SHEATH BLIGHT OF RICE CAUSED BY *Rhizoctonia* spp. AND IT'S MANAGEMENT

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By
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DECLARATION OF STUDENT

I hereby declare that, the experimental work and its interpretation of the thesis entitled **"SHEATH BLIGHT OF RICE CAUSED BY *Rhizoctonia spp.* AND IT's MANAGEMENT"** or part thereof has neither been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis / publication of any university or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola. **(USENDI PUNAM NAMDEO)**

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CERTIFICATE

This is to certify that, the thesis entitled **" SHEATH BLIGHT OF RICE CAUSED BY *Rhizoctonia spp*. AND IT'S MANAGEMENT "** submitted in partial fulfilment of the requirements for the degree of **"Master of Science in Agriculture (Plant Pathology)"** of Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafied research work carried out by **USENDI PUNAM NAMDEO** under my guidance and supervision.

The subject of thesis has been approved by the Student's Advisory Committee.

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"……..Gratitude is the due respect, right from the heart"

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Abbreviations		Full form
%	:	Per cent
/	:	Per
@	:	At the rate of
^{0}C	:	Degree Celsius
mm	:	Milli meter
ml	:	Mililitre
C.D.	:	Critical difference
Cm	:	Centimetre(s)
Hrs	:	Hours
Deptt.	:	Department
PDA	:	Potato dextrose agar
PGI	:	Per cent growth of inhibition
e.g.	:	Exampli gratia (For example)
SE(M)±	:	Standard error
et al.	:	Et alia (and associates)
etc.	:	Et cetera
Fig.	:	Figure
G	:	Gram
i.e.	:	That is
J.	:	Journal
Sig.	:	Significant
spp.	:	Species
viz.,	:	Vice licet (namely)
Hrs	:	Hours
IIRR	:	Indian Institute of Rice Research

F) Thesis Abstract

a)	**Title of the thesis**	**:**	**SHEATH BLIGHT OF RICE CAUSED BY *Rhizoctonia spp*. AND IT'S MANAGEMENT**
b)	**Full name of student**	**:**	**Usendi Punam Namdeo**
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j)	**Signature, name and address of forwarding authority**	**:**	

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ABSTRACT

Rice production worldwide is affected by various biotic and abiotic stresses. Sheath blight of rice caused by a soil-borne fungal pathogen, *Rhizoctonia solani* (Kuhn) is a destructive disease in all crop growing areas of the world. It is reported to survive through soil and collateral hosts. It can lead to severe losses in rice productivity and grain quality by infecting and destroying rice sheath and leaves.

Considering the economic importance of the cereal crop, as well as disease, present study was undertaken to conduct collection of

disease samples, isolation, identification, pathogenicity test, mode of infection through seed and soil inoculation and efficacy of fungicides, botanicals and bio-agents against *R. solani*.

R. solani pathogen were isolated from sheath blight diseased samples collected from Agriculture research station, Sakoli (Bhandara). Pathogenicity of isolate was proved by artificial inoculation method.

Studied the mode of infection through sick soil method which shows seed rot (43.0%) and seedling infection (57.0%). and the mode of infection through seed inoculation recorded seed rot (62.0%) and seedling infection (38.0%).

The fungicides Propiconazole 13% + Difenoconazole 13.9% SC, Propiconazole 25% EC, Tebuconazole 50% + Trifoxystrobin 25% WG, Hexaconazole 5% EC and Carbendazim 50% WP completely inhibited the radial growth of *R. solani*. The fungicide Azoxystrobin (21.11%) and Validamaycin (30.37%) found least effective.

In vitro studies revealed that, all the four bio-agents were effective against the pathogen. The order of per cent inhibition of the fungus are *Trichoderma reesei* (73.04%) > *Trichoderma asperellum* (71.38%) > *Pseudomonas fluorescens* (64.44%) > *Bacillus subtilis* (61.38%).

Among six botanical extracts (10%) evaluated against *R. solani*, complete inhibition of the fungal mycelium was observed in garlic bulb extract which was significantly superior to rest of the treatments. It was followed by tulsi leaves (70.37%), neem leaves (68.88%), lemon grass leaves (68.52%), aloe vera leaves (68.15%) and karanj leaves (64.14%) respectively.

CHAPTER I

INTRODUCTION

1.1 Background Information

Rice is an important crop worldwide, serving as the staple food for half of humanity and additionally being used in industry and for animal feed. Rice is grown in various agro-ecological zones in tropical and subtropical areas, especially in Asia, the continent accounting for 90% of the world production (IRRI, 2015a)**.**

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. The production of rice to be achieved by 2020 is 128 Mt to feed the growing population in India. To meet the global demand, it is estimated that about 114 Mt of additional milled rice needs to be produced by 2035 with an increase of 26% in next 25 years. (Anonymous,2016)

In Maharashtra, rice is cultivated on 15.13 lakh hectares in regions viz. Vidarbha (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.

Rice is a monocotyledonous annual grass, and belongs to the family Gramineae and the genus *Oryza*. It includes 20 wild species and two cultivated species: *Oryza sativa* (grown throughout the world) and *Oryza glaberrima* (grown only in Africa) [Pareja *et al*., 2011]. Globally, more than 3 billion people have rice as staple food, and it accounts for 50 to 80% of their daily calorie intake [Delseny *et al*., 2001].

1.2 Importance of study

Rice production worldwide is affected by various biotic and abiotic stresses (Richa *et al*., 2016). Among biotic stresses, diseases are considered as major constraints for rice production as 10 to 30 per cent of the annual rice harvest is lost due to infection by many diseases. (Skamnioti and Gurr, 2009). Of different diseases, the most common and severe diseases in rice are blast and sheath blight (Wopereis *et al*., 2009). Rice sheath blight disease caused by *Rhizoctonia solani* has led to large scale yield losses, especially in U.S.A, Japan, China, and India, where intensive agricultural practices are being followed (Gautam *et al*., 2003). While, under favourable environmental conditions, sheath blight fungus can reduce yield by up to 50 per cent (Richa *et al*., 2016).

Sheath blight, also known as 'oriental sheath and leaf blight' of rice caused by *Rhizoctonia solani* Kuhn [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] was first reported in Japan by Miyake (1910) as *Sclerotium irregulare* was the causal organism of the disease. Subsequently, its occurrence was recorded throughout the temperate and tropical rice growing areas including Africa, Bangladesh, Brazil, Burma, Colombia, China, Cuba, Germany, Fiji, Formosa, India, Indonesia, Iran, Korea, Liberia, Madagascar, Malaya, Malaysia, Netherland, Nigeria, Papua New Guinea, Philippines, Russia, Senegal, Sri Lanka, Surinam, Taiwan, Thailand,Trinidad, Tobago, UK, USA, Venezuela and Vietnam (Sing *et al*., 2016).

In India, Butler (1918) first noticed the sheath blight disease, with symptoms similar to those of banded sclerotial disease of sugarcane. Later, Paracer and Chahal (1963) reported the presence of this disease from Gurdaspur district in Punjab. They described the disease as sheath blight of rice caused by *Rhizoctonia solani.*

Chahal (2005) reported that sheath blight of rice caused severe loss in Punjab in the years 1978, 2003 and 2004. The yield loss due to sheath blight ranges between 20-50 per cent depending on the severity of infection (Rao, 1995). The estimation of losses due to sheath blight of

rice in India has been reported to be up to 54.3 per cent (Chahal, 2003). It has been reported that in China 15 to 20 million hectares of rice field affected by sheath blight lead to yield losses of 6 million tonnes every year (Chen et al., 2012). In 2012, sheath blight affected about 491,932 ha of rice in Japan (JPPA 2013). In the USA, crop losses due to rice sheath blight have been recorded up to 50 % in susceptible cultivars (Prasad and Eizenga 2008). A crop loss of up to 40 % has been recorded in Bangladesh (Shahjahan *et al*., 1986).

The pathogen *R*. *solani* is a versatile soilborne saprophyte with high competitive saprophytic ability and wide host range. It survives in soil as sclerotia or thick walled mycelia. They remain viable in soil for several months over a wide range of temperature and moisture (Park and Bertus, 1932). The disease also spreads through airborne basidiospores (Kozaka, 1970; Lee and Rush, 1983) and reports are available on their seedborne nature (Kannaiyan and Prasad, 1978).

1.3 Objectives

1. To study the mode of infection of *Rhizoctonia spp*. through soil and seed inoculation.
2. Evaluation of fungicides, bio agents and botanicals against *Rhizoctonia spp*.

1.4 Hypothesis

Sheath blight of rice caused by *Rhizoctonia solani* is one of the major biotic constraints in India and reduce rice yield ranging from 20-50% depending on the severity of the disease and stages of infection. The disease has spread widely in terms of both occurrence and intensity over past 20 years. At present it is one of the major production constraints in the states of Punjab, Haryana, Uttarakhand, Eastern Uttar Pradesh, Bihar, West Bengal, Odisha, Chhattishgarh, coastal areas of Andhra Pradesh, Tamil Nadu and Kerala and parts of Karnataka. The *R*. *solani* emerged as an economically important rice pathogen, due to the intensification of rice cropping systems with the development of new dwarf, high-tillering, high yielding varieties, high plant densities and an increase in nitrogen

fertilization. These factors promote disease spread by providing a favorable microclimate, due to a denser leaf canopy with an increased leaf-to leaf and leaf-to-sheath contact. That's the reason it is necessary to study the pathogen *R*. *solani* causes sheath blight of rice and improve the method which overcome the problem of sheath blight.

1.5 Scope and limitation

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 favor 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). Use of fungicide to control diseases causes several adverse effects i.e. development of resistance in the pathogen, residual toxicity, pollution to the environment etc. (Grasela *et al*. 1990). 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).

Combi fungicides are costly and not available in small pockets. Timely availability of bio control agents of their application in paddy ecosystem are some of the limitations of the studies.

CHAPTER II

REVIEW OF LITERATURE

Sheath blight caused by *Rhizoctonia solani* is very destructive disease which develop under favorable weather conditions in rice growing countries causing substantial yield losses. In this chapter the available literature on the disease reports caused by *Rhizoctonia spp*., their isolation method, pathogenicity and mode of infection, efficacy of fungicides, bio-agents and botanicals are reviewed.

2.1 Collection of disease samples and isolation of pathogen

2.1.1 Symptomatology of rice sheath blight

Kozaka (1970) reported that the lesions on leaf sheaths are first greenish-grey and ellipsoid, 2 to 3 cm long or more, gradually becoming greyish-white with a blackish-brown narrow margin. On leaf blades the lesions are larger and somewhat irregular in shape, which are first watery greenish-grey and gradually enlarging and becoming greyish-white with brown margin.

Ou (1985) reported that Sheath blight causes spots on the leaf sheath. The spots were at first ellipsoid or ovoid, somewhat irregular, greenish-grey and varying from 1 to 3 cm long. Sclerotia are formed on or near these spots, but were easily detached. Depending upon environmental conditions, mycelium of the fungus may grow on the surface of the leaf sheaths and spread to a considerable distance.

Singh *et al*. (1988) reported that the pathogen normally attacked the leaf sheath and leaf blade but the symptoms were also found on emerging panicles which chaffy, greyish brown and matted together by fungal mycelium. Numerous white and brown sclerotia were found on diseased panicles.

Rush and Lee (1992) observed the symptoms of sheath blight do not appear until plants were in the late tillering or early internode elongation growth stages. Initial symptoms consist of circular, oblong or ellipsoid, green-grey, water-soaked spots about 1 cm long that occur on the

leaf sheath near the water line. The lesions enlarged to approximately 2-3 cm in length and 1 cm in width and the center of the lesions become pale green and white and are surrounded by an irregular purple brown border.

Rangaswamy and mahadevan (1998) reported that sometimes the disease produce large lesions which are irregularly elongated and appear on any part of the leaf sheath and sometimes, extending on to the leaf blade. The disease attacks the leaf sheath, leaf blades and in severe cases symptoms also observed on emerging panicles.

Singh *et al.* (2016) noticed that under favorable conditions, the infection spreads rapidly to the upper plant parts and also to the neighboring plants by means of normal emergence and expansion of the ears and result in poor filling of the grains. The pathogen is also known to cause panicle infection resulting in production of unfilled or partially filled discolored seed bearing brownish black spots or black to ashy gray patches.

Turaidar *et al*. (2018) reported that the disease was named as 'sheath blight' because of its primary infection on leaf sheath. The most critical stage for the infection to occur was at maximum tillering stage, while leaf sheath becomes discolored at or above water level. Initially the disease appears on leaf sheath as elliptical or oval to irregular, 1-3 cm long, greenish grey spots with brown margin at or above the water line. Presence of many such spots on the leaf sheath gives the appearance of snake skin.

2.1.2 Isolation, identification and Pathogenicity of *Rhizoctonia solani*

2.1.2.1 Isolation

Sonakar *et al*. (2014) reported that the pathogen *R. solani* isolated from diseased plants of soybean affected by aerial blight by tissue isolation method.

Singh *et al.* (2015) isolated *Rhizoctonia solani* from the infected sheath of rice and isolate was incubated on Potato dextrose agar medium for further studies.

Neha *et al.* (2016) isolated *R. solani* from diseased rice plants showing typical symptoms of sheath blight from fifteen different location. The fungus was subsequently purified, maintained on PDA slants and used for further studies.

Sifat and Monjil (2017) isolated *Rhizoctonia spp*. from infected tillers of rice sheath showing typical symptoms of sheath blight.

Mushineni *et al.* (2017) isolated *Rhizoctonia solani* from infected rice sheath and leaf, by using Modified Ko and Hora medium and pure culture was maintained on PDA for further use.

Biljana (2018) obtained pure culture of *Rhizoctonia solani* from an infected tobacco seedling by a standard laboratory method, on potato dextrose agar medium.

2.1.2.2 Identification

Parmeter (1970) reported that constriction of branch hyphae at the point of origin and formation of septum at right angle in the point of origin appear to be stable and reliable characteristics of *R. solani*.

Ou (1973) observed that initially the young hyphae of *R. solani* was colorless but become yellow and ultimately brown with age, 8-12 µm in diameter with a slight constriction at the branch which tend to branch at right angles.

Hashiba and Mogi (1975) reported that individual sclerotia of *R. solani* may be up to 5 mm in diameter. The shape of the sclerotia is roughly spherical or somewhat flattened and irregular. Young sclerotia are composed of compact masses of hyphal cells about 5 µm wide, the cell wall 0.9 µm thick.

Sneh *et al.* (1991) studied the basic characteristics of *R. solani* the mycelium branching at right angles, characteristic constriction at the point of branching and formation of septum near the point of origin of the branch. It was an obvious observation for the mycelial branching at right angles as a known feature of *R. solani*.

Meena *et al*. (2001) reported that the mycelial and sclerotial characters of *R. solani* produced dark brown mycelium, which larger sized sclerotia dark brown in color.

Kumar *et al.* (2008) observed sclerotial characters of *R. solani* causing sheath blight of rice and stated that among the sclerotial characters, variation was observed in distribution pattern, color size and weight of sclerotia. Most of the isolates were fast growers having dark brown mycelium.

Singh *et al*. (2014) reported that the color of sclerotia varied from light brown to dark brown in *R. solani* from rice.

Lal *et al*. (2014) studies 25 isolates of *R. solani* revealed that all the isolates had hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical importance.

Sifat and Monjil (2017) isolated *Rhizoctonia spp*. from infected tillers of rice sheath and recorded variability in morphological characters include colony color, colony growth, sclerotial color compactness etc. among four isolates of *Rhizoctonia solani.*

2.1.2.2 Pathogenicity

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. Inoculated plants were regularly examined for symptom appearance of rice sheath blight. Inoculated plants shows typical sheath blight symptoms.

Peterson *et al*. (2004) studied the pathogenicity of *R. solani* on four days old corn seedlings. Non inoculated seedlings served as control.

Chakraborty *et al*. (2006) used different inoculation technique such as single grain insertion, single sclerotium insertion and injection of mycelium suspension for assessing the pathogenicity of sheath blight of rice. It was found that single sclerotium insertion was the most effective.

Park *et al*. (2008) inoculated rice plants at late tillering stage with *R. solani* by placing a mycelial ball beneath the leaf sheath. The inoculated sheath was covered immediately with aluminum foil. When typical lesions appeared after 3 days, the aluminum foil was removed. *R. solani* infected plants were left in a humidity chamber made of clear plastic for 3 weeks to allow for disease development. Mycelial balls produced the longest typical lesions on rice sheaths 7 days after inoculation.

Chaudhary (2015) obtained local isolate of *R. solani* from rice and used to prepare inoculum. When local isolate inoculated on rice, produced typical sheath blight symptoms on sheath and leaves. Mycelial growth and sclerotia production was also typical of this *R. solani* isolate.

Moni *et al*. (2016) proved the pathogenicity of *Rhizoctonia solani* causing sheath blight of rice by artificial inoculation method and confirmed all the collected isolates based on pathogenicity test.

Mughal *et al*. (2017) isolated the *Rhizoctonia solani* rice sheath bits (3-4 mm) containing diseased and healthy sheath portions. The hyphal tips emanating from the tissue bits were cut off and transferred to PDA in Petri plates for purification and further growth of the pathogen. Pathogenicity point of view, inoculation of rice and maize plants with different morphological groups under poly house conditions produced typical sheath blight symptoms on rice and banded sclerotial disease on maize.

2.1.3 Mode of infection of *Rhizoctonia solani*

Ou (1987) studied rice sheath blight characteristics with other diseases caused by *Rhizoctonia spp*. in that the primary inoculum is mainly soilborne.

Sivalingam *et al*. (2006) studied the transmission of *Rhizoctonia solani* causing sheath blight of rice from seed to emerging seedling of rice. Inspite of good survival of the pathogen in seed, its transmission to rice plant under field condition was very poor.

Singh *et al.* (2016) observed the pathogen *R. solani* is also known to cause panicle infection resulting in production of unfilled or partially filled discolored seed bearing brownish black spots or black to ashy gray patches in rice.

Richa *et al.* (2016) recorded that *Rhizoctonia solani* is a soil borne necrotic pathogen of rice and it survives either as sclerotia or mycelia in the debris of host flooding water in the rice fields and germinate on rice sheaths forming infection cushions or appressoria during the infection process.

2.2 Management of *Rhizoctonia solani*

2.2.1 Efficacy of fungicides

Lore *et al*. (2005) tested different fungicides, both *in vitro* and *in vivo* against *R*. *solani* in order to control sheath blight disease of rice. It was observed that Propiconazole 25% EC (0.1%), Carbendazim 50% WP and Hexaconazole 5% EC (0.1%) were effective against sheath blight and sheath rot of rice.

Sundravadana *et al*. (2007) evaluated the effect of Azoxystrobin against *R*. *solani* causing rice sheath blight. The result revealed that Azoxystrobin at 1, 2, and 4 ppm concentration completely inhibited mycelial growth of *R*. *solani*.

Kotamraju *et al*. (2010) observed that Propiconazole (0.1%) was the most effective fungicide against sheath blight of rice under laboratory and field condition.

Reddy *et al*. (2010) reported that hexaconazole, carbendazim, mancozeb and copper oxychloride (150 ppm) inhibited mycelial growth of *Rhizoctonia solani* causing sheath blight of rice by 92%, 81%, 70% and 60% respectively using poisoned food technique.

Hunjan *et al*. (2011) evaluated five fungicides *in vitro* namely Trifoxystrobin + Tebuconazole (Nativo 75% WG), Tebuconazole (Folicur 25% EC), Propiconazole (Tilt 25% EC), Pencycuron (Monceren 25% SC) and Thifluzamide (Spencer 24% SC) against *R*. *solani* of 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 Trifoxystrobin + Tebuconazole at 0.04 % concentration.

Agrawal and Sunder (2012) observed complete inhibition of sclerotial formation at 2.0 ppm a.i. of Hexaconazole, 2.5 ppm a.i. of Carbendazim and >10 ppm a.i. of Trifoxystrobin + Tebucoazole and Tebuconazole while, Metominostrobin and Azoxystrobin were least toxic to fungal growth of *R*. *solani* in rice.

Begum *et al*. (2014) reported *in vitro* efficacy of eight fungicides and two combi fungicide against *R*. *solani*. Copper oxychloride was the best effective fungicide against the pathogen.

Singh *et al*. (2015) observed that, Validamaycin 3%L showed 25% and 45% inhibition of radial growth at 10 and 20 µg/ml concentration and 60% inhibition of radial growth at 50 µg/ml concentration against *Rhizoctonia solani* in rice causing sheath blight.

Raji *et al*. (2016) recorded that tebuconazole + trifoxystrobin 75 WG were statistically on par with the standard check fungicide, Hexaconazole 5% EC in inhibiting *Rhizoctonia solani* of rice.

Subhash Chandra *et al*. (2016) reported that Propiconazole, Carbendazim (0.2%), Hexaconazole (0.3%) completely inhibited the mycelial growth of *Rhizoctonia solani* in rice and Validamaycin (0.3%) was found least effective.

Kumar *et al*. (2017) evaluated fungicides, propiconazole and carbendazim were individually effective against the pathogen even at the lowest concentration of 200 ppm by maximum inhibiting the mycelial growth and sclerotia formation. At 1000 ppm these fungicides completely inhibited the mycelial growth of *Rhizoctonia solani* of rice.

Mushineni *et al*. (2017) detected the use of Propiconazole 25% EC at 40 , 80 and 160 ppm concentration which inhibited the growth of *Rhizoctonia solani* by 100% and Tebuconazole 25% EC at 80% and

160% ppm conc. Inhibited 100% growth of *R*. *solani*. Hexaconazole 5% SC and Tebuconazole 50% +Trifloxystrobin 25% WG showing 89.26% and 83.15% inhibition at just 8 ppm concentration.

Rajput and Zacharia (2017) recorded that Propiconazole (0.1%) was effective in the inhibition of mycelial growth (82.21%) of *Rhizoctonia solani* causing sheath blight of rice.

i. Efficacy of bioagents

Kazempour *et al.* (2003) observed that, *P. fluorescens* were found to inhibit the rice sheath blight pathogen under *in vitro* conditions. All the strains of the bioagent (Biovar 2) produced siderophores on King"s B media. The volatile metabolites, extra cellular secretions and antibiotics of these isolates were inhibitory to R. solani. All the antagonists could reduce germination and caused lysis of sclerotial bodies.

Khan and Sinha (2007) observed that the radial growth of different isolates of *Trichoderma spp*. against *R*. *solani* the causal agent of rice sheath blight. Volatile compounds produced by *T*. *harzianum* resulted in maximum inhibition (59.7%) of mycelial growth of *R*. *solani* after 48 hours.

Reddy *et al*. (2010) reported that *Pseudomonas fluorescens (*P.f. 003 strain) inhibited the growth of *Rhizoctonia solani* by 77.78% and P.f. 008 by 20%.

Rani *et al*. (2011) recorded that the per cent growth inhibition was maximum (67.02%) in case of *Trichoderma harzianum* followed by *T*. *viride* against *R. solani* causing sheath blight of rice.

Seema and Devaki (2012) reported the efficacy of four fungal and one bacterial bio-agent viz., *Trichoderma spp. Aspergillus niger, Penicillium spp.* and *Bacillus subtilis* against *R. solani* of rice. 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%).

Saranya Devi and Sowndaram (2014) studied the effectiveness of *Pseudomonas fluorescens* which inhibited the 78% of mycelial growth of *R. Solani*.

Mezeal (2014) evaluated the antagonistic activity of bacterial bio-agents, four strains of *B. subtilis* and five strains of *P. fluorescens* against *R. solani*. *P. fluorescens* (strain 5) exhibited 81.3 per cent growth inhibition, while *B. subtilis* 77.4 (strain 1) per cent of growth inhibition of the test pathogens.

Srinivas et al. (2014) studied the antagonistic nature of *Trichoderma spp., Penicillium notatum, Aspergillus niger* and observed the restricted growth of rice sheath blight pathogen *R. solani* after contact with each other.

Neha *et al*. (2016) showed among the antagonists *P*. *fluorescens* was found to be more antagonistsic to *R*. *solani* of rice as it recorded maximum per cent inhibition (73.88%), which was followed by *Bacillus subtilis* (73.88%) and *Serratia marcescens* (62.71%).

Rajput and Zacharia (2017) reported that the maximum reduction in colony growth of *Rhizoctonia solani* causing sheath blight of paddy was recorded in *Trichoderma harzianum* (63.37%) followed by *Trichoderma asperellum* (58.16%).

2.2.3 Efficacy of botanicals

Sehajpal *et al.* (2009) reported that the effect of different concentrations of plant extracts on the pathogen *R. solani* isolated from paddy. It was observed that *Allium sativum* gave strong inhibition i.e. 5.75 mm at 1000 ppm concentration, followed by *Allium cepa* and *Emblica officinalis* i.e. 3.25 mm of each.

Seint San Aye and Matsumoto (2011) tested the plants had the ability to control *Rhizoctonia solani* which showed 100.0% inhibition of *R. solani*, whereas the lowest inhibitions were recorded for the extracts of pennywort (14.3%), Sage (14.3%), lemongrass (14.3%) and Aloe (16.7%).

Koma *et al*. (2014) tested the efficacy of leaf extracts derived from garlic, eucalyptus, lemongrass, gokhru, van tulsi which completely inhibited the growth of *R. solani* causing web blight of groundnut followed by lantana (72.4%) and neem (60.37%).

Sonakar *et al.* (2014) evaluated seven plant extracts against *R*. *solani* causing aerial blight of soybean and found that Garlic (88.47%) and Madar (87.98%) are effective against *R. solani* followed by Ginger (52.62%), Aloe vera (47.37%). The per cent inhibition of the fungal growth is very less in the case of Neem (36.47%), Makoy (23.07%) and Datura (21.17%) as compared to the remaining botanical extracts.

Negi *et al.* (2014) onion extract was found to be the most effective (100%) followed by Garlic (75%) and Turmeric (69.9%), while Eucalyptus extract was least effective in checking the mycelial growth of *R. solani* the causal agent of aerial blight of soybean.

Srinivas *et al*. (2014) tested botanicals which are effective in inhibiting the fungus *R*. *solani,* Garlic (91.82%) and Calotropis (84.75%) were significantly superior over the control in inhibiting the growth of the fungus while Aloe vera (51.30%), Tulsi (47.22%) and Neem (41.64%).

Sifat and Monjil (2017) tested the efficacy of botanicals against rice sheath blight pathogen *Rhizoctonia solani* , highest per cent inhibition of radial mycelial growth over control was found for Isolate 1 by Garlic (97.50%), Neem (96.75%), and Biskatali (94.25%).

Kumar *et al*. (2017) reported that the bulb extract of *Allium sativum* and rhizome extract of *Zinziber officinales* suppressed the mycelia growth 80.19 and 76.32% respectively at 10% concentration followed by leaf extract of *Azadiracta indica (*72.78%*).*

Sharma *et al*. (2018) reported that the *Rhizoctonia solani* was recordet clove extract of garlic produce maximum inhibition (71.85%) followed by leaf extract of Karanj (38.88%), Bulb extract of onion (37.03%), leaf extract of Jetropha (30%) and leaf extract of Tulsi (24.81%) against *R. solani* on incident of sheath blight of rice.

CHAPTER III

MATERIAL AND METHODS

The present investigations on sheath blight of rice caused by *Rhizoctonia spp*. and its management was conducted 2018-2019 at the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

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

3 .1 Material required

3.1.1 Collection of diseased samples

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

3.1.2 Collection of seeds

Seeds were collected from Krishi Vidyan Kendra, Gadchiroli.

3.1.3 Source of fungicides, bioagents and botanicals

The fungicides *viz*. Propiconazole 13%+ Difenconazole 13.9% SC, Propiconazole 25% EC, Tebuconazole 50% + Trifoxystrobin 25% WG, Azoxystrobin 23 % EC, Validamaycin 3% L, Hexaconazole 5% EC and Carbendazim 50 % WP and bio-agents *Trichoderma asperellum*, *Trichoderma reesei*, *Bacillus subtilis* and *Pseudomonas fluorescens* were procured from Department of Plant Pathology Dr. P.D.K.V., Akola and bulb of *Allium sativam*, leaves of *Azadirachta indica*, *Aloe vera*, *Oscimum sanctum, Cymbopogon flexuosus and Pongamia pinnata* were collected from University campus of Dr. P.D.K.V., Akola.

3.1.4 Glassware, plastic wares and other materials

Petri plates, glass petri dishes, conical flasks, test tubes, beakers etc. were used in the present studies.

3.2 Method adopted

3.2.1 Preparation of culture media

1. Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) medium was used for isolation and maintenance of cultures. The medium was prepared with following ingredients and sterilized in autoclave at 15 psi (1.04 kg/cm^2) for 15 minutes.

Peeled potato	-	200gm
Dextrose	-	20gm
Agar agar	-	20gm
Distilled water	-	1000 ml

Healthy peeled potatoes 200 g were cut into pieces and boiled in 500 ml of sterilized distilled water in sauce pan for 30 min. The extract was strained through muslin cloth and quantity was measured. In remaining 500 ml water, 20 g agar-agar and 20 g dextrose were dissolved by heating. Then both mixed and volume was made to one liter. The medium was filtered through muslin cloth and poured into conical flasks and test tubes. Then plugged with non-absorbent cotton and autoclaved at 1.04 kg/cm^2 for 15 min. Autoclaved tubes were kept in slanting position to obtain PDA slants for maintenance of cultures.

2. Nutrient Agar (NA)

Nutrient Agar (NA) medium was used for the maintenance of pure culture of bacterial bio control agents i.e. *Pseudomonas fluorescens* and *Bacillus subtilis*.

The composition of NA

Beef extract	-	3.0 g
Yeast extract	-	2.0 g
Peptone	-	5.0 g
Sodium chloride	-	5.0 g

Agar-agar - 20 g

Distilled water - 1000 ml

Peptone, beef extract, yeast extract and sodium chloride were dissolved in 500 ml distilled water by heating and agar-agar in remaining water. Both the solutions were mixed together, filtered through muslin cloth, distributed in flasks and test tubes as per requirements. The flasks and tubes were plugged with cotton and autoclaved at 1.04 kg/cm^2 for 15 min. Autoclaved tubes were kept in slanting position to obtain NA slants for maintenance of bacterial cultures.

3.2.2 Disinfection / sterilization of laboratory materials

The glasswares were washed with cleaning powder under running water, dried and then sterilized in hot air oven at 180°C for 1 hr. before use. The other material were disinfected with denature spirit.

3.3 Isolation of fungal pathogen by tissue isolation method

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 inert 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 sodium hypo chloride solution 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. This fungal growth around the bits was transferred to PDA slants and maintained as stock culture for further studies.

3.3.1 Identification, Purification and maintenance of fungal culture

The fungal culture identified based on morphological characters and published literature and was purified by following hyphal tip method and culture obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

3.3.2 Pathogenicity test

The pathogenicity of the isolates was determined by injection method on 40-day-old susceptible rice cultivar Swarna. The culture with fungal mycelia (and sclerotia) was taken in a disposable syringe of 0.5ml capacity then placed in between the tillers in the central region of the hill, 5-10 cm above the water line. Care was taken that no air bubble was trapped in the suspension. Bigger wounds were avoided at the point of injection.

Healthy uninoculated plants served as control. The pathogen was reisolated on the PDA medium from the symptomatic plants and microscopic observations made were found similar to that of the organism isolated from naturally diseased rice plants. Thus, the test pathogen was confirmed as *Rhizoctonia solani* and pathogenicity of *Rhizoctonia solani* was proved.

3.4 Efficacy of fungicides against *Rhizoctonia solani* by poisoned food method

The test fungus were evaluated by employing poison food method. Potato dextrose agar (PDA) medium was prepared, equally distributed measuring 100 ml in 250 ml conical flask and sterilized in autoclave. Requisite quantity of each of the fungicides (as per concentration) was added in sterilized melted (45 ^{o}C) PDA separately so as to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20ml of melted poisoned PDA was poured in each sterilized petri plate and allow to solidify. These petri plates were inoculated by test fungus separately. Five mm disc of one week old fungus culture was cut with sterilized cork borer, lifted and transferred aseptically in the center of petri plate containing the medium poisoned with test fungicide. The control plates were kept where the culture disc grown in same condition on PDA without fungicides. Treated plates were incubated at room temperature (28± 2^{0}C) for a period of seven days. Colony diameter was recorded in mm and per cent mycelial growth inhibition was calculated as per Vincent's

formula (1927) based on the average colony diameter. The data was subjected to statistical analysis wherever necessary.

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

Where,

PI = Per cent Inhibition

C = Growth of fungi in control (mm)

T = Growth of fungi in treatment (mm)

3.5 Efficacy of bio-agents against *Rhizoctonia solani* by dual culture method

The lawn culture of test fungi and bioagents i.e. *Trichoderma asperellum* and *Trichoderma reesei* were prepared. Autoclaved, melted potato dextrose agar was poured in petri plates and allowed to solidify for obtaining leveled surface. The plates were inoculated with the culture of test fungi and bioagents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bioagents, *Bacillus subtilis* and *Pseudomonas fluorescens* were prepared by inoculating a loopful culture in sterilized conical flask containing 100 ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bioagent lawn culture was cut with the help of cork borer lifted and transferred in petri plates, containing autoclaved solidified PDA medium. In each petri plates, four discs of bioagents were inoculated at four peripheral points of the plates and the test fungi was placed in the center of petri plates. In case of *Pseudomonas fluorescens* and *Bacillus subtilis*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on potato Dextrose Agar without bioagents served as control. All these plates were incubated at room temperature for seven days. After an expiry of seven days incubation period the mycelial inhibition was calculated as per formula mentioned in the poisoned food method.

3.6 Efficacy of botanicals against *Rhizoctonia solani* by poisoned food method

The poisoned food method was employed to evaluate the efficacy of various botanicals against different isolates of *Rhizoctonia solani* as per the procedure given below.

3.6.1 Preparation of aqueous leaf extract of botanicals

Aqueous leaf extracts of the test botanicals were obtained by grinding the washed plant leaves (100 g) in mortal and pestle with equal volume (100 ml) of sterilized distilled water. The macerate obtained was filtered through the folds of muslin cloth and the filtrate obtained formed 100% phytoextracts, which were evaluated by poisoned food technique.

3.7 The mode of infection through soil inoculation

Stems of 35-40 days old rice plants were cut into small pieces of about 2 cm size and filled in to 500 ml conical flask upto one third. Flasks were autoclaved at 1.04 Kg/ cm^2 for 30 minutes. Mycelial discs of 5 mm diameter cut from the margin of 48 hours old culture of the pathogen were inoculated into the flask and incubated at $28 \pm 2\ ^0$C up to fifteen days for full growth of fungus and sclerotia formation. Meanwhile flasks were shaken to avoid clumping and to facilitate early growth of the fungus.

After 15 days of incubation, the inoculums was taken out from flask and mixed thoroughly with sterilized sand plus soil mixture (1:1) at 100 g inoculums per kg soil. This potting mixture (sand +soil +inoculum) was filled in the earthen pots and watered lightly and incubated for four days. Then seeds were sown (at 40 seeds / pot) in the earthen pot. The pots with uninoculated soil served as control. All these pots were then watered lightly and kept in a green house for further recording of observations on seed rot and seedling infection etc. the observation was recorded up to 20 days from the date of inoculation of the seeds.

3.8 The mode of infection through seed inoculation

The seeds of rice plant were disinfected with sodium hypochloride solution for two minutes, followed by three subsequent washing with the sterilized distilled water. These were dried on sterile tissue paper. After that inoculum was properly mixed having mycelia and sclerotia from 9 days old culture. Seeds (40 seeds/pot) were sown in separate pots containing 1/3 rd of sterilized sand + soil mixture for raising of seedlings. Inoculated pots were kept in green house . Inoculated plants were observed for the appearance of disease symptoms up to 20 days. The pot contains seeds without culture served as control pot. All these pots were then watered lightly and observations was recorded on seed rot and seedling infection.

CHAPTER IV

RESULT AND DISCUSSION

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

4.1 Collection of disease samples

4.1.1) The symptoms

The sheath blight symptoms appeared in the form of lesions on sheath 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.

Result on same line observed by Rangaswamy and mahadevan (1998) reported that sometimes the disease produce large lesions which are irregularly elongated and appear on any part of the leaf sheath. Singh *et al.* (2016) recorded that under favorable conditions, the infection spreads rapidly to the upper plant parts and also to the neighboring plants by means of normal emergence and expansion of the ears and result in poor filling of the grains. Turaidar *et al*. (2018) reported that the disease was named as ‘sheath blight’ because of its primary infection on leaf sheath. The most critical stage for the infection to occur was at maximum tillering stage, while leaf sheath becomes discolored at or above water level.

4.1.2) Isolation and purification of the fungal pathogen

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 3-4 days of inoculation. Initially, the fungal mycelium formed white, sparse mat on the surface of the medium. Later on the tips of hyphae entwined to form white, young sclerotia. As the mycelium grew old, its colour changed to buff brown.

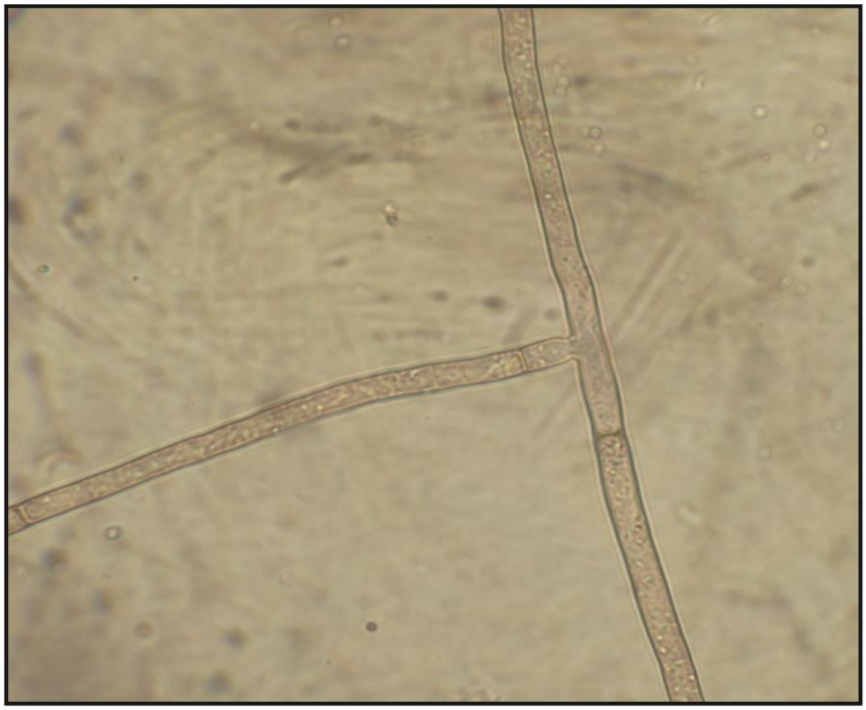
Within a week, many brown to dark brown, irregular, hard sclerotia were formed on the mycelial mat. The pure culture of the test fungus, obtained by the transferring mycelial bits was maintained by periodic transfer after every 20 days on PDA slants. The slants were stored in the refrigerator and this culture was used as stock culture for further studies.

Similar results were observed by Mughal *et al.* (2017) isolated *Rhizoctonia solani* from rice sheath bits by tissue isolation method. Neha *et al*. (2016) collected the diseased rice plants showing typical symptoms of sheath blight and isolated by tissue isolation method. The fungus was subsequently purified and maintained on PDA slants. Sifat and Monjil (2017) isolated *Rhizoctonia spp*. from the infected tillers of rice sheath showing typical symptoms of sheath blight.

4.1.3) Identification of the fungal pathogen

The isolated fungi were identified on the basis of following morphological characteristics and published literature. The genus *R. solani* belongs to form class Deuteromycetes that does not make vegetative spores and present as mycelium and sclerotia. It produces shade of brown hypha, constriction at the point of branching and right angle branching in matured hyphae. The isolate shared typical characteristics of *R. solani* (a) branching a right angle near the distal septum of the cell in young vegetative hyphae, (b) formation of the septum in the branch near the point of origin, Sclerotia were differentiated aggregations of thick-walled cells, small (1-3-mm diameter) irregular-shaped, brown to black structures.

Various earlier worker have been reported the identification of pathogen *R. solani.* Singh *et al*. (2014) reported that the color of sclerotia varied from light brown to dark brown in *R. solani* from rice. Lal *et al*. (2014) studies 25 isolates of *R. solani* revealed that all the isolates had hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical important.



***R. solani* hyphae showing the distinguishing right angle**

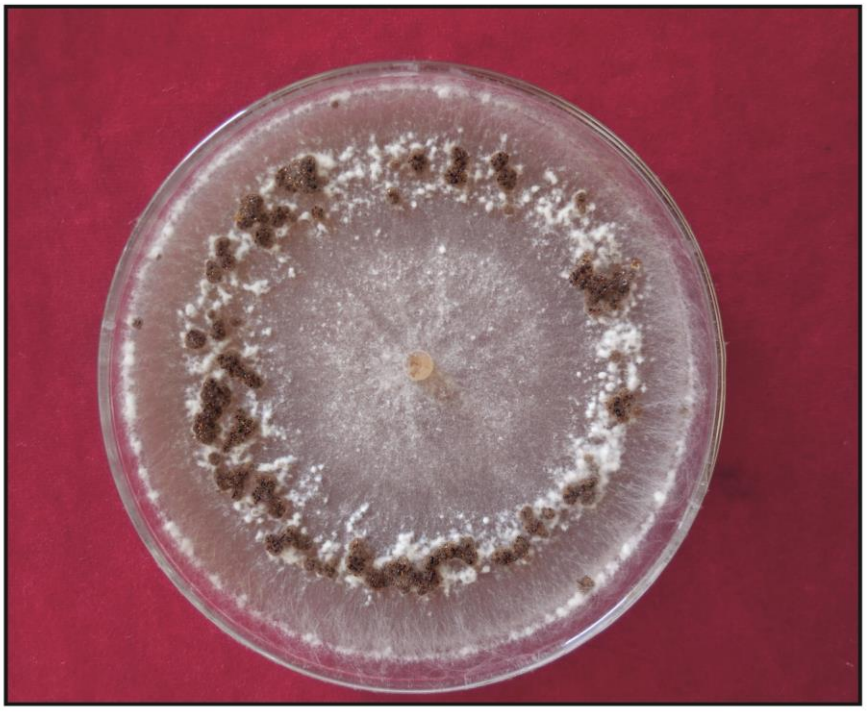


Plate 1 : Mycelial growth and sclerotia of *Rhizoctonia solani*

4.2. Pathogenicity test

Symptoms development on artificial 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 lesion enlarged gradually covering 60-65 per cent area of the leaf sheath. On an average, the lesions were 1.5-3 cm length. These symptoms resembled to those observed on naturally infected sheaths. Non-inoculated seedlings remained healthy.

Similar results were observed by Park *et al*. (2008) rice plants at late tillering stage were inoculated with *Rhizoctonia solani* by placing mycelial ball beneath the leaf sheath shows mycelial ball produced the longest lesions on rice sheath. Moni *et al*. (2016) proved the pathogenicity of *Rhizoctonia solani* causing sheath blight of rice by artificial inoculation method and confirmed all the collected isolates based on pathogenicity test.

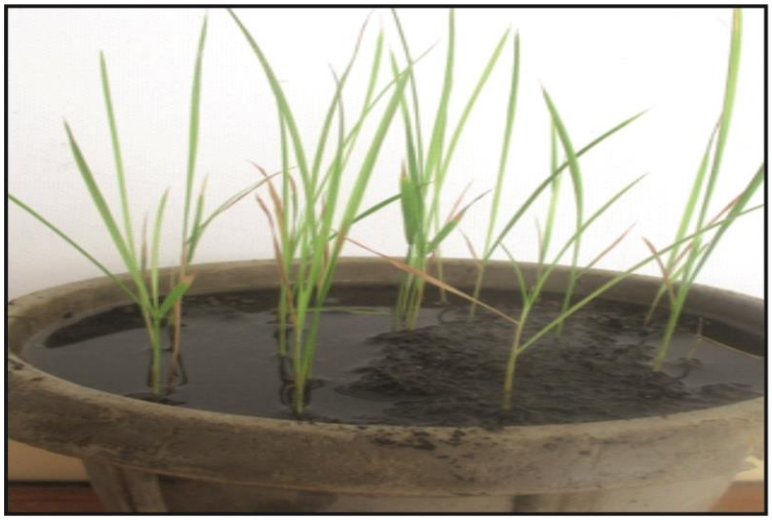
4.2.1) Re-isolation

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

4.3 The mode of infection through soil inoculation

Soil inoculation is done by sick soil method as mentioned in methodology. The pathogen *R. solani* inoculated in earthen pots containing sterilized soil. 200 seed were used and observations were recorded on seed rot and seedling blight.

From the data presented in Table-4, it is seen that the pathogen inoculated soil caused seed rot (43.0%) and seedling infection (57.0%).



CONTROL

Initial symptoms of sheath blight

Irregular greyish white spots of sheath blight

Plate 2 : Pathogenicity test

Table 1 : Mode of infection of *R. solani* through soil inoculation:

Sr No.	Fungi	Number of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling infection	Seed rot	Seedling infection
1.	*R. solani*	200	140	43.00	57.00	*R. solani*	*R. solani*

4.4 The mode of infection through seed inoculation

Seeds of rice were inoculated with the culture of *Rhizoctonia solani* and sown in earthen pots containing sterilized soil. Observations were recorded on seed rot and seedling blight.

From the data presented in Table-5, it is seen that the pathogen inoculated seeds caused seed rot (62.0%) and seedling blight (38.0%).

Table 2 : Mode of infection of *R. solani* through seed inoculation :

Sr. no	Fungi	Number of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling infection	Seed rot	Seedling infection
1.	*R. solani*	200	138	62.00	38.00	*R. solani*	*R. solani*

The present result similar with Sivalingan *et al*. (2006) studied the transmission of *Rhizoctonia solani* causing sheath blight of rice from seed to emerging seedling of rice. Inspite of good survival of the pathogen in seed, its transmission to rice plant under field condition was very poor. Singh *et al.* (2016) observed the pathogen *R. solani* is also known to cause panicle infection resulting in production of unfilled or partially filled discolored seed bearing brownish black spots or black to ashy gray patches in rice. Richa *et al.* (2016) recorded that *Rhizoctonia solani* is a soil borne necrotic pathogen and it survives either as sclerotia or mycelia in the debris of host flooding water in the rice fields and germinate on rice

Seedling blight symptoms

Plate 3 : Seedling infection of rice plant due to soil inoculation

Seed rot caused by *R. solani*

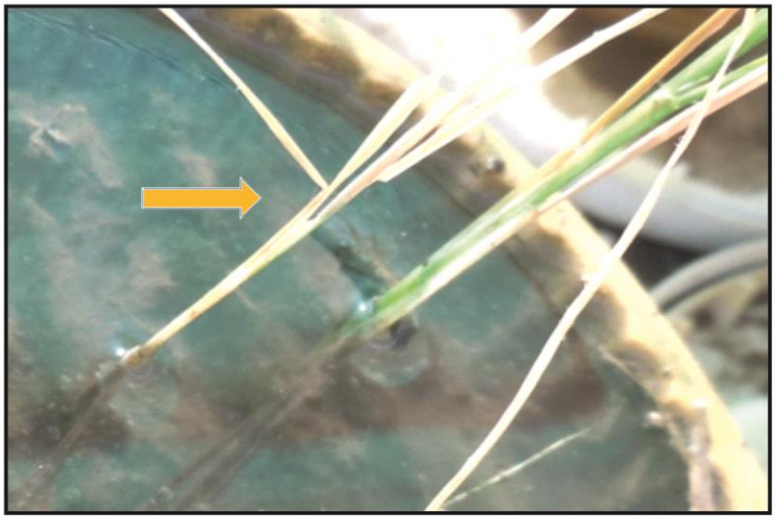


Plate 4 : Seedling infection of rice plant due to seed inoculation

sheaths forming infection cushions or appressoria during the infection process.

4.5 Efficacy of fungicides against *Rhizoctonia solani.*

The fungicides *viz.,* Propiconazole 13% + Difenconazole 13.9% SC (Taspa) , Propiconazole 25% EC (Tilt), Tebuconazole 50% + Trifoxystrobin 25% WG (Nativo), Azoxystrobin 23% SC (Amistar), Validamaycin 3% L (Vamcin), Hexaconazole 5% EC (Contaf) and Carbendazim 50 WP (Bavistin) was evaluated against *Rhizoctonia solani* causes sheath blight of rice.

Seven fungicides were tested in laboratory against *Rhizoctonia solani* for their efficacy against radial growth. Data presented in table 1., clearly showed that all the fungicides taken in this investigation were effective against *R*. *solani* and significantly inhibited the radial growth *in vitro* at different concentrations. Out of which Propiconazole 13% + Difenoconazole 13.9% SC, Propiconazole 25% EC, Tebuconazole 50% + Trifoxystrobin 25% WG, Hexaconazole 5% EC and Carbendazim 50 WP completely inhibited the radial growth of the fungus at test concentrations on Potato dextrose agar medium. The fungicide Azoxystrobin and Validamaycin found least effective i.e., 21.11% and 30.37% respectively.

Similar results were observed by Hunjan *et al*. (2011) also reported that fungicides viz., Trifoxystrobin+Tebuconazole and Propiconazole showed higher level of efficacy against *Rhizoctonia solani*. Subhash Chandra *et al*. (2016) reported that Propiconazole, Carbendazim and Hexaconazole completely inhibited the mycelial growth of *Rhizoctonia solani* followed by Validamaycin at different concentrations.

Table 3. Efficacy of fungicides against *Rhizoctonia solani*

Sr. No.	Fungicides	Conc. (%)	Mean colony diameter (mm) *	Mycelial inhibition (%)
1	Propiconazole 13% + Difenconazole 13.9% SC	0.1	0.00	100.0
2	Propiconazole 25% EC	0.1	0.00	100.0
3	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1	0.00	100.0
4	Azoxystrobin 23% SC	0.1	71.00	21.11
5	Validamaycin 3% L	100 ppm	63.00	30.00
6	Hexaconazole 5% EC	0.2	0.00	100.0
7	Carbendazim 50 WP	0.2	0.00	100.0
8	Control	-	90.00	
	F ' test	-	Sig.	-
	SE(m)±	-	0.45	-
	CD(P=0.01)	-	1.88	

*Average of three replications.

4.6 Efficacy of bio-agents against *Rhizoctonia solani.*

Bio-agents were evaluated for their inhibiting effect against the pathogen *in vitro* by dual culture technique as described in "Material and Methods". The results of average diameter of fungal colony incubated at 27 $\pm$ 1^0c after 7 days are presented in table 2, revealed that all the bio-agents suppressed colony growth of *R*. *solani*.

The order of percent inhibition of the fungus as follows, *Trichoderma reesei* (73.04%) > *Trichoderma asperellum* (71.38%) > *Pseudomonas fluorescens* (64.44%) > *Bacillus subtilis* (61.38%).

Result on same line observed by Reddy *et al*. (2010) reported that *Pseudomonas fluorescens* (P.f.003) strain effectively controlled the pathogen *Rhizoctonia solani* by 77.78% and (P.f. 008) by 20% causing sheath blight of rice. Rajput and Zacharia (2017) recorded inhibition of *Rhizoctonia solani* with *Trichoderma asperellum* (58.16%). Neha *et al*. (2016) reported that *P*. *fluorescens* found more effective to the *Rhizoctonia*

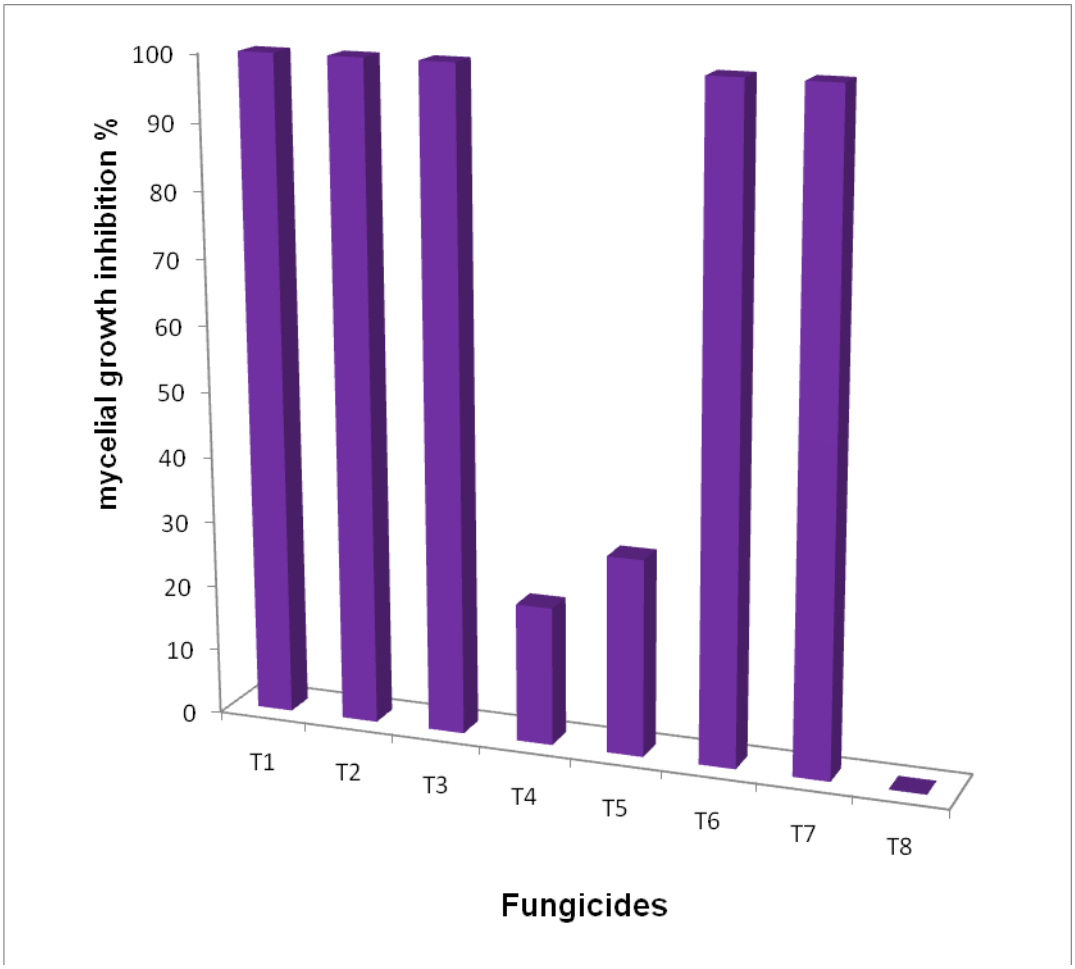


Fig. 1 - Efficacy of fungicides against *Rhizoctonia solani*

T1- Propiconazole+Difenconazole (0.1%)
T2- Propiconazole (0.1%)
T3- Tebuconazole+Trifoxystrobin (0.1%)
T4- Azoxystrobin (0.1%)
T5- Validamaycin (100 ppm)
T6- Hexazonazole (0.2%)
T7- Carbendazim (0.2%)
T8- Control

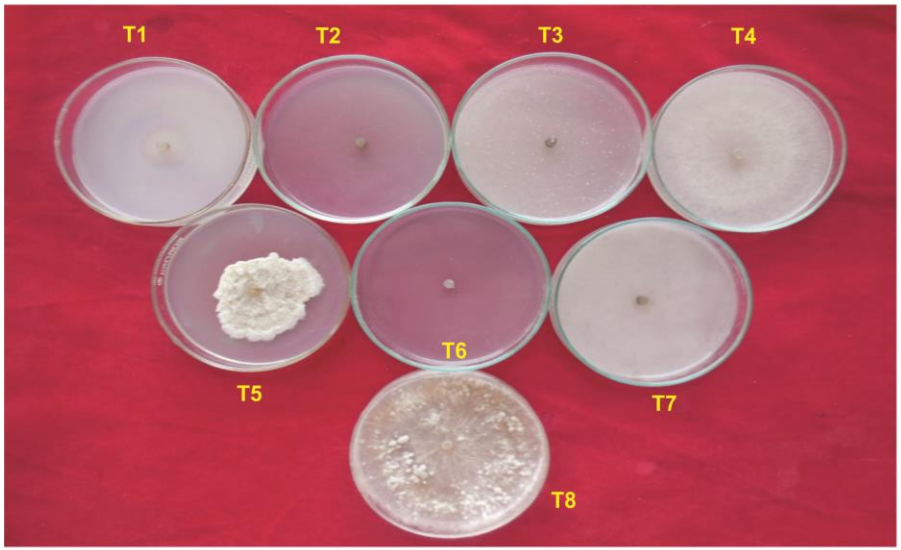


Plate 5. : Efficacy of fungicides against *Rhizoctonia solani*

T1- Propiconazole+Difenconazole (0.1%)
T2- Propiconazole (0.1%)
T3- Tebuconazole+Trifoxystrobin (0.1%)
T4- Azoxystrobin (0.1%)
T5- Validamaycin (100 ppm)
T6- Hexazonazole (0.2%)
T7- Carbendazim (0.2%)
T8- Control

solani as it recorded mazimum per cent inhibition (75.86%), which was followed by *Bacillus subtilis* (73.88%) in rice crop.

Table 4. Efficacy of bio-agents of *Rhizoctonia solani* :

Sr. No.	Bioagents	Mean radial mycelial growth (mm)*	Per cent mycelial inhibition (%)
1	*Trichoderma asperellum*	25.75	71.38
2	*Trichoderma reesei*	24.25	73.05
3	*Pseudomonas fluorescens*	32.00	64.44
4	*Bacillus subtilis*	34.75	61.38
5	Control	90.00	-
	F' test	Sig.	-
	SE(m)±	1.24	-
	CD (P=0.01)	3.66	-

*Average of four replication

4.7 Efficacy of botanicals against *Rhizoctonia solani*.

The effect of plant extracts of six plant species was studied against *R*. *solani* to test their antifungal properties. All the plant extracts were tested at 10 per cent concentration by poisoned food technique. All the plant extracts under study exhibited antifungal properties against *R*. *solani*. The data on the effect of different plant extracts on mycelial growth of the pathogen are presented in Table 3.

The complete inhibition of the fungal mycelium (100.0%) was observed in garlic bulb extract which was significantly superior to rest of the treatments. It was followed by tulsi leaves (70.37%), neem leaves (68.88%), lemon grass leaves (68.52%), aloe vera leaves (68.15%) and karanj leaves (64.14%) respectively.

The present results of botanicals similar with Koma *et al*. (2014) who reported complete mycelial inhibition of *Rhizoctonia solani* with Garlic bulb extract in groundnut followed by Neem leaves (60.37%). Srinivas *et al*. (2014) recorded maximum mycelial inhibition of *Rhizoctonia solani* in rice by Garlic bulb extract (91.82%) followed by Aloe vera (51.30%), Tulsi leaves (47.22%) and Neem leaves (41.64%). Similar

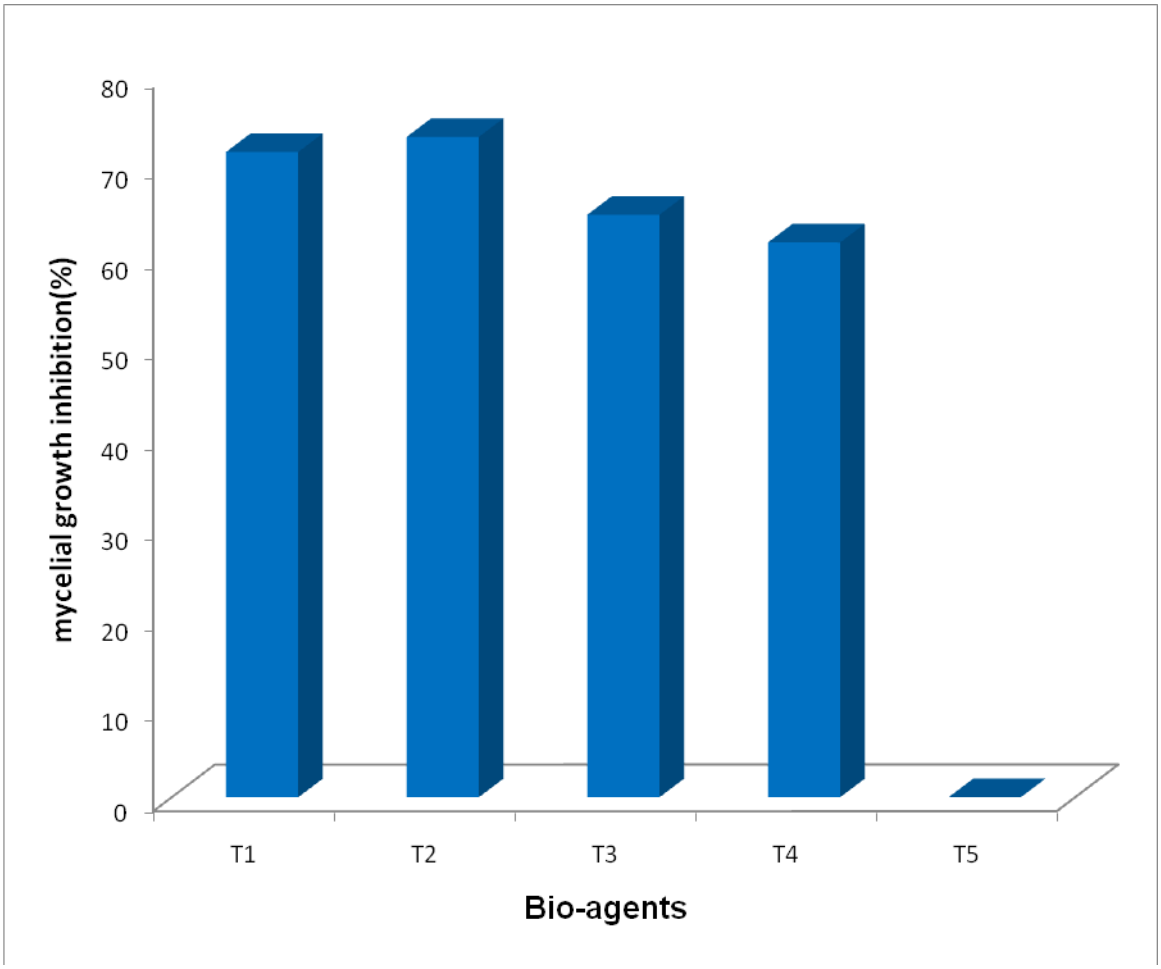


Fig. 2 - Efficacy of bio-agents of *Rhizoctonia solani*

T1- *Trichoderma asperellum*
T2- *Trichoderma reesei*
T3- *Pseudomonas fluorescens*
T4- *Bacillus subtilis*
T5- Control

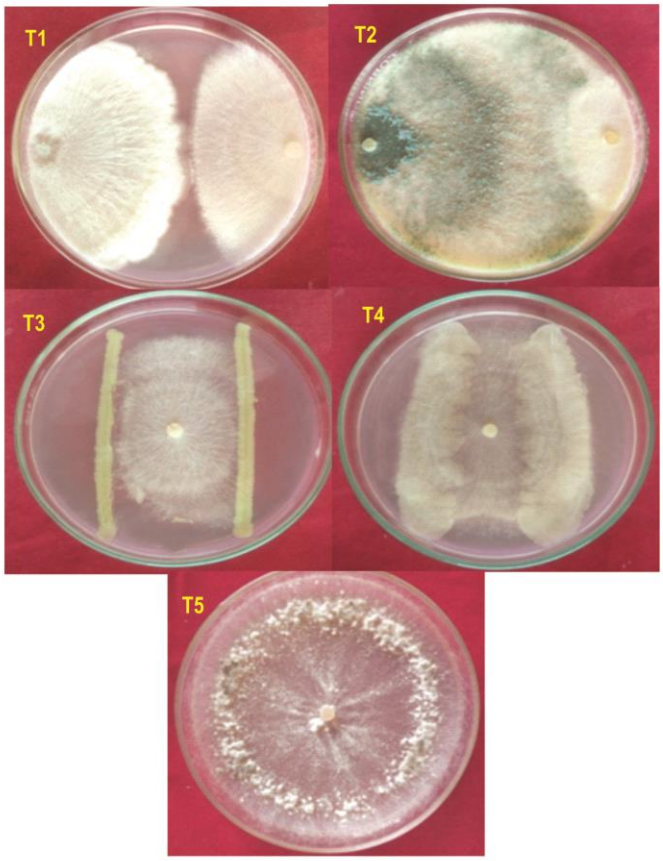


Plate 6 : Efficacy of bio-agents against *Rhizoctonia solani*

T1- *Trichoderma asperellum*
T2- *Trichoderma reesei*
T3- *Pseudomonas fluo*rescens
T4- *Bacillus subtilis*
T5- Control

finding was also recorded by Sharma *et al*. (2018) in respect of Garlic bulb extract (71.85%) followed by Karanj leaves (38.88%), Neem leaves (26.66%) and Tulsi leaves (24.81%) against *Rhizoctonia solani* causing sheath blight of rice.

Table 5. Efficacy of botanicals against *Rhizoctonia solani*

Sr. No.	Botanicals	Conc. (%)	Mean radial mycelial growth (mm)*	Mycelial inhibition (%)
1	Tulsi leaves extract (*Ocimum sanctum*)	10.00	26.66	70.37
2	Neem leaves extract (*Azadiracta indica*)	10.00	28.00	68.88
3	Aloe vera leaves extract (*Aloe vera*)	10.00	28.66	68.15
4	Karanj leaves extract (*Pongamia pinnata*)	10.00	32.00	64.44
5	Garlic bulb extract (*Allium sativum*)	10.00	0.00	100.0
6	Lemon grass extract (*Cymbopogon flexuosus*)	10.00	28.33	68.52
7	Control	-	90.0	
	F' test	-	Sig.	-
	SE(m)±	-	0.81	-
	CD(P=0.01)		3.43	

*Average of three replication

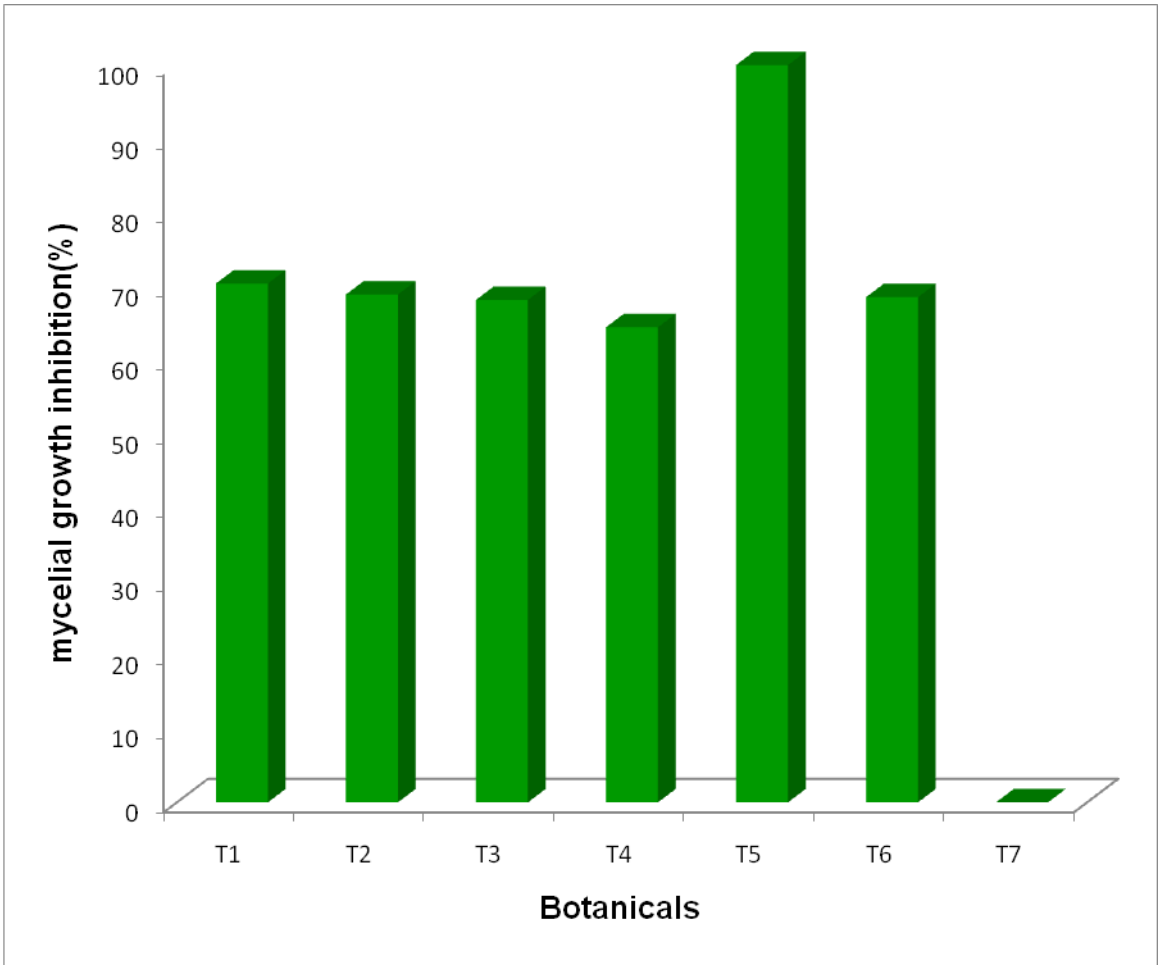


Fig. 3- Efficacy of botanicals against *Rhizoctonia solani*

T1- Tulsi leaves (*Ocimum sanctum*)
T2- Neem leaves (*Azadiracta indica*)
T3- Aloe vera leaves (*Aloe vera*)
T4- Karanj leaves (*Pongamia pinnata*)
T5- Garlic bulb (*Allium sativum*)
T6- Lemon grass (*Cymbopogon flexuosus*)
T7- Control

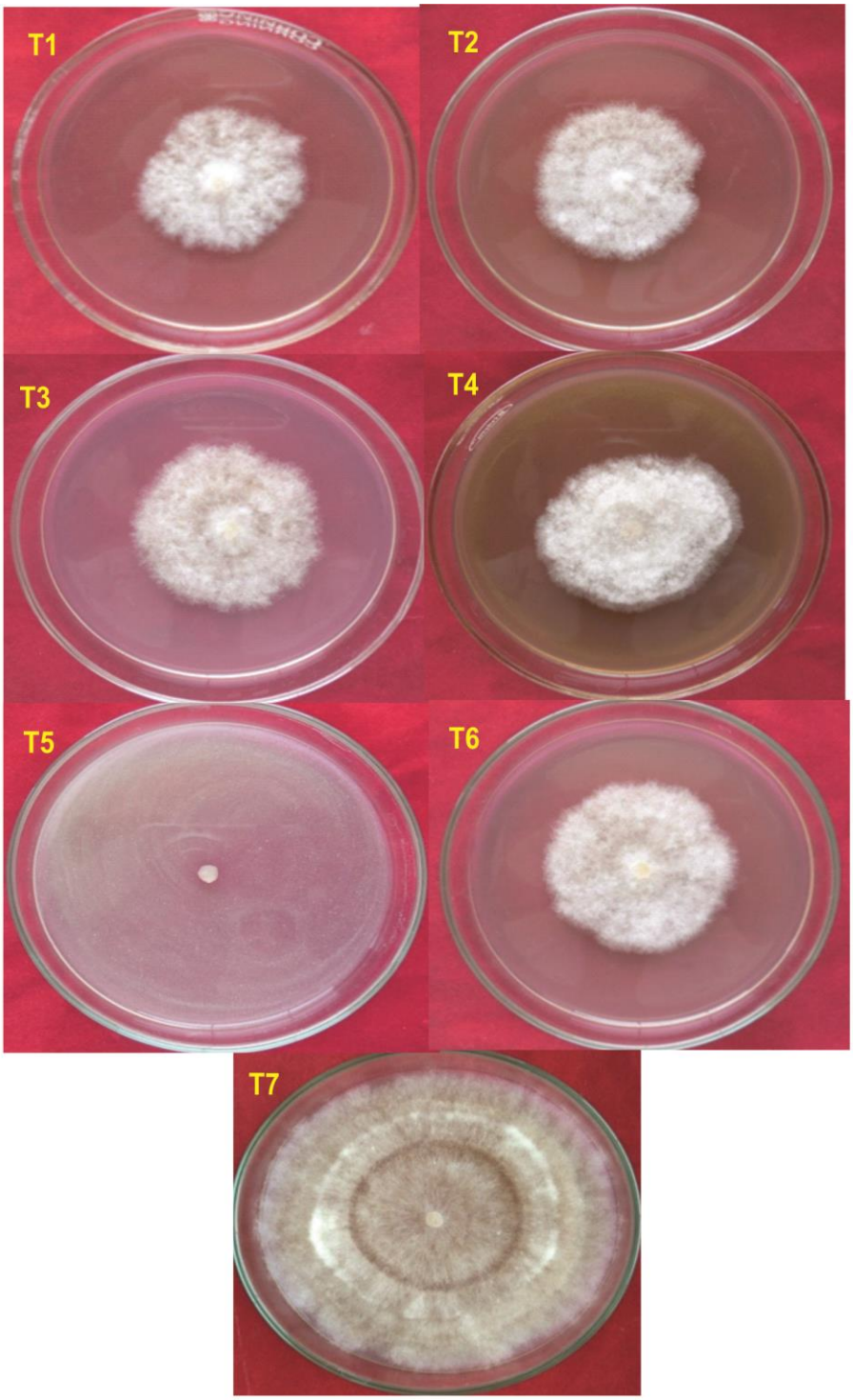


Plate 7: Efficacy of botanicals against *Rhizoctonia solani*

T1- Tulsi leaves (*Ocimum sanctum*)
T2- Neem leaves (*Azadirachta indica*)
T3- Aloe vera leaves (*Aloe vera*)
T4- Karanj leaves (*Pongamia pinnata*)
T5- Garlic bulb (*Allium sativum*)
T6- Lemon grass (*Cymbopogon flexuosus*)
T7- Control

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study entitled “SHEATH BLIGHT OF RICE CAUSED BY *Rhizoctonia spp.* AND IT’s MANAGEMENT” was undertaken in which isolation, identification, pathogenicity and mode of infection of pathogen *R. solani* and efficacy of fungicides, bio-agents and botanicals against *Rhizoctonia solani* were studied.

Sheath blight caused by fungal pathogen, *Rhizoctonia solani* is the second important disease reported in rice next to blast in terms of economic concern and food security that causes up to 25 per cent yield losses. As rice is the staple food for 65 per cent of the population, it is very much essential to avoid monetary loss due to effect of this disease. The complexity of the ecosystem poses problem in practicing traditional mode of protective measures in overcoming the disease. Even the steps taken with advanced technology like genetic engineering is not fool proof. Hence, use of chemical control, use of bio-agents and botanicals is the last resort among the odds.

The pathogen *Rhizoctonia solani* isolated from infected rice sheath and leaf by tissue isolation method on PDA media. The isolated pathogen identified by morphological characteristics of *R. solani* that the mycelial and sclerotial characters of *R. solani* produced dark brown mycelium, which larger sized sclerotia and dark brown in color.

The pathogenicity test done by injection method showed symptoms development on artificial inoculated leaf sheaths of young rice seedlings commenced on 5^{th} 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 lesion enlarged gradually covering the larger area of the leaf sheath.

The mode of infection through soil and seed inoculation causes the seed rot and seedling infection to the rice plant that indicates

the pathogenic nature of *Rhizoctonia solani* through soil and seed inoculation.

The results of the present study revealed that, all the seven fungicides tested inhibited the mycelial growth of *Rhizoctonia solani* when compared to the control. Of these, Propiconazole 13% + Difenoconazole 13.9% SC, Propiconazole 25% EC, Tebuconazole 50% + Trifoxystrobin 25% WG, Hexaconazole 5% EC and Carbendazim 50% WP completely inhibited the radial growth of the fungus at test concentrations on Potato dextrose agar medium. The fungicide Azoxystrobin and Validamaycin found least effective inhibiting 21.11% and 30.37% radial mycelial growth respectively.

Among the bio-agents against, *Trichoderma reesei* (73.04%) was the best performer followed by *Trichoderma asperellum* (71.38%), *Pseudomonas fluorescens* (64.44%) and *Bacillus subtilis* (61.38%) against *R. solani*.

The complete inhibition of the fungal mycelium of *R. solani* (100.0%) was observed in garlic bulb extract which was significantly superior to rest of the treatments. It was followed by tulsi leaves (70.37%), neem leaves (68.88%), lemon grass leaves (68.52%), aloe vera leaves (68.15%) and karanj leaves (64.14%) respectively.

Conclusions:

1. *Rhizoctonia solani* was predominantly associated in collected disease samples.
2. *Rhizoctonia solani* was pathogenic to rice crop causing sheath blight of rice.
3. In soil and seed inoculation method, seed rot (43% and 62%) and seedling infection (57% and 38%) was observed respectively.
4. Among the fungicides tested Propiconazole+Difenconazole , Propiconazole , Tebuconazole+Trifoxystrobin , Hexaconazole and Carbendazim are the most effective fungicides against *Rhizoctonia solani* which shows 100% mycelial inhibition.

5. Highest growth inhibition of *Rhizoctonia solani* was recorded with *Trichoderma reesei* (73.05%) followed by *Trichoderma asperellum* (71.38%) and *Pseudomonas fluorescens* (64.44%) .
6. Among the botanicals tested complete growth inhibition of *R*. *solani* was observed in Garlic bulb extract (100.0%) followed by tulsi leaves extract (70.37%).

CHAPTER VI

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1.	B.Sc. (Agri.)	2016	First Class	Dr. P.D.K.V., Akola	Agriculture

6. Research papers published (if any) : Nil

7. Field of Interest : Research and Development in Plant Pathology.

Date: / / 2019

Place: Akola **(USENDI PUNAM NAMDEO)**

APPENDIX-I

Fungicides

Sr.No.	Trade name	Common name	Active ingredients	Formulation
1	Taspa	Propiconazole+Difenconazole	13%+13.9%	SC
2	Tilt	Propiconazole	25%	EC
3	Nativo	Tebuconazole+Trifoxystrobin	50%+25%	WG
4	Amistar	Azoxystrobin	23%	SC
5	Vamcin	Validamaycin	3%	L
6	Contaf	Hexaconazole	5%	EC
7	Bavistin	Carbendazim	50%	WP

APPENDIX-II

Media and broth used with their composition

1. Potato Dextrose Agar

Sr. No.	Composition	g/liter
1.	Potato	200.0
2.	Agar-Agar	20.0
3.	Dextrose	20.0
4.	Distilled water	1000ml

2. Nutrient agar

Sr. No.	Composition	g/liter
1.	Beef extract	3.0
2.	Yeast extract	2.0
3.	Peptone	5.0
4.	Sodium chloride	5.0
5.	Agar	20.0
6.	Distilled water	1000ml