Ecofriendly management of sheath blight disease of rice

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(2017-11-049)

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by

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2020

DECLARATION

I, hereby declare that this thesis entitled **"Ecofriendly 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.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
^{0}C	Degree Celsius
AMF	Arbuscular mycorrhizal fungi
CD	Critical difference
CRD	Completely Randomized Design
cm	Centimeter
DAI	Days after inoculation
et al	And other co workers
g	Gram
h	Hour
L	Litre
M	Molar
mM	Milli molar
ml	Milliliter
mg	Milligram
min.	Minute
PDA	Potato dextrose agar
PO	Peroxidase
PPO	Polyphenol oxidase
PAL	Phenylalanine ammonia lyase
PR-proteins	Pathogenesis related protein
PVP	Poly vinyl pyrrolidone
rpm	Rotations per minute
SE (m) $\pm$	Standard error of mean
Viz.,	Namely

Introduction

1. INTRODUCTION

Rice is the second important cereal after wheat in the world. It is considered to be a staple food crop of the South Asian countries (Shasmita *et al*., 2019). It occupies around 11.7 percent of total cropped area with a production of 574.6 million tonnes and productivity of 4.5 tonnes per hectare in the world. India ranks second in production of rice, next to china (FAO, 2018). About 80 per cent of the calorific intake of the people is obtained from rice in the Asian countries.

India, despite having larger area under cultivation of rice, the major problems faced by rice farmers include inefficient utilization of applied nitrogen (N), lack of awareness of high yielding varieties, poor nutrient management, poor seed quality, widely varying climatic conditions, adverse effects of soil alkalinity and salinity as well as susceptibility to pests and diseases (Yellareddy, 2014).

Pests and diseases are considered to be the major limiting factors in yield reduction of the crop. Several diseases caused by bacteria, fungi, viruses, nematode and phytoplasmas result in yield reduction of the crop. Diseases alone are estimated to result in yield losses of about 2 to 40 per cent (Talukdar, 1968). Ou (1985) has described 60 rice diseases, of which 37 are of fungal origin. Among the fungal diseases, sheath blight disease is the most devastating disease which drastically reduces the grain and straw yield (Kumar *et al*., 2009).

Rice sheath blight disease was initially reported from Japan (Miyake, 1910) and later researchers from several countries reported the occurrence of disease. It is also referred as oriental leaf and sheath blight disease (Kozaka, 1975). In India, it was first reported from Gurdaspur in Punjab (Paracer and Chahal, 1963), and later from Uttar Pradesh (Kohli, 1966). Further, the disease was reported from Tamil Nadu, Kerala, Andhra Pradesh and Kashmir (Reddy and Reddy, 1986). Management of the disease is often difficult due to wider host range of *R. solani* and longer duration of survival of sclerotia in rice stubbles and in the soil (Singh *et al.,* 2019).

Use of chemical pesticides is the most common practice followed for plant protection. However, application of fungicides for disease management is neither economical nor environmentally safe and there is the possibility of development of enhanced pathogen population which are resistant to fungicides (Yellareddy, 2014). The indiscriminate and disproportionate use of pesticides may lead to residues in food chain, harmful effects on humans, animals and environment (Chahal *et al*., 2011). Hence, farmers prefer integrated strategies for management of the diseases. In recent years, wider application of liquid organic preparations and lesser use of new generation fungicides for disease management are gaining importance.

Plants develop enormous amount of secondary substances with antimicrobial property to withstand the diseases (Jun-Dong *et al.,* 2006). Several organic products and plant extracts have proven their usefulness against numerous plant pathogens.

The present study entitled "Ecofriendly management of sheath blight disease of rice" was undertaken with an aim to develop an effective ecofriendly strategy for the management of sheath blight disease of rice by using organic preparations, botanicals and non- hazardous chemicals.

The major objectives of the study were,

- Studies on the pathogenicity and cultural characters of *R. solani*.
- *In vitro* disease management studies.
- *In vivo* management studies.
- Enzyme assay in the treated rice plants.

Review of Literature

2. REVIEW OF LITERATURE

Sheath blight disease of rice is one of the major fungal diseases of rice caused by *Rhizoctonia solani* Kuhn. In India, the yield loss due to sheath blight disease is around 54.3% (Chahal *et al*., 2003). A yield loss of about 20 - 50 per cent is recorded worldwide depending up on the severity of the disease (Dey *et al*., 2019). There are no resistant genes identified for the sheath blight disease till date. Hence, management of the disease is a difficult task. Thus, the current work "Ecofriendly management of sheath blight disease of rice" was carried out with an objective to develop an effective ecofriendly strategy for the management of sheath blight disease of rice. The literature pertaining to the pathogenicity and cultural studies of *R. solani*, *in vitro* and *in vivo* management of the disease have been reviewed and detailed in this chapter.

2.1. STUDIES ON THE PATHOGENICITY OF *Rhizoctonia solani*, THE RICE SHEATH BLIGHT PATHOGEN

Pathogenicity of *R. solani* was tested by artificial inoculation of the pathogen on the healthy rice plants. 0.2 mg of inoculum was placed inside the leaf sheath of the healthy plants (Singh *et al*., 2002). The lesion length of four rice cultivars inoculated with *R. solani* was studied and found that lesion length was smaller in kavya and swarndhan cultivars, where as it was larger in PD- 4 and ARC- 10635 (Singh *et al*., 2002).

The pathogenicity of *R. solani* isolates from rice was tested and the isolate of AG1-IA produced typical rice sheath blight symptoms of ellipsoidal shape with a lesion length of 1.5 to 6 cm which are initially light green in colour, later turned to greyish center with dark brown margin (Taheri *et al*., 2007, Adhipathi *et al*., 2013).

Most of the *R. solani* isolates collected from rice plants produced were elliptical or elongated lesion with lesion length varying from 0.15- 3.15 cm^2

(Bhukal *et al*., 2015). Manjunatha *et al*. (2018) studied the pathogenicity of 20 isolates of *R. solani* on the susceptible cultivar *viz.,* TN-1 and observed that all the isolates took five days to exhibit the characteristic sheath blight symptoms. Pralhad *et al*. (2019) reported that inoculated plants produced symptoms of sheath blight disease from third day in all the plants.

2.2. CULTURAL CHARACTERS OF *R. solani*

According to Meena *et al* (2001), time taken for the formation of sclerotia ranged from 3-11 days. Lal and Kandhari (2009) studied the cultural and morphological characteristics of *R. solani* isolates from different rice growing regions in India. They reported that the isolates took three to five days for the initiation of sclerotia.

Twenty five isolates of *R. solani* affecting rice were collected from five different states of India including Karnataka, Tamil Nadu, Kerala, Odisha and Andhra Pradesh and evaluated their diversity in virulence on PDA medium. The collected isolates significantly differed in colour, colony texture, size of sclerotia, number of sclerotia produced and time taken for the formation of sclerotia. (Singh *et al*., 2015).

Gopireddy *et al*. (2017) evaluated sixty isolates of *R. solani* collected from different districts of Andhra Pradesh. The isolates took ten to fifteen days for the initiation of sclerotia. Sclerotial formation was not found in RS 58 and RS 59 isolates. Only 15 isolates were found to produce good number of sclerotia after fifteen days of inoculation. 21 isolates were found to have dark brown colour sclerotia.

Mughal *et al*. (2017) grouped the *R. solani* isolates based up on the sclerotial colour. Isolates with dark brown colour of the sclerotia were in one group and light brown sclerotia producing sclerotia in other group. Gurav *et al*. (2018) isolated 11 cultures of *R. solani* from potato causing black scurf disease and studied both cultural and morphological characteristics in PDA medium. All

the isolates produced brown to dark brown sclerotia which were either in the form of concentric ring at the centre or scattered throughout the colony.

Pralhad *et al*. (2019) studied the morphology and sclerotial characteristics of three *R. solani* isolates causing sheath blight disease of rice in Karnataka on PDA medium. All the isolates produced mycelium of yellowish brown colour with sclerotial formation varying from four to eight days with either round or mustard sclerotia. Sandoval *et al*. (2019) studied the morphological characters of 42 isolates of *R. solani* collected from rice, mung bean and grasses. Sixteen isolates developed white to brown coloured small sclerotia, whereas 20 isolates developed light to dark brown sclerotia and six isolates did not produce any sclerotia.

Recently, Divya *et al*. (2019) studied the cultural and morphological characters of *R. solani* isolates collected from rice, soybean, mung bean, groundnut and maize under *in vitro* conditions in PDA medium. Significant differences in shape, size and colour of sclerotia were observed in all the isolates. The sclerotia were big sized, globular in the isolates collected from rice plants, whereas the sclerotia were spherical to oval shaped and irregular shaped in the isolates collected from maize and soybean respectively. Dark brown mature sclerotia were observed in the isolates of rice, soybean and mung bean, whereas grey coloured sclerotia were observed in maize and groundnut.

2.3. *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS, BOTANICALS, NON-HAZARDOUS CHEMICALS AND THEIR COMBINATIONS AGAINST *R. solani*

2.3.1. Effect of organic preparations, botanicals, non-hazardous chemicals and there combinations on *in vitro* inhibition of mycelium of *R. solani*

2.3.1.1. Organic preparations

Mdee (2009) studied the effect of seven weed species against phytopathogenic fungi *Lantana camera* extract recorded the greater mycelial inhibition (85 %) against *R. solani* at 15 per cent concentration. Khoa *et al*. (2010) observed the antifungal effect of fresh and dried leaves of the common weed plant *Chromolaena odorata* against *R. solani* in PDA medium. Dried leaf extracts at ten per cent concentration inhibited 68 per cent of mycelial growth even after 21 days in PDA medium.

Pal *et al*. (2013) studied the antifungal activity of some weed extracts. They reported that *Ageratum conyzoides* and *Parthenium hysterophorus* have antifungal against *Alternaria spp.* Pal and Kumar (2013) studied the antifungal activity of 11 weed extracts against wilt causing pathogen, *Fusarium oxysporum* under *in vitro* conditions by poisoned food technique. From the tested weed extracts, the methanol extracts of *Cannabis sativa* (84.21 %), *Ageratum conyzoides* (75.22 %) and *Argemone maxicana* (61.84 %) were observed as most effective in inhibiting the mycelial growth of phytopathogenic fungi, *Fusarium oxysporum.*

Rodino *et al*. (2014) studied the antifungal effect of four weed plants like absinth *(Artemisia absinthium*), rosemary (*Rosmarinus officinalis*), jimson weed (*Datura stramonium*) and cocklebur (*Xanthium strumarium*) against *Alternaria alternata.* Ethanolic and aqueous extract of jimson weed recorded maximum inhibition (100 %) of the mycelial growth of fungi at 10 per cent concentration.

Pathak *et al*. (2017) studied the efficacy of weed extracts from castor and datura against *R. solani* causing root rot disease of buckwheat. Maximum inhibition of mycelial growth was observed in castor than datura under *in vitro* conditions. Devkota and Sahu (2017) studied the antifungal activity of plant extracts of *Parthenium* against *Alternaria brassicae, Botrytis cinerea, Fusarium*

oxysporum, *Phytophthora capsici* and *Sclerotium rolfsii* at different concentrations (50 mg ml^{-1}, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml) and found that parthenium extract exhibited maximum per cent inhibition with *A. brassica* at 100 mg/ml.

2.3.1.2 Botanicals

A wide range of microorganisms including bacteria, fungi, protozoa and viruses were found to be sensitive to crushed garlic preparations (Delaha and Garagusi 1985).

Majority of the fungi and bacteria evaluated were found to be sensitive to garlic extract. Garlic was the most effective botanical in inhibiting the radial mycelial growth (91.82 %) of sheath blight fungus of rice (Meena *et al*., 1998). Dutta *et al.* (2004) reported that 10 per cent of crude *Allium sativum* extract exhibited maximum inhibition of sclerotial production and 20 per cent showed excellent mycelial inhibition of *R. solani* causing sheath blight of rice. The leaf extracts of *Zizyphus jujuba* and *Ipomoea carnea* inhibited the *in vitro* mycelial growth of *R. solani,* and effectively reduced the occurrence of sheath blight disease in rice (Kagale *et al*., 2004).

Yadav (2007) reported that out of eight plant extracts tested against *R. solani* causing web blight of french bean, garlic extract was having cent percent inhibition of the mycelial growth followed by ginger, neem, onion, datura and tulsi. Zainab *et al*. (2009) studied the effect of seed powder of ten local trees against *Macrophomina phaseolina, R. solani* and *Fusarium solani* causing root rot disease in mung bean *(Vigna radiata L.)* and chick pea *(Cicer arietinum L.)* plants and found that neem at 10 per cent was found effective in the mycelial inhibition of the pathogens.

Afifi *et al*. (2009) studied the antifungal effect of neem and other plant extracts against *A. solani*, the cause of tomato early blight disease. Among the treatments, maximum inhibition of the mycelial growth was recorded with neem

extract at 15 per cent concentration, followed by garlic extract at 10 per cent concentration. Sinha *et al*. (2009) reported that garlic and ginger extracts had maximum (100 %) inhibition against *R. solani* under *in vitro* condition followed by neem (70 %) when ten botanicals were tested. Forty four plant extracts were tested for their antifungal activity against *R. solani* and found that clove extract of garlic exhibited fungicidal property at 100 ppm (Shejpal *et al*., 2009).

Emanual *et al.* (2010) observed the reduced infection of *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina* on mung bean and okra along with enhanced plant growth parameters when treated with neem and datura at 10 per cent concentration. Askar and Rashad (2010) studied the antifungal activity of anise (*Pimpinella anisum* L.), black seed (*Nigella sativa* L.) and clove (*Syzygium aromaticum* L. Merr. & Perry.) against pea (*Pisum sativum* L.) root-rot fungus *Rhizoctonia solani* and found that clove and black seed extracts were found to have maximum mycelial inhibition of the pathogen.

Nine botanicals *viz.,* Bhang (*Cannabis sativa*), Bael (*Aegle marmelos*), Eucalyptus (*Eucalyptus citridora*), curry leaves (*Murraya koenghii*), congress grass (*Parthenium hysterophorus*), paanch phooli (*Lantana camara*), tulsi (*Ocimum sanctum*), drek (*Melia azedirach*) and onion (*Allium cepa*), each at 5, 10, 15 and 20 per cent were tested against *R. solani* and found that drek extract was most effective in inhibiting the mycelial growth (46.5 %) followed by bhang (29.7 %), onion (25.4 %), tulsi (23.9 %), bael (20.6 %), paanch phooli (17.9 %), curry leaves (14.1 %), congress grass (13.4 %) and eucalyptus (10.4 %) at 10 per cent concentration (Dutta and Kalha, 2011).

Khair and Nadia (2011) studied the antifungal effect of aqueous extracts of chilli, lantana, lemon grass and onion seeds and found that extracts of onion and lemon grass have shown maximum disease suppression of *F. solani* and of *R. solani* in *Phaseolus vulgaris* plants. Sallam (2011) studied the effect of six plant extracts at 5 per cent concentration against *Alternaria solani* under *in vitro*

conditions. Plant extracts of datura, neem and garlic at 5 per cent concentration recorded maximum inhibition of mycelial growth (44.4%, 43.3%, 42.2%) respectively. Seema *et al*. (2011) studied the antifungal principles of ten plant extracts against *R. solani* using poisoned food technique. *Piper betel* was found to have maximum inhibition of the mycelial growth (80 %).

Shukla and Dwivedi (2012) studied the antifungal efficacy of different plant extracts like bitter guard, turmeric, garlic and black pepper against fusarium species *viz. Fusarium udum* (wilt of pigeonpea) and *Fusarium oxysporum* f.sp.*ciceri* (wilt of chickpea).The extracts were evaluated at 5, 10 and 15 per cent concentrations. The maximum reduction of mycelial growth of *F. udum* was recorded with turmeric (89.2 %) at 15 per cent concentration followed by garlic extract (88.26 %) at 10 per cent concentration. The maximum reduction of mycelial growth of *F. oxysporum* was recorded with garlic (94.63 %) at 10 per cent concentration followed by turmeric and black pepper.

Gurjar *et al*. (2012) highlighted the significant role of important plant botanicals including Neem (*Azadirachta indica*, A. Juss), Garlic (*Allium sativum*, Linn.), Eucalyptus (*Eucalyptus globulus*, Labill.), Turmeric (*Curcuma longa*, Linn.), Tobacco (*Nicotiana tabacum*, Linn.) and Ginger (*Zingiber officinale*, Rosc.) in plant disease management. Mishra *et al*. (2005) and Srinivas (2013) screened thirteen plant extracts and found that garlic extract (10 %) was the most effective botanical in inhibiting the mycelial growth of *R. solani* followed by *Calotropis* (10 %).

The antifungal efficacy of six botanical extracts *viz.*, *Cannabis sativa L.*, *Peganum harmala* L., *Datura starmonium* L., *Artemisia brevifolium* L., *Capparis spinosa* L., *Mentha royleana* L. were evaluated under *in vitro* conditions against sclerotial isolates of *R. solani* causing black scurf of potato from five to fifteen per cent concentrations and found that the highest antifungal property was in *C. sativa* with a mycelial inhibition of 36.43 - 80.00 per cent which was followed by *P.*

harmala (26.71 % - 69.20 %) and *D. starmonium* (26.44 % - 70.28 %), while the least was by *Capparis spinose* (21.70 % -50.16 %). (Hussain *et al*., 2014).

Sriraj *et al.* (2014) found seed and oil extract of *Madhuca longifolia* had greater mycelial inhibition among the nine botanicals evaluated against *R. solani* causing leaf blight of turmeric. Mokhtar (2014) evaluated the antifungal effect of three botanicals in powdered form and their extracts (powder of chilli pods, cabbage leaves, eucalyptus leaves) at 2, 4 and 8 per cent under *in vitro* condition against root rot disease of bean. They found that the extract form had shown greater inhibition on radial mycelial growth than the powdered forms. All the treatments have shown maximum inhibition at 8 per cent concentration.

Srinivas *et al*. (2014) reported that among thirteen plant extracts tested against *R. solani*, garlic extract was the most effective in inhibiting the mycelial growth of the fungus followed by *Calotropis sp*. at 10 per cent concentration. Zaker (2014) studied the antifungal effect of six plant extracts against *Fusarium solani*, the causal agent of potato dry rot. Maximum mycelial inhibition (85 %) was seen in artemisia at 15 per cent concentration, followed by garlic extract (84.8 %) in PDA medium.

The efficacy of 13 plant extracts *viz* leaf extracts of *Azadirachta indica, Catharanthus roseus, Lantana camara, Ocimum sanctum, Ricinus communis, Saraca indica* and *Thuja occidentalis* , latex yielding plants, *Calotropis procera, Nerium indicum, Datura, Ficus religiosa* and bulbs of *Allium cepa* and *Allium sativum* were evaluated to control root rot pathogen under *in vitro* conditions in sponge gourd and found that leaf extract of *Ricinus communis* and bulb extract of *Allium sativum* resulted in maximum control of *R. solani*. (Sadda and Varma, 2015). Ghany *et al.* (2015) studied the antifungal effect of four plant extracts against *Fusarium oxysporum, Alternaria alternate* and *R. solani* and recorded the maximum disease reduction with neem and jatropa at ten per cent concentration. Lekshmi *et al*. (2015) evaluated the effectiveness of garlic extract against a range

of plant pathogenic fungi and bacteria under *in vitro* conditions and *in vivo* conditions.

Rajput *et al*. (2016) evaluated seven plant extracts namely neem, jatropha, annona, curcuma, garlic, pongamia and datura for antagonistic effect against *R.solani* causing banded leaf and sheath blight disease in maize, and found that the maximum inhibition of mycelial growth was recorded by neem followed by garlic at both five and ten per cent concentrations. Sifat and Monjil (2017) studied the efficacy of six plant extracts for mycelial growth inhibition of *R. solani*. The maximum per cent inhibition of radial mycelial growth over control was recorded with garlic (97.50 %), followed by neem (96.75 %), and biskatali (94.25 %).

Kumar *et al*. (2017) reported that the bulb extract of *Allium sativum* and rhizome extract of *Zingiber officinale* inhibited the mycelial growth (80.19 and 76.32 % respectively) at ten per cent concentration followed by the leaf extract of *Azadiratcha indica* (72.78 %) when neem, tulsi, garlic, onion and ginger were tested for their antifungal property against *R. solani*.

Karthika *et al*. (2017) reported that garlic extract (10 %), fermented weed (*Setaria barbata)* extract (100 %), fermented egg-lemon juice extract (10 %), potassium silicate (1 %), lime solution (12.5 %) and panchagavya (5 %) resulted in cent per cent mycelial inhibition of *R. solani* in potato dextrose agar medium. Among the three plant extracts which were evaluated for the inhibition of mycelial growth of *R. solani*, neem leaf extract at 10 per cent was found effective in the inhibition of mycelial growth (48.71 %) followed by garlic bulb extract at 10 per cent (46.13 %) and tulsi leaf extract at 10 per cent (34.38 %) (Rajput *et al*., 2017).

A total of 11 plants were evaluated against *Colletotrichum musae* (anthracnose of banana) and *Rhizoctonia solani* (sheath blight of rice) at 0.2 per cent concentration and found that the leaves of *Clerodendrum*, *Polyalthia* and

rhizomes of ginger were found most effective against both the pathogens (Choudhury *et al*., 2017). Jain *et al*. (2017) found that the aqueous bud extract of garlic and rhizome extract of ginger completely inhibited the radial mycelial growth of *R. solani* causing banded leaf and sheath blight of little millet followed by onion bulb extract (92.5 %), neem leaf extract (76.7 %), datura leaf extract (75.3 %), tulsi leaf extract (70.8 %), parthenium leaf extract (69.3 %) and bel leaf extract (51.4 %) at nine per cent concentration.

Sharma (2018) reported that the clove extract of garlic (*Allium sativum*) at ten per cent resulted in maximum inhibition (71.85 %) when compared to the other commonly available aqueous extracts of ten plants, when tested against *R. solani*. Mahmud *et al*. (2018) conducted an experiment to evaluate extracts of garlic, neem, BAU- biofungicide (*Trichoderma* based formulation), bavistin and potent 250 EC for the management of four rice diseases like narrow brown leaf spot, sheath blight, sheath rot and false smut. Significant reduction in mycelial growth of *Cercospora oryzae, R. solani, Sarocladium oryzae* and *Ustilaginoidea virens* were observed with BAU- biofungicide (82 %) at three per cent concentration, followed by neem and garlic at three per cent concentration.

Karem *et al*. (2019) studied the antifungal effect of three plant extracts against *R. solani* causing sheath blight disease of rice and observed that the extract of *Tamarix mannifera* recorded the maximum mycelial inhibition (85 %) than other plant extracts. Persuad *et al*. (2019) studied the effect of eleven plant extracts under *in vitro* conditions against *R. solani*. The extracts of lemon grass, thick leaf thyme, marigold and clove of garlic at 15 per cent showed greater inhibition of mycelial growth over control.

In vitro efficacy of 5, 10 and 20 per cent concentrations of six plant extracts *viz*., *Adhatoda vasica* (Nees), *Azadirachta indica* (A Juss). *Ocimujm sanctum* (L), *Allium sativum* (L), *Datura metel* (Linn) and *Zingiber officinale* (Rose) were tested against *A. solani*, causing early blight disease of tomato.

Allium sativum was the most effective botanical in inhibiting the radial mycelial growth (74.07 %), followed by *Zingiber officinale* (70.05 %) (Roy *et al*., 2019).

2.3.1.3. Non- hazardous chemicals

Silicon is found to enhance the disease resistance of monocot and dicot plants. Negative interactions between the silicon content of plant tissues and disease severity were observed 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).

Kaiser *et al*. (2005) reported that all concentrations from 5 to 80 ml per litre PDA of soluble silicon completely suppressed the mycelial growth of *Colletotrichum coccodes, Mucor pusillus, Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Phytophthora cinnamomi*. Bekker *et al*. (2006) conducted an experiment to evaluate the efficacy of soluble potassium silicate against *Phytophthora cinnamomi, Sclerotinia sclerotiorum, Pythium, Mucor pusillus, Drechslera, Fusarium oxysporum, F. solani, Altemaria solani, Colletotrichum coccodes, Verticilium theobromae, Curvularia lunata* and *Stemphylium herbarum*. Cent per cent inhibition of mycelial growth was observed at 100 ppm for all fungi tested with the exception of *Drechslera* sp. and *F. oxysporum*.

2.3.2. Effect of organic preparations, botanicals, non-hazardous chemicals and their combinations on *in vitro* inhibition of mycelial regeneration from sclerotia of *R. solani*

Imtiaj *et al*. (2005) studied the antifungal effect of 13 plant extracts like *Curcuma longa, Ocimum sanctum, Adhatoda vasica, Polygonum hydropiper, Azadirachta indica, Tagetes erecta, Zingiber officinales, Acalypha indica, Datura metel, Allium sativum* and *Vinca rosea* against the conidial germination of *Colletotrichum gloeosporioides* causing anthracnose of mango. The maximum conidial inhibition was observed in *C. longa* (leaf and rhizome), *A. indica* (bark), *T. erecta* (leaf) and *Z. officinale* (rhizome) after 15, 15, 30, 15 and 10 minutes of

dipping in treatment, respectively. The lowest conidial inhibition was recorded in *V. rosea* (leaf) (94 %) and *A. sativum* (bulb) (78 %).

Seema *et al*. (2011) studied the antifungal principles of ten plant extracts against *R. solani* using poisoned food technique. *Piper betel* was found to have the maximum inhibition of mycelial regeneration from sclerotia (70 %). Zaker (2014) studied the antifungal effect of six plant extracts against *Fusarium solani*, the causal agent of potato dry rot. Methanolic extracts of artemisia at 15 per cent concentration resulted in the maximum inhibition of spore germination (75 %), followed by extracts of thyme and eucalyptus in PDA medium.

Karthika *et al.* (2017) reported that dipping sclerotia of *R. solani* for 24 h in fermented egg-lemon juice extract (10 %), Panchagavya (5 %), lime (12.5 %) and fermented weed extract (100 %) resulted in complete inhibition of mycelial regeneration from sclerotia of *R. solani.*

2.4. *IN VIVO* EXPERIMENTAL STUDIES

2.4.1. Organic preparations

Screening of fresh and dried leaves of common weed plant *Chromolaena odorata* against *R.* solani under semi field conditions was carried out. The maximum reduction of the disease severity (52 %) was recorded by dried leaf extracts of the weed at 10 per cent concentration. This extract was also found to reduce the severity of other diseases of rice like blast (*Pyricularia oryzae*) (45 %), brown spot (*Bipolaris oryzae*) (57 %), and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) (50 %) (Khoa *et al*. 2010).

2.4.2. Botanicals

Obagwu and Korsten (2002) studied the management of *Penicillium digitatum* and *P. italicum*, the cause of citrus green and blue moulds respectively with extract of garlic cloves mixed with vegetable (sunflower) cooking oil or fruit wax in (0.1% v/v), using two orange cultivars (Valencia and Shamouti), and

grapefruit. Use of one per cent garlic extract along with oil was found to be effective in managing the disease.

Leaf extract of garlic was used to control stem rot of chilli (Mathur and Gurjar 2002), basal stem rot of arecanut (Iyer *et al*., 2004) and maydis leaf blight of maize (Tomar and Chandel 2006). Afifi *et al*. (2009) studied the antifungal effect of neem and other plant extracts against *A. solani*, the cause of tomato early blight disease. All the treatments were evaluated at 5, 10 and 15 per cent concentrations. The maximum disease reduction was observed with neem extract at 15 per cent concentration, followed by garlic extract at 10 % concentration.

Sallam (2011) studied the effect of six plant extracts against *Alternaria solani* under greenhouse conditions. The maximum reduction in disease severity was observed with fungicide followed by extracts of garlic at five per cent and Datura at one and five per cent concentrations. Nashwa and Abo-Elyousr (2012) studied the effect of various plant extracts against the early blight disease of tomato plants caused by *Alternaria solani* under greenhouse and field conditions. Under greenhouse conditions, greater reduction of disease severity was observed in the plants treated with garlic at five per cent concentration (46.1 %) followed by datura at five per cent concentration. (45.2 %). Under field conditions, the maximum disease reduction was observed in plants treated with garlic (76.1 %) followed by datura (66.7%).

Ezeh *et al*. (2013) studied the antifungal efficacy of garlic (*Allium sativum*) and uziza (*Piper guineense*) for the control of tuber rot fungi of potato and carrot. Greater reduction of disease severity was recorded in the plants treated with uziza (91.7 %) followed by garlic (87.06 %). Chourasiya *et al*. (2013) studied the effect of botanicals against *Alternaria solani*, causing early blight of tomato under field conditions. Neem leaf extract recorded lower disease incidence (30.66 PDI), followed by garlic bulb extract (32.44 PDI) and also increased the fruit yield.

Zaker (2014) studied the antifungal effect of six plant extracts against *Fusarium solani*, the causal agent of potato dry rot under *in vivo* conditions. Artemisia treated plants at 15 per cent recorded cent per cent disease reduction, followed by eucalyptus and garlic extracts. Ten botanicals were tested against *R. solani* in soybean causing root rot disease under glass house conditions and minimum PDI was recorded by zinger (31.48 %) followed by garlic (35.18 %) and neem (40.18 %) (Patole *et al*., 2016).

Rajput and Zacharia (2017) studied the antifungal effect of 14 botanicals against *R. solani*. Among the different treatments, foliar spray of neem leaf extract recorded the maximum per cent inhibition (44.24 %), followed by garlic bulb extract (37.61 %) and tulsi leaf extract (27.60 %) at 10 per cent. Choudhury *et al*. (2017) studied the fungicidal property of 11 plants against *Colletotrichum musae* (anthracnose of banana) and *Rhizoctonia solani* (sheath blight of rice) at 0.2 per cent concentration, in pot culture and field experiment on rice plant. They found that all plant extracts showed significant inhibition of radial growth of all the test pathogen. The most effective plant extract was *Clerodendron* extract against rice sheath blight pathogen both under *in vitro* and *in vivo* conditions.

Clove extract of garlic was used to manage several diseases like alternaria blight of mustard, sheath blight of rice, early and late blight of potato, wilt of cowpea, grey mildew of cotton, basal stem rot of coconut (Kumar and Singh 2018). Among six plant extracts *viz*., aak, datura, garlic, neem, kheep and tumba, garlic at 10 per cent concentration was found most effective in reducing root rot incidence of chickpea caused by *Macrophomina phaseolina* followed by neem leaf extract (Lakhran *et al*., 2018). Among the botanicals evaluated Achook, Neem, Neem gold and Tricure showed significant reduction in disease severity, along with improvement in yield attributes (Pandey 2018).

Recently, Persuad *et al*. (2019) studied the effect of eleven plant extracts under field and greenhouse conditions against *R. solani*, causing sheath blight disease in rice. The extracts of lemon grass (7.21, 8.04, 4.85 %) and bulb extract of garlic (6.71, 7.28, 4.71 %) at 15 per cent recorded the minimum percent disease severity compared to untreated control (26.25, 31.16, 20.43 %) in greenhouse.

2.4.3. Non- hazardous chemicals

Savanth *et al*. (1997) demonstrated the use of potassium silicate for the management of sheath blight disease of rice. Rodrigues *et al*. (1998) evaluated the effects of Silicon on three different rice cultivars (high level of resistance, moderately susceptible and susceptible) towards their resistance to sheath blight disease and found that Silicon application significantly reduced the development of lesions and final disease intensity in all the varieties.

Rodrigues *et al*. (2001) studied on the application of calcium silicate as a Si source in reducing sheath blight disease for susceptible and moderately susceptible US rice cultivars and found that the application significantly reduced the severity and occurrence of the disease. Total number of sheath blight lesions, severity of sheath blight disease, and the highest relative lesion height on the main tiller of rice plants decreased by 37, 40, 52 and 24 per cent respectively when the rate of Si increased from 0 to 1.92 g pot^{-1} (Rodrigues *et al*., 2003).

Meiqin *et al*. (2005) conducted a study to compare MR 219 and MR 253 rice varieties to sheath blight inoculations and micronutrient like zinc, silicon and copper applications along with the impact of disease on rice yield. Silica gel, copper sulphate and zinc sulphate were applied to the soil prior to planting at the rate of 360, 0.30, 0.45 g per 15 kg soil respectively. When Si was applied, sheath blight severity was reduced by 17.16 per cent for MR 219 and 29.04% for MR 253 variety compared to the respective control treatments (Rodrigues *et al*., 2005). Fertilization with Si was significantly more effective than Cu and Zn treatments in minimizing yield loss due to sheath blight in both varieties (Khaing *et al*., 2014).

Polanco *et al*. (2014) studied the management of anthracnose in common bean by the foliar sprays of potassium silicate and sodium molybdate. Among all the treatments, the plants treated with potassium silicate resulted in maximum disease reduction (47 %) and also resulted in enhanced yield.

Ratnayake *et al*. (2016) studied the soil application of potassium silicate for the management of downy mildew in bitter gourd and found that application of potassium silicate reduced the disease intensity by 37 to 53 per cent over the control. Yongqiang *et al*. (2018) studied the effects of soil amendment for the management of pests and diseases of rice crop. The pots which were amended with 300 kg Sio2 per ha resulted in lower incidence of sheath blight disease.

2.4.4. Arbuscular mycorrhizal fungi (AMF)

Mycorrhizal enrichment greatly improved the uptake of soil nutrients such as nitrogen and phosphorus as well as growth of rice plants (Yeasmin *et al*., 2007). AMF was mainly used for the management of soil borne fungi like *Fusarium, Rhizoctonia, Phytophthora, Cochliobolus/ Bipolaris, Verticillium, Olpidium, Cylindrocladium, Pythium, Fusarium, Aphanomyces, Macrophomina, etc.,* (Arnaud *et al*., 2007). AMF improved the nutrient status of plants, increased plant growth and development, protected plants against pathogens and also aided in the development of resistance to drought and salinity (Bhattacharjee *et al*., 2011). Lidia *et al*. (2012) observed that the rice plants colonized by AMF *Glomus intraradices* showed higher resistance to rice blast disease caused by *Magnaparthe oryzae*.

A study was conducted by Kumar *et al*. (2016) to evaluate different AMF for their ability to reduce wilt disease complex of tomato caused by the bacterial pathogen *Ralstonia solanacearum,* fungal pathogen *Phytophthora capsici* and root-knot nematode *Meloidogyne incognita.* Among 11 AMF screened, *Glomus*

bagyarajii was found to be the best AMF in enhancing plant height, stem girth, total plant dry weight, mycorrhizal root colonization and most importantly recorded least per cent disease index (50.20 %) as compared to control (82.14 %).

Bharath (2017) conducted an experiment with AMF for the bio control of various soil borne diseases in apple like root rot and replant diseases. Results indicated that host plant inoculated with indigenous AMF increased tolerance to a wide range of root diseases in apple. Infection with mycorrhizal fungi elicited a resistance mechanism in inoculated plant which suppressed subsequent infection by fungal pathogens. Inoculation of apple root stock with a potent AM fungal isolate increased growth in apple replant disease.

A study was conducted to evaluate the influence of AMF on rice against pests and diseases. The pests evaluated include rice water weevil (RWW) (*Lissorhoptrus oryzophilus*) and the fall armyworm (*Spodoptera frugiperda*) and the disease evaluated was sheath blight (*Rhizoctonia solani*). The experiment was carried out in both in field and in greenhouse conditions. In the field, inoculation of the rice plants with AMF resulted in higher numbers of RWW larvae on rice roots. In the greenhouse, more RWW first instars emerged from AMF-colonized rice plants than from non-colonized control plants. Lesion length and susceptibility to sheath blight infection were higher in rice plants colonized by AMF (Bernaola *et al*., 2018).

2.5. BIOCHEMICAL STUDIES FOR ASSESSING THE MECHANISMS OF DISEASE MANAGEMENT IN VARIOUS TREATMENT SPRAYED *R. solani* INOCULATED PLANTS

Paranidharan *et al*. (2003) and Zhang *et al*. (2006) reported that peroxidase, superoxide dismutase and catalase were involved in the defense response to *R. solani* infection in rice. Jayaraj *et al*. (2010) recorded a significant increase in the activities of oxalic acid, phenylalanine ammonia lyase and peroxidase from two days after treatment application of garlic and neem in rice. They suggested enhanced activities of defense enzymes and defense-related

compounds in oxalic acid-treated rice plants which contributed to resistance against *R. solani.*

Foliar application of the aqueous leaf extracts of *Ziziphus jujuba* and *Ipomea carnea* followed by challenge inoculation with *R. solani* induced systemic resistance in rice which is clearly evident from significantly enhanced accumulation of pathogenesis-related proteins such as chitinase, β-1,3-glucanase and peroxidase, as well as enzymes and phenolic substances (Kagale *et al.*, 2011). Peroxidase activity reached at its peak at 36 h after inoculation and maximum reduction of its activity was observed 72 h after inoculation. Polyphenol oxidase activity reached its peak at 48 h after inoculation and afterwards a steady decrease was recorded up to 120 h in rice crop against blast disease. (Mondal *et al*., 2012).

Enhanced activities of phenylalanine ammonia lyase, peroxidases, polyphenol oxidases and chitinases were observed in the leaf sheaths of rice plants supplied with silicon resulting in reduced progress of sheath blight lesions. (Schurt *et al*., 2014). Shamim *et al*. (2018) observed that there was greater increase in the activities of catalase, polyphenol oxidase and phenylalanine ammonia lyase in *R. solani* inoculated leaf sheaths of IR 42 rice cultivar than healthy plants. Maximum enzymes activities were recorded in between the 48 h to 96 h after inoculation in all the host and non-hosts.

Materials and Methods

3. MATERIALS AND METHODS

The thesis work on the "Ecofriendly management of sheath blight disease of rice" was conducted during 2017-2019 at the department of Plant Pathology, College of Agriculture (COA), Vellayani and Integrated Farming System Research Station (IFSRS), Karamana. All laboratory studies were conducted at IFSRS, Karamana and the glass house experiment was performed at COA, Vellayani. The materials which were used and the methodology followed are detailed in this chapter.

3.1 STUDIES ON THE PATHOGENICITY OF *Rhizoctonia solani*, THE RICE SHEATH BLIGHT PATHOGEN

The culture of *R. solani* (accession no. KX674527) which was maintained at the department of Plant Pathology, COA, Vellayani was used for the laboratory as well as the glass house studies.

3.1.1 Purification of *R. solani*

The culture of *R. solani* was purified by hyphal tip method (Rangaswamy and Mahadevan, 2006) in potato dextrose agar (PDA) (APPENDIX - I) medium. The purified culture was maintained at room temperature (27±2°C) and was used to undertake further *in vitro* and *in vivo* experimental studies.

3.1.2 Testing the pathogenicity of *R. solani* in rice

The rice variety, Uma was used for the pathogenicity study. Twenty one days old rice seedlings were transplanted into polypropylene grow bags which were filled with sandy clay loam soil. Adequate level of water was maintained in each grow bag. Artificial inoculation of rice plants was carried out at maximum tillering stage of the plants which were free from disease or pest infection using the procedure developed by Jia *et al*. (2013). Five mm mycelial discs of actively growing seven days old culture of *R. solani* were inoculated on to the sheath portion of the rice plants. The plants which were inoculated with uninoculated

PDA culture discs were maintained as the control. The inoculated sheath portion was covered by using a thin layer of moist cotton and all the inoculated as well as uninoculated control plants were covered with finely perforated polypropylene covers to maintain optimum conditions of humidity for the disease development. The days taken for symptom development, nature of the symptoms expressed in the inoculated plants and the length of lesions were recorded in the plants inoculated with the pathogen.

3.2 CULTURAL CHARACTERS OF *R. solani*

To evaluate the cultural characters, PDA medium was prepared and sterilized in an autoclave. Then, 15 ml of the medium was added on to the sterilized plates under aseptic conditions. One week old culture was used for this experiment. From these plates, five mm mycelial bits were cut with the help of sterile cork borer and kept at the centre of Petri plates containing PDA medium. These plates were incubated at room temperature (27±2°C). Cultural characteristics of the pathogen including mycelial growth, number of days taken for the formation of sclerotia, number of sclerotia produced, colour of sclerotia and shape of sclerotia were observed and recorded.

3.3 *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS, BOTANICALS, NON-HAZARDOUS CHEMICALS AND THEIR COMBINATIONS AGAINST *R. solani*

Organic preparations, botanicals, non-hazardous chemicals and their combinations were tested for their efficiency in inhibition of the mycelial growth and regeneration of the mycelium from sclerotia of *R. solani* under *in vitro* conditions at IFSRS, Karamana by poisoned food technique developed by Nene and Thapliyal (1979).

Design : Completely Randomized Design (CRD)

Replications : Three

Treatments : 14

Table 1. Treatments selected for *in vitro* studies of *Rhizoctonia solani* (accession no. KX674527)

Treatments	Description
T1	Fermented egg - lemon juice extract (FEE) (10%)
T2	Fermented weed (*Setaria barbata*) extract (FWE) (10%)
T3	Potassium silicate (PS) (0.5%)
T4	Garlic extract (GE) (10%)
T5	FEE+FWE (1:1)
T6	FEE+ PS (1:1)
T7	PS+FWE (1:1)
T8	FEE+FWE+ GE + PS (1:1:1:1)
T9	FEE+GE (1:1)
T10	FWE+GE (1:1)
T11	FEE+FWE+PS (1:1:1)
T12	PS+GE (1:1)
T13	Tebuconazole 50%+Trifloxystrobin 25% WG (0.04%) (treated control)
T14	Pathogen alone (untreated control)

3.3.1. Preparation of organic preparations

3.3.1.1. *Fermented egg-lemon juice extract (FEE)* (Sajeena *et al*., 2016)

Raw hen eggs, lemon juice and jaggery powder were required for preparing this extract. Twelve eggs were kept in a clean container. Lemon juice was added over the eggs to immerse them in the juice. On the tenth day, jiggery

powder (500g) was added, mixed the contents well and maintained for ten days. The muslin cloth filtered extract on the 21st day was used for subsequent studies at a dilution of ten per cent with water.

3.3.1.2 *Fermented weed extract (FWE)* (Sajeena *et al*., 2016)

Fermented weed extract was prepared by using the weed *viz*., *Setaria barbata* (East Indian bristle grass), salt powder, tamarind pulp, powdered jaggery and water. Two hundred and fifty g of the weed was washed, cut into small bits and taken in a container. Salt, tamarind and jaggery (20 g each) were added. One litre of water was then added to the mixture. The preparation was filtered through muslin cloth on 21st day and used for subsequent studies at ten per cent concentration.

3.3.2 Preparation of botanicals

3.3.2.1 *Garlic extract (GE) (Kumar and Tripathi, 2012)*

The extract was prepared using garlic bulbs (100 g) which were ground using 100 ml of sterile distilled water. The muslin cloth filtered extract was used at ten per cent concentration for the *in vitro* evaluation.

3.3.3 Preparation of non-hazardous chemicals

3.3.3.1 *Potassium silicate (PS) (Devi and Nayar, 2016)*

Potassium silicate (24% silica and 10% potassium) was used at 0.5 per cent concentration for subsequent studies.

3.3.4 Preparation of combination treatments

3.3.4.1 *FEE+FWE (1:1)*

FEE and FWE in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.2 *FEE+PS (1:1)*

FEE and PS in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.3 *PS+FWE (1:1)*

PS and FWE in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.4 *FEE+FWE+GE+PS (1:1:1:1)*

FEE, FWE, GE and PS in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.5 *FEE+GE (1:1)*

FEE and GE in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.6 *FWE+GE (1:1)*

FWE and GE in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.7 *FEE+FWE+PS (1:1:1)*

FEE, FWE and PS in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.4.8 *PS+GE (1:1)*

PS and GE in equal proportions (v/v basis) were mixed and used for subsequent studies.

3.3.5 Effect on *in vitro* inhibition of mycelium

Fermented egg- lemon juice extract, fermented *S. barbata* extract, garlic extract, potassium silicate and their combinations were tested *in vitro* for the mycelial inhibition of the rice sheath blight pathogen, *R. solani* by poisoned food technique (Nene and Thapliyal, 1979) using double strength PDA medium. The media was poisoned using each of the treatment which were filtered through both

Whatman No. 1 as well as bacterial proof filters and diluted with required amount of sterile distilled water. The *in vitro* evaluation study was performed as explained in 3.2. The mycelial growth inhibition in percentage was measured as per the formula developed by Vincent (1947) which is as follows,

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

I - Percentage inhibition

C – Mycelial growth (diameter in cm) of *R. solani* in control plates

T - Mycelial growth (diameter in cm) of *R. solani* in treatment amended plates

3.3.6 Effect on *in vitro* inhibition of mycelial regeneration from sclerotia

The treatments were tested for their potential to inhibit the mycelial regeneration from sclerotia. The sclerotia from one week old *R. solani* culture plate were dipped in the different treatments for 24, 48 and 72 h time periods. Double strength PDA medium was prepared, autoclaved and poured onto the sterilized petriplates and allowed to solidify. One sclerotia which was dipped for each time interval was placed in each petri plate containing PDA medium. Control was maintained by placing the sclerotia dipped in sterile water. The mycelial growth from the sclerotia as well as the per cent reduction of the mycelial growth over control were measured as in 3.3.5.

3.3.7 Selection of the best treatments for *in vivo* study

The two best treatments which exhibited the maximum inhibition of the mycelial growth and mycelial regeneration from sclerotia of *R. solani* during *in vitro* suppression studies were selected for the pot culture experiment.

3.4 *IN VIVO* STUDIES ON THE MANAGEMENT OF RICE SHEATH BLIGHT DISEASE

A pot culture experiment was laid out at COA, Vellayani to study the potential of the best treatments selected from *in vitro* studies in reducing the incidence and severity of sheath blight disease under *in vivo* conditions. The experiment was laid out in completely randomized design (CRD) with eight treatments and four replications per treatment. The rice variety "Uma" was used for the study.

Twenty one days old rice seedlings were transplanted to grow bags (40 cm x 35 cm x 32 cm) which were filled with sandy clay loam soil. Adequate level of water was maintained in the grow bags. Manuring, pest control practices and other cultural operations were done as per the package of practices (POP) recommendations of KAU (2016).

The two best treatments selected from *in vitro* studies singly as well as in combination with the soil application of arbuscular mycorrhizal fungus (AMF) (KAU formulation) which was applied at the rate of 200g/m^2 of nursery area, AMF (soil) application alone, the combination fungicide *viz*., tebuconazole 50% + trifloxystrobin 25% WG (foliar spray @ 0.04%), inoculated untreated control and uninoculated untreated control were the treatments selected for the study.

3.4.1 Preparation and artificial inoculation of *R. solani* (IRRI, 1986)

The inoculum of *R. solani* prepared using rice bran and water in 2:1 ratio was used to induce sheath blight disease symptoms in rice plants. The propylene covers containing the mixture of rice bran and water (250g) was autoclaved at 121 °C, 1.2 kg/cm^2 pressure for two h. Culture discs of one week old *R. solani* were inoculated into each bag and incubated to obtain sufficient mycelial growth of the fungus.

Two hundred and fifty gram of *R. solani* inoculum multiplied in rice bran was inoculated in to the soil contained in the grow bags at one week after transplanting as per the methodology developed by IRRI (1986). Another

challenge inoculation of the pathogen was given at 45 DAS by placing a mixture of mycelia and sclerotia inside the outer most leaf sheath for all the treatments except in the un-inoculated untreated control.

3.4.2 Preparation and application of effective treatments

The effective treatments were prepared as described under 3.3.1. The plants were sprayed with the corresponding treatments on 35th, 55th and 75th DAS. The observations including days taken for symptom development, days taken for the production of sclerotia, plant height (cm) and lesion height (cm) were noted. The intensity of sheath blight disease was found by calculating the percent disease index (PDI) (Yoshimura and Nishizawa, 1954) and relative lesion height (RLH) (Sharma *et al.*, 1990) methods using the formulae

$$PDI = \frac{\text{Sum of individual grades} \times 100}{\text{Total no. of leaves observed} \times \text{Maximum grade}}$$

$$RLH = \frac{\text{Lesion height} \times 100}{\text{Plant height}}$$

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 (0-9) 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

3.5 BIOCHEMICAL STUDIES FOR ASSESSING THE MECHANISM OF DISEASE MANAGEMENT IN VARIOUS TREATMENT SPRAYED *R. solani* INOCULATED PLANTS

The activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase will be recorded at 0, 24, 48 and 72 h after inoculation of *R. solani* on the treated and untreated rice plants

3.5.1 Peroxidase (PO)

It was estimated by the protocol elucidated by Srivasta (1987). One gram of leaf was homogenised with 5 ml of sodium phosphate buffer (pH 6.5) with a pinch of polyvinyl pyrrolidone in pre-chilled pestle and mortar. It was filtered using cheese cloth and centrifuged at 6,000 rpm for 15 min at 4ºC. The supernatant was collected as enzyme extract for analysis of PO activity. The reaction mixture consists of 3 ml of 0.05M pyrogallol, 500µl of enzyme extract in sample cuvettes and pyrogallol in reference cuvettes in which both were mixed uniformly and placed in spectrophotometer. The reaction was initiated by adding 1ml of one per cent hydrogen peroxide into sample cuvettes and changes in absorbance was measured at 420 nm in 30 seconds interval up to 180 seconds.

3.5.2. Polyphenol oxidase (PPO)

It was estimated by using the procedure described by Mayer (1965). One gram of leaf was grinded in 5ml of sodium phosphate buffer (pH 6.5) with a pinch of polyvinyl pyrrolidone in pre-chilled pestle and mortar at 4ºC. It was filtered through cheese cloth and centrifuged at 6000rpm for 15min at 4ºC. The supernatant was used as enzyme extract for estimation of PPO activity. The reaction mixture contained 1ml of sodium phosphate buffer, 200µl enzyme extract for sample and buffer alone for reference, kept in spectrophotometer. The reaction was initiated by adding 1ml of 0.01M Catechol. Change in absorbance was measured at 495 nm with 30 seconds interval for 180 seconds.

3.5.3. Phenylalanine ammonia lyase (PAL)

It was accounted by using a procedure devised by Dickerson *et al*. (1984). One g of leaf sample was grinded in 5ml of sodium borate buffer (pH 8.8) with a pinch of polyvinyl pyrrolidone in pre-chilled pestle and mortar. It was centrifuged at 10,000 rpm for 10 min at 4ºC and supernatant was used as enzyme activity. Reaction mixture consists of 3 ml of sodium borate buffer, 0.2 ml of enzyme extract and 0.1 ml of L- phenylalanine. A blank consisting of 3ml sodium borate buffer and 0.1ml of L-phenylalanine was also prepared. Both the reaction mixture and blank were incubated at 40ºC for 30 minutes. Reaction was stopped by adding 0.2 ml of 3N Hcl and kept in spectrophotometer. Change in absorbance was measured at 290 nm.

3.6 EFFECT OF THE TREATMENTS ON BIOMETRIC 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 plant

The total number of tillers per plant was counted.

3.6.1.3 Productive tillers per plant

The number of productive tillers per plant was counted.

3.6.1.4 Infected tillers

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

3.6.1.5 Lesion height

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

3.6.1.6 Lesion width

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

3.6.1.7 Lesion area

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

3.7 STATISTICAL ANALYSIS

The data obtained from the studies conducted under laboratory and field conditions were subjected to the analysis of variance techniques (ANOVA) and were applied to completely randomized design (CRD). The data obtained on per cent inhibition were transformed using angular (arc sine) transformation. The entire data sets of all the experiments were analyzed statistically by Duncan's Multiple Range Test (DMRT) as per the procedure given by Steel and Torrie (1960).

Results

4. RESULTS

The study on the "Ecofriendly management of sheath blight disease of rice" was carried out during 2017-2019 at the department of Plant Pathology, College of Agriculture, Vellayani (*in vivo* studies) and Integrated Farming System Research Station (IFSRS), Karamana (*in vitro* studies*)*. The results which were obtained in these experiments are detailed in this chapter

4.1 STUDIES ON THE PATHOGENICITY OF *R. solani*, THE RICE SHEATH BLIGHT PATHOGEN

4.1.1 Purification of *R. solani*

The culture *R. solani* which was purified and maintained at room temperature was used for the entire experiments.

4.1.2 Studies on the pathogenicity of *R. solani* in rice

Pathogenicity of the *R. solani* was assessed by artificial inoculation of the pathogen on healthy plants. The development of sheath blight disease symptoms were observed on the inoculated plants. The symptoms were observed initially on three days after inoculation (DAI) which were manifested as small, water soaked grey coloured lesions on the sheath. The lesions enlarged in the subsequent days and finally on seven DAI, coalition of the lesions occurred which resulted in the complete drying of the leaf and the sheath (Plate 1). The length of the lesion on the inoculated plants was observed to be 0.79 cm on three DAI which enlarged to 3.86 cm on seven DAI (Table 3). White coloured and mustard shaped sclerotia were observed on five DAI (Plate 2).

4.2 CULTURAL CHARACTERS OF *R. solani*

Cultural characters of *R. solani* were studied on PDA medium. The fungus completed its mycelial growth (9 cm) on the medium on three DAI. Pin head initiation of sclerotia was observed on four DAI. The number of sclerotia increased from five on six DAI to 34 on ten DAI. The colour of the sclerotia appeared initially as white which later turned from light brown to dark brown. The sclerotia were round and mustard shaped (Table 4).

R. solani in PDA on
7 days of growth

Symptom development on
sheath (3 days after inoculation)

Reisolated fungus

Plate 1. Pathogenicity of *R. solani* in rice var. Uma

Table 3. Pathogenicity of *Rhizoctonia solani* (accession no. KX674527) in rice variety Uma

Days for symptom development	Symptoms observed on sheath	Length of lesion (cm)*
1 DAI	No symptoms observed	0
2 DAI	No symptoms observed	0
3 DAI	Small, water soaked, grey coloured lesions	0.79
4 DAI	Large, water soaked lesions with greyish brown, irregular margin	1.73
5 DAI	Enlarged lesions with few white sclerotia	2.80
6 DAI	Enlarged lesions with numerous white sclerotia	3.40
7 DAI	Coalition of lesions resulting in drying of sheath and leaves	3.86
SE m (±)	-	0.09
CD (0.05)	-	0.29

DAI- Days after inoculation

* Average of six replications

Water soaked lesions on 3 DAI

Sclerotia formation on 5 DAI

Drying of leaves on 7 DAI

Plate 2. Symptomatology of sheath blight disease in rice var. Uma

Table 4. Cultural characters of *Rhizoctonia solani* (accession no. KX674527), the sheath blight pathogen of rice

Days after inoculation (DAI)	Mycelial growth (cm) (3 DAI)*	Number of sclerotia and days of formation	Colour of sclerotia	Shape of sclerotia
1	1.83	No sclerotia	-	-
2	4.71	No sclerotia	-	-
3	9	No sclerotia	-	-
4	9	Pin head initiation of sclerotia	-	-
5	9	Pin head initiation of sclerotia	-	-
6	9	5 sclerotia	White	Round and mustard shaped
7	9	9 sclerotia	White	Round and mustard shaped
8	9	17 sclerotia	Light Brown	Round and mustard shaped
9	9	29 sclerotia	Light Brown	Round and mustard shaped

10	9	34 sclerotia	Dark brown	Round and mustard shaped

* Average of three replications, * DAI- Days after inoculation

4.3 EFFECT ON *IN VITRO* EVALUATION OF ORGANIC PREPRATIONS, BOTANICLAS AND NON-HAZARDOUS CHEMICALS AND THEIR COMBINATIONS AGAINST *R. solani*

4.3.1 Effect on *in vitro* inhibition of mycelial growth

Fermented egg-lemon juice extract (10%), fermented *S. barbata* extract (10%), garlic extract (10%), potassium silicate (0.5%) and their combinations were evaluated for assessing their potential to inhibit the mycelial growth of *R. solani in vitro.* The study revealed that all the treatments at their respective concentrations resulted in cent per cent (100%) inhibition of the mycelial growth of *R. solani.* Sclerotia were not observed in any of the Petri plates containing PDA media poisoned with the treatments, whereas, in the untreated control plates, eight sclerotia were observed on five DAI (Table 5; Plate 3).

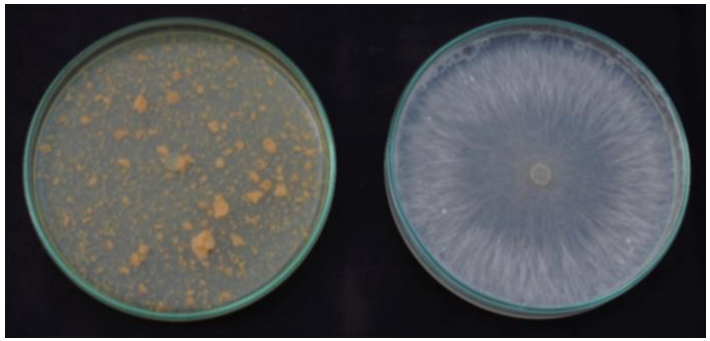
4.3.2 Effect on *in vitro* inhibition of mycelial regeneration from sclerotia

Sclerotia collected from one week old culture of *R. solani* were dipped in the treatments for different time intervals *viz.*, 24, 48 and 72h. The treatments were then evaluated for their potential in inhibiting the mycelial regeneration from sclerotia which were placed in PDA media. Among the individual four treatments, dipping of sclerotia in fermented egg- lemon juice extract (10%) for 24 and 48 h resulted in cent per cent inhibition of mycelial regeneration from sclerotia. However, dipping for 72 h in the extract resulted in 96.60 per cent inhibition of mycelial regeneration. Mycelial inhibition of 76.60, 83.30 and 83.66 per cent were recorded when the sclerotia were dipped in fermented *S. barbata* extract (10%) for 24, 48 and 72 h respectively. Thus, there was a gradual increase in the inhibition of mycelial regeneration when sclerotia were dipped in fermented *S. barbata* extract for different time intervals. Garlic extract (10%) did not have any inhibitory effect when sclerotia were dipped for 24h. However, a reduction of 62.20 and 79.96 per cent were recorded when sclerotial dipping was done for 48

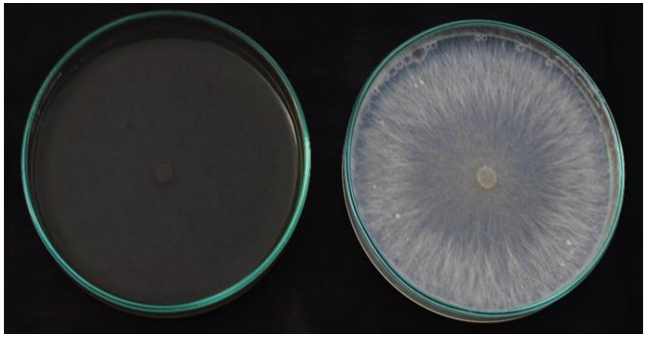
Table 5. Organic preparations, botanicals, non-hazardous chemicals and their combinations on *in vitro* inhibition of *R. solani* in PDA medium

Treatments	Mycelial growth (cm) (3 DAI)*	Percentage inhibition	Days for formation of sclerotia*	Number of sclerotia (5 DAI)
T1 (FEE) (10%)	0	100	Nil	Nil
T2 (FWE) (10%)	0	100	Nil	Nil
T3 (PS) (0.5%)	0	100	Nil	Nil
T4 (GE) (10%)	0	100	Nil	Nil
T5 (FEE+FWE) (1:1)	0	100	Nil	Nil
T6 (FEE+PS) (1:1)	0	100	Nil	Nil
T7 (PS+FWE) (1:1)	0	100	Nil	Nil
T8 (FEE+FWE+GE+PS) (1:1:1:1)	0	100	Nil	Nil
T9 (FEE+GE) (1:1)	0	100	Nil	Nil
T10 (FWE+GE) (1:1)	0	100	Nil	Nil
T11 (FEE+FWE+PS) (1:1:1)	0	100	Nil	Nil
T12 (PS+GE) (1:1)	0	100	Nil	Nil
T13 (Tebuconazole 50%+Trifloxystrobin 25% WG) (0.04%)	0	100	Nil	Nil
T14 (Untreated control)	9	0	5	8

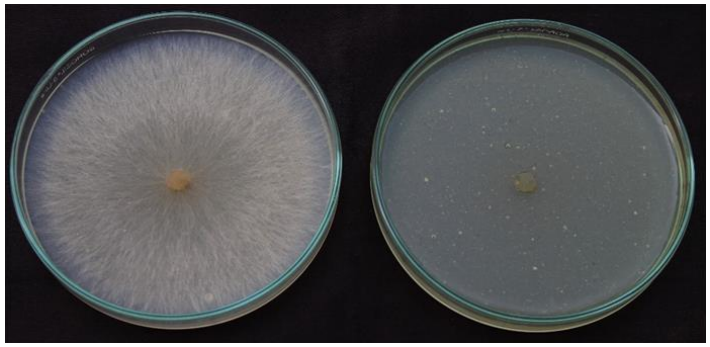
*Average of three replications



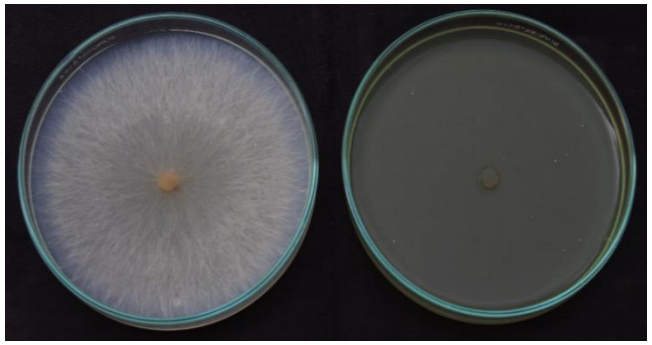
FEE (10%) Control



FWE (10%) Control



Control GE (10%)



Control PS (0.5%)

Plate 3. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* mycelial inhibition of *R. solani* in PDA medium at room temperature

and 72h respectively in garlic extract. No inhibitory effect on mycelial regeneration from sclerotia was observed when sclerotia were dipped in potassium silicate (0.5%) for 24 and 48h. However, an inhibition of 66.60 per cent in the mycelial regeneration was recorded when sclerotia were dipped for 72 h in the extract (Table 6).

Among the different combination treatments, FEE+FWE, FEE+GE, FEW+GE, FEE+FEW+PS and FEE+FEW+GE+PS resulted in cent per cent inhibition of the mycelial regeneration from sclerotia when dipped for 24, 48 and 72h. Sclerotial dipping in the treated control *viz*., tebuconazole 50% + trifloxystrobin 25% (0.04%) also resulted in the complete inhibition of the mycelial regeneration when dipped for 24, 48 and 72h (Plate 4, 5 and 6).

4.3.3 Selection of best treatments for *in vivo* study

All the treatments resulted in cent per cent inhibition of the mycelial growth of *R. solani in vitro* in PDA medium. When the treatments were evaluated for their potential in inhibiting the mycelial regeneration from sclerotia by dipping them in the treatments for 24, 48 and 72h, it was observed that dipping in fermented *S. barbata* extract (10%) resulted in an appreciable and steady increase in the inhibition rate. Among the treatments, potassium silicate which was effective in completely inhibiting the mycelial growth as well as resulting in 66.60 per cent inhibition of mycelial regeneration from sclerotia was the treatment in its lowest concentration, viz., 0.05 percentage. Hence, these two treatments, *viz*., fermented *S. barbata* extract (10%) and potassium silicate (0.05%) were selected as the best two treatments for evaluating their efficacy against sheath blight disease *in vivo*.

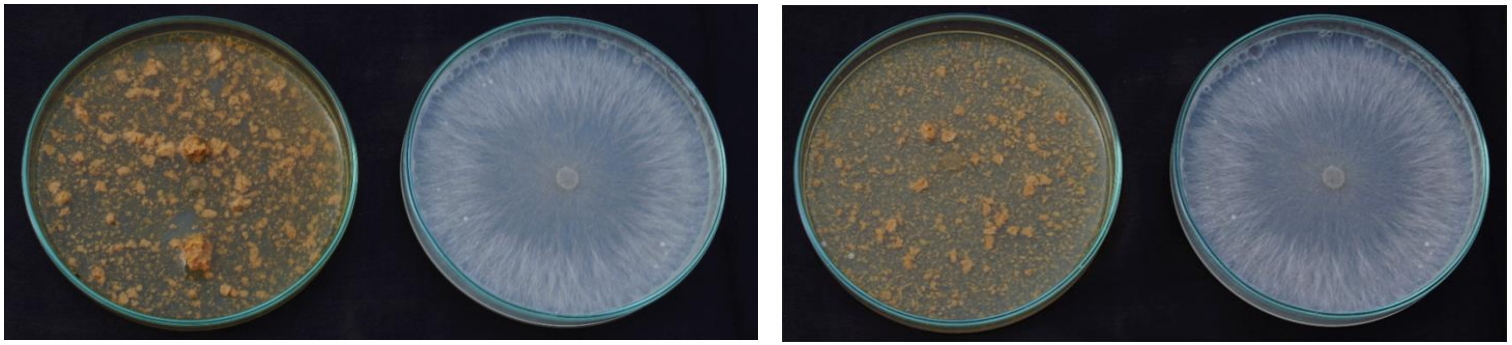
4.4 *IN VIVO* EXPERIMENTAL STUDIES

A pot culture experiment was laid out at the College of Agriculture, Vellayani with eight treatments (Table 7) to manage the sheath blight disease of rice. The rice variety “Uma” was used for the study (Plate 7). The study revealed

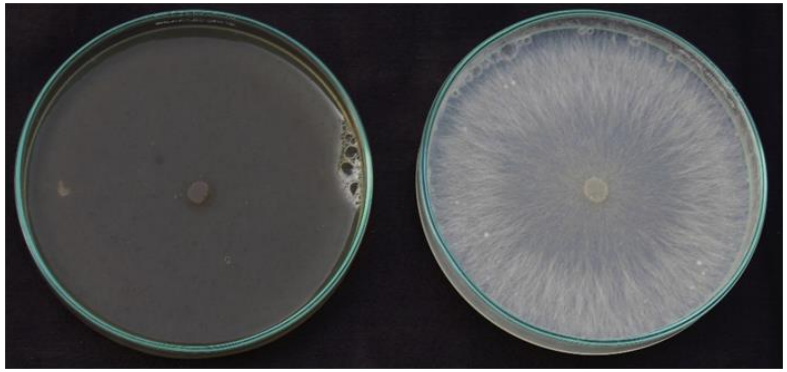
Table 6. Organic preparations, botanicals, non-hazardous chemicals and their combinations on inhibition of mycelial regeneration from sclerotia of *R. solani*

Treatments	Soaking duration of sclerotia in treatments before sclerotial germination assay (hours)					
	24 h		48 h		72 h	
	MRS (cm)	S (%)*	MRS (cm)	S (%)*	MRS (cm)	S (%)*
T1 (FEE) (10%)	0	[a] 100 (89.71)	0	[a] 100 (87.71)	0.3	[a] 96.60 (79.46)
T2 (FWE) (10%)	2.1	[b] 76.60 (61.08)	1.5	[b] 83.30 (65.93)	1.46	[b] 83.66 (66.19)
T3 (PS) (0.5%)	9	[c] 0.0 (0.28)	9	[d] 0.0 (0.28)	3.0	[e] 66.60 (54.70)
T4 (GE) (10%)	9	[c] 0.0 (0.28)	3.4	[c] 62.20 (52.06)	1.8	[c] 79.96 (63.52)
T5 (FEE+FWE) (1:1)	0	[a] 100 (89.71)	0	[a] 100 (87.71)	0	[a] 100 (89.71)
T6 (FEE+PS) (1:1)	9	[c] 0.0 (0.28)	9	[d] 0.0 (0.28)	3.1	[e] 65.66 (54.71)
T7 (FWE+PS) (1:1)	9	[c] 0.0 (0.28)	9	[d] 0.0 (0.28)	2.03	[c] 77.33 (61.50)
T8 (FEE+FWE+GE+PS) (1:1:1:1)	0	[a] 100 (89.71)	0	[a] 100 (89.71)	0	[a] 100 (89.71)
T9 (FEE+GE) (1:1)	0	[a] 100 (89.71)	0	[a] 100 (89.71)	0	[a] 100 (89.71)
T10 (FWE+GE) (1:1)	0	[a] 100 (89.71)	0	[a] 100 (89.71)	0	[a] 100 (89.71)
T11 (FEE+FWE+PS) (1:1:1)	0	[a] 100 (89.71)	0	[a] 100 (89.71)	0	[a] 100 (89.71)
T12 (GE+PS) (1:1)	9	[c] 0.0 (0.28)	9	[d] 0.0 (0.28)	2.36	[d] 73.66 (59.15)
T13 (Tebuconazole 50 % + Trifloxystrobin 25 %)	0	[a] 100 (89.71)	0	[a] 100 (87.71)	0	[a] 100.0 (89.71)
T14 (Control)	9	[c] 0.0 (0.28)	9	[d] 0.0 (0.28)	9	[f] 0.0 (0.28)
SE m (±)		0.39		0.47		1.29
CD (0.05)		1.25		1.77		3.44

MRS- Mycelial regeneration from sclerotia, S- Percentage of suppression, Values in the parenthesis are arc sine transformed, Treatments with same alphabet do not differ significantly

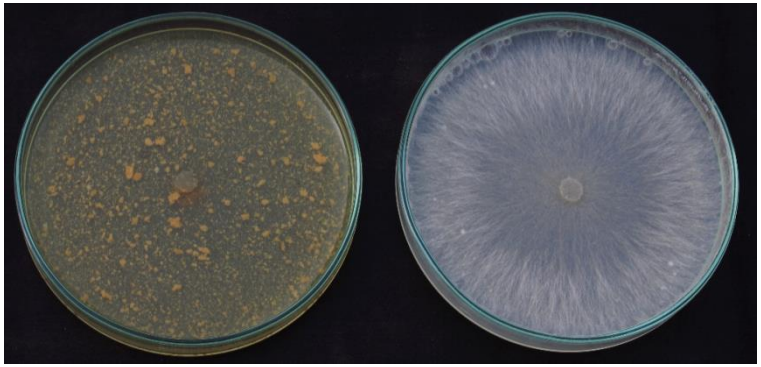


FEE+FWE (1:1) Control FEE+PS (1:1) Control

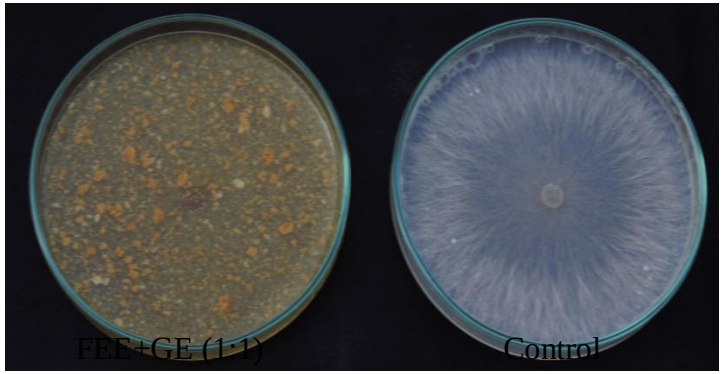


PS+FWE (1:1) Control

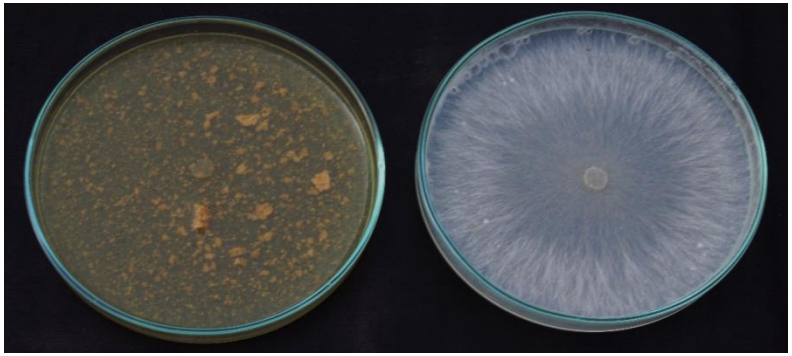
Plate 4. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* mycelial inhibition of *R. solani* in PDA medium at room temperature (continued).



FWE+FEE+GE+PS (1:1:1:1)



Control



FWE+GE (1:1)

Control

Plate 5. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* mycelial inhibition of *R. solani* in PDA medium at room temperature (continued).

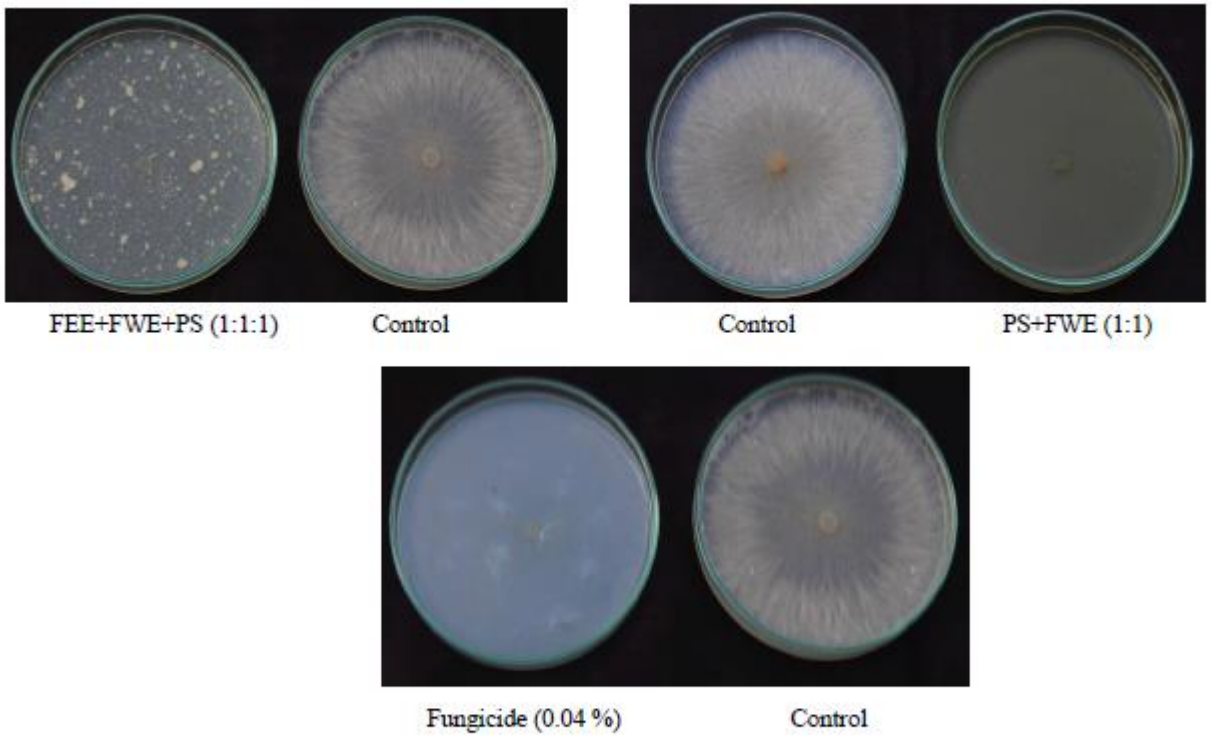


Plate 3. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* mycelial inhibition of *R. solani* in PDA medium at room temperature (continued).

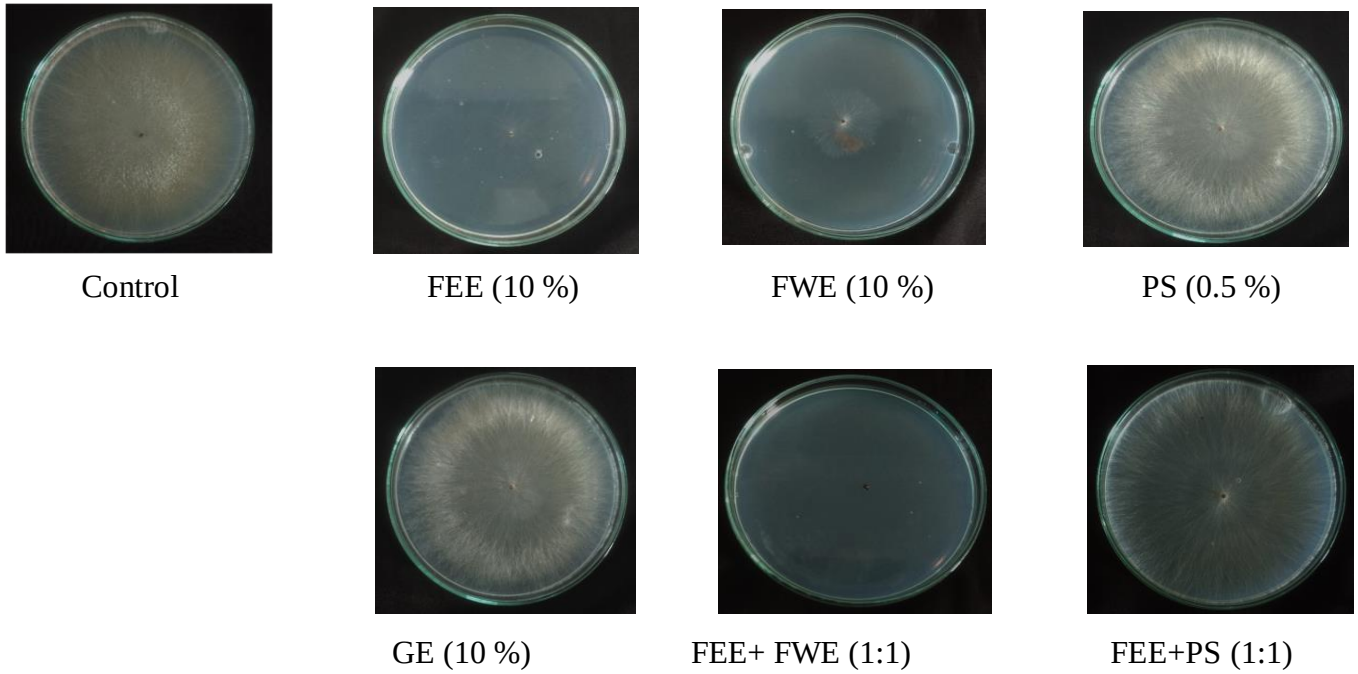


Plate 7. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* inhibition of sclerotial germination after soaking in treatments for 24 h.

Table 7. Treatments selected for *in vivo* management studies of sheath blight disease

Treatments	Description
T1	Fermented *Setaria barbata* extract (10%)
T2	Potassium silicate (0.5%)
T3	AMF (KAU formulation) (200g/m^2 of nursery area)
T4	Fermented *S. barbata* extract (10%) + AMF
T5	Potassium silicate (0.5%) + AMF
T6	Tebuconazole 50%+ Trifloxystrobin 25%WG (0.04%)
T7	Inoculated untreated control
T8	Uninoculated untreated control

that typical symptoms of rice sheath blight disease were developed after two or three days after inoculation in the infected rice plants (Table 8). Sclerotial formation was observed on seven to eight days after inoculation in the infected rice plants. The development of disease symptoms coincided with the maximum tillering stage of the crop.

The vertical spread (the progression of the disease along the infected tiller) of the disease was estimated for all the plants sprayed with the respective treatments (Table 9; Plate 8). The vertical spread of the disease was estimated by measuring the relative lesion height (RLH) which was calculated by using the plant height and lesion height. All the treatments recorded significant reduction in the vertical spread of the disease and were on par with each other when compared to the inoculated untreated control. The lowest per cent disease index (PDI) was recorded in the plants sprayed with potassium silicate (0.05%) (12%) and a combination of AMF application at nursery stage and foliar application of fermented *S. barbata* extract (10%) at 35, 55 and 75 DAS (14%) which were on par with each other as well as with the foliar spray of the treated control *viz*., tebuconazole 50% + trifloxystrobin 25% (0.04%) (Fig 1).

The horizontal spread (disease spread to adjacent tillers) was recorded in all the plants which was expressed as the total number of infected tillers per plant. The minimum number of infected tillers per plant was recorded in the plants sprayed with tebuconazole 50% + trifloxystrobin 25% WG (4.74). Among the treatments, foliar application of potassium silicate (0.5%) revealed the minimum number of infected tillers per plant (5.55) which was on par with the foliar application of fermented *S. barbata* extract (5.6). The least per cent horizontal spread was recorded in the plants sprayed with potassium silicate (23.81 %) which was followed by the plants which were treated with tebuconazole 50% + trifloxystrobin 25% WG (25.2 %) (Table 10, Plate 9 and Fig 2).

The total tillers per plant was the maximum in the plants sprayed with potassium silicate (0.05%) (23.3) which was on par with the plants applied with AMF at nursery stage (23.1) (Fig 3). The maximum number of productive tillers was recorded in the plants sprayed with potassium silicate (0.05%) (22.1) which

was on par with the plants where nursery application of AMF @ 200g/m^2 (21) was performed as well as in the plants applied with AMF in the nursery stage and sprayed with fermented *S. barbata* extract (10%) (20.9) (Fig 4).

There was significant reduction in the lesion height in all the treatment applied plants and were on par with each other compared to untreated control plants. The lesion width was the least in the plants sprayed with fermented *S. barbata* extract (10%) (0.48 cm) followed by the plants sprayed with potassium silicate (0.55) (0.55 cm). The lesion area was also lesser in all the treatment applied plants which were on par with each other compared to untreated control plants (Table 11).

4.5 BIOCHEMICAL STUDIES FOR ASSESSING THE MECHANISM OF DISEASE MANAGEMENT IN VARIOUS TREATEMENT SPRAYED PLANTS

The activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were recorded at 0, 24, 48 and 72 hours after inoculation of *R. solani* on the treated and untreated rice plants.

The maximum activity of peroxidase on 72 h after the third spray was observed in the plants sprayed with potassium silicate (45.1 mg $g^{-1}min^{-1}$) and in the plants where nursery application of AMF was carried out (45.1 mg $g^{-1}min^{-1}$) as described in Table 12. The least activity of peroxidase was recorded in the uninoculated untreated control plants (28.8 mg $g^{-1}min^{-1}$) (Fig 5). The highest activity of polyphenol oxidase was recorded in the plants sprayed with potassium silicate (3.89) which was on par with the plants treated with tebuconazole 50% + trifloxystrobin 25% WG (3.81) after 72 h of treatment application (Table 13; Fig 6). The lowest polyphenol oxidase was recorded in the plants maintained as uninoculated untreated control (1.17 mg $g^{-1}min^{-1}$). The maximum activity of phenylalanine ammonia lyase was observed in the plants sprayed with tebuconazole 50% + trifloxystrobin 25% WG (110.5 mg $g^{-1}min^{-1}$) followed by plants where AMF application at nursery stage was carried out (104.2 mg $g^{-1}min^{-1}$) after 72 h of treatment application (Table 14; Fig 7).

Table 8. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on symptom development of rice sheath blight disease at active tillering stage

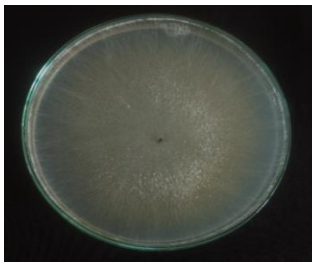
Treatments	Days for symptom development*	Days for formation of sclerotia*
FWE (10%)	2 DAI	7 DAI
PS (0.5%)	2 DAI	8 DAI
AMF (KAU formulation) (200g/m^2)	3 DAI	7 DAI
FWE (10%)+ AMF	3 DAI	7 DAI
PS (0.5%) + AMF	2 DAI	7 DAI
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	3 DAI	8 DAI
Inoculated untreated control	3 DAI	6 DAI
Uninoculated untreated control	-	-

*Average of four replications, DAI – Days after inoculation

Table 9. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on the vertical spread of sheath blight disease

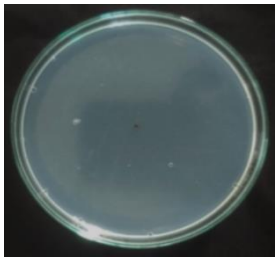
Treatments	Plant height (cm)* on 90 DAS	Lesion height (cm)* on 90 DAS	RLH (%)*	Percentage suppression over control (%)	PDI (%)*
FWE (10%)	58	7.83^{b}	13.50^{b}	69.03	29^{b}
PS (0.5%)	60.60	6.33^{b}	10.46^{b}	76.05	12^{c}
AMF (KAU formulation) (200g/m^2)	60.66	6.87^{b}	11.34^{b}	74.03	20.90^{bc}
FWE (10%)+ AMF	58.66	6.41^{b}	10.97^{b}	75.06	14^{c}
PS (0.5%) + AMF	57.33	7.16^{b}	12.52^{b}	70.25	22^{bc}
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	58	6.27^{b}	10.81^{b}	75.20	13^{c}
Inoculated untreated control	57.33	24.78^{a}	43.38^{a}	-	63^{a}
Uninoculated untreated control	54	0.00^{c}	0.00^{c}	-	
SE m (±)		0.68	1.08	-	1.33
CD (0.05)	NS	2.04	3.28	-	4.01

*Average of four replications, Treatments with same alphabet do not differ significantly, RLH- Relative lesion height, PDI- Per cent disease index, DAS- Days after sowing

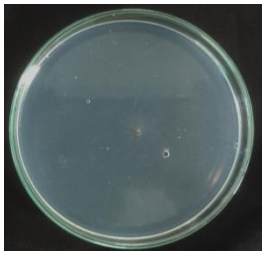


Control

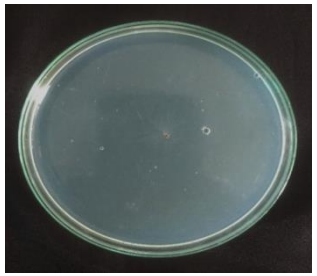
PS+FWE (1:1)



FEE+FWE+GE+PS (1:1:1:1)



FEE+GE (1:1)



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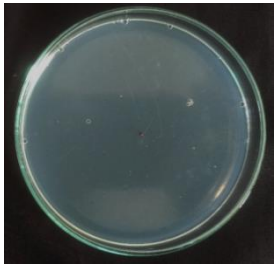
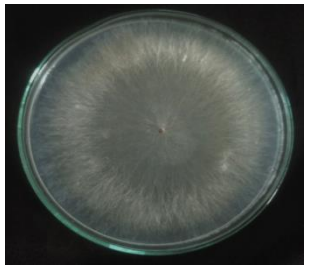
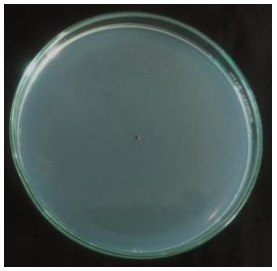


FEE+FWE+PS (1:1:1)



PS+GE (1:1)



Fungicide (0.04 %)

Plate 8. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* inhibition of sclerotial germination after soaking in treatments for 24 h (continued).

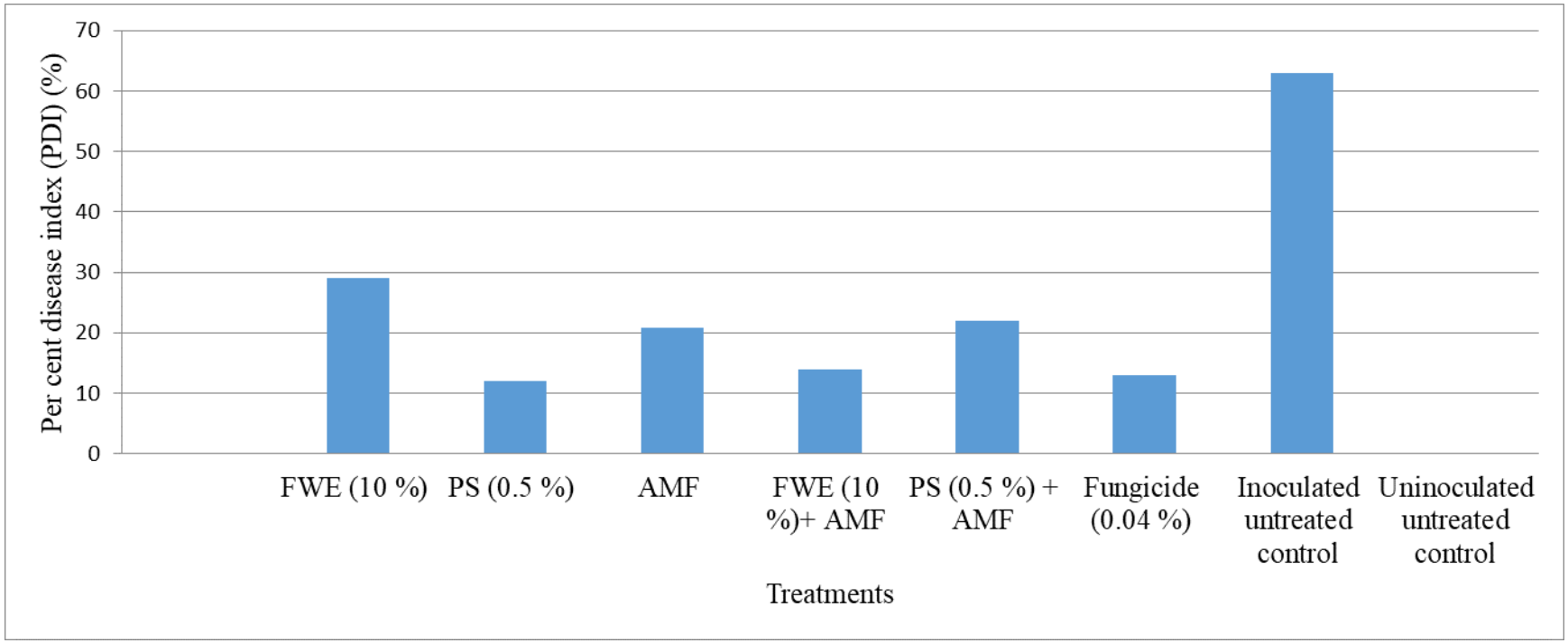


Figure 1. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on per cent disease index of sheath blight on *R. solani* inoculated rice plants, variety Uma at 60 DAI.

Table 10. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on the horizontal spread of rice sheath blight

Treatments	Tillers/plant*	Productive tillers/ plant*	Infected tillers/ plant*	Percent horizontal spread (%)*
FWE (10%)	17.8 d	16.5 c	5.66 d	31.79 b
PS (0.5%)	23.3 a	22.1 a	5.55 d	23.81 d
AMF (KAU formulation) (200g/m^2)	23.1 a	21 a	6.33 c	27.33 c
FWE (10%) + AMF	22.1 ab	20.9 a	7.50 b	34.15 b
PS (0.5%) + AMF	19.8 c	18.2 bc	6.66 c	34.24 b
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	19.15 cd	17.5 bc	4.74 e	25.2 cd
Inoculated untreated control	19.06 cd	18.5 b	9.50 a	47.91 a
Uninoculated untreated control	20.3 bc	19 b	0.00 f	0.00 e
SE m (±)	0.63	0.58	0.16	1.12
CD (0.05)	1.89	1.74	0.492	3.36

Average of four replications, Treatments with same alphabet do not differ significantly

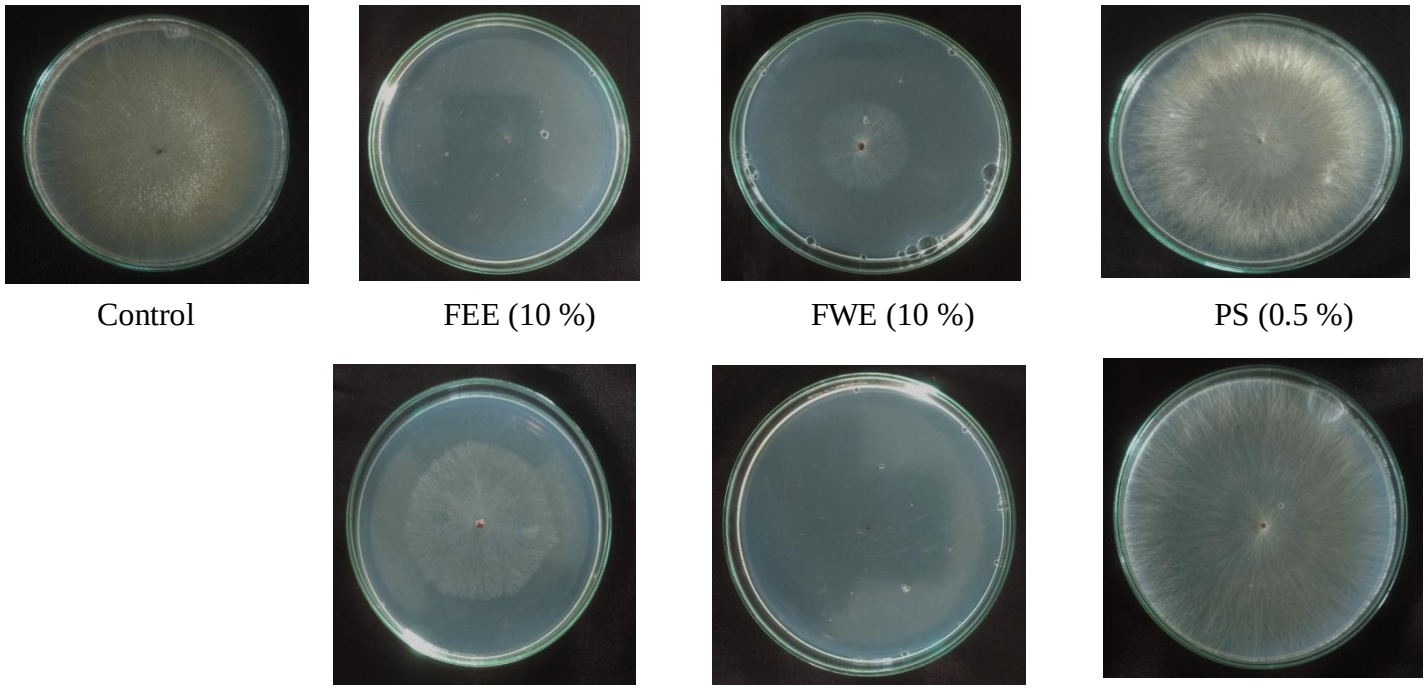


Plate 9. Organic preparations, botanicals and non-hazardous chemicals on *in vitro* inhibition of sclerotial germination after soaking in treatments for 48 h.

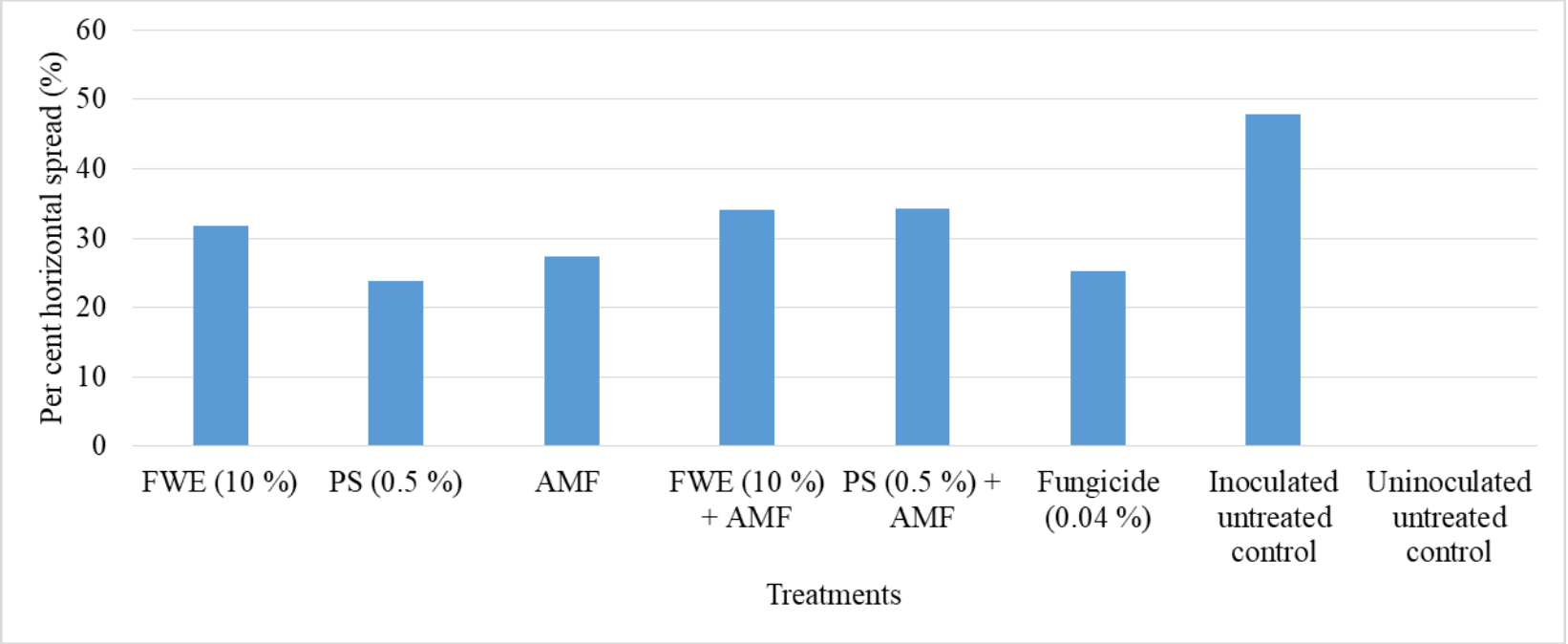


Figure 2. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on per cent horizontal spread of sheath blight on *R. solani* inoculated rice plants, variety Uma at 60 DAI.

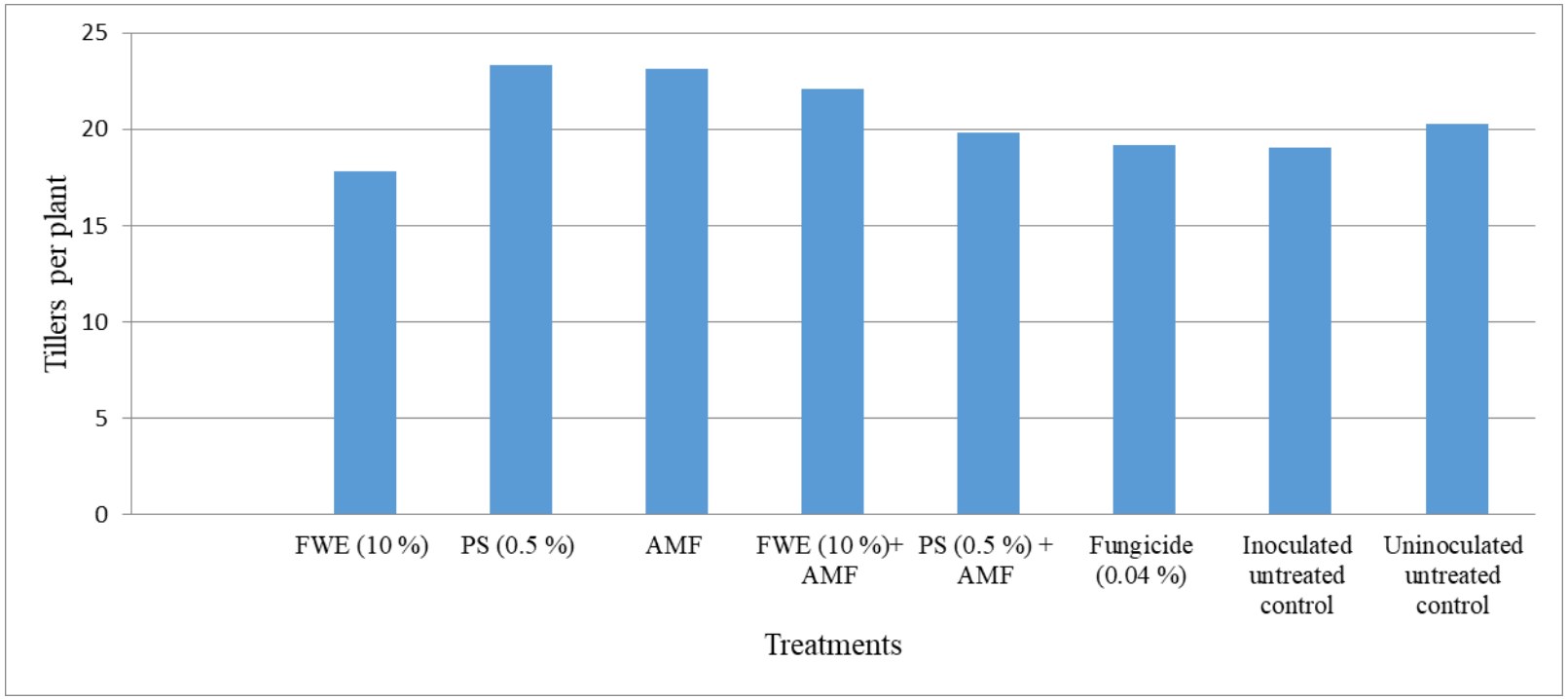


Figure 3. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on tillers per plant of *R. solani* inoculated rice plants, variety Uma at 60 DAI.

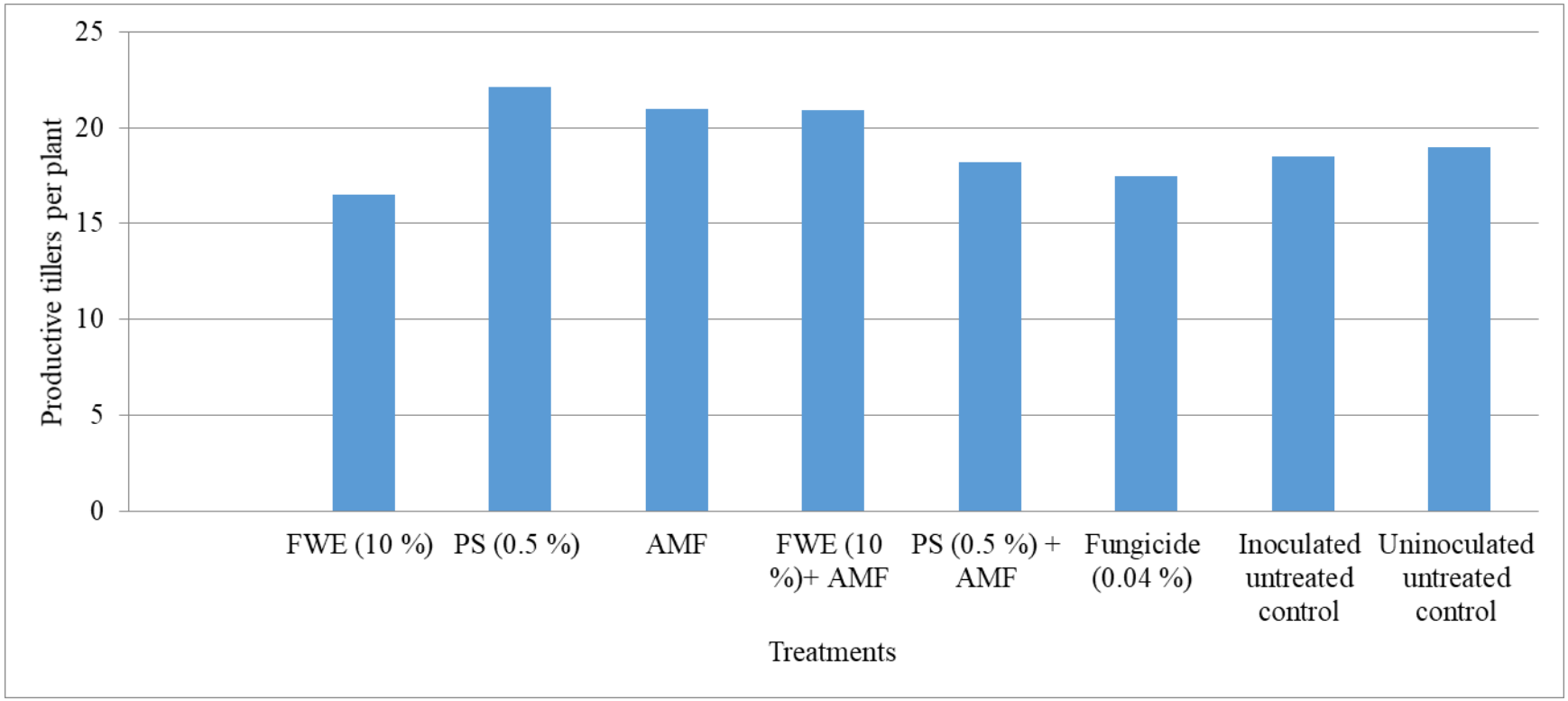


Figure 4. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on productive tillers per plant of *R. solani* inoculated rice plants, variety Uma at 60 DAI.

Table 11. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on the lesion area of sheath blight disease

Treatments	Lesion height (cm)*	Lesion width (cm)*	Lesion area (cm^2)*
FWE (10%)	7.83[b]	0.48[c]	3.75[b]
PS (0.5%)	6.33[b]	0.55[b]	3.47[b]
AMF (KAU formulation) (200g/m^2)	6.87[b]	0.68[a]	4.69[b]
FWE (10%)+ AMF	6.41[b]	0.67[a]	4.28[b]
PS (0.5%) + AMF	7.16[b]	0.65[a]	4.64[b]
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	6.27[b]	0.62[a]	3.92[b]
Inoculated untreated control	24.78[a]	0.65[a]	16.09[a]
Uninoculated untreated control	0.00[c]	0.00[d]	0.00[c]
SE m (±)	0.68	0.01	0.42
CD (0.05)	2.04	0.05	1.26

*Average of four replications, Treatments with same alphabet do not differ significantly

Table 12. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on activity of peroxidase (PO)

Treatments	PO activity (mg g^{-1} fresh weight of tissues min^{-1})*			
	0h	24h	48h	72h
FWE (10%)	21.1 bc	42.7 ab	43.2 ab	42.9 ab
PS (0.5%)	23.8 a	42.9 ab	43.9 ab	45.1 a
AMF (KAU formulation) (200g/m^2)	23.0 ab	44.2 a	44.5 a	45.1 a
FWE (10%)+ AMF	19.0 d	39.7 bc	39.3 c	40.1 b
PS (0.5%) + AMF	15.7 e	38.1 c	37.9 c	40.2 b
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	20.8 bc	40.1 abc	40.6 bc	39.4 b
Inoculated untreated control	20.0 cd	31.3 d	29.7 d	32.1 c
Uninoculated untreated control	18.0 d	28.7 d	28.7 d	28.8 c
SE m (±)	0.64	0.71	0.71	0.71
CD (0.05)	1.94	3.67	3.65	3.66

*Average of four replications, Treatments with same alphabet do not differ significantly

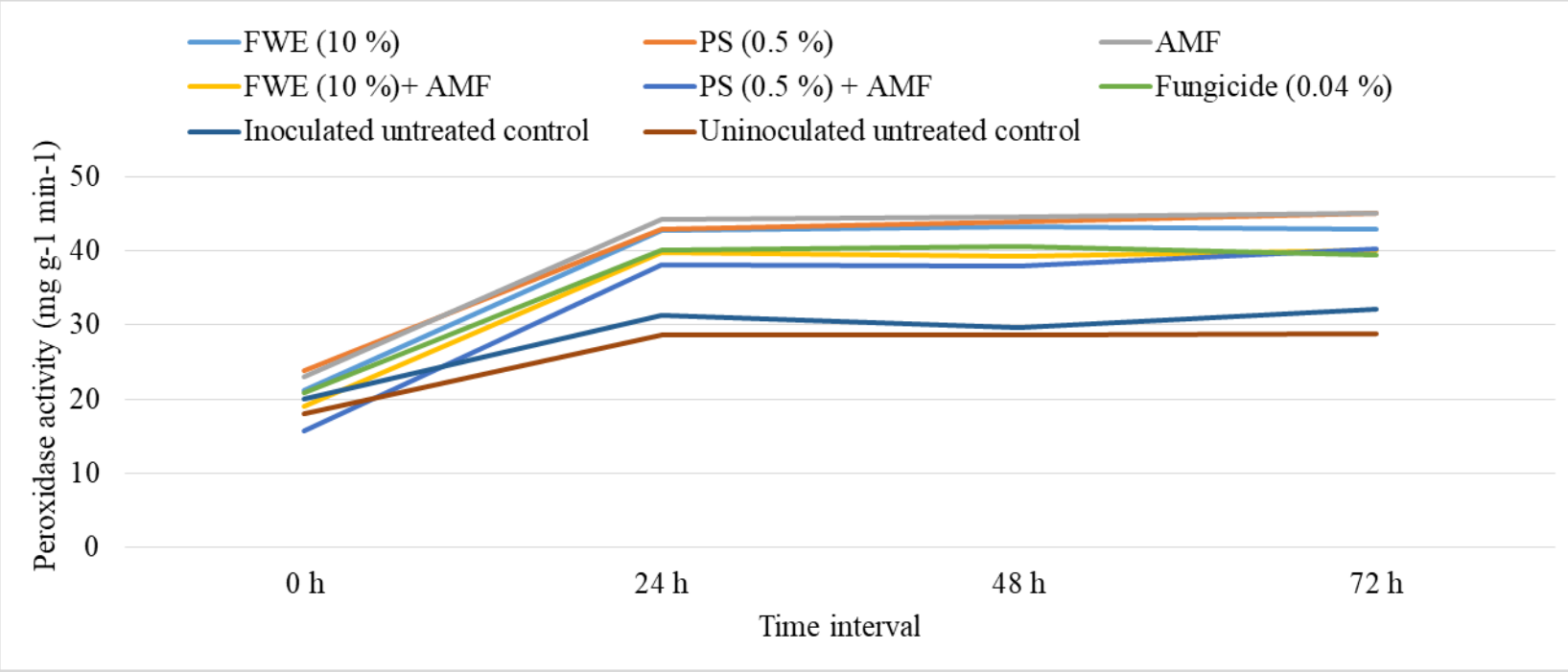


Figure 5. Peroxidase activity at different intervals in *R. solani* inoculated rice plants, variety Uma treated with fermented *S. barbata* extract, potassium silicate, AMF and their combinations.

Table 13. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on activity of polyphenol oxidase (PPO)

Treatments	PPO activity (mg g^{-1} fresh weight of tissues min^{-1})*			
	0h	24h	48h	72h
FWE (10%)	1.9 [a]	3.81 [a]	3.7 [a]	2.97 [c]
PS (0.5%)	1.61 [c]	3.62 [a]	3.72 [a]	3.89 [a]
AMF (KAU formulation) (200g/m^2)	1.12 [d]	3.2 [b]	3.3 [b]	3.41 [b]
FWE (10%)+ AMF	1.21 [d]	3.22 [b]	3.2 [b]	3.3 [b]
PS (0.5%) + AMF	1.1 [d]	3.3 [b]	3.1 [b]	3.33 [b]
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	1.72 [b]	3.79 [a]	3.85 [a]	3.81 [a]
Inoculated untreated control	0.12 [f]	1.13 [d]	1.15 [d]	1.17 [e]
Uninoculated untreated control	0.77 [e]	1.72 [c]	1.81 [c]	1.86 [d]
SE m (±)	0.04	0.15	0.15	0.16
CD (0.05)	0.12	0.29	0.3	0.28

*Average of four replications, Treatments with same alphabet do not differ significantly

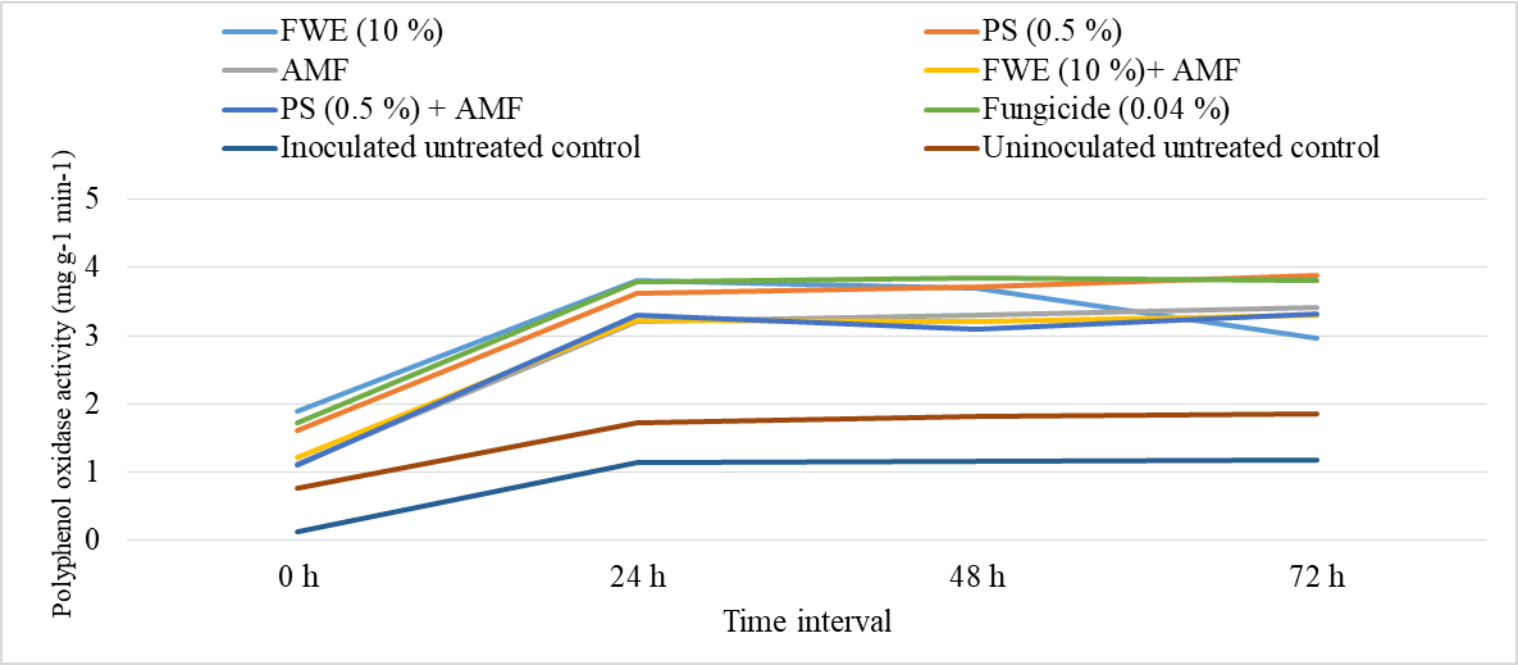


Figure 6. Polyphenol oxidase activity at different intervals in *R. solani* inoculated rice plants, variety Uma treated with fermented *S. barbata* extract, potassium silicate, AMF and their combinations.

Table 14. Effect of fermented *S. barbata* extract, potassium silicate, AMF and their combinations on activity of phenylalanine ammonia lyase (PAL)

Treatments	PAL activity (mg g^{-1} fresh weight of tissues min^{-1})*			
	0h	24h	48h	72h
FWE (10%)	104^{ab}	104.1^{a}	103.3^{a}	103.9^{ab}
PS (0.5%)	101^{b}	102.1^{a}	101.5^{a}	101.7^{ab}
AMF (KAU formulation) (200g/m^2)	103.1^{ab}	103.5^{a}	103.3^{a}	104.2^{ab}
FWE (10%)+ AMF	102.8^{ab}	101.7^{a}	100.9^{a}	100^{b}
PS (0.5%) + AMF	104.1^{ab}	104.2^{a}	103.9^{a}	103.9^{ab}
Tebuconazole 50%+ Trifloxystrobin 25% WG (0.04%)	110.84^{a}	109.7^{a}	110.03^{a}	110.5^{a}
Inoculated untreated control	81.1^{c}	82.1^{b}	83.1^{b}	83.3^{c}
Uninoculated untreated control	85.98^{c}	86.6^{b}	85.9^{b}	86.3^{c}
SE m (±)	2.93	2.92	2.92	2.94
CD (0.05)	9.26	9.30	9.29	9.35

*Average of four replications, Treatments with same alphabet do not differ significantly

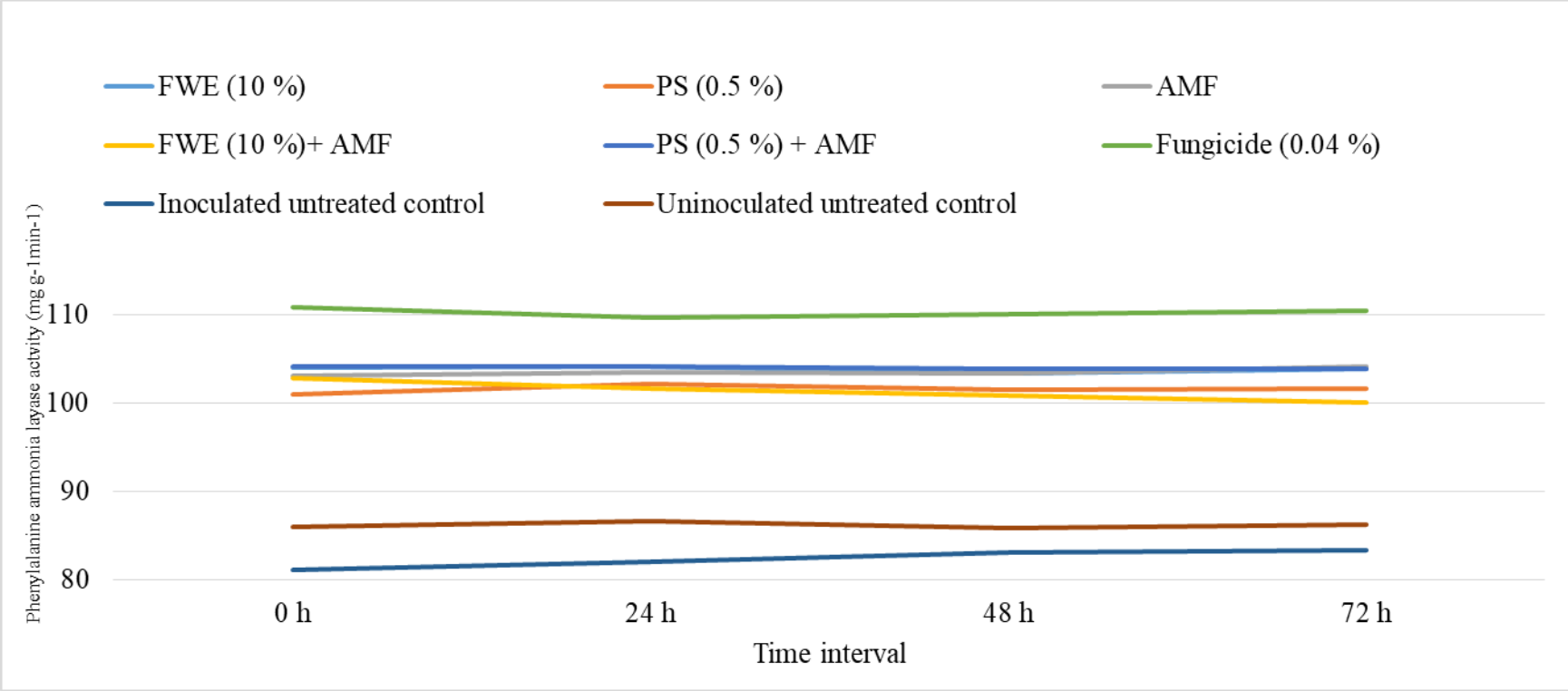


Figure 7. Phenylalanine ammonia lyase activity at different intervals in *R. solani* inoculated rice plants, variety Uma treated with fermented *S. barbata* extract, potassium silicate, AMF and their combinations

4.6 EFFECT OF TREATMENTS ON THE VARIOUS BIOMETRIC ATTRIBUTES OF RICE

The treatments did not reveal any significant difference in plant height between each other and with he treated control plants. Foliar spray of potassium silicate (0.05%) as well as AMF application at nursery stage were the superior treatments as far as the number of total tillers per plant was concerned. The maximum number of total tillers were recorded in the plants applied with the above two treatments. These two treatments along with another treatment *viz*., AMF application at nursery stage and foliar application of fermented *S. barbata* extract recorded the maximum number of productive tillers per plant revealing that these treatments had a positive influence on improving the biometric attributes of rice plants.

Thus, the study revealed that foliar spray of potassium silicate (0.5%) at 35, 55 and 75 DAS or application of AMF at nursery stage @ 200 g/m^2 or application of AMF at nursery stage @ 200 g/m2 combined with the foliar application of fermented *S. barbata* extract (10%) at 35, 55 and 75 DAS improved the number of productive tillers of rice plants. As far as the management of sheath blight disease of rice was concerned, foliar spray of potassium silicate (0.05%) at 35, 55 and 75 DAS was revealed to be the best treatment which recorded the least vertical and horizontal disease spread as well as the minimum number of sheath blight infected tillers per plant. There was an increase in the activity of peroxidase and poly phenol oxidase enzymes when the plants were sprayed with potassium silicate which may be attributed to the observed decrease in the sheath blight incidence in the sprayed plants

Discussion

5. DISCUSSION

Rice (*Oryza sativa* L.) is a major cereal which is consumed throughout the world as staple food. However, the crop yield was significantly reduced due to several diseases like blast, sheath blight, brown spot, and bacterial leaf blight. Sheath blight disease of rice caused by *Rhizoctonia solani* is a major cause in the reduction of rice yield in India. Management of the disease was highly difficult due to lack of resistant genes available to the pathogen, existence of numerous races and its ability to infect various crops and weeds. Application of fungicides and pesticides resulted in various harmful effects on human beings and other life forms. So management of the disease through naturally available compounds and ecofriendly products provides a good scope. In this context the present experiment on the "Ecofriendly management of sheath blight disease of rice" was carried out with an objective to develop an effective ecofriendly management strategy for the management of the disease. Discussion of the obtained results is presented in this chapter

5.1. STUDIES ON THE PATHOGENICITY OF *R. solani*, THE RICE SHEATH BLIGHT PATHOGEN

Pathogenicity of *R. solani* was carried out by Koch's postulates. Small water soaked grey coloured lesions were observed on the inoculated plants on third day after inoculation (DAI). Earlier studies conducted by Adhipathi *et al*. (2013), Bhukal *et al*. (2015) and Manjunatha *et al*. (2018) also reported that small water soaked lesions were developed on the inoculated leaf sheaths due to infection by the *R. solani*. A toxin was identified which was responsible for appearance of symptoms caused by *R. solani* in rice, cotton and tomato (Vidhyasekhran *et al*., 1997). Growth, development and pathogenicity of *R. solani* was determined by G protein encoding gene which is involved in signal transduction pathway in the pathogen (Charoensopharat *et al*., 2008). The ability to damage host cell wall effectively and survival in the environment by managing oxidative stress, cytotoxic compounds, etc. was being proposed to be important for pathogenesis of *R. solani* in rice (Ghosh *et al*., 2017).

The length of the lesion on the inoculated plants was 0.79 cm on three DAI. Many authors reported that the lesion length produced by *R. solani* were initially 1.5 cm and may go up to 6 cm as the disease progressed. The lesions were initially light green in colour and later turned to greyish centered with dark brown margin (Taheri *et al*., 2007, Adhipathi *et al*., 2013). Manjunath *et al*. (2018) recorded the lesion size varying from 0.15 – 0.35 cm^2. White coloured and mustard shaped sclerotia were observed on five DAI. Gondal *et al*. (2019) also reported the white and mustard shaped sclerotia and proposed that the possible mechanism behind the colour of sclerotia was due to differential pigmentation by the pathogen.

5.2. CULTURAL CHARACTERS OF *R. solani*

Cultural characters of the *R. solani* were studied on PDA medium. The fungi completely covered the petri plates (90 mm) within 72 h of inoculation. Sharma *et al*. (2013) also reported that the pathogen grown completely on Petri plates within three days after inoculation.

Sclerotia started to develop from four DAI reaching its maximum number by ten DAI. According to Meena *et al.* (2001), time taken for the formation of sclerotia ranged from three to eleven days after artificial inoculation. The time taken for the formation of sclerotia ranged from 4 to 15 days among the sixty and tweleve isolates of *R. solani* as reported by Lal and Kandhari (2009) and Gopireddy *et al*. (2017). Recently, Pralhad *et al*. (2019) reported that the time taken for the formation of sclerotia varied from four to eight days.

The observed sclerotia were round mustard shaped, dark brown in colour. Earlier studies by Gopireddy *et al*. (2017), Munghal *et al*. (2017), Gondal *et al*. (2019), Sandoval *et al.* (2019) and Divya *et al*. (2019) also reported the appearance of mustard shaped and brown to dark brown sclerotia

5.3. *IN VITRO* EVALUATION OF ORGANIC PREPARATIONS, BOTANICALS, NON-HAZARDOUS CHEMICALS AND THEIR COMBINATIONS AGAINST *R. solani*

5.3.1. Effect on *in vitro* inhibition of mycelium

Fermented egg-lemon juice extract, fermented weed (*Setaria barbata)* extract, garlic extract, potassium silicate and their combination treatments were accessed for their potential in inhibition of mycelial growth of *R. solani.* All the treatments at respective concentrations resulted in cent per cent inhibition of the mycelial growth of the *R. solani.* Karthika *et al*. (2017) reported the cent per cent mycelial inhibition of *R. solani* when treated with fermented egg-lemon juice extract (10 %), fermented weed (*Setaria barbata)* extract (100 %), garlic extract (10 %) and potassium silicate (1 %).

Egg white, shell and yolk possess biologically active compounds which imparts antibacterial and antiviral properties (Ibrahim *et al.,* 2002). Flavanoids present in the egg were found to play a significant role in the plant defense mechanism against a variety of fungi, bacteria and viruses (Sohn *et al.,* 2004). Phenolic compounds present in the citrus fruits were found to be responsible for providing resistance to the plants against pathogenic fungi as reported by Ortuno and Rio (2009). Antifungal property of the egg against human pathogen *Candida* spp was due to cystatin, a compound present in the membrane and yolk of the eggs as reported by Kolaczkowska *et al.* (2010). The peel of the citrus fruits found to have rich source of flavones, which were very unique to the citrus fruits which posses antibacterial property against *Pencillium* and *Salmonella* spp (Dhanavade *et al.,* 2011). Citrus juice is found to have antibacterial effect on several plant pathogenic bacteria (Hindi and chabuck, 2013).

Kagale *et al.* (2004) reported that the antifungal effect of datura against *R. solani* in rice was due to induction of systemic resistance in the plants. Rodino *et*

al. (2014) recorded the maximum inhibition (100 %) of the mycelial growth of *Alternaria alternata* by jimson weed at 10 per cent concentration. Bhattacharya *et al*. (2013) reported that the leaf extract of *Datura metel* inhibited the growth of *R. solani.* They found that there was enhanced activity of the defense related enzymes like peroxidase, polyphenol oxidase, catalase and tyrosinases which clearly indicated the induction of systemic resistance in the plants. The maximum inhibition of mycelial growth against *R. solani* causing root rot disease of buckwheat was observed with castor when compared to datura under *in vitro* conditions (Pathak *et al*., 2017). Devkota and Sahu (2017) performed the phytochemical analysis of several weed extracts and revealed the presence of terpenoids, saponins, flavonoids, tannins and alkaloids which were responsible for antifungal activity. There are no evidences depicting the antifungal effect of fermented egg lemon juice extract and fermented weed (*Setaria barbata*) extract.

A wide range of microorganisms including bacteria, fungi, protozoa and viruses were recorded to be sensitive to garlic preparations (Delaha and Garagusi 1985). Garlic extract at 10 per cent concentration recorded cent per cent mycelial inhibition of *R. solani*. The studies conducted by Meena *et al*., 1998, Sinha *et al*., 2009 and Srinivas *et al.,* 2014 also reported the cent per cent mycelial inhibition of *R. solani* by garlic extract. Several *Allium* sp possess cysteine sulphoxides which when crushed releases the enzymes which were responsible for convertion of sulphoxides into thiosulfates having unpleasant odour which restricts the growth of the fungal pathogens (Block *et al.*, 1992). Kyung and Lee (2001) found that several biologically active compounds were present in garlic extract which affected a wide range of soil borne fungal pathogens. The strong inhibition of *R. solani* by garlic extract was due to the presence of sulphur compounds and its active antimicrobial component *viz*., allicin as proposed by Singh and Singh (2005) and Perry *et al*. (2009). Polyphenols present in the garlic were found to be responsible for inhibition of the fungal growth of *R. solani* as reported by Chung *et al.* (2006), Bozin *et al.* (2008) and Wan *et al*. (2009).

Potassium silicate at 0.5 per cent concentration resulted in maximum (100 %) inhibition of mycelial growth of *R. solani*. Menzies *et al*. (1992) also observed the maximum inhibition of *R. solani*, who proposed that potassium silicate at one per cent concentration had a direct effect on the mycelial growth of the fungi. Epstein (1999) and Liang *et al*. (2005) proposed that potassium silicate application enhanced the host defense system thereby limiting the growth of the fungi. Bekker *et al*. (2006) recorded that the potassium silicate at 100 ppm concentration resulted in cent per cent mycelial inhibition of *Phytophthora cinnamomi, Sclerotinia sclerotiorum, Pythium, Mucor pusillus, Fusarium solani, Alternaria solani, Colletotrichum coccodes, Verticilium theobromae, Curvularia lanata* and *Stemphylium herbarum*. The possible mechanism put forth by them was due to change in pH which makes the pathogen to survive at higher pH levels.

5.3.2. Effect on *in vitro* inhibition of mycelial regeneration from sclerotia

Inhibition of regeneration of mycelium from sclerotia was evaluated. The results showed that the dipping of sclerotia in fermented egg-lemon juice extract for 24, 48 and 72 hours resulted in maximum inhibition of mycelial regeneration from sclerotia (100, 100 and 96.6 % respectively) followed by fermented weed extract (76.6, 83.3 and 83.6 % respectively). Dipping the sclerotia in potassium silicate and garlic extract resulted in 66.6 and 79.96 per cent inhibition respectively after 72 h of dipping in the treatments. Potassium silicate resulted in lesser inhibition of the mycelial regeneration from sclerotia. Earlier studies carried out by Wainwright (1993) and Wainwright *et al*. (1997) also observed the visible mycelial growth of the fungi on silicic acid amended media.

5.4. *IN VIVO* EXPERIMENTAL STUDIES

A pot culture experiment was carried out with rice variety “Uma” to evaluate the efficacy of treatments in managing the sheath blight disease of rice. The treatments which were found to be promising under *in vitro* conditions were selected for this study.

Typical symptoms of sheath blight disease were observed three days after inoculation and sclerotial formation was observed seven to eight days after inoculation in the rice plants. The stage of disease development was coincided with the active tillering stage. Adhipathi *et al*. (2013), Bhukal *et al*. (2015), Manjunatha *et al*. (2018) and Yongqiang *et al*. (2018) also reported the appearance of small water soaked grey coloured lesion on the inoculated leaf sheath by three days after artificial inoculation of *R. solani*. Sclerotia were observed after seven days after inoculation (Bhukal *et al*., 2015, Manjunatha *et al*., 2018 and Yongqiang *et al*., 2018).

The vertical spread of the disease was estimated for all treated plants in terms of relative lesion height (RLH). The least RLH and the maximum percentage suppression over control were observed in the plants sprayed with potassium silicate (10.46 and 76.05 % respectively). Total number of sheath blight lesions, severity of sheath blight disease, and the highest relative lesion height on the main tiller of rice plants decreased by 37, 40, 52 and 24 per cent respectively when the rate of Silicon increased from 0 to 1.92 g pot^{-1} (Rodrigues *et al*., 2003). Moreover, glycosidically bound phenolics extracted from silicon treated rice plants display a strong fungistatic activity (Ma, 2004). Silicon acts as physical barrier by depositing on the surface of plant tissues (Fauteux *et al*., 2005, Datnoff *et al*., 2007, Van Bockhaven *et al*., 2013, Pozza *et al*., 2015). Foliar application of Silicon reported to be effective in preventing powdery mildew development on grape leaves, cucumber, and muskmelon by the deposition of callose, which was an indicator of biotic and abiotic stresses (Menzies *et al*., 1992 and Brugiére and Exley, 2017).

Per cent disease index (PDI) was also calculated for all the treatments and it was found that lowest PDI (12 %) was recorded for the plants which were sprayed with potassium silicate which were on par with the plants sprayed with tebuconazole 50 % + trifloxystrobin 25 % WG (13 %) and plants treated with fermented weed extract along with AMF application in the nursery stage (14 %) (Fig. 1). Potassium silicate was found to reduce the intensity of the sheath blight disease in rice as reported by Savanth *et al*. (1997), Rodrigues *et al*. (2001) and

Meiqin *et al*. (2005). Fertilization with Si was significantly more effective than Cu and Zn in minimizing yield loss due to sheath blight disease in rice (Khaing *et al.*, 2014). Vivancos *et al.* (2015) showed that silicon application increases the resistance against powdery mildew infection caused by *Golovinomyces cichoracearum*. They proposed that silicon interfers with the effector proteins released by *Golovinomyces* suggesting that mechanisms other than salicylic acid defense were also involved in silicon mediated defense in plants. Interestingly, studies revealed that silicon mediated brown spot tolerance to the rice plants not solely dependent on the immune hormones, salicylic acid and jasmonic acid, but silicon increased the tolerance by interfering action and production of fungal ethylene and kept the rice innate immune system active (Van-Bockhaven *et al.*, 2015).

Similarly, the horizontal spread was also evaluated for all the plants. The number of infected tillers per plant were found to be minimum for the plants sprayed with tebuconazole 50 % + trifloxystrobin 25 % WG which was followed by plants treated with potassium silicate and fermented weed extract. The per cent horizontal spread was recorded least for the plants sprayed with potassium silicate (23.81 %) which was followed by the plants treated with tebuconazole 50% + trifloxystrobin 25% WG (25.2 %). (Fig. 2). Silicon treated plants exhibited localized cell defense mechanisms like papilla formation, callose production and buildup of glycosylated phenolics in response to infection by the fungus *Blumeria graminis* (Belanger *et al*., 2003).

5.5. BIOCHEMICAL STUDIES FOR ASSESSING THE MECHANISMS OF DISEASE MANAGEMENT IN VARIOUS TREATMENT SPRAYED *R. solani* INOCULATED PLANTS

The activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was recorded at 0, 24, 48 and 72 hours after inoculation of *R. solani* on the treated and untreated rice plants

The maximum activity of PO was observed in the plants sprayed with potassium silicate (45.1 mg $g^{-1}min^{-1}$) on 72 h and for the plants where nursery application of AMF was carried out (45.1 mg $g^{-1}min^{-1}$) (Fig. 3). The highest activity of PPO was recorded in the plants sprayed with potassium silicate (3.89) which were on par with the plants treated with tebuconazole 50 % + trifloxystrobin 25 % WG (3.81) after 72h of treatment application (Fig. 4). The maximum activity of PAL was observed in the plants sprayed with tebuconazole 50 % + trifloxystrobin 25 % WG (110.5 mg $g^{-1}min^{-1}$) followed by plants where AMF application at nursery stage was carried out (104.2 mg $g^{-1}min^{-1}$) after 72 h of treatment application (Fig. 5). Cherif *et al*. (1994) also reported the greater activities of PO, chitinase, polyphenol oxidases and β-1, 3 glucanase in response to infection by *R. solani* in rice. Similarly, ryegrass amended with silicon recorded higher concentrations of chlogenic acid and flavanoids and greater activities of PO and PPO (Farroq *et al*., 2015).

The leaves of cucumber plants infected by *Podosphaera xanthii* showed increased PO activity when supplied with silicon as compared to the leaves of untreated plants (Liang *et al*., 2005). Manila *et al.,* (2014) recorded the maximum reduction of wilt disease in AMF (*Glomus fasiculatum* and *Acaulospora laevis*) colonized tomato plants. Biochemical analyses of the treated plants revealed greater production of total sugars, soluble sugars, phenols, proteins and higher activities of PO, PPO and serine. Rahman *et al.* (2015) recorded the greater activity of lipoxygenase and PAL when the plants treated with potassium silicate infected by *Maganaparthe oryzae*. Contrasting results were obtained by Durner and Klessig (1995) who observed that the increase in the PO activity was correlated with increase in susceptibility of the plants to the pathogen.

Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). It is involved in production of reactive oxyzen species (ROS), regulation of cell wall elongation, wound healing and resistance against pathogens. It is considered to be important PR proteins produced during host-pathogen interaction. (Vanloon *et al.,* 1994). Increase in the

PO and PPO activity may contribute to cross linking of hydroxyproline rich glycoproteins (HRGPs), lignifications that will acts as barriers against pathogen entry and establishment. Hydrogen peroxide generated by peroxidase may function as antifungal agent in disease resistance. Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals that are antimicrobial in nature (Chen *et al.*, 2000).

The product of phenylalanine ammonia lyase is trans-cinnimic acid, which is an immediate precursor for the biosynthesis of salicylic acid, a molecule in systemic acquired resistance (SAR) (Klessing and Malamy, 1994). Phenylalanine ammonia lyase is found to play a key role in shikimate and secondary phenyl propanoid pathway by synthesis of phenols, which in turn enhances the resistance in plants. (Yao *et al.*, 1995). Chittoor *et al.* (1999) proposed that polyphenol oxidase was involved in the oxidation of polyphenols into quinones using molecular oxygen as an electron acceptor and lignification of plant cells during microbial infections. Peroxidase enzymes found to participate in defense reactions by conferring to hypersensitivity to the plants resistant to diseases (Shamim *et al.*, 2018).

Foliar spray of potassium silicate (0.5 %) or application of AMF at nursery stage @ 200 g/m^2 or application of AMF at nursery stage @ 200 g/m^2 combined with the foliar application of fermented *S. barbata* (10 %) at 35, 55 and 75 DAS improved the number of tillers, productive tillers of rice plants (Fig. 6 and Fig. 7). Lalithya *et al.*, (2014) reported that application of silicon on sapota plants resulted in the increased yield of the fruit. The possible reason behind the enhanced yield may be due to increased cell division, more uptake of nutrients and water.

The study entitled “Ecofriendly management of sheath blight disease of rice” developed with an objective to develop an effective ecofriendly strategy for the management of the disease yielded salient findings in managing the disease in an ecofriendly approach. Application of potassium silicate (0.5 %) at 35, 55 and 75 DAS resulted in increased biometric parameters like number of tillers and productive tillers per plant and also resulted in reduced incidence of horizontal

and vertical spread of the disease. The activity of defense related enzymes like peroxidase, polyphenol oxidase were found to be greater for the plants sprayed with potassium silicate, which is a clear indication of effective management of disease through effective defense signaling. Potassium silicate at 0.5 per cent concentration in managing the disease need to be evaluated at the field level for final recommendation to the farmers. The active principle in the weed, *Setaria barbata* need to be evaluated.

Summary

6. SUMMARY

Sheath blight disease caused by *Rhizoctonia solani* is an economically important disease of rice. Management of the disease is very difficult due to the wide host range of the fungus and lack of disease resistant varieties. Use of fungicides for the management of the disease results in various problems both to the environment and life forms. Hence use of naturally available fermented organic compounds and plant products is found to be a sustainable approach for the management of the disease. With this regard, a study entitled 'Ecofriendly management of sheath blight disease of rice' was carried out during 2017 - 2019 at the Department of Plant Pathology, College of Agriculture (COA), Vellayani and Integrated Farming System Research Station (IFSRS), Karamana with the objective to develop an effective ecofriendly strategy for the management of sheath blight disease of rice.

R. solani (Accession no. KX674527) which was maintained at the Department of Plant Pathology, COA, Vellayani, was used for both *in vitro and in vivo* experiments. Pathogenicity of the fungus was proved by Koch's postulates in the rice variety 'Úma'. Typical symptoms of sheath blight disease including small, water soaked lesions with greyish brown irregular margin were observed on the inoculated leaf sheaths on three days after inoculation (DAI). The lesion length was 0.79 cm on three DAI which increased to 3.86 cm by seven DAI. Sclerotia started to develop from fifth DAI which were initially white in colour later turning to brown in colour.

Cultural characters of *R. solani* were studied. The fungi inoculated on the Petri plates containing PDA medium produced 9 cm radial growth on the third DAI. Sclerotia started to develop from fifth DAI which were few in number, white coloured later turning to brown in colour. The sclerotia were round and mustard shaped.

In vitro evaluation of organic preparations *viz.*, fermented egg lemon-juice extract (10 %) and fermented *Setaria barbata* extract (10 %), botanicals *viz.*, garlic extract (10 %), non-hazardous chemicals *viz.*, potassium silicate (0.5 %) and their combinations were tested for their potential in mycelial inhibition of the

fungus at IFSRS, Karamana. The study revealed that all the treatments individually and in combinations resulted in complete mycelial inhibition of the fungus. On fifth DAI, sclerotial formation was completely inhibited by all the treatments except in the control plates.

A study conducted to evaluate the various treatments for their potential in inhibiting the mycelial regeneration from sclerotia dipped in different treatments for different time periods revealed that dipping sclerotia in fermented egg-lemon juice extract for 24, 48, 72 h resulted in maximum inhibition of mycelial regeneration from sclerotia (100, 100 and 96.60 % respectively), followed by fermented *Setaria barbata* extract (76.6, 83.3 and 83.66 % respectively). No inhibitory effect on mycelial regeneration from sclerotia was observed when sclerotia were dipped in potassium silicate (0.5 %) for 24 and 48 h. However, an inhibition of 66.6 per cent in the mycelial regeneration was recorded when sclerotia were dipped for 72 h in the extract.

A pot culture experiment was conducted in the rice variety Uma for the management of sheath blight disease using eight treatments and four replications in completely randomized design (CRD). All the inoculated plants developed typical sheath blight symptoms on three DAI. Sclerotia started to develop from seventh to eight DAI in all the pathogen inoculated plants.

The lowest per cent disease index (PDI) and hence the least vertical disease spread was recorded in the plants sprayed with potassium silicate (0.5 %) (12 %) and a combination of AMF application at nursery stage and foliar application of fermented *S. barbata* extract (10 %) at 35, 55 and 75 DAS (14 %) which were on par with each other as well as the foliar spray of the treated control *viz.*, tebuconazole 50 % and trifloxystrobin 25 % WG. Lesion length was found to be lesser than inoculated control in all the treated plants.

The least per cent horizontal spread was recorded in plants sprayed with potassium silicate (23.81 %) which was followed by plants which were sprayed with tebuconazole 50 % and trifloxystrobin 25 % WG (25.2 %). Among the treatments, foliar application of potassium silicate (0.5 %) revealed the minimum number of infected tillers per plant (5.55) which was on par with the foliar

application of fermented *Setaria barbata* extract (5.6). The total tillers per plant was the maximum in the plants sprayed with the potassium silicate (0.5 %) (23.3) which was on par with the plants applied with AMF at nursery stage (23.1) (Fig. 3). The maximum number of productive tillers was recorded in the plants sprayed with potassium silicate (0.5 %) (22.1) which was on par with the plants where nursery application of AMF @ 200 g/m^2 (21) was performed as well as in the plants applied with AMF in the nursery stage and sprayed with fermented *S. barbata* extract (10 %) (20.9).

There was significant reduction in the lesion height in all the treatment applied plants and were on par with each other compared to untreated control plants. The lesion width was least in the plants sprayed with fermented *S. barbata* extract (10 %) (0.48 cm) followed by the plants sprayed with potassium silicate (0.5 %) (0.55 cm). The lesion area was also lesser in all the treatment applied plants which were on par with each other compared to untreated control plants.

The activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were recorded on 0, 24, 48 and 72 h after foliar spray. The study revealed that the maximum activity of peroxidase at 72 h after the third spray was observed in plants sprayed with potassium silicate and the plants where nursery application of AMF was undertaken. Next to these, the plants sprayed with potassium silicate along with AMF application and plants treated with weed extract along with AMF application recorded higher activity of peroxidase.

The highest activity of polyphenol oxidase was recorded in the plants sprayed with potassium silicate and tebuconazole 50 % and trifloxystrobin 25 % WG, which were on par at 72 h after the third spray. The plants treated with AMF and the plants applied with AMF along with the application of potassium silicate and plants applied with AMF and treated with fermented weed extract recorded the next highest activity of polyphenol oxidase.

The foliar application of tebuconazole 50 % and trifloxystrobin 25 % WG recorded the maximum activity of phenylalanine ammonia lyase followed by AMF application at nursery stage at 72 h after the third spray. Lowest

phenylalanine ammonia lyase activity was recorded for the plants treated with AMF at the nursery stage along with application of fermented weed extract.

Thus, the study revealed that foliar spray of potassium silicate (0.5 %) at 35, 55 and 75 DAS or application of AMF at nursery stage @ 200 g/m^2 or application of AMF at nursery stage @ 200 g/m^2 combined with the foliar application of fermented *S. barbata* (10 %) at 35, 55 and 75 DAS improved the number of productive tillers of rice plants. As far as the management of sheath blight disease of rice was concerned, foliar sprays of potassium silicate (0.5 %) at 35, 55 and 75 DAS was revealed to be the best treatment which recorded the least vertical and horizontal disease spread as well as the minimum number of sheath blight infected tillers per plant.

Future prospects:

- Efficacy of the potassium silicate (0.5 %) need to be evaluated under field conditions
- Identification of the bioactive antifungal principles of the weed, *Setaria barbata* need to be studied.

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Appendices

APPENDIX - I

COMPOSITION OF MEDIA USED

1. **Potato Dextrose Agar**

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

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

APPENDIX - II

Buffers for enzyme analysis

1. **0.1 M**

Sodium phosphate buffer (pH 6.5)

Stock solutions

A: 0.2 M solutions of monobasic sodium phosphate (27.8 g in 1 litre)

B: 0.2 M solutions of dibasic sodium phosphate (53.65 g in 1 litre)

68.5 ml of A mixed with 31.5 ml of B diluted to a total of 200 ml.

Abstract

Ecofriendly management of sheath blight disease of rice

by

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Abstract of the thesis

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ABSTRACT

The study entitled “Ecofriendly management of sheath blight disease of rice” was carried out at College of Agriculture (COA), Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2017-19 with the objective to develop an effective eco-friendly strategy for the management of sheath blight disease.

The sheath blight fungus *viz., Rhizoctonia solani* (Accession No. KX674527) maintained at the Department of Plant Pathology, College of Agriculture, Vellayani was used for both *in vitro* and *in vivo* studies. Pathogenicity of the fungus was proved in the rice variety Uma. Characteristic sheath blight symptoms appeared on the inoculated sheath as water soaked, grey coloured lesions on the third day after inoculation (DAI) with a lesion length of 0.79 cm which increased to 3.86 cm on the seventh DAI. Development of white coloured sclerotia was observed on the fifth DAI on the inoculated leaf sheath which gradually turned to brown.

In vitro evaluation of organic preparations namely fermented egg lemon juice extract (10 %) and fermented *Setaria barbata* extract (10 %), botanicals namely garlic extract (10 %), non-hazardous chemicals namely potassium silicate (0.5 %) and their combinations in mycelial inhibition of the fungus was undertaken at IFSRS, Karamana in completely randomised design (CRD) with 14 treatments and three replications. The study revealed that all the treatments individually and in combinations resulted in complete mycelial inhibition of the fungus. Dipping sclerotia in fermented egg-lemon juice extract for 24, 48, 72 h resulted in maximum inhibition of mycelial regeneration from sclerotia (100, 100 and 96.60 % respectively) followed by fermented *Setaria barbata* extract (76.6, 83.3 and 83.66 % respectively).

The pot culture experiment in the rice variety Uma in CRD using eight treatments and four replications revealed that the maximum suppression of vertical spread of the disease was observed in plants sprayed at 35, 55 and 75 days after sowing (DAS) with potassium silicate (76.05 %) and was followed by the nursery application of AMF (200 g m^{-2}), foliar spray of fermented *Setaria barbata* extract (75.06 %) and tebuconazole 50 % and trifloxystrobin 25 % WG (75.20 %). The minimum horizontal spread was observed in plants sprayed with potassium silicate (23.81 %) which was followed by plants treated with tebuconazole 50 % and trifloxystrobin 25 % WG (25.20 %).

The activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were recorded on 0, 24, 48 and 72 h after foliar spray. The study revealed that the maximum activity of peroxidase at 72 h after the third spray was observed in plants sprayed with potassium silicate and the plants where nursery application of AMF was undertaken. The highest activity of polyphenol oxidase was recorded in the plants sprayed with potassium silicate and tebuconazole 50 % and trifloxystrobin 25 % WG, which were on par. The foliar application of tebuconazole 50 % and trifloxystrobin 25 % WG revealed the maximum activity of phenylalanine ammonia lyase followed by AMF application at nursery stage. Thus the present study revealed that rice sheath blight disease could be effectively managed by three foliar sprays of potassium silicate (0.5 %) or tebuconazole 50 % and trifloxystrobin 25 % WG (0.04 %) at 35, 55 and 75 DAS as evident from the maximum number of tillers per plant, the minimum vertical and horizontal spread of the disease as well as the highest activity of peroxidase and polyphenol oxidase in the inoculated plants.