIELD LOSS, SYMPTOMATOLOGY AND ETIOLOGY

2.1.1 Importance and Yield Loss

Sheath blight is a major constraint adversely affecting rice production worldwide (Ou, 1985). The disease is present in almost all the rice growing tracts of the world (Dasgupta, 1992). In the global scenario, sheath blight is ranked as the second most important disease of rice (Lee and Rush, 1983). Khan and Sinha (2006) also reported that sheath blight is a major threat in rice cultivation.

Sheath blight disease was first reported in the world in Japan in 1910 (Lee and Rush, 1983). The first report of the disease in India was from Gurudaspur in Punjab (Paracer and Chahal, 1963). In Kerala, sheath blight incidence was first recorded in 1969 by Prabhath (1971) at Central Rice Research Institute, Pattambi (Das, 1986).

A yield loss of 54.3 per cent was estimated to be caused due to the disease (Rajan, 1987; Roy, 1993). Yield losses to the tune of 69 per cent in grain yield under favourable environmental conditions were reported by Sivalingam *et al.* (2006). In the absence of plant protection, the disease is reported to result in 10 to

30 per cent yield loss (Xie *et al.*, 2008). Depending upon the crop stage during the infection, the disease severity as well as the prevailing environment, a yield loss of 4 to 50 per cent has been estimated (Singh *et al.*, 2004; Bhunkal *et al.*, 2015).

2.1.2 Symptomatology

Sheath blight disease infects the crop at any stage of growth from seedling to flowering (Acharya, 1997 and Kumar *et al.*, 2009).

The symptoms first appear on the leaf sheath near the water level. Under humid conditions, the infection may spread to upper leaf sheaths and leaf blades leading to rotting of the leaf sheath and drying up of the whole leaves (Dasgupta, 1992). In severe cases, most of the leaves in the plants may get blighted. Heavily infected plants will die with poorly filled grains. During panicle initiation or flowering, the disease results in lower percentage of filled spikelets and significant yield losses (Nagarajkumar *et al.*, 2004).

The disease symptoms initially appear as circular, oblong or ellipsoid, greenish grey, water soaked lesions on the leaf sheath near water level (Ou, 1972). The lesions enlarge, become oblong and irregular in outline (Webster and Gunnel, 1992). Initial symptoms consist of lesions on the lower leaf sheaths during the late tillering or early internodal elongation growth stages (Rush and Lee, 1992). Later, the centre of the lesions turns greyish white with brown margins. Several lesions will be produced on entire tillers from the water line to the flag leaf (Vidyasekharan *et al.*, 1997) and may unite to encircle the whole culm resulting in complete death of the plants.

2.1.3 Etiology

Rice sheath blight is caused by a plant pathogenic fungus *viz.*, *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk). The pathogen has a wide host range as well as worldwide distribution (Sneh *et al.*, 1996). The fungus is known to cause severe damages to field and horticultural crops, worldwide. Webster and Webster (2007) classified the teleomorph of the fungus to be under the kingdom Fungi; subkingdom Eumycota; phylum

Basidiomycota; class Heterobasidiomycetes; order Ceratobasidiales and family Ceratobasidiaceae.

The fungus is seed borne in nature. Muller (1924) observed that the resting structure of the fungus, *viz.*, sclerotia remained viable in the soil for more than eighteen months. Palo (1926) also reported that the sclerotia survive in the soil for several months. Sclerotia are thus considered to be the most important source of inoculum of *R. solani* (Allison, 1951). Kobayashi *et al.* (1997) reported that the sheath blight fungus survives from one crop season to another through the sclerotia and mycelia in plant debris and also through weed hosts under tropical environmental conditions. Sivalingam *et al.* (2006) reported that the fungus gets transmitted from seeds to seedlings in the form of brownish black to blackish lesions on the coleoptile, radicle, leaf and sheath. Kumar *et al.*, (2009) studied that mostly the sheath blight fungus survives through its resting structures, *viz.*, sclerotia in the field which infect the crop during the next season.

Hu *et al.* (2010) found that among the 186 *Rhizoctonia* like fungal strains isolated in China, *R. solani* was the most virulent and common species. Lore *et al.* (2015) observed that sheath blight can be caused by *R. solani* and *R. oryzae sativae* occurring singly or in combination with each other in different parts of India, with *R. solani* being the dominant species. The mixed inoculation of *Rhizoctonia* species aggravates the disease complex (Lore *et al.*, 2015). Thus, besides *R. solani,* two other species of *Rhizoctonia viz.*, *R. oryzae* and *R. oryzae sativae* have been reported to be associated with the sheath blight complex (Singh *et al.*, 2016).

2.1.3.1 Pathogenicity Studies

The procedure for the artificial inoculation of sheath blight pathogen on the leaf sheath has been studied and described in detail by Singh *et al.* (2003).

R. solani successfully colonized on typha pieces (Bhaktavalsalam *et al.*, 1978). Maximum infection and yield loss due to sheath blight incidence was observed in the plants at active tillering, less at early tillering and the least at booting stages (Tiwari and Choure, 1997). Hollier *et al.* (2009) reported that sheath blight appears at tillering stage. Lore *et al.* (2009) reported that the

maximum disease severity and incidence was observed at booting stage indicating that this was the most susceptible stage of rice under pot and field conditions, followed by tillering stage. The disease severity and incidence was the lowest at seedling and grain filling stages under both field and pot culture conditions (Lore *et al.*, 2009).

Susheela (2012) reported that rice plants of the susceptible variety IR 50 were inoculated at the maximum tillering stage with *R. solani* multiplied on typha pieces to reproduce the sheath blight symptoms. Adipathi *et al.* (2013) reported that the isolates of *R. solani* induced typical rice sheath blight symptoms of ellipsoidal shape with greenish grey centre and dark brown margin upon artificial inoculation. Moni *et al.* (2016) conducted artificial inoculation at the maximum tillering stage using the mycelial block of five days old culture and the symptom development was observed on five days after inoculation under field conditions. They also reported that artificial inoculation of *R. solani* isolates in the rice cultivar, Purbachi induced typical symptoms of ellipsoid, dark brown lesions.

2.1.3.2 *Virulence Rating*

The diversity of various isolates of *R. solani* can be assessed based on the pathogenicity testing and virulence diversity (Banniza *et al.*, 1996). The virulence pattern of the pathogen will help in the evaluation of pathogenic races and to identify disease susceptibility and resistant genotypes (Adhipathi *et al.*, 2013).

Adipathi *et al.,* (2013) reported that among the twelve isolates studied, the isolate D-14 was the most virulent and the isolates *viz.*, A-4, A-7 and A-10 were less virulent. Similar kind of virulence pattern of *R. solani* isolates were observed and reported by Singh *et al.* (2001) and Kumar *et al.* (2008).

2.2 CHARACTERIZATION AND IDENTIFICATION OF PATHOGEN

The isolates of *R. solani* are genetically diverse in their cultural, morphological and physiological characteristics as well as in their pathogenic range of host plants (Kuninga *et al.*, 1997; Gonzalez *et al.*, 2006). Sunder *et al.* (2003) reported that the colony colour of *R. solani* ranged from brown, light brown to yellowish brown. The brown pigment appears to be a stable diagnostic character of *R. solani*. Lal and Kandhari (2009) conducted variability studies in

several isolates of *R. solani* and found six isolates as light brown, five isolates as yellowish brown, four isolates as whitish brown, six isolates as dark brown and four isolates as very pale brown. Debbarma and Dutta (2015) reported that the colony colour and mycelial growth of six isolates of *R. solani* showed great diversity. The isolates were assigned into four groups based on the colony pigmentation as white, yellow, grey and orange.

Taheri *et al.* (2007) reported that among the 110 rice isolates, 96 isolates belonging to AG-1 IA produced dark brown mycelium on PDA with barrel shaped moniliod cells. Eleven isolates produced white colonies, which later turned to pale brown within three weeks. Kaur and Kaur (2013) studied the morphological and molecular variability in 22 *R. solani* isolates and reported that the mycelium showed aerial or appressed growth and were light brown to dark brown in colour. They also studied the type of sclerotia and reported that they varied from minute to large. One isolate from rice produced large (94 mm) sclerotia, ten isolates produced sclerotia up to 2 mm and eleven isolates produced sclerotia up to 3 mm size. Out of the 22 isolates, 11 isolates produced sclerotia in a scattered manner. Round to oval sclerotia with flattened bottom were found in most of the isolates (Moni *et al.* 2016). Singh *et al.* (2015) revealed that all the 25 *R. solani* isolates showed great variation in the time taken for initiation of sclerotia, ranging from 3 to 6 days. They found that the sclerotia of 8 isolates were formed towards the centre and 7 isolates in scattered pattern in PDA media.

Parmeter and Whitney (1970) attempted to organize and group the isolates of *R. solani* based on morphological, physiological and pathological characteristics. Sneh *et al.* (1991) reported that the mycelial branching at right angles is a known feature of *R. solani*. They also reported that the isolates of *R. solani* had hyphal branching at right angle, constriction at the point of branching of the mycelium and the presence of a septum near the branching junction which are all of immense taxonomical importance. Although the morphology and physiology based classification is a standard and useful technique, it is laborious and time consuming. DNA based analysis has been attempted to understand the genetic relatedness among the different *R. solani* species (Sharma *et al.*, 2005).

Based on the sclerotial size, shape and DNA based sequence homology, *R. solani* AG isolates are sub divided in to IA, IB and IC (Sneh *et al.*, 1991). The isolates of *R. solani* identified as AG1-IA were more virulent on grasses than other AG groups of *R. solani* and other *Rhizoctonia* species (Tsukibushi and Kimigafukauro, 1992). The classification of AG-1 IA is supported by morphological characterization, analysis of rDNA ITS sequences (Johanson *et al.,* 1998; Gillemaut *et al.,* 2003; and Taheri *et al.*, 2007). The predominant causal agent of rice sheath blight is AG-1 IA sub group and has been reported from rice growing regions worldwide (Taheri *et al.*, 2007; Bernardes-De-Assis *et al.*, 2009; Gonzalez-vera *et al.*, 2010). Among the 14 anastomosis group of *R. solani*, sheath blight pathogen is placed in AG-1 IA (Gonzalez-vera *et al.*, 2010).

2.3 *IN VITRO* MANAGEMENT OF THE PATHOGEN

In the absence of plant protection, sheath blight can result in 10 to 30 per cent yield loss and may reach up to 50 per cent (Xie *et al.*, 2008). Sheath blight pathogen is reported to have a very wide host range and has considerable pathogenic and molecular variability which makes its management a difficult task (Singh *et al.*, 2016).

Use of resistant varieties is the most effective and safe method for the management of the disease. However, until today no known rice variety exists, which is either immune or having a high degree of resistance to the disease. No appreciable amount of resistance was observed in any of the existing rice varieties in India (Adipathi et al., 2013). Chemical control is the most widely adopted management strategy for the disease (Dev and Mary, 1986). However, the indiscriminate and disproportionate use of pesticides may lead to their residues in food chain and may exert harmful effects in human beings and other life forms (Chahal *et al.*, 2016). Excessive use of fungicides for a very long time has resulted in resistance development in pathogen and a negative impact on both human health and environment (Zhang *et al.*, 2016). Hence, replacement of synthetic fungicides by natural products which are non- toxic and specific in their action is of importance in the present context.

2.3.1 Organic Preparations

Natural fungicides are cost effective and easily accessible. Egg has been considered to be a rich source of many minerals, proteins, fats, essential vitamins, and omega-3 fatty acids. Zambrowicz *et al.* (2012) reported that many of the proteins present in egg such as lysozyme, ovotransferin, ovomucin, flavoproetien, avidin *etc.* have been known to possess antimicrobial properties. Several biological activities have been associated with the egg protein derived peptides including antihypertensive, antimicrobial, immuno modulatory, anticancer and antioxidant activities highlighting the importance of these bio peptides in human health and disease prevention as well as treatment (Zambrowicz *et al.*, 2012). Proteins such as lysozyme, ovotransferin and avidin have proven to exert numerous biological activities (Abdou *et al.,* 2013). Specific immuno globulin in the yolk (IgY) have been found to be effective against many bacterial and viral infections (Abdou *et al.,* 2013). Recently, products based on the natural components as an ecofriendly method for combating phytopathogenic agents is an area of interest (Rodino *et al.*, 2014).

Citrus fruit is known for its medicinal properties due to the presence of flavanoids, flavanones and other compounds which are rare in other plants (Burt, 2004). Citrus flavonoids have a broad spectrum of antibacterial, antifungal, antidiabetic, anticancerous and antiviral activities (Ortuno *et al.*, 2006). They appear to play a defensive role against the invading pathogens, including bacteria, fungi and viruses in plants. The juice of ripe as well as unripe citrus fruits could inhibit *Pseudomonas aeriginosa, Staphyllococcus aureus*, *Bacillus subtilis* and *E. coli* (Sarmah and Kumari, 2013). The extract of *Citrus lemon* exhibited the highest antimicrobial activity against six gram positive and eight gram negative bacteria (Hindi and Chabuck, 2013).

Webber *et al.* (1999) opined that alternative uses of the weed species should be considered before expensive eradications are undertaken. Dold and Cocks (2000) reported that new bio medical products from problem plants and weeds are being discovered. Hostettman *et al.* (2000) reported that many plant species contain potential renewable source of antifungal agents and that invasive

or weedy species may be a useful source for developing antifungal products useful on commercial scale. Eloff *et al.* (2007) elaborated that invasive plant species have increased resistance to fungal pathogens which help them to thrive under conditions where most other species fail. Requirement of sufficiently large quantity of raw materials is the major drawback in developing antifungal products from plants (Mdee *et al.,* 2009). Weeds may therefore become a readily available source of raw materials for plant based fungicidal agents (Aderogba *et al.*, 2014). Exploration of the antifungal activity of weeds remains an area of interest, but not many reports are available on the exploitation of the antifungal property of weed plants. The data regarding the use of weeds as antifungal agents are scanty.

Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non-phytotoxic, unlike chemical fungicides (Alam *et al.*, 2002). If the antifungal property resides in weeds, that will be an added advantage. Rodino *et al.* (2014) reported that 10 per cent ethanolic extract of the weed, *Xanthium strumarium* inhibited the mycelial growth (86.7%) of *Alternaria alternata*. According to Aderogba *et al.* (2014), invasive and weedy species may serve as a source of biologically active extracts or compounds with application in plant protection. They reported that the acetone crude extract of *Pseudognaphalium luteoalbum* had strong antifungal activity against *Aspergillus niger, Aspergillus parasiticus, Colletotrichum gloeosporioides, Fusarium oxysporum, Penicillium expansum, Penicillium janthinellium, Phytophthora nicotiana, Pythium ultimum and Trichoderma harzianum.* Two antifungal compounds, *viz.*, hispidulin-7-o-glucopyranoside and stigmastero-3-o-beta-glucopyranoside were isolated from the weedy species.

Prasad and Niza (2014) reported that jeevamrutha at 10 per cent concentration showed maximum (85.18 %) inhibition of the mycelial growth of *R. solani* which was on par with jeevamrutha 20 per cent (79.62 %) during *in vitro* studies.

2.3.2 Botanicals

Natural products from many plants are known to control many plant pathogens (Khan *et al.*, 1979). Vijayan (1989) reported that garlic bulb extract

inhibited the spore germination and mycelial growth of *Alternaria solani*. Hippe (1991) reported that the accumulation of lipid bodies, thickening of cell walls and undulations of plasmalemma of the cells caused by garlic extract are similar to those produced by some synthetic fungicides. Ten ml of the garlic bulb extract caused physiological alterations of the hypha of *Pythium ultimum* that appeared collapsed. 100 ml of garlic extract resulted in serious damage of the mycelium of *R. solani, Colletotrichum lindemuthianum*, and *F. solani* appeared fragmented under Standard Electron Microscope. When observed under Transmission Electron Microscope, 10 and 100 ml/L aqueous extract concentration caused alterations in the cell structure of *R. solani, P. ultimum* and *C. lindemuthianum.* Increase in the number and size of the vacuoles was the most frequent modification observed. *R. solani* showed a marked thickening in the cell walls up to twice the size of control (Bianchi *et al.*, 1997).

The *Allium sativum* extracts caused inhibition of *Curvularia penneseti* in pearl millet (Singh, 2008). 10 per cent clove extract of *Allium sativum* was reported to be the most effective treatment in suppressing the mycelial growth of seed borne fungi of green gram, *viz.*, *Fusarium oxysporum* (62.8%), *Aspergillus niger* (62.1%) and *R. solani* (61.2%) (Swami and Alane, 2013). Ravi *et al.* (2014) reported that garlic extract at 15 per cent concentration caused 78.89 per cent inhibition of mycelial growth of *R. solani* inciting leaf blight of *Andrographis paniculata.* Srinivas *et al.* (2014) reported that among the 13 plant extracts evaluated *in vitro* against *R. solani*, garlic extract (10%) was the most effective. Sugha (2005) reported that panchagavya was found effective against *R. solani* (95.31%), *F. solani* (98.96%), *F. oxysporum* f. sp. *capsici* (92.89%) and *Colletotrichum capsici* (97.91%) at 10 per cent concentration. Panchagavya resulted in 86.3 per cent inhibition of the mycelial growth and 95.9 per cent inhibition of spore germination of *Curvularia lunata in vitro* (Sumangala and Patel, 2009). Joseph and Sankarganesh (2011) reported that 1000 µL dilution of panchagavya showed 100 per cent antifungal activity against *F. oxysporum, F. solani* and *Rhizopus oligosporus.* Adhao (2013) reported that under *in vitro* conditions four per cent panchagavya suppressed the mycelial growth of

Fusarium oxysporum. Anees (2014) reported that panchagavya at 2.5, 5 and 10 per cent concentrations completely inhibited the mycelial growth of *Pythium aphanidermatum*. Panchagavya was tested against the major pathogens of bell pepper and was found most effective against *Sclerotinia sclerotiorum* with 100 per cent inhibition followed by *Sclerotium rolfsii* (99.73%) and *Phytophthora nicotiana* (98.71%) at all the tested concentrations (Ashlesha and Paul, 2014).

The botanicals *viz*., *Chromolaena odorata* and *Ocimum sanctum* as well as the neem based formulation, nimbicidine were found to be effective against sheath blight disease (Saifunneesa and Niza, 2001). Cow urine at 100 per cent concentration resulted in 87.53 per cent reduction in mycelial growth of *R. solani* causing black scurf of potato (Sirari *et al*., 2015). The mycelial growth of *Rhizoctonia solani* causing leaf blight disease of amaranthus was completely inhibited *in vitro* by fermented egg - lemon juice extract as well as lime solution, followed by fermented tapioca - rind extract, fermented weed (*Setaria barbata*) extract, fermented papaya leaf extract, fish amino acid as well as turmeric powder – baking soda mixture (Sajeena *et al*., 2015).

Neem oil produced maximum inhibition zone of 7.2 mm at 20 µg/ ml against *Ralstonia solanacearum* (Pankaj *et al.,* 2015). Turmeric powder and baking soda (10:1) combination inhibited the maximum growth of *R. solani*, causing the leaf blight of amaranthus by 64.40 percentage (Gireesh and Radhakrishnan, 2016). They also reported that fish amino acid at 5 percentage concentration resulted in 29 percent inhibition of the mycelial growth of *R. solani* causing leaf blight of amaranthus.

2.3.3 Non-hazardous chemicals

Data on the inhibitory effect of calcium carbonate on the mycelial growth of fungal pathogens is scanty. The calcium content of nutrient solution influenced infection as well as club root development and was influenced by pH (Myers and Campbell, 1985). Incubation of the resting spores with 1 M calcium chloride reduced the spore viability. Calcium carbonate (250 µg/ml) nano particles showed good antibacterial effect and after 16 hours, the *Agrobacterium tumefaciens* totally diminished (Ataee *et al*., 2011).

Silicon has been reported to increase the disease resistance of monocot and dicot plants. Negative correlations between the silicon content of plant tissues and disease severity have been reported for blast and sheath blight of rice (Mathai *et al.*, 1978; Aleshin *et al.*, 1986), powdery mildew disease of barley (Jiang *et al.*, 1989), wheat (Leusch and Buchenauer, 1989) and cucumber (Menzies *et al.*, 1991). Biggs *et al.* (1997) reported 65 per cent growth reduction of *Monilinia fructicola*, the causal fungus of brown rot of peach on PDA amended with calcium silicate. According to Kaiser *et al.* (2005) all concentrations from 5 to 80 ml/L PDA of soluble silicon completely suppressed *Colletotrichum coccodes, Mucor pusillus, Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Phytophthora cinnamomi*. Bekker *et al.* (2009) reported that soluble potassium silicate inhibited the mycelial growth of *Phytophthora cinnamomi* and *P. capsici* at all the concentrations (20 – 80 ml/L) tested. He proposed that silicon may act as the first protective barrier in silicon treated plants and inhibit pathogen colonization and infection by inhibiting the fungal growth on plant surface.

2.3.4 Soil amendments

Geogy and Rajan (1990) reported that the soil amendments *viz*, neem cake and lime reduced the disease severity and increased the grain and straw yield. FYM, Glyricidia, neem cake, *T. harzianum*, beejamrutha, neem seed kernel extract and *Pseudomonas fluorescens* have been reported to be very effective against sheath blight (Sridhar and Patil, 2009). Neem cake was most effective in reducing the sheath blight incidence from 77.5 to 27.5 per cent and the disease severity from 23.75 to 2.55 per cent (Senapoty, 2010). 5 per cent concentration of neem oil cake caused 20.41 per cent inhibition of the mycelial growth of *R. solani* (Lenka and Pun, 2014).

2.4 INHIBITION OF SCLEROTIA OF PATHOGEN

Palo (1926) reported the survival of sclerotia of the sheath blight fungus for several months in the soil. Sclerotia are considered to be the most important source of inoculum of *R. solani* (Allison, 1951). Myers and Campbell (1985) reported that 1 M calcium chloride reduced the spore viability of club root pathogen. Calcium chloride and calcium hydroxide at 1.5 and 2 per cent decreased

the spore germination and germ tube growth *of Colletotrichum acutatum, C. gloeosporioides, Alternaria alternate* and *Penicillium* (Stosic *et al.*, 2014).

Garlic extract at 10 per cent concentration showed total inhibition of sclerotial production of the sheath blight pathogen, *R. solani* (Dutta *et al.*, 2004). Production of conidiophores and oospores by *Hyaloperenospora parasitica*, the causal agent of downy mildew of *Arabdopsis* was inhibited by garlic juice (Curtis *et al.,* 2004). Portz *et al.* (2008) found that garlic extract inhibited the germination of sporangia and cysts and germ tube growth by *Phytophthora infestans* both *in vitro* and *in vivo* on the leaf surface of cucumber. The disease severity was reduced by 45 to 100 per cent on tomato seedlings when sprayed with garlic juice containing allicin at 55- 100 µg/L. The survival of the sheath blight fungus is through the sclerotia dropped in fields during harvest, which will infect the next season crop (Kumar *et al.*, 2009). Panchagavya at 5 per cent concentration resulted in 95.9 per cent inhibition of the spore germination of *Curvularia lunata in vitro* (Sumangala and Patel, 2009).

2.5 *IN VIVO* MANAGEMENT OF THE DISEASE

2.5.1 Organic Preparations

The development of resistance to common fungicides and increasing restrictions on the use of toxic materials in the environment has given an impetus to the search for novel plant protectants that interfere with the fungal pathogenicity (Srivastava and Singh, 2011). No scientific reports are there regarding the plant disease management by foliar application of fermented egg-lemon juice extract. Few reports are available regarding plant disease management using the extracts of weeds. Foliar spraying with the methanol extract of *Datura metel* was controlled rice sheath blight through direct antimicrobial effect of this extract as well as by induced resistance (Kagale *et al.*, 2004). Khoa *et al.* (2011) reported that under controlled and semi field conditions, foliar spray and seed soaking application of either fresh or dried leaf extract of the weed, *Chromalaena odorata* (10%) gave up to 68 per cent reduction in sheath blight lesion length.

Patil (2009) reported that 3 sprays of vermiwash at 50 per cent and panchagavya at 3 per cent were the most effective indigenous technology knowledge for controlling soyabean rust and for increasing grain yield. Anuja (2010) opined that the application of panchagavya could suppress the foliar blight of amaranthus caused by *R. solani* by 47.29 per cent. Chadha *et al.* (2012) concluded that the application of panchagavya, compost tea and jeevamruth were effective in enhancing the productivity of the crop as well as in suppressing the growth of various pathogens. Panchagavya (10%) suppressed *Choanephora* pod rot of cowpea by 71.9 percentage (George, 2015).

2.5.2 Botanicals

Portz *et al.* (2008) found that garlic extract reduced the disease severity by 45 to 100 per cent on tomato seedlings infested by *Phytophthora infestans* when sprayed with garlic juice containing allicin (55 to 100 microgram per L). The leaf extract (1% and 5%) of *Allium sativum* was effective treatment in reducing early blight disease severity (20.8% and 15.3% respectively) caused by *Alternaria solani* under greenhouse conditions (Sallam, 2011). The disease severity of early blight was greatly reduced by Metalaxyl-M (2g/L) followed by *Allium sativum* (5%) in field conditions by 57.6 percentage. Arzoo *et al.* (2012) reported that the minimum disease intensity (8.93%) was reported in garlic extract (10%) treated plants against *Fusarium* wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*.

2.5.3 Non-hazardous chemicals

Field and green house trials have shown that calcium or magnesium in lime may affect disease development independent of pH (Fletcher *et al.*, 1982). Liming is considered to be one of the most widely used control measures for club root of crucifers caused by *Plasmodiophora brassicae* (Myers and Campbell, 1985). Liming raises the soil pH to 7.2 or above for adequate control. Myers and Campbell (1985) proposed that calcium and a high pH may have little fungicidal effect on resting spores, but may affect the pathogen penetration or development in the host. Soils treated with calcium carbonate particles significantly lowered the population of *Ralstonia solanacearum* (bacterial wilt of tobacco) nearly 100

times and after 60 days, the disease incidence was less than one per cent (He *et al.*, 2014).

Foliar application of silicon to field plants is a potentially viable alternative to root zone application (Menzies *et al.*, 1992). Inanaga *et al.* (1995) reported that silicic acid competes with calcium for binding sites on the cell wall after forming complexes with polyhydroalcohols, organic acids, phenol carbohydrates and lignin. Sun *et al.* (2002) reported reduction in the incidence of anthracnose disease in cucumber caused by *Colletotrichum orbiculare* by the application of silica. Studies conducted in cucumber leaves investigating the process of infection in plants showed that resistance to infection can be acquired by the expression of a protein rich in proline together with the presence of silica at the site of pathogen penetration (Kauss *et al.*, 2003). An average of 63 per cent reduction of angular leaf spot of bean by the application of potassium silicate was reported by Moraes *et al.* (2005). Silicon amendments proved to be effective in controlling both soil borne and foliar fungal diseases in cucumber, rice, sugarcane, turf and several other plant species (Datnoff *et al.*, 2007). Silicon-enhanced disease resistance is hypothesized in two ways *viz.*, the accumulated silicon on tissues act as a physical barrier and prevents penetration thereby making the cells less susceptible to enzymatic degradation by fungal pathogens (Heine *et al.*, 2007) and that it acts as a signal that induces different chemical defenses against pathogens.

Polanco *et al.* (2014) reported that foliar application of potassium silicate resulted in the control of anthracnose in common bean, probably due to the formation of a physical barrier as a result of silicon deposition on leaf surface or due to the osmotic effect of silicate sprayed on the leaf and thereby, the disease severity was reduced by 41 percentage by the foliar application of potassium silicate. Liquid potassium silicate at 200 mg silicon/kg treatment resulted in highly significant disease suppression of 37 to 53 per cent reduction of downy mildew in bitter gourd caused by *Pseudoperonospora cubensis* (Ratnayake *et al.*, 2016). Devi and Nayar (2016) reported that snake gourd plants sprayed with 0.5

per cent potassium silicate had low incidence (10.71%) and disease severity (0.71%) of anthracnose and recorded 89.12 percentage disease suppression.

2.6 EFFECT OF THE TREATMENTS ON BIOMETRIC AND YIELD ATTRIBUTES OF RICE

Wallingford (1980) mentioned that potassium is involved in the activation of more than 60 enzymes necessary for energy utilization, starch synthesis, nitrogen metabolism and respiration. Four sprays of panchagavya at three per cent and moringa leaf extract spray at 25 ml/plant resulted in higher plant height, number of branches per plant in tomato. Potassium is needed for stimulating plasma lemma ATPase that produces the necessary conditions for the metabolites like sucrose and amino acids (Barker and Pilbeam, 2007). Rathore *et al.* (2009) reported that foliar application of sea weed extract significantly enhanced the yield parameters of soyabean.

The fruit characters of sapota like fruit weight (99.6g), fruit length (5.55cm), fruit diameter (5.58cm), volume (102.38cm^3) and maximum shelf life (10.9 days) were recorded in treatments with foliar application of potassium silicate @ 8ml per litre (Lalithya *et al.*, 2014). The highest grain yield in transplanted rice was recorded in the treatment with application of 15 percentage sap of *Kappaphykus* (a sea weed) (in 6.55 tons) together with the recommended dose of fertilizers (RDF) followed by 15 percentage sap of *Gracilaria* together with the RDF (6.25 tons) which resulted in 41.47 and 34.99 percent increase respectively (Pramanick *et al.* (2014). Ratnayake *et al.* (2016) reported that the growth parameters such as number of leaves, flowers and fruits per plant were significantly higher in silicon treated plants at 200 ppm.

2.7 CHEMICAL ANALYSIS

Fermented liquid organic manures contain plant growth promoting substances nutrients and microbial load that help in improving plant growth, metabolic activities and resistance to pest and diseases (Gore and Sreenivasa, 2011). They analyzed the pH and the nutrient status of organic liquid manures and found that panchagavya contained the maximum total nitrogen (1000 ppm), phosphorous (175.40 ppm) and potassium (194.1 ppm) with a near neutral pH of

6.82. They reported that there was significant improvement in growth and yield with the combined application of liquid organic manures and RDF as compared to RDF alone.

In addition to mineral fertilization, bio-stimulants can enhance the effectiveness of fertilizers as well as nutrient utilization from soil (Frankenberger and Arshad, 1995). Rathore *et al.* (2009) reported that improved nutrient uptake of N, P, K and S was observed with the application of sea weed extract at 12.5 and 15 percentage in soyabean. Growth enhancing potential of the sea weed extract might be due to the presence of macro and micro nutrients. The highest value of total potassium content in wheat leaves were noticed by potassium silicate at 400 ppm (Salim *et al.*, 2011; Salim *et al.*, 2013). They also proposed that the increment in the percentage of potassium in leaves is due to potassium silicate application rate.

All potassium treatments have strongly stimulating effect on mineral nutrients N, P, K, Mg, Zn, Mn and Fe as well as the protein concentration (Salim *et al.*, 2014). Lalithya *et al.,* (2014) observed that the nutrients like nitrogen (1.583%), phosphorus (0.175%), potassium (1.2%) and silicon (1.2%) in leaves were recorded highest with potassium silicate spray at 8ml/L. Silicon application avoided leaching loss of nitrogen and helped in more accumulation of nitrogen in leaves. Silicon rendered more phosphorous availability to the plants reversing its fixation as silicon itself competed for phosphorous fixation and the slowly released phosphorous helped in more accumulation of P content in the leaf. Pramanick *et al.* (2014) studied that the use of sea weed extract increased N, P and K uptake significantly by grains at higher concentrations and was the maximum at 15 percentage of sea weed extract. The saps also enhanced the nutrient uptake by the crop.

The perusal of the literature clearly indicated that not much work have been taken up regarding the antifungal potential or the mode of action of potassium silicate or fermented weed extract or fermented egg-lemon juice extract against *R. solani* causing sheath blight of rice.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present work entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken during 2015-2017. The laboratory experiments were conducted at Integrated Farming System Research Station (IFSRS), Karamana and the pot culture experiment was laid out at College of Agriculture, Vellayani. Different isolates of the sheath blight fungus were collected from which the most virulent isolate was identified, which was used for the management of the disease under laboratory and pot culture conditions. The relevant details of the materials used and the experimental procedures that were followed are presented in this chapter.

3.1 ISOLATION OF SHEATH BLIGHT PATHOGEN

3.1.1 Collection of Samples for Isolation

Rice plants showing typical symptoms of sheath blight disease were collected from seven rice growing tracts of Thiruvananthapuram district to isolate the rice sheath blight fungus. A detailed study of the symptoms of the disease in the infected plants was made following standard procedures (Lee and Rush, 1983). Detailed observations such as the location from where the disease samples were collected, the stage of the crop affected, nature of the symptoms observed, presence or absence of sclerotia under natural conditions as well as the characters of the sclerotia were recorded for each of the disease affected plant samples collected from the different rice growing tracts.

3.1.2 Isolation of the Pathogen

Isolations were made from seven sheath blight samples collected from different rice growing tracts of Thiruvananthapuram following standard procedure (Rangaswami, 1958) from the sheath blight infected plant samples on potato dextrose agar (PDA) medium. Leaf sheaths showing characteristic symptoms of sheath blight were washed thoroughly and cut into small bits of 1.0 to 1.5 cm. The infected leaf sheath portions along with healthy regions were selected for the isolation of the fungus. The bits of leaf sheaths of each sample were separately surface sterilized using 0.1 per cent mercuric chloride for one min and washed in

three changes of sterile distilled water. The surface sterilized leaf bits were transferred to 90 mm sterile petri plates containing PDA medium amended with streptomycin sulphate and incubated at room temperature (28 ± 2°C). The plates were observed for mycelial growth of the fungal isolates. The fungal colonies exhibiting typical characters of the sheath blight fungus, which were consistently obtained during the isolation from the disease specimens, were transferred to PDA slants (Aneja, 2003).

3.1.3 Purification of the Isolates

The pure culture of each isolate of the fungus was obtained by hyphal tip culture method (Rangaswamy and Mahadevan, 2006) and stored at room temperature (28 ± 2°C) for conducting subsequent studies.

3.1.4 Pathogenicity Studies

Pathogenicity tests of the seven isolates obtained and maintained as in 3.1.3 were conducted at the Department of Plant Pathology, College of Agriculture, Vellayani on the rice variety Uma. Healthy rice plants were transplanted into UV stabilized 600 gauge, 150 μ grow bags (40 cm x 24 cm x 24 cm) which were filled with sandy clay loam soil (10 kg) having a pH of 5.5 with the water level maintained at five cm. Pathogenicity of each of the seven isolates of *R. solani* was tested by sheath inoculation method (Sharma and Thrimurty, 2004). Healthy rice plants at active tillering stage (45 days after sowing (DAS)) were artificially inoculated in the leaf sheath by inserting five mm mycelial bits of seven days old culture of each isolate along with sclerotia. The inoculated portion of the sheath was then covered with a thin layer of moist cotton. Rice plants inoculated with PDA culture discs alone (without the pathogen) were maintained as the control for each isolate. The artificially inoculated plants were covered with finely perforated polythene covers to maintain humidity in order to induce sheath blight symptom development. All the inoculated plants were observed daily for the appearance of typical symptoms of sheath blight. The pathogen isolates were re-isolated when the characteristic symptoms of the disease appeared on the inoculated leaf sheaths. The number of days taken for symptom

development, lesion size as well as the sclerotial formation and their characters were also recorded for the plants inoculated with each of the isolate.

3.1.5 Virulence Rating

Virulence rating was done to determine the most virulent isolate of the pathogen among the seven isolates collected from the different rice growing tracts. Virulence was assessed for each of the isolate by two methods.

3.1.5.1 ***Cut Stalk Assay Method (Pillai, 1990)***

The susceptible rice variety Uma was used for this study. Rice stalks each of 15 cm length were excised during the active tillering stage and were kept on moist cotton placed in 19.5 cm diameter petri plates. A single sclerotium along with five mm mycelial disc of seven days old culture of each fungal isolate was placed inside the sheath of each cut stalk with the help of a sterile forceps. Un-inoculated control was also maintained by inoculating the sheath with five mm PDA disc alone without any inoculum. The inoculated stalks were covered with a thin layer of moist cotton and sprinkled with water periodically to maintain humidity. Three replications were maintained for each isolate. On the seventh day after inoculation (DAI), the length of the lesions produced by each isolate on the cut stalk was recorded.

3.1.5.2. Direct Inoculation on Rice Plants (Singh et al., 2003)

Healthy rice (variety Uma) seedlings were transplanted into UV stabilized 600 gauge, 150 μ grow bags (40 cm x 24 cm x 24 cm) which were filled with sandy clay loam soil having a pH of 5.5. The water level in the soil was maintained constantly for attaining optimum growth conditions.

Each of the seven fungal isolates was artificially inoculated on potted rice plants at 45 days after transplanting. Three pots were maintained for each isolate. The plants were artificially inoculated by placing one sclerotium along with five mm mycelial bits of seven days old culture of each isolate inside the outer most sheath portion. Rice plants inoculated with PDA culture discs alone (without the pathogen) were maintained as the control for each isolate. The inoculated portion was then covered with a thin layer of moist cotton and the plants were covered using finely perforated polythene covers to maintain adequate humidity for

disease development. The plants were observed daily for symptom development until lesions were produced on the sheath. The observations on symptom development, relative lesion height (RLH) on the 5th, 7th, 10th and 15th DAI as well as the total number of disease affected tillers produced by each isolate were recorded. The RLH (Sharma *et al.*, 1990) was calculated using the formula;

RLH = (Lesion height in cm/Plant height in cm) x 100

3.1.5.3. Selection of the Most Virulent Isolate

The most virulent isolate of the pathogen was selected based on the results of both the cut stalk assay and direct inoculation method.

3.2 CHARACTERIZATION OF PATHOGEN

A comparative study of the morphological and cultural characteristics of the different isolates of *R. solani* obtained as in 3.1.3 was conducted in detail.

3.2.1 Morphological Characterization of Pathogen

The morphological characters of the pathogen were studied by preparing slides stained with cotton blue (Appendix II) and observing under 45X and 100X magnification of Leica DM 750.

3.2.2 Cultural Characterization of Pathogen

The cultural characteristics of the pathogen were studied by growing them on PDA medium and observing the mycelial characters such as nature of mycelial growth, colour of the colony, texture and sclerotial characters such as the days for pin head formation, the number and average size of sclerotia and the distribution pattern of the sclerotia of each isolate.

PDA medium (15-20 ml) was poured into 90 mm diameter sterile petri plates under aseptic condition and was allowed to solidify. Five mm discs of seven days old culture of each isolate was placed at the centre of the petri plates. The plates were incubated at room temperature till the completion of mycelial growth. Three replications were maintained for each of the isolate.

3.2.3 Tentative Identification of Pathogen

The pathogen was tentatively identified based on the morphological and cultural characteristics.

3.2.4 Confirmation of Pathogen

The pure culture of the most virulent isolate obtained as in 3.1.5.2 was submitted to the National Fungal Culture Collection of India (NFCCI), Agarkhar Research Institute, Pune for morphological and molecular identification of the pathogen.

3.2.4.1 Morphological Identification

The identification was based on morphological characters of the fungus under *in vitro* culture.

3.2.4.2 Molecular Identification

The identification was based on the ITS (Internal Transcribed Space) region of rDNA of the fungus. The genomic DNA was isolated in pure form from the culture. The ITS region of rDNA was successfully amplified using the fungal universal primers ITS4 & ITS5. The sequencing PCR was set up with ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit. The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency. The sequence data was aligned with publicly available sequences and analyzed to reach the identity (Altshul *et al.,* 1990).

3.3 *IN VITRO* PATHOGEN SUPRESSION

Indigenous organic preparations, botanicals, non-hazardous chemicals and soil amendments were tested for their potential in inhibiting the mycelial growth of *R. solani* under *in vitro* conditions at IFSRS, Karamana by poisoned food technique (Nene and Thapliyal, 1979) as follows,

Design : Completely Randomized Design (CRD)
Replications : Three
Treatments : 20

Table 1. Details of treatments selected for *in vitro* studies

Treatment	Description
T_1	Fermented tapioca (*Manihot esculenta* L.) leaf and rind (1:1) extract in cow's urine diluted in water (1:5)
T_2	Fermented papaya *Carica papaya* L.) leaf in cow's urine diluted in water (1:1:5)
T_3	Fermented weed (*Setaria barbata* L.) extract (100%)
T_4	Neem cake (500 kg/ha)
T_5	Fish amino acid (5%)
T_6	Fermented egg – lemon juice extract (10%)
T_7	Panchagavya (5%) (KAU, 2009)
T_8	Cow dung supernatant (10%)
T_9	Biogas slurry (10%)
T_{10}	Turmeric (*Curcuma longa* L.) + baking soda (4:1) mixture (5g/L water)
T_{11}	Turmeric (*Curcuma longa* L.) + baking soda (4:1) mixture (5g/L rice gruel water (kanjivellam))
T_{12}	Turmeric (*Curcuma longa* L.) + baking soda (4:1) mixture (5g/L cowdung supernatant)
T_{13}	Potassium silicate (1%)
T_{14}	Lime solution (12.5%)
T_{15}	Jeevamruth (10%)
T_{16}	Diluted cow's urine (10%)
T_{17}	Neem (*Azadiracta indica* A. Juss.) oil (2%)
T_{18}	Garlic (*Allium sativum* L.) extract (10%)
T_{19}	Tulsi (*Ocimum sanctum* L.) extract (10%)
T_{20}	Control

3.3.1. Preparation of Indigenous Organic Preparations

3.3.1.2 *Fermented tapioca leaf and rind extract (Sajeena et al., 2015)*

Fermented tapioca leaf and rind extract in cow's urine diluted in water was prepared by weighing out fresh leaves and rind of tapioca (one kg each on w/w basis) and chopping them into small pieces. The chopped bits were then immersed in cow's urine (1 L) diluted five times with water. The preparation was stirred daily once and kept for fermentation for a period of seven days and then filtered through muslin cloth to obtain the required extract for the *in vitro* study.

3.3.1.2 *Fermented papaya leaf extract (Sajeena et al., 2015)*

One kg fresh leaves of papaya was weighed and immersed in cow's urine (1 L) diluted five times with water. The preparation was stirred daily once and kept for fermentation for seven days and then filtered through muslin cloth to obtain the required extract for the *in vitro* study.

3.3.1.3 Fermented weed extract (Sajeena et al., 2016).

Two and a half kg of the weed *viz*., (*Setaria barbata*) (mary grass/ corn grass) was weighed, washed thoroughly to remove soil and dirt and was cut into small pieces. 20 g each of salt powder, tamarind (pulp) and powdered jaggery (sugarcane product) were added to the cut bits of the weed taken in a container and the mixture was diluted using 10 L of water. The mixture was stirred daily once and was kept for fermentation for a period of 21 days. The fermented preparation was filtered through muslin cloth and used directly for further studies.

3.3.1.4 Fish amino acid (Weinert et al., 2014)

Sardine fish and jaggery were used for the preparation of fish amino acid. One kg of fresh sardine fish (*Sardina pilchardus*) was cut into small pieces. One kg of jaggery (sugarcane product) was powdered. The cut pieces of fish and powdered jaggery (1:1 ratio on w/w basis) were filled in a container as layers, one above the other. The container was covered tightly using a muslin cloth for provision for air circulation. The mixture was kept undisturbed for 21 days after

which it was filtered through muslin cloth. The filtered extract was used at the rate of five per cent concentration for the inhibition studies against *R. solani*.

3.3.1.5 Fermented egg-lemon juice extract (Sajeena et al. 2016)

Fermented egg-lemon juice extract was prepared using hen eggs, lemon juice and powdered jaggery. Twelve raw eggs were taken in a container. Lemon juice was squeezed and poured over the eggs such that the eggs got immersed in the juice. This preparation was kept undisturbed for ten days. On the tenth day, 500 g of powdered jaggery (sugarcane product) was added to the mixture and stirred thoroughly. This was kept undisturbed for ten more days. The preparation was ready for use after 21 days. This was filtered through muslin cloth and used at the rate of ten per cent concentration for further studies.

3.3.1.6 Panchagavya (KAU, 2009)

Panchagavya is an organic preparation based on the five products obtained from cow along with certain other natural materials. Initially, fresh cow dung (7 kg) and cow ghee (1 kg) were mixed thoroughly in a clean container and was kept aside for three days. The mixture was stirred thoroughly twice a day during morning and evening hours. After three days, cow urine (10 L) and water (10 L) were added to this mixture and was kept aside for another 15 days, with regular mixing twice a day. After 15 days, cow's milk (3 L), cow's curd (2L), tender coconut water (3L), jaggery (3 kg) and well ripened Poovan banana (12 Nos.) were added to the above mixture. The mouth of the container was properly covered and the container was kept under shade with regular stirring. The prepared Panchagavya mixture was ready for use after 30 days and was used at the rate of five per cent concentration.

3.3.1.7 Cow dung supernatant (KAU, 2009)

One kg of fresh cow dung was weighed and diluted with 10 L of water. The clear solution obtained after filtering the supernatant liquid, was used directly for *in vitro* inhibition studies.

3.3.1.8 Biogas slurry (KAU, 2009)

Biogas slurry collected from the biogas plant installed at IFSRS, Karamana was diluted to ten times with water and was used directly for *in vitro* inhibition studies.

3.3.1.9 Turmeric - baking soda mixture in water (Bhadrasree, 2007)

Turmeric powder mixed with baking soda in 4:1 ratio (on w/w basis) was diluted with water (1 L) and the fresh preparation was used directly for inhibition studies.

3.3.1.10 Turmeric - baking soda mixture in rice gruel water

Turmeric powder mixed with baking soda in 4:1 ratio (on w/w basis) was diluted with fresh rice gruel water (kanjivellam) (1 L) and the fresh preparation was used directly for inhibition studies.

3.3.1.11 Turmeric - baking soda mixture in cow dung supernatant

Turmeric powder mixed with baking soda in 4:1 ratio (on w/w basis) was diluted with cow dung supernatant (1 L) as described in 3.3.1.7 and the fresh preparation was used directly for inhibition studies.

3.3.1.12 Jeevamruth (Chadha et al., 2012)

Fresh cow dung (10 kg), fresh cow urine (10 L), powdered jaggery (sugarcane product) (2 kg), pulse (green gram) flour (2 kg) and a hand full of fertile soil (soil collected from cultivated field without any accumulation of chemical fertilizers/pesticides) were taken in a vessel and mixed with 20 L of water. The mixture was filtered through muslin cloth after one week and was used at a rate of ten per cent concentration for the *in vitro* studies.

3.3.1.13 Fresh cow urine (KAU, 2009)

Fresh cow urine was diluted ten times with water and was used directly for *in vitro* inhibition studies.

3.3.2 Botanicals

3.3.2.1 Neem oil (KAU, 2009)

Commercial neem oil @ 2 per cent concentration was prepared and used for *in vitro* studies against *R. solani*.

3.3.2.2 Garlic extract (Kumar and Tripathi, 2012)

Garlic (*Allium sativum*) extract was prepared by weighing out 100 g of clean, descaled fresh garlic bulbs which were homogenized in 100 ml (1:1 ratio) of distilled water. This extract was filtered through muslin cloth and used at the rate of ten per cent concentration for inhibition studies.

3.3.2.3 Tulsi extract (Saifunneesa and Niza, 2001)

Basil/Tulsi (*Ocimum sanctum*) extract was prepared by weighing out 100 g fresh, clean leaves of the plant and chopping them into small bits. The bits were macerated with water (1:1 ratio on w/v basis) and filtered through two layers of muslin cloth. This extract was used at the rate of ten per cent concentration for *in vitro* studies.

3.3.3 Non-hazardous Chemicals

3.3.3.1 Potassium silicate (Devi and Nayar, 2016)

Potassium silicate with 25 per cent silica and seven per cent potassium was used at the rate of one per cent concentration for *in vitro* inhibition studies.

3.3.3.2 Lime solution (Sajeena et al., 2015)

Lime containing calcium @ 12.5 per cent concentration was used for *in vitro* inhibition studies against *R. solani*.

3.3.4 Soil Amendments

3.3.4.1 Neem cake (Lenka and Pun, 2014)

Fifty grams of neem cake was weighed and soaked in 100 ml distilled water for 24 h. Subsequently, it was filtered through double layered muslin cloth. The extract (20 ml) was mixed with 80 ml of molten PDA in 250 ml conical flasks to get ten per cent concentration which was used for *in vitro* inhibition studies.

3.3.5 *In vitro* Evaluation of the Treatments against *R. solani*

In vitro evaluation studies were performed to assess the potential of the various treatments in inhibiting the mycelial growth of rice sheath blight fungus, *R. solani* by poisoned food technique (Nene and Thapliyal, 1979). Double strength PDA (40 g agar, 40 g dextrose and 400 g potato in 1 L water) was prepared and sterilized in 250 ml conical flasks. All the treatments except the non-hazardous chemicals were filtered by passing through Whatman No 1 filter

paper and further through bacterial filters (0.2 µm) before their amendment to PDA medium. Each of these treatments were mixed with sterile water in required quantity under aseptic conditions and added to double strength PDA medium to get the desired concentration for the *in vitro* suppression studies. 15 mL of each amended medium was poured into 90 mm sterile petri plates and allowed to solidify. Five mm discs of seven days old culture of the most virulent isolate of *R. solani* was placed at the centre of petri plates containing each treatment amended media under aseptic conditions. The plates were sealed and incubated under room temperature (28 ± 2° C). The un-amended PDA medium inoculated at the centre with the pathogen served as the control. Observations were taken when full growth of the fungus was attained in the control plates. The percentage inhibition of the mycelial growth of *R. solani* in the amended medium over that in the control plate was calculated using the formula (Vincent, 1947) as follows;

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

I - Percentage inhibition

C - Growth (cm) of the fungus in control plates

T - Growth (cm) of fungus in treatment amended plates

3.3.6 Selection of Best Treatments

The best five treatments exhibiting the maximum inhibition of the mycelial growth of sheath blight pathogen in *in vitro* suppression studies were selected for further studies.

3.4 EFFECT OF SELECTED TREATMENTS ON SCLEROTIA

A study was undertaken to find the potential of the best effective treatments selected from *in vitro* suppression studies for their potential in inhibiting the mycelial regeneration from sclerotia. For this, the sclerotia were dipped in the treatments separately for different time intervals *viz*., 24, 48 and 72 h. The sclerotia collected from seven days old culture plates of *R. solani* were used for this assay. The sclerotia that were dipped in each treatment for different

time intervals were placed separately on solidified PDA medium poured in sterile petri plates at the rate of one sclerotium per plate. The sclerotia dipped in sterile distilled water served as the control. The mycelial growth (regeneration) from the sclerotia was observed from 24 h after inoculation. The mycelial growth from the sclerotia as well as the per cent reduction of the mycelial growth over control were measured using the following formula (Vincent, 1947)

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

I - percentage inhibition/per cent reduction over control

C – mycelial growth (cm) from sclerotia in control plates

T - mycelial growth (cm) from sclerotia in treated plates

3.5 *IN VIVO* MANAGEMENT STUDIES

A pot culture experiment was conducted at College of Agriculture, Vellayani to evaluate the efficacy of the effective treatments screened from the *in vitro* study for the management of sheath blight disease. The experiment was laid out as described below:

Design	Completely Randomized Design (CRD)
Replications	Three
Treatments	11
Variety	Uma

The treatments selected for the study were the five best treatments selected from *in vitro* suppression studies along with five checks *viz.*, hexaconazole (1ml/L), *Trichoderma viride* (KAU isolate) (20 g/L), *Pseudomonas fluorescens* I (KAU isolate) (20 g/L), inoculated untreated control and un-inoculated untreated control.

The rice seedlings were transplanted to UV stabilized 600 gauge, 150 μ grow bags (40 cm x 35 cm x 32 cm) which were filled with 20 kg sandy clay loam soil having a pH of 5.5. The water level in the grow bags were constantly maintained at five cm. Twenty days old seedlings were transplanted from the nursery into

grow bags. The fertilizer application and pest control practices were followed according to the Package of Practices (POP) recommendations of KAU (2016).

3.5.1 Preparation of inoculum of *R. solani* (IRRI, 1986)

R. solani was artificially inoculated in the soil for pot culture studies to obtain uniform symptom development and for evaluating the potential of different treatments in managing the disease. The inoculum of *R. solani* was prepared using rice bran, which was mixed with water in 2:1 ratio. The mixture (250 g) was filled in polypropylene covers. The covers were sealed and sterilized at 121 °C at 1.2 kg/cm^2 pressure for two h. Each bag was inoculated with five mm mycelial discs of seven days old culture of *R. solani* and was incubated at room temperature for two weeks.

3.5.2 Artificial inoculation of the prepared inoculum

Seven days after transplanting, the soil in the grow bags was top dressed (inoculated) with 250 gram of rice bran inoculum of *R. solani* (rice bran inoculum of *R. solani*: sand in the proportion 1:2 ratio on w/w basis) (IRRI, 1986). A second challenge inoculation of the pathogen was given at 40 DAS by inserting the mycelium along with one sclerotium in between the outer most leaf sheath of the rice plant in all the treatments except the un-inoculated untreated control.

3.5.3 Preparation and application of treatments

The effective treatments were prepared as described under 3.3. The plants were sprayed with the respective treatments on 45th, 60th, 75th and 90th DAS. The observations on the stage of the crop at which the disease appears, days for symptom development, days for the production of sclerotia, number of infected tillers, plant height (cm) and lesion height were taken. The intensity of sheath blight disease was rated by calculating the RLH (Sharma *et al.*, 1990) using the formula;

$$RLH = (\text{Lesion height}/\text{Plant height}) \times 100$$

Based on the RLH, the plants were scored according to 0 - 9 scale of Standard Evaluation System for Rice (IRRI, 2002) as described in Table 2, as follows:-

Table 2. Disease scale for scoring sheath blight disease of rice

Scale based on RLH	Description
0	No infection observed
1	Lesions limited to lower 20 per cent of the plant height
3	Lesions limited to lower 20 to 30 per cent of the plant height
5	Lesions limited to lower 31 to 45 per cent of the plant height
7	Lesions limited to lower 46 to 65 per cent of the plant height
9	Lesions limited to more than 65 per cent of the plant height

3.6 EFFECT OF THE TREATMENTS ON BIOMETRIC AND YIELD ATTRIBUTES OF RICE

The following biometric observations were recorded in the pot culture experiment

3.6.1 Biometric observations

3.6.1.1 Plant height

Plant height was measured as the distance from the ground level to the tip of the tallest leaf/panicle.

3.6.1.2 Total tillers per hill

The total number of tillers per hill was counted.

3.6.1.3 Productive tillers per hill

The number of productive tillers per hill was counted at the time of harvest.

3.6.1.4 Infected tillers

The number of sheath blight disease affected tillers was also recorded.

3.6.1.5 Number of filled grains per panicle

The number of fully developed grains (filled grains) per panicle was counted.

3.6.1.6 Number of spikelets per panicle

The number of spikelets per panicle was recorded.

3.6.1.7 Lesion height

The height of the top most lesion (cm) from the ground level was observed.

3.6.1.8 Lesion width

The width of the lesion (cm) in the sheath was also noted.

3.6.1.9 Lesion area

The lesion area (cm^2) was calculated as the product of lesion height (cm) and lesion width (cm).

3.6.2 Yield attributes

3.6.2.1 Grain yield

All the plants in each treatment were harvested and threshed separately. The grain yield was measured as the weight (expressed as gram per plant) of the grains in all the treatments.

3.6.2.2 Thousand grain weight

Thousand grain weight (g) was recorded for the plants in each treatment.

3.6.2.3 Straw yield

The weight of the straw (expressed as gram per plant) was recorded separately for each treatment.

3.7 CHEMICAL ANALYSIS

3.7.1 Nutrient content of the selected organic preparations/ botanicals

The best treatments selected for the pot culture experiment were analyzed for their total N, P, K and pH as described in Table 3.

Table 3. Methods for the Analysis of Total N, P, K and pH

SL. No.	Parameter	Method	References
1.	Total N	Kjeldahl method	Jackson, 1958
2.	Total P	Vanado molybdate yellow colour method	Piper, 1966
3.	Total K	Flame photometric method	Jackson, 1958
4.	pH	pH meter	Jackson, 1958

3.7.2 Analysis of the Plants for Major Nutrients at the Time of Harvest

The plants at the time of harvest were analyzed for their total N, P and K, as detailed in table 3.

3.8 STATISTICAL ANALYSIS

The data generated from the experiments were statistically analyzed using the analysis of variance techniques (ANOVA) and were applied to completely randomized design (CRD). The data recorded on per cent inhibition were transformed using per cent (arc sine) transformation. The entire data sets of the various experiments were analyzed statistically by Duncans Multiple Range Test (DMRT) as per the procedure given by Steel and Torrie (1960).

RESULTS

4. RESULTS

The work entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken at Integrated Farming System Research Station (*in vitro* studies) and at College of Agriculture, Vellayani (*in vivo* studies) during 2015-17 to develop an ecofriendly and safe management strategy against sheath blight disease of rice. The experimental data collected from the laboratory and pot culture experiments were statistically analyzed. The results obtained are presented under the following heads:

4.1 ISOLATION OF SHEATH BLIGHT PATHOGEN

4.1.1 Collection of Samples

Sheath blight affected rice plants were collected from seven different rice growing tracts of Thiruvanathapuram district *viz*., Chenkal, Attingal, Punchakari, Nagaroor, Vellayani, Karode and Karamana. Uma was the rice variety cultivated in the above mentioned tracts. The symptoms observed in the infected plants of each rice growing tract are described in Table 4. The sheath blight disease incidence was observed during the active tillering stage of the crop in the six rice growing tracts (Chenkal, Attingal, Punchakari, Nagaroor, Vellayani and Karamana), whereas, it was observed during the late tillering stage of the crop at Karode tract. The symptoms of the disease were exhibited as grey coloured lesions, with the colour of the margin of the lesions varying from brown, dark brown, greyish brown to purplish brown. Moreover, water soaked lesions were noticed in the samples collected from Attingal and Punchakari tracts. The presence of numerous, chocolate brown, and mustard like sclerotia was noticed only in the disease affected plants collected from Punchakari tract.

4.1.2 Isolation of Pathogen

Seven isolates of *R. solani* were isolated from the disease affected rice plants collected from the seven rice growing tracts of Thiruvananthapuram *viz*., Chenkal, Attingal, Punchakari, Nagaroor, Vellayani and Karamana on potato dextrose agar (PDA) medium following standard procedures.

Table 4. Details of sheath blight affected rice plants collected and their field characteristics

Sl. No	Location	Variety	Crop stage affected	Observed symptoms	Sclerotia	
					Presence / absence	Description
I_1	Chenkal	Uma	Active tillering	Grey lesions with brown margin	Absent	-
I_2	Attingal	Uma	Active tillering	Grey, water soaked lesions with greyish brown margin.	Absent	-
I_3	Punchakari	Uma	Active tillering	Grey, water soaked lesions with greyish brown margin	Present	Numerous, chocolate brown, mustard like sclerotia
I_4	Nagaroor	Uma	Active tillering	Grey lesions with purplish brown margin.	Absent	-
I_5	Vellayani	Uma	Active tillering	Grey lesions with confined dark brown margin	Absent	-
I_6	Karode	Uma	Late tillering	Grey lesions with brown margin	Absent	-
I_7	Karamana	Uma	Active tillering	Grey lesions with greyish brown margin	Absent	-

4.1.3 Purification of the Isolates

The seven isolates of *R. solani* were purified following hyphal tip culture method and maintained on PDA slants for further studies.

4.1.4 Pathogenicity Studies

The isolates were used for pathogenicity studies on healthy rice plants (variety Uma) under artificial conditions for symptom development. Typical sheath blight symptoms were produced by each isolate on the artificially inoculated plants (Plate 1). The days for symptom development in the plants artificially inoculated with the seven isolates, lesion size and the sclerotial characters were studied and are described in Table 5. In the rice plants artificially inoculated with the isolate collected from Karode (I_6), the symptom development was observed on the seventh day after inoculation. In the plants inoculated with the other six isolates (I_1, I_2, I_3, I_4, I_5 and I_7), the symptom development was noticed on the third day after inoculation. The size of the lesions inoculated with the seven isolates on the third day after inoculation (DAI) varied from a minimum of 0.07 cm^2 in the case of isolate from Karode (I_6) to a maximum of 2.19 cm^2 in the case of isolate from Attingal (I_2). Production of sclerotia was noticed only in the plants inoculated with the isolates collected from Chenkal (I_1) (one sclerotium) and Attingal (I_2) (two sclerotia) on the 11^{th} and 12^{th} DAI respectively. In rice plants inoculated with isolates from Chenkal (I_1) and Attingal (I_2), the sclerotia appeared to be chocolate brown and dark brown respectively.

4.1.5 Virulence Rating

Virulence rating was undertaken to find out the most virulent isolate of *R. solani* using two techniques.

4.1.5.1. Cut Stalk Assay Method

In the cut stalk assay method of virulence rating, it was revealed that the lesion length on the excised stalks were the maximum for the three isolates *viz*, Chenkal (I_1) (11.65cm), Attingal (I_2) (12.58cm) and Karamana (I_7) (11.00cm) (Table 6)on the 7^{th} day after inoculation and were on par with each other (Figure 1).

Table 5. Pathogenicity related attributes of *R. solani* isolates on artificial inoculation

Isolate	DSD	Lesion Size 3 DAI (cm^2)	Sclerotial characters			
			Presence/ absence	Days for formation	No.	Colour and shape
I_1	3	1.33^{b} (1.15)	Present	11	1	Round, mustard like chocolate brown
I_2	3	2.19^{a} (1.47)	Present	12	2	Round, mustard like dark brown
I_3	3	0.67^{c} (0.78)	Absent	-	-	-
I_4	3	0.95^{bc} (0.95)	Absent	-	-	-
I_5	3	1.10^{bc} (1.04)	Absent	-		-
I_6	7	0.07^{d} (0.26)	Absent	-	-	-
I_7	3	1.23^{b} (1.11)	Absent	-	-	-
CD (0.05)	-	0.30	-	-	-	-
SE m(±)		0.08				

Values in parenthesis are square root transformed Treatments with same alphabet do not differ significantly
Average of three replications DSD – days for symptom development, DAI – days after inoculation

Table 6. Length of lesion on inoculation by cut stalk assay method

R. solani Isolate	Lesion length (cm) (7 DAI)
I_1	11.65[a]
I_2	12.58[a]
I_3	7.63[b]
I_4	7.00[b]
I_5	6.63[b]
I_6	7.23[b]
I_7	11.00[a]
Control	0.00[c]
CD (0.05)	2.68
SE m(±)	0.72

Treatments with same alphabet do not differ significantly
Values are average of three replications
DAI - Days after inoculation

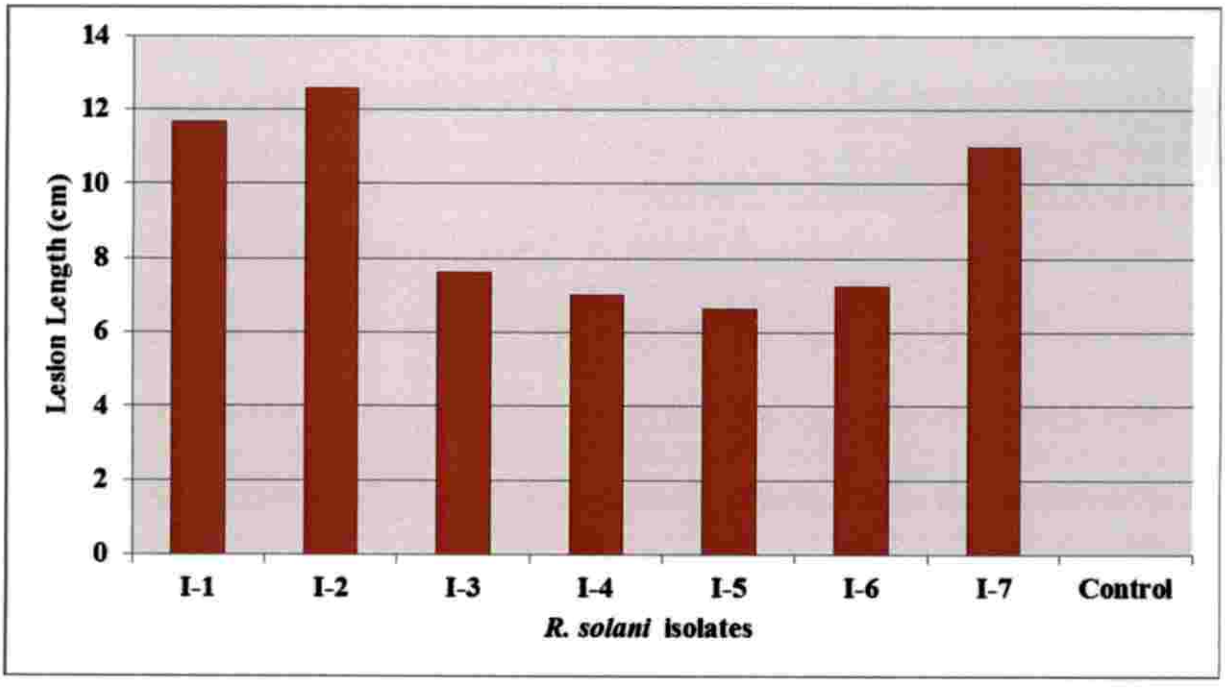


Figure 1. Lesion length on inoculation by cut stalk assay method

I_1

I_2

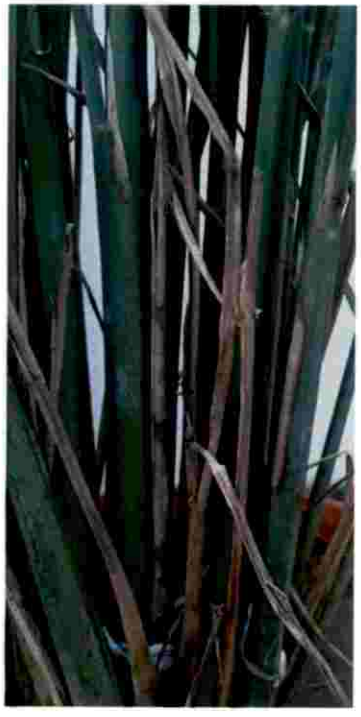
I_3

I_4

Plate 1: Lesion development by *R. solani* isolates on artificial inoculation

I_5

I_6



I_7

Control

Plate 1: Lesion development by *R. solani* isolates on artificial inoculation (continued)

4.1.5.2 Direct Inoculation on Rice Plants

Sheath blight symptom development was observed on the 3rd DAI on the leaf sheaths inoculated with all the isolates except I_6 (Karode isolate) which took seven days for symptom development. The *R. solani* isolate collected from Attingal (I_2) produced the maximum relative lesion height on 5th (9.20%), 7th (15.70%), 10th (19.03%) and 15th (20.70%) DAI. The number of affected tillers was also maximum (4.66) in the case of isolate from Attingal (I_2) (Table 7). Thus the virulence rating of the seven isolates of *R. solani* revealed that Attingal (I_2) isolate was the most virulent one (Figure 2).

4.1.5.3 Selection of the Most Virulent Isolate

Three isolates *viz*, Chenkal (I_1), Attingal (I_2) and Karamana (I_7) were revealed to be the most virulent isolates based on the cut stalk assay method. Further, direct inoculation on potted plants revealed that from among all the seven isolates, Attingal (I_2) isolate produced the maximum RLH as well as the number of affected tillers and thus was confirmed to be the most virulent. This isolate was thus used for further studies.

4.2 CHARACTERIZATION OF PATHOGEN

4.2.1 Morphological Characterization of Pathogen

Microscopic studies (100 X) of the mycelium of the fungus revealed the presence of right angled branching, septum at the origin and constriction at the base of branching, which are all the characteristic features of *R. solani*. The sclerotia when crushed and observed under the microscope revealed the presence of moniliod cells which further confirm the fungus to be *R. solani*.

4.2.2 Cultural Characterization of Pathogen

The mycelial colour of the isolates ranged from light brown to dark brown with the isolate from Karode (I_6) exhibiting white coloured mycelium. The mycelial texture also ranged from fluffy to cottony in the various isolates. The mycelial growth was found to be the maximum in the case of isolate from Attingal (I_2) on 1st (3.60cm) and 2nd (8.26cm) DAI (Table 8). The sclerotia produced by five isolates (I_1, I_2, I_3, I_4 and I_7) exhibited the maximum average size (2.13, 2.11, 1.99, 1.91 and 2.01mm respectively). The isolate from Karode (I_6) produced the

Table 7. Lesion development by *R. solani* isolates on artificial inoculation *in vivo*

Isolate	DSD	Relative Lesion Height (%) (RLH)				No. of tillers affected
		Days after inoculation (DAI)				
		5	7	10	15	
I_1	3	2.57^{c}	4.67^{c}	5.97^{c}	7.33^{c}	3.00^{b}
I_2	3	9.20^{a}	15.70^{a}	19.03^{a}	20.97^{a}	4.67^{a}
I_3	3	0.93^{d}	1.67^{de}	3.30^{cd}	5.17^{c}	2.33^{b}
I_4	3	1.90^{cd}	3.37^{cd}	4.77^{cd}	6.50^{c}	2.33^{b}
I_5	3	2.20^{c}	3.57^{cd}	4.33^{cd}	5.60^{c}	3.00^{b}
I_6	7	0.00^{e}	0.23^{e}	4.93^{cd}	2.17^{d}	1.33^{c}
I_7	3	5.03^{b}	11.13^{b}	13.67^{b}	16.07^{b}	3.00^{b}
Control	0.00	0.00	0.00	0.00	0.00	0.00
CD (0.05)	-	1.04	2.63	5.08	2.27	0.99
SE m (±)		0.28	0.72	1.38	0.62	0.27

Treatments with same alphabet do not differ significantly, Average of 3 replications, DSD Days for symptom development

Table 8. Morphological and cultural characteristics of *R. solani* isolates

Isolate	Mycelial characteristics						Sclerotial characteristics			
	Colour	Texture	Mycelial growth (cm) Days after inoculation			Days for completion of growth	Days for pin head formation	Number (DAI)	Average Size (mm)	Distribution pattern
			1	2	3					
I_1	Dark brown	Fluffy	2.93^{b}	7.23^{b}	9	3	4	26.00^{cd}	2.13^{a}	Scattered
I_2	Light brown	Cottony	3.60^{a}	8.26^{a}	9	3	4	59.33^{b}	2.11^{a}	Scattered
I_3	Light brown	Cottony	1.83^{c}	5.06^{d}	9	3	4	27.00^{cd}	1.99^{ab}	Central
I_4	Light brown	Cottony	2.56^{b}	6.33^{c}	9	3	4	34.66^{c}	1.91^{bc}	Scattered
I_5	Light brown	Fluffy	2.96^{b}	7.33^{b}	9	3	4	24.00^{d}	1.76^{c}	Scattered
I_6	White	Cottony	3.00^{b}	5.96^{c}	9	3	5	104.66^{a}	0.98^{d}	Scattered
I_7	Light brown	Cottony	2.96^{b}	8.23^{a}	9	3	4	53.66^{b}	2.01^{ab}	Central
CD (0.05)	-	-	0.53	0.606	NS	NS	NS	9.030	0.164	
SE m(±)	-	-	0.14	0.16	-	-	-	2.43	0.04	

Values in parenthesis are arc sine transformed,
Treatments with same alphabet do not differ significantly
Average of three replications

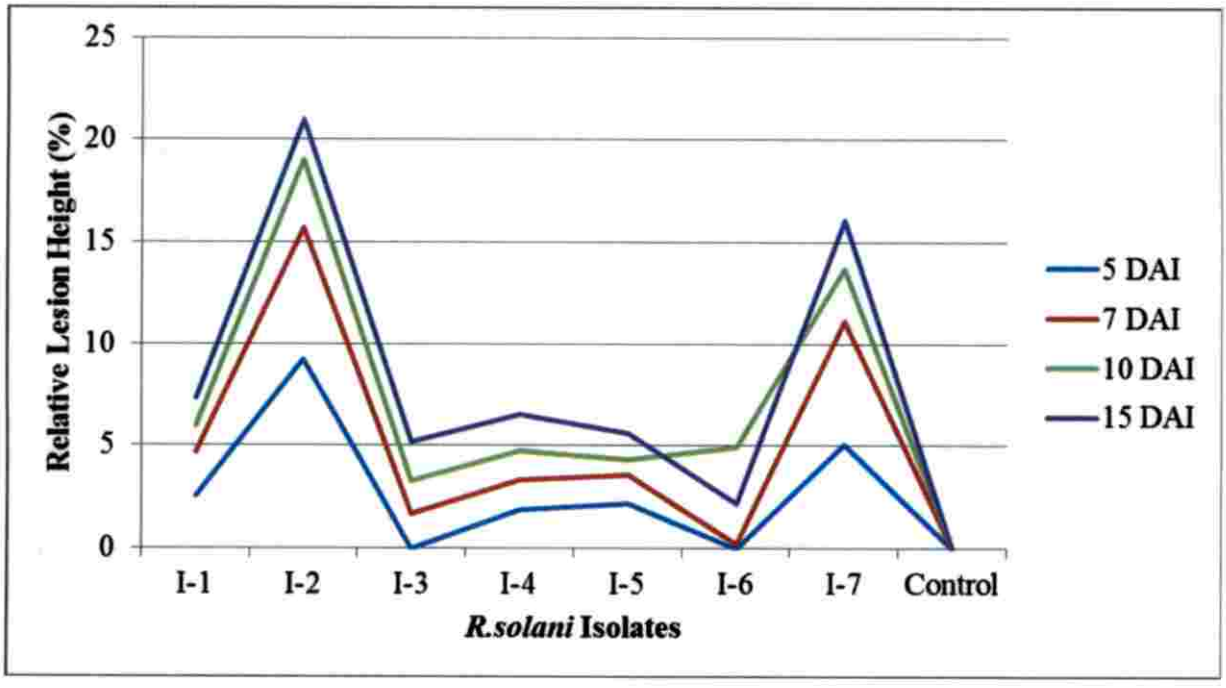


Figure 2. Lesion development by *R. solani* isolates on direct inoculation on rice plants

maximum number of minute sclerotia (104.66) which was followed by the isolate from Attingal (I_2) (59.33) and isolate from Karamana (I_7) (53.66). The growth pattern of the sclerotia in the Petri plates ranged from scattered to central (Plate 2).

4.2.3 Tentative Identification of Pathogen

The presence of right angled branching, septum at the origin of branching, constriction at the base as well as the presence of moniliod cells in the sclerotia tentatively confirmed the pathogen to be *R. solani*. The brown colour of the mycelia also could confirm it to be *R. solani* (Plate 3).

4.2.4. Confirmation of Pathogen

4.2.4.1 Morphological Identification

The *in vitro* identification of the isolate based on morphological characters revealed it to be *Rhizoctonia solani* J.G. Kuhn of the family Ceratobasidiaceae with its current name as *Thanatephorus cucumeris* (A. B. Frank) Donk (http://www.indexfungorum.org/names/Names.asp).

4.2.4.2 Molecular Identification

The identification of the isolate based on the ITS (Internal Transcribed space) region of rDNA showed 100 per cent similarity with *Rhizoctonia solani,* current name *Thanatephorus cucumeris* (A. B. Frank) Donk. The sequence analyses with NCBI accession number KX674527, *Rhizoctonia solani* AG-1 IA isolate CSU8 resulted in the alignment statistics of Query length of 557, Score of 1005 bits (1114), Expect 0.0, Identities – 557/557 (100%), Gaps – 0/557 (0%) and Strand Plus/ Minus (Table 9).

4.3 *IN VITRO* PATHOGEN SUPRESSION

In vitro studies for evaluating the potential of 19 treatments in inhibiting the mycelial growth of *R. solani* revealed that six treatments *viz*, fermented egg–lemon juice extract (10%), fermented weed (*Setaria barbata*) extract (100%), panchagavya (5%), garlic extract (10%), potassium silicate (1%) and lime solution (12.5%) resulted in complete (100%) inhibition of the mycelial growth of *R. solani* as described in Table 10. The next best effective treatment was fermented tapioca leaf-rind extract (91.85%) followed by neem cake (87.04%). The next

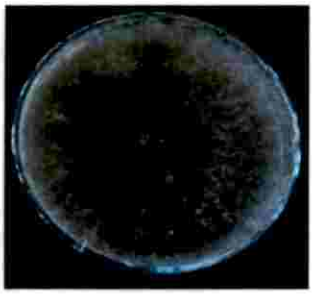
Table 9. Molecular identification of the most virulent isolate based on ITS region of rDNA

Gene Bank Accession No.	Description	Maximum score	Query cover	Query coverage	E value	Identity (%)
KX674527.1	*Rhizoctonia solani* AG-1 IA isolate CSU8	1005	1005	100 %	0.0	100 %
KX674526.1	*Rhizoctonia solani* AG-1 IA isolate CSU4	1005	1005	100 %	0.0	100 %
KX674525.1	*Rhizoctonia solani* AG-1 IA isolate CSU1	1005	1005	100 %	0.0	100 %
KX674524.1	*Rhizoctonia solani* AG-1 IA isolate RKH	1005	1005	100 %	0.0	100 %
KX674523.1	*Rhizoctonia solani* AG-1 IA isolate RKL	1005	1005	100 %	0.0	100 %

Table 10. Effect of treatments on *in vitro* suppression of *R. solani*

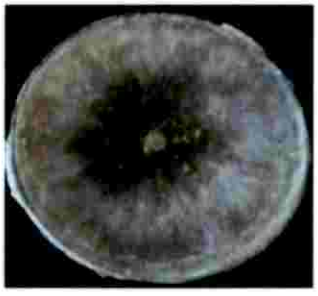
Treatment	Mycelial growth (3DAI) (cm)	Percentage inhibition	Sclerotial characters	
			Number	Days for PHI
T_1	0.73	91.85^{b} (76.59)	0	0
T_2	1.67	81.48^{d} (64.52)	0	0
T_3	0.03	99.63^{a} (87.34)	0	0
T_4	1.17	87.04^{c} (68.99)	0	0
T_5	3.30	63.33^{gh} (52.73)	0	0
T_6	0.00	100.00^{a} (89.04)	0	0
T_7	0.00	100.00^{a} (89.04)	0	0
T_8	3.43	61.85^{gh} (51.85)	0	0
T_9	2.97	67.04^{fgh} (54.96)	3	10
T_{10}	4.73	47.41^{j} (43.51)	0	0
T_{11}	4.37	51.48^{ij} (45.84)	0	0
T_{12}	2.10	76.67^{de} (61.12)	0	0
T_{13}	0.00	100.00^{a} (89.04)	0	0
T_{14}	0.00	100.00^{a} (89.04)	0	0
T_{15}	2.30	74.44^{def} (59.66)	2	11
T_{16}	2.70	70.00^{efg} (56.82)	2	10
T_{17}	3.77	58.15^{hi} (49.72)	0	0
T_{18}	0.00	100.00^{a} (89.04)	0	0
T_{19}	4.50	50.00^{ij} (45.01)	0	0
T_{20}	9.00	—	36	5
CD (0.05)	-	5.857	-	-
SE m (±)		1.67	-	-

Treatments with same alphabet do not differ significantly
Values in parenthesis are arc sine transformed
Average of 3 replications, PHI - Pin Head Initiation



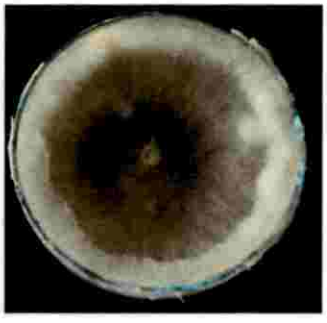
I_1

I_2

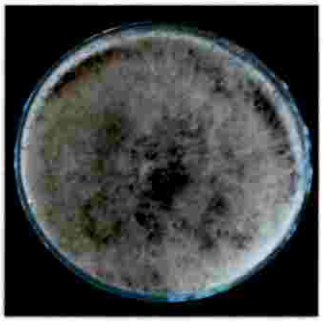


I_3

I_4



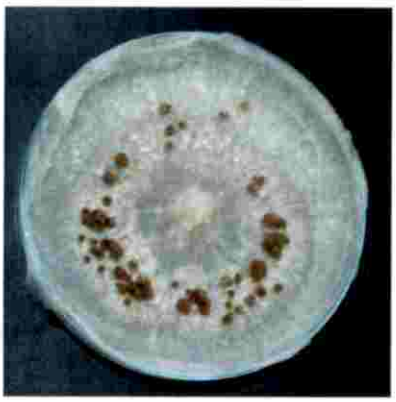
I_5



I_6

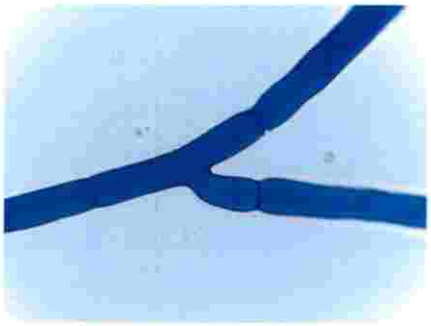
I_7

Plate 2. Isolates of *R. solani* collected from seven rice growing tracts of Thiruvananthapuram district



a. Mycelial growth of *R. solani* on PDA Media

b. Symptom development on artificial inoculation



c. Presence of septum at branching and constriction at the base

d. Presence of moniliod cells in sclerotia

Plate 3. The virulent isolate of *R. solani* (I_2), symptom development and morphological characters

best effectiveness was exhibited by fermented papaya leaf extract (81.48%) which was on par with turmeric-baking soda mixture in cowdung supernatant (76.67%) and jeevamruth (74.44%). The least effectiveness in suppressing the mycelial growth was exhibited by turmeric-baking soda mixture (4:1) in water (47.41%). Sclerotial formation was observed only in the plates amended with biogas slurry (10%), jeevamruth (10%) and diluted cow's urine (10%). Thus the six treatments *viz*., fermented egg–lemon juice extract, fermented weed extract, panchagavya, garlic extract, potassium silicate and lime solution, which resulted in the complete inhibition of the mycelial growth of the pathogen were selected for the *in vivo* management studies of the pathogen (Plate 4)

4.4 EFFECT OF SELECTED TREATMENTS ON SCLEROTIA

The study revealed that dipping of sclerotia in lime solution (12.5%), fermented weed extract (100%), fermented egg-lemon juice extract (10%) and panchagavya (5%) for 24 hours itself resulted in complete (100%) inhibition of the mycelial regeneration from the sclerotia (Plate 5). The garlic extract (10%) resulted in complete inhibition of the mycelial regeneration from the sclerotia only after 72 hours after dipping in it whereas, dipping in potassium silicate resulted only in less inhibition (27.28%) of the mycelial regeneration from the sclerotia even after 72 hours (Table 11; Plate 6 & 7).

4.5 *IN VIVO* MANAGEMENT STUDIES

R. solani was multiplied in rice bran media for artificial inoculation in the rice plants during the pot culture study (Plate 8). White mycelial growth of the fungus was observed in the rice bran media on five DAI which covered the entire media within two weeks. Numerous white sclerotial initials were observed in the media after two weeks which later turned into brown colour (Plate 9).

The pot culture study conducted at College of Agriculture, Vellayani revealed that the symptoms of rice sheath blight disease developed on 45 DAS, which coincided at the active tillering stage of the crop. Sclerotial formation was not observed in any of the infected rice plants (Table 12).

The vertical spread (the upward progress of the disease along the infected tiller) was observed for the plants sprayed with each treatment as described in

Table 11. Effect of treatments on inhibition of mycelial regeneration from sclerotia

Treatments	Soaking duration of sclerotia in selected treatments before sclerotial germination assay (hours)					
	24		48		72	
	MR (cm)	S (%)	MR (cm)	S (%)	MR (cm)	S (%)
T_3	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)
T_6	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)
T_7	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)
T_{13}	9.00	0.00	7.79	13.34[c] (19.46)	7.17	21.03[b] (27.29)
T_{14}	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)	0.00	100.00[a] (89.04)
T_{18}	7.50	16.66[b] (23.89)	4.16	53.71[b] (46.88)	0.00	100.00[a] (89.04)
Control	9.00	0.00	9.00	0	9.00	0.00
CD (0.05)	-	3.53	-	16.77		0.51
SE m (±)	-	0.91	-	4.44	-	0.13

Values in parenthesis are arc sine transformed, Treatments with same alphabet do not differ significantly
Average of 3 replications, MR - Mycelial regeneration from sclerotia, S - Suppression

Table 12. Sheath blight symptom development as affected by the treatments

Treatment	DSD	DFS	SDD
T_1 (Fermented weed extract)	45 DAS	-	ATS
T_2 (Panchagavya)	45 DAS	-	ATS
T_3 (Fermented egg-lemon juice extract)	45 DAS	-	ATS
T_4 (Garlic extract)	50 DAS	-	ATS
T_5 (Lime solution)	45 DAS	-	ATS
T_6 (Potassium silicate)	50 DAS	-	ATS
T_7 (Hexaconazole)	52 DAS	-	ATS
T_8 (*Trichoderma viride*)	50 DAS	-	ATS
T_9 (*Pseudomonas fluorescens*)	50 DAS	-	ATS
T_{10} (Inoculated control)	45 DAS	-	ATS
T_{11} (un inoculated control)	-	-	-
CD (0.05)			

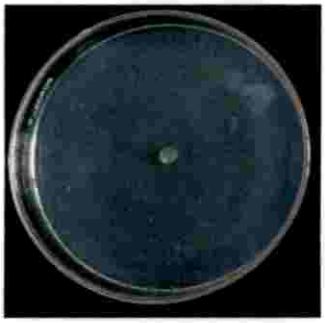
ATS – active tillering stage, DFS – days for formation of sclerotia

SDD – stage of disease development

a. Fermented weed extract

b. Fermented egg-lemon juice extract

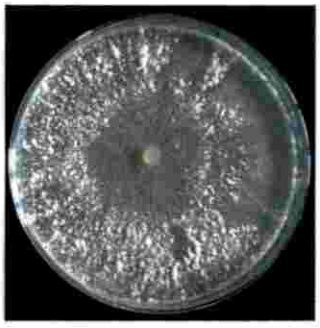
c. Panchagavya



d. Potassium silicate

e. Lime solution

f. Garlic extract



g. Control

Plate 4. Effective treatments selected from *in vitro* studies against *R. solani*

a. Fermented weed extract

b. Fermented egg lemon juice extract

c. Panchagavya

d.Potassium silicate

e. Lime solution

f.Garlic extract

g.Control

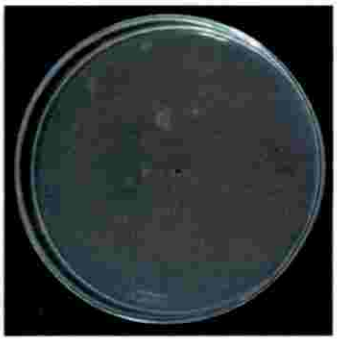
Plate 5. Effect of treatments *in vitro* on the inhibition of mycelial regeneration from sclerotia after 24 h

Fermented weed extract

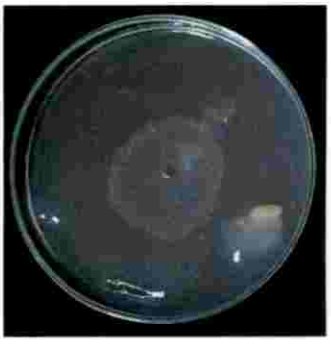
Fermented egg-lemon juice extract

Panchagavya

Potassium silicate



Lime solution

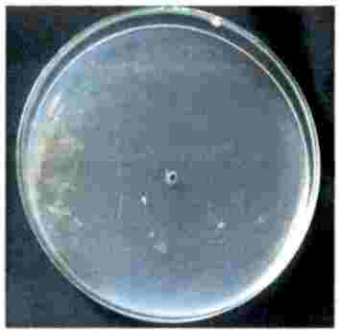


Garlic extract

Control

Plate 6. Effect of treatments *in vitro* on the inhibition of mycelial regeneration from sclerotia after 48 h

Fermented weed extract



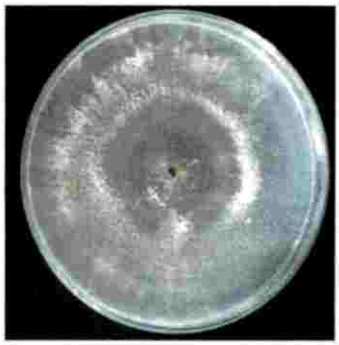
Fermented egg-lemon juice extract

Panchagavya

Potassium silicate

Lime solution

Garlic extract



Control

Plate 7. Effect of treatments *in vitro* on the inhibition of mycelial regeneration from sclerotia after 72 h

Plate 8. General view of pot culture experiment

a.Mycelial growth of *R. solani*

b. Sclerotia formation

Plate 9 a-b. Multiplication of *R. solani* in rice bran media for artificial inoculation

Table 13. The vertical spread was expressed as the relative lesion height (RLH) which was obtained from the height of the plant and lesion height. All the treatments significantly reduced the vertical spread of the disease compared to the inoculated control. The least RLH and the maximum percentage suppression over control were observed in the plants sprayed with fermented egg-lemon juice extract (15.24% and 79.42% respectively) and garlic extract (15.29% and 79.36% respectively), which were on par with the fungicide check hexaconazole (15.29% and 79.36% respectively) (Plate 10)

The horizontal spread (the spread of the disease to the adjacent tillers) was also observed. The horizontal disease spread was represented in terms of the total number of infected tillers per hill. The number of infected tillers per hill and the percentage of horizontal spread were the least for the plants sprayed with the fungicide check (1 No. and 10.44% respectively) which was on par with the plants sprayed with fermented egg-lemon juice extract (1.2 No. and 11.12% respectively) and potassium silicate (1.8 and 16.93% respectively) (Table 14) (Plate 11).

The lesion area (cm^2), which is a representation of the length and breadth of the lesion, was also observed for each plant as detailed in Table 15. The lesion area was the least for the plants sprayed with the fungicide check (4.73 cm^2) which was on par with those sprayed with fermented egg-lemon juice extract (6.85 cm^2) and potassium silicate (7.61cm^2). Thus the study revealed that among the nine treatments tested *in vivo* for the management of sheath blight, three treatments *viz.*, fermented egg-lemon juice extract, potassium silicate and garlic extract significantly reduced the number of infected tillers, disease spread and disease severity in terms of RLH.

4.6 EFFECT OF THE TREATMENTS ON BIOMETRIC AND YIELD ATTRIBUTES OF RICE

As far as the biometric observations of the rice plants sprayed with the different treatments were concerned, the plant height was observed to be maximum for the plants sprayed with garlic extract (98.5cm), fermented weed extract (96.7cm), fermented egg-lemon juice extract (93.8cm), *Pseudomonas*

Table 13. Effect of treatments on the vertical spread of sheath blight disease

Treatment	Height (cm)	Lesion height (cm)	RLH (%)	PSC (%)
T_1 (Fermented weed extract)	85.24^{abc}	17.06^{b}	20.01^{b}	72.99
T_2 (Panchagavya)	73.48^{de}	15.18^{bcd}	20.90^{b}	71.79
T_3 (Fermented egg-lemon juice extract)	85.38^{abc}	13.04^{d}	15.24^{c}	79.42
T_4 (Garlic extract)	90.64^{a}	13.87^{d}	15.29^{c}	79.36
T_5 (Lime solution)	79.22^{bcde}	14.82^{bcd}	18.70^{b}	74.76
T_6 (Potassium silicate)	72.14^{e}	13.34^{d}	18.48^{b}	75.06
T_7 (Hexaconazole)	59.60^{f}	9.02^{e}	15.29^{c}	79.36
T_8 (*Trichoderma viride*)	82.12^{abcd}	16.38^{bc}	19.97^{b}	73.04
T_9 (*Pseudomonas fluorescens*)	77.06^{cde}	14.5^{cd}	18.88^{b}	74.52
T_{10} (Inoculated control)	85.94^{ab}	63.64^{a}	74.15^{a}	-
T_{11} (un inoculated control)	85.60^{abc}	0	0	-
CD (0.05)	8.85	2.46	3.09	-
SE m (±)	1.96	0.54	0.69	-

Treatments with same alphabets do not differ significantly PSC – percentage suppression over control
Average of three replications

Table 14. Effect of treatments on the horizontal spread of sheath blight disease

Treatment	Tillers/ hill	Infected tillers/ hill	PHS (%)
T_1 (Fermented weed extract)	11.40[a]	3.60[b]	32.14[b]
T_2 (Panchagavya)	10.60[abc]	3.60[b]	34.60[b]
T_3 (Fermented egg-lemon juice extract)	11.00[ab]	1.20[e]	11.12[d]
T_4 (Garlic extract)	9.40[cde]	2.60[cd]	27.78[bc]
T_5 (Lime solution)	10.00[abcd]	3.40[bc]	34.12[b]
T_6 (Potassium silicate)	10.60[abc]	1.80[de]	16.93[cd]
T_7 (Hexaconazole)	9.60[bcd]	1.00[e]	10.44[d]
T_8 (*Trichoderma viride*)	9.60[bcde]	3.4[bc]	36.00[b]
T_9 (*Pseudomonas fluorescens*)	9.40[cde]	3.00[bc]	33.33[b]
T_{10} (Inoculated control)	8.40[de]	6.80[a]	82.22[a]
T_{11} (un inoculated control)	8.80[e]	-	-
CD (0.05)	1.55	0.955	11.958
SE m(±)	0.34	0.21	2.64

Treatments with same alphabets do not differ significantly
Average of three replications, PHS – percentage horizontal spread

Table 15. Effect of treatments on the lesion size of sheath blight disease

Treatment	Lesion height (cm)	Lesion width (cm)	Lesion area (cm^2)
T_1 (Fermented weed extract)	17.06 [b]	0.64 [bc]	11.16 [b] (3.29)
T_2 (Panchagavya)	15.18 [bcd]	0.62 [bc]	9.63 [bc] (3.07)
T_3 (Fermented egg-lemon juice extract)	13.04 [d]	0.52 [c]	6.85 [cd] (2.61)
T_4 (Garlic extract)	13.87 [d]	0.64 [bc]	8.88 [bc] (2.96)
T_5 (Lime solution)	14.82 [bcd]	0.56 [bc]	8.45 [bc] (2.87)
T_6 (Potassium silicate)	13.34 [d]	0.56 [bc]	7.61 [bcd] (2.71)
T_7 (Hexaconazole)	9.02 [e]	0.52 [c]	4.73 [d] (2.17)
T_8 (*Trichoderma viride*)	16.38 [bc]	0.62 [bc]	10.28 [bc] (3.16)
T_9 (*Pseudomonas fluorescens*)	14.5 [cd]	0.74 [ab]	10.16 [bc] (3.15)
T_{10} (Inoculated control)	63.64 [a]	0.88 [a]	56.01 [a] (7.46)
T_{11} (un inoculated control)	-	-	-
CD (0.05)	2.46	0.19	0.65
SE m(±)	0.54	0.04	0.14

Treatments with same alphabets do not differ significantly
Average of three replications

a.Fermented egg lemon juice extract

b. Potassium silicate

c. Hexaconazole

d. Inoculated control

Plate10 a-d. Effect of treatments on vertical spread of sheath blight disease

a.Fermented egg-lemon juice extract

b. Potassium silicate

c. Hexaconazole

d. Inoculated control

Plate 11 a-d. Effect of treatments on horizontal spread of sheath blight disease

fluorescens (93.5cm), lime solution (92.9cm) and *Trichoderma viride* (91.6cm) (Figure 3). The maximum number of tillers per hill was recorded for the plants sprayed with fermented weed extract (11.40), fermented egg-lemon juice extract (11), garlic extract (9.40), potassium silicate (10.60), lime solution (10) and panchagavya (10.60). The number of productive tillers per hill was the maximum for the plants sprayed with fermented weed extract (11.40), panchagavya (10.60), fermented egg-lemon juice extract (10.60), lime solution (10) and potassium silicate (10.40) (Figure 4). The number of infected tillers per hill was the least for hexaconazole (1), fermented egg-lemon juice extract (1.20) and potassium silicate (1.8) sprayed plants. The number of filled grains per panicle was the maximum in the plants sprayed with garlic extract (113.8), fermented egg-lemon juice extract (110.80), fermented weed extract (88.6), lime solution (92.40) and *Pseudomonas fluorescens* (102). The number of spikelets per panicle was the maximum in the case of plants which received the foliar application of fermented egg-lemon juice extract (118.80), garlic extract (115.40) and fermented weed extract (115) (Figure 5). The lesion height was observed to be the least for the plants sprayed with the fungicide check, *viz.*, hexaconazole (9.02cm) followed by fermented egg-lemon juice extract (13.04cm), garlic extract (13.87cm) and potassium silicate (13.34cm). There was no significant difference in the width of the lesions produced in the plants sprayed with the different treatments. The lesion area was the least for the plants sprayed with hexaconazole (4.73cm^2), fermented egg-lemon juice extract (6.85cm^2) and potassium silicate (7.61cm^2) (Table 16).

When the yield attributes of rice plants sprayed with the different treatments were analyzed, it was observed that the maximum grain yield was observed in the case of the plants sprayed with fermented weed extract (15.98g) which was on par with fermented egg-lemon juice extract (15.33g), garlic extract (14.66g), lime solution (13.65g) and potassium silicate (13.32g) (Table 17). There was no significant difference in 1000 grain weight among the treatments except for the inoculated and un-inoculated controls. The straw yield was recorded to be the maximum in the case of the plants sprayed with fermented

Table 16. Effect of treatments on biometric observations of rice.

Treatment	Plant height(cm)	Total tillers/hill	Productive tillers/hill
T_1 (Fermented weed extract)	96.70[a]	11.40[a]	11.40[a]
T_2 (Panchagavya)	86.10[bc]	10.60[abc]	10.60[ab]
T_3(Fermented egg-lemon juice extract)	93.80[ab]	11.00[ab]	10.60[ab]
T_4 (Garlic extract)	98.50[a]	9.40[cde]	9.4[bc]
T_5 (Lime solution)	92.90[ab]	10.00[abcd]	10[ab]
T_6 (Potassium silicate)	84.50[c]	10.60[abc]	10.40[ab]
T_7 (Hexaconazole)	57.50[d]	9.60[bcd]	9.40[bc]
T_8 (*Trichoderma viride*)	91.60[abc]	9.60[bcde]	9.40[bc]
T_9(*Pseudomonas fluorescens*)	93.50[ab]	9.40[cde]	8.40[c]
T_{10} (Inoculated control)	86.10[bc]	8.40[de]	8[c]
T_{11} (un inoculated control)	86.30[bc]	8.80[e]	8[c]
CD (0.05)	7.965	1.55	1.59
SE m(±)	1.76	0.34	0.35

Treatments with same alphabets do not differ significantly
Average of three replications

Table 17. Effect of treatments on the yield attributes of rice

Treatments	No. of filled grains/panicle	No. of spikelets/ panicle	Grain yield (g/plant)	1000 grain weight(g)	Straw yield (g/plant)
T_1 (Fermented weed extract)	88.6 ^{abcd}	115 ^{abc}	15.98 ^{a}	26.52 ^{a}	17.73 ^{a}
T_2 (Panchagavya)	77.6 ^{cde}	113.80 ^{bcd}	10.31 ^{d}	24.30 ^{abcd}	10.86 ^{e}
T_3(Fermented egg-lemon juice extract)	110.80 ^{ab}	118.80 ^{a}	15.33 ^{ab}	24.07 ^{bcd}	15.46 ^{abc}
T_4 (Garlic extract)	113.8 ^{a}	115.40 ^{ab}	14.66 ^{abc}	26.45 ^{ab}	14.53 ^{bcd}
T_5 (Lime solution)	92.40 ^{abcd}	112 ^{cdef}	13.65 ^{abcd}	24.87 ^{abc}	16.13 ^{ab}
T_6 (Potassium silicate)	84.60 ^{bcd}	112.20 ^{cde}	13.32 ^{abcd}	25.51 ^{ab}	12.66 ^{cde}
T_7 (Hexaconazole)	68.00 ^{de}	110.80 ^{defg}	10.59 ^{d}	24.80 ^{abc}	12.13 ^{de}
T_8(*Trichoderma viride*)	82.80 ^{bcd}	109.60 ^{efg}	11.67 ^{cd}	26.28 ^{ab}	10.19 ^{ef}
T_9(*Pseudomonas fluorescens*)	102.00 ^{abc}	110.40 ^{efg}	12.31 ^{bcd}	26.66 ^{a}	12.53 ^{cde}
T_{10} (Inoculated control)	65.80 ^{de}	108.40 ^{g}	5.664 ^{e}	22.01 ^{d}	7.22 ^{fg}
T_{11}(uninoculated control)	49.60 ^{e}	109 ^{fg}	6.33 ^{e}	22.54 ^{cd}	5.86 ^{g}
CD (0.05)	28.189	3.03	3.63	2.435	3.13
SE m(±)	6.25	0.67	0.81	0.54	0.69

Treatments with same alphabets do not differ significantly
Average of three replications

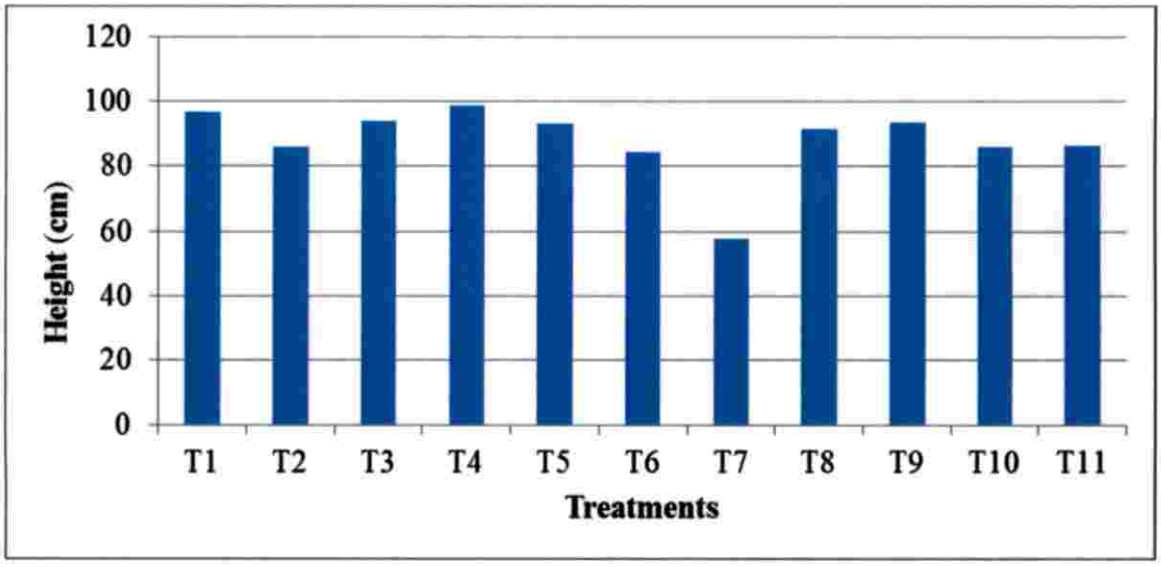


Figure 3. Effect of selected treatments on plant height

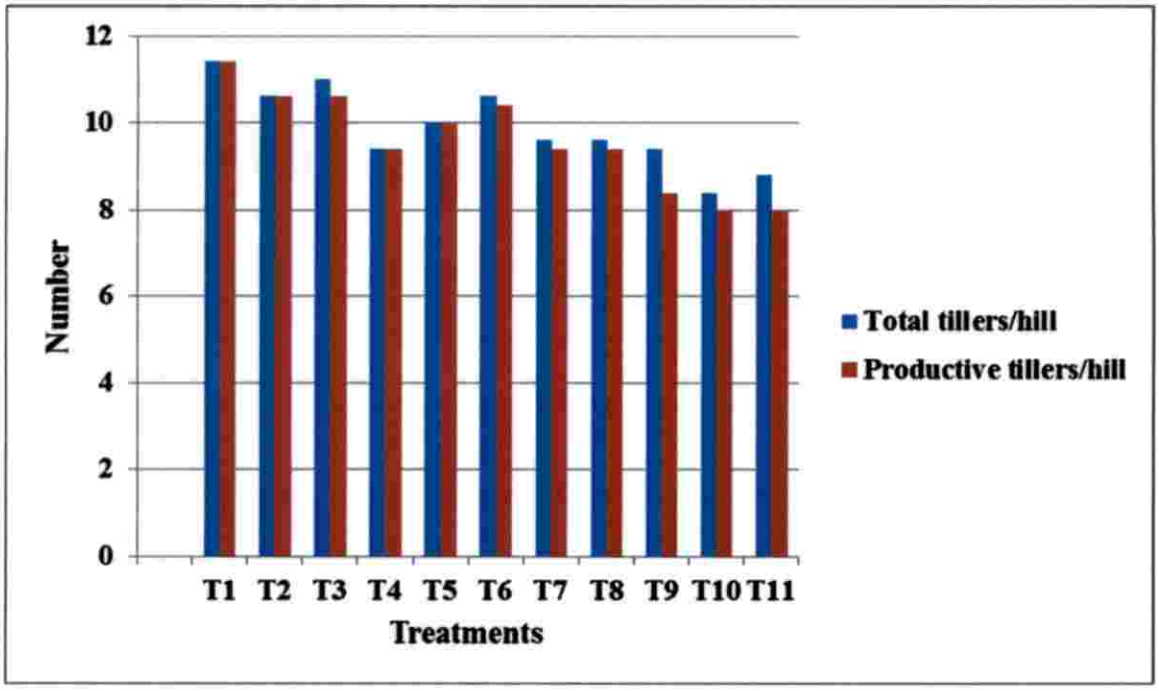


Figure 4. Effect of treatments on total tillers and productive tillers/ hill

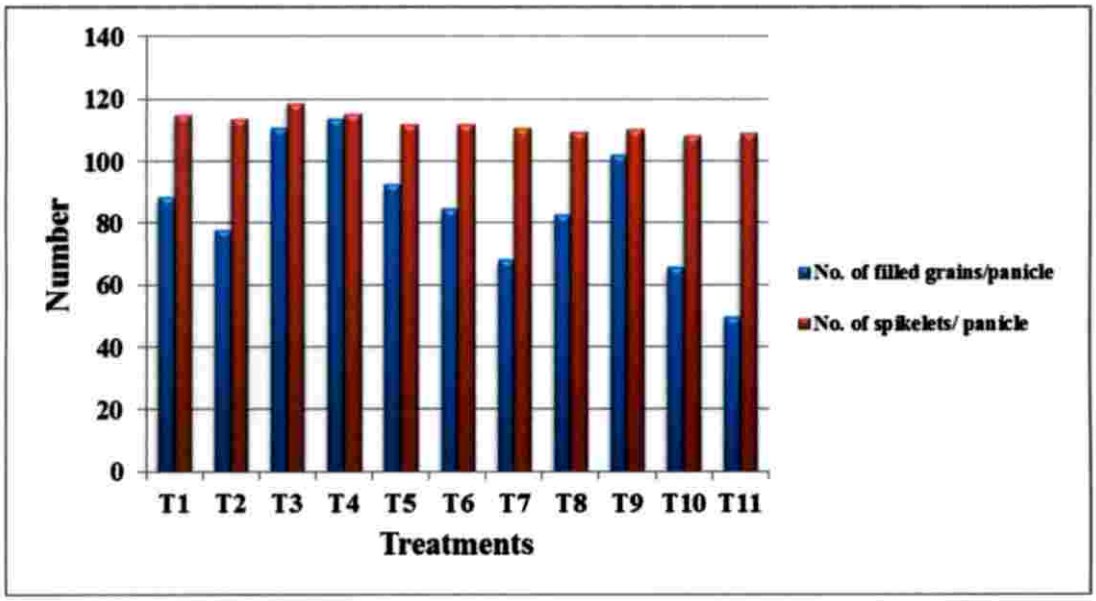


Figure 5: Effect of treatments on no. of filled grains and spikelets/panicle

weed extract (17.73g) which was on par with those sprayed with fermented egg-lemon juice extract (15.46g) and lime solution (16.13g) (Figure 6).

From this study, it is evident that the treatments *viz*., fermented egg-lemon juice extract (10%), potassium silicate (1%), garlic extract (10%) and fermented weed extract (100%) significantly improved both the biometric as well as the yield parameters of rice plants.

4.7 CHEMICAL ANALYSIS

4.7.1 Analysis of Nutrients and pH in Selected Treatments

The treatments selected for *in vivo* studies were analyzed for pH and total N, P and K contents as described in Table 18. The study revealed that fermented weed extract (4.9 pH), panchagavya (4.2 pH) and fermented egg–lemon juice extract (4.5 pH) were highly acidic. The garlic extract was found to be slightly acidic (5.2 pH). The lime solution (11.9 pH) and potassium silicate (10.8 pH) showed alkaline nature. The total nitrogen content was the maximum for panchagavya (1870 mg/l) followed by fermented egg lemon juice extract (1100 mg/l). The total nitrogen content was the least in the case of lime solution (33 mg/L). The total phosphorous content was observed to be the maximum for panchagavya (730 mg/l) followed by fermented egg-lemon juice extract (610 mg/l) among the various treatments. The total potassium content was the maximum for panchagavya (3160 mg/l) followed by potassium silicate (1080 mg/l). The total potassium content was observed to be the least in the case of lime solution (32 mg/L).

4.7.2 Analysis of Major Nutrients in the Plants at Harvest

All the plants sprayed with the different treatments were analyzed for total N, P and K content at harvest. The total nitrogen content was observed to be the maximum for the plants sprayed with garlic extract (1.65%) and the least for inoculated control (1.32%). There was no significant difference in the total phosphorous content among the plants sprayed the different treatments. The total potassium content was maximum for plants sprayed with potassium silicate (3.60%) followed by garlic extract (3.12%), fermented egg-lemon juice extract (3.011%) and lime solution (2.99%) (Table 19).

Table 18. Content of major nutrients and pH in the effective treatments

Treatment	pH	N (mg/l)	P (mg/l)	K (mg/l)
T_1 (Fermented weed extract)	4.9	530.0	81.0	320.0
T_2 (Panchagavya)	4.2	1870.0	730.0	3160.0
T_3 (Fermented egg-lemon juice extract)	4.5	1100.0	610.0	760.0
T_4 (Garlic extract)	5.2	750.0	41.0	96.0
T_5 (Lime solution)	11.9	33.0	8.3	32.0
T_6 (Potassium silicate)	10.8	99.0	3.7	1080

Table 19. Content of major nutrients in rice at harvest

Treatment	Total N (%)	Total P (%)	Total K (%)
T_1 (Fermented weed extract)	1.03^{f}	0.189	2.732^{e}
T_2 (Panchagavya)	1.46^{cd}	0.256	2.889^{d}
T_3 (Fermented egg-lemon juice extract)	1.46^{cd}	0.237	3.011^{c}
T_4 (Garlic extract)	1.65^{a}	0.192	3.121^{b}
T_5 (Lime solution)	1.46^{cd}	0.167	2.991^{c}
T_6 (Potassium silicate)	1.48^{cd}	0.224	3.601^{a}
T_7 (Hexaconazole)	1.52^{bc}	0.216	2.511^{f}
T_8 (*Trichoderma viride*)	1.46^{cd}	0.267	1.899^{i}
T_9 (*Pseudomonas fluorescens*)	1.48^{cd}	0.301	2.134^{g}
T_{10} (Inoculated control)	1.32^{e}	0.185	2.106^{h}
T_{11} (un inoculated control)	1.38^{de}	0.166	1.916^{i}
CD (0.05)	0.11	NS	0.025
	0.02	-	0.02

Treatments with same alphabets do not differ significantly

Average of three replications

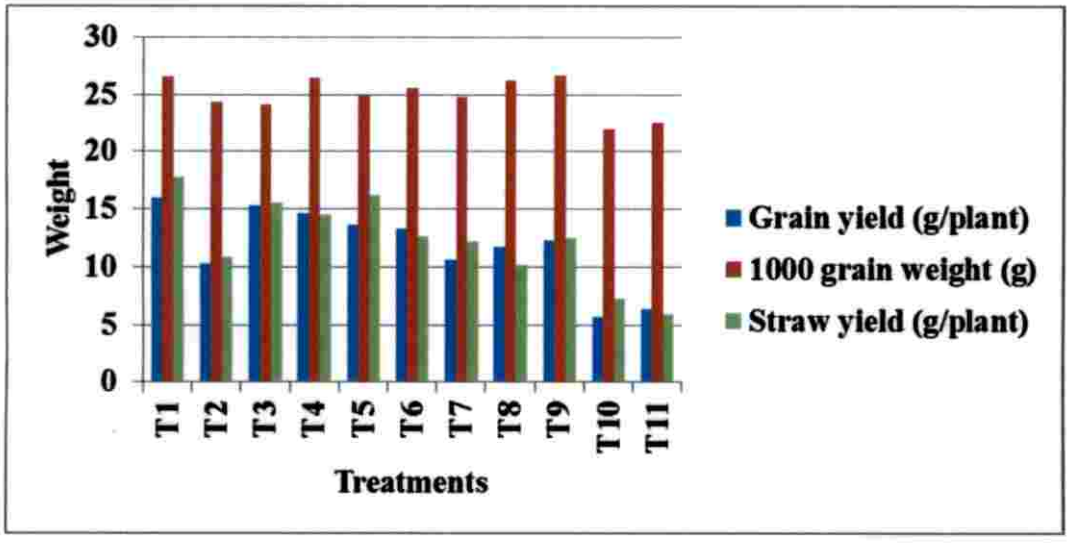


Figure 6: Effect of treatments on yield attributes

DISCUSSION

5. DISCUSSION

Rice is the most economically important food crop of India. Diseases are one of the most important reasons for its decline in production and quality. The annual losses due to rice diseases are estimated to be 10 to15 per cent worldwide. Among the diseases, sheath blight caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) causes significant yield loss (Lee and Rush, 1983). According to Feakin (1977) and Meng *et al.* (2001), yield loss ranging from 25 to 50 per cent occurs due to the disease. The use of fungicides is the most common management practice undertaken against the disease (Dev and Mary, 1986). Indiscriminate use of fungicides may lead to environmental pollution, health hazards, residual toxicity and pathogen resistance. Besides, the perpetuating and resting structures of the fungus *viz.*, the sclerotia survive in the soil for longer duration (Dath, 1990). Soil drenching with fungicides is highly expensive and impractical under flooded conditions. Hence, the search for alternative strategies for the management of the disease has gained momentum (Jacobsen and Backman, 1993). Thus, the present study entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken at Integrated Farming System Research Station, Karamana and College of Agriculture, Vellayani, during 2015-17 for developing an organic and ecofriendly disease management strategy for sheath blight. The results obtained in the study are discussed in this chapter.

In the present study, seven rice growing tracts *viz.*, Chenkal, Attingal, Punchakari, Nagaroor, Vellayani, Karode and Karamana were identified for the collection of sheath blight infected rice plants. It was observed that sheath blight incidence was present in all the tracts which implicate the importance of the disease. Among the seven tracts, the symptoms were observed during the active tillering stage of the crop in six rice tracts, whereas in Karode tract, the disease appeared during the late tillering stage. Thus active tillering stage can be concluded as the most susceptible stage for sheath blight incidence. Similar results were reported by Yoshimera and Nishizawa (1954) who suggested that

maximum tillering stage is the most susceptible growth stage for sheath blight incidence. Rodrigues *et al.* (2003) also reported that all the growth stages of rice were susceptible to sheath blight infection, but the most susceptible stage was 45 days after emergence which is in confirmation with the results of this study. In contrary, Adhipathi *et al.* (2013) observed that the disease occurrence and its spreads were severe during the late tillering, internode elongation, booting and flag leaf emergence stages.

The seven isolates of the sheath blight pathogen collected from the different tracts were isolated and pathogenicity studies were conducted to prove Koch's postulates. Typical sheath blight symptoms were produced by each isolate on the artificially inoculated plants. The studies also revealed that the maximum lesion size (2.19 cm^2) was produced by the isolate collected from Attingal (I_2) on three days after inoculation (DAI). Similar results were reported by Chakraborty *et al.* (2006) who found that artificial inoculation of rice plants by sheath blight pathogen resulted in a maximum lesion length of 2.45 to 4.75 cm. Rodrigues *et al.* (2003) also proposed that regardless of the growth stage of rice, the incubation period for sheath blight disease development was from 48 to 96 hours after inoculation.

The symptoms of sheath blight were exhibited as grey coloured lesions, with the colour of the margin of the lesions varying from brown, dark brown, greyish brown to purplish brown. The result is in concurrence with the findings of Growth and Nowick (1992), Vidyasekharan *et al.* (1997) and Hollier *et al.* (2009) who reported the presence of elliptical or ovoid to irregular greenish, grey lesions with brown margin at or above the water level, on the base of the leaf sheath and on leaf blades of sheath blight affected rice plants. Round, mustard like, dark brown sclerotia were produced by I_2 and I_1 which is in confirmation with the findings of Ou (1985) who reported the presence of dark brown sclerotia attached to the lesions on the leaf sheaths of infected rice plants.

Johanson *et al.*, (1998) reported that sheath blight disease diagnosis is extremely difficult and often inaccurate by visual observation, particularly during the early stages of lesion development. This necessitates the study on virulence as

well as the morphology and physiology of different strains of *R. solani* collected and isolated from varied geographical locations. Virulence rating of the seven isolates was carried out by two methods, *viz*., cut stalk assay as well as by direct inoculation on potted plants. In the cut stalk assay method, three isolates (I_1, I_2 and I_7) produced the maximum lesion length. Further, virulence rating by direct inoculation on potted plants identified Attingal isolate (I_2) of *R. solani* as the most virulent one as it exhibited the maximum relative lesion height (RLH) and number of affected tillers. Thus, the Attingal isolate (I_2) was identified to be the most virulent isolate by both the above mentioned methods of virulence rating. The result is in confirmation with the works of Lal *et al.* (2012) who characterized the virulence pattern and revealed that, upon artificial inoculation using 25 isolates of *R. solani*, two isolates produced the maximum RLH of 69.66 and 34.55 per cent during the active tillering and panicle initiation stages. The study also revealed the reliability of cut stalk assay method which is easy and time saving.

Characterization of the different isolates of the pathogen was undertaken *in vitro*. Morphological characterization revealed the presence of right angled branching, septum at the origin and constriction at the base of branching, which are all the characteristic features of *R. solani*. The sclerotia when crushed and observed under the microscope revealed the presence of moniliod cells which is also a characteristic feature of *R. solani*. Duggar (1915) partly characterized the *Rhizoctonia* spp. based on the diameter of vegetative hyphae, constriction at the point of branching and right angled branching of mature hyphae. Parmeter and Whitney (1970) gave a detailed description of the various characteristics specific to *R. solani*. The observations are similar to the findings of Moni *et al.* (2016) who revealed that the 18 isolates *of R. solani* they collected had hyphal branching at right angle, constriction at the point of branching of the mycelium and the presence of septum near the branching junction and were confirmed as *R. solani.* Ontogenetical studies have shown that in *Rhizoctonia,* monilioid cells are the precursors of sclerotia (Tu and Kimbrough, 1975) and that their presence is an indication of their identity (Saksena and Vaartaja, 1961; Taheri *et al.*, 2007).

Among the cultural characteristics studied, the mycelial colour of the isolates varied from light brown to dark brown and the mycelial texture from fluffy to cottony. The results are in consensus with the findings of Palo (1926) who reported that the mycelia permanently remaining white or showing pigmentation other than any shades of brown are not considered as *R. solani* and that the brown pigment appears to be a stable diagnostic colony character of *R. solani* under *in vitro* conditions. The discolouration of the growth media of *R. solani* may be due to the production of pigments by the pathogen as proposed by Sunder *et al.* (2003) and the difference in the colour intensity among the isolates may be due to the amount of the pigments released by the isolates in the media.

The present study also revealed that the growth pattern of the sclerotia produced by the seven isolates in petri plates ranged from scattered to central. This is in confirmation with the works of Susheela and Reddy (2013) who collected 35 isolates of *R. solani* from different rice growing agro ecological regions of India and found that the sclerotial positions varied from peripheral, central, peripheral & central as well as scattered.

Thus there was significant variation in the virulence pattern, morphological and cultural characteristics among the seven isolates of *R. solani* collected from the different tracts. This result is in concurrence with the findings of Jayaprakashvel and Mathivanna (2012) who proposed that among the isolates of *R. solani*, there was considerable variation in the mycelial growth, sclerotial emergence, sclerotial colour and their pattern of distribution. The findings are in confirmation with that of Taheri *et al.* (2007) who proposed that geographical region was the dominant factor determining the population structure of *R. solani.*

The morphological and cultural characterization of the isolates could tentatively identify them as *R. solani* isolates. Further to confirm the identification, molecular identification based on the ITS region of rDNA of the most virulent isolate of the pathogen was carried out and it revealed 100 per cent similarity with *Rhizoctonia solani* AG-1 IA isolate CSU8. This is in consensus with the reports of Taheri *et al.* (2007) who proposed that among the 110 *R. solani* isolates, 96 isolates belonged to AG-1 IA.

Among the 19 treatments tested for their potential in inhibiting the mycelial growth of *R. solani*, six treatments namely, fermented egg-lemon juice extract (10%), fermented weed (*Setaria barbata*) extract (100%), panchagavya (5%), garlic extract (10%), potassium silicate (1%) and lime solution (12.5%) resulted in complete (100%) inhibition of the mycelial growth of *R. solani*. Studies on the potential of egg proteins against human diseases have been reported by some scientists. The biologically active functional molecules on the egg white, yolk, shell and membrane possessing unique functional properties such as antiviral, antibacterial, enzymatic and immunological activities have been reported by Ibrahim *et al.* (2002). Cystatin, the affinity purified from the chicken egg white has been reported to have antifungal activity against human fungal pathogen, *viz.*, *Candida* species (Kolaczkowska *et al.*, 2010). Citrus juice has been reported to have significant antimicrobial activity (Hindi and Chabuck, 2013). However, no reports are there proving the antifungal potential of fermented egg-lemon juice extract against plant pathogenic fungi. The antifungal potential of this extract may be due to the activity of the microbes produced during its preparation and fermentation or due to the presence of metabolites which need to be further confirmed.

Fermented weed (*Setaria barbata*) extract was also revealed to result in 100 per cent inhibition of the mycelial growth of *R. solani*. The exploration of the antifungal activity of weeds remains an area of interest, but not many reports are available on the exploitation of the antifungal property of weed plants against plant diseases. Srivastava and Singh (2011) reported that the dried leaf powder (20 mg/ml) of two weeds *viz.*, *Lantana camara* and *Parthenium hysterophorus* inhibited the mycelial growth (59.5 and 45.9% respectively) of *Alternaria* species. Pal *et al.* (2013) studied on the effect of eleven weed plants for their antifungal activity against the seed borne phytopathogenic fungi, viz., *Alternaria* sp and found that the extracts of the weeds *viz.*, *Ageratum conyzoides* and *Parthenium hysterophorus* showed the most potential antifungal activity against the fungus. The antifungal potential of *Setaria barbata* need to be further confirmed.

Lime solution (12.5%) also resulted in 100 per cent mycelial inhibition of *R. solani*. The results are in consensus with the findings of Stosic *et al.* (2014) who reported that calcium chloride and calcium hydroxide at 1.5 and 2 per cent concentrations significantly decreased the spore germination and germ tube growth of *Colletotrichum acutatum, C. gloeosporioides, Alternaria alternata and Penicillium expansum*.

Potassium silicate at one per cent concentration also completely inhibited the mycelial growth of *R. solani*. The study is in confirmation with findings of Menzies *et al.* (1992) who proposed that potassium silicate has a direct inhibitory influence on fungal growth. Epstein (1999) also proposed that potassium silicate increases the host defense system or strengthens the plant cell walls thereby, inhibiting infection. The inhibition of fungal growth by potassium silicate was due to pH effect.

Garlic extract at ten per cent concentration also completely inhibited the mycelial growth of *R. solani*. The findings are in confirmation with the works of Avato *et al.* (2000) and Kyung and Lee (2001) who proposed that several biologically active compounds are present in garlic extract which affect a wide range of soil borne fungal pathogens. The strong inhibition of *R. solani* by garlic extract may be due to the presence of sulphur compounds and its active antimicrobial component *viz.*, allicin as proposed by Singh and Singh (2005) and Portz *et al.* (2008).

Panchagavya could result in 100 per cent inhibition of *R. solani in vitro*. The finding is in consensus with that of Sugha (2005) who reported that panchagavya resulted in 40 to 100 per cent inhibition of the mycelial growth of *R. solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, Phytophthora colacasiae* and *Fusarium solani*. The antifungal property of panchagavya may be attributed due to the presence of antimicrobial substances in cow dung such as patulodin like compounds, CK2108A and CK2108B produced by *Eupenicillium bovifimosum* present in cow dung as proposed by Dorothy and Frisvad (2002). The release of the antimicrobial compounds may be due to the microorganisms like bacteria,

fungi and actinomycetes present in panchagavya as reported by Swaminathan *et al.* (2007).

In the study for the inhibition of mycelial regeneration from sclerotia, sclerotia were dipped in the best six treatments selected from *in vitro* studies for 24, 48 and 72 h. The study revealed that fermented egg-lemon juice extract, fermented weed extract, panchagavya and lime completely inhibited the mycelial regeneration from sclerotia after 24 h of dip. Garlic extract could completely inhibit the mycelial regeneration 72 h after sclerotial dip. The reason whether high pH and calcium were fungistatic or fungicidal to the resting spores have not been resolved. Myers and Campbell (1985) proposed that calcium and a high pH may have little fungicidal effect on resting spores, but may affect the pathogen penetration or development in the host. He *et al.*, 2014 proposed that the effect of calcium carbonate on *Ralstonia solanacearum* of tobacco was mainly related to the role of calcium (Ca^{2+}). Jiang *et al.* (2013) reported that the mechanism of action of calcium against bacterial wilt of tomato may be due to the increased activity of peroxidase and poly phenol oxidase.

Potassium silicate resulted only in less inhibition of the mycelial regeneration for sclerotia. Similar results were proposed by Wainwright *et al.* (1997) who reported that visible mycelial growth was present on silicic acid amended media and that of Wainwright (1993) who suggested that nutrient free silica gel supported the fungal growth with the gel itself acting as a nutrient source, stimulating its spore germination.

Foliar application of fermented egg-lemon juice extract (10%), potassium silicate (1%) and garlic extract (10%) significantly reduced the number of infected tillers, the disease spread and the disease severity in terms of RLH. No scientific reports are there regarding plant disease management by fermented egg-lemon juice extract. The results are in concurrence with the findings of Menzies *et al.* (1992) who reported that foliar application of silicon in field plants is a potentially viable alternative to root zone application. The effect of foliar application of potassium silicate may be due to the fact that it activates the host defense mechanisms by increased accumulation of phenolic compounds, chitinases,

peroxidases and poly phenol oxidases as reported by Cheriff *et al.* (1994). The presence of low molecular weight metabolites like phytoalexins *viz.*, flavanone aglycone rhamnetin may be the reason for the enhanced disease resistance as studied by Fawe *et al.* (1998) who worked in the powdery mildew disease of cucumber. Silicon has an active role in the induction of plant defense mechanisms by the production of compounds such as flavonoids (Fawe *et al.*, 1998), diterpenoids (Rodrigues *et al.*, 2004) and pathogenesis related proteins (Liang *et al.*, 2005). Kettlewell *et al.* (2000) suggested that potassium reduces the disease severity by various modes of action like regulation of photosynthesis, respiration and osmotic pressure regulation.

No reports are there on the *in vivo* antifungal potential of garlic extract against rice sheath blight disease. However, Obagwu and Korsten (2003) proposed that garlic extract has a significant effect (*in vitro* and *in vivo*) on the growth of both *Penicillium digitatum* and *P. italicum*.

Foliar application of fermented egg-lemon juice extract (10%), potassium silicate (1%) and garlic extract (10%) were observed to significantly improve both the biometric as well as the yield parameters of rice plants. No scientific reports are there on the effect of fermented egg-lemon juice extract or garlic extract on the growth and yield improvement. The results are in concurrence with those of Blunden (1991) and Mooney and Banstaden (1986) who reported that the increased growth of soyabean plants sprayed with sea weed extract may be due to the presence of some growth promoting substances present in the extract. The increase in the yield of rice plants sprayed with sea weed extract might be due to the fact that the extract was a biostimulant which can provide micro and macro nutrients as well as significant amount of cytokinins, auxins and betaines as suggested by Blunden, (1991), ultimately increasing the production of chlorophyll by boosting the photosynthesis process and stimulating vegetative growth. Lalithya *et al.,* (2014) proposed that foliar application of silicon in sapota plants have helped in increasing the cell division, more nutrient and water uptake thereby resulting in the production of more number of fruits. It might have helped

in improving the fruit quality due to the suppression of respiration and reduction in ethylene evolution and thus minimizing the physiological loss in fruit weight.

The analysis of the pH and major nutrients in the treatments selected for *in vivo* studies revealed that fermented weed extract, panchagavya and fermented egg–lemon juice extract were highly acidic. The total N content was the maximum for panchagavya. The total P content was observed to be the maximum for panchagavya followed by fermented egg-lemon juice extract. The total K content was the maximum for panchagavya followed by potassium silicate. Similar findings were reported by Gore and Sreenivasa (2011) who proposed that fermented liquid organic manures contain microbial load and plant growth promoting substances in addition to the nutrients that help in improving plant growth, metabolic activities and resistance to pests and diseases.

In the present study, fermented egg-lemon juice extract (10%), potassium silicate (1%) and garlic extract (10%) were found to be the three promising treatments in improving the growth and yield parameters of rice as well as in reducing the sheath blight incidence. The studies on the analysis of the total N, P and K contents revealed that fermented egg-lemon juice extract had a higher content of total N and P, indicating a positive role towards improving the vegetative and root growth of rice plants. Potassium silicate was observed to have a higher content of K, which indicates a possible role in enhancing flower and fruit development as well as in imparting host resistance.

The total N content was the maximum in the plants sprayed with garlic extract. There was no significant difference in the total P content among the treatments. The total K content was the maximum in the plants sprayed with potassium silicate followed by garlic extract, fermented egg-lemon juice extract and lime solution. The findings are similar to those of El-Fouly and El-Sayed (1997) who reported that foliar feeding of a nutrient promotes the root absorption of the same nutrient or other nutrients through improving the root growth and that of Lalithya *et al.,* (2014) who studied that Si application can avoid leaching loss of N and can help in more accumulation of N in leaves and also of Pramanick *et al.* (2014) who reported that the presence of micro elements and plant growth

regulators especially cytokinins in the sea weed extract is responsible for the increased yield and improved nutrition of rice receiving foliar application of sea weed extract.

Thus, the present study reveals the scope of use of indigenous organic preparations/ botanicals/ non-hazardous chemicals for disease management in plants. From this study, it was revealed that three treatments *viz*., fermented egg-lemon juice extract (10%), potassium silicate (1%) and garlic extract (10%) resulted in improved biometric and yield attributes of rice plants and also resulted in reduced sheath blight incidence. Narrowing down to a single, most effective and economical treatment, it can be concluded that four foliar sprays with potassium silicate at one per cent concentration at 45^{th}, 60^{th}, 75^{th} and 90^{th} DAS appreciably reduced the sheath blight disease incidence which was found to be as effective as the fungicide check (hexaconazole) compared to other two treatments.

SUMMARY

6. SUMMARY

The thesis work entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken at Integrated Farming System Research Station (*in vitro* studies) and at College of Agriculture, Vellayani (*in vivo* studies) during 2016-17 to develop an ecofriendly and safe management strategy against sheath blight disease of rice. Sheath blight affected rice plants were collected from seven different rice growing tracts of Thiruvanathapuram district namely, Chenkal, Attingal, Punchakari, Nagaroor, Vellayani, Karode and Karamana based on the extent of rice cultivation. The variety of the rice cultivated in all the tracts was Uma.

The sheath blight disease incidence was observed during the active and late tillering stage. The symptoms of the disease were exhibited as grey coloured lesions, with the colour of the lesion margin varying from brown, dark brown, greyish brown to purplish brown. The presence of numerous and chocolate brown coloured sclerotia was observed only in the infected rice samples collected from the Punchakari tract under natural conditions.

The pathogenicity studies revealed that in the rice plants artificially inoculated with the isolate collected from Karode (I_6), the symptom development was observed on the seventh day after inoculation (DAI) whereas the symptom development was noticed on the third day after inoculation for the other six isolates (I_1, I_2, I_3, I_4, I_5 and I_7). The lesion size observed was a minimum of 0.07 cm^2 the case of Karode isolate (I_6) to a maximum size of 2.19 cm^2in the case of Attingal (I_2) isolate. Formation of sclerotia was noticed only in the plants inoculated with the isolates I_1 (Chenkal) and I_2 (Attingal) on the 11^{th} and 12^{th} DAI respectively. The sclerotia appeared to be chocolate brown in the case of Chenkal (I_1) and dark brown in the case of Attingal (I_2) isolate inoculated rice plants respectively.

In the cut stalk assay method of virulence rating, it was revealed that the lesion length on the excised stalks produced by the three isolates namely, Chenkal

(I_1), Attingal (I_2) and Karamana (I_7) were the maximum (11.65, 12.58 and 11.00 cm respectively) on the 7th day after inoculation and were on par with each other.

In the virulence rating by artificial inoculation on intact leaf sheath of 45 days old potted rice plants, the symptom development was observed on 3rd day after inoculation on the leaf sheaths inoculated with all the isolates except I_6(Karode). The isolate from Attingal (I_2)produced the maximum relative lesion height (RLH) on 5th (9.20 %), 7th (15.70 %), 10th (19.03 %) and 15th (20.96 %) DAIas well asthe maximum number of infected tillers(4.66).Thus virulence rating revealed that Attingal (I_2) isolate was found to be the most virulent isolate among the seven isolates.

The morphological and cultural characters of the seven isolates of *R. solani* were studied. It was observed that the mycelial colour of the isolates ranged from light brown to dark brown whereas the isolate from Karode (I_6) exhibited white coloured mycelium. The mycelial texture also ranged from fluffy to cottony in the various isolates. The mycelial growth was found to be the maximum in the case of Attingal isolate (I_2) on 1st (3.60 cm) and 2nd (8.26) days after inoculation. The sclerotia produced by five isolates (I_1, I_2, I_3, I_4 and I_7) exhibited the maximum average size (2.13, 2.11, 1.99, 1.91 and 2.01 mm respectively). Karode isolate (I_6) produced the maximum number of minute sclerotia (104.66) which was followed by the Attingal isolate (I_2) (59.33) and Karamana isolate (I_7). The growth pattern of the sclerotia in Petri plates ranged from scattered to central in nature.

The morphological and molecular identification of the most virulent isolate of *R. solani*done at the National Fungal Culture Collection of India (NFCCI), Pune revealed the fungus to be *Rhizoctonia solani* J.G. Kuhn of family Ceratobasidiaceae. The identification of the isolate based on the ITS (Internal Transcribed space) region of rDNA showed 100 per cent similarity with *Rhizoctonia solani,* current name *Thanatephorus cucumeris* (A. B. Frank) Donk (1956) and was assigned the NCBI accession number KX674527. Further, microscopic studies (100 X) of the mycelium of the virulent isolate of the fungus

revealed the presence of right angled branching, septum at the origin of branching and constriction at the base which are the characteristic features of *R. solani*. The sclerotia when crushed and observed under the microscope revealed the presence of monilioid cells whichfurther confirm the fungus to be *R. solani*.

In vitro studies for evaluating the potential of 19 treatments in inhibiting the mycelial growth of *R. solani* revealed that six treatments namely, fermented egg–lemon juice extract (10%), fermented weed (*Setariabarbata*) extract (100%), panchagavya (5%), garlic extract (10%), potassium silicate (1%) and lime solution (12.5%) resulted in complete (100%) inhibition of the mycelial growth of *R. solani*. The next best effective treatment was fermented tapioca leaf – rind extract (91.85%) followed by neem cake (87.04%). Sclerotial formation was observed in plates amended with biogas slurry (10%), jeevamruth (10%) and diluted cow's urine (10%).

The study on the dipping of sclerotia for different time intervals revealed that dipping in lime solution (12.5%), fermented weed extract (100%), fermented egg-lemon juice extract (10%) and panchagavya (5%) for 24 hours itself resulted in complete (100%) inhibition of the mycelial regeneration from the sclerotia. Garlic extract (10%) resulted in complete inhibition of the mycelial regeneration from the sclerotia only after 72 hours after dipping. Potassium silicate resulted in only less inhibition (27.28%) of the mycelial regeneration even after 72 hours.

A pot culture experiment was conducted to evaluate the efficacy of six treatments along with five checks. White mycelial growth was observed 5 DAI, which covered the rice bran medium within 2 weeks. Numerous white sclerotial initials were observed in rice bran medium after 2 weeks which later turned brown in colour.The pot culture study revealed that the symptoms of rice sheath blight disease developed during 45-52 DAS, which coincided during the active tillering stage of the crop.

The least RLH and the maximum percentage suppression over control were observed in the plants sprayed with fermented egg-lemon juice extract

(15.24% and 79.42% respectively) and garlic extract (15.29% and 79.36% respectively), which were on par with the fungicide check hexaconazole (15.29% and 79.36% respectively).

The number of infected tillers per hill and the percentage of horizontal spread were the least for the plants sprayed with the fungicide check (1 No. and 10.44% respectively) which was on par with the plants sprayed with fermented egg-lemon juice extract (1.2 No. and 11.12% respectively) and potassium silicate (1.8 and 16.93% respectively).

The lesion area was the least for the plants sprayed with the fungicide check (4.73 cm^2) which was on par with those sprayed with fermented egg-lemon juice extract (6.85 cm^2) and potassium silicate (7.61cm^2).

Among the biometric observations, the plant height was observed to be the maximum for the plants sprayed with garlic extract (98.5 cm), fermented weed extract (96.7 cm), fermented egg-lemon juice extract (93.8 cm), *Pseudomonasfluorescens* (93.5 cm), lime solution (92.9 cm) and *Trichodermaviride* (91.6 cm). The maximum number of tillers per hill was recorded for the plants sprayed with fermented weed extract (11.40), fermented egg-lemon juice extract (11), garlic extract (9.40), potassium silicate (10.60), lime solution (10) and panchagavya (10.60). The number of productive tillers per hill was the maximum for the plants sprayed with fermented weed extract (11.40) panchagavya (10.60), fermented egg-lemon juice extract (10.60), lime solution (10), potassium silicate (10.40) and hexaconazole (10).

The number of filled grains per panicle was the maximum in the plants sprayed with garlic extract (113.8), fermented egg-lemon juice extract (110.80), fermented weed extract (88.6), lime solution (92.40) and *Pseudomonasfluorescens* (102). The number of spikelets per panicle was the maximum in the case of plants which received the foliar application of fermented egg-lemon juice extract (118.80,) garlic extract (115.40) and fermented weed extract (115).

The number of infected tillers per hill was the least for hexaconazole (1), fermented egg-lemon juice extract (1.20) and potassium silicate (1.8) sprayed plants. The lesion height was observed to be the least for the plants sprayed with the fungicide check, *viz.*, hexaconazole (9.02 cm) followed by fermented egg-lemon juice extract (13.04 cm), garlic extract (13.87 cm) and potassium silicate (13.34 cm). There was no significant difference in the width of the lesions among the treatment. The lesion area was the least for the plants sprayed with hexaconazole (4.73 cm^2), fermented egg-lemon juice extract (6.85 cm^2) and potassium silicate (7.61 cm^2).

As far as the yield attributes were concerned, the maximum grain yield was observed in the case of the plants sprayed with fermented weed extract (15.98g) which was on par with fermented egg-lemon juice extract (15.33g), garlic extract (14.66g), lime solution (13.65g) and potassium silicate (13.32 g). There was no significant difference in 1000 grain weight among the treatments except for inoculated and un-inoculated control. The straw yield was recorded to be the maximum in the case of the plants sprayed with fermented weed extract (17.73g) which was on par with those sprayed with fermented egg-lemon juice extract (15.46g) and lime solution (16.13g).

Fermented egg-lemon juice extract was recorded to have the maximum plant height, total tillers per hill, productive tillers per hill, number of filled grains and spikelets per panicle, grain yield and straw yield with the least number of infected tillers per hill, lesion height and lesion area. Spraying with potassium silicate spray was recorded the maximum total tillers and productive tillers per hill, grain yield with the least number of infected tillers per hill, lesion height and lesion area. The garlic extract spray was found to have the maximum plant height, total tillers per hill, number of filled grains and spikelets per panicle, grain yield with the least lesion height and lesion area.

The effective six treatments when analyzed for pH and the total N, P and K content, it was revealed that the pH was highly acidic for fermented weed

extract (4.9), panchagavya (4.2) and fermented egg–lemon juice extract (4.5). Garlic extract was found to be slightly acidic (5.2). The lime solution (11.9) and potassium silicate (10.8) showed alkaline nature.

The total nitrogen content was the maximum for panchagavya (1870 mg/l) followed by fermented egg lemon juice extract (1100 mg/l). The total nitrogen content was the least in lime solution (33 mg/L). The total phosphorous content was observed to be the maximum for panchagavya (730 mg/l) followed by fermented egg-lemon juice extract (610 mg/l) among the various treatments. The total potassium content was the maximum for panchagavya (3160 mg/l) followed by potassium silicate (1080 mg/l). The total potassium content was observed to be the least in lime solution (32 mg/L).

Rice plants were analyzed for their total N, P and K at harvest. The total nitrogen content was observed to be the maximum in the plants sprayed with garlic extract (1.65%) and the least for inoculated control (1.32%). There was no significant difference in the total phosphorous content among the treatments. The total potassium content was maximum in plants sprayed with potassium silicate (3.60%) followed by garlic extract (3.12%), fermented egg-lemon juice extract (3.011%) and lime solution (2.991%).

Detailed studies on the rice sheath blight disease, pathogenicity, variability in the virulence pattern of the different *R. solani*isolates, identification of the fungus and management of the disease have been investigated in the present study. Preliminary trials conducted under both *in vitro* and *in vivo* conditions for evaluating the efficacy of organic preparations, botanicals, soil amendments and non-hazardous chemicals against the disease revealed the conspicuous effect of potassium silicate (1%), fermented egg lemon juice extract (10%) and garlic extract (10%) in restraining *R solani, the* causal agent of sheath blight of rice. However, considering the different aspects includingthe biometric and yield attributes, disease suppression as well as the economy of application, the foliar spray of potassium silicate turned out to be the most potential treatment for the

management of the disease. The study opens up a new realm for the use of non-hazardous chemicals, organic preparations and botanicals for the development of an organic strategy for disease management which is eco- friendly, economically viable and safe.

REFERENCES

7. REFERENCES

Abdou, A. M., Kim, M., and Sato, K. 2013. Functional proteins and peptides of hen's egg origin. *Bioactive food peptides in health and disease*. pp. 115-143.

Acharya, S., Basu, A., and Sengupta, P. K. 1997. Symptom types in rice plants infected with *Rhizoctonia solani* Kuhn. *J. Mycopathol. Res.* 35(2): 115-118.

Aderogba, M. A., McGaw, L. J., Bagla, V. P., Eloff, J. N., and Abegaz, B. M. 2014. *In vitro* antifungal activity of the acetone extract and two isolated compounds from the weed, *Pseudognaphalium luteoalbum*. *S. Afr. J. Bot.* 940: 74-78.

Adhao, A. D. 2013. Effect of panchagavya on soilborne plant pathogen, *Fusarium oxysporum*. *Indian Streams Res*. J. 3: 1-3.

Adhipathi, P., Singh, V., and Meena, S. C. 2013. Virulence diversity of *Rhizoctonia solani* causing sheath blight disease in rice and its host pathogen interaction. *Bioscan.* 8(3): 949-952.

Alam, S., Akther, N., Begum, M. F., Banu, M. S., Islam, M. R., Chowdary, A. N., and Alam, M. S. 2002. Antifungal activities (*in vitro*) of some plant extracts and smoke on four fungal pathogen of different hosts. *Pakist. J. Biol. Sci.* 5(3): 307-309.

Aleshin, N. E., Avakyan, E. R., Dyakunchak, S. A., Aleshkin,E.P., Baryshok, V. P., and Voronkov, M. G. 1986. Role of silicon in resistance of rice to blast. *Dokl. Akad. Nauk.* 291(2): 499-502.

Allison, J. L. 1951. *Rhizoctonia* blight of forage legumes and grasses. *Plant Dis. Rep.* 35: 372-373.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215(3): 403-410.

Anees, M. 2014. Integrated management of *Pythium* stem rot of vegetable cowpea (*Vigna unguiculata* sub specie *sesquipedalis*. (L.)) Verdcourt. M.Sc (Ag.) thesis, Kerala Agricultural University, Thrissur, 65p.

Aneja, K. R. 2003. *Experiments in Microbiology, Plant Pathology and Biotechnology* (4th Ed.). New Age International (P) Ltd. Publishers, New Delhi. 607p.

Anuja, R. 2010. Biological potential of organic growth promoters for management of leaf blight of Amaranthus. M. Sc. (Microbiology) thesis (Dissertation), School of Bioscience, Mahatma Gandhi University, Kottayam, Kerala, 89 p.

Arzoo, K., Biswas, S. K., and Rajik, M. 2012. Biochemical evidence of defense response in tomato against *Fusarium* wilt induced by plant extracts. *Plant Pathol. J.* 11(2): 42-50.

Ashlesha. and Paul, Y. S. 2014. Antifungal bioefficacy of organic inputs against fungal pathogens of bell pepper. *Indian J. Res.* 3(6): 4-9.

Ataee, R. A., Derakhshanpour, J., Tavana A. M., and Eydi, A. 2011. Antibacterial effect of calcium carbonate nanoparticles on *Agrobacterium tumefaciens*. *Iranian J. Military Med.* 13(2): 65-70.

Avato, P. F., Tursi, C., Vitali, V., Miccolis., and Candido, V. 2000. Allysulfide constituents of garlic volatile oil as antimicrobial agents. *Phytomedicine* 7: 239–243.

Banniza, S., Rutherford, M. A., Bridge, P. D., Holderness, M., and Mordue, J. E. 1996. Biological characterization of *Rhizoctonia solani* in rice-based cropping systems. Proceedings of Brighton Crop Protection Conference. Pests and Diseases. 1: 399-404.

Barker, A.V. and Pilbeam, D. I. 2007. *Handbook of Plant Nutrition*. Taylor & Francis Group, LLC CRC Press: 244 p.

Bekker, T. F., Kaiser, C., and Labuschange, N. 2009. The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi *in vitro*. *S. Afr. J. Plant Soil.* 26(1): 25-26.

Bernandes-De-Assis, J., Storari, M., and Zala, M. 2009. Genetic structure of populations of the rice infecting pathogen *Rhizoctonia solani* AG-1 IA from china. *Phytopathol.* 99(9): 1090-1099.

Bhadrasree, S. 2007. Ecofriendly Management of Collar rot and Web blight of Cowpea. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 102p.

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A. P. K., and John, V. T. 1978. Evaluation for sheath blight resistance in rice. *Int. Rice Res. Newsletter.* 3: 9-10.

Bhunkal, N., Ram, S., and Mehta, N. 2015. Assessment of lossess and identification of slow blighting genotypes against sheath blight of rice. *J. Mycol. Plant Pathol.* 45: 285-292.

Bianchi, A., Zambonelli, A., D'Aulerio, A. Z., and Bellesia, F. 1997. Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi *in vitro*. *Plant Dis.* 81: 1241-1246.

Biggs, A.R., El-Kholi, M.M., and El-Neshawy, S. 1997. Effects of calcium salts on growth polygalacturonase activity and infection of peach fruit by *Monilinia fructicola*. *Plant Dis*. 81: 399-403.

Blunden, G. 1991. Agricultural uses of seaweeds and seaweed products. In: Guiry, M. D. and Blunden, G. (eds.), Seaweed Resources in Europe: Uses and Potential. John Wiley and Sons, Chichester. pp. 65–81.

Burt, S. A. 2004. Essential oils, their antibacterial properties and potential applications in food – a review. *Int. J. Food. Microbiol.* 94: 223-253

Chadha, S., Rameshwar., Ashlesha., Saina, J. P., and Paul, P. S. 2012. *Vedic Krishi*: Sustainable livelihood option for small and marginal farmers. *Indian J. Tradit. Knowl.* 480-486.

Chahal, K. K., Kumar, A., Kataria, D., Singh, T., Kaur, B., and Chadha, S. 2016. Dynamics of demand and consumption of pesticides in Agriculture – An overview. *Indian J. Econ. Dev.* 12(1): 171-177.

Chakraborty, P., Chowdhury, A. K., and Chakraborty, B. N. 2006. Effectiveness of different methods of inoculation against sheath blight of rice. *Crop Res.* 31(3): 481-482.

Cherif, M., Asselin, A., and Belanger, R. R. 1994. Defense response induced by soluble silicon in cucumber roots infected by *Pythium* sp. *Phytopathol.* 84 (3): 236-242.

Curtis, H. U., Noll, U., Stormann, J., and Slusarenko, A. J. 2004. Broad spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and oomycetes. *Physiol. Mol. Plant Pathol.* 65: 79–89.

Das, L. 1986. Effect of application of plant protection chemicals on the survival *of Rhizoctonia solani* Kuhn. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 105p.

Dasgupta, M. K. 1992. *Plant Diseases of International importance:* In: Diseases of cereals and pulses, Volume 1. Singh, U. S. (Ed.), Prentice Hall Englewood Cliffs, New Jersey. pp. 130-150

Dath, A. P. 1990. Sheath blight of rice and its management. Associated publishing Co., Shidipura, Karol Bagh, New Delhi, 129 pp.

Datnoff, L. E., Elmer, W.H., and Huber, D. M. 2007. *Mineral nutrition and plant disease*. APS Press. The American Phytopathological Society, St. Paul, Minnesota, U. S. A. 278p.

Debbarma, M. and Dutta, P. 2015. Cultural and morphological variability in *Rhizoctonia solani* isolates of different hosts of Assam. *J. Appl. Res.* 5(2): 878-884.

Dev, V. P. S. and Mary, C. A. 1986. Sheath blight control. *Int. Rice Res. News*, 11: 22.

Devi, A. and Nayar, K. 2016. Integrated management of anthracnose of snake gourd. M. Sc. (Ag.) thesis, Kerala Agricultural University. Thrissur, 170p.

Dold, A. P. and Cocks, M. L. 2000. The medicinal use of some weed, problem and alien plants in the Grahams town and Peddie districts of the Eastern cape, South Africa. *S. Afr. J. Sci*. 96: 467-473.

Dorothy, E. T. and Frisvad, J. C. 2002. *Eupenicillium bovifimosum*, a new species from dry cow manure in Wyoming, *Mycologia*. 94(2): 240-246.

Duggar, B. M. 1915. *Rhizoctonia crocorum* (Pres.) D C and *R. solani* Kuhn (*Corticium vagum* B. and C) with notes on other species. *Ann. Mole. Bot. Gard.* 2: 403-458.

Dutta, S., Chaudhury, A., Chaudhury, A. K., and Laha, S. K. 2004. *In vitro* fungi toxicity of plant extracts against *Pyricularia oryzae* and *Rhizoctonia solani* incitant of blast and sheath blight of rice. *Indian Phytopathol.* 57: 344.

El-Fouly, M. M. and El-Sayed, A. A. 1997. Foliar fertilization: An environmentally friendly application of fertilizers. Dahlia Greidinger. International Symposium on "Fertilization and Environment". 24-27th March, Haifa, Israel, John, first (ed.). 346-357.

Eloff, J. N., Mdee, L. K., and Masoko, P. 2007. Invasive and weedy species can be used as a source of antifungal compounds to control plat fungal pathogens. *S. Afr. J. Bot.* 73: 287.

Epstein, E.1999. Silicon. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50: 641-664.

Fawe, A., Abou-Zaid, M., Menzies, J. G., and Bélanger, R. R. 1998. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathol.* 88: 396-401.

Feaking, D. S. 1977. *Pest control in rice.* PANS Manual. 3: 1-270.

FIB [Farm Information Bureau]. 2017. Farm Guide. Area, production, productivity of principal crops. Thiruvananthapuram, Kerala. 337p.

Fletcher, J. T., Hims, M. J., Archer, F. C., and Brown, A. 1982. Effects of adding calcium and sodium salts to field soils on the incidenc of clubroot. *Ann. Appl. Biol.* 100: 245-251.

Frankenberger, W. T. and Arshad, M. 1995. *Phytohormones in Soils.* Marcel Dekker, New York, USA. 503p.

Geogy, Z. and Rajan, K. M. 1990. Integrated management of sheath blight disease of rice. M.Sc (Ag.) thesis, Kerala Agricultural University, Thrissur, 107p.

George, M. 2015. *Choanephora* pod rot of cowpea and its ecofriendly management. MSc. thesis, Kerala Agricultural University, Vellanikkara, Thrissur, 105p.

Gireesh and Radhakrishnan. N. V. 2016. Ecofriendly management of *Rhizoctonia* leaf blight of amaranthus. *Int. J. appl. Pure Sci. Agric.* 2(8): 22-26.

Gonzalez, D., Cubeta, M. A., and Vilgalys, R. 2006. Phylogenetic utility of indels within ribosomal DNA and b-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. *Mol. Phylogen. Evol.* 40: 459-470.

Gonzalez-vera, A. D., Bernardes-de-Assis, J., Zala, M., Mcdonald, B. A., Correa-victoria, F., Graterol-matute, E. J., and Ceresini, P.C. 2010. Divergense between sympatric rice and maize infecting populations of *Rhizoctonia solani* AG-1-IA from Latin America. *Phyopathol.* 100: 172-182.

Gore, N. S. and Sreenivasa, M. N. 2011. Influence of liquid organic manures on growth, nutrient content and yield of tomato *(Lycopersicon esculentum* Mill.) in the sterilized soil. *Karnataka J. Agric. Sci.* 24(2): 153-157.

Groth, D. E. and Nowick, E. M. 1992. Selection for resistance to rice sheath blight through number of infection cushions and lesion type. *Plant Dis.* 76: 721-723.

Guillemaut, C., Herman, V. E., Camporota, P., Alabouvette, C., Molard, M. R., and Steinberg, C. 2003. Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. *Can. J. Microbiol.* 49: 556-568.

He, K., Yang, S., Li, H., Wang, H,. and Li, Z. 2014. Effects of calcium carbonate on the survival of *Ralstonia solanacearum* in soil and control of tobacco bacterial wilt. *Eur J Plant Pathol.* 140: 665–675.

Heine, G., Tikum, G., and Horsi, W. J. 2007. The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. *J. Exp. Bot.* 58(3): 569-577.

Hindi, N. K. K. and Chabuck, Z. A. G. 2013. Antimicrobial activity of different aqueous lemon extracts. *J. Appl. Pharma. Sci.*, 3: 074-078.

Hippe, S. 1991. Influence of fungicides on fungal fine structure; electron microscopy of plant pathogens. Mendgee, K. and Lesemann, D. E. (Ed.). Springer Verlag. Berlin. 317-331.

Hollier, C. A., Rush, M. C., and Groth, D. E. 2009. Sheath blight of rice *Thanetophorous cucumeris* (A. B. Frank) Donk *Rhizoctonia solani* Kuhn. Lousiana, *Plant pathology disease identification and management series publication*. 3123p.

Hostettmann, K., Marston, A., Ndjoko, K., and Wolfender, J. L. 2000. The potential of African plants as a source of drugs. *Curr. Org. Chem.* 4: 973-1010.

Hu, C., Wei, Y.W., Huang, S.L., Shi, G., and Li, Y. R. 2010. Identification and characterization of fungal strains involved in rice sheath blight complex in Guangxi Province. *Acta Agr. Boreali-occidentalis Sinica*, 19: 45-51.

Ibrahim, H. R., Aoki, T., and Pellegrini, A. 2002. Strategies for new antimicrobial proteins and peptides: Lysozyme and aprotinin as model molecules. *Curr. Pharmacol. Design*. 8: 671.

Inanaga, S., Okasaka, A., and Tanaka, S. 1995. Does silicon exist in association with organic compounds in rice plant? *Soil Sci. Plant Nutr*. 11: 111-117.

IRRI. 1986. Annual report for 1985. Los Banos, Philippines. pp. 143-146.

IRRI. 2002. *Standard evaluation system for rice*. 5th Edition., Nov. 2002, INGER, Genetic Resources Centre, IRRI, Manila, Philippines. 56p.

Jackson, M. L. 1958. *Soil Chemical Analysis.* Prentice - Hall of India (Pvt.) Ltd, New Delhi, 498p.

Jacobsen, B. J. and Backman, P. A. 1993. Biological and cultural plant disease controls: alternatives and supplements to chemicals in IPM systems. *Plant Dis.* 77: 311–315.

Jayaprakashvel, M. & Mathivanan, N. 2012. Morphological and pathological variations of rice sheath blight inciting south Indian *Rhizoctonia solani* isolates. *Arch. Phytopathol. Plant Prot*. 45(4): 455-467.

Jiang, D., Zeyen, R. J., and Russo. V. 1989. Silicon enhances resistance of barley to powdery mildew. *Phytopathol.* 79: 1198.

Jiang, J. F., Li, J. G., and Dong, Y. H. 2013. Effect of calcium nutrition on resistance of tomato against bacterial wilt induced by *Ralstonia solanacearum*. *Eur. J. Plant Pathol.* 136: 547–555.

Johanson, A., Turner, H. C., McKay, G. J., and Brown, A. E. 1998. A PCR-based method to distinguish fungi of the rice sheath blight complex, *Rhizoctonia solani, R. oryzae, R. oryzae-sativae*. *FEMS Microbiol. Lett.* 162: 289-294.

Joseph, B. and Sankarganesh, P. 2011. Antifungal efficacy of Panchagavya. *Int. J. Pharmtech Res.* 3(1): 585-588.

Kagale, S., Marimuthu, T., Thayumanavan, B., Nandakumar, R., and Samiyappan, R. 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. *Physiol. Mol. Plant Pathol.*65: 91-100.

Kaiser, C., vander Merwe, R., Bekker, T. F., and Labuschagne, N. 2005. *In vitro* inhibition of mycelial growth of several phytopathogenic fungi, including *Phytophthora cinnamomi* by soluble silicon. *S. Afr. J. Plant Soil.* 23: 169-172.

KAU [Kerala Agricultural University]. 2009. *Package of practices recommendations (Adhoc) for organic farming*: Crops. Kerala Agricultural university, Thrissur. 200p.

KAU [Kerala Agricultural University]. 2016. *Package of practices recommendations*: Crops (15th Ed.). Kerala Agricultural University, Thrissur, 393p.

Kaur, J. and Kaur, A. 2013. Morphological and molecular variability in *Rhizoctonia solani* isolates infecting potato and rice. *Plant Dis. Res.* 28(2): 163-168.

Kauss, K. R., Franke, S., Gilbert, A., Dietrich., and Kroger., N. 2003. Silica deposition by a strongly cationic proline -rich protein from ssystemically resistant cucumber plants. *Plant J.* 33:87-95.

Kettlewell, P. S., Cook, J. W., and Parry, W. D. 2000. Evidence for an osmotic mechanism in the control of powdery mildew disease of wheat by foliar – applied potassium chloride. *Eur. J. Plant Pathol.*106: 297-300.

Khan, A. A. and Sinha, A. P. 2006. Integration of fungal antagonists and organic amendments for the control of rice sheath blight. *Indian Phytopathol.* 363-365.

Khan, I. A., Subhan, A., and Ahmad, A. 1979. Inhibition of spore germination of *Helminthosporium turcicum*, the incitant of sorghum leaf blight by chemicals and plant extracts. *Indian J. Plant prot.* 7: 77-81.

Khoa, N. D., Thuy, P. T. H., Thuy, T. T. T., Collinge, D. B., and Jorgensen, H. J. L. 2011. Disease-reducing effect of *Chromolaena odorata* extract on sheath blight and other rice diseases. *Phytopathol.* 101: 231-240.

Kobayashi, T., Mew, T. W., and Hashiba, T. 1997. Relationship between incidence of rice sheath blight and primary inoculum in the Philippines: Mycelia in plant debris and sclerotia. *Ann. Phytopathol. Soc. Jpn.* 63: 324-327.

Kolaczkowska, A., Kolaczkowski, M., Sokolowska, A., Miecznikowska, H., Kubiak, A., Rolka, K., and Polanowski, A. 2010. The antifungal properties of chicken egg cystatin against *Candida* yeast isolates showing different levels of azole resistance. *Mycoses*. 314-320.

Kumar, K. V. K., Reddy, M. S., Kloepper, J. W., Lawrence, K. S., Groth, D. E. and Miller, M. E. 2009. Sheath blight disease of rice (*Oryza sativa*) - An overview. *Biosci. Biotech. Res. Asia.* 6(2): 465-480.

Kumar, M., Singh, V., Singh, K. N., and Vikram, P. 2008. Morphological and virulence characterization of *Rhizoctonia solani* causing sheath blight of rice. *Environ. Ecol.* 26(3): 1158-1166.

Kumar, S. and Tripathi, H. S. 2012. Evaluation of plant extracts against *Rhizoctonia solani* Kuhn, the incitant of web blight of urd bean. *Plant Dis. Res.* 27(2): 190-193.

Kuninga, S., Natsuaki, T., Takeuchi, T., and Yokosawa, R. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. *Curr. Gen.* 32: 237-243.

Kyung, K.H. and Y.C. Lee. 2001. Antimicrobial activities of sulfur compounds derived from S-alkyl-L-cysteine sulfoxides in *Allium* and *Brassica*. *Food Rev. Int.* 17: 183–198.

Lal, M. and Kandhari, J. 2009. Cultural and morphological variability in *Rhizoctonia solani* isolates causing sheath blight of rice. *J. Mycol. Plant Pathol.* 39(1): 77-81.

Lal, M., Kandhari, J., and Singh, V. 2012. Characterization of virulence pattern in *Rhizoctonia solani* causing sheath blight disease of rice. *Indian Phytopathol.* 65: 60-63.

Lalithya, K. A., Bhagya, H. P., and Choudhary, R. 2014. Response of silicon and micro nutrients on fruit character and nutrient content in leaf of sapota. *Biolife.* 2(2): 593-598.

Lee, F. N. and Rush, M. C. 1983. Rice sheath blight: A major rice disease. *Plant Dis.* 67: 829-832.

Lenka, S. and Pun, K. B. 2014. *In vitro* effect of organic amendment through oil cakes on *Rhizoctonia solani* Kuhn causing sheath blight disease in rice. *J. Plant Prot. and Environ.* 11(2): 88-90.

Leusch, H. J. and Buchenauer. H.1989. Effect of soil treatments with silica; rich lime fertilizers and sodium trisilicate on the incidence of wheat by *Erysiphe graminis* and *Septoria nodorum* depending on the form of N-fertilizer. Z. *Pflanzenkr. Pflanzensshutz* 96(2): 154-172.

Liang, Y.C., Sun, W.C., and Si, J. 2005. Effects of foliar and root applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant Pathol.* 54: 678-685.

Lore J. S., Tejdeep, K., and Hunjan, M. S. 2009. Effect of different growth stages on the development of sheath blight in rice. *Plant Dis. Res.* 24 (1): 63-64.

Lore, J. S., Jain, J., Hunjan, M. S., Gargos, G., Mangat, G. S., and Sandhu, J. S. 2015. Virulence spectrum and genetic structure of *Rhizoctonia* isolates

associated with rice sheath blight in the northern region of India. *Eur.ean J. Plant Pathol.* 143: 847-860.

Mathai, G., Paily, P.V., and Menon, M. R. 1978. Effect of fungicides and silica in the control of sheath blight disease of rice caused by *Corticum sasakii* (Shiriai). *Agri. Res. J. Kerala.* 19(1): 79-83.

Mdee, L. K., Mosoko, P., and Eloff, J. N. 2009. The activity of extracts of seven common invasive plant species on fungal phytopathogens. *S. Afr. J. Bot.* 75: 375-379.

Meng, Q. Z., Liu, Z. H., Wang, H. Y., Zhang, S. S., Wei, S. H. 2001. Research progress in rice sheath blight. (in Chinese with English abstract). *J. Shenyang Agric Univ.* 32: 376–381.

Menzies, J. G., Ehret, D. L., Glass, A. D. M., and Samuels, A. L. 1991. The influence of silicon on cytological interactions between *Sphaerotheca fulginea* and *Cucumis sativus*. *Physiol. Mol. Plant Pathol.* 39: 403-414.

Menzies, J., Bowen, P., Ehret, D., and Glass, A. D. M. 1992. Foliar application of potassium silicate reduces severity in powdery mildew on cucumber, musk melon and Zucchini squash. *J. Am. Soc. Hort. Sci.* 117: 902-905.

Moni, Z. R., Ali, M. A., Alam, M. S., Rahman, M. A., Bhuiyan, M. R., Mian, M. S., Iftekharuddaula, K. M., Latif, M. A., and Khan, M. A. I. 2016. Morphological and genetical variability among *Rhizoctonia solani* isolates causing sheath blight disease of rice. *Rice Sci.* 23(1): 42−50.

Mooney, P.A. and Van Staden, J. 1986. Algae and cytokinins. *J. Plant Physiol.* 123: 1–2.

Moraes, S. R. G., Pozza, E. A., Alves, E., Pozza, A. S. A. , Carvalho, J. G., Lima, P. H., and Botelho. 2005. Effect of silicon on incidence and severity of anthracnose in common bean. *Phytopathol.* 31(2):69-75.

Muller, K. O. 1924. Unterruchugen Zur Enteickel ungsgeschichte and Biologie Von *Hypochnus solani* P.U.D. (*Rhizoctonia solani* K.). *Arbeit Biol. Reichsanst Lnad –U_Forstw.* 13: 198-262.

Myers, D. F. and Campbell, R. N. 1985. Lime and the control club root of crucifers: Effect of pH, calcium, magnesium and their interactions. *Phytopathol.* 670-673.

Nagarajkumar, M., Bhaskaran, R., and Velazhahan, R. 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani,* the rice sheath blight pathogen. *Microbiol. Res.* 159(1): 73-81.

Nene, Y. L. and Thapliyal, P. N. 1979. Fungicides in plant disease control. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. 691p.

Obagwu, J. and Korsten, L. 2003. Control of citrus green and blue molds with garlic extracts. *Eur. J. Plant Pathol.* 109: 221–225.

Ortuno, A. A., Baidez, P., Gomez, M. C., Arcas, I., Porras, A. G. and Del Rio, J. A. 2006. *Citrus paradisi* and *Citrus sinensis* flavonoids: Their influence in the defense mechanism against *Penicillium digitatum. Food Chem.*, 98(2): 351-358.

Ou, S. H. 1972. Rice diseases. (1st Ed.). Commonwealth Mycological Institute, Kew, England. 368p.

Ou, S. H. 1985. Rice diseases. (2nd Ed.), Commonwealth Mycological Institute, Kew, England. 380p.

Pal, G. K., Kumar, B., and Shahi, S. K. 2013. Antifungal activity of some common weed extracts against seed- borne phytopathogenic fungi *Alternaria* spp. *Int. J. Universal Pharm. Life Sci.* 6-14.

Pal, T. K., Bhattacharya, S., and Chakraborty, K. 2011. Induction of systemic resistance in rice by leaf extract of *Cymbopogan citrus* and *Ocimum sanctum* against sheath blight disease. *Arch. Appl. Sci. Res.* 3(1): 392-400.

Palo, M. A. 1926. *Rhizoctonia* diseases of rice - A study of the disease and of the influence of certain conditions upon viability of the sclerotial bodies of the causal fungus. *Philippine Agric.* 15: 361-376.

Pankaj., Sood, A. K., and Kumar, P. 2015. Evaluation of essential oils against *Ralstonia solanacearum* causing bacterial wilt of solanaceous crops. *Plant Dis. Res.* 30(1): 67-72.

Paracer, C. S. and Chahal, D. S. 1963. Sheath blight of rice caused by *Rhizoctonia solani* Kuhn – A new record in India. *Curr. Sci.* 32: 328-329.

Parmeter, J. R. and Whitney, H. S. 1970. *Taxonomy and nomenclature of the imperfect state.* In: *Rhizoctonia solani*: Biology and Pathology. University of California Press: Berkely. pp. 7-19.

Patil, P. V. 2009. Eco friendly approaches for the management of soybean rust. *Karnataka. J. Mycopathol. Res.* 47(2): 145-151.

Pillai, P. 1990. Laboratory screening of rice cultivars for sheath blight resistance. Extended summary, Proceedings of International Symposium on Rice Research - New frontiers, November 15-18, 1990. Directorate of Rice Research, Hyderabad. pp. 337-339.

Piper, 1966. Aging of crystalline precipitates. *Analyst.* 77: 1000-1011.

Polanco, L. R., Rodrigues, F. A., and Moreria, E. N. 2014. Management of anthracnose in common bean by foliar sprays of potassium silicate, sodium molybdate and fungicide. *Plant Dis.* 98: 84-89.

Portz, D., Koch, E., and Slusarenko, A. 2008. Effect of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *Eur. J. Plant Pathol.* 197-206.

Prabhath, M. C. A. 1971. Studies on sheath blight of rice caused by *Corticium sasakii* (Shirai) Matsumoto. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 80p.

Pramanick, B., Brahmachari, K., Ghosh, A., and Zodape S. T. 2014. Effect of seaweed saps on growth and yield improvement of transplanted rice in old alluvial soil of West Bengal. *Bangladesh J. Bot.* 43(1): 53-58.

Prasad, V. R. and Niza, R. T. J. 2014. Integrated management of sheath blight disease of rice. M.Sc (Ag.) thesis, Kerala Agricultural University, Thrissur, 90p.

Rajan, C. P. D. 1987. Estimation of yield losses due to sheath blight of rice. *Indian PhytoPathol.* 40: 174-177.

Rangaswami, G. and Mahadevan, A. 2006. Diseases of crops plants in India. 4th Edition. Prentice Hall of India Pvt. Ltd. New Delhi. pp. 335- 336.

Rangaswamy, G. 1958. An agar blocks technique for isolating soil microorganisms with special refrence to Pythiaceous fungi. *Sci. Culture.* 24: 85.

Rathore, S. S., Chaudhary, D. R., Boricha, G. N., Ghosh, A., Bhatt, B. P., Zodape, S. T., and Patolia, J. S. 2009. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *S. Afr. J. Bot.* 75: 351–355.

Ratnayake, R. M. R. N. K., Daundasekera, W. A. M., Ariyarathne, H. M., and Ganehenege, M. Y. U. 2016. Soil application of potassium silicate reduces the intensity of downy mildew in bitter gourd (*Momordica charantia* L.) leaves. *Ceylon J. Sci.* 45(1): 23-31.

Ravi, S., Vishwanath., Kumar, S., and Kumar, P. 2014. Evaluation of botanicals, cow urine and biocontrol agents against *Rhizoctonia solani* inciting leaf blight of Kalmegh (*Andrographis paniculata*). *Plant Dis. Res.* 29(1): 46-49.

Rodino, S., Butu, M., Petrache, P., Butu, A., and Cornea, C. P. 2014. Antifungal activity of four plants against *Alternaria alternata*. *Biotech.*18: 60-65.

Rodrigues, F. A., Vale, F. X. R., Dtanoff, L. E., Prabhu, A. S., and Korndorfer, G. H. 2003. Effect of rice growth stages of rice and silicon on sheath blight development. *Phytopathol.* 93: 256-261.

Rodrigues, F.A., McNally, D.J., Datnoff, L.E., Jones, J. B., Labbe, C., Benhamou, N., Menzies, J.G., and Belanger, R. R. 2004. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. *Phytopathol.* 94: 177-183.

Roy, A. K. 1993. Sheath blight of rice in India. *Indian Phytopathol.* 46: 97-205.

Rush, M. C. and Lee, F. N. 1992. Sheath blight. In: *Compendium of Rice Diseases*. Webster, R. K. and Gunnell, P. S. (eds.). The American Phytopathological Society, St. Paul, MN. pp. 22-23.

Saifunneesa, T. K. and Niza, R. T. J. 2001. Efficacy of selected biopesticides for the management of sheath blight of rice. M.Sc (Ag.) thesis, Kerala Agricultural University, Thrissur. 97p.

Sajeena, A., Sukumari, P., Jacob John., and Kamala Nayar. 2015. Indigenous organic preparations for management of leaf blight disease of amaranthus. In: Proceedings of the National Seminar on Biological Products for Crop, Animal and Human Health - problems and prospects, 21-22nd August 2015, Mysore. National Academy of Biological Science. pp. 29-30.

Sajeena, A., Sukumari, P., Jacob John., and Kamala Nayar. 2016. Fermented extracts of *Setaria barbata* and egg-lemon juice for eco-friendly disease management and crop growth. *Indian Phytopathol.* 69: 590-593.

Saksena, H. K. and Vaartaja, O. 1961. Taxonomy, morphology, and pathogenicity of *Rhizoctonia* species from forest nurseries. *Can. J. Biotech.* 39: 627-647.

Salim B. B. M., Eisa, S. S., Ibrahim; I. S., Girgis M.G.Z., and Abdel-Rassoul, M. 2013. Effect of biofertilizers, mycorrhiza and foliar spraying of some micronutrients (Fe+, Mn+, Zn) and potassium silicate on enhancing salt tolerance of wheat plant. *Int. J. Environ.* 2(2): 35-45.

Salim, B. B. M., Abd El-Gawad, H. G., and Abou El-Yazied, A. 2014. Effect of foliar spray of different potassium sources on growth, yield and mineral composition of potato (*Solanum tuberosum* L.) *Middle East J. Appl. Sci.* 4(4): 1197-1204.

Salim, B. B. M., Eisa, S. S., Ibrahim, I. S., Girgis, M. G. Z., and Abdel- Rasoul, M. 2011. Enhanced salt tolerance of wheat plant. *J. Biol. Chem. Environ. Sci.* 6(4): 30-52.

Sallam, N. M. A. 2011. Control of tomato early blight disease by certain aqueous plant extracts. *Plant Pathol. J.* 10(4): 187-191.

Sarmah, N. and Kumari, S. 2013. Comparitive study of antibacterial activity of ripen and unripen indigenous citrus union of assam, India. *Int. J. Advmt. Res. Technol.* 9:25-31.

Senapoty, D. 2010. Efficacy of soil amendments for the management of rice sheath blight. *Indian Phytopathol.* 63(1): 94-95.

Sharma, D. and Thrimurty, V. S. 2004. Effect of age of mycelium and sclerotia in sheath blight of rice (*Oryza sativa* L.) disease development. *Plant Archives*. 4(2): 363-365.

Sharma, N. R., Teng, P. S., and Olivares. F. M. 1990. Comparison of assessment methods for rice sheath blight disease. *Philippines Phytopathol.* 26: 20-24.

Sharma, M., Gupta, S. K., and Sharma. T. R. 2005. Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. *Phytopathol*, 153: 449-456.

Singh, A. 2008. Evaluation of bioagents and phyto extracts against *Curvularia penniseti* in pearl millet. *J. Mycol. Plat Pathol.* 38(3): 687-688.

Singh, A., Rohila, R., Singh, U. S., Savary, S., Willoocquel, L., and Duvoillor, E. 2001. An improved inoculation technique for sheath blight of rice caused by *Rhizoctonia solani. Can. J. Plant Pathol.* 24: 64-68.

Singh, M. and Singh, R. P. 2005. Management of mushroom pathogens through botanicals. *Indian Phytopathol.* 189-193.

Singh, R., Murti, S., Mehilalm., Tomer, A., and Prasad, D. 2015. Virulence diversity in *Rhizoctonia solani* causing sheath blight in rice. *J. Plant Pathol. Microbiol.* 6(8): 296-303.

Singh, R., Singh, M., and Lore, J. S. 2016. Morphological characterization of *Rhizoctonia* species associated with sheath blight complex in rice. *Plant Dis. Res.* 31(2): 130-137.

Singh, S. K., Shukla, V., Singh, H., and Sinha, A. P. 2004. Current status and impact of sheath blight in rice *(Oryza sativa)*-a review. *Agric. Rev.* 25(4): 289-297.

Singh, V., Singh, U. S. and Singh, K. P. 2003. Relative virulence of rice isolates of *Rhizoctonia solani* on rice and wheat. *Indian Phytopathol.* 56(2): 186-187.

Sirari, K., Mehta, C. M., and Pundhir, V. S. 2015. Biological management of black scurf of potato caused by *Rhizoctonia solani* Kuhn. *Res. J. Pharma., Biol. Chem. Sci.* 6: 754.

Sireesha, O. 2013. Effect of plant products, panchagavya and bio-control agents on rice blast disease of paddy and yield parameters. *Int. J. Res. Biol. Sci.* 3(1): 48-50.

Sivalingam, P. N., Vishwakarma, S. N., and Singh, U. S. 2006. *Indian Phytopathol.* 59: 445-452.

Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. American Phytopathological Society, Press, St. Paul, Minnesota, 133p.

Sneh, B., Hare, S. J., Neate, S., and Djist, G. 1996. *Rhizoctonia* species: *Taxonomy, molecular biology, ecology, pathology, and disease control.* Kliuwer Academic Publishers, Dordrecht, Netherlands. 547p.

Sridhar, D. and Patil, M. B. 2009. Impact of organics on major foliar diseases of paddy. *J. Plant Dis. Sci.* 4(1): 64-67.

Srinivas, P., Ratan, V., Reddy P. N., and Bindumadhavi, G. 2014. *In vitro* evaluation of fungicides, bio control agents and plant extracts against rice sheath blight pathogen *Rhizoctonia solani*. *Int. J. Appl. Biol. Pharma. Technol.* 5(1): 121-126.

Srivastava, D. and Singh, P. 2011. Antifungal potential of two common weeds against plant pathogenic fungi - *Alternaria* sp. *Asian J. Exp. Biol. Sci.* 2(3): 525-528.

Steel, G. D. R. and Torrie, J. H. 1960. Principles and Procedures of Statistics, Mc Graw-Hill Book company, Inc. New york. 481p.

Stosic, S., Stojanovic, S., Milosavljevic, A., Dolovac, E. P., and Zivkovic, S. 2014. Effect of calcium salts on postharvest fungal pathogens *in vitro*. *Plant Prot.* 65(1): 45-46.

Sugha, S. K. 2005. Antifungal potential of panchagavya. *Plant Dis. Res.* 20(2): 156-158.

Sumangala, K. and Patil, M. B. 2009. Panchagavya, an organic weapon against plant pathogens. *J. Plant Dis. Sci.* 147-151.

Sun, W., Liang, Y., and Yang, Y. 2002. Influences of silicon and inoculation with *Colletotrichum lagenarium* on peroxidase activity in leaves of cucumber and their relation to resisance to anthracnose. *Sci. Agric. Sin.* 35: 1560-1564.

Sunder, B., Kataria, H. R., Satyavir., and Sheoran, O. P. 2003. Characterization of *Rhizoctonia solani* associated with root/collar rots and blights. *Indian Phytopathol.* 27-33.

Susheela, K. 2012. Characterization, virulence and genetic variation of *Rhizoctonia solani* AG-1 IA in India. *Indian J. Plant Prot.* 40 (4): 318-328.

Susheela, K. and Reddy, C. S. 2013. Variability in *Rhizoctonia solani* (AG-1 IA) isolates causing sheath blight of rice in India. *Indian Phytopathol.*66: 341-350.

Swami, C. S. and Alane, S. K. 2013. Efficacy of some botanicals against seed - borne fungi of green gram (*Phaseolus aureus* roxb.). *Biosci. Discovery.* 4(1): 107-110.

Swaminathan, C., Swaminathan, V., and Vijayalakshmi . 2007. Panchagavya – A boon to organic farming. International Book Distributing Co., India.

Taheri, P. and Hofte, M. 2007. Riboflavin induced resistance against sheath blight of rice functions through the potentiation of lignin formation and jasmonic acid signaling pathway. *Commun. Agric. Appl. Biol. Sci.* 72: 309-313.

Taheri, P., Gnanamanickam, S., and Hofte, M. 2007. Characterization, genetic structure, and pathogenicity of *Rhizoctonia* spp. associated with rice sheath diseases in India. *Phytopathol.* 97: 373-383.

Tiwari, R. K. S. and Chaure, N. K. 1997. Studies on factors influencing appearance and severity of sheath blight (*Rhizoctonia solani* f sp. *sasakii)* of rice. *Adv. Plant Sci.* 10: 223-226.

Tsukiboshi, T. and Kimigafukuro, T. 1992. Characterization and pathogenicity of *Rhizoctonia* spp. causal agent of summer blight in pasture grasses and

legumes in Japan. *Sochi Shikenjo Kenkyu Hokoku*. Bulletin of the National Grassland Research Institute. 47: 29-36.

Tu, C. C. and Kimbrough, J. W. 1975. Morphology, development and cytochemistry of the hyphae and sclerotia of species in the *Rhizoctonia* complex. *Can. J. Bot.* 53: 2282-2296.

van Bruggen, A. H. C., Gamliel, A., and Finckh, M. R. 2015. Plant disease management in organic farming systems. *Pest Manag. Sci.* 72: 30-44.

Vidhyasekaran, P., Ruby Ponmalar, T., Samiyappan, R., Velazhahan, R., Vimala, R., Ramanathan, A., Paranidharan, V., and Muthukrishnan, S. 1997. Host-specific toxin production by *Rhizoctonia solani,* the rice sheath blight pathogen. *Phytopathol.* 87: 1258-1263.

Vijayan, M. 1989. Studies on early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. M Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Vincent, J. M. 1947. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*. 159(4051): 850.

Wainwright, M. 1993. Oligotrophic growth of fungi. Stress or natural state? In: D.H. Jennings (ed.), Stress Tolerance of Fungi, Academic Press, London. pp. 172-184.

Wainwright, M., Al-Wajeeh, K., and Grayston, S. J. 1997. Effect of silicic acid and other silicon compounds on fungal growth in oligotrophic and nutrient rich media. *Mycol. Res.* 101: 933-938.

Wallingford, W. 1980. Function of potassium in plants In: *Potassium for Agriculture.* Potash and Phosphate Inst., Atlanta. Georgia. pp. 10-27.

Webber, L. N., Magwa, M. L., and Vanstaden, J. 1999. Alternative uses for some invader plants: Turning liabilities into assets. *S. Afr. J. Sci.* 95: 329-331.

Webster, J. and Webster, R. W. S. 2007. *Introduction to fungi*. (3rd Ed.). Cambridge University press. 167p.

Webster, R.W. and Gunnel, P.S. 1992. *Compendium of Rice Diseases.* American Phytopathological Society, Minnesota, U. S. A. pp. 22-23.

Weinert, E. J., Miller, S. A., Ikeda, D. M., Chang, K. S., McGinn, J. M., and Duponte, M. W. 2014. *Natural Farming: Fish Amino Acid*. Sustainable Agriculture, SA-12. University of Hawaii, College of Tropical Agriculture and Human Resources, Honolulu. 104p.

Xie, X. W., Xu, M. R., Zang, J. T., Sun, Y., and Zhu, L. H. 2008. Genetic background and environmental effects on QTLs for sheath blight resistance revealed by reciprocal introgression lines in rice. *Acta Agronomica Sinica*. 34: 1885-1893.

Yoshimera, S. and Nishizawa, T. 1954. Studies on the method of testing of varietal resistance of upland rice plants caused by sheath blight, *Hypochmus sasakii*, shirari bull. *Yushnu Agric. Exp. Stn*. 2: 361-376.

Zambrowicz, A., Pokora, M., Eckert, E., Szołtysik, M., Dąbrowska, A., Chrzanowska, J., and Trziszka, T. 2012. Antioxidant and antimicrobial activity of lecithin free egg yolk protein preparation hydrolysates obtained with digestive enzymes. Functional Foods in Health and Disease. pp. 487-500.

Zhang, C., Hu, R., Huang, J., Hunag, X., Shi, G., Li, I., Yin, Y., and Chen, Z. Health effects of agricultural pesticide use in China- implications for the development of GM crops. 2016. *Sci. Rep*. 6: 34918

Zheng, A., Lin, R., Zhang, D., Qin, P., Xu, L., Ai, P., Ding, L., Wang, Y., Chen, Y., and Liu, Y. 2013. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. *Nat. Commun*. 4: 1424.

APPENDICES

Appendix- I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Potato - 200 g

Dextrose ($C_6H_{12}O_6$) - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved into the mixture. The volume was made up to 1000 ml with distilled water and medium was sterilized at 15 psi and 121^0 C for 15 min.

Appendix- II

COMPOSITION OF STAINS USED

1. Lactophenol –Cotton blue

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

Anhydrous lactophenol prepared by dissolving 20 g phenol in 6 ml lactic acid in 3 ml glycerol.

महाराष्ट्र असोसिएशन फॉर द कल्टिव्हेशन ऑफ सायन्स
आघारकर अनुसंधान संस्थान
(विज्ञान और प्रौद्योगिकी विभाग, भारत सरकार के अधिन स्वायत्त संस्थान)
गो. ग. आगरकर पथ, पुणे - ४११ ००४.
Maharashtra Association for the Cultivation of Science
AGHARKAR RESEARCH INSTITUTE
(An Autonomous Body under the Department of Science and Technology, Govt. of India)
G. G. Agarkar Road, Pune - 411 004.

दूरभाष/Tel : 020-2532 5000, 2565 3680
फॅक्स/Fax : 020-2565 1542
वेब/Web : www.aripune.org
ई-मेल/E-mail : director@aripune.org

MOLECULAR IDENTIFICATION REPORT

SENDER :**Dr. V.K. Girija, Head**
INSTITUTE/ORGANIZATION : Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram – 6955522, Kerala
ACKNOWLEDGEMENT CODE : **2633**
JOB TITLE : Molecular identification of the fungal isolate.

PROCEDURE:

- Genomic DNA was isolated in pure form, from the culture provided by the sender.
- The **ITS region of rDNA** was successfully amplified using fungal universal primers ITS4 & ITS5.
- The sequencing PCR was set up with ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit.
- The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency.
- The sequence data was aligned with publicly available sequences &analyzed to reach identity.

Results of Molecular Identification:

- The tested fungal isolate showed **100 %** sequence similarity with ***Rhizoctonia solani*** current name ***Thanatephorus cucumeris* (A.B. Frank) Donk 1956.**
- Sequence analyses with NCBI accession number KX674527, ***Rhizoctonia solani*** AG-1 IA isolate CSU8 resulted in following alignment statistics.
- Alignment statistics: Query Length - 557, Score - 1005 bits (1114), Expect - 0.0, Identities - 557/557 (100%), Gaps - 0/557 (0%), Strand - Plus/Minus

```
Query  1    AAAATAGAATATTGTCCAAGTCAATGGACTATTAGAAGCGGTTCATCTGCATTTACCTTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  605  AAAATAGAATATTGTCCAAGTCAATGGACTATTAGAAGCGGTTCATCTGCATTTACCTTG  546

Query  61   GCCACCTTTTTTACGGGTGTCCTCAGCGATAGATAACTTATCACGCCGAGTGGAACCAAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  545  GCCACCTTTTTTACGGGTGTCCTCAGCGATAGATAACTTATCACGCCGAGTGGAACCAAG  486

Query  121  CATAACACTGAGATCCAGCTAATGCACAAAGAGGAGCAGGTGTGAAGCTGCAATAAGATC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  485  CATAACACTGAGATCCAGCTAATGCACAAAGAGGAGCAGGTGTGAAGCTGCAATAAGATC  426

Query  181  CTCCAAAACCAAAGTAAAAAAGACCAATTGAATTAACAAAAGGTTTACTTTGAAGATTTC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  425  CTCCAAAACCAAAGTAAAAAAGACCAATTGAATTAACAAAAGGTTTACTTTGAAGATTTC  366

Query  241  ATGATACTCAAACAGGCATGCTCCAAGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  365  ATGATACTCAAACAGGCATGCTCCAAGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGAT  306

Query  301  TCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  305  TCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCAT  246

Query  361  CGATGCGAGAGCCAAGAGATCCGTTGTTGAAACTTAGTATTAGATGCGTTACATCAATTA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  245  CGATGCGAGAGCCAAGAGATCCGTTGTTGAAACTTAGTATTAGATGCGTTACATCAATTA  186

Query  421  CATTCAGTTTAAATTAAGTAGAGTGTGTGTAAATTAAGTAGACAGCAAATGGATGATGGA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  185  CATTCAGTTTAAATTAAGTAGAGTGTGTGTAAATTAAGTAGACAGCAAATGGATGATGGA  126
```

```
Query  481  ATTAAATCCACCAACTATTGCTGTCTCACAAGTGCACAGGGGGTGTGATGGATGAAAGAG  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  125  ATTAAATCCACCAACTATTGCTGTCTCACAAGTGCACAGGGGGTGTGATGGATGAAAGAG  66

Query  541  AAGGTGTGCACATGCCC  557
            |||||||||||||||||
Sbjct  65   AAGGTGTGCACATGCCC  49
```

Top five hits upon BLAST analysis

Gene Bank Accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
KX674527.1	*Rhizoctonia solani* AG-1 IA isolate CSU8	1005	1005	100%	0.0	100%
KX674526.1	*Rhizoctonia solani* AG-1 IA isolate CSU4	1005	1005	100%	0.0	100%
KX674525.1	*Rhizoctonia solani* AG-1 IA isolate CSU1	1005	1005	100%	0.0	100%
KX674524.1	*Rhizoctonia solani* AG-1 IA isolate RKH	1005	1005	100%	0.0	100%
KX674523.1	*Rhizoctonia solani* AG-1 IA isolate RKL	1005	1005	100%	0.0	100%

Reference:

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. &Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

CONDITIONS AND REMARKS:

1. THE PARTY HAS DELIVERED THE SAMPLE AT ARI.
2. THE RESULTS HAVE BEEN OBTAINED ON CAREFUL ANALYSIS AND EXAMINATION OF THE SAMPLE ONLY AND IN THE CONDITION RECEIVED.
3. THIS REPORT SHOULD BE USED ONLY FOR ACADEMIC AND RESEARCH PURPOSES. IT SHOULD NOT BE USED AS AN EVIDENCE OF AUTHENTICITY IN ANY OFFICIAL/ GOVERNMENTAL/ LEGAL/STATUTORY CORRESPONADANCE OR CERTIFICATION. THE INSTITUTE SHALL NOT BE BOUND TO CONFIRM THE AUTHENTICITY BEFORE ANY LEGAL FORUM.
4. THE CONTENTS OF THIS REPORT ARE CONFIDENTIAL AND BEING DISCLOSED ONLY TO THE PARTY / SUPPLIER OF SAMPLE.
5. THE PARTY NEEDS TO ACKNOWLEDGE THE SERVICE (S) PROVIDED/RENDERED BY NFCCI-ARI (IN THESIS/REPORTS/PUBLICATION/BOOKS/MONOGRAPHS ETC.).
6. THE PARTY IS REQUESTED TO SUBMIT COPY/REPRINT OF PUBLICATION TO CURATOR, NFCCI BASED ON FUNGAL STRAINS DEPOSITED & ACCESSIONED IN NFCCI FOR OFFICIAL RECORD.

(S. K. Singh)

Kind Attn:
Dr. V.K. Girija, Professor & Head
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram – 6955522, Kerala

NFCCI/2017-08/AKC 2633-5/AB/SKS/DKM

ORGANIC STRATEGY FOR THE MANAGEMENT OF SHEATH BLIGHT DISEASE OF RICE

by

KARTHIKA S. R.

(2015-11-073)

Abstract of the thesis

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2017

ABSTRACT

The study entitled "Organic strategy for the management of sheath blight disease of rice" was undertaken at the Department of Plant Pathology, College of Agriculture, Vellayani and IFSRS, Karamana during 2015-2017 with the objective to develop an eco-friendly disease management package for sheath blight disease of rice using organic preparations, botanicals, soil amendments and non-hazardous chemicals.

Seven isolates of *Rhizoctonia solani* was isolated from sheath blight infected rice plants collected from seven different rice growing tracts of Thiruvananthapuram district during Kharif, 2016. The symptoms of the disease were observed during the active tillering stage of the crop. Pathogenicity was confirmed by proving Koch's postulates. The isolate collected from Karode (I_6) took seven days and the other six isolates took three days for symptom development. Virulence rating by artificial inoculation on potted plants proved that the isolate collected from Attingal (I_2) produced the maximum number of infected tillers (4.66) as well as the maximum relative lesion height at 5^{th} (9.20%), 7^{th} (15.70%), 10^{th} (19.03%) and 15^{th} (20.96%) days after inoculation. Thus, I_2 was found to be the most virulent isolate. Based on the morphological and cultural characteristics, the pathogen was identified to be *Rhizoctonia solani* Kuhn (Accession no: KX674527). Further, molecular identification by ITS (Internal Transcribed Space) sequencing revealed 100 per cent similarity with *R. solani* AG-1 IA isolate CSU8.

In vitro pathogen suppression studies revealed that fermented egg-lemon juice extract (10%), panchagavya (5%), garlic extract (10%), potassium silicate (1%), lime (12.5%) and fermented weed (*Setaria barbata*) extract (100%) resulted in 100 per cent inhibition of the mycelial growth of *R. solani*. Dipping sclerotia for 24 hours in fermented egg-lemon juice extract (10%), panchagavya (5%), lime (12.5%) and fermented weed (*Setaria barbata*) extract (100%) resulted in complete (100%) suppression of mycelial regeneration from sclerotia. Analysis of the major nutrients (N, P, K) and pH of the various treatments revealed that

fermented weed extract, fermented egg-lemon juice extract and panchagavya were highly acidic (pH of 4.9, 4.5 and 4.2 respectively). The total N content was high in panchagavya (0.02 %) followed by fermented egg-lemon juice extract (0.01%) whereas the total K content was high in panchagavya (0.03%) followed by potassium silicate (0.01%). The P content was comparatively high in panchagavya (0.007%) followed by fermented egg-lemon juice extract (0.006%).

The pot culture experiment for the management of sheath blight in rice (var. Uma) revealed that the maximum suppression of the vertical spread of the disease was observed in fermented egg-lemon juice extract (79.43%) and garlic extract (79.36%) sprayed plants which were found to be on par with the fungicide check, hexaconazole (79.36%). The percentage horizontal spread was the least for fermented egg-lemon juice extract (11.12%) and potassium silicate (16.93%) sprayed plants which were also on par with the fungicide check (10.44%). However, the highest grain yield was recorded in the plants sprayed with fermented weed (*Setaria barbata*) extract (15.98g) followed by fermented egg-lemon juice extract (15.33g) and garlic extract (14.66g) sprayed plants. Analysis of the major nutrients in the plants at the time of harvest revealed that the total N content was the maximum for the plants sprayed with garlic extract (1.645%) whereas the total K content was the maximum for the plants sprayed with potassium silicate (3.601%).

Thus, the present study revealed that three treatments *viz*., fermented egg-lemon juice extract (10%), potassium silicate (1%) and garlic extract (10%) resulted in improved biometric and yield attributes of rice plants as well as in the reduction of sheath blight disease incidence. However, four foliar sprays at 45^{th}, 60^{th}, 75^{th} and 90^{th} DAS of potassium silicate at one per cent concentration was found to be the most economical and effective treatment in improving the yield as well as in reducing the sheath blight disease incidence and was found to be on par with the fungicide check.

സംഗ്രഹം

"നെല്ലിന്റെ പോളരോഗ നിയന്ത്രണം ജൈവ ഉപായത്തിലൂടെ" എന്ന പേരിൽ 2015 -17 കാലയളവിൽ വെള്ളായണി കാർഷിക കോളേജ് സസ്യരോഗശാസ്ത്ര വിഭാഗത്തിലും കരമന IFSRS ലുമായി ഒരു പഠനം നടത്തുകയുണ്ടായി ജൈവക്കൂട്ടുകൾ പച്ചിലസത്ത്, മണ്ണ് ഭേദഗതികൾ, നിർദോശകരമായ രാസപദാർത്ഥങ്ങൾ എന്നിവ ഉപയോഗിച്ച് പരിസ്ഥിതി സൗഹൃദമായി നെല്ലിന്റെ പോളരോഗത്തെ ചെറുക്കുക എന്നതായിരുന്നു ലക്ഷ്യം.

തിരുവനന്തപുരം ജില്ലയിലെ 2016 വിരിപ്പുകൃഷിസമയത്ത് 7 വിവിധ പ്രദേശങ്ങളിൽ നിന്നും പോളരോഗം ബാധിച്ച നെൽചെടികൾ ശേഖരിക്കുകയും അവയിൽ നിന്ന് രോഗഹേതുവായ *റൈസ്ക്ടോണിയ സൊളാനി* എന്ന ഫംഗസ് വേർപ്പെടുത്തിയെടുക്കുകയും ചെയ്തു. നെല്ലിന്റെ ചിനപ്പ് പൊട്ടുന്ന സമയത്താണ് രോഗലക്ഷണങ്ങൾ കണ്ടുതുടങ്ങുന്നത്. കോച്ചിന്റെ അനുശാസനങ്ങൾ പ്രകാരം രോഗഹേതുവായ ഫംഗസിന്റെ രോഗോല്പാദനക്ഷമത സ്ഥിതീകരിച്ചു. കാരോടിൽ നിന്ന് ശേഖരിച്ച (16) ഫംഗസ് 7 ദിവസം കൊണ്ടും മറ്റ് 6 പ്രദേശങ്ങളിൽ നിന്നുള്ളവ 3 ദിവസം കൊണ്ടും രോഗലക്ഷണങ്ങൾ കാണിച്ചു തുടങ്ങി. മൺചട്ടികളിൽ വളർത്തിയ നെൽ ചെടികളിൽ കൃത്യമമായി ഫംഗസിനെ കുത്തിവയ്ച്ച് രോഗകാഠിന്യം തരംതിരിച്ചതു വഴി , ആറ്റിങ്ങലിൽ നിന്നും വേർതിരിച്ച ഫംഗസാണ് (12) ഏറ്റവും അധികം രോഗം ബാധിച്ച ചിനപ്പുകൾ (4.66) ഉണ്ടാക്കിയത് എന്ന് കണ്ടെത്തി. മാത്രവുമല്ല രോഗലക്ഷണത്തിന്റെ തീവ്രതയും ആറ്റിങ്ങലിൽനിന്ന് ശേഖരിച്ചതിനായിരുന്നു. അതിനാൽ ഏറ്റവും കൂടുതൽ രോഗം ഉണ്ടാക്കാൻ ശേഷിയുള്ളതായി ആറ്റിങ്ങലിൽ നിന്നും വേർതിരിച്ച ഫംഗസിനെ തിരഞ്ഞെടുത്തു.

രൂപശാസ്ത്രപരവും തനതുമായ പ്രത്യേകതകൾ മനസ്സിലാക്കി രോഗഹേതു *R. solani* (A. NO.KX674527) റൈസ്ക്ടോണി സൊളാനി എന്ന് തിരിച്ചറിഞ്ഞു. കൂടാതെ ITS അനുവർത്തനം വഴി കണിക പഠനം നടത്തിയപ്പോൾ *R. solani* AG - 1 IA CSU 8 വുമായി 100% സാദൃശ്യം ഉണ്ടെന്ന് കണ്ടെത്തുകയും ചെയ്തു.

ലാബ് പഠനത്തിൽ രോഗഹേതുവിന്റെ തന്തുജാലവളർച്ചയെ മുട്ടമിശ്രിതം (10:1) പഞ്ചഗവ്യം (5:1) വെളുത്തുള്ളി സത്ത് (10:1) സെറ്റേറിയസത്ത് (100:1) പൊട്ടാസ്യം സിലിക്കേറ്റ് (1:1) എന്നിവകൊണ്ട് 100% നിരോധിക്കാൻ കഴിഞ്ഞു. സ്ക്ലലീറോഷിയത്തെ മേൽപ്പറഞ്ഞ ലായനികളിൽ 24 മണിക്കൂർ മുക്കിവച്ചപ്പോൾ പൊട്ടാസ്യയം സിലിക്കേറ്റ് ഒഴികെ ഉള്ളവ 100% തന്തുജാല പുനരുജ്ജീവനം തടഞ്ഞു. മുഖ്യപോഷകങ്ങളുടെയും (N,P,K) അമ്ലതയുടേയും അളവുകൾ സൂക്ഷ്മമായിപരിശോധിച്ചതുവഴി സിറ്റേറിയസത്ത് (4.9), മുട്ടമിശ്രിതം (4.5), പഞ്ചഗവ്യയം (4.2)

എന്നിവയ്ക്ക് അമ്ലത വളരെ കൂടുതലാണെന്ന് കണ്ടെത്തി. മൊത്തം N ന്റെ അളവ് ഏറ്റവും കൂടുതൽ പഞ്ചഗവ്യയത്തിലും (0.02%) തുടർന്ന് മുട്ടമിശ്രിതത്തിലും (0.01%) ആണ്. എന്നാൽ K ഏറ്റവും അധികം പഞ്ചഗവ്യയത്തിലും (0.03%) തുടർന്ന് പൊട്ടാസ്യയം സിലിക്കേറ്റിലും (0.01%) കൂടാതെ Pയുടെ അളവ് പഞ്ചഗവ്യയത്തിൽ ഏറ്റവുംകൂടുതലും (0.007%) മുട്ടമിശ്രിതത്തിൽ (0.006%) 2-ാമതും കണ്ടെത്താൻ സാധിച്ചു.

മൺച്ചട്ടികളിൽ പരിപാലിച്ച നെൽചെടികളിൽ (Varഉമ) പരീക്ഷിച്ചതിന്റെ അടിസ്ഥാനത്തിൽ രോഗത്തിന്റെ മുകളിലോട്ടുള്ള വളർച്ചയെ മുട്ടമിശ്രിതത്തിന് 79.43 % വും വെളുത്തുള്ളി സത്തിന് 79.36% വും നിരോധിക്കുവാൻ സാധിച്ചിട്ടുണ്ട്. ഇത് പരമ്പരാഗത കുമിൾനാശിനി ഹെക്സാകൊനാസോളി (79.36%) നോട് കിടപിടിക്കുന്ന ഫലം തന്നെയാണ്. രോഗത്തിന്റെ സമന്തരവ്യാപനം ഏറ്റവും കുറവ് മുട്ടമിശ്രിതം തളിച്ച നെൽ ചെടികളിലും (11.12 %) തുടർന്ന് പൊട്ടാസ്യയം സിലിക്കേറ്റിലും (16.93%) തളിച്ചവയിലും ആയിരുന്നു. ഇതും ഹെക്സാകൊനാസോളിന്റെ ഫലവുമായി വളരെ അടുത്ത ഒന്നാണ് (10.44%) എന്നാൽ ഏറ്റവും കൂടുതൽ ധാന്യവിളവ് കാണിച്ചത് സിറ്റേറിയസത്ത് (15.98%) തളിച്ച നെൽചെടികളിലും തുടർന്ന് മുട്ടമിശ്രിതത്തിലും (15.33%) വെളുത്തുള്ളിസത്ത് (14.66g) പൊട്ടാസ്യയം സിലിക്കേറ്റ് (13.32g) എന്നിവ തളിച്ച നെൽചെടികളിലും ആയിരുന്നു.

ഒിളവെടുപ്പ് സമയത്തെ പോഷകങ്ങളുടെ അളവ് പരിശോധിച്ചപ്പോൾ N ഏറ്റവും കൂടുതൽ വെളുത്തുള്ളി തളിച്ചവയിലാണെന്നും (1.645%) K ഏറ്റവും പൊട്ടാസ്യം സിലിക്കേറ്റ് തളിച്ചവയിലാണെന്നും (3.60%) കണ്ടെത്താൻ സാധിച്ചു.

അുട്ടമിശ്രിതം (10%), പൊട്ടാസ്യം സിലിക്കേറ്റ് (1%), വെളുത്തുള്ളി സത്ത് (10%) എന്നിവ കൊണ്ട് നെല്ലിന്റെ സ്വാഭാവിക ഗുണങ്ങളും വിളവും വർദ്ധിപ്പിക്കുവാനും സാധിച്ചു. നെല്ല് വിതച്ച് 45, 60, 75, 90 എന്നീ ദിനങ്ങളിൽ 1% വീര്യമുള്ള പൊട്ടാസ്യം സിലിക്കേറ്റ് തളിക്കുന്നത് ഏറ്റവും ചിലവ് കുറഞ്ഞതും, ഫലപ്രദവുമായ പരിചരണമാണെന്നും ഈ പഠനം തെളിയിച്ചു. മാത്രവുമല്ല ഏതൊരു പരമ്പരാഗത രാസകുമിൾനാശിനിയോടും കിടപിടിക്കുന്ന ഫലവുമാണ് ഈ പഠനം