STUDIES ON SHEATH BLIGHT OF RICE (*Oryza sativa* L.) INCITED BY *Rhizoctonia solani* Kuhn.

By

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DAPOLI - 415 712, DIST. RATNAGIRI (M.S.)

May, 2015

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CERTIFICATE

This is to certify that the thesis entitled, **"Studies on Sheath Blight of Rice (*Oryza sativa* L.) Incited by *Rhizoctonia solani* Kuhn."** submitted to the Faculty of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, Maharashtra State, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (Agriculture)** in **PLANT PATHOLOGY**, embodies the results of a piece of bona-fide research carried out by **Mr. PAWAR SANDESH VYANKATESH** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma or published in other form. All the assistance and help received during the course of investigation and the sources of literature have been duly acknowledged by him.

Place: Dapoli
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Title of the Thesis	:	"Studies on Sheath Blight of Rice (*Oryza sativa* L.) incited by *Rhizoctonia solani*"
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THESIS ABSTRACT

Incidence of sheath blight disease of rice incited by *Rhizoctonia solani* Kuhn. was observed at Agricultural Research Station Shirgaon, Dist. Ratnagiri (M.S.) during third week of September in the year 2014.

The pathogenic fungus was isolated on potato dextrose agar medium and its pathogenicity was proved by following standard procedure. All the fungicides tested against the pathogen *viz.*, Mancozeb (0.2% and 0.3%), Hexaconazole (0.1% and 0.2%), Propiconazole (0.1% and 0.2%), Difenconazole (0.1% and 0.2%), Azoxystrobin (0.1% and 0.2%), and combination product of flusilazole and carbendazim (0.05% and 0.1%) completely inhibited the mycelial growth of *R. solani*. Thiophanate methyl completely inhibited the mycelial growth of *R. solani* at higher concentration (0.2%) however, it was found to be comparatively less effective at lower concentration (0.1%). The antibiotic Validamycin was ineffective at lower as well as higher concentration.

In vitro studies revealed that, 20 per cent plant extracts of garlic, mustard, soap nut and marigold inhibited mycelial growth of *R. solani* to the tune of 81.70, 80.36, 76.25 and 75.25 per cent respectively, in comparison with control. All the three bio-agents (*Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens*) were effective against the pathogen. Tillering stage was found to be the most susceptible stage to the infection of *R. solani*.

पादप व्याधिविज्ञान विभाग
कृषी महाविद्यालय, दापोली

प्रबंध का शीर्षक	:	रायझोकटोनीया सोलानी इस फफुंदी के कारण चावलपर (ओरायझा सटायवा एल.) आनेवाले पत्तोंके कोषका जलना इस रोग का अभ्यास।
छात्र का नाम	:	कु. संदेश व्यंकटेश पवार
पंजीकृत क्रमांक	:	२२७०
संशोधन मार्गदर्शक	:	***विद्यावाचस्पती प्रमोद गो. बोरकर*** सहाय्यक आचार्य, पादप व्याधिविज्ञान विभाग, कृषि महाविद्यालय, दापोली- ४१५ ७१२
पदवी के पुरस्कार का वर्ष	:	मई, २०१५

सार

चावलपर रायझोकटोनीया सोलानी इस फफुंदी के कारण आनेवाले पत्तोंके कोषका जलना इस रोग का प्रादुर्भाव वर्ष २०१४ सितंबर महिनेके तिसरे साप्ताह मे कृषी संशोधन केंद्र शिरगाव जिला रत्नागिरी के सस्य विज्ञान विभाग क्षेत्रमे दिखाई दिया|

यह फफुंदी पोटेटो डेक्सट्रोज अगर इस माध्यमपर बढाई गई और इस फफुंदी की व्याधीजन्यता चावल के पौधेपर सिद्ध की गई. प्रयोगशालेके नियंत्रित वातावरण मे रायझोकटोनीया सोलानी इस फफुंदीपर मॅन्कोझेब (०.२ और ०.३ %), हेक्झाकोनझोल (०.१ और ०.२ %), प्रोपिकोनझोल (०.१ और ०.२ %), डायफेन्कोनझोल (०.१ और ०.२ %), ॲझोक्झीस्ट्रोबीन (०.१ और ०.२%) और फ्लूसिलाझोल और कॅर्बेडान्झीम (संयोजीत फफुंदीनाशक) यह फफुंदीनाशक के कारण रायझोकटोनीया सोलानी इस फफुंदी के कवकजाल की वृद्धी पूर्णरूप से रोक दी गई| थायोफेनेट मिथाईल इस फफुंदनाशक के जादा सांद्रता (०.२%) मे रायझोकटोनीया सोलानी इस फफुंदके कवकजाल की वृद्धी पूर्णरूप से रोक दी गई, लेकीन कम सांद्रता (०.१%) मे इस फफुंदके कवकजाल की वृद्धी कम रूप से रोक दी गई| जादा और कम सांद्रतामे वालिडामायसीन ये अनुपयोगी दिखाई दी|

लहसून, सरसो, गेंदा और रिठा ईस वनस्पतींके २० प्रतीशत निचोड से तयार की गई माध्यमपर रायझोकटोनीया सोलानी इस फफूंद की क्रमशः ८१.७०, ८०.३६, ७६.२५ आणि ७५.२५ प्रतिशत वृद्धी कम हुई पायी गई| रोगनियंत्रण करने के लिए इस्तमाल किये गये तिन्हो जैवनियंत्रके (ट्रायकोडर्मा विरीडी, ट्रायकोडर्मा हर्झीयानम आणि प्सूडोमोनास फ्लूरेन्सस) यह रोगप्रतिकारक पाये गये| चावलके तिल्लेरिंग अवस्था में रायझोकटोनीया सोलानी यह रोगकरक संक्रमण के लिये जादा सवेंदनशील था|

वनस्पती रोगशास्त्र विभाग

कृषी महाविद्यालय, दापोली

प्रबंधाचे नाव : रायझोकटोनीया सोलानी या बुरशीमुळे भात पिकावर (ओरायझा सटायवा एल.) येणाऱ्या पर्णकोष करपा ह्या रोगाचा अभ्यास .

विद्यार्थ्याचे नाव : कु. पवार संदेश व्यंकटेश

नोंदणी क्रमांक : २२७०

प्रमुख मार्गदर्शक :

विद्यावाचस्पती प्रमोद गो. बोरकर,

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सादरीकरणाचे वर्ष : मे, २०१५

सारांश

रायझोकटोनीया सोलानी या बुरशीमुळे भात पिकावर येणाऱ्या पर्णकोष करपा ह्या रोगाचा प्रादुर्भाव हा सन २०१४ सप्टेंबर महिन्याच्या तिसऱ्या आठवडयात कृषी संशोधन केंद्र शिरगाव, जिल्हा रत्नागिरी यांच्या प्रक्षेत्रावर आढळून आला.

ही बुरशी पोटेटो डेक्सट्रोज अगर या माध्यमावर वाढवण्यात आली आणि या बुरशीची रोगकारकता भाताच्या रोपावर सिद्ध करण्यात आली. प्रयोगशाळेत नियंत्रित वातावरणात रायझोकटोनीया सोलानी या बुरशीवर मँन्कोझेब (०.२ आणि ०.३ %), हेक्झाकोनझोल (०.१ आणि ०.२ %), प्रोपिकोनझोल (०.१ आणि ०.२ %), डायफेन्कोनझोल (०.१ आणि ०.२ %), ॲझोक्झीस्ट्रोबीन (०.१ आणि ०.२%) आणि फ्लूसिलाझोल व कॅर्बेडान्झीम (संयुक्त बुरशीनाशक) या बुरशीनाशकांमुळे रायझोकटोनीया सोलानी या बुरशीची कवकजालाची वाढ पूर्णता खुंटण्यात आली असे आढळून आले. थायोफेनेट मिथाईल या बुरशीनाशकाच्या जास्त तीव्रतेत (०.२%) रायझोकटोनीया सोलानी या बुरशीची कवकजालाची वाढ पूर्णता खुंटण्यात आली, परंतु, कमी तीव्रतेत (०.१%) या बुरशीनाशकामुळे बुरशीची कवकजालाची वाढ कमी खुंटण्यात आली. जास्त आणि कमी तीव्रतेत वालिडामायसीन हे निरर्थक दिसून आले.

लसूण, मोहरी, झेंडू आणि रिठा या वनस्पतींच्या २० टक्के अर्कापासून तयार करण्यात आलेल्या माध्यमांवर रायझोकटोनीया सोलानी या बुरशीची अनुक्रमे ८१.७०, ८०.३६, ७६.२५ आणि ७५.२५ टक्के वाढ खुंटलेली दिसून आली. रोगनियंत्रण करण्यासाठी वापरण्यात आलेले तिन्ही जैवनियंत्रके (ट्रायकोडर्मा विरीडी, ट्रायकोडर्मा हर्झीयानम आणि प्सूडोमोनास फ्लूरेन्सस) हे रोगकारकाविरुद्ध प्रभावी होती. भात फुटव्यांच्या अवस्थेत रायझोकटोनीया सोलानी हा रोगकारक संक्रमण करण्यासाठी सर्वात संवेदनाक्षम टप्पा होता.

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Place: Dapoli
Date: 22/05/2015 ***(Pawar Sandesh Vyankatesh)***

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food grain crop of the world which constitutes the principle food for about 60 per cent of the world's population. Rice contributes 43 per cent of total food grain production and 46 per cent of total cereal production in India. Rice based production system provides the income and employment for more than 50 million households. China ranks first in rice production followed by India. One third of Asia's rice production is consumed in China and one fifth in India.

India is the world's second largest rice producer and consumer next to China. About 90 per cent of rice grown in the world is produced and consumed in Asian continent. Globally, rice is cultivated on about 163.1 million hectares of area with total production of 722.5 million tonnes and productivity up to 4.4 tonnes ha^{-1} (Anonymous, 2012 a, b). Rice has unique position in Indian economy.

India's rice production reached to a record yield of 104.32 million tonnes from an area of 43.17 million hectares with productivity of 2.42 tones ha^{-1} in 2011-2012 (Anonymous, 2013). Though the production is high, the average productivity poor as compared to other rice growing countries like Spain, Japan, Australia and China. The main reasons for low productivity are vagaries of nature, low fertilizer use efficiency, poor management of diseases, pests and weeds.

In Maharashtra, rice is cultivated on 15.13 lakh hectares in regions *viz.* Vidharbha (7.95 lakh ha.), Konkan (3.83 lakh ha.), Western Maharashtra (3.23 lakh ha.) and Marathwada (0.12 lakh ha.) with annual production of 41.71 lakh tonnes. The major rice

growing districts in Maharashtra are Thane, Raigad, Ratnagiri and Sindhudurg along the west coast and Bhandara, Chandrapur in the eastern parts of the states and minor areas such as Kolhapur, Satara and Pune. Enhancing rice production is becoming a challenging task especially in the background of burgeoning population, declining agricultural land, less availability of agricultural labour and yield losses due to abiotic and biotic stresses such as diseases and pests.

Diseases not only reduce yield but also greatly impair the quality and stability of production and affect the agricultural sustainability. The rice cultivation practices adopted in different parts of country vary widely depending upon the climatic conditions, soil, and availability of water and crop variety. All these factors greatly influence the susceptibility of the variety to one or more diseases.

Rice crop is affected by a number of fungal, bacterial and viral diseases. Among these, diseases caused by fungal pathogens are responsible for major causes of crop losses. The major diseases of rice is listed below which limits its production.

Blast- *Pyricularia grisea*

Brown spot- *Helminthosporium oryzae*

False smut- *Ustilaginoidea virnens*

Sheath rot- *Sarocladium oryzae*

Sheath blight- *Rhizoctonia solani*

BLB- *Xanthomonas oryzae* pv. *oryzae*

Among these diseases, Sheath blight is becoming a major constraint in rice production in India. The disease is caused by *Rhizoctonia solani* Kuhn (Telemorph: *Thanetophorus cucumeris* Frank Donk), a fungal pathogen of rice and many other agronomical

and horticultural crops. It is very destructive disease under favourable weather conditions in rice growing areas of the world which ultimately causes substantial yield losses (Gautam *et al.* 2003).

The yield losses due to this disease are reported to be in a range of 5.2 to 50 per cent depending on the environmental conditions, crop stage at which the disease occurs cultivation practices and cultivars used (Marchetti and Bolllich 1991). Grain losses to an extent of 40 per cent have been able to report annually with this disease (Tan Wan Zhong *et al.,* 2007). The disease was first recorded in Japan, and also found to be widespread in East and South East Asian countries hence it is name 'Oriental sheath and leaf blight'. The disease occurs worldwide in all rice producing areas (Savary *et al.* 2006).

The widespread adoption of new, susceptible, high-yielding cultivars with large numbers of tillers, and the changes in cultural practices associated with these cultivars, favour the development of sheath blight and contribute greatly to the rapid increase in the incidence and severity of this disease. Furthermore, environmental conditions such as low light, cloudy days, high temperature and high relative humidity also favour the disease (Ou, 1985). The pathogen overwinters as soil-borne sclerotia and mycelium in plant debris which serve as primary inoculum. Control of the pathogen is difficult because of its ecological behavior it's extremely broad host range and the high survival rate of sclerotia under various environmental conditions (Groth *et al.* 2006). Although expensive severity of rice cultivars and germplasm has been done so far, none of them has been found to be resistant to this disease (Oard *et al.* 2004). In the absence of a desired level of host resistance, the disease is currently managed by excessive application of chemical

fungicides, which eventually impair the soil biota and the atmosphere. Some potentially effective fungicides are highly phytotoxic to rice and, if the disease is not severe, these fungicides may reduce yield (Groth *et al.* 1990). In this situation, biological control seems to be an effective in minimizing the incidence of sheath blight (Das and Hazarika 2000).

Rice based cropping system is the tradition of Konkan region. Rice is the sole crop cultivated in *Kharif* season. During, 2013-14 severe incidence of sheath blight disease was noticed on Agricultural Research Station, Shirgaon (Ratnagiri). Considering increasing severity of this disease, it was necessary thought to undertake investigations on the disease with following objectives.

1) To isolate the causal organism and prove Koch's postulates.
2) To test the efficacy of different fungicides *in vitro* against the pathogen.
3) To study the antifungal properties of certain herbal extracts against the pathogen *in vitro*.
4) To study the effect of bio-control agent against the pathogen *in vitro*.
5) To study the infectivity of the pathogen in relation to crop age.

CHAPTER II

REVIEW OF LITERATURE

Rhizoctonia is a soil borne basidiomycetes which causes diseases in many crop plants. The *Rhizoctonia solani* was reported for the first time on potato tuber by Kühn (1858). It is a highly destructive facultative parasite causing leaf spot, leaf blight, root and shoot or shoot or fruit rots, formation of cankers on sprouts and stolon's or damping off and occasionally wilting of numerous plant species. The fungus is able to survive in soil for long period of time. In this chapter the available literature on the disease reports caused by *Rhizoctonia* spp., their isolation method, and pathogenicity, efficacy of fungicides, plant extract and bio agents and infectivity of pathogen in relation to crop age against the pathogen has been reviewed.

2.1 Disease report:

In Baton Rouge, LA *R. solani* causing web blight of verbena was reported for the first time by Holcomb *et al.* (2000).

Root rot of pea caused by *Rhizoctonia oryzae* was reported by Paulitz (2002).

First report of *Petunia* root rot caused by *R. solani* in Argentina by Wright *et al.* (2003).

Kurt *et al.* (2005), for first time reported the crater rot of carrot (*Daucus carota*) caused by *Rhizoctonia carotae* in Turkey.

Schillinger *et al.* (2006) studied the bare patch disease on the roots of wheat caused by *R. solani* (AG 8) near Ritzvelle, Washington.

Post-harvest rot of okra pods incited by *R. solani* was observed in Brazil by Henz *et al.* (2007).

Association of *R. solani* on roots of torch ginger (*Etlingera elation*) in the State of Para was reported by Verzignassi *et al.* (2008).

Rhizome blight of ginger (*Zingiber officinale*) incited by *Rhizoctonia* spp. was reported from china by Yang *et al.* (2008).

Muzhinji *et al.* (2014) give first reported that *R. solani* (Ag 4 HG III) is responsible for potato stem canker in South Africa.

Leaf blight disease of cardamom caused by *R. solani* in Konkan region was first time reported by Waghrulkar (2014).

Crown and root rot of sugar beet caused by *R. solani* (AG 2) was studied by Zhao *et al.* (2014) in Shanxi Province of China.

2.2 Isolation of causal organism:

Botha *et al.* (2003) reported the characterization and pathogenicity of *R. solani* associated with black root rot of strawberries and described the procedure of isolation of the pathogen. Infected roots were cut and disinfected by dipping in 1 per cent sodium hypochlorite (NaOCl) solution for 1 minute followed 30 seconds dip in 50 per cent ethyl alcohol. Such disinfected root pieces were rinsed twice with distilled sterile water and air dried in a laminar air flow cabinet. The pieces were then transferred to PDA and WA (Water agar). On both the media, the mycelial growth of the fungus was obtained within 5 days and sclerotia emerged within 7 days after isolation.

Eken *et al.* (2003) isolated *Rhizoctonia* spp. from roots and crowns of alfalfa in Turkey.

R. solani anastomosis group (AG13) was isolated from asymptomatic root tissues of a corn seedling grown at Brooksville.

The isolate was cultured on potato dextrose agar (PDA) and incubated at 27 ± 2° C. (Peterson *et al.* 2004).

Sivalingam *et al.* (2006) studied the role of seed borne inoculum in rice sheath blight development and observed no correlation between degree of seed discoloration and isolation frequency of pathogen.

R. solani was isolated from the naturally infected leaf samples (Sriraj *et al.* 2014). A small portion of sheath showing typical lesion was cut into small bits of 0.5 to 1 cm, surface sterilized with 0.1 per cent mercuric chloride and rinsed three times with sterile distilled water. Then, they were transferred to Petri dish containing PDA.

2.3 To prove the pathogenicity of isolated organism:

Studies on pathogenicity factors of *R. solani* indicated that melanin producing cultures are more virulent than non-melanin producing cultures. (Tae *et al.* 2001)

Singh *et al.* (2002) proved the pathogenicity of *R. solani* on rice. The leaf sheath was opened carefully and a single sclerotial body was placed inside. A few drops of sterile water were sprinkled on the inoculated sheath. Inoculation was done in the evening and inoculated plants were sprayed with water next morning. Inoculated plants were maintained in a greenhouse at 25 ± 3° C and were regularly examined for symptom appearance of rice sheath blight.

Pathogenicity of *R. solani* was proved on four days old corn seedlings by placing the bits of 7 day old culture in mesocotyl tissues contact with below the soil. Non inoculated seedlings served as control. All the seedlings were maintained under controlled condition. (Peterson *et al.* 2004)

Different inoculation techniques such as single grain insertion, single sclerotium insertion, and injecting mycelial suspension were

used by Chakraborty *et al.* (2006) for assessing the pathogenicity of sheath blight of rice. It was found that single sclerotium insertion was the most effective

Gilmar *et al.* (2007) confirmed the pathogenicity test of *R. solani* on okra fruits by making pin prick injury followed by deposition of a plug of mycelial culture of *R. solani* (AG 1-IB) on the injured portion.

Park *et al.* (2008) reported the method for inoculation and evaluation of rice sheath blight disease. Rice plants at late tillering stage were inoculated with *R. solani* by placing a mycelial ball beneath the leaf sheath. The inoculated sheath was covered immediately with an aluminum foil. When typical lesions appeared after 3 days, the aluminum foil was removed. Freshly inoculated plants were kept in a plastic humid chamber for 3 weeks to aid the disease development. The humidity in the chamber was maintained between 80 – 100 percent from the time of inoculation till the development of symptoms.

To prove pathogenicity, 40-day old maize plants were inoculated by inserting 2 to 3 grains covered with mycelial growth of *R. solani*, separately, between the rind and the leaf sheath of test plants. High humidity was maintained during disease development by frequent watering. (Akhtar *et al.* 2009).

In order to prove pathogenicity of leaf blight samples of the cardamom were washed with running tap water, and cut into small bits of half healthy and diseased portion of infected leaf. Such bits were disinfected with 0.1 per cent mercuric chloride solution for 1 minute followed by three washing in distilled sterile water to remove traces of mercuric chloride, then bits are transferred aseptically in

sterile petri plate containing sterilized, solidified PDA medium. (Waghrulkar, 2014).

Sriraj *et al.* (2014) conducted the pathogenicity test of *R. solani* on turmeric. Disease free turmeric rhizomes (Erode local 8) were inoculated with *R. solani* by inserting young immature sclerotia (two sclerotia per sheath) and incubated for seven days for the development of typical blight symptoms on the plants.

2.5 *In vitro* evaluation of fungicides against the pathogen:

Efficacy of different fungicides *viz.;* Bavistin 50 WP (Carbendazim), Contaf 5 EC (Hexaconazole), Forastin 50 WP (Carbendazim), Hexaconazole (RIL 01 WG) and Tilt 25 EC (Propiconazole) against sheath blight of rice was tested by Akter *et al.* (2001). Contaf 5 EC (Hexaconazole) was found to be the best in Propiconazole 25 EC.

Eight fungicides such as Carbendazim (0.1%), Thiophanate methyl (0.1%), Iprodione (0.3%), Triadimefon (0.1%), Chlorothalonil (0.3%), Carboxin (0.2%), Propiconazole (0.1 %) and Edifenphos (0.1 %) each at three concentrations 0.1, 0.2 and 0.3 per cent were evaluated against sheath blight of rice (Chahal *et al.* 2003). The inoculum grown on rice husk plus maize meal medium was broadcasted in the standing crop. After five days of inoculation, plants were sprayed with fungicides. They found that Propiconazole (0.1%) was the best fungicide.

In order to control sheath blight disease of rice Lore *et al.* (2005) tested different fungicides, both *in vitro* and *in vivo* against R. *solani*. It was observed that Propiconazole 25 EC (0.1%), Carbendazim 50 WP and Hexaconazole (0.1%) were effective against sheath blight and sheath rot of rice.

Windels *et al.* (2005) observed that early season application of Azoxystrobin to sugar beet crop was significantly effective to control root rot caused by *R. solani.*

In a trial conducted by Reddy *et al.* (2007) Luster 37.5 SE (Flusilazole + Carbendazim) was tested at three doses (240, 300 and 360 g a.i/ha) along with sole Flusilazole and Carbendazim against *R. solani* inciting sheath blight of rice. In addition to these fungicides Hexaconazole and Validamycin were used as standard checks. The trial was conducted in greenhouse as well as in field conditions. The result indicated that, a prophylactic spray of Luster at all the doses was superior to rest of the fungicides under greenhouse conditions. In the field trial, both the standard checks (Hexaconazole and Validamycin) were statistically at par with all the concentrations of Luster and all these significantly superior to sole Flusilazole and Carbendazim.

The effect of Azoxystrobin on *R. solani* (*in vitro*) and its efficacy against rice sheath blight, under field conditions was studied by Sundravadana *et al.* (2007). The results revealed that Azoxystrobin at 1, 2, and 4 ppm concentration completely inhibited mycelial growth of *R. solani.* Field study showed that foliar spray of Azoxystrobin at 125, 250, and 500 g/ha significantly suppressed the development of sheath blight and enhanced yield level can be controlled by spraying Azoxystrobin at a minimum dose of 125 g/ha.

Kandhari (2007) evaluated *in vivo* efficacy of Armure 30EC (Propiconazole + Difenconazole), RIL-FA 200SC (Kresoxim-methyl), Antracol 75WP (Propineb)-Dithiocarbamate, Baycor 25WP (Tridemfon)-Triazole, Contaf 5EC (Hexaconazole)-Triazole, Saaf 75WP (Carbendazim + Dithiocarbamate) and Result 25EC (Propiconazole) - Triazole at different concentration against sheath

blight of rice. All the fungicides showed inhibitory effect on disease severity at all concentrations. Armure 30EC (Propiconazole + Difenconazole) was the best among seven fungicides used.

Bhanu *et al.* (2007) reported that, Validamycin (antibiotic) 2.5 ml/L and Propiconazole 1ml/L were statistically at par is controlling sheath blight of rice in Andhra Pradesh.

Seven fungicides were evaluated in laboratory and field condition to test efficacy against *R. solani* (Swamy *et al.* 2009). The treatments consisted of two new formulations *viz.,* Filia 52.5 SE (Tricyclazole 400 g + Propiconazole 125 g) and Nativo75 WG (Trifloxystrobin 25% + Tebuconazole 50%) and three standard checks fungicides such as Hexaconzole (Contaf 5 EC), Validamycin (Rhizocin 3L) and Propiconazole (Tilt 25 EC) were used. Rice seedlings in tillering stage were artificially inoculated by inserting mycelial bits in sheath. Three sprays of each fungicide with desired concentration were given on 50^{th}, 65^{th} and 80^{th} days after transplanting. Among the new formulations, application of Filia 52.5 SE and Nativo 75 WG 0.4g was found equally effective in controlling the sheath blight. Among the ruling fungicides Contaf 5 EC was found very effective.

Bolton *et al.* (2010) tested different fungicides to manage *Rhizoctonia* root and crown rot of sugar beet. They found that triazole fungicides were more effective against the disease.

Propiconazole (0.1%) was the most effective fungicide against sheath blight of rice under laboratory and field conditions. (Kotamraju, 2010)

Hamada *et al.* (2011) evaluated the sensitivity of 89 *Rhizoctonia cerealis* isolates to different fungicides by using mycelial growth inhibition assays. The results showed that all *R. cerealis*

isolates tested were sensitive to Iprodione, Difenoconazole and Fludioxonil.

Hunjan *et al.* (2011) evaluated five fungicides (*in vitro* and *in vivo)* namely Trifloxystrobin + Tebuconazole (Nativo 75 WG), Tebuconazole (Folicur 25 EC), Propiconazole (Tilt 25 EC), Pencycuron (Monceren 250SC) and Thifluzamide (Spencer 24SC) against *R. solani* in rice at 0.04 and 0.1 per cent concentration. All the treatments significantly inhibited mycelial growth of the test pathogen as compared to control. Among the fungicides, maximum inhibition was observed in treatment with Trifloxystrobin + Tebuconazole (Nativo 75 WG) at 0.04 percent concentration both *in vitro* and *in vivo* condition. Thifluzamide was the least effective fungicide.

A combination fungicide comprising Azoxysrobin (0.125%) plus Difenconazole (0.1%) while comparing the efficacy a combination fungicide with a recommended fungicide Hexaconazole 5% EC against sheath blight of rice. Bhuvaneswari *et al.* (2012) found that both are equally effective against the disease.

Efficacy of new combination fungicide comprising Kresoxim methyl (40%) plus Hexaconazole (8%) at concentration of 0.1 per cent against sheath blight of rice was compared with that of sole fungicide such as Hexaconazole (0.2%) and Propiconazole (0.1%) under laboratory as well as field condition by Lore *et al.* (2012). The result of the experiment revealed that, all the fungicides were statistically at par in laboratory while in the field trial, the combination fungicides was superior to both the sole fungicide.

Azoxystrobin at five concentration (500, 250, 50, 5, 0.5, and 0 ppm mg a.i/ml) against *R. solani* was tested by LaMondia (2012).

Efficacy of Hexaconazole 5SC was tested against sheath blight of rice under field as well as glass house conditions. Hexaconazole at different doses viz., 500, 1000, 1500 and 2000 ml/ha effectively reduced the sheath blight incidence in all the three field trials, but Hexaconazole 2000 ml/ha was superior to rest of the treatments. (Johanson *et al.* 2013).

Five fungicides such as Thifluzamide 24% SC, RIL-068/F1 48 WG (Kresoxim methyl 40% + Hexaconazole 8% WG), Propiconazole 25% EC (Tilt), Tricyclazole 75% WP (Beam) and a new combination fungicide, Fluxapyroxad 62.5 g/l + Epoxiconazlle 62.5 g/l EC (Adexar w/v EC) were tested against *R. solani* (under field condition) by Prasanna Kumar and Veerabhadraswamy (2014). All the fungicides significantly reduced the disease severity.

Shahid *et al.* (2014) tested different fungicides against the *R. solani* and reported that Cordate, Precurecombi, Curon, Bendict, Nativo, Validamycin and Tilt were effective in restricting the growth and spread of the pathogen.

In vitro efficacy of eight sole fungicide and two combination fungicide was tested against *R.solani* (Begum *et al* 2014).Copper-oxy-chloride was the best effective fungicide against the pathogen.

2.6 *In vitro* evaluation of plant extract against the pathogen:

Eight botanicals each at three concentrations (2.5, 5.0 and 10.0%) were tested against *R. solani* causing banded leaf and sheath blight of maize (Meena *et al.* 2003). Bulb extract of *Allium sativum* was effective against the pathogen under laboratory as well as field conditions.

Dutta *et al.* (2004) reported that 10 per cent concentration of crude *Allium sativum* extract totally inhibited sclerotia production

and 20 per cent concentration inhibited mycelial growth of *R. solani* inciting sheath blight of rice.

The maximum inhibition of *R. solani* by using *Allium sativum* clove extract may be due to the presence of sulphur and allicin content. (Singh and Singh 2005).

Field application of neem formulations (300 ppm and 1500 ppm azadirachtin) @ 4.5 ml/L during afternoon hours was very effective in reducing sheath blight incidence (Biswas 2007).

Biswas *et al.* (2008) observed that plant extract of *Azadirachta indica* and *Allium sativum* reduced mycelial growth of *R. solani* by 70 per cent and 68.55 per cent respectively.

Amongst the different plant extracts such as *Allium cepa, Allium sativum, Aloe vera, Azadirachta indica, Brassica compesrtis, Datura stramonium, Lantana camara, Psidium guajava, Ocimum basilicum, Jasminum officinale, Tagetes erecta* tried against *R. solani. A. indica, A.sativum, Brassica compesrtis* and *T. erecta* were the most effective (Sehajpal *et al.* 2009)

Aye *et al.* (2011) tested *in vitro* potential of phyto-extracts against four pathogens of rice *viz. R. solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum*. Clove extract supressed cent per cent mycelial growth of all the pathogen under study Neem leaf extract inhibited the growth of *R. solani* by 87.5 per cent, *R. oryzae* by 92.5 per cent and *R. oryzae-sativae* by 80 per cent.

In an experiment conducted by Hadian (2012) five plants extracts such as *Azadirachta indica*, *Melia azadirach*, *Allium sativum*, *Curcuma longa* and *Caryophilium aromaticus* were tested against *R. solani.* Among them, *Allium sativum* and *Azadirachta*

indica were the most effective against *R. solani* followed by *Melia azadirach* and *Curcuma longa*.

Extracts of ten plants *Artemisia vulgaris*, *A. integefolia, Coix lacryma jobi*, *C. lachrymajobi, C. maxima, H.coronarium, Lantana camera*, *Michelia champaka*, *Passiflora foetida*, *Punica granatum*, and *Strobilanthes flaccidifolius* were tested against *R. solani* causing root rot of French bean (Mangang *et al.* 2012). The extract of *Coix lacryma jobi* was superior to rest of the treatments under controlled conditions. But *Lantana camera* was effective under field conditions.

Begum *et al.* (2014) evaluated ten plant extract, *viz., Allium sativum* at 3 per cent concentration and *Zingiber officinale, Vinca rosea, Azadirachta indica, Tagetes erecta, Ageratum conyzoides, Centella asiatica* and *Allium cepa* each at 20 per cent concentration for their efficacy against *R. solani* through Poisoned food technique. Among them *Allium sativum* extract at 3 per cent concentration showed maximum inhibition (100%), followed by extract of *Azadirachta indica* (77.8%), *Zingiber officinale* (70.0%), *Allium cepa* (42.2%).

Leaves of nine plants *viz., Lawsonia inermis* L. (Maruthani), *Ocimum tenuiflorum* L. (Tulsi), *Azadirachta indica* L. (Neem), *Morinda citrifolia* L. (Noni), *Vinca major* (Periwikle), *Gloriosa suberba* (Glory lilly), *Justicia adhatoda* (Adathoda), *Vitex nigundo* (Nochi) and *Madhuca longifolia* (Mahuva seed extract) and seven oilcakes mahuva, pungum, sesamum, groundnut, castor, neem and sunflower were tested against *R. solani* by Poisoned food technique (Sriraj *et al.* 2014). Among them *Madhuca longifolia* (Mahua) seed extract showed maximum inhibition (34.81%) at 20 per cent

concentration and it was found significantly superior to other extracts.

2.7 *In vitro* evaluation of bio control agents against the pathogen:

Das *et al.* (1996) reported that foliar sprays with *Trichoderma harzianum*, *Trichoderma viride* and *Aspergillus terreus* significantly reduced sheath blight severity.

The effect of *Trichoderma* spp. on the germination of *R. solani* sclerotia was determined by Mukherjee *et al.* (1999).

Laha and Venkataraman (2001) reported that fluorescent *Pseudomonas* spp. was most effective in reducing the disease severity either alone or in combination with one spray of Bavistin (0.1%).

The volatile substances released by *Trichoderma* spp. inhibit the growth of *Thanatephorus cucumeris* (Dubey and Patel 2001).

In an *in vitro* study conducted by Tang *et al.* (2002) it was observed that *Trichoderma* have a distinct antagonistic ability against *R. solani.*

Sharma *et al.* (2004) demonstrated that the foliar application of selected PGPR strains *viz. Pseudomonas fluorescens*, *P. putida* and *P. aeruginosa* and *Bacillus subtilis* and *B. megaterium* resulted in reduced sheath blight incidence in the field.

Kazempour *et al.* (2003) showed antagonistic effect of *T. harzianum, T. viride* and *Gliocladium virens* against *R. solani.*

The influence of different factors on the effectivity of fungal bio-agent to manage rice sheath blight in nursery was investigated by Khan *et al.* (2005). Soil treatment with *T. harzianum* was found more effective in reducing sheath blight. Application of *T. harzianum,* 7 days before inoculation of *R. solani* resulted in maximum reduction (78.98%) of the disease.

A study conducted by Singh *et al.* (2005) revealed maximum reduction in disease severity (56.6%) and infected tillers/hill (32.2%) was recorded in the treatment comprising (*Pfr* 1+ talc + C.M.C) formulation. The treatment not only reduced disease but also increase grain yield.

Singh *et al.* (2005) reported the effect of time of application of *Pseudomonas fluorescens* in suppressing sheath blight of rice. Foliar sprays of cultural suspension of the bacterial bio-agent 7 days before inoculation of the pathogen resulted in maximum reduction in sheath blight severity (59.6-64.4%).

Spore suspension of *Trichoderma harzianum* sprayed on the rice leaves of sheath blight significantly reduced the disease severity and incidence and was more effective than soil treatment or seedling root dip. (Tewari and Singh, 2005).

Spraying of talc based formulation of bioagents *viz., Trichoderma harzianum* and *Trichoderma virens* was found effective against sheath blight of rice. (Khan and Sinha, 2006).

Khan *et al.* (2007) screening of *Trichoderma* spp. against *R. solani* the causal agent of rice sheath blight. In present investigation they observed that the radial growth of different isolates of *Trichoderma* spp. showed that *T. harzianum* was the fastest growing fungus is followed by *T. viride.* After 144 hours of co-culture with *R.*

solani, *T. harzianum* and *T. viride* attained a growth of 90 mm and 89 mm respectively. Volatile compounds produced by *T. harzianum* resulted in maximum inhibition (59.7%) of mycelial growth of *R. solani* after 48 hours and also showed that the *T. harzianum* was most effective reduction in disease severity and incidence of rice sheath blight.

Biswas *et al.* (2008) observed that there was 42.7 per cent of reduction in the radial growth of *R. solani* when it was grown with by dual culture technique.

Soil treatment with *T. harzianum* was found more effective in reducing sheath blight as compared to that of *T. virens* (Johanson *et al.* 2008).

Akhtar *et al.* (2010) found that *T. harzianum* was the most effective bio-control agent as it caused significant suppression of growth and sclerotia formation of *R. solani*.

According to the *in vitro* experiment, several strains of *Trichoderma* spp. such as *T. harzianum*, *T. virens*, and *T. atroviride* showed excellent bio-control agent (Naeimi *et al.* 2010). They found that *T. harzianum* AS12-2 was the most effective strain in controlling rice sheath blight.

In an *in vitro* experiment conducted by Rani *et al.* (2011) two fungal bio-control agents (*Trichoderma harzianum* and *T. viride)* and four bacterial bio-control agents (*Pseudomonas fluorescens, Pseudomonad* spp, *Bacillus subtilis* and phylloplane bacterial isolate 1) were tested against sheath blight of rice. The per cent growth inhibition was maximum (67.02%) in case of *Trichoderma harzianum* followed by *T. viride.*

Seema *et al.* (2012) evaluated the efficacy of four fungal and one bacterial bio-agent *viz., Trichoderma viride*, *T. harzianum*, *Aspergillus niger*, *Penicillium* spp. and *Bacillus subtilis* evaluated under *in vitro* condition against *R. solani*. Maximum inhibition of mycelial growth of the pathogen (70%) was recorded by *T. viride* followed by *T. harzianum* (67%), *A. niger* (57%), *B. subtilis* (50%) and *Penicillium* spp. (44%).

In vitro screening of rhizosphere micro-flora, exotic and commercial formulations of fungal and bacterial antagonists against *R. solani* were studied by Babu *et al.* (2013). *Trichoderma viride* (Native Tv1), *T. harzianum* (DOR Th), *Pseudomonas fluorescens* (ANGRAU Pf1) and *Bacillus subtilis* (NBAII Bs) were found to be the best among all the isolates tested in inhibiting the growth of the pathogen.

Biswas *et al.* (2013) tested four isolates of *Trichoderma viride* (TV-01, TV-02, TV-03 and TV-04), one each of *T. harzianum* (TH-01) and *Pseudomonas fluorescens* (PF-01) against sheath blight. Only *Pseudomonas fluorescens*, when applied both in soil as a supplement along with FYM and on foliage as spray (5% solution of 2 ×109 cfu), was effective in minimizing the disease.

Bio-control agents either alone or in various combinations against were evaluated against *Rhizoctonia* seedling rot of Naga king chilli in green house as well as field conditions. Among all tested combination, the treatment containing combination of *T. viride* +*P. fluorescens* was found to be the most effective in reducing the incidence of seedling rot in both greenhouse and field conditions (Ngullie *et al.* 2013).

Begum *et al.* (2014) evaluated four bio-control agents (*T. harzianum, T. hamatum, T. viride* and *Penicillium glabrum*) by dual culture technique against *R. solani. T. hamatum* and *T. harzianum* completely over grew the pathogen. Mycelium and sclerotial formation was completely inhibited by all the four antagonists.

In order to know the antagonistic activity of bacterial bio-agents, four strains of *B. subtilis* and five strains of *P. fluorescens* were tested against *R. solani* by Mezeal (2014). *P. fluorescens* (strain5) exhibited 81.3 per cent growth inhibition, while *B. subtilis* 77.4 (strain1) per cent of growth inhibition of the test pathogens.

2.8 Study the infectivity of the pathogen in relation to crop age:

Castilla *et al.* (1996) reported that, leaf to leaf contacts represents an important epidemiological factor for sheath blight disease.

Lakepal *et al.* (1996) observed that the maximum tillering stage was more susceptible to the disease as compared to booting and milking stage.

Fabricio *et al.* (2003) reported that effect of growth stages and silicon accumulation on development of rice sheath blight. They observed that even though, the plants is all growth stages are more or less susceptible, they are highly susceptible 45 days after emergence. All growth stages were susceptible to infection but most susceptible stage was 45 days after emergence. The total number of sheath blight lesions was more on rice plants grown in pots without silicon amendment.

Lore *et al.* (2009) studied effect of different growth stages on the development of sheath blight in rice. Five different growth stages under study were, seedling stage, tillering stage, panicle initiation stage, booting stage, flowering stage and grain filling stage. Maximum disease incidence was observed at booting stage, indicating that the most susceptible stage of the host. Similar results were also reported by Singh *et al.* (2010).

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CHAPTER III
MATERIALS AND METHODS

The various aspects of present investigation on sheath blight disease of rice (*Oryza sativa* L.) incited by *Rhizoctonia solani* Kuhn. were undertaken in the Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, during 2013 to 2015.

The materials used and methods or techniques adopted during the course of present investigations are mentioned as below.

Materials:

The following materials were used during the course of the present studies.

Disease sample:

The infected plant samples showing typical symptoms of sheath blight of rice were collected in the paper bags from the rice crop grown in the Agricultural Research Station, Shirgaon (Ratnagiri), and brought to the laboratory for further studies.

Culture Media:

Common laboratory culture medium i.e Potato dextrose agar (PDA) medium was used for isolation of the causal organism from infected plant samples.

Chemicals:

The chemicals used for the studies were of analytical grade. The chemicals required for the investigation were obtained from Department of Plant Pathology, Dr. B. S. K. K. V., Dapoli and other reliable sources.

Glassware's:

Standard Borosil brand glassware was used during the course of research work.

Equipment's:

Laboratory equipment's *viz.* Autoclave, Laminar air flow bench, BOD incubator, Refrigerator, Research microscope, Centrifuge, Sintered glass filter, Hot air oven, etc. were used.

Fungicides:

The fungicides such as Mancozeb, Thiophanate methyl, Hexaconazole, Luster (Flusilazole plus Carbendazim), Difenconazole, Propiconazole, Azoxystrobin, and one antibiotic Validamycin were used during present studies. All the fungicides used for the investigations were from fresh stock.

Bio-control agents:

The fresh cultures of fungal bio-agents *viz. Trichoderma harzianum, T. viride, and Pseudomonas fluorescens* available with the Department of Plant Pathology, were used.

Plant extracts:

The extracts of garlic, neem, wild sage, guava, mustard, crown flower, soap nut and marigold were used during the studies.

Seed material:

Rice seeds used for pathogenicity test were obtained from the Agricultural Research Station, Shirgaon (Ratnagiri).

Miscellaneous materials:

Electronic weighing balance, cork borer, polythene bags, forceps, inoculation needles, spirit lamps, cotton, *etc.* were used during the course of investigation.

Methods:

3.1. Examination of disease samples:

3.1.1 Visual observation:

Visual observations on initial and final disease symptoms were recorded in the nursery to know the development of the disease in a plant population under natural conditions.

3.1.2 Microscopic examination:

Fresh diseased samples showing typical symptoms of sheath blight were collected and brought to the laboratory. These samples were then washed under tap water to remove extraneous material. Temporary mounts were prepared from the diseased specimens in lacto phenol cotton blue and examined under compound microscope for presence of microorganism if any.

3.2 Isolation of causal organism:

Fresh samples of diseased leaves, showing sheath blight symptoms were brought to the laboratory in paper bags. These samples were washed with running tap water to remove extraneous material. Small bits of desired size were cut by taking care that each bit contained half infected and half healthy portion. Such bits were then disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride. These bits were then placed on sterilized blotters for drying. Properly dried bits were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium. The plates were incubated in BOD incubator at 26 ± 1° C till the fungal mycelium fully covered the surface of the medium. This fungal growth was transferred to PDA slants and maintained as stock culture for further studies.

3.3 Proving the pathogenicity of isolated organism:

3.3.1 Inoculation:

Healthy rice seedlings collected from the Botany field were transplanted in earthen pots containing desired potting mixture. The transplanted seedlings with vigorous growth were selected for artificial inoculation. The young sheath of such seedlings were disinfected with 0.1 per cent mercuric chloride solution with the help of a cotton swab followed by washing with sterile water to remove traces of mercuric chloride and allowed to dry. The disinfected sheaths were injured by gently pressing with a sand paper (No. 40) so as to facilitate easy penetration by the test fungus. The young sclerotia formed in 4 days old culture on PDA were placed on injured sheath surface and immediately covered with moist cotton. After inoculation, artificially inoculated seedlings along with non-inoculated control were transferred to a humid chamber comprising a wooden frame covered with a muslin cloth. Proper humidity (85-90%) was maintained in the chamber by frequently spraying sufficient clean water on the muslin cloth. Seedlings were watered as and when required till the development of typical disease symptoms.

3.3.2 Development of symptoms:

Pots (both inoculated and non-inoculated) were incubated in the moist chamber. Prepared with a wooden frame covered with a muslin cloth. Proper humidity (85-90%) was maintained in the chamber by frequently spraying sufficient clean water on the muslin cloth. Seedlings were watered as and when required till the development of typical disease symptoms.

3.3.3 Re-isolation:

The causal organism was re-isolated from the artificially inoculated sheaths showing typical symptoms. The fungal growth obtained on PDA medium on re-isolation was compared with the original culture obtained from naturally infected sheaths under field condition.

3.3.4 Identification of the causal organism:

The re-isolated, pure fungal culture was identified by comparing its morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification. The culture was sent to Chief Mycologist, Agharkar Research Institute, Pune for identification of the fungus up to species level.

3.4 *In vitro* evaluation of fungicides against the pathogen:

Seven fungicides and one antibiotic belonging to different groups were tested against the pathogen by 'Poisoned Food Technique' (PFT) as described by Nene and Thapliyal (1997). The details of the fungicides used were mentioned below.

List of fungicides tested against causal organism:

Tr. No.	Treatments	Trade Name	Manufacturing company	Conc (%)
T 1	Mancozeb 75 % WP	Uthane M-45	United Phosphorus Ltd., Gujrat.	0.20 and 0.30
T 2	Thiophenate methyl 70 % WP	Maxim	Chemtura Chemicals India Pvt. Ltd., J.P. Nagar, (UP)	0.10 and 0.20

T 3	Hexaconazole 25 EC	Topper	Gharda Chemical Ltd. Mumbai-40	0.10 and 0.20
T 4	Propiconazole 25 % EC	Tilt	Syngenta India Ltd., Mumbai – 20	0.10 and 0.20
T 5	Difenconazole 25 % EC	Score	Syngenta India Ltd., Mumbai – 20	0.10 and 0.20
T 6	Flusilazole+ 12.5% Carbendazim 25% SE	Luster	Dhanuka Agrotech Pvt. Ltd.	0.05 and 0.10
T 7	Azoxystrobin 23% EC	Amistar	Syngenta India Ltd., Mumbai – 20	0.10 and 0.20
T 8	Validamycin 3% L	Validacin	Sumitomo Chemical India Pvt. Ltd., Mumbai	0.10 and 0.20
T 9	Control	-	-	-

Potato dextrose agar medium (PDA) was used as basal medium and distributed in 100 ml aliquots in each 250 ml Erlenmeyer conical flasks, which were sterilized at 1.054 kg/cm^2 pressure for 20 minutes. The quantity of every fungicides/ antibiotic for each concentration was calculated for 100 ml medium separately. The weighed quantity of each fungicide was added in lukewarm PDA at 40 ± 2 °C, mixed thoroughly and poured into sterilized Petri plates and allowed to solidify. The mycelial discs of 5 mm diameter were cut from 4 days old culture with the help of sterile cork borer. A single disc was transferred aseptically to the center of each plate already poured with poisoned medium. The plates with PDA without fungicide but inoculated with fungal culture, served as control.

The plates were incubated at room temperature (26 ± 1 °C). Three replication per treatments were maintained. The observations

on colony diameter were recorded when Petri plates in control treatment were fully covered with mycelial growth.

Per cent inhibition of growth of the test fungus was calculated by the following formula (Horsfall, 1956).

$$X = \frac{Y - Z}{Y} \times 100$$

Where,

X = Per cent inhibition

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm

3.5 *In vitro* evaluation of phyto-extracts against the pathogen:

3.5.1 Evaluation of phyto-extracts:

In all, 8 locally available plants with defined antifungal properties were used in the present study. The details as mentioned below.

Details of plant extracts tested against the pathogen:

Tr. No	Local name of the plant	Botanical name	Plant part used	Conc. (%)
1.	Neem	*Azadirachta indica*	Leaves	20
2.	Garlic	*Allium sativum*	Bulb	20
3.	Wild sage	*Lantana camara*	Leaves	20
4.	Guava	*Psidium guajava*	Leaves	20
5.	Mustard	*Brassica compestris*	Seeds	20
6.	Crown flower	*Calotropis gigantean*	Leaves	20
7.	Soap nut	*Sapendus tripholiarus*	Fruits	20
8.	Marigold	*Tagetes erecta*	Leaves	20
9.	Control	-	-	-

3.5.1.1 Preparation of phyto-extracts:

Aqueous phyto-extracts were obtained as per the method described by Bhatti (1988). A 100 gram sample of each plant was washed with distilled, sterile water. Then each sample was ground separately by using sterile pestle and mortal in 100 ml distilled sterile water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material is the filtrate. Then this extract filtered through a filter paper (Whatman No.1). The filtered extracts were centrifuged at 4000 rpm for 5 minutes to get homogenous aqueous solution. After centrifuging, the supernatant of each extract was collected. The extracts thus collected were passed separately through a Sintered glass filter to avoid bacterial contamination. This formed the standard plant extracts solution (100%).

3.5.1.2 Effect of phyto-extracts against the causal pathogen:

The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1997). All glassware used in the study was sterilized before their use. All the plant extracts were tested at 20 per cent concentration against the test pathogen using PDA as a basal medium. To obtain 20 per cent concentration of plant extracts, 80 ml of lukewarm PDA was mixed with 20 ml of standard plant extracts in 250 ml conical flask, separately, and then it was stirred well to obtain homogenized mixture. Twenty milliliter of such poisoned medium was then poured in each sterilized Petri plate and allowed to solidify. Mycelial discs of 5 mm diameter were cut from seven day old culture of test pathogen with the help of sterilized cork borer and transferred aseptically to the center of each Petri plate already poured with

poisoned medium. Medium devoid of plant extract served as control. The inoculated Petri plates were incubated as room temperature (27 ± 2 ºC) for further growth of the fungus.

Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test pathogen was calculated as described earlier under 3.4.

3.6 Evaluation of bio-agents:

In order to study the antagonism of the bio-agents against the test pathogen, two fungal bio-agents (*Trichoderma harzianum* and *T. viride*) and one bacterial bio-agent (*Pseudomonas fluorescens*) were used. The experiment was carried out by dual culture technique (Padder *et al.* 2010). Both the fungal bio-agents and the test pathogen were grown on PDA and the bacterial bio-agent was grown on nutrient agar medium. Seven days old culture of each organism was used.

A 5 mm culture disc of the test pathogen was placed in the center of solidified medium. In the same plate three culture discs of *T. harzianum* were cut in similar way and placed around the pathogen disc in such a way that all the three discs were at equal distance away from the pathogen. Appropriate space was provided for the growth of the pathogen. The procedure was repeated by using the culture of *T. viride.*

In case of the bacterial antagonist, a loopful of bacterial culture was streaked around the culture disc of the pathogen in the centre. The experiment was repeated by placing the culture of bio-agents is the center and three discs of the pathogen around the bio-agent.

Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when

Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test pathogen was calculated as described earlier under 3.4

Treatment wise placement details for bio-agent against *R. solani*

Tr. No.	**Placement details**
T1	*Th* *Rs* *Th* *Th*
T2	*Tv* *Rs* Tv Tv
T3	*Pf* *Rs* *Pf* *Pf*
T4	*Rs* *Th* *Rs* *Rs*
T5	*Rs* *Tv* *Rs* *Rs*
T6	*Rs* *Pf* *Rs* *Rs*
T7	Control

Where,

Rs = Rhizoctonia solani

Tv = Trichoderma viride

Th = T. harzianum

Pf = Pseudomonas fluorescens

3.7 Study the infectivity of the pathogen in relation to crop age:

The objective of this experiment was to determine the effect of rice seedling on susceptibility. The rice seedlings grown in pots were inoculated with sclerotia of *R. solani* at seedling stage, four leaf stage (45 DAS), eight leaf stage (65 DAS), tillering stage (85 DAS), booting stage (117 DAS) and panicle excertion stage (130 DAS). (Fabricio *et al.* 2003).

3.7.1 Inoculation of plants:

The young sheath of growing seedlings in pots were disinfected with 0.1 per cent mercuric chloride solution with the help of a cotton swab followed by washing with sterile water to remove traces of mercuric chloride and allowed to dry. The disinfected sheaths were injured by gently pressing with a sand paper (No. 40) so as to facilitate easy penetration by the test fungus. The young sclerotia formed in 4 days old culture on PDA were placed on injured sheath surface and immediately covered with moist cotton. After inoculation, artificially inoculated seedlings along with non-inoculated control were transferred to a humid chamber comprising a wooden frame covered with a muslin cloth. Proper humidity (85-90%) was maintained in the chamber by frequently spraying sufficient clean water on the muslin cloth. Seedlings were watered as and when required till the development of typical disease symptoms.

3.8 Statistical analysis:

The data obtained in all the experiments were statistically analyzed using methods suggested by Gomez and Gomez (1986). Completely Randomized Design (CRD) was used for radial growth, poisoned food technique and dual cultural technique. The standard

error (S.Em.) and critical difference (C.D.) at level P = 0.01 were worked out and results obtained were compared statistically.

CHAPTER IV
EXPERIMENTAL RESULTS

Sheath blight of rice, caused by a soil borne pathogen *Rhizoctonia solani* is a disease of worldwide occurrence. At present it is confined to limited pockets in the Konkan Region. The climatic conditions of the region are conducive for perpetuation and spread of fungal diseases of many crops. Under such circumstances, in near future, the disease may pose a major threat to the rice cultivation in the region.

In lieu of this, the present study was taken up to initiate the work on isolation, pathogenicity and evaluation of fungicides, botanicals and bio-control agents against the pathogen under *in vitro* conditions. The results of the experiments conducted on these lines are presented in this chapter.

4.1 Examination of diseased samples:

4.1.1 Visual observation:

The sheath blight incidence was observed in the field of Agricultural Research Station, Shirgaon (Ratnagiri), during third week of September 2014. It was noticed that the disease occurred when the prevailing temperature was within range of 24 to 29 ºC, relative humidity about 81 to 88 per cent and the average rainfall around 10.80 mm per day. The disease appeared in the form of lesions on sheaths of lower leaves near the water line when plants were in the late tillering or early internode elongation stage. The lesion enlarged to form irregular, water soaked, light brown-grey patches on the sheath. On close examination of the infected sheaths white mycelial growth of fungus with scattered development of brownish sclerotial bodies were observed. Infected sheaths became

flaccid, and remained drooping on the seedling. In later phase of the disease, the infected seedlings dried and died. (PLATE I).

4.1.2 Microscopic examination:

The bit of infected sheaths were placed in lacto-phenol cotton blue and observed under compound microscope. Microscopic examination revealed the presence of fungal mycelium in diseased tissues. The hyphae were distinctly branched at right angle with a conspicuous constriction at the point of branching (PLATE II).

4.2 Isolation of causal organism:

The fungus associated with infected sheath was isolated on potato dextrose agar medium in the laboratory. The visible mycelial growth developed around the artificially inoculated mycelial bits within 2-3 days of inoculation. Initially, the fungal mycelium formed, white, sparse mat on the surface of the medium. Fungal colony on PDA grew up to the rim of petri plate (9 cm diameter) in 4 days at room temperature (27 ± 1° C). Later on the, tips of hyphae entwined to from white, young sclerotia. From the fourth day of incubation, formation of watery droplets, on and around the young sclerotia was observed. Within 24-30 hours these droplets were transferred into, white encrustation which later on merged with sclerotia, consequently increasing the size of sclerotia. As the mycelium grew old, its colour changed to buff brown. At this stage, instead of watery droplets, honey coloured droplets began to appear on and around the young sclerotia. Within a week, many, brown to dark brown, irregular, hard sclerotia were formed on the mycelial mat (PLATE III). The pure culture of the test fungus, obtained by transferring mycelial bits was maintained by periodic transfer after every 20 days on PDA slants. The slants were stored in the refrigerator and this culture was used as stock culture for further studies.

4.3 Proving of pathogenicity of isolated organisms:

4.3.1 Inoculation:

Leaf sheaths of young rice seedlings (25 day old) were slightly injured with sand paper and young sclerotial bodies of the test fungus were placed on the injured surface. The sclerotial bodies were then covered with moist cotton. About 85 -90 per cent humidity was maintained in humid chamber. Typical symptoms of the disease were observed 10-12 days after inoculation (PLATE IV).

4.3.2 Development of disease:

Symptom development on artificially inoculated leaf sheaths of young rice seedlings commenced on 5th day after inoculation. Initially greenish grey to light brown, water soaked, ellipsoid to oval lesions with irregular brown margin, appeared on the leaf sheaths. Within 10-12 days after inoculation, the lesions enlarged gradually covering 65 -80 per cent area of the leaf sheaths. On an average, the lesions were 1.5-3 cm in length. Dark brown sclerotia appeared on the leaf sheaths within 17-18 days of inoculation. These symptoms resembled to those observed on naturally infected sheaths. Non-inoculated seedlings remained healthy (PLATE V).

4.3.3 Re-isolation:

The fungus was re-isolated from artificially inoculated leaf sheaths, on PDA and the growth of this isolate was compared with original culture.

4.3.4 Identification of the causal organism:

The symptoms produced on artificially inoculated seedlings were identical to the symptoms produced on naturally infected plants. Microscopic observation as well as growth and colony characters of the re-isolated fungus were similar to those of original culture. On the basis of these observations the pathogen was

identified as *Rhizoctonia* spp. This confirmed the pathogenicity of *Rhizoctonia* spp. The Chief Mycologist Agharkar Research Institute, Pune, identified the fungus as *Rhizoctonia solani* Kuhn.

4.4 *In vitro* efficacy of different fungicides against the pathogen:

The data obtained on the efficacy of different fungicides and antibiotic at different concentration on the vegetative growth of *Rhizoctonia solani* are presented in TABLE 1, Figure 1and 2 (PLATE VI, VII)

Table 1: *In vitro* efficacy of fungicides on radial and sclerotial inhibition of *Rhizoctonia solani*.

Sr. No.	Common name	Conc. (%)	Mean colony diameter (mm)	Per cent inhibition
T1	Mancozeb 75% WP	0.20	00.00	100.00
		0.30	00.00	100.00
T2	Thiophanate methyl 70% WP	0.10	18.66	79.25
		0.20	00.00	100.00
T3	Hexaconazole 25% EC	0.10	00.00	100.00
		0.20	00.00	100.00
T4	Propiconazole 25% EC	0.10	00.00	100.00
		0.20	00.00	100.00
T5	Difenconazole 25% EC	0.10	00.00	100.00
		0.20	00.00	100.00
T6	Flusilazole 12.5% + Carbendazim 25% SE	0.05	00.00	100.00
		0.10	00.00	100.00
T7	Azoxystrobin 23% EC	0.10	00.00	100.00
		0.20	00.00	100.00
T8	Validamycin 3% L	0.10	84.46	6.14
		0.20	64.83	27.96

T9	Control	-	90.00	-

It is revealed from the data presented in Table-1 that, all the fungicides except Thiophanate methyl completely inhibited the mycelial growth of *R. solani* at both the concentrations. Thiophanate methyl was less effective at lower concentration, but at higher concentration it could completely inhibit the mycelial growth of the pathogen like other fungicides. The combination fungicide (Flusilazole 12.5% + Carbendazim 25% SE) was effective at the lower concentration (0.05%). Validamycin the only antibiotic used in the experiment was ineffective at lower concentration and slightly effective at higher concentration.

4.5 *In vitro* evaluation of phyto-extracts against *R. solani:*

The effect of plant extracts of eight plant species was studied against *R. solani* to test their antifungal properties. All the plant extracts were tested at 20 per cent concentration by poisoned food technique. All the plant extracts under study exhibited antifungal properties against *R. solani.* The data obtained on the effect of different plant extracts on mycelial growth of the pathogen are presented in Table 2, Figure 3 (PLATE VIII).

Table 2: *In vitro* effect of plant extracts on growth of *R. solani*:

Tr. No	Treatments	Conc. (%)	Mean colony dia. (mm)	Per cent inhibition
T1	Neem (*Azadirachta indica*)	20	33.40	62.88
T2	Garlic (*Allium sativum*)	20	16.26	81.70
T3	Wild sage (*Lantana camara*)	20	46.13	48.74
T4	Guava (*Psidium guajava*)	20	57.96	35.59
T5	Mustard (*Brassica compestris*)	20	17.66	80.36
T6	Crown flower (*Calotropis gigantea*)	20	84.56	6.03
T7	Soap nut (*Sapendus tripholiarus*)	20	22.26	75.25
T8	Marigold (*Tagetes erecta*)	20	21.36	76.25
T9	Control	-	-	-
	S.Em. ±		**1.15**	
	C.D. at 1%		**4.66**	

It is revealed from the data presented in Table-2 that the maximum inhibition of the fungal mycelium (81.70%) was observed in garlic bulb extract which was significantly superior to rest of the treatments. It was followed by mustard (80.36%), marigold (76.25%), soap nut (75.25%), neem (62.88%), wild sage (48.74%), guava (35.59%) and crown flower (6.03%) respectively. Among the eight extracts used, garlic, mustard, marigold, soap nut and neem were more effective as the inhibition of fungal mycelium in these extracts was more than 50 per cent. Though the garlic extract was numerically superior to mustard, the inhibition percentage in both these extracts was statistically at par and also marigold and soap

nut extract was statistically at par. The extracts of remaining three plants i.e. wild sage, guava and crown flower were less effective as the mycelium inhibition in them was less than 50 per cent. Crown flower extract was the least effective treatment as it recorded only 6.03 per cent inhibition of the fungal mycelium.

4.6 Evaluation of bio-agents:

Dual culture technique was employed for testing the efficacy of bio agents against *Rhizoctonia solani*. The data obtained on the effect of bio agents on mycelial growth of *R. solani* are presented in Table 3, Figure 4 (PLATE IX).

Table 3: *In vitro* effect of bio-agents on growth of *R. solani*:

Tr. No.	Placement details	Mean colony diameter (mm)	Per cent inhibition
T1	Th *Rs* *Th* *Th*	15.18	83.13
T2	Tv *Rs* *Tv* *Tv*	10.79	88.00
T3	Pf *Rs* *Pf* *Pf*	14.33	84.07
T4	Rs *Th* ***Rs*** ***Rs***	17.66	80.36
T5	Rs *Tv* *Rs* *Rs*	18.68	79.23
T6	Rs *Pf* *Rs* *Rs*	19.11	78.76
T7	*Control*	-	-

	S.Em	**0.13**	
	C.D. at 1%	**0.57**	

Where,

Rs = Rhizoctonia solani

Tv = Trichoderma viride

Th = T. harzianum

Pf = Pseudomonas fluorescens

The data presented in Table 3 indicate that all the bio-agents were effective against *R. solani*. When the pathogen was placed at the centre; *T. viride* (T_2) recorded maximum inhibition (88.00%). It was followed by *P. fluorescens* (T_3), and *T. harzianum* (T_1) respectively. In the other method where in the bio-agent was placed at the centre, the colony diameter of the pathogen was slightly more. In order of merit, T_2 was the best treatment followed by T_3, T_1, T_4, T_5 and T_6 respectively.

4.7 Infectivity of the pathogen in relation to crop age:

The pure culture of the pathogen was inoculated in six different growth stages of rice crop such as seedling stage, four leaf stage, eight leaf stages, tillering stage, booting stage and panicle excertion stage. The data obtained on the infectivity of the pathogen at different stages of crop growth are presented in Table 4.

Table 4: Infectivity of pathogen on different crop age

Sr. No.	Crop Stage	PDI
1.	Seedling	12.60
2.	Four leaf	23.34
3.	Eight leaf	42.78
4.	Tillering	70.56
5.	Booting	68.43
6.	Panicle	65.89

Typical symptoms of sheath blight appeared on the seedlings of eight leaf stage, booting stage, tillering stage and panicle excertion stage. Initially typical brown lesions were formed within 10 days of artificial inoculation. The symptoms were identical to those observed under natural conditions in the field. In case of seedling and four leaf stages, there was browning of the inoculated area but the lesions did not extend further (PLATE X, XI).

The results of this experiment indicate that the seedlings in tillering stage are more susceptible to the infection of *R. solani*.

CHAPTER V

DISCUSSION

Rice is one of the major cereal crops of the world. Sheath blight of rice caused by *R. solani* is a well established disease in many rice growing regions. Climatic conditions of Konkan region are suitable for cultivation of rice and therefore, it is cultivated throughout the region during rainy season. In Raigad district where irrigation facilities are available, it is also cultivated in summer season. Severe incidence of sheath blight of rice was observed on the rice seedlings at Agricultural Research Station, Shirgaon (Ratnagiri) in the third week of September 2014. Considering the importance of this crop and the emerging incidence of the disease, it was thought essential to undertake the systematic studies on some aspects like isolation, pathogenicity and *in vitro* control measures against the pathogen.

The infected leaf sheaths of the rice were collected from the field and brought to the laboratory for further studies. The pathogenic fungus was isolated from infected sheath tissues and was successfully cultured on PDA in the laboratory. Peterson *et al.* (2004), Sivalingam *et al.* (2006), Sriraj *et al.* (2014) and Waghrulkar (2014) reported that PDA favours both mycilial growth and sclerotia formation of *R. solani*. Results of the present study confirmed the findings of earlier workers.

In order to prove the pathogenicity of *R. solani* some workers had used the sclerotia while some used bits of mycelial mat for inoculation. Singh *et al.* (2002) and Park *et al.* (2008) proved the pathogenicity of *R. solani* on rice by placing a sclerotium in the leaf sheath and the inoculated sheath was immediately covered with an aluminum foil. Chakraborty *et al.* (2006) assessed three inoculation

methods such as single grain insertion, single sclerotium insertion and injecting mycelial suspension to confirm the pathogenicity of *R. solani*. They found that among these three, single sclerotium insertion was the most effective. Akhtar *et al.* (2009) successfully proved the pathogenicity of *R. solani* on rice by insertion of an infected grain in the leaf sheath. Insertion of young sclerotia on injured leaves of cardamom leads to early development of symptoms of cardamom leaf blight incited by *R. solani* (Waghrulkar, 2014). However, all these workers emphasized that; the inoculated seedlings should be necessarily transferred to a humid chamber with 80-100 per cent humidity to facilitate easy penetration of the pathogen. In the present study, young sclerotia formed in 4 days old culture on PDA were placed in injured leaf sheaths and immediately covered with moist cotton which was then wrapped with aluminum foil. The artificially inoculated seedlings were transferred to the humid chamber. Typical disease symptoms appeared within 10-12 days of inoculation. This confirmed that the inoculation method suggested by earlier workers and used in the present study was accurate and appropriate. These observations indicate that the sclerotia of *R. solani* germinate to form new mycelium under highly humid conditions.

The symptoms produced on the inoculated plants were identical to those observed in the field. Initially the irregular, water soaked, light brown-grey lesion developed on the inoculated sheaths. The infection progressed further covering maximum sheath area which eventually resulted in withering and drying of the seedlings. Within 2-3 days, developing sclerotial bodies were observed on the infected sheaths.

During present investigation, six sole fungicides, one combination fungicide and one antibiotic were evaluated to test their efficacy against *R. solani*. The results of this *in vitro* experiment revealed that Mancozeb (0.2 and 0.3%), Thiophenate methyl (0.2%), Propiconazole (0.1 and 0.2%), Hexaconazole (0.1 and 0.2%), Difenconazole (0.1 and 0.2%), Azoxystrobin (0.1 and 0.2%) and Flusilazole plus Carbendazim (0.05 and 0.1%) completely inhibited the mycilial of the pathogen. Obviously, sclerotia did not develop in these treatments. Thiophenate methyl did support sparse development of the mycelium at lower concentration (0.1%) but at higher concentration it was equally effective like other fungicides. The only antibiotic - Validamycin was ineffective in inhibiting growth of mycelium both at lower as well as higher concentration.

Lore *et al.* (2005) observed that Propiconazole (0.1%), Carbendazim, Hexaconazole (0.1%) and Validamycin (0.25%) were effective against sheath blight and sheath rot of rice, both *in vitro* and *in vivo*. The results of present study are in confirmation with them as far as trizole are concerned, but not in case of Validamycin. This may be due to the fact that they had used higher concentration of the antibiotic. The efficacy of trizole group fungicides against *R. solani* may be attributed to the activity of these fungicides in inhibiting sterol synthesis in fungi. Ergosterol- the major fungal sterol is involved in maintaining the integrity of fungal cell membrane. The trizole fungicides reduce ergosterol synthesis which results in membrane disruption and leakage of electrolytes in fungal cells.

In respect of Flusilazole plus Carbendazim, the results are in concurrence with those reported by Reddy *et al.* (2007), who demonstrated that sheath blight of rice can be effectively controlled with Flusilazole plus Carbendazim.

Swamy *et al.* (2009) studied *in vitro* and *in vivo* control of *R. solani*, the cause of sheath blight of rice and reported that Contaf 5 EC was very effective fungicide. The results of present study also confirmed that Hexaconazole (Contaf) is effective in inhibiting mycelial growth of the pathogen under study.

In a field trail conducted by Bhuvaneshwari and Krishnam Raju (2012) it was found that Difenconazole (0.5%) was inferior to other fungicides in controlling sheath blight of rice. This is contradictory with the findings of present study wherein this fungicide was effective against *R. solani*.

The results of present study are in conformity with those of Prasannakumar and Veerbhadraswamy (2014) who reported that Propiconazole is effective against *R. solani* under field conditions and Begum *et al.* (2014) who reported that Propiconazole and Hexaconazole completely inhibited mycelial growth of the pathogen *in vitro*.

The members of dithiocarbamate group of fungicides alter the permeability of the fungal cells and also inactivate the fungal enzymes. The fungicides of this group are strong chelating agents. The metals in chelate complex easily permeate the cell wall of fungal pathogens and this leads to transport and accumulation of heavy metals in the cells which subsequently inhibit the enzyme activity of the cells. Therefore, the performance of Mancozeb was excellent against the pathogen.

During present investigation, eight plant extract were used to test their antifungal properties against *R. solani* by poisoned food technique. Among all the plant extracts tried, garlic bulb extract (20%) was significantly superior (81.70% inhibition), in comparison to rest of the treatments. It was followed by mustard (80.36%),

marigold (76.25%), soap nut (75.25%), neem (62.88%), and wild sage (48.74%).

These results are in agreement with the findings of many earlier workers.

Dutta *et al.,* 2004, found that, *Allium sativum* extract (20 % concentration) totally inhibited mycelial growth of *R. solani* inciting sheath blight of rice. The fungicidal effect of garlic bulb extract may be due to presence of sulphur and allicin content in the extract (Singh and Singh 2005).

Similar results were also reported by Biswas *et al.* (2008) in respect of neem and garlic extract against the same pathogen. The Sehajpal *et al.* (2009) also reported that the extracts of, garlic, mustard, neem and marigold were the most effective against *R. solani*.

Aye *et al.* (2011) evaluated sixteen plant extracts *in vitro* against four pathogens of rice such as *R. solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum* by poisoned food technique. Among the plant extracts, maximum inhibition was observed in Neem leaf extract treatment.

Among the five plant extracts used by Hadian (2012), neem *and* garlic were the most effective plant extracts against *R. solani*. Begum *et al.* (2014) also revealed that garlic was effective against *R. solani*.

The results of present study are in agreement with those of Dutta *et al.,* 2004, Sehajpal *et al.* (2009), Hadian (2012) and Begum *et al.* (2014).

In the experiment on *in vitro* evaluation of bio-agents it was observed that all the three bio-agents were promising antagonists of

R. solani. They inhibited mycelial growth of the pathogen and therefore sclerotia could not be formed.

T. harzianum resulted in maximum inhibition (70.1%) of mycelial growth of *R. solani*. The culture filtrate of the bio-agent was also effective under glasshouse conditions (Khan *et al.*, 2007).

Begum *et al.* (2014) reported that mycelium and sclerotia formation of *R. solani* was completely inhibited by *T. harzianum, T. hamatum* and *T. viride* by dual culture technique.

Mezeal (2014) observed maximum mycelial inhibition (81.30%) of *R. solani* by *P. fluorescens*.

The findings of present study are in consensus with the reports of earlier workers.

Trichoderma species not only produce a large variety of volatile secondary metabolites such as ethylene and hydrogen cyanide, which are detrimental to the fungal pathogens but, they also utilize the protoplasmic contents of the pathogenic organisms as a source of food(Khan et al., 2007).

Enzymes secreted by *P. fluorescens*, destroy the cell wall of the fungal pathogens and annihilate them.

It was evident from the experiment conducted to assess the most susceptible stage of the rice seedling to the infection of *R. solani* that, tillering stage is the most susceptible stage for penetration and colonization of *R. solani*. Similar results were recorded by Lakepal *et al.* (1996) who also reported that, maximum tillering stage was more susceptible to disease as compared to booting and milking stage.

The results are similar to those of Lore *et al.* (2009) and Singh *et al.* (2010) who demonstrated that incidence of sheath blight was

higher when plants were inoculated at booting stage. But they are contradictory to the findings Fabracio *et al.* (2003) who reported that 45 days old seedlings (4 leaf stage) are more susceptible to the infection.

Disease proneness of a variety depends upon canopy microclimate, canopy structure and tissue wetness (Castilla *et al.,* 1996). Infection and disease intensification are influenced by physiology of the plant. Researchers in different regions use different cultivars for experimentation. This divergence might have reflected in the results reported by different workers regarding susceptibility and age of the crop.

CHAPTER VI

SUMMARY AND CONCLUSION

The sheath blight disease of rice caused by *Rhizoctonia solani* was noticed in severe form at farm of Agricultural Research Station, Shirgaon during second week of September in year 2014.

The pathogenic fungus was isolated on potato dextrose agar medium from infected sheath of rice. The pathogen was identified as *Rhizoctonia solani* on the basis of microscopic observations, cultural characteristics and symptoms observed in field as well as produced on artificially inoculated seedlings. The pathogenicity of the fungus was proved by Koch's postulates.

The disease appeared initially as irregular water soaked light brown-grey lesions on sheaths. Later, these lesions increased in size and formed large patches. When the infected sheaths were closely examined, the whitish mycelial growth of fungus with scattered sclerotial bodies was noticed. In due course of time, the infected sheaths became flaccid, dried and remained drooping on the seedlings. Mortality of severely infected seedlings was observed in the field.

Efficacy of fungicides was assessed by poisoned food technique. In this it was revealed that, except Validamycin (0.05 and 0.1 per cent) all the fungicides gave excellent inhibition (100 per cent) of *Rhizoctonia solani* at both higher and lower concentration.

All the plant extracts tested against the pathogen had inhibitory effect on mycelial growth of the pathogen. Maximum inhibition of mycelial growth and sclerotia was recorded in garlic bulb extract of followed mustard, marigold, soap nut and neem. Leaf extracts of wild sage and guava were moderately effective while that of crown flower was the least effective.

In the *in vitro* experiment on evaluation of bio-agents against the pathogen, *T. viride* was the best performer followed by *P. fluorescens* and *T. harzianum*.

It was realized from results of the experiment conducted to know the most vulnerable growth stage of the crop, that, the plants in tillering stage are severely attacked by the pathogen.

On the basis of the results of present study it can be concluded that sheath blight of rice caused by *R. solani* is an emerging threat to the rice cultivation in Konkan region of Maharashtra. Trizole group fungicides are very effective against this soil borne pathogen. Plant extracts of garlic, mustard, marigold and soap nut and bio-agents such as *T. viride, T. harzianum*, *and P. fluorescens* and are useful in restricting mycelial growth of the pathogen. Tillering stage was the most susceptible stage for infection of the pathogen.

These results need to be confirmed under field conditions to recommend appropriate strategy for management of the disease.

APPENDIX - I

ABBREVIATION'S USED

%	Per cent
/	Per
@	At the rate
^{0}C	Degree celsius
BOD	Biological Oxygen Demand
C.D.	Critical Difference
cm	Centimeter
Co.	Company
Conc.	Concentration
d.f.	Degree of freedom
Dist.	District
Dr.B.S.K.K.V.	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
E.M.S.	Error mean sum of square
et al.	And others
etc.	Etcetera
Fig.	Figure
FYM	Farm yard manure
g	Gram
ha	Hectare
i.e.	That is
Kg	Kilogram
lbs	Pounds
m	Meter
M.S.S.	Mean sum of square
mg	Milligram
Min	Minutes
P.D.A.	Potato Dextrose Agar
Pf	*Pseudomonas fluorescens*
P.F.T.	Poisoned Foot Technique
Psi	Per square inch
Pvt.	Private
Rs	*Rhizoctonia solani*
S.E.	Standard error
Sig.	Significant
Syn.	Synonymous
Th	*Trichoderma harzianum*
Tv	*Trichoderma viride*
viz.	Namely
w/w	Weight / weight
WP	Wettable Powder

APPENDIX – II

Analysis of variance (Phyto-extract x ***Rhizoctonia solani*)**

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F-cal	F-tab	Result
Treatment	8	19548.95	2443.619	620.3245	3.28840	Sig.
Error	18	70.90667	3.939259			
Total	26	19619.86				
S.Em.±	**1.25**					
C.D at 1 %	**4.66**					

APPENDIX –III

Analysis of variance (Bio-agents x ***Rhizoctonia solani*)**

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F-cal	F tab	Result
Treatment	6	14246.88	2374.481	42794.45	3.8742	Sig.
Error	14	0.7768	0.055486			
Total	20	14247.66				
S.Em.±	**0.13**					
C.D at 1 %	**0.57**					

APPENDIX –IV

LABORATORY MEDIA USED

Potato Dextrose Agar (PDA) medium

i) Peeled potato : 200.00 g
ii) Dextrose : 20.00 g
iii) Agar-agar : 20.00 g
iv) Distilled water : 1000 ml

LEGENDS

(For Fig. 1)

***In vitro* evaluation of fungicides at lower concentrations against *Rhizoctonia solani* Kuhn.**

Tr. No.	Treatments	Conc. (%)
T_1	Mancozeb 75 % WP	0.2
T_2	Thiophanate methyl 70 % WP	0.1
T_3	Hexaconazole 25% EC	0.1
T_4	Propiconazole 25 % EC	0.1
T_5	Difenconazole 25 % EC	0.1
T_6	Flusilazole 12.5 % + Carbendazim 25 % SE	0.05
T_7	Azoxystrobin 23% EC	0.1
T_8	Validamycin 3 % L	0.1
T_9	Control	-

LEGENDS

(For Fig. 2)

***In vitro* evaluation of fungicides at higher concentrations against *Rhizoctonia solani* Kuhn.**

Tr. No.	Treatments	Conc. (%)
T_1	Mancozeb 75 % WP	0.3
T_2	Thiophanate methyl 70 % WP	0.2
T_3	Hexaconazole 25% EC	0.2
T_4	Propiconazole 25 % EC	0.2
T_5	Difenconazole 25 % EC	0.2
T_6	Flusilazole 12.5 % + Carbendazim 25 % SE	0.1
T_7	Azoxystrobin 23% EC	0.2
T_8	Validamycin3 % L	0.2
T_9	Control	-

LEGENDS

(For Fig. 3)

***In vitro* evaluation of plant extracts against *Rhizoctonia solani* Kuhn.**

Tr. No	Local name of the plant	Plant part used	Conc. (%)
1.	Neem	Leaves	20
2.	Garlic	Bulb	20
3.	Wild sage	Leaves	20
4.	Guava	Leaves	20
5.	Mustard	Seeds	20
6.	Crown flower	Leaves	20
7.	Soap nut	Fruits	20
8.	Marigold	Leaves	20
9.	Control	-	-

LEGENDS

(For Fig. 4)

In vitro evaluation of bio-agent against *R. solani* Kuhn.

Tr. No.	Placement details
T1	Th *Rs* *Th* *Th*
T2	Tv *Rs* *Tv* *Tv*
T3	Pf *Rs* *Pf* *Pf*
T4	Rs *Th* *Rs* *Rs*
T5	Rs *Tv* *Rs* *Rs*
T6	Rs *Pf* *Rs* *Rs*
T7	Control

Where,

Rs = Rhizoctonia solani *Pf = Pseudomonas fluorescens*

Tv = Trichoderma viride *Th= Trichoderma harzianum*

LITERATURE CITED

Anonymous, (2012a). htttps://www.eands.dacnet.nic.in.

Anonymous, (2012b). Report of the review committee meeting on weed management held on 28th and 29th Feb. Dr. B.S.K.K.V Dapoli. pp. 15-16.

Anonymous, (2013). All India Coordinated Research Project on Weed Control, Dr. B.S.K.K.V., Dapoli Centre. Seventeenth Annual Progress Report Jan to Dec. 2013 pp. 76-87.

Akter, S.; Mian, M. S. and Mia, M. A. T. 2001. Chemical control of sheath blight disease (*Rhizoctonia solani*) of rice. *Bangladesh J. Pl. Pathol.* **17**: 35-38.

Akhtar, J.; Jha, V. K. and Lal, H. C. 2009. Occurrence of banded leaf and sheath blight of maize in Jharkhand with reference to diversity in *Rhizoctonia solani. Asian Journal of Agricultural Sciences.* **1**(2): 32-35.

Akhtar, J.; Kumar, V.; Tiu, K. R. and Lal, H. C. 2010. Integrated disease management of banded leaf and sheath blight of maize. *Pl. Dis. Res.* **25**: 35-38.

Aye, S. S. and Matsumoto, M. 2011. Effect of some plant extracts on *Rhizoctonia* spp. and *Sclerotium hydrophilum. Journal of Medicinal Plants Research.* 5(16): 3751-3757.

Babu, T. K.; Kumar, C. P. C.; Reddy, D. R. R. and Singh, T. V. K. 2013. Biological Control of Root Rot of China Aster. *Ind. J. of Pl. Prot.* **41**(1): 50-60.

Begum, S.; Devi, R. K. T. and Singh, N. I. 2014. Management of damping-off of vegetable seedlings caused by *Rhizoctonia solani. Ind. J. of Pl. Prot.* **42**(3): 248-254.

Bhanu, V.; Rao, N. S. and Reddy, P. S. 2007. Compatibility of Certain Promosing Pesticides Against Planthoppers and Sheath blight in Rice. *Ind. J. of Pl. Prot.* **35**(2): 279-282.

Bhatti, B. S. 1988. Utilization of toxic plants for the control of nematode pest of economic crop. Final Technical Report April 1, 1983 to March 31, 1988, HAU Hissar, India, pp.56.

Bhuvaneswari, V. and Krishnam Raju, S. 2012. Efficacy of new combination fungicide against rice sheath blight caused by *Rhizoctonia solani* (Kuhn). *Journal of Rice Research.* Vol. **5** No.1 & 2.

Biswas, A. 2007. Evaluation of Neem Formulations Against Sheath Blight Disease of Rice. *Ind. J. of Pl. Prot.* **35**(2): 296-298.

Biswas, S. and Datta, M. 2013. Evaluation of biological control agents against sheath blight of rice in Tripura. *Indian Phytopath.* **66**(1): 77-80.

Biswas, S. K.; Ratan, V.; Srivastava, S. S. L. and Singh, R. 2008. Influence of seed treatment with biocides and foliar spray with fungicides for management of brown leaf spot and sheath blight of paddy. *Ind. Phytopath.* **61**(1): 55-59.

Bolton, M. D.; Panella, L.; Campbell, L. and Khan, M. F. R. 2010. Temperature, Moisture, and Fungicide Effects in Managing *Rhizoctonia* Root and Crown Rot of Sugar Beet. *Phytopath.* **100**(7): 689-697.

Botha, A.; Denman, S. and Mazzola, M. 2003. Characterization and pathogenicity of *Rhizoctonia* isolates associated with black root rot of strawberry in the Western Cape Province, South Africa. *Australasian Plant Pathology*. **32**: 195-201.

Castilla, N. P.; LeanÄo, R. M.; Elazegui, F. A.; Teng, P. S. and Savary, S. 1996. Effects of plant contacts, inoculation pattern, leaf wetness regime, and nitrogen supply on inoculum efficiency in rice sheath blight. *Journal of Phytopathology* **144**, 187 ± 92.

Chahal, K. S.; Sokhi, S. S. and Rattan, G. S. 2003. Investigations on sheath blight of rice in Punjab. *Ind. Phytopath.* **56:** 22-26.

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

Das, B. C.; Bora, L. C.; Phookan, A.K. and Bhagabati 1996. Antagonistic effects of *Aspergillus terreus*, *Trichoderma harzianum* and *Trichoderma viride* on sheath blight of rice. *Oryza.* **33**: 62-65.

Das B.C. and D.K. Hazarika, 2000. Biological management of sheath blight of rice. *Indian Phytopath.* **53**: 433–435.

Dubey, S. C. and Patel, B. 2001. Evaluation of fungal antagonist against *Thanetophorus cucumeris* causing web blight in urd and mung bean. *Ind. Phytopath* **54**: 206-209.

Dutta, S.; Chaudhury, A. K. and Laha, S. K., 2004. *In vitro* fungitoxicity of plant extract against *Pyricularia oryzae*, *Rhizoctonia solani* incitant of blast and sheath blight disease of rice. *Ind. Phytopath* **57**: 344.

Eken, C. and Demirci, E. 2003. Identification and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* anastomosis groups isolated from forage legumes in Erzurum, Turkey. *Phytoparasitica.* **31**(1): 76-80.

Fabricio, A.; Rodrigues.; Vale, F. X. R.; Datnoff, L. E.; Prabhu, A. S. and Gaspar, H. K. 2003. Effect of Rice Growth Stages and Silicon on Sheath Blight Development. *Phytopath.* **93**(3): 256-261.

Gautam, K.; Rao, P. B. and Chauhan S. V. S. 2003. Efficacy of some botanicals of the family compositae against *Rhizoctonia solani* Kuhn. *Journal of Mycology and Plant Pathology* **33**: 230-35.

Gilmar, P. H.; Carlos, A. L. and Reis, A. 2007. A novel post-harvest rot in okra pods caused by *Rhizoctonia solani* in Brazil. *Fitopatologia Brasileira.* **32**(3): 237-240.

Groth D.E.; Rush M. C. and Lindberg G. D. 1990. Foliar fungicides for control of rice diseases in the United States. In: Pest Management in Rice (B.T. Grayson, M.B. Green, L.G. Copping, ed.), Elsevier, London, UK, 31–52.

Groth D.E. and J.A. Bond, 2006. Initiation of rice sheath blight epidemics and effects of application timing of azoxystrobin on disease incidence, severity, yield and milling yields. *Plant Disease.* **90**: 1073–1078.

Hadian, S. 2012. Antifungal Activity of Some Plant Extracts against Some Plant Pathogenic Fungi in Iran. *Asian. J. Exp. Biol. Sci.* **3**(4): 714-718.

Hamada, M. S; Yin, Y. and Ma, Z. 2011. Sensitivity to iprodione, difenoconazole and fludioxonil of *Rhizoctonia cerealis* isolates collected from wheat in China. *Crop Protection,* 30**(8)**: 1028-1033.

Henz, G. P.; Lopes, C. A. and Reis, A. 2007. A novel postharvest rot in okra pods caused by *Rhizoctonia solani* in Brazil. *Fitopatologia Brasileira.* **32**: 237-240.

Holcomb, G. E and Carling, D. E. 2000. First report of web blight on verbena caused by *Rhizoctonia solani. Plant Disease.* **84**(4): 492.

Horsfall, J. G. 1956. Principles of fungicidal action. *Cronical Botanica*. pp. 207.

Hunjan, M. S.; Lore, J. S.; Pannu, P. P. S. and Thind, T. S. 2011. Performance of some new fungicides against sheath blight and brown spot of rice. *Pl. Dis. Res.* **26**(1): 61-67.

Johanson, I.; Marimuthu, T.; Rajegathesh, R.; Raguchander, T.; Karthikeyan, M. and Samiyappan, R. 2013. Hexaconazole 5 EC for the management of rice sheath blight. *Journal of Today's Biological Sciences: Research & Review (JTBSRR).* **2**(2): 29-35.

Johnson, M.; Reddy, P.N. and Reddy, D.R. 2008. Effective management of stem rot of groundnut through application of *Pseudomonas fluorescens*. *Ann. Pl. Protec. Sci.* **16**: 428-432.

Kandhari, J. 2007. Management of sheath blight of rice through fungicides and botanicals. *Indian Phytopath.* **60**(2): 214-217.

Kazempour, M. N.; Pedramfar, H. and Elahinia, S. A. 2003. Effect of certain fungicides and isolates of antagonistic fungi on *Rhizoctonia solani*, the causal agent of rice sheath blight. *J. Sci. Technol. Agri. Natural Resour.* **6**: 151-158.

Khan, A. A. and Sinha, A. P. 2005. Influence of different factors on the affectivity of fungal bio-agent to manage sheath blight of rice, in nursery. *Indian Phytopath.* **58**:289-293.

Khan, A.A. and Sinha, A.P. (2006). Influence of formulations, doses and time of application of fungal bio-agents on rice sheath blight. *Annl. Pl. Protec. Sci.* **14**: 157-161.

Khan, A. A. and Sinha, A. P. 2007. Screening of *Trichoderma* spp. against *Rhizoctonia solani* the causal agent of rice sheath blight. *Indian Phytopath.* **60**(4): 450-456.

Kotamraju, V. K. K., 2010. Management of Sheath Blight and Enhancement of Growth and Yield of Rice with Plant Growth-Promoting Rhizobacteria, Dissertation (Ph.D.), Auburn University, Auburn, Alabama, PP. 15-17.

*Kühn, J. G. 1858. Die krankheíten der kulturgewächse íhre ursachen und íhre verhütung. Gustav Bosselmann, Berlín. 312 p.

Kurt, S.; Soylu, E. M.; Soylu, S. and Tok, F. M. 2005. First report of crater rot caused by *Rhizoctonia carotae* on carrots (*Daucus carota*) in Turkey. *Plant Pathology*. **54**(2): 251.

Laha, G.S. and Venkataraman, S. (2001). Sheath blight management in rice with bio-control agents. *Indian Phytopath.* **54**: 461-464.

Lakepal, N.; Kothasthane, A.S.; Thrimurty, V.S. and Agrawal, K.C. 1996. *Indian J. Mycol. Pl. Pathol.* **26**: 193-195.

LaMondia, J. A. 2012. Efficacy of Azoxystrobin fungicide against sore shin of shade tobacco caused by *Rhizoctonia solani. Tobacco Science.* **49**: 1-3.

Lore, J. S.; Hunjan, M. S. and Sharma, N. 2012. Evaluation of some new fungicide formulations to control sheath blight and brown spot of rice. *Indian Phytopath.* **65**(3): 244-248.

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

Lore, J. S.; Thind, T. S. and Mohan, C. 2005. Performance of some new fungicide formulations against sheath blight of rice. *Pesticide Research Journal.* **17**(2): 49-51.

Mangang, H. C. and Chhetry, G. K. N. 2012. Antifungal Properties of certain Plant Extracts against *Rhizoctonia Solani* Causing Root Rot of French bean In Organic Soil of Manipur. *International Journal of Scientific and Research Publications.* **2**(5): 1-4.

Meena, R. L.; Rathore, R. S. and Mathur, K. 2003. Evaluation of Fungicides and Plant Extract against Banded Leaf and Sheath Blight of Maize. *Ind. J. of Pl. Prot.* **31**(1): 94-97.

Mezeal, I. A. 2014. Study biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* and *Fusarium oxysporum* causing disease in tomato (lycopersicon esculentum l.). *Indian Journal of Fundamental and Applied Life Sciences.* **4**(4): 175-183.

Mukherjee, P.K.; Mukhopadhyay, A. N.; Sarmah, D. K. and Shrestha, S. M. 1999. Comparative antagonistic properties of *Gliocladium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani* – its relevance to understanding the mechanisms of biocontrol. *Journal of Phytopath.* **143**, 275–279.

Muzhinji, N.; Woodhall, J. W. and Truter, M. 2014. First report of *Rhizoctonia solani* AG 4 HG-III causing potato stem canker in South Africa. *Plant Disease.* http://dx.doi.org/10.1094/PDIS-11-13-1131-PDN.

Naeimi, S.; Okhovvat, S. M.; Javan-Nikkhah, M.; Vagvolgyi, C.; Khosravi, V. and Kredics, L. 2010. Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathol. Mediterr.* **49**: 287–300.

Nene, Y. L. and Thapliyal, P. N. 1997. Fungicides in plant disease control. *Oxford and IBH Publishing Co., New Delhi.* pp. 531-532.

Oard, S.; Rush, M. C. and Oard, J. H. 2004. Characterization of antimicrobial peptides against a US strain of the rice pathogen *Rhizoctonia solani*. *Journal of Applied Microbiology*. **97**: 169–180.

Padder, B. A.; Sharma, P. N.; Kapil, R.; Pathania, A. and Sharma, O. P. 2010. Evaluation of Bio-agents and Bio-pesticides against *Colletotrichum lindemuthianum* and its Integrated management in Common Bean. *Not Sci Biol.* **2** (3): 72-76.

Park, D. S.; Sayler, R. J.; Hong, Y.G.; Nam, M. H. and Yang, Y., 2008. A method for inoculation and evaluation of rice sheath blight disease. *Plant Disease*. **92**: 25-29.

Paulitz, T. C. 2002. First report of *Rhizoctonia oryzae* on pea. *Plant Disease.* **86**(4): 442.

Peterson and Trevathan L. E. 2004. *Rhizoctonia solani* AG-13 Isolated from Corn in Mississippi. *Plant Disease.* **88**(8): 908.

Prasanna Kumar, M. K. and Veerabhadraswamy, A. L. 2014. Appraise a combination of fungicides against blast and sheath blight diseases of paddy. *Journal of Experimental Biology and Agricultural Sciences*. **2**(1): 49-57.

Rani, V. D.; Reddy, P. N.; Devi, R. G. and Reddy, S. S. 2011. Evaluation of Biological Agents Against *Rhizoctonia solani* f.sp. *sasakii* Causing Banded Leaf and Sheath Blight of Maize. *Ind. J. of Pl. Prot.* **39**(3): 208-211.

Reddy, C. S. and Muralidharan, K. 2007. Lustre 37.5 SE- An Effective Combination Product of Flusilazole and Carbendazim Against Sheath Blight of Rice. *Ind. J. of Pl. Prot.* **35**(2): 287-290.

*Savary, S.; Teng, P.S.; Willocquet, L. and Nutter, F.W. Jr. 2006. Quantification and modeling of crop losses: a review of purposes. *Annual Review of Phytopathology* **44**: 89-112.

Schillinger, W. F. and Paulitz, T. C. 2006. Reduction of *Rhizoctonia* bare patch in wheat with barley rotations. *Plant Disease*. **90**: 302-306.

Seema, M. and Devaki, M. S. 2012. *In vitro* evaluation of biological control agents against *Rhizoctonia solani. Journal of Agricultural Technology*. **8**(1): 233-240.

Sehajpal, A.; Arora, S. and Kaur, P. 2009. Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *The Journal of Plant Protection Sciences.* **1**(1): 25-30.

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

Shahid, A. A.; Shahbaz, M. and Ali, M. 2014. A Comparative Study of the Commercially Available Fungicides to Control Sheath Blight of Rice in Lahore. *Journal of Biology, Agriculture and Healthcare.* **4**(20): 185-189.

Singh, A.; Rohilla, R.; Singh, U. S.; Savary, S.; Willocquet, L. and Duveiller, E. 2002. An improved inoculation technique for sheath blight of rice caused by *Rhizoctonia solani*. *Can. J. Plant Pathol.* **24**: 65–68.

Singh, R. and Sinha, A. P. 2005. Influence of time of application of *Pseudomonas fluorescens* in suppressing sheath blight of rice. *Indian Phytopath.* **58**(1): 30-34.

Singh, R. and Sinha, A. P. 2005. Management of rice sheath blight by *Pseudomonas fluorescens* and grain yield. *Ann. Pl. Protec. Sci.* **13:** 410-414.

Singh, M. and Singh, R. P. 2005 Management of mushroom pathogens through botanicals. *Indian Phytopath.* **57**: 189-93.

Singh, R.; Singh, L. S.; Prasad, D.; Kureel, R. S.; Sengar, R. and Singh, A. 2010. Relationship of susceptibility and growth stages of plant for development of epidemic of sheath blight in rice. *Journal of Applied and Natural Science*. **2** (2): 230-233.

Sivalingam, P. N.; Vishwakarma, S. N., and Singh,U. S., 2006 Role of seed-borne inoculum of *Rhizoctonia solani* in sheath blight of rice. *Indian Phytopath.*. **59**(4): 445-452.

Sriraj, P. P.; Sundravadana, S.; Adhipathi and Alice. D. 2014. Efficacy of fungicides, botanicals and bioagents against *Rhizoctonia solani* inciting leaf blight on turmeric. *Afr. J. of Micro. Res.* **8**(36): 3284-3294.

Sundravadana, S. and Alice, D. 2007. Azoxystrobin Activity on *Rhizoctonia solani* and its Efficacy Against Rice Sheath Blight. *Tunisian J. of Pl. Prot.* **2**(2): 79-84.

Swamy, H. N.; Sannaulla, S. and Kumar, M. D. 2009. Sreening of new fungicides against the sheath blight disease. *Karnataka J. Agric. Sci.* **22**(2): 448-449.

Tang, J.B.; Ma, B.T.; Li, L.X.; Wang, P.; Zheng, A.P. and Chen, H. 2002. Biological control of rice sheath blight with *Trichoderma* and *Trichoderma*-like species. *Chinese J. Rice Sci.* **16**: 63-66.

Tewari, L. and Singh, R. 2005. Biological control of sheath blight of rice by *Trichoderma harzianum* using different delivery systems. *Indian Phytopath.* **58**: 35-40.

Verzignassi, J. R.; Poltronieri, L. S. and Benchimol, R. L. 2008. Presence of *Rhizoctonia solani* AG1 in torch ginger in the State of Pará. *Summa Phytopathologica*. **34**(3): 290.

Waghrulkar, P. S. 2014. Studies on *Rhizoctonia* leaf blight of cardamom (*Elletaria cardamomum*). M.Sc. (Ag.) Thesis (Unpub.) submitted Dr. B. S. K. K. V., Dapoli (M. S)

Windels Carol, E. and Brantner Jason, R. 2005. Early-season Application of Azoxystrobin to Sugerbeet for Control of *Rhizoctonia solani* AG4 and AG2-2. *J. of Sugar beet Res.* **42**(1, 2): 1-16.

Write, E. R.; Rivera, M. C. and Asciutto, K. 2003. First report of Petunia Root Rot Caused by *Rhizoctonia solani* in Argentina. *Plant Disease.* **88**(1): 86.

Yang, G. H.; Conner, R. L.; Cai, H.; Li, F. and Chen, Y. 2008. First report of rhizome blight of ginger caused by binucleate *Rhizoctonia* AG-R in China. *Plant Disease.* **92**(2): 312.

Zhao, C. and Wu, X. H. 2014. First report of sugar beet *Rhizoctonia* crown and root rot caused by *Rhizoctonia solani* AG-2-2IIIB in Shanxi Province of China. *Plant Disease.* **98**(3): 149.

*Original not seen

TREATMENT DETAILS

(For Plate VI)

***In vitro* evaluation of fungicides at lower concentrations against *Rhizoctonia solani* Kuhn.**

Tr. No.	Treatments	Conc. (%)
T_1	Mancozeb 75 % WP	0.2
T_2	Thiophanate methyl 70 % WP	0.1
T_3	Hexaconazole 25% EC	0.1
T_4	Propiconazole 25 % EC	0.1
T_5	Difenconazole 25 % EC	0.1
T_6	Flusilazole 12.5 % + Carbendazim 25 % SE	0.05
T_7	Azoxystrobin 23% EC	0.1
T_8	Validamycin 3 % L	0.1
T_9	Control	-

TREATMENT DETAILS

(For Plate VII)

***In vitro* evaluation of fungicides at higher concentrations against *Rhizoctonia solani* Kuhn.**

Tr. No.	Treatments	Conc. (%)
T_1	Mancozeb 75 % WP	0.3
T_2	Thiophanate methyl 70 % WP	0.2
T_3	Hexaconazole 25% EC	0.2
T_4	Propiconazole 25 % EC	0.2
T_5	Difenconazole 25 % EC	0.2
T_6	Flusilazole 12.5 % + Carbendazim 25 % SE	0.1
T_7	Azoxystrobin 23% EC	0.2
T_8	Validamycin3 % L	0.2
T_9	Control	-

TREATMENT DETAILS

(For Plate VIII)

***In vitro* evaluation plant extracts against *Rhizoctonia solani* Kuhn.**

Tr. No	Local name of the plant	Plant part used	Conc. (%)
1.	Neem	Leaves	20
2.	Garlic	Bulb	20
3.	Wild sage	Leaves	20
4.	Guava	Leaves	20
5.	Mustard	Seeds	20
6.	Crown flower	Leaves	20
7.	Soap nut	Fruits	20
8.	Marigold	Leaves	20
9.	Control	-	-

TREATMENT DETAILS
(For Plate IX)

***In vitro* evaluation of bio-agent against *Rhizoctonia solani* Kuhn.**

Tr. No.	Placement details
T1	Th *Rs* *Th* *Th*
T2	Tv *Rs* ***Tv*** ***Tv***
T3	Pf *Rs* *Pf* *Pf*
T4	Rs *Th* *Rs* *Rs*
T5	Rs *Tv* *Rs* *Rs*
T6	Rs *Pf* *Rs* *Rs*
T7	Control

Where,

Rs = Rhizoctonia solani

Th = Trichoderma harzianum

Tv = Trichoderma viride

Pf = Pseudomonas fluorescens

WEATHER DATA DURING GROWTH PERIOD *KHARIF* 2014

Week No.	Metrological Period	Rainfall (mm)	Rainy days	Max. Temp. (^{o}C)	Min. Temp. (^{o}C)	Hum. % Mor	Hum. % Eve
21	21-05-14 to 27-05-14	0.30	0	33.4	26.1	75.1	66.0
22	28-05-14 to 03-06-14	11.10	1	33.8	26.0	75.9	70.3
23	04-06-14 to 10-06-14	25.10	3	33.0	25.5	83.9	70.0
24	11-06-14 to 17-06-14	98.30	7	32.0	24.1	87.1	78.6
25	18-06-14 to 24-06-14	65.80	6	30.7	24.3	82.0	72.7
26	25-06-14 to 01-07-14	1.40	0	31.8	25.4	78.0	68.6
27	02-07-14 to 08-07-14	176.80	4	30.2	23.4	87.7	92.0
28	09-07-14 to 15-07-14	547.10	7	27.2	22.9	96.3	96.3
29	16-07-14 to 22-07-14	240.40	7	29.0	23.9	90.0	90.3
30	23-07-14 to 29-07-14	100.00	7	28.6	24.4	86.9	90.3
31	30-07-14 to 05-05-14	197.50	7	29.2	24.4	88.6	86.0
32	06-08-14 to 12-08-14	85.30	7	29.0	24.0	88.4	86.0
33	13-08-14 to 19-08-14	10.80	1	30.0	24.4	87.9	81.1
34	20-08-14 to 26-08-14	20.00	1	26.9	24.4	91.3	88.7
35	27-08-14 to 02-09-14	438.60	7	29.0	22.6	94.6	92.6
36	03-09-14 to 09-09-14	170.90	7	29.6	23.9	87.7	93.1
37	10-09-14 to 16-09-14	115.90	5	30.4	23.2	78.3	92.0
38	17-09-14 to 23-09-14	0.70	0	32.4	23.2	76.7	87.6
39	24-09-14 to 30-09-14	58.80	2	32.3	23.6	81.9	82.4
40	01-10-14 to 07-10-14	29.00	1	32.6	23.1	77.6	83.7
41	08-10-14 to 14-10-14	1.00	0	35.5	23.6	71.3	86.6
42	15-10-14 to 21-10-14	0	0	32.2	23.7	64.6	72.1
43	22-10-14 to 28-10-14	30.00	3	35.1	22.3	69.4	76.9
44	29-10-14 to 04-11-14	0	0	34.1	19.9	57.1	62.7
45	05-11-14 to 11-11-14	0	0	33.2	21.0	68.4	72.6
46	12-11-14 to 18-11-14	21.40	1	34.7	23.2	70.6	70.0
47	19-11-14 to 25-11-14	9.40	1	34.3	21.4	60.4	70.3
48	26-11-14 to 02-12-14	0	0	30.4	19.0	50.3	55.3
	Total / Average	**2455.6**	**85.0**	**31.4**	**23.5**	**78.9**	**79.8**