

**STUDIES ON SHEATH ROT OF RICE CAUSED BY  
*Sarocladium oryzae* (Sawada) Gams and Hawksworth**

**Thesis submitted to the  
University of Agricultural Sciences, Dharwad  
in partial fulfillment of the requirements for the  
Degree of**

**MASTER OF SCIENCE (AGRICULTURE)**

**IN**

**PLANT PATHOLOGY**

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**JUNE, 2012**

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# 1. INTRODUCTION

Rice (*Oryza sativa* L.) commonly known as Asian rice is an important cereal crop grown throughout the world. It is a self pollinated crop belongs to the family *Poaceae*, sub family *Bambusoideae* or *Ehrhartoideae*, tribe *Oryzeae*. It is a staple food crop of 60 percent of world's population. The rice was first domesticated in the region of the Yangtze River valley in China. Today, the majority of all rice produced comes from China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Japan. More than 90 percent of the world's rice area is in Asia, which is the home for more than half of world's poor and more than half of world's rice cultivators (Rao *et al.*, 2010).

Rice is normally grown as an annual crop, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years. The edible seed of rice is a grain (caryopsis). Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by the humans. In 100 g weight of rice grains, it has a nutritional value of 365 k cal of energy which is higher than any other cereal crop, easily digestible Carbohydrates 80 g, Sugars 0.12 g, Dietary fiber 1.3 g, Fat 0.66 g, Protein 7.13 g, Thiamine 0.0701 mg, Riboflavin 0.0149 mg, Niacin 1.62 mg, Pantothenic acid 1.014 mg, Vitamin B<sub>6</sub> 0.164 mg, Calcium 28 mg, Iron 0.80 mg, Magnesium 25 mg, Manganese 1.088 mg, Phosphorus 115 mg, Potassium 115 mg, Zinc 1.09 mg and 11.6 g of water. Its biological value is as high as egg protein, due to high content of essential amino acids.

The edible use of rice includes *viz.*, rice flakes, puffed rice, and canned rice. It is used in starch and brewing industries. By product by rice milling *i.e.*, rice husk and bran are used as cattle and poultry feed. Rice bran is used for extracting the cooking oil.

Rice is one of the diverse crop grown in different agro-climatic conditions and it is the second largest produced cereal in the world. At the beginning of 1990s, annual production was around 350 million tons by the end of the century it had reached 410 m tonnes. More than 550 million tons of rice is produced annually around the globe. Area under rice is 37 percent of the total area under grains as against 20 percent under wheat, another important cereal crop. Most of the world's rice is grown in tropics which include South and South East Asia, West Africa, Central and South America (Panda, 2009).

Rice is an important food crop in India, cultivated in the *kharif*, *rabi* and summer seasons. The area under cultivated rice in India accounts for 39.47 m ha with a production of 87.10 m tonnes and productivity 2207 kg/ha (Anon., 2011). Major rice growing states in India are West Bengal, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka, Tamil Nadu and Uttar Pradesh.

In Karnataka rice cultivated in an area of 14.47 lakh ha, with a production of 33.28 lakh tonnes and productivity of 2.3 tonnes/ha (Anon., 2011). In Karnataka it is largely grown in Koppal, Davangere, Bellary, Chamaraj Nagar, Mandya, Mysore, Raichur, Tumkur, Kolar, Gadag, Chitradurga under irrigated condition. In the high rain fall areas it is being grown under drill sown condition (Dharwad, Belgaum, Haveri and Uttar Kannada).

Rice suffers from many of the diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non parasitic disorders. Among the fungal foliar diseases such as blast caused by *Pyricularia grisea* (Cooke) Sacc., brown spot of rice caused by *Drechslera oryzae* (Brada, de Haan) Subram., leaf scald caused by *Rhynchosporium oryzae* Hashioka & Yokogi are important and fungal diseases of stem, leaf sheath and root such as stem rot caused by *Sclerotium oryzae* Catt., bakanae disease and foot rot disease caused by *Gibberella fujikuroi* (Sawada) Ito., sheath blight caused by *Rhizoctonia solani* Kuhn, sheath rot caused by *Sarocladium oryzae* (Sawada) Gams and Hawksworth are important. Among the fungal diseases of grain and inflorescence false smut caused by *Claviceps oryzaesativa* and udbatta disease caused by *Ephelis oryzae* are important.

Sheath rot of rice is present in all rice growing countries worldwide. Sheath rot has become more prevalent in recent decades. Densely planted fields and those infested by stem borer are susceptible to *Sarocladium oryzae* infection. The fungus tends to attack the leaf sheaths enclosing young panicles, which retards or aborts the emergence of panicles.

Seeds from infected panicles become discolored and sterile, thereby reducing grain yield and quality significantly. Sheath rot of rice caused by *S. oryzae* is a seed borne pathogen mostly observed on the entire seed (about 46%) and on the lemma and/or palea (about 31%) (Mew and Gozales, 2002). Since, the pathogen attacks the crop at maturity during panicle initiation stages, its impact is direct to minimize the crop yields. The yield losses from 20 to 85 per cent in Taiwan and 30 to 80 per cent in Vietnam, the Philippines and India (Anon., 2012).

Survey on the disease in the field gives information about the extent of sheath rot disease affecting the crop. Sheath rot disease is very common in rainfed rice or rice during rainy season; however, systematic survey on the incidence and severity in northern Karnataka is lacking. Considering the above facts, survey was undertaken to know the incidence and severity of sheath rot in Dharwad, Belgaum, Uttara Kannada, Raichur, Yadagir, Koppal districts and parts of Andhra Pradesh (East Godavari and West Godavari).

Studies on cultural, morphological, and physiological features of the pathogen are of immense use in understanding the nature of the pathogen.

Variation is the rule in most of the fungi. The variation may arise following change in crop cultivation, genetic modification of hosts, environment or accidental induction of new genetic material into a region or local gene pool. It may also be a way of survival of the pathogen under adverse conditions. There is a need to identify the molecular variability in *S. oryzae* so that, the breeding for disease resistance can be taken up to a specific race in the locality. Hence identifying variability of different isolates by morphology and at molecular level through RAPD was added.

The screening for disease resistance is essential to identify the resistant variety/source. Resistant variety is one of the best ways in reducing loss due to disease. However, there is a need to screen the genotypes against sheath rot of rice. Hence, screening of genotypes was included.

Management of the sheath rot disease depends on application of fungicides. Effective management of disease can be achieved by applying recommended fungicides at recommended intervals. There are large number of chemicals available in the market as fungicides and their bioefficacy and suitability needs to be verified by *in vitro* and in field studies. Fungicides are heterogeneous group of compounds used to manage the loss due to fungal pathogens. Hence, screening of fungicides to control sheath rot disease is most essential, so as to incorporate the effective ones in the management package.

In view of the above facts, the present investigation was therefore initiated to elucidate some of the aspects of the pathogen, host and relative damage caused by pathogen with the following objectives.

1. Survey for the incidence of sheath rot disease in north Karnataka and parts of Andhra Pradesh.
2. To study the morphological, cultural, physiological and molecular variability of the pathogen.
3. To screen for disease resistance in different rice genotypes.
4. To study the management of the disease by using chemicals.

## 2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is an important staple food crop of predominant population of the country and it is grown in tropical and sub tropical areas. One of the limiting factor in its cultivation is the loss due to various diseases. Among them, sheath rot caused by *Sarocladium oryzae* (Sawada) Gams and Hawksworth, is an economically important disease in many rice growing countries of the world. The yield losses from 20% 85% in Taiwan and 30% to 80% in Vietnam, the Philippines and India (Anon., 2012). The literature on the history, symptomatology, survey, pathogenicity, cultural, morphological, physiological studies, variability, screening for disease resistance and chemical control of the disease is reviewed hereunder.

### 2.1 History and symptomatology

#### 2.1.1 History

Rice sheath rot was first reported by Sawada (1922) from Taiwan and the causal organism was named as *Acrocylindrium oryzae* Sawada. Subsequently Kawamura (1940) reported its occurrence in Japan. Tasuki and Ikeda (1956) reported the sheath rot from Japan, Chen and Chien (1964) from south east Asia, Jimenez and Panizos (1974) from Peru and Shahjahan *et al.* (1977) also reported from United States of America. Agnihotrudu (1973) first reported the occurrence of sheath rot of rice in India. Subsequently several workers reported sheath rot incidence from different parts of the country.

Prabhakaran *et al.* (1973) observed the disease in south Arcot district of Tamil Nadu; Amin *et al.* (1974) reported this disease from parts of Andhra Pradesh and West Bengal; Subramanian and Ramakrishnan (1975) recorded sheath rot in parts of Tiruchinapalli district of Tamil Nadu; Nair and Rajan (1976) reported from Kerala; Nair (1976) from Orissa; Koteswara rao (1977) from Andhra Pradesh and Ghuffran *et al.* (1980) from Bihar.

Muralidharan and Venkata Rao (1980), Raina and Singh (1980) reported the outbreak of sheath rot on rice from Nellore districts of Andhra Pradesh and Punjab respectively. Seneviratne (1980) first observed the disease in Sri Lanka during early part of the 1970's. Purkayastha and Amin Ghosal (1982); Sadasiva reddy and Ghosh (1985); and Suriachandra Selven and Ranganathan (1989) reported the occurrence of sheath rot disease from West Bengal, Andhra Pradesh, Rajasthan and Tamil Nadu, respectively. Gill *et al.* (1993) reported the occurrence of sheath rot for the first time from Pakistan on susceptible cultivar Basumati 385.

#### 2.1.2 Symptomatology

Tasuki and Ikeda (1956) observed that the pathogen mainly infected the upper most leaf sheath of rice and the presence of wound on the upper most leaf sheath at the boot leaf stage facilitated easy infection. Ou (1972) reported that the lesions started as an oblong or irregular spots of 0.5-1.5 cm in length with brown margins and grey centre or grayish brown throughout. The spots enlarged, coalesce and covered most of the leaf sheath. The young panicles remained within the sheath or partially emerged. An abundant whitish powdery growth was found inside the affected sheath and the panicles choked.

Agnihotrudu (1973) reported that grayish brown lesions occurred on the upper most leaf sheath enclosing the young panicles with powdery masses of conidia at the centre and the panicles did not emerge or partially emerged or distorted.

Amin *et al.* (1974) observed that the occurrence of choking and sheath rot symptoms with discolored grains was common on dwarf varieties but varied with varieties, locations and seasons. Subramanian and Ramakrishnan (1975) reported that the occurrence of dark brown to chocolate brown spots on upper sheaths resulting in either partial or incomplete emergence of panicles and seed sterility were the chief symptoms of the disease. Koteswara rao (1977) reported that *A. oryzae* produced oblong chocolate colored spots on the top of the leaf sheath and the spots coalesced to form dark brown areas. Chen and Thseng (1981) observed that the number of infected panicles as well as brown spots and sterile grains were higher in indica varieties than in japonica rice varieties.

Tripathi (1982) reported that rotting occurred on the upper leaf sheath enclosing young panicles; oblong lesions on the flag leaf coalesce quickly and covered most of the sheaths. Powdery growth of the fungus was observed inside the affected leaf sheath. The spots produced by *S. oryzae* were oblong or irregular measuring 5-20 mm long with reddish brown lesions and grayish centre or grayish throughout the upper leaf sheath. Lower sheaths were also affected and superficial pink powdery growth of the fungus was visible on all the sheaths. Infection reached the stem and caused brownish and rotting of the panicle. Panicles partially emerged or remained inside the sheath. Emerged panicles had chaffy grains or partially filled thin grains. Upadhyay and Diwakar (1984) reported that the disease was associated with reduced spikelets per panicle as well as grain weight. Extensive rotting of the sheaths caused significant losses and glume discoloration.

Lakshmanan (1991) reported that nearly seven percent of rice plants at the boot leaf stage showed disease symptoms mostly on the sheath enclosing the young panicle, irregular dark brown lesions covered much of the sheath, panicle emergence from diseased plants were mostly blocked. Narain (1992) said that the disease could be diagnosed by non emergence and partial emergence of rice panicle along with dark to chocolate colored unfilled grains. Dark diseased lesions may be surrounded by light brown yellow lesions. The disease first appears on boot leaf and topmost sheath covering the panicles.

## 2.2 Survey

Sudarshan rao (1975) stated that survey and surveillance form the basis for any successful plant protection strategy. Successful plant protection depends upon early detection of the disease incidence followed by timely adoption and application of preventive measures. Chao yen Hsich (1977) reported empty – head due to association of *Stenotarsonemus madacassus* Gutierrez and sheath rot fungus *Acrocyllindrium oryzae* Sawada severe in southern Taiwan, and occurred sporadically in central and northern areas. About 17,000 ha was affected in some major rice growing areas.

Shahjahan *et al.* (1977) conducted survey in five major rice growing areas of south eastern Louisiana. He observed sheath rot of rice caused by *Acrocyllindrium oryzae* was widely distributed in all the five rice growing areas of Louisiana. Of the tillers examined, 11.4% showed symptoms of sheath rot. Srinivasan (1981) reported that heavy infection by *S. oryzae* resulted in greater yield loss in Thanjavur delta of Tamil Nadu. Singh (1988) surveyed eastern Uttar Pradesh and found incidence of sheath rot in all 5000 visited sites.

Girish (1999) conducted survey in different locations of Karnataka and he identified sheath rot incidence ranged from 26.4 to 44.5% in Bangalore area, and observed the maximum incidence in Tumkur 47.3%.

Devaraju *et al.* (2011) collected seeds from various places of Southern transition zone of Karnataka and used for the study by employing blotter paper method. Twelve fields and five storage fungi were found associated with 41 varieties sampled, out of these 36 varieties were infected. The fungus *Sarocladium oryzae* was totally absent.

## 2.3 Isolation and proving pathogenicity

### 2.3.1 Isolation

Sakthivel and Gnanamanickam (1987) isolated *S. oryzae* from naturally infected rice plants with severe sheath rot symptoms. Pure cultures were maintained on Potato Glucose Agar. Gnanamanickam and Mew (1991) isolated sheath rot pathogen *S. oryzae* from infected rice tissues and seeds.

Lakshmanan *et al.* (1992) isolated *S. oryzae* on Potato Dextrose Agar medium. Leaf pieces taken from the edge of lesions were surface disinfected with commercial sodium hypochlorite (30% a.i.) for five minutes, rinsed with three changes of sterile deionized water and placed on Potato Dextrose Agar. Further purification of cultures was carried out by a single spore method.

Sakthivel *et al.* (2002) isolated *S. oryzae* from diseased rice sheaths and purified through single spore cultures. Vivekananathan and Rabindran (2008) isolated the fungus *S. oryzae* from portions of infected boot leaf sheath showing typical lesions. The fungus was purified by using single hyphal tip technique.

### 2.3.2 Proving pathogenicity

Amin *et al.* (1974) inoculated plants with stem tape inoculation method or by spraying a conidial suspension of *S. oryzae* on the plants. Symptoms observed and pathogen was reisolated from the inoculated plant. Reisolated cultures from artificially infected plants were found to be similar to cultures that were used in inoculations.

Raju and Singh (1978) tested five methods of inoculation. Out of five methods tested inoculation by the fungal culture grown on bajra grains was observed to be the best and gave maximum percentage of sheath rot affected panicles.

Pratima (1993) observed maximum discolored grains when inoculated at flowering stage of the rice plants, with sprayed spore suspension of *S. oryzae* and she identified the fungus as the virulent grain discoloring pathogen compared to other pathogens. Deka and Phookan (1997) used rice grain culture of the isolated fungus for proving pathogenicity. Susceptible cultivar IR 50 were inoculated using single grain inoculation technique and symptoms of sheath rot disease were observed.

Hazarika and Phookan (1998) tested three inoculation techniques *viz.* single grain insertion, spraying and injecting spore suspension. Out of three, inoculation with single grain culture inside flag leaf sheath was found most effective and recorded highest sheath infection (96.7%). Ghosh *et al.* (2002) and Vivekananathan and Rabindran (2008) proved the pathogenicity by placing the infected grains in between boot leaf sheath and panicle. The inoculated plants were observed for development of symptoms under glass house.

## 2.4 Cultural and morphological studies

### 2.4.1 Cultural studies

Shahjahan *et al.* (1977) reported that colonies of *S. oryzae* appear white and cottony with a light pinkish orange color at the reverse on potato dextrose agar. Mohan and Subramanian (1978) observed the fungus *Acrocylium oryzae* Sawada, grew and sporulate well in potato dextrose agar and oats agar media. Among the four liquid media selected, maximum growth and sporulation were in Czapek's medium.

Boa and Brady (1987) reported *S. oryzae* produces whitish pale pink or saffron colonies on oat meal agar at 21°C day light, reverse pink to orange ochre. Bridge *et al.* (1989) observed all the strains isolated from bamboo blight caused by *Sarocladium* spp. in Bangladesh showed a bright yellow pigmentation in the colony and colony reverse on nitrite sucrose agar and a buff brown pigmentation in colonies on ammonium oxalate agar.

Mithrasena and Wijesundera (1992) observed the maximum growth of the fungus *S. oryzae* on the seed extract agar medium and highest sporulation was on potato dextrose agar followed by the host extract media. Brady (1998) reported cultures of *S. oryzae* on 2% malt extract agar are pale saffron, compacted with cottony growth.

Vivekananathan and Rabindran (2008) identified the cultures of *S. oryzae* on PDA was pale, saffron coloured, compact and cottony.

### 2.4.2 Morphological studies

Tasuki and Ikeda (1956) reported differences in conidial measurements of *Acrocylium oryzae* obtained from the host plant (2.1-8.5 x 0.5-1.6 µm) and from culture (1.8-13 x 1-1.6 µm). Ahamed *et al.* (1974) described *S. oryzae*. The mycelium is white, septate, sparsely branched. Conidiophores are once or twice branched each time having 3-4 branches in a whorl. Conidia were borne on the tip and these were hyaline, smooth, cylindrical and single celled.

Shahjahan *et al.* (1977) gave morphological description of *Acrocyndrium oryzae* fungus as colorless, septate mycelium with 1.5-3  $\mu\text{m}$  in diameter. Conidiophores were single or branched, 15-25  $\mu\text{m}$  long, secondary branches in whorls of 2-5 phialides, 13-19  $\mu\text{m}$  long. Conidia are borne singly at the conidiophores tip, hyaline, smooth, cylindrical, single celled, 3-7 x 1-2  $\mu\text{m}$  in cultures on PDA inoculated at 32<sup>o</sup>c. Variations in the size of conidia were observed, when the fungus grown in different culture media.

Ou (1972) and Mew and Gozales (2002) described the fungus *S. oryzae* morphologically. Mycelium white, sparsely branched, septate with 1.5-2  $\mu\text{m}$  in diameter. Conidiophores are shortly thicker than the vegetative hyphae, branched once or twice, each time with 3-4 branches in a whorl. Conidia borne simply on the tip, produced consecutively, hyaline, smooth, single celled, cylindrical and 4-9x 1-2.5  $\mu\text{m}$  in dimension.

Brady (1998) described microscopic characters of *S. oryzae*. Hyphae are 1-1.5  $\mu\text{m}$  wide with some irregular granulated hyphae upto 2.5  $\mu\text{m}$  diameter, conidiophores abundant, sometimes with several branches arising in whorls.

Conidiogenous cells were phialidic, arising from the conidiophores or directly from undifferentiated hyphae, 30-40 x 1.5-2  $\mu\text{m}$  when arising from the former or 6-20 x 1-1.5  $\mu\text{m}$  when borne directly on the hyphae, tapering gradually to 0.5-1  $\mu\text{m}$  at the tips which have no collarette. Conidia formed singly, cylindrical with rounded ends, sometimes slightly curved, hyaline, smooth, non septate, 3.5-7 x 1-1.5  $\mu\text{m}$ , collecting in slimy masses at the tips of the phialides.

Vivekananathan and Rabindran (2008) reported microscopic structures of *S. oryzae*. Hyphae measured 1-1.5  $\mu\text{m}$  wide, conidiophores were verticillate, abundant and bearing conidia singly. Conidia were cylindrical with rounded ends, hyaline, one celled and 3.5-7 x 0.8-1.5  $\mu\text{m}$ .

## 2.5 Physiological studies

### 2.5.1 Effect of pH

Tasuki and Ikeda (1956) stated good mycelia growth of sheath rot fungus at 6.4 pH. Cochrane (1958) stated an initial pH of 5 to 7 was satisfactory for majority of fungi. Mohan and Subramanian (1978) reported that growth of the pathogen was maximum at pH 6.5 followed by 7.0 and 6.0, while poor growth was recorded at pH 7.5 and 5.5 levels on PDA.

Swapan and Purkayastha (1978) observed the spores of *S. oryzae* germinated at a pH range of 5 to 8 with an optimum range between 5.5 to 6.0. Shahjahan *et al.* (1977) reported maximum growth and sporulation of *S. oryzae* obtained at pH of 7.5 in PDA and pH 6.5 in potato dextrose broth.

Mithrasena and Wijesundera (1992) observed growth of *S. oryzae* maximum at 3.5 and sporulation highest at pH 5.5 on czepeck- dox liquid medium at 30<sup>o</sup>C.

### 2.5.2 Effect of temperature

Kawamura (1940) reported that *Acrocyndrium oryzae* grew well at 30-31<sup>o</sup>C. While Tasuki and Ikeda (1956) stated 20-28<sup>o</sup>C as optimum temperature for the growth of the pathogen, and very slow growth at 37<sup>o</sup>C with virtually no growth at 13<sup>o</sup>C. It was killed when incubated for 5 minutes at 50<sup>o</sup>C.

Shahjahan *et al.* (1977) reported the fungus *S. oryzae* grew and form conidia best on PDA at 32<sup>o</sup>C and on corn meal agar at 28<sup>o</sup>C. Mohan and Subramanian (1978) reported maximum growth and sporulation of the fungus *S. oryzae* on oat agar medium at 30 $\pm$ 1<sup>o</sup>C.

Mithrasena and Wijesundera (1992) reported the optimum temperature for growth and sporulation of *S. oryzae* varied with the medium. On potato dextrose agar it was 30<sup>o</sup>C and on the host extract media it was 25<sup>o</sup>C and spore germination was highest at 25<sup>o</sup>C.

### 2.5.3 Effect of different sugars

Mohan and Subramanian (1978) reported sucrose and starch recorded maximum growth and sporulation of *S. oryzae* followed by glucose and maltose in Czapek's dox medium.

Mahapatra *et al.* (1977) recorded maximum growth of *Altrnaria sesami* on mannitol followed by lactogen starch. Mathur and Sarbay (1977) reported pentose sugars poorly supported the growth of *Alternaria alternata* while sucrose supported maximum growth.

## 2.6 Toxin studies

The term vivotoxins, pathotoxins, phytotoxins and specific toxins have been defined by early workers (Dimmond and Waggoner, 1953; Wheeler and Luke, 1963). Several phytotoxins produced by microorganisms were reported to be responsible for the production of disease in plant (Owens, 1969). Many plant pathogenic microorganisms produced toxic metabolites in culture media and plant tissues, which were involved in the disease syndrome (Wood *et al.*, 1972).

Production of toxin by any fungus *in vitro* had been provided by a number of bioassay methods of plant cutting seed germination bioassay, root and shoot elongation bioassay (Anahosur, 1976). Sakthivel and Gnanamanickam (1986) found to produce cerulenin which, when applied to rice sheaths, induced rotting similar to that produced by *S. oryzae*.

Gnanamanickam and Mew (1991) observed cerulenin, the toxin produced by *S. oryzae* showed a toxicity pattern towards rice pathogens similar to that of *S. oryzae*. The stem rot pathogen was most sensitive to cerulenin. Velazhahan (1991) reported the culture filtrate of *S. oryzae* showed pronounced inhibitory effect on seed germination in all varieties. The shoot and root lengths of all races were also reduced significantly. Maximum reduction in the percent germination, shoot length and root length was observed in IET-8611.

Reddy *et al.*, (2000) observed the culture filtrates of *S. oryzae* reduced seed germination to the extent of 33% in paddy variety Prakash, shoot length to the extent of 42% in IR20 and root length to the extent of 27.5% in Prakash. Ghosh *et al.* (2002) detected heloviolic acid and cerulenin in infected rice grains by *S. oryzae* and these metabolites induced chlorosis and reduced the seed viability and seedling health of rice.

Sakthivel *et al.* (2002) detected the secondary metabolites; helovic acid and cerulenin were detected in liquid culture of *S. oryzae*, causal agent of sheath rot of rice and also in diseased sheath tissues.

## 2.7 Variability studies

### 2.7.1 Morphological variability

Shahjahan *et al.* (1986) tested two isolates 283-P, 1183-W of *S. oryzae* to obtain information on the variation in growth and virulence. No difference in morphology was found. 1183-W grew better than 283-P in both semisolid and liquid media. The mean disease severity of the 37 entries was significantly higher for isolate 283-P.

Phookan and Hazarika (1994) collected eight isolates of sheath rot pathogen *S. oryzae* from different localities of North – East India. Morphometric variation among the isolates was insignificant.

The isolates responded differently to different media, temperature and pH levels. Pathogenic variability among the isolates was significant. Based on the observed variability, isolates categorized into three distinct groups.

Reddy *et al.* (2000) collected nine isolates of *S. oryzae* from different locations. RNR, AMB & RYP isolates exhibited similarities in conidiophores morphology, physiological requirements, virulence and protein and ester banding patterns. MTU- NLR and CO-ADT isolates also exhibited similarities in the characters studied. Finally, the isolates were categorized into five groups.

## 2.7.2 Molecular variability studies

The genetic variability in 36 isolates of *Fusarium udum* Butler, collected from four pigeonpea growing states in India and assessed using Random Amplified Polymorphic DNA (RAPD) and Amplified Length Polymorphism (AFLP) techniques. Cluster analysis of similarity index data from the two DNA markers classified the isolates into three major groups suggesting the existence of a minimum of three specific races of pathogen prevailing in pigeonpea growing areas in India (Sivaramakrishna *et al.*, 2002). The pathogenic and genetic diversities among the isolates of *Sclerospora graminicola*, the pearl millet downy mildew pathogen, collected from various pearl millet cultivars in India. The dendrogram based on mean disease incidence data classified the 15 isolates into 5 major virulence groups. Genetic diversity was assessed using RAPD & AFLP (Sivaramakrishna *et al.*, 2003).

Niraikulam *et al.* (2005) detected the variability in pathogenicity phytotoxic metabolite production and DNA polymorphisms among the *S. oryzae* isolates. According to their level of similarity, *S. oryzae* isolates from north east and south India were grouped separately into two major clusters and 13 genotypes. A high level of genetic variability within *S. oryzae* isolates was identified.

Nine isolates of *Rhizoctonia bataticola* the incitant of dry root rot of chickpea were assessed for genetic diversity /variability using RAPD markers. Isolates differed in their RAPD banding pattern. The similarity value of RAPD profiles ranged from 0.062 (UPR & DUR) to 0.5 (NHR & HAR) with an average of 0.283 among the isolates. The cluster analysis using unweighed pair group method with arithmetic average distinguished *R. bataticola* isolates into different fingerprinting groups (Lekraj *et al.*, 2007). The genetic variation of isolates of *Fusarium oxysporum* fsp *ricini* was studied by Random Amplified Polymorphic DNA analysis. The isolates were grouped into five clusters based on molecular polymorphism generated by RAPD primers. The grouping of isolates based on pathogenic variation has no correlation with grouping based on RAPD analysis with most of the test isolates (Santhalakshmi Prasad *et al.*, 2008).

Seven isolates of *Macrophomina phaseolina* the incitant of maize charcoal rot from major maize growing agro-ecological zones of India, were analyzed through RAPD markers for genetic diversity. The UPGMA cluster analysis for 706 loci score permitted identification of three main clusters. Similarity matrix and Jaccard's similarity coefficients between the isolates indicated that the maximum genetic variation among the isolates of Arabhavi and Coimbatore with 70.8 per cent followed by Ludhiana and Coimbatore with 69.5 per cent. The most closely related isolates were Hyderabad and Delhi with an affinity percentage of 65.5 followed by Udaipur and Bangalore isolate with a percentage of 62.9 per cent (Meena *et al.*, 2006).

## 2.8 Screening of rice genotypes to sheath rot disease resistance

Chen and Chien (1964) reported that incidence of sheath rot disease varied with rice varieties in Taiwan. Amin *et al.* (1974) observed that high yielding dwarf rice cultivars were severely affected by sheath rot pathogen. Ahmed *et al.* (1974) reported that the rice varieties *viz.*, Karikalan, Karuna and Culture 7711 were susceptible, while BJ1, TKM6 and Sigadis were completely free from disease at Aduthurai (Tamil Nadu).

Subramanian and Ramakrishnan (1975) reported the reaction of released varieties and promising cultivars for sheath rot under field conditions at their booting stage. It was found that Annapoorna, IET-2234, Kannagi, Soorya, Cauvery, ADT-30, Jayj, Ratna and Karuna were most susceptible. The tall rice varieties *viz.*, Ramtulasi, Monoharasali, ARC-7117 and Raminand cultivars were highly resistant to sheath rot. Animapal (1975) reported that out of ten varieties studied, Kanchi was the least susceptible, while IR-8 exhibited maximum infection at Chinsurah, West Bengal.

Kannaiyan *et al.* (1978) tested 130 varieties under field conditions and found that five varieties *viz.*, AS-5821, AS-2992, AS-1287, AS-3226 and 6403 were free from sheath rot while 18 cultivars were found resistant. Raju and Singh (1978) reported that among different varieties of rice screened against sheath rot, Sigadis and Homthong were resistant, VRR1-71-21 and Ratna were moderately resistant, Jayantha and Vijay were susceptible.

Singh and Raju (1981) reported that among different varieties screened, five varieties viz., Basumati-370, Chinsurah, Boro-II, homthong, sigadis and TKM-6 were resistant to sheath rot disease on artificial inoculation. Upadhyay and Diwakar (1984) reported that among the several important commercially grown rice varieties tested Asha, Usha, Sabri17, Dibraj and Madhuri showed resistant reaction to sheath rot.

Paromithamukerjee *et al.* (1985) studied the response of indigenous rice cultivars against sheath rot under artificial inoculation and reported varieties viz., Kasha, Tinpakhya, Sonkharcha and Mutri from early maturing group and Masuri small, Anjani and Motipandy from the late maturing group were found resistant to the disease.

Saroja *et al.* (1987) evaluated rice entries resistant to sheath rot under field conditions and reported that out of 64 entries screened, 13 were resistant, 14 intermediate and 37 susceptible to sheath rot. Lewin and Vidyasekaran (1987) evaluated 59 rice varieties for sheath rot incidence under very high disease pressure and reported that among 59 varieties evaluated 10 were resistant to *S. oryzae*.

Singh (1988) reported that among 134 different genotypes screened for sheath rot, only four genotypes viz., Bishinbhog, Gajgaur, Kalanamak, and Kodya gave highest resistant score. Nagaraju *et al.* (1991) tested 49 rice genotypes for the sheath rot incidence and reported that 15 genotypes showed good level of resistance. Lakshman and Lelusamy (1991) evaluated 87 lines with their pedigree in field and green house experiments and reported that among 87 lines tested 15 showed no symptoms under natural infection while, only few exhibited high level of resistance on artificial inoculation.

Mohanty *et al.* (1996) screened 60 deep water rice selections to sheath rot disease and reported that the cultivars viz., MDR40049, CN 1035-61 and OR-090-3-158 recorded resistant reaction to this disease having less than one per cent infection. Of the rest, 32 showed moderate reaction and other 25 showed moderately susceptible reaction. Mishra *et al.* (1996) evaluated 76 lines of diverse origin for sheath rot disease. 15 were found to be resistant. Early maturing and dwarf genotype Shakti 1 identified as a source of resistance to sheath rot.

Sahu and Parida (1997) studied the response of 60 rice breeding lines against sheath rot. Out of 60 lines tested, three lines were highly resistant, 18 were resistant and 33 were moderately resistant to the disease. Reddy *et al.* (2002) evaluated 76 rice cultures from different locations in Andhra Pradesh belonging to three duration groups, i.e. early, medium and long for the resistance against sheath rot pathogen under field conditions. Among them Swarnadhan showed resistance reaction. Yadav and Thrimurthy (2006) screened wild races to know the resistant donor. Out of 260 entries screened only two viz., VS99 and *Oryza latifolia*-100963 scored resistant.

## 2.9 Management of the disease by using chemicals

### 2.9.1 *In vitro* evaluation of fungicides

Mohan (1976) found that systemic fungicides like benlate and bavistin were more effective than any other group of fungicides in inhibiting the pathogen. However diathane M-45 and hinosan both at 1000 and 2000 ppm concentrations were found satisfactory in inhibiting the growth of the pathogen.

Vijayaraghavan (1976) found that growth of *A. oryzae* was completely inhibited by benlate and bavistin followed by hinosan, demoston, maneb and zineb. Pourkayastha and raychoudri (1977) evaluated six systemic fungicides viz., benomyl, MBC, vitavax, DDVP and aureofungin against *S. oryzae* and reported that MBC was the most effective fungicide being toxic to pathogen even at a concentration of 5 ppm.

Reddy *et al.* (1983) reported that bavistin was the best inhibitor of *A. oryzae*. Reddy *et al.* (1985) reported that bavistin proved as the most effective fungicide, followed by blue copper and anthracol among 11 fungicides evaluated against *S. oryzae*. Mithrasena and Wijesundera (1992) tested several fungicides benlate was very effective at low concentrations in inhibiting conidia germination, growth and sporulation of the fungus.

Pandiarajakumar *et al.* (1998) reported that propiconazole was the effective fungicide in inhibiting the mycelia growth and spore germination followed by penconazole and metalaxyl, wettable sulphur and pyrethrin were ineffective. Further they also found that mancozeb significantly inhibited the spore germination, germ tube elongation, mycelia growth and sporulation of *S. oryzae*.

### 2.9.2 *In vivo* evaluation of fungicides

Efficacy of carbendazim and benlate (benomyl) was also recorded by Chen and Huang (1979). Raina and Singh (1980) and Chinnaswamy *et al.* (1981) reported that the carbendazim was the most effective fungicide for the control of sheath rot followed by MBC, aureofungin and difolaton.

Viswanathan and Miriappan (1980) reported that the pathogen *S. oryzae* was controlled by prophylactic and therapeutic spray of carbendazim, carboxin, kitazin, wet ceresin and PCNB in pot culture studies. Vasan *et al.* (1984) observed from field tests that foliar application of benthocarb at 2 kg a.i. /ha controlled the disease.

Singh and Raju (1981) found that three sprays of bavistin, carboxin, benlate or brestinol gave good control of the disease and also increased grain yield. Lakshmanan (1984) reported that carbendazim 100g plus calixin 100 ml/ha or calixin 200 ml/ha controlled sheath rot incidence effectively. Application of carbendazim was most effective in controlling the disease followed by mancozeb and copper oxychloride. The highest average yield was obtained from carbendazim sprayed plots (Dhal and Choudhary, 1987).

Vidhyasekaran and Lewin (1987) reported that application of captafol 0.125 per cent had lowest sheath rot intensity compared to other fungicides. Murthy (1986) reported that hinosan, bavistin and dithane M-45 significantly reduced the *S. oryzae* infection compared to other fungicides tested. Pratima ray (1993) tested six fungicides at 0.05, 0.1 and 0.2 per cent concentration against *S. oryzae* and reported carbendazim was found effective in controlling the infection at all concentrations.

Viswanathan and Narayanaswamy (1993) studied the efficacy of mancozeb, edifenphos and tricyclazole against sheath rot and reported that application of mancozeb plus tricyclazole two times and mancozeb four times were superior in controlling sheath rot. Dodan *et al.* (1996) reported that application of carbendazim and propiconazole once at boot leaf stage and 15 days later significantly reduced the sheath rot incidence and increased the yield.

Das and Nayak (1997) evaluated six fungicides against sheath rot and observed that two sprays of thiophanatemethyl or carbendazim at 0.1 per cent were found highly effective in controlling the disease with significant increase in grain yield.

Thapak *et al.* (2003) studied nine fungicide formulations. Among them the fungicides tilt, bavistin and antracol were found to be superior in reducing the severity of sheath rot disease.

Lore *et al.* (2007) measured efficacy of eight fungicides namely tilt 25 EC, bavistin 50WP, contaf 5EC, dithane Z-78, kitazin 48 EC, saaf 75 WP and rhizocin 3L were compared. Tilt 25 EC effective against sheath rot followed by bavistin 50WP.

Phookan (2011) formulated a location-specific eco-friendly viable Integrated Disease Management (IDM) module for managing sheath rot disease of rice under the dominant rice-rice cropping system in Assam. Wet seed treatment with salicylic acid (100 ppm) for 24 hr. + root dip treatment with salicylic acid (100 ppm) for 4 hours before planting + application of Rice Husk Ash (RHA) at the time of land preparation @ 5 ton/ha + need based foliar spray with carbendazim (0.1%) upon initial symptom appearance was very effective in managing the disease to the extent of 90.42 per cent.

### 3. MATERIAL AND METHODS

The present studies on sheath rot of rice were carried out during 2011-12 in the Department of Plant Pathology, College of Agriculture, Dharwad. Field experiment was carried out at Agricultural Research Station, Mugad, University of Agricultural Sciences Dharwad. Mugad is situated at 15° 15' North latitude and 74° 40' East longitude and at an altitude of 697 m above the mean sea level. The station lies in Northern transitional tract (Zone-VIII) of Karnataka, representing the rainfed drill sown rice belt.

The details of materials used and the methodology followed during the course of investigation are described here under.

#### 3.1 General laboratory procedure

##### 3.1.1 Glassware cleaning

For all laboratory experimental studies Corning and Borosil glasswares were used. The glasswares were kept for 24 hrs in cleaning solution containing 60.0 g of potassium dichromate ( $K_2Cr_2O_7$ ), 60.0 ml of concentrated sulphuric acid ( $H_2SO_4$ ) in 1000 ml of water and were washed with soap powder followed by washing in running tap water and then finally rinsed with distilled water.

##### 3.1.2 Sterilization

All the glasswares were sterilized in an autoclave at 1.1  $kg/cm^2$  pressure for 20 minutes and further sterilized in hot air oven at 160° C for two hrs. Sterilization of both solid and liquid media was achieved by autoclaving at 1.1  $kg/cm^2$  (121.6° C) pressure for 20 minutes for all the laboratory studies.

#### 3.2 Disease survey and surveillance

The intensive roving survey was conducted during *kharif* 2011-12, to know the incidence and severity of sheath rot in the farmers fields in Belgaum, Dharwad, Uttara Kannada, Yadagir, Koppal and Raichur districts in Karnataka, East Godavari and West Godavari districts in Andhra Pradesh. In each district, five villages were selected, in each village two fields were surveyed. In field plants were selected in zig-zag manner and the severity of sheath rot disease of rice was recorded by following 0-9 scale (Anon., 1996).

Grade	Reaction
0	No infection
1	Small lesions
3	5% area of boot leaf sheath with lesions
5	6-25% area of boot leaf sheath with lesions
7	26-50% area of boot leaf sheath with lesions
9	>50% area of boot leaf sheath with lesions

$$\text{Percent disease incidence} = \frac{\text{Number of infected tillers}}{\text{Total number of tillers}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\text{Area of leaf sheath infected}}{\text{Total leaf sheath area}} \times 100$$

Per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

### 3.2.1 Collection and isolation of the pathogen

Rice boot leaf sheaths and grains infected with sheath rot were collected from different places *viz.*, Belgaum, Dharwad, Uttar Kannada, Yadgir, Koppal, Raichur, East Godavari and West Godavari during survey and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique as described below.

The infected portions along with some healthy parts were cut and surface sterilized using 1 per cent sodium hypochlorite solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants and incubated at room temperature ( $27 \pm 1^\circ\text{C}$ ) and observed periodically for fungal growth and sporulation. Colonies, which developed from the bits, were identified by microscopic observation by taking mycelial and spore character as means for identifying the pathogen. After identification they were transferred to new PDA slants and incubated at  $27 \pm 1^\circ\text{C}$  for further use.

### 3.2.2 Single spore isolation

Ten ml of clear filtered two per cent water agar was poured into the sterile Petriplates and allowed to solidify. Diluted spore suspension was prepared in sterilized distilled water from 12 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at  $27 \pm 1^\circ\text{C}$  for 12 hrs. Then such plates were examined under microscope so as to locate germination of conidia. Single isolated germinated conidium was marked with ink on the lower surface of the plates.

The growing hyphal tip portion was transferred to fresh PDA slants with the help of cork borer under aseptic conditions and incubated at  $27 \pm 1^\circ\text{C}$ . Such obtained pure culture tubes were used for further studies.

### 3.2.3 Maintenance of the culture

The fungus was sub cultured on PDA slant and allowed to grow at  $27 \pm 1^\circ\text{C}$  for 12 days. Such slants were preserved in refrigerator at  $5^\circ\text{C}$  and renewed once in a month. This pure culture was used for future study.

### 3.2.4 Identification of the fungus

The morphological characters of the fungus such as mycelial and cultural characters, length and breadth of conidia were studied by using microscope at 400X magnification.

### 3.2.5 Proving the pathogenicity

Artificial inoculation of the fungus was carried out to prove the pathogenicity. The rice seedlings were raised in pots and inoculated with grains incubated in fungal culture.

Rice seeds of 30 g were autoclaved in 250 ml of erlenmayer flasks after overnight presoaking. The flasks were inoculated with the fungal culture and incubated for ten days at room temperature. For inoculating the plants, the grains along with the inoculum were placed singly in between the flag leaf sheath and unemerged panicles at the time of panicle initiation stage.

The inoculated pots were incubated in growth chamber to maintain high relative humidity (90%) for expression of symptoms. The plants were monitored for expression of symptoms which appeared one week after incubation. Sheath rot appeared as brown spots with yellow halo on boot leaf sheath. The fungus was re-isolated from infected boot leaf of the plant and the culture obtained was compared with original culture for confirmation.

### 3.3 Cultural and morphological studies

#### 3.3.1 Growth phase of *S. oryzae* on Potato dextrose broth (PDB) and Richard's broth (RB)

Thirty ml of potato dextrose broth (PDB) and richard's broth (RB) were added into each of 100 ml conical flask and sterilized at 1.1 kg/cm<sup>2</sup> pressure for 20 minutes at 121°C. After strelization these flasks were allowed to cool and then inoculated with 5 mm disc from 12 days old culture and incubated at room temperature. Each treatment was replicated three times. The culture was filtered through Whatman No.1 filter paper of 9 cm diameter, which were dried to a constant temperature at 60°C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to remove traces of salts likely to be associated with it. Three flasks were harvested at 72 hrs after incubation and subsequent harvesting was done at an interval of three days up to 30 days.

The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed immediately on an analytical balance. The difference between final and initial weight of filter disc was taken as the weight of the mycelial mat. The data were analyzed statistically.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

#### 3.3.2 Growth characters of *S. oryzae* on different solid media

The cultural characters of single spore Dharwad isolate of *S. oryzae* was studied on eight non synthetic/semi-synthetic and two synthetic solid and liquid media.

- I. Non synthetic or semi synthetic media
  - i. Potato dextrose agar
  - ii. Oatmeal agar
  - iii. Host leaf extract agar
  - iv. Potato sucrose agar
  - v. Host leaf extract agar with sucrose
  - vi. Potato dextrose agar with biotin
  - vii. Host leaf extract agar with biotin
  - viii. Sabouraud dextrose agar
- II. Synthetic media
  - i. Richards' agar
  - ii. Czapek (Dox) agar

The composition and preparation of the above mentioned synthetic and semi-synthetic media were obtained from Ainsworth and Bisby's 'Dictionary of the Fungi' by Ainsworth (1971) and plant pathological methods, fungi and bacteria by Tuite (1969). The modified media were prepared either by changing the sugar or by adding the vitamin Biotin. The composition of the media is given below.

##### I. Potato dextrose agar (PDA)

In most of the experimental studies the Potato dextrose agar (PDA) was used. The composition of PDA is as follows

Potato (peeled) 200.00 g  
Dextrose 20.00 g  
Agar-agar 20.00 g  
Distilled water 1000.00ml (to make up)

Two hundred grams of peeled and cleaned potato was made into small pieces. Later these pieces were boiled in tap water and then extract was collected by filtering through muslin cloth. Dextrose 20.0 g and agar 20.0 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water. Known quantity of such medium was dispensed into a number of conical flasks and plugged with non-absorbent cotton and finally wrapped with brown paper. The flask containing dispensed medium were sterilized at 1.1 kg/cm<sup>2</sup> pressure for 20 minutes.

#### II. Oatmeal agar

Oatmeal 60.00 g  
Agar-agar 12.50 g  
Distilled water 1000.00 ml (to make up)

The ingredients were suspended in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized.

#### III. Host leaf agar (HLA)

Host leaf 200.00 g  
Dextrose 20.00 g  
Agar-agar 20.00 g  
Distilled water 1000.00 ml

Two hundred grams of rice leaves were cut into small bits, boiled in 500 ml tap water for 30 minutes and extract was collected by filtering through muslin cloth. Dextrose 20 g and agar-agar 20 g each were dissolved in the leaf extract and the final volume was made up to 1000 ml with distilled water and sterilized.

#### IV. Potato sucrose agar

Potato (peeled) 200.00 g  
Sucrose 20.00 g  
Agar-agar 20.00 g  
Distilled water 1000.00 ml (to make up)

Two hundred grams of peeled and cleaned potato was made into small pieces. Later these pieces were boiled in tap water and then extract was collected by filtering through muslin cloth. Sucrose 20.0 g and agar-agar 20.0 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized as described earlier.

#### V. Host leaf extract with sucrose

Host leaf 200.00 g  
Sucrose 20.00 g  
Agar-agar 20.00 g  
Distilled water 1000.00 ml

Two hundred grams of rice leaves were cut into small bits, boiled in 500 ml tap water for 30 minutes and extract was collected by filtering through muslin cloth. sucrose 20 g and agar-agar 20 g each were dissolved in the leaf extract and the final volume was made up to 1000 ml with distilled water and sterilized.

VI. Potato dextrose agar with biotin (modified)

Potato (peeled)	200.00 g
Dextrose	20.00 g
Biotin	0.05 mg
Agar-agar	20.00 g
Distilled water	1000.00ml (to make up)

Two hundred grams of peeled and cleaned potato was made into small pieces. Later these pieces were boiled in tap water and then extract was collected by filtering through muslin cloth. Dextrose 20.0 g, agar-agar 20.0 g and biotin 0.05 mg each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water.

VII. Host leaf extract agar with biotin (modified)

Host leaf	200.00 g
Dextrose	20.00 g
Biotin	0.05 mg
Agar-agar	20.00 g
Distilled water	1000.00 ml

Two hundred grams of rice leaves were cut into small bits, boiled in 500 ml tap water for 30 minutes and extract was collected by filtering through muslin cloth. Dextrose 20 g, agar-agar 20 g and biotin 0.05 mg each were dissolved in the leaf extract and the final volume was made up to 1000 ml with distilled water and sterilized.

VIII. Sabouraud dextrose agar

Dextrose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	40.00 g
Peptone	10.00 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to one litre and sterilized as described earlier.

IX. Richards' agar

Sucrose (C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	20.00 g
Potassium nitrate (KNO <sub>3</sub> )	10.00 g
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	5.00 g
Magnesium sulphate (MgSO <sub>4</sub> ·7H <sub>2</sub> O)	2.50 g
Ferric chloride (FeCl <sub>3</sub> ·6H <sub>2</sub> O)	0.02 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

All the ingredients except Potassium dihydrogen phosphate was dissolved separately in 450 ml of distilled water. Agar melted in 500 ml distilled water was mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml water and mixed together to get and final volume of 1000 ml and sterilized.

X. Czapek (Dox) agar

Sucrose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	30.00 g
Sodium nitrate (NaNO <sub>3</sub> )	2.00 g
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.00 g
Magnesium sulphate (MgSO <sub>4</sub> ·2H <sub>2</sub> O)	0.50 g
Potassium chloride (KCl)	0.50 g
Ferric chloride (FeCl <sub>3</sub> ·6H <sub>2</sub> O)	0.01 g
Agar-agar	20.00 g
Distilled water	1000.00 ml

Agar-agar was melted in 500 ml distilled water. Ingredients were dissolved in remaining 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml and sterilized.

Twenty ml of each medium listed above was poured aseptically into 90 mm diameter Petriplates. After solidification, five mm discs of the *S. oryzae* were selected from actively growing culture using a cork borer and a single disc placed at the center of Petridish. Each set of experiment replicated five and they were incubated at  $27 \pm 1^\circ \text{C}$  for 40 days. Cultural characters such as the colony diameter, colony colour and sporulation were recorded. The sporulation was graded as follows.

Sl. No.	Score	Grade	Conidia/microscopic field (400x)
1.	++++	Excellent	> 75
2.	+++	Good	51-75
3.	++	Moderate	26-50
4.	+	Poor	1-25
5.	-	No sporulation	-

### 3.3.3 Growth studies of *S. oryzae* on different liquid media

The composition and preparation of different liquid media used, were the same as that of solid media except that the agar was not added. All the liquid media were sterilized and the flasks were inoculated under aseptic condition and were incubated at  $27 \pm 1^\circ \text{C}$  for 24 days. The mycelia growth harvested, dried and weighed. The best non-synthetic/ semi-synthetic and synthetic media was found and used as a basal medium for further studies.

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

### 3.3.4 Spore Morphology

A loopful of culture of *S. oryzae* obtained from twelve days old culture was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread and cover slip was placed. Length and breadth of one hundred spores were measured using motic images in computer (400 X). The average size of the spore was calculated. Similarly, the spores produced in the host leaf tissue were also measured and the average size was calculated.

## 3.4 Physiological studies

### 3.4.1 Effect of pH on the growth of *S. oryzae*

Effect of on pH on the growth of fungus was tested at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Thirty ml of potato dextrose broth (PDB) was added into each of 150 ml conical flask and sterilized at  $1.1 \text{ kg/cm}^2$  pressure for 20 minutes at  $121^\circ \text{C}$ . After sterilization these flasks were allowed to cool and then inoculated with 5 mm disc from 12 days old culture and incubated at  $27 \pm 1^\circ \text{C}$  for 24 days. For each pH level three replications were maintained. After incubation period the culture was filtered through Whatman No.1 filter paper of 9 cm diameter. Dry mycelial weight was recorded for each flask as described earlier.

### 3.4.2 Effect of temperature on the growth of *S. oryzae*

Effect of temperature on the growth of fungus was tested at 15, 20, 25, 30, 35 and  $40^\circ \text{C}$ . Thirty ml of potato dextrose broth (PDB) was added into each of 150 ml conical flask and sterilized at  $1.1 \text{ kg/cm}^2$  pressure for 20 minutes at  $121^\circ \text{C}$ . After sterilization these flasks were allowed to cool and then inoculated with 5 mm disc from 12 days old culture and incubated at their respective temperatures for 24 days.

For each temperature level five replications were maintained. After incubation period the culture was filtered through Whatman No.1 filter paper of 9 cm diameter. Dry mycelial weight was recorded for each flask.

### 3.4.3 Effect of sugars on spore germination of *S. oryzae*

The effect of different forms of sugars *viz.*, glucose, sucrose, lactose and maltose at different concentrations 1.0, 1.5 and 2.0 percent on spore germination of *S.oryzae* was observed by preparing the spore suspensions of  $1 \times 10^2$  dilution with the above sugars at given concentrations. Each treatment replicated three times. The observations on percentage spore germination were recorded at 24 h after incubation. The per cent germination was calculated using the formula:

$$PG = \frac{A}{B} \times 100$$

Where,

PG = Per cent germination

A = Number of conidia germinated

B = Total number of conidia examined

## 3.5 Toxin studies

### 3.5.1 Production of toxic metabolites by *S. oryzae*

Culture filtrates of *S. oryzae* obtained by using potato dextrose broth as basal medium. Aliquots of thirty ml of basal medium was dispensed in 100 ml conical flask and sterilized. Each flask was inoculated with 5 mm disc from 12 days old culture. The flasks were incubated for 30 days at room temperature. Each treatment replicated three times. One set of flasks were harvested on third day after incubation and subsequent harvesting was done at an interval of three days up to 30 days. The culture was filtered through Whatman No.1 filter paper. The culture filtrate obtained was used for further studies.

### 3.5.2 Effect of culture filtrate on germination, shoot and root length of sorghum seeds

This experiment was conducted as per the procedure followed by Anahosur (1976). One hundred healthy sorghum seeds were surface sterilized in 1% sodium hypochlorite solution and washed with sterile distilled water to remove the traces of sodium hypochlorite. The sorghum seeds were soaked in culture filtrates for 30 minutes. They were spread on moistened germination paper. Equal numbers of healthy seeds were soaked in the sterile distilled water and potato dextrose broth, which served as control.

Observations on germination of sorghum seeds were recorded after seven days. Per cent inhibition of seed germination was calculated by the formula given by Vincent (1947). Further root and shoot lengths were recorded in each treatment and seedling vigour index was calculated.

$$\text{Seedling vigour} = (\text{Shoot length} + \text{Root length}) \times \text{Germination percentage}$$

## 3.6 Variability studies

### 3.6.1 Morphological variability of different isolates of the pathogen

The experiment was conducted in order to study the variation in the morphological characters of different isolates of *S. oryzae*. For this purpose, 12 ml of oat meal agar was poured into 70 mm petriplates. Five mm mycelial disc of twelve day old culture of respective isolates was placed at the centre of the plate. Three replications were maintained at room temperature ( $27 \pm 1^\circ\text{C}$ ) for 15 days and colony character like; i) radial colony diameter, ii) color, iii) size of conidia iv) size of conidiophores for each isolates were noted.

A loopful of culture of each isolate was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread and cover slip was placed. Length and breadth of conidia and conidiophores were measured using motic images in computer (400 X). The average size was calculated.

### 3.6.2 Molecular variability

#### 3.6.2.1 RAPD (Genetic variability)

Random Amplified Polymorphic DNA (RAPD) analysis was used to detect the variations among the eight isolates of *S. oryzae* from different locations abbreviated as below. Standard protocols were used for the isolation of DNA and RAPD analysis. This technique uses a Polymerase Chain Reaction (PCR) method to amplify DNA with random primers. Using PCR, a random primer of arbitrary nucleotide sequence binds to the DNA at two different sites on opposite strands of the DNA templet. The primer sites are located at a close amplifiable distance from each other. This results in a DNA product by a thermocyclic reaction. This technique detects genetic polymorphism.

Designation of different isolates of *S. oryzae* obtained from different locations

Isolates of <i>S. oryzae</i>	Designation of isolates of <i>S. oryzae</i>
Belgaum	BGM
Dharwad	DWD
East Godavari	EGV
Koppal	KPL
Raichur	RCR
Uttar Kannada	UTK
West Godavari	WGV
Yadgir	YDG

### 3.6.2.2 DNA extraction

#### Requirements/stock solutions

Extraction buffer	: 1M Tris HCL 5M NaCl 0.5M EDTA 0.1% Mercaptoethanol 2% C-TAB 1% PVP 10% SDS
10% SDS	: Dissolved 10g of sodium dodecyl sulphate in 100 ml of distilled water
5M NaCl	: Dissolved 292g of NaCl in 1 litre of distilled water
Chloroform	: Isoamyl alcohol – 24:1
70% Ethanol	: Dilute 70 ml of alcohol in 30 ml of distilled water
T10E1	: 10 mM Tris 1 mM EDTA pH 8.0
100 µM random primers	
25 ng/ µl template DNA	
3 U/ µl taq DNA polymerase	
Rnase 10 µg/ml	

#### Procedure

The total genetic DNA of *S. oryzae* isolates was extracted from vegetative mycelial using the procedure Murry and Thompson (1980) with slight modifications.

1. Fungal mat of 0.5g grounded to fine powder in liquid nitrogen and transferred to sterile eppendorf tube.
2. To this, 1 ml of extraction buffer (1M Tris-5.0 ml, 5M NaCl-14.0 ml, 0.5M EDTA-0.2 ml, 0.1% Mercaptoethanol – 50 µl, 2% C-TAB-1.0 g, % PVP-0.5 g) was added and incubated for 1 hr in water bath at 65°C. A gentle inversion was done at every 10 minutes interval. Then, the tubes were centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was transferred into other tubes.
3. To the supernatant equal ml of chloroform and isoamyl alcohol (24:1) and RNase (1 µl /100 µl) was added and incubated at room temperature for 10-20 min. The tubes were centrifuged at 10,000 rpm for 10 min, separated the supernatant and added 0.6 volume of ice cold isopropanol + 0.1 volume of sodium acetate and incubated at -20°C for overnight.
4. Next day, the tubes were centrifuged at 13,000 rpm for 20 min at 4°C, the supernatant was discarded and the pellet washed with 70 per cent ethanol and centrifuged at 13,000 rpm for 20 min at 4°C.
5. Again the supernatant discarded, the pellet air dried and dissolved in 100 µl of sterile distilled water. The DNA samples were stored at -20°C for further studies.

### 3.6.2.3 Polymerase chain reaction

#### Requirements for PCR

##### 1. Random primers

Total 10 random primers were used which were ordered from Sigma Ltd.

##### 2. dNTP's

The four individual dNTP's such as dATP, dGTP, dCTP, dTTP were obtained from M/S Bangalore Genei, Pvt. Ltd., Bangalore.

### 3. Taq DNA polymerase

Taq DNA polymerase and 10X Taq buffer A were obtained from M/S Bangalore, Genei, Pvt. Ltd., Bangalore.

### 4. Thermocycler

Corbett Research Gradient PCR supplied by JH B10 Innovation Pvt. Ltd., Bangalore was used for cyclic amplification of DNA.

The thermoprofile for PCR

Sl. No.	Step	Temperature (°C)	Duration (min)	Number of cycles
1	Initial denaturation	95	5.0	1
2	Denaturation	95	1.0	40
3	Annealing	36	1.0	
4	Extension	72	2.0	
5	Final extension	72	8.0	1
6	Hold temperature	4	Forever	-

The PCR amplification for RAPD analysis was performed according to Williams *et al.* (1990) with certain modifications. The optimum conditions for DNA amplifications used were as follows. After the completion of the PCR, the products were stored at 4°C until the gel electrophoresis was done.

A total of 10 primers with the following sequences were used in the study as shown below.

Sequences of random primers used in RAPD analysis of different isolates of *S. oryzae*

Primers	Sequence
OPA-01	5'- CAGGCCCTTC-3'
OPA-05	5'-AGGGGTCTTG-3'
OPA-07	5'-GAAACGGGTG-3'
OPA-10	5'-GTGATCGCAG-3'
OPA-11	5'-CAATCGCCGT-3'
OPA-16	5'-AGCCAGCGAA-3'
OPA-17	5'-GACCGCTTGT-3'
OPB-11	5'-GTAGACCCGT-3'
OPB-13	5'-TTCCCCCGCT-3'
OPB-18	5'-CCACAGCAGT-3'

### Master mix for PCR

Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing following components. The total volume of each reaction mixture was 20  $\mu$ l. The cocktail for PCR amplification was found to be optimum were

1. 10X assay buffer with 15mM MgCl<sub>2</sub> 2.5  $\mu$ l
2. dTNP's mix (2.5 mM each ) 1.0  $\mu$ l
3. Primer (5 pM/  $\mu$ l) 1.0  $\mu$ l
4. Template DNA (25 mg/  $\mu$ l) 1.0  $\mu$ l
5. Sterile distilled water 14.30  $\mu$ l
6. Taq DNA polymerase (3.0 U/  $\mu$ l) 0.2  $\mu$ l

Extract template, the master mix was distributed to PCR tubes (19  $\mu$ l /tube) and later 1  $\mu$ l of template DNA from the respective isolates was added making the final volume of 20  $\mu$ l.

Separation of amplified products by agarose gel electrophoresis.

### Requirements

1. Electrophoretic unit : Gel casting through gel combs, power pack, UV-transilluminator
2. Agarose (1.2%)
3. Bromophenol blue
4. Ethidium bromide (0.5  $\mu$ g/ml<sup>-1</sup>)
5. 50X TAE (Stock): Tris-base-60.5 g, Glacial acetic acid – 14.25 ml, 0.5M EDTA – 25 ml and make up the volume to 250 ml, pH 8.0
6. Working solution (1X TAE): 20 ml by using distilled water

### Procedure

- Three grams of agarose was weighed and added to a conical flask containing 250 ml of 1X TAE buffer
- The agarose was melted by heating the solution on a oven and solution was stirred to ensure even mixing and complete dissolution of agarose.
- Three drops of ethidium bromide (0.5  $\mu$ g/ml) was added.
- The solution was mixed and poured into the gel casting platform after inserting the comb in the trough, while pouring sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1X TAE) so as to cover the wells completely.
- The amplified products (20  $\mu$ l) to be analyzed were carefully loaded into the sample wells, after adding bromophenol blue with the help of micro pipette.
- Electrophoresis was carried out at 60 volts, until the tracking dye migrated to the end of the gel.
- The gel was taken out from electrophoretic apparatus and stained by placing it in distilled water containing ethidium bromide (0.5  $\mu$ g /ml) for 10 min.
- Ethidium bromide stained DNA bands were viewed under transilluminator and photographed for documentation.

### 3.6.2.4 Scoring the amplified fragments

The amplified profiles for all the primers were compared with each other and the bands of DNA fragment were scored as '1' for the presence and '0' for the absence of a band generating the '0', '1' matrices.

The genetic similarity coefficient was estimated using NTSYS PC 2.0 software programme (Nei and Li, 1979). The clustering was done and dendrogram was drawn by following unweighted pair group with arithmetic mean (UPGMA) routine, using the above programme other parameters computed were

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

### 3.6.2.5 Quantitative verification of DNA

The quality of DNA was analyzed by running 2 µl of each sample mixed with 2 µl of 10X loading dye in 1% agarose gel. The DNA from all isolates produced clear sharp bands in one per cent agarose gel indicating the good quality of DNA.

## 3.7 Screening of genotypes

Experiment conducted during *kharif* 2011 at ARS, Mugad. Fourty four rice cultivars were selected and sown during August. Each entry was planted in one line of two meter length with spacing of 20 cm X 10 cm and two replications were made. The cultivars were used for screening under artificial inoculation by single grain insertion method (Hazarika and Phookan, 1998). Susceptible check IET-19396 was sown after every five test rows and in border areas. The varieties were grouped according to their reaction as per Standard Evaluation System.

## 3.8 Disease management

### 3.8.1 *In vitro* evaluation of fungicides against *S. oryzae*

The efficacy of five non-systemic (three combi- products) and five systemic fungicides were tested against *S. oryzae*. The non-systemic fungicides were tried at 0.1, 0.2 and 0.3 per cent concentration, whereas systemic fungicides were tried at 0.05, 0.1, 0.15 per cent concentrations. The list of fungicides used along with their chemical and trade names are given below.

Sl. No.	Non-systemic / Combi fungicides		Systemic fungicides	
	Common name	Trade name	Common name	Trade name
1.	Copper oxychloride	Blue copper-50%WP	Carbendazim	Bavistin 50% WP
2.	Mancozeb	Dithane M- 45 75 %WP	Hexaconazole	Contaf 5% EC
3.	Carbendazim 12%+ Mancozeb 63%	SAAF 75 %WP	Propiconazole	Tilt 25% EC
4.	Hexaconazole 4% + Zineb 64%	Avtar	Tricyclazole	Beam 75 WP
5.	Carbendazim 25% + Iprodion 25%	Quintal	Thiophanate methyl	Topsin M 70 WP

Various fungicides were evaluated under *in vitro* conditions by poison food technique against *S. oryzae*. Required concentrations were prepared by dissolving known quantity of fungicides in potato dextrose broth under aseptic conditions. The loopful of fungus inoculated into the poisoned broths and incubated for 24 days. The experiment was conducted in two factor completely randomised design (CRD). In each treatment three replications were maintained.

Observations are taken at 24 days after incubation. The dry mycelia weight in each treatment was taken and the per cent inhibition over the control was calculated by using the formula given by Vincent (1947).

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

Where,

PI = Per cent inhibition.

C = Dry mycelial weight in control.

T = Dry mycelial weight in treatment.

Angular transformations were made for data and analyzed statistically.

### 3.8.2 *In vivo* evaluation of fungicides against *S. oryzae*

An experiment was conducted in ARS, Mugad during 2011 *kharif*. The susceptible variety IET – 19396 was used for the studies. The experiment includes 10 foliar applications of fungicides one seed treatment and one untreated check with three replications. The artificial inoculation was done to get maximum disease with single grain insertion method at booting stage. After inoculation when the symptoms were observed the first spray was given. Second spray was given after 10 days of first spray.

Details of treatments

Treatments		Dosage
T <sub>1</sub>	Carbendazim	0.1%
T <sub>2</sub>	Mancozeb	0.25%
T <sub>3</sub>	Copper oxychloride	0.25%
T <sub>4</sub>	Propiconazole	0.1%
T <sub>5</sub>	Hexaconazole	0.1%
T <sub>6</sub>	Tricyclazole	0.1%
T <sub>7</sub>	Thiophanate methyl	0.1%
T <sub>8</sub>	Carbendazim+Mancozeb	0.2%
T <sub>9</sub>	Hexaconazole+Mancozeb	0.2%
T <sub>10</sub>	Carbendazim+Iprodione	0.2%
T <sub>11</sub>	Control with seed treatment	0.2%
T <sub>12</sub>	Control without seed treatment	-

Observations on disease severity was scored using 0-9 scale .The per cent disease index (PDI) and per cent disease reduction over control (PDC) was calculated and angular transformed data were analyzed statistically.

$$\text{PDC} = \frac{\text{PDIC} - \text{PDIT}}{\text{PDIC}} \times 100$$

Where,

PDC = Per cent disease index over control

PDIC = PDI in control

PDIT = PDI in treatment

### 3.8.3 Yield

At the time of harvest, yield from all the treatments were weighed as per replication and analysed statistically.

## 3.9 Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for completely randomized design and randomized block design and interpretation of data was carried out in accordance with Walter (1997). The level of significance used in 'F' and 'T' test was P=0.05 and P=0.01. Critical differences were calculated wherever 'F' test was significant. The values in percentages were subjected to angular transformation according to the table given by Sundarraj *et al.* (1974).

## 4. EXPERIMENTAL RESULTS

The results of the experiments conducted on various aspects on sheath rot of rice (*Oryza sativa* L.) during the period 2011 to 2012 with reference to survey and surveillance of disease; cultural, morphological, physiological aspects of pathogen; morphological and molecular variability of the pathogen; screening of rice genotypes against disease; management of disease by fungicides are presented here under.

### 4.1 Disease survey and surveillance

Roving survey was undertaken during 2011 – 2012 to assess the severity of sheath rot of rice in major rice growing areas of Dharwad, Belgaum, Koppal, Raichur, Uttar Kannada, Yadgir districts of Karnataka and East Godavari and West Godavari districts of Andhra Pradesh by taking at least two plots in each village, five villages in each district as explained in the “Material and Methods” and results are presented in Table 1a.

Percent disease incidence ranged from 8.5 to 35.59 with highest per cent incidence of 35.59 was recorded in Khanapur village of Belgaum district followed by 34.88 per cent in Kustagi (Koppal) and 31.37 per cent in Kelageri (Dharwad). The least per cent incidence 8.5 observed in Samalkot (East Godavari).

Among different locations per cent disease index (PDI) ranged from 6.67 to 37.78. Arabhavi (Belgaum) recorded highest PDI (37.78) followed by 33.33 in Daddikamalapur (Dharwad) and 29.00 in Nigadi (Dharwad). Least PDI 6.67 was recorded in Veeravalli (West Godavari), 8.89 in Timmapuram (East Godavari), Lingasgoor (Raichur) villages.

District wise severity of sheath rot of rice surveyed during 2011-12 given in Table 1b and Fig.1. The maximum per cent disease incidence (27.89) and per cent disease index (22.42) recorded in Dharwad followed by Belgaum (27.01 and 21.52). Lowest incidence (13.51) was recorded in East Godavari and lowest PDI (14.22) recorded in West Godavari.

Among the varieties grown in different districts (Table 1c) maximum per cent incidence was observed in sona masuri (29.69) followed by Sali batta (29.63) and Sirsi sanna (28.57) and least incidence was observed in swarna (10.41) and samba mahsuri (14.54). The PDI was more in Sali batta (33.33) followed by basumati rice (29.64), hybrid rice (28.89) and intan (28.89). The lowest PDI was observed in vani (10.00) and abhilash (11.11).

Among different types of soils on which the rice was grown, the incidence was more in black laterite soils (24.24) followed by red soils (23.25). PDI was maximum in black soils (20.68). Least incidence and PDI were observed in alluvial soils.

Within the two different sowing conditions, drill sowing showed highest per cent disease incidence of 27.29 and PDI of 21.21 compared to transplanting with per cent disease incidence 20.43 and PDI 17.08.

### 4.2 Symptomatology

The typical sheath rot symptoms were observed on boot leaf sheaths. Light brown colored necrotic spots with yellow halo appeared on the boot leaf sheaths. In advanced stages, spots covered the entire area of boot leaf sheath and panicle exertion was aborted. Brown discolored grains in infected panicle were also noticed.

### 4.3 Isolation of the pathogen

Standard tissue isolation technique was followed to obtain causal agent from the rice leaf sheaths showing typical sheath rot symptoms. Repeated isolation yielded a species of *Sarocladium*.

#### 4.3.1 Proving pathogenicity

Fungus was isolated from infected boot leaf sheath and pure culture was obtained by single spore isolation method as described in “Material and Methods” and such culture was used for pathogenicity test. Single grain insertion method with rice seeds incubated in fungal culture was used to prove the pathogenicity showed in Plate 1 and as explained in “Material and Methods”.

**Table1a: Survey on severity of sheath rot of rice caused by *Sarocladium oryzae* in northern Karnataka and Andhra Pradesh during *kharif* 2011-12**

Sl. No.	District	Village	No. of plots	Soil	Type of cultivation	Cropping system	Varieties	Per cent disease incidence	Per cent disease index	Other diseases
1	Dharwad	Honnapur	2	Black soil	Drill sowing	Rice + vegetables	Doddiga	23.64	15.56	Blast, BLB in traces
		Daddikamalapur	2	Black soil	Drill sowing	Rice + vegetables	Siri 1253	26.23	13.33	Blast, BLB in traces
		Kelageri	2	Red soil	Drill sowing	Rice + Fallow	Sona masuri	31.37	20.98	Blast, Leaf blight
		Nigadi	2	Black soil	Drill sowing	Rice + vegetables	Basumati	28.57	28.89	Blast
		Khalagatigi	2	Red soil	Drill sowing	Rice + Vegetables and pulses	Sali batta	29.63	33.33	BLB, Blast
Mean								27.89	22.42	
2	Belgaum	Khanapur	2	Black soil	Drill sowing	Rice + vegetables	Sona masuri	35.59	20.89	Blast, BLB in traces
		Arabhavi	2	Black soil	Drill sowing	Rice + vegetables	Basumati	29.63	37.78	Blast, BLB
		Bailhangal	2	Red soil	Drill sowing	Rice + Rice	Abhilash	24.00	11.11	Blast, BLB
		Gundenatti	2	Black soil	Drill sowing	Rice + Rice	Doddiga	20.37	15.56	Blast, BLB
		Bidi	2	Black soil	Drill sowing	Rice + vegetables	Basumati	25.49	22.27	Blast, BLB
Mean								27.01	21.52	
3	Uttara Kannada	Sirsi	2	Black soil	Transplanting	Rice + Rice	Asha	25.00	18.89	Blast, BLB
		Sirsi farm	2	Red soil	Transplanting	Rice + Rice	Sona masuri	25.49	14.44	BLB
		Alnavar	2	Red soil	Drill sowing	Rice + vegetables	Rasi	27.08	28.89	Blast

**Survey on severity of sheath rot of rice caused by *Sarocladium oryzae* in northern Karnataka and Andhra Pradesh during *kharif* 2011-12**

		Yellapur	2	Black soil	Drill sowing	Rice + vegetables	Jaya	28.21	20.00	Blast
		Siddapur	2	Red soil	Transplanting	Rice + Rice	Sirsi sanna	28.57	17.78	Blast, BLB
Mean								26.87	20.00	
4	Yadgir	Shalapur	2	Black soil	Transplanting	Rice + Rice	Jaya	22.50	28.89	Blast, BLB, Leaf blight
		Surpur	2	Red soil	Transplanting	Rice + vegetables	Jaya	17.86	11.11	Blast, BLB
		Yadagir	2	Red soil	Transplanting	Rice + vegetables	Sona masuri	26.32	17.78	Blast, Leaf blight
		Kembhavi	2	Black soil	Transplanting	Rice + vegetables	Rasi	16.13	20.00	Blast
		Surpur campus	2	Red soil	Transplanting	Rice + Rice	Jaya	30.56	13.33	BLB, Blast
Mean								22.67	18.22	
5	Koppal	Kukanur	2	Black laterite soil	Transplanting	Rice + vegetables	Shakti	24.24	17.78	BLB, Blast
		Gangavati farm	2	Black soil	Transplanting	Rice + Rice	Hybrid rice	27.27	28.89	False smut in traces, BLB
		Sivaguppa	2	Black soil	Transplanting	Rice + Rice	Vani	21.05	11.11	Blast
		Gangavati	2	Black soil	Transplanting	Rice + Rice	Mangala	17.65	20.00	Blast
		Kustagi	2	Black soil	Transplanting	Rice + Rice	Intan	34.88	28.89	BLB, Blast
Mean								25.01	21.33	
6	Raichur	Raichur farm	2	Black soil	Transplanting	Rice + Rice	Shakti	21.49	17.78	BLB, Leaf blight
		Lingasgoor	2	Black soil	Transplanting	Rice + Rice	Vani	22.27	8.89	BLB, Blast
		Asihal	2	Black soil	Transplanting	Rice + vegetables	Jaya	20.00	22.22	Blast, sheath blight
		Kavital	2	Red soil	Transplanting	Rice + Rice	Shakti	16.57	14.44	BLB, Blast, Sheath blight
		Siravar	2	Black soil	Transplanting	Rice + Rice	Vani	26.92	28.89	BLB, Blast
Mean								21.45	16.44	

**Survey on severity of sheath rot of rice caused by *Sarocladium oryzae* in northern Karnataka and Andhra Pradesh during *kharif* 2011-12**

7	East godavari	Timmapuram	2	Black soil	Transplanting	Rice + Rice	Samba mahsuri	10.5	8.89	Blast, Sheath blight, BLB
		Penumarti	2	Black alluvial soil	Transplanting	Rice + Rice	Swarna	12.0	13.33	Blast, Sheath blight, BLB
		Samalkot	2	Black soil	Transplanting	Rice + Rice	Swarna	8.5	11.11	Blast, BLB
		VKpuram	2	Black soil	Transplanting	Rice + Rice	Samba mahsuri	16.57	24.44	Blast , BLB, Leaf blight
		Sirivada	2	Black soil	Transplanting	Rice + Rice	MTU 1001	20.00	17.78	Blast , BLB
Mean								13.51	15.11	
8	West godavari	Vatluru	2	Black soil	Transplanting	Rice + Rice	Swarna	12.5	13.33	Blast , BLB, Leaf blight
		Kalaparru	2	Black soil	Transplanting	Rice + Rice	MTU 1001	14.93	15.56	Blast, Sheath blight, BLB
		Veluru	2	Black soil	Transplanting	Rice + Rice	MTU 1001	20.00	20.0	Blast , BLB
		Veeravalli	2	Alluvial soil	Transplanting	Rice + Rice	Swarna	8.67	6.67	Blast, Sheath blight, BLB
		Satrapadu	2	Black soil	Transplanting	Rice + Rice	Samba mahsuri	16.57	15.56	Blast , BLB, Leaf blight
Mean								14.53	14.22	

**Table 1b: District wise mean severity of sheath rot of rice caused by *S. oryzae* in northern Karnataka and Andhra Pradesh during *kharif* 2011-12**

District	Mean percent incidence	Mean Percent disease index
Dharwad	27.89	22.42
Belgaum	27.01	21.52
Uttara Kannada	26.87	20.00
Yadgir	22.67	18.22
Koppal	25.01	21.33
Raichur	21.45	16.44
East godavari	13.51	15.11
West godavari	14.53	14.22

**Table 1c: Variety wise mean severity of sheath rot of rice caused by *S. oryzae* in northern Karnataka and Andhra Pradesh during *kharif* 2011-12**

Variety	Percent disease incidence	Percent disease index
Doddiga	22.0	15.56
Siri 1253	26.23	13.33
Sona masuri	29.69	18.52
Basumati	27.89	29.64
Sali batta	29.63	33.33
Abhilash	24.00	11.11
Asha	25.00	18.89
Rasi	21.60	24.45
Jaya	22.73	16.56
Sirsi sanna	28.57	17.78
Shakti	20.76	13.33
Hybrid rice	27.27	28.89
Vani	21.66	10.00
Mangala	17.65	20.00
Intan	34.88	28.89
Samba mahsuri	14.54	16.29
Swarna	10.41	11.11
MTU 1001	18.31	17.78



**Fig. 1: Severity of sheath rot of rice in major rice growing areas of northern Karnataka and Andhra Pradesh**

Fig 1: Severity of sheath rot of rice in major rice growing areas of northern Karnataka and Andhra Pradesh

On fourth day of inoculation, brown colored water soaked spot was seen on boot leaf sheath at the place of inoculation. On sixth day the spot turned light brown surrounded by chlorotic halo, while on eighth day the spot enlarged with chlorotic halo having irregular margins and were not delimited by veins. Later the spot started enlarging further as shown in Plate 2. In the cases, where the panicles inserted with the rice grains incubated with fungus before panicle exertion stage, more discoloration of grains was observed. The fungus was re-isolated and pathogenic culture thus obtained was compared with the original culture of *S. oryzae*.

#### 4.3.2 Identification of the pathogen

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The fungus in the present study produced septate mycelium. Later it produced verticillate conidiophores. The conidia were cylindrical, straight or slightly curved, hyaline, aseptate.

### 4.4 Morphological characters

#### 4.4.1 Spore morphology

The conidia of *S. oryzae* obtained from infected leaf sheaths and from culture were measured and compared in respect of their spore morphology. The conidia were cylindrical with rounded ends; straight or sometimes slightly curved; hyaline, aseptate. The conidia collected from potato dextrose agar measured 2.13- 7.14  $\mu\text{m}$   $\times$  1.0-3.1  $\mu\text{m}$  and average being 4.63  $\mu\text{m}$   $\times$  2.1  $\mu\text{m}$  where as the conidia from host leaf sheath measured 2.0-8.2  $\mu\text{m}$   $\times$  1.2-3.3  $\mu\text{m}$  and average being 5.1  $\mu\text{m}$   $\times$  2.25  $\mu\text{m}$

#### 4.4.2 Growth phase studies

The experiment was conducted as mentioned in "Material and Methods" to ascertain the period for the maximum growth of the fungus by dry mycelial weight method, starting from the 3<sup>rd</sup> day to 30<sup>th</sup> day. The growth on two different liquid media one semi synthetic (Potato Dextrose Broth) and another synthetic media (Richards Broth) was compared simultaneously. The results obtained are presented in the Table 2 and Fig. 2.

It is evident from the data that there were significant differences in the different incubation periods. Dry mycelial weight of *S. oryzae* recorded gradual increase starting from third day (89.67 mg in PDB and 145.47 mg in RB) and reached peak growth on 24<sup>th</sup> day (294.27mg in PDB and 268.83 mg in RB), and remained significantly superior over remaining treatments. Later the dry mycelia weight declined to reach 215.00mg in PDB and 230.33 mg in RB on 30<sup>th</sup> day of incubation. Although the initial dry weight of fungus is more in RB, the gradual increase in dry mycelial weight is more in PDB. The dry mycelia weight of 18<sup>th</sup> and 27<sup>th</sup> day in RB remained on par with each other.

As highest growth of the fungus was recorded at 24<sup>th</sup> day it was taken for further studies and as the growth phases were studied in clear in PDB, it was selected as basal medium for further studies.

#### 4.4.3 Cultural characteristics of *S. oryzae* on different solid media

Diversity in cultural and morphological characters of *S. oryzae* were studied in eight non synthetic / semi synthetic and two synthetic media at room temperature  $27 \pm 1^\circ \text{C}$  as described in "Material and Methods" and the results obtained are presented in Table 3, Fig. 3 and Plate 4.

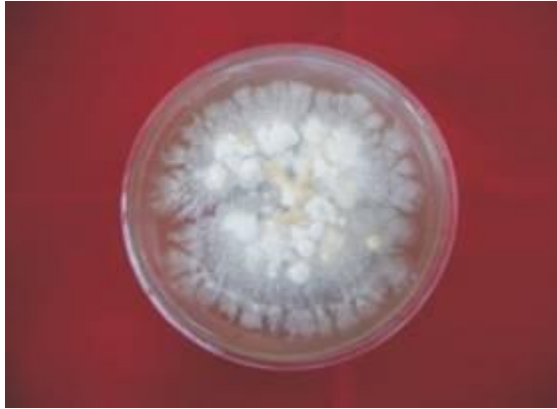
The observations on radial growth of the fungus at 20 days after inoculation and the radial growth, colony characters and sporulation of the fungus were recorded, when the maximum growth was attained on any one of the tested media which was at 40 days after inoculation. The effect of different culture media on the growth of fungi differed significantly. At 20 days after incubation maximum radial growth of *S. oryzae* was recorded on oat meal agar (5.73 cm), which was found to be significantly superior to all other media. The least radial growth was recorded in Sabouraud dextrose agar (3.33 cm) and remaining other media are almost on par with each other.



**Plate 1: Artificial inoculation using single grain insertion method**



**Plate 2. Proving pathogenicity**



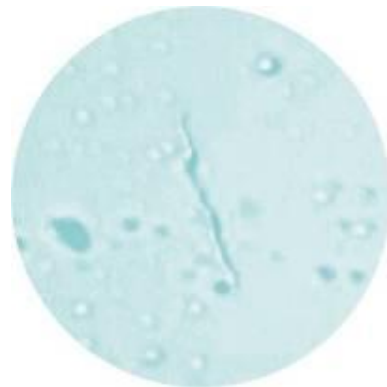
**Culture**



**Conidiophore**



**Conidia**

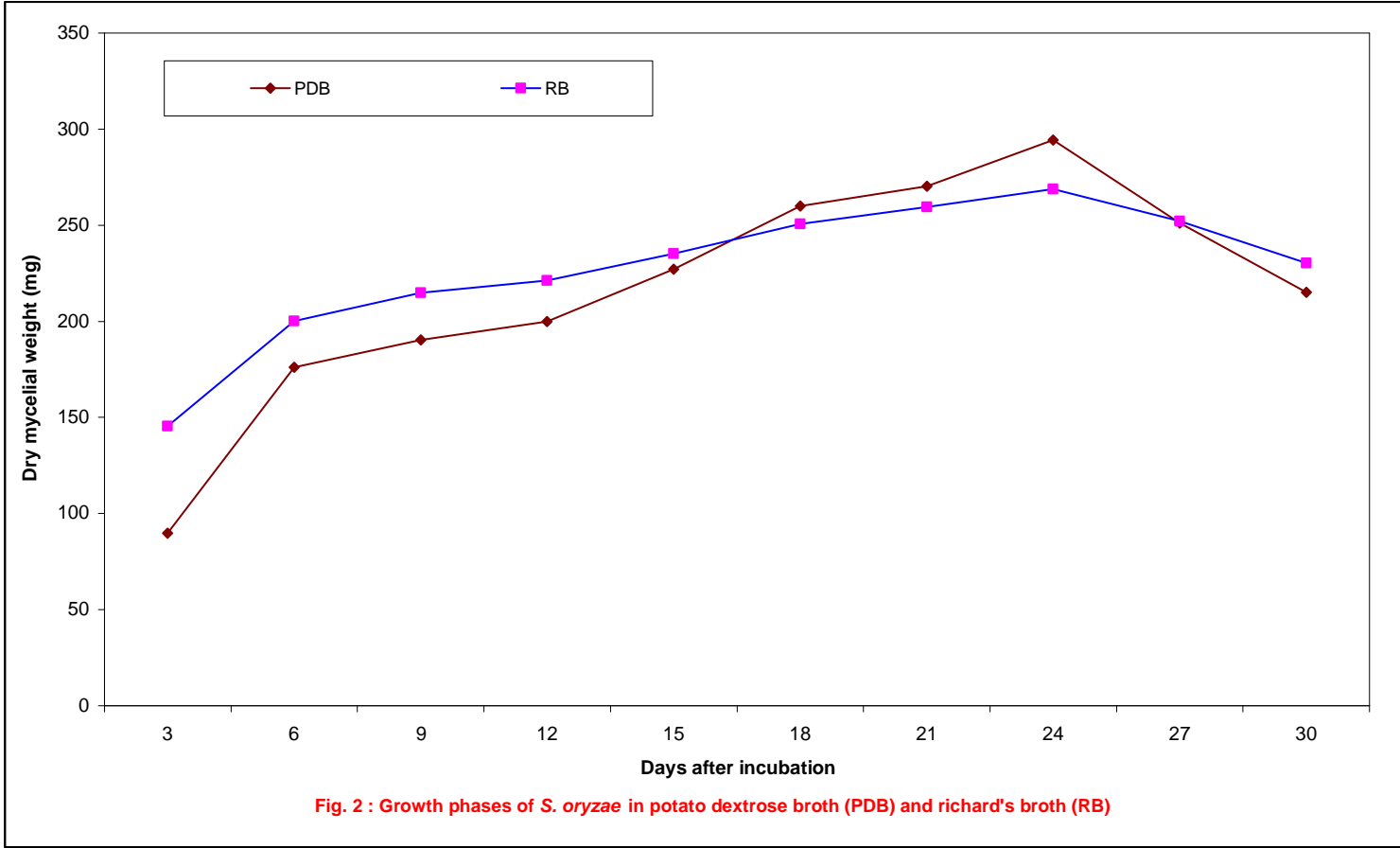


**Spore germination**

**Plate 3. Culture and morphology of *Sarocladium oryzae***

**Table 2: Growth phases of *S. oryzae* in Potato dextrose broth (PDB) and Richard's broth (RB)**

Days after incubation	Dry mycelial weight (mg)	
	PDB	RB
3	89.67	145.47
6	176.17	200.20
9	190.30	214.77
12	200.00	221.17
15	227.23	235.17
18	260.00	250.57
21	270.33	259.43
24	294.27	268.83
27	251.23	252.20
30	215.00	230.33
CD at 1%	1.37	1.15
S.Em. $\pm$	5.53	4.64



**Fig. 2 : Growth phases of *S. oryzae* in potato dextrose broth (PDB) and richard's broth (RB)**

After 40 days the maximum radial growth was observed in oat meal agar (8.97 cm) and on par results were obtained with host leaf extract agar (8.83 cm), host plant extract agar with biotin (8.70 cm) followed by host leaf extract agar with sucrose (8.57 cm) and potato dextrose agar (7.40 cm). The radial growth on Richard's agar (6.87 cm), potato dextrose agar with biotin (6.70 cm) and czapek (dox) agar (6.53 cm) were on par with each other. The least growth was observed on Sabouraud dextrose agar (4.93 cm). The non synthetic/semi synthetic media recorded maximum growth compared to synthetic media.

Mycelium colour varied from white to dark orange. The growth varied from flat, raised fluffy to sparse. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation.

Excellent sporulation was recorded on potato sucrose agar and poor sporulation was recorded in Sabouraud dextrose agar.

#### 4.4.4 Effect of different liquid media on growth of *S. oryzae*

The results of the experiment pertaining to identification the best liquid media for growth under laboratory conditions are presented in Table 4, Fig. 4 and Plate 5. For this two synthetic and eight non synthetic / semi synthetic broth were tested as described in "Material and Methods".

The data revealed that, there were significant differences among the different liquid media on growth of *S. oryzae*. Maximum dry mycelial weight of *S. oryzae* was recorded in oat meal broth (361.67mg), which was found to be significantly superior to all the tested broths. Next best basal medium was Host plant extract broth (351.47mg) followed by Host plant extract with biotin (318.67mg) and host plant extract with sucrose broth (302.33mg). Further host plant extract with sucrose broth and potato dextrose broth remain on par with each other. Least dry mycelial weight of *S. oryzae* was recorded in sabouraud dextrose broth (147.23mg). Between synthetic and non/ semi synthetic media maximum growth was recorded in non / semi synthetic media.

### 4.5 Physiological studies

#### 4.5.1 Effect of pH on growth of *S. oryzae* on liquid media

*S. oryzae* was grown on potato dextrose broth at ten pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 to know the optimum pH required for maximum dry mycelial weight. The results obtained are presented in Table 5, Fig 5 and Plate 6.

The pH of 6.5 and 7.0 were found to be significantly superior to other pH levels by recording the maximum dry mycelial weight (303.67 mg) followed by pH 6.0 (297.53 mg). The least dry mycelial weight was recorded at pH 9.0 (218.33 mg). Further the dry mycelial weight at pH levels 5.5 (293.00 mg), 5.0 (291.00 mg) and 7.5 (290.00 mg) remained on par with each other.

As the pH levels 6.5 and 7.0 supported for good mycelia growth the pH 7.0 was maintained for further studies.

#### 4.5.2 Effect of temperature on growth of *S. oryzae* on liquid media

*S. oryzae* was grown on potato dextrose broth at six temperature levels viz., 15, 20, 25, 30, 35 and 40°C to know the optimum temperature required for maximum dry mycelial weight. The results obtained are presented in Table 6, Fig.6 and Plate 8.

The temperature of 30°C was found to be significantly superior to other temperature levels by recording the maximum dry mycelial weight (304.00 mg) followed by, 25°C (295.12 mg). The least dry mycelial weight was recorded at 40°C (88.02 mg).

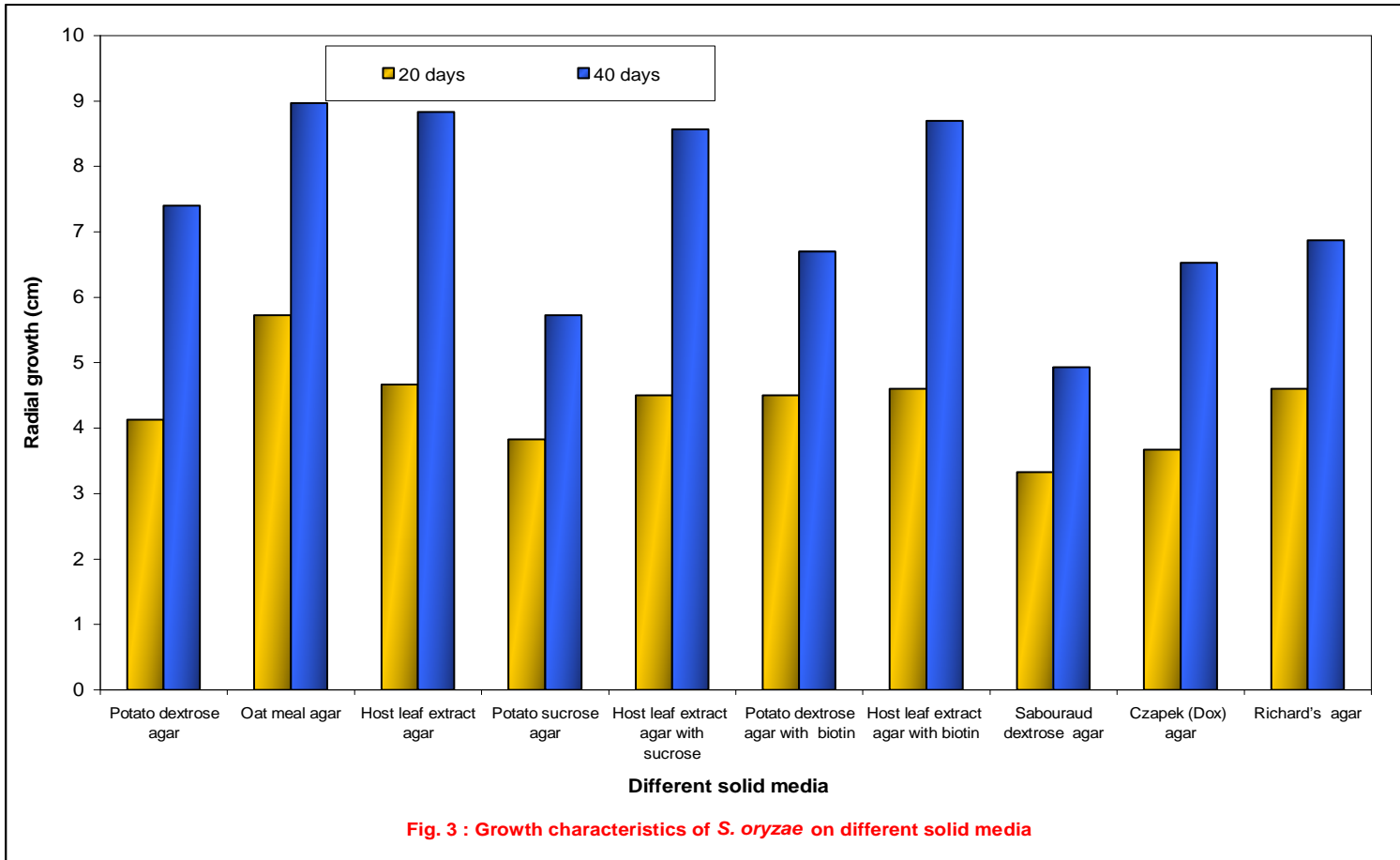
#### 4.5.3 Effect of different sugars on spore germination of *S. oryzae*

The experiment was conducted by using different sugars (dextrose, sucrose, maltose and lactose) at three different concentrations (1%, 1.5% and 2.0%) to know the best sugar which enhances the conidial germination of *S.oryzae*. The results were given under Table 7, Fig.7 and Plate 8.

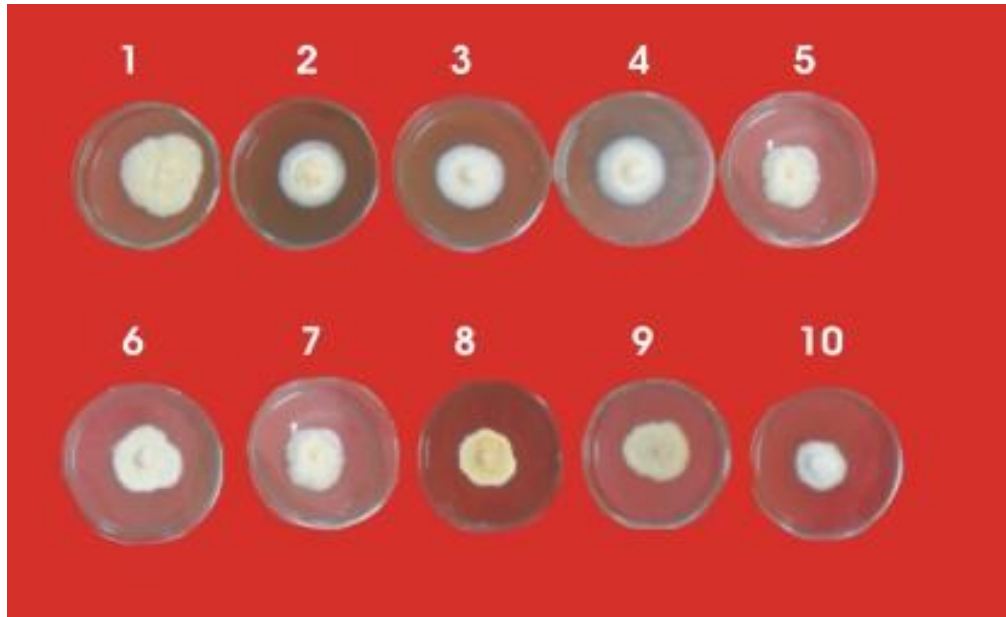
**Table 3: Growth characteristics of *S. oryzae* on different solid media**

Different Media	Mycelium				Sporulation
	Radial growth (cm)		Color	Type of growth	
	20 days	40 days			
Non synthetic/ semi synthetic media					
Potato dextrose agar	4.13	7.40	White with pale orange	Fluffy circular	+++
Oat meal agar	5.73	8.97	White with pale orange tinge	Fluffy circular	+++
Host leaf extract agar	4.67	8.83	White with pale orange tinge	Sparse circular	++
Potato sucrose agar	3.83	5.73	White and pale orange	Fluffy circular	++++
Host leaf extract agar with sucrose	4.50	8.57	White and pale orange	Sparse circular	++
Potato dextrose agar with biotin	4.50	6.70	White and pale orange	Fluffy and irregular	++
Host leaf extract agar with biotin	4.60	8.70	White and pale orange	Sparse and circular	++
Sabouraud dextrose agar	3.33	4.93	white	Flat and circular	+
Synthetic media					
Czapek (Dox) agar	3.67	6.53	White and orange	Sparse circular	++
Richard's agar	4.60	6.87	Dark orange	Fluffy and irregular	+++
CD at 1%	0.13	0.07			
S.Em.±	0.54	0.30			

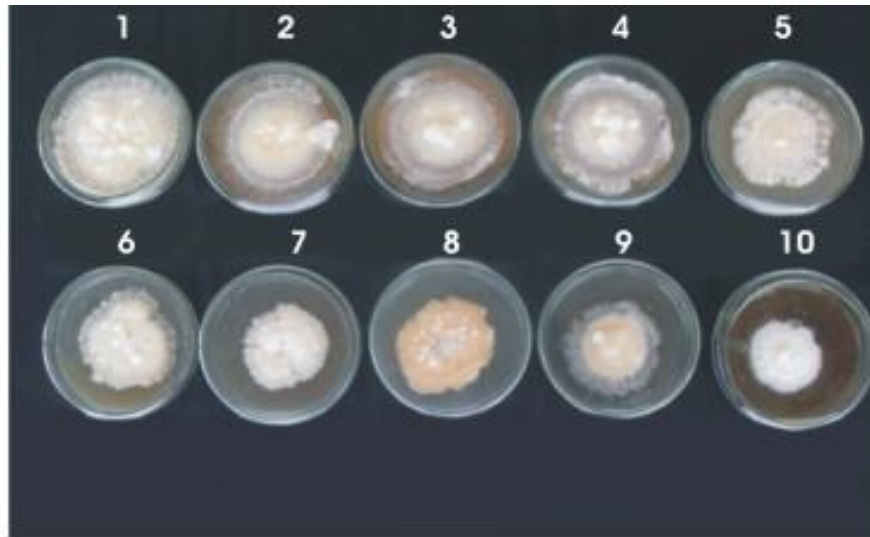
++++ (Excellent), +++ (Good), ++ (Moderate), + (Poor)



**Fig. 3 : Growth characteristics of *S. oryzae* on different solid media**



20 days after inoculation



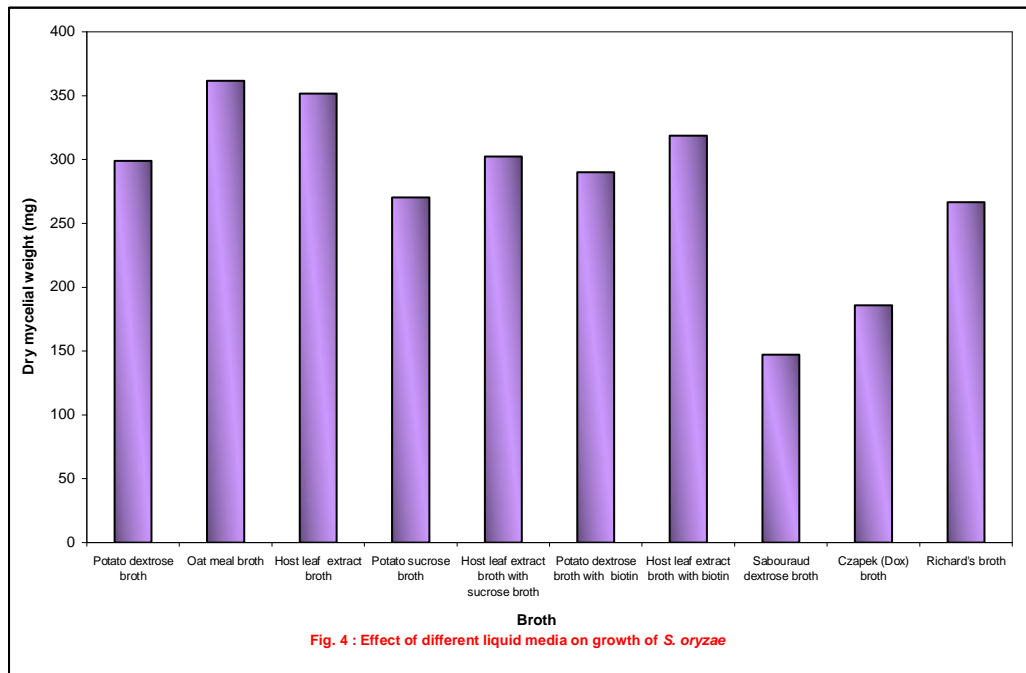
40 days after inoculation

- |   |  |
|---|--|
| 1 - Oat meal agar                       | 2 - Host leaf extract agar             |
| 3 - Host leaf extract agar with sucrose | 4 - Host leaf extract agar with biotin |
| 5 - Potato dextrose agar                | 6 - Potato dextrose agar with biotin   |
| 7 - Potato sucrose agar                 | 8 - Richard's agar                     |
| 9 - Czapek (Dox) dextrose agar          | 10 - Sabouraud's dextrose agar         |

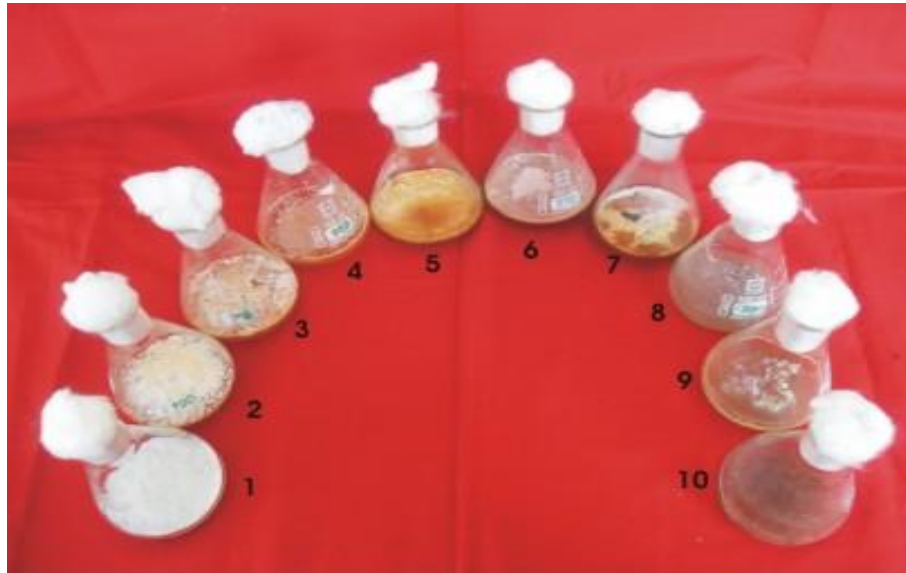
Plate 4. Growth of *S. oryzae* on different solid media

**Table 4: Effect of different liquid media on growth of *S. oryzae***

Broth	Dry mycelial weight (mg)
<b>Non synthetic/ semi synthetic media</b>	
Potato dextrose broth	299.00
Oat meal broth	361.67
Host leaf extract broth	351.47
Potato sucrose broth	270.23
Host leaf extract with sucrose broth	302.33
Potato dextrose broth with biotin	290.00
Host leaf extract broth with biotin	318.67
Sabouraud dextrose broth	147.23
<b>Synthetic media</b>	
Czapek (Dox) broth	185.70
Richard's broth	266.47
CD at 1%	0.84
S.E.m.±	3.36



**Fig. 4 : Effect of different liquid media on growth of *S. oryzae***



- |  |   |
|--|---|
| 1 - Oat meal broth                       | 2 - Potato dextrose broth               |
| 3 - Potato dextrose broth with biotin    | 4 - Potato sucrose broth                |
| 5 - Host leaf extract broth              | 6 - Host leaf extract broth with biotin |
| 7 - Host leaf extract broth with sucrose | 8 - Czapek (Dox) broth                  |
| 9 - Richard's broth                      | 10 - Sabouraud's dextrose broth         |

#### Plate 5. Growth of *S. oryzae* on different liquid media

Among different sugars tested sucrose showed the maximum increase in percent germination of conidia (78.65) compared to other sugars, followed by dextrose (66.26) and maltose (61.22). The least percent germination was recorded with maltose (53.01) among the tested sugars where as the control maintained with sterile distilled water showed the 40.00 per cent germination.

Among the different concentrations of sugars tested the concentration at 2.0 percent showed maximum germination percentage of conidia (76.12). Sucrose at 2% the enhanced the germination up to 90.13 percent followed by dextrose at 80.23 percent.

#### 4.6 Effect of culture filtrate on seed germination, shoot and root length of sorghum

The details of this experiment are given in the "Material and Methods" and results are given in Table 8, Fig. 8 and Plate 9.

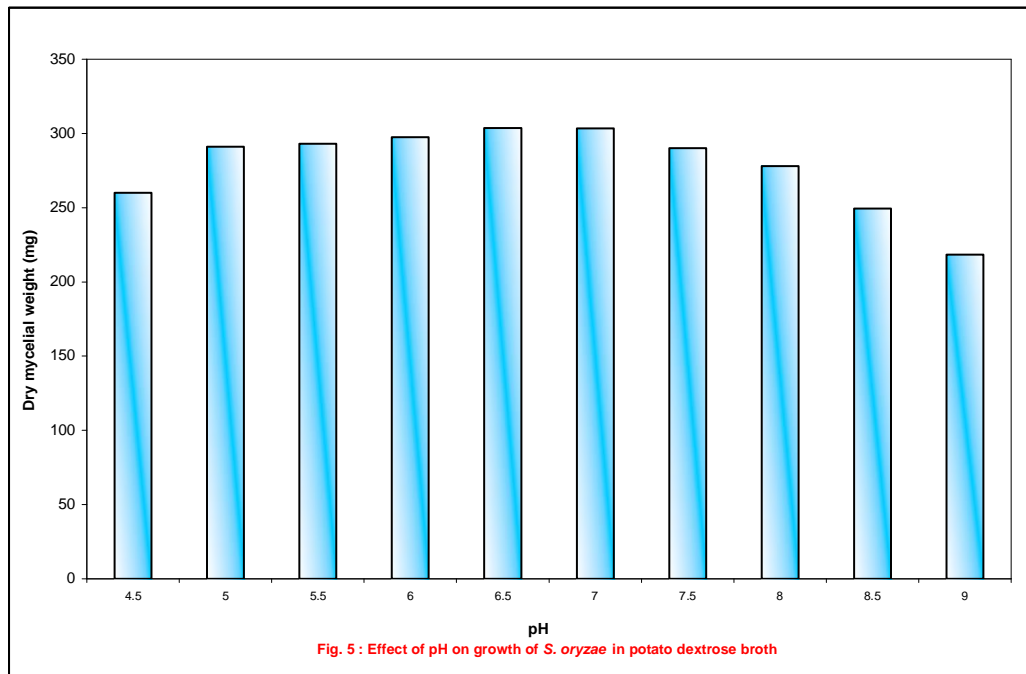
From the Table 8, it is clear that culture filtrate of *S. oryzae* affected the germination of sorghum seeds, seedling growth (shoot and root length) and vigor index. The maximum per cent inhibition of seed germination was recorded in both 24 and 27 days culture filtrate (9.62 %) which was on par with 30 days culture filtrate (8.24%). Least per cent seed germination inhibition was recorded in 3 days and 6 days (2.06 %) culture filtrate which was on par with 9 days (3.43%) culture filtrate.

Maximum inhibition of sorghum shoot length (37.60%) and root length (45.45%) were recorded in 27 days old culture filtrate followed by 24 and 21 days. Least per cent shoot length and root length inhibition was recorded in 3 days culture filtrate (17.18 and 6.23%)

Further, the seedling vigor index was calculated and presented in the Table 8 and Fig. 8a. It was noticed that higher seedling vigor index was noticed in sterile distilled water (3150) followed by 3 days old culture filtrate (2720.12). While, the least vigor index was noticed in 27 days old culture filtrate (1667.27) followed by 24 days culture filtrate (1848.64).

**Table 5: Effect of pH on growth of *S. oryzae* in potato dextrose broth**

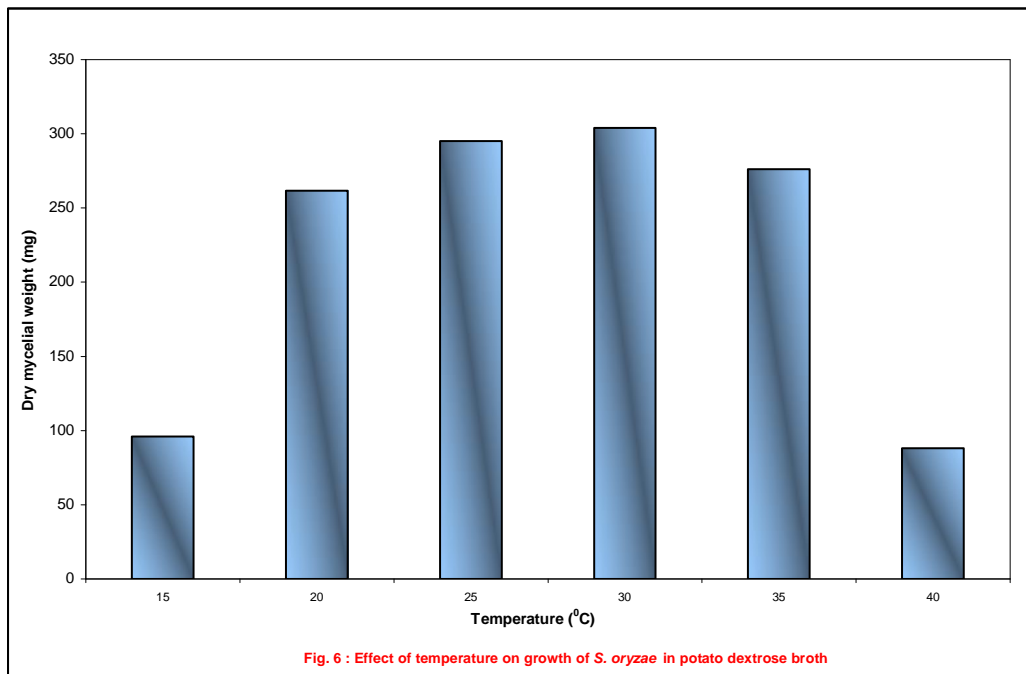
pH	Dry mycelia weight (mg)
4.5	260.00
5.0	291.17
5.5	293.00
6.0	297.53
6.5	303.67
7.0	303.33
7.5	290.00
8.0	278.00
8.5	249.53
9.0	218.33
CD at 1%	1.14
S.Em. <sub>±</sub>	3.37



**Fig. 5 : Effect of pH on growth of *S. oryzae* in potato dextrose broth**

**Table 6: Effect of temperature on growth of *S. oryzae* in potato dextrose broth**

Temperature (°C)	Dry mycelial weight (mg)
15	96.00
20	261.60
25	295.12
30	304.00
35	276.20
40	88.02
CD at 1%	1.47
S.Em.±	5.81



**Fig. 6 : Effect of temperature on growth of *S. oryzae* in potato dextrose broth**

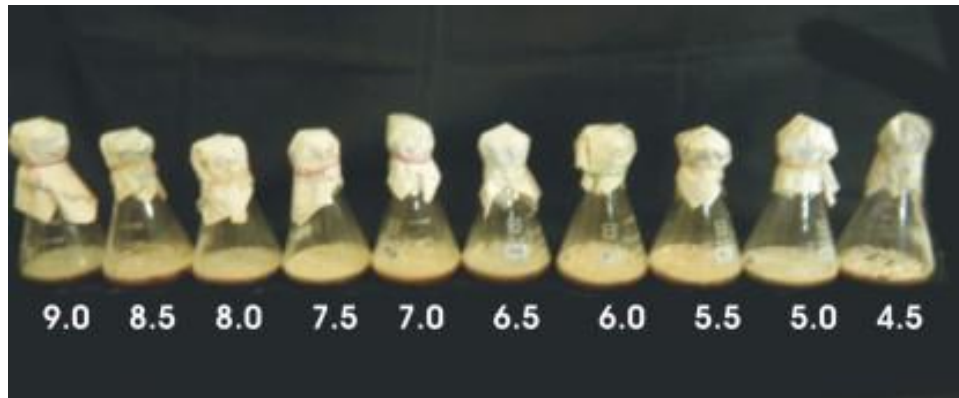


Plate 6. Effect of pH on growth of *S. oryzae* in potato dextrose broth

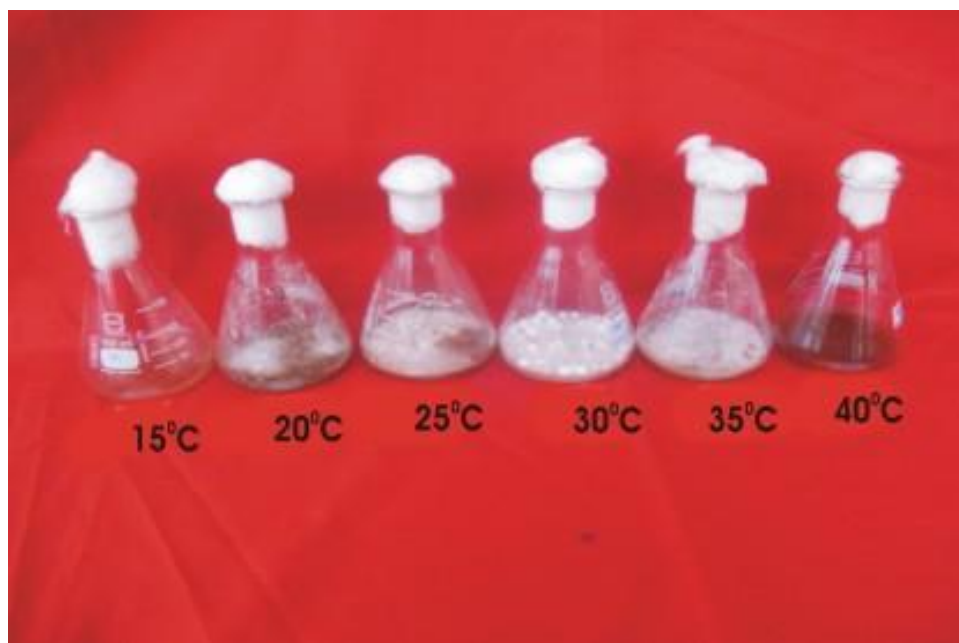
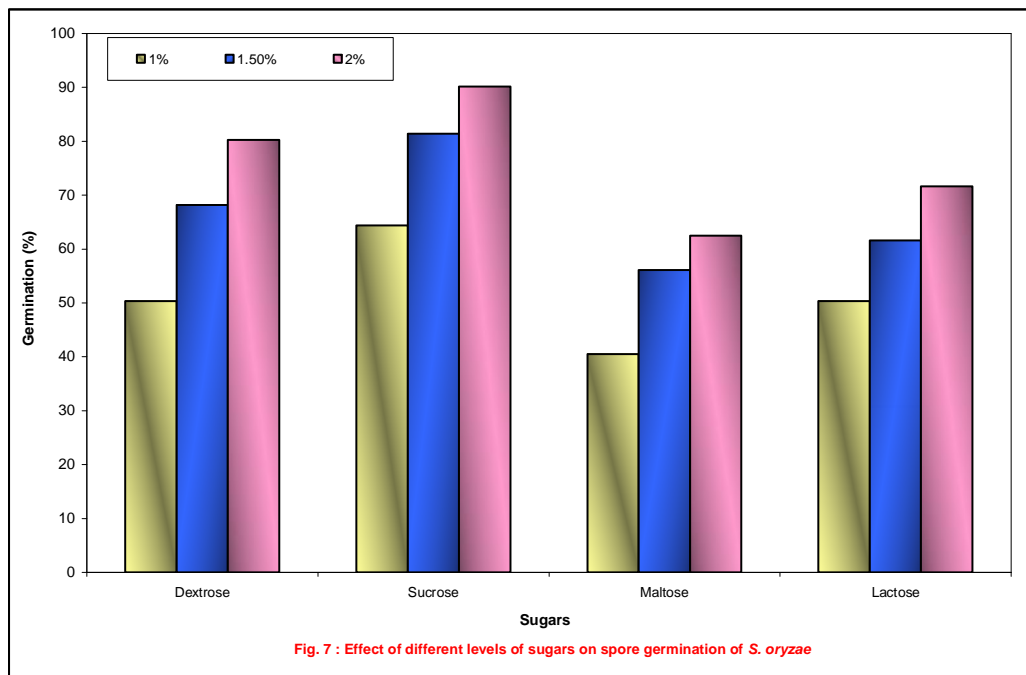


Plate 7. Effect of temperature on growth of *S. oryzae* in potato dextrose broth

**Table 7: Effect of different levels of sugars on spore germination of *S. oryzae***

Sugars	Germination (%)			
	Concentrations (%)			
	1	1.5	2	Mean
Dextrose	50.36 (45.23)*	68.20 (55.71)	80.23 (63.77)	66.26 (54.90)
Sucrose	64.4 (53.41)	81.43 (64.62)	90.13 (71.75)	78.65 (63.26)
Maltose	40.48 (39.49)	56.1 (48.53)	62.46 (52.26)	53.01 (46.76)
Lactose	50.36 (45.23)	61.63 (51.77)	71.67 (57.89)	61.22 (51.63)
Mean	51.40 (45.84)	66.84 (55.16)	76.12 (61.42)	64.79 (54.14)
Control	40.00			
	Sugars (S)	Concentration (C)	SXC	
CD at 1%	1.08	0.94	1.88	
S.Em.±	4.34	3.75	7.51	

\*Arcsine values



**Fig. 7 : Effect of different levels of sugars on spore germination of *S. oryzae***

## 4.7 Variability studies

### 4.7.1 Morphological variability of different isolates of *S. oryzae*

#### 4.7.1a Morphological variability of different isolates on solid media

Morphological variability of eight isolates Belgaum, Dharwad, East Godavari, Koppal, Raichur, West Godavari, Uttar Kannada and Yadgir was studied on oat meal agar. Results are given in Table 9a and Fig. 9.

The observations on colony diameter, conidiophores size, and conidia size were taken on 15 days after inoculation and observations of colony diameter, color of the colony and type of growth were taken on 29 days when one of the isolates reached maximum growth in 7.0 cm Petriplate.

After 15 days of incubation maximum colony diameter on oat meal agar was noticed in Koppal isolate (5.63 cm) and there was no significant difference in colony diameter of different isolates. But after 29 days of incubation, maximum colony diameter (6.97cm) was noticed in Koppal isolate which is on par with colony diameter of East Godavari (6.8 cm) followed by Belgaum (6.53 cm), West Godavari (6.43 cm), Uttar Kannada (6.27 cm) and Dharwad (6.23 cm) which are on par with each other. The least growth was observed in Yadgiri isolate (5.2 cm). The isolates were grouped into three groups based on colony diameter. Group I consists of two isolates Koppal and East Godavari ranging from 6.97cm to 6.8cm. Group II consists of four isolates Belgaum, West Godavari, Uttar Kannada and Dharwad ranging from 6.53 cm to 6.23 cm and group III consists of two isolates Raichur and Yadgir ranging from 5.9 cm to 5.2 cm.

The isolates showed four types of color patterns. Belgaum, Dharwad and Koppal isolates showed whitish mycelium with pale orange color. East Godavari isolate showed whitish mycelium with pale orange and light pink tinge. Uttar Kannada and West Godavari showed light grayish tinge. Raichur and Yadgiri isolates showed white mycelium with pale orange and grayish tinge. The growth varied from fluffy irregular to circular.

Other morphological aspects taken into present investigation like conidiophore size and conidia size. The large conidiophores were observed in Koppal isolate (37.52 X 6.22  $\mu\text{m}$ ) followed by West Godavari (35.89 X 5.60  $\mu\text{m}$ ). The least conidiophores size was observed in Raichur isolate (22.84 X 3.89  $\mu\text{m}$ ). The maximum conidia size was observed in West Godavari (4.96 X 1.86  $\mu\text{m}$ ) followed by Yadgiri (4.81 X 1.62  $\mu\text{m}$ ). The least conidia size was observed in Belgaum (4.04 X 1.66  $\mu\text{m}$ ). The variation among the sizes of conidiophores and conidia were not significant as there is variation in sizes within each isolate.

#### 4.7.1b Morphological variability of different isolates on liquid media

All the isolates of *S. oryzae* were grown in potato dextrose broth and the average dry weight of mycelium after 24 days of growth is given in Table 9b, Fig. 10 and Plate 10.

The variation in the growth of the isolates was significant. Koppal isolate produced maximum growth with mean dry weight of 330.33 mg which is on par with East Godavari isolate (326.33 mg) followed by Belgaum (316.67 mg), West Godavari (311.00 mg) and Uttar Kannada (309.00 mg) isolates. Similarly there was significant difference between Dharwad (296.3 mg) and Raichur (291.00 mg) isolates. Minimum growth was observed in Yadgiri (280.00 mg) isolate. The isolates were grouped into three groups based on average dry mycelial weight on potato dextrose broth. Group I consisted of two isolates Koppal and East Godavari ranging from 330.33 mg to 326.33 mg. Group II consisted of Belgaum, West Godavari and Uttar Kannada ranging from 316.67 to 309.00 mg. Group III consisted of three isolates Dharwad, Raichur and Yadgiri ranged from 296.3 mg to 280.00 mg.

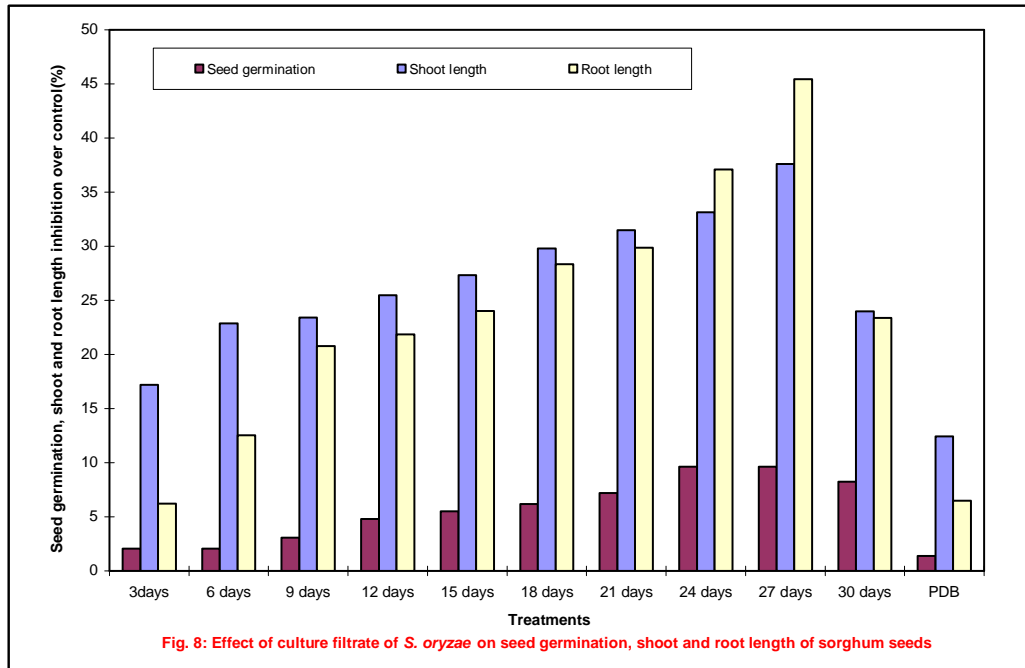
### 4.7.2 Molecular variability of different isolates of *S. oryzae*

Genetic variations were detected among all eight isolates of *S. oryzae* using RAPD markers (Table 10a, Plate 11). Ten primers were used for amplification. Among ten primers OPA 10 showed 100 per cent polymorphism. OPA 7, OPB 11, OPA 16, OPA17, OPB 18, OPB 13, OPA 1 showed 91.67, 85.71, 83.33, 80, 77.77, 75 and 75 per cent, respectively.

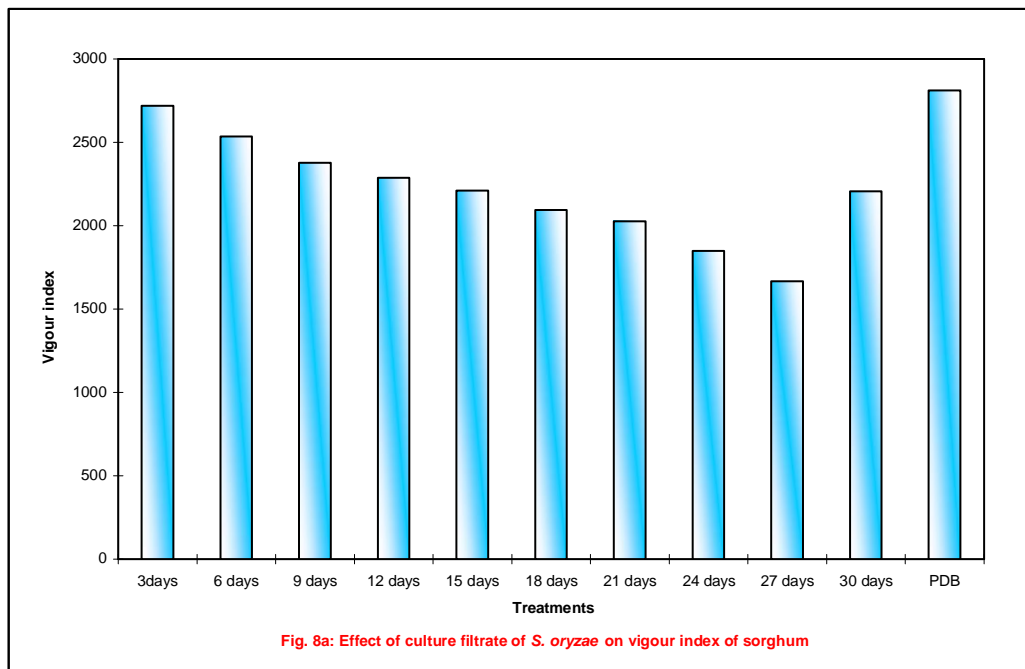
**Table 8: Effect of culture filtrate of *S. oryzae* on seed germination, shoot and root length of sorghum seeds**

Treatments	Inhibition over control (%)			Vigour index
	Seed germination	Shoot length	Root length	
3days	2.06 (8.26)*	17.18 (24.46)	6.23 (14.41)	2720.12
6 days	2.06 (8.26)	22.86 (28.57)	12.55 (20.76)	2535.34
9 days	3.09 (10.13)	23.40 (28.93)	20.78 (27.13)	2377.52
12 days	4.81 (12.65)	25.47 (30.32)	21.86 (27.89)	2287.81
15 days	5.49 (13.55)	27.33 (31.53)	24.03 (29.36)	2211.45
18 days	6.18 (14.37)	29.81 (33.10)	28.35 (32.19)	2095.31
21 days	7.21 (15.56)	31.47 (34.14)	29.87 (33.15)	2025.91
24 days	9.62 (18.04)	33.13 (35.15)	37.10 (37.54)	1848.64
27 days	9.62 (18.04)	37.60 (37.84)	45.45 (42.41)	1667.27
30 days	8.24 (16.67)	23.98 (29.33)	23.38 (28.93)	2205.91
PDB	1.37 (6.64)	12.42 (20.62)	6.49 (14.72)	2810.86
Control (sterile distilled water)	-	-	-	3150
CD at 1%	0.60	0.61	0.50	
S.Em.±	2.38	2.45	1.98	

\*Arcsine values



**Fig. 8: Effect of culture filtrate of *S. oryzae* on seed germination, shoot and root length of sorghum seeds**



**Fig. 8a: Effect of culture filtrate of *S. oryzae* on vigour index of sorghum**



**Sucrose at 2 per cent**



**Control**

Plate 8. Effect of different levels of sugars on spore germination of *S. oryzae*



**27 days old culture filtrate**

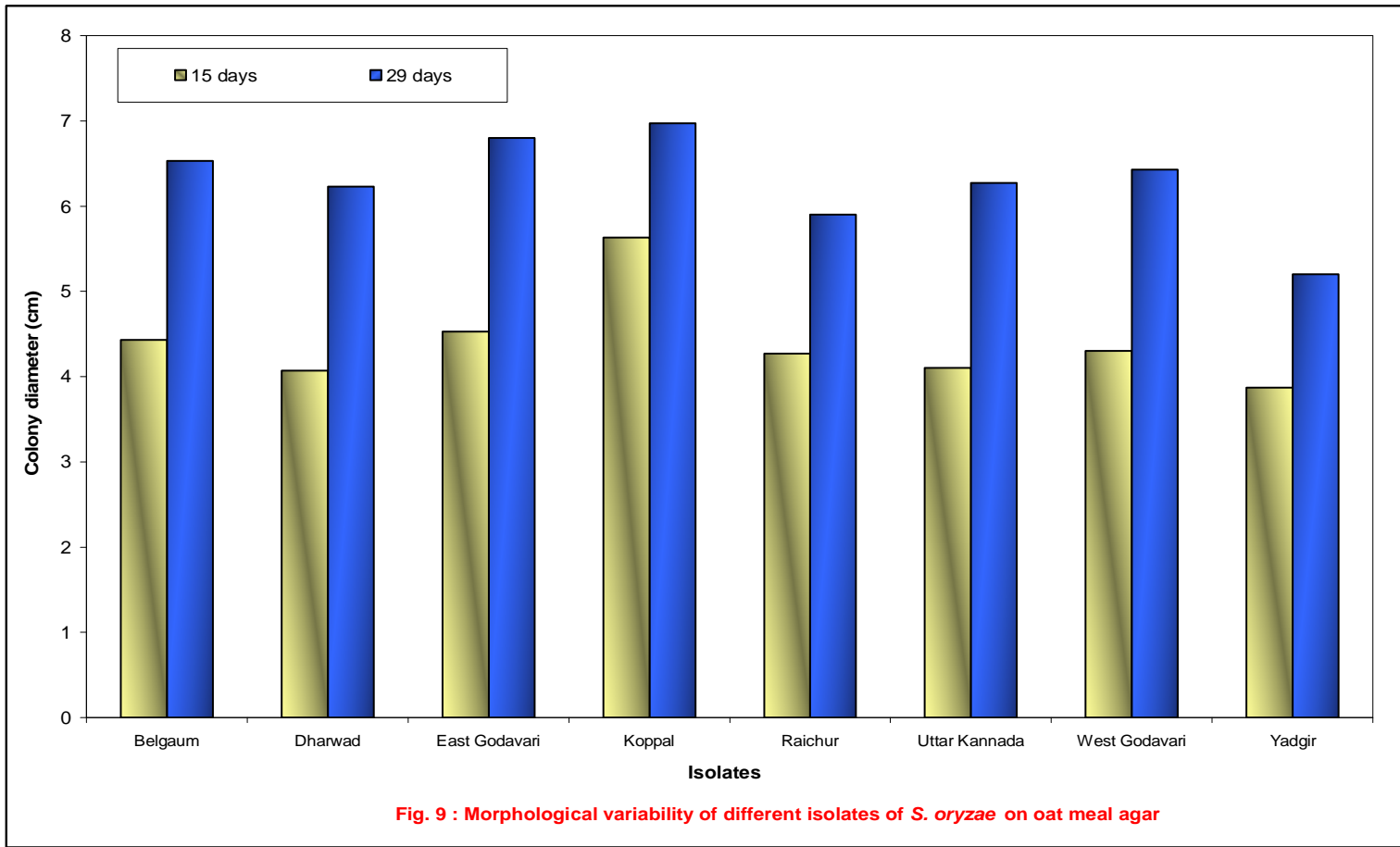


**Control**

Plate 9. Effect of culture filtrate of *S. oryzae* on germination, shoot and root length of sorghum seeds

**Table 9a: Morphological variability of different isolates of *S. oryzae* on oat meal agar**

Isolates	Mycelium			Conidiophores size (µm)	Conidia size (µm)	
	Colony diameter (cm)		Color			Type of growth
	15 days	29 days				
Belgaum	4.43	6.53	Whitish with pale orange tinge	Fluffy circular	30.00x5.32	4.04x1.66
Dharwad	4.07	6.23	Whitish with pale orange tinge	Fluffy circular	28.39x4.40	4.52x1.64
East Godavari	4.53	6.8	Whitish with pale orange and light pink tinge	Fluffy irregular	27.58x4.70	4.66x1.88
Koppal	5.63	6.97	Whitish with pale orange tinge	Fluffy irregular	37.52x6.22	4.16x1.60
Raichur	4.27	5.9	White with pale orange and grayish tinge	Fluffy irregular	22.84x3.89	4.22x1.97
Uttar Kannada	4.10	6.27	White with light grayish tinge	Fluffy irregular	31.80x5.74	4.65x1.60
West Godavari	4.30	6.43	White with light grayish tinge	Fluffy circular	35.89x5.60	4.96x1.86
Yadgir	3.87	5.2	White with pale orange and grayish tinge	Fluffy irregular	25.14x5.20	4.81x1.62
CD at 1%	0.08	0.06				
S.Em.±	0.32	0.23				

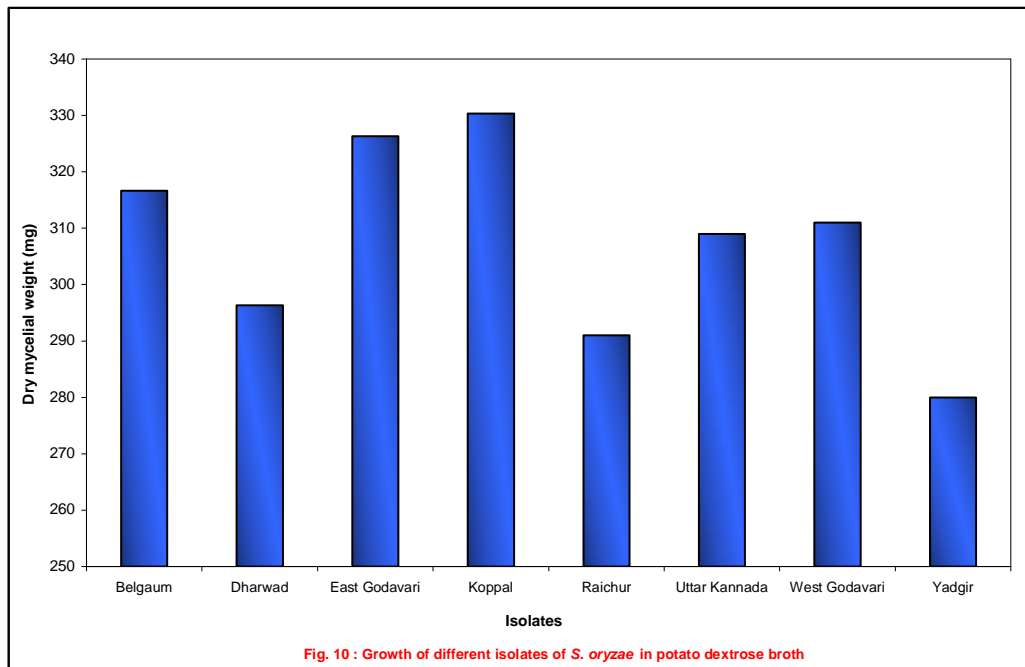


**Fig. 9 : Morphological variability of different isolates of *S. oryzae* on oat meal agar**

**Fig. 9 : Morphological variability of different isolates of *S. oryzae* on oat meal agar**

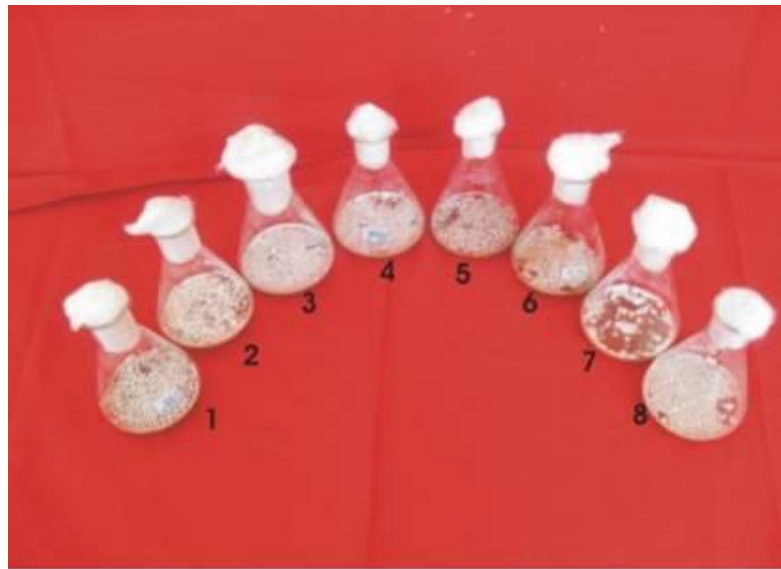
**Table 9b: Growth of different isolates of *S. oryzae* in potato dextrose broth**

Isolate	Dry mycelia weight (mg)
Belgaum	316.67
Dharwad	296.3
East Godavari	326.33
Koppal	330.33
Raichur	291.00
Uttar Kannada	309.00
West Godavari	311.00
Yadgir	280.00
CD at 1%	1.08
S.Em. <sub>±</sub>	4.45



**Fig. 10 : Growth of different isolates of *S. oryzae* in potato dextrose broth**

**Fig. 10 : Growth of different isolates of *S. oryzae* in potato dextrose broth**



1 - Belgaum      2 - Dharwad      3 - East Godavari  
 4 - Koppal      5 - Raichur      6 - Uttar Kannada  
 7 - Yadgir      8 - West Godavari

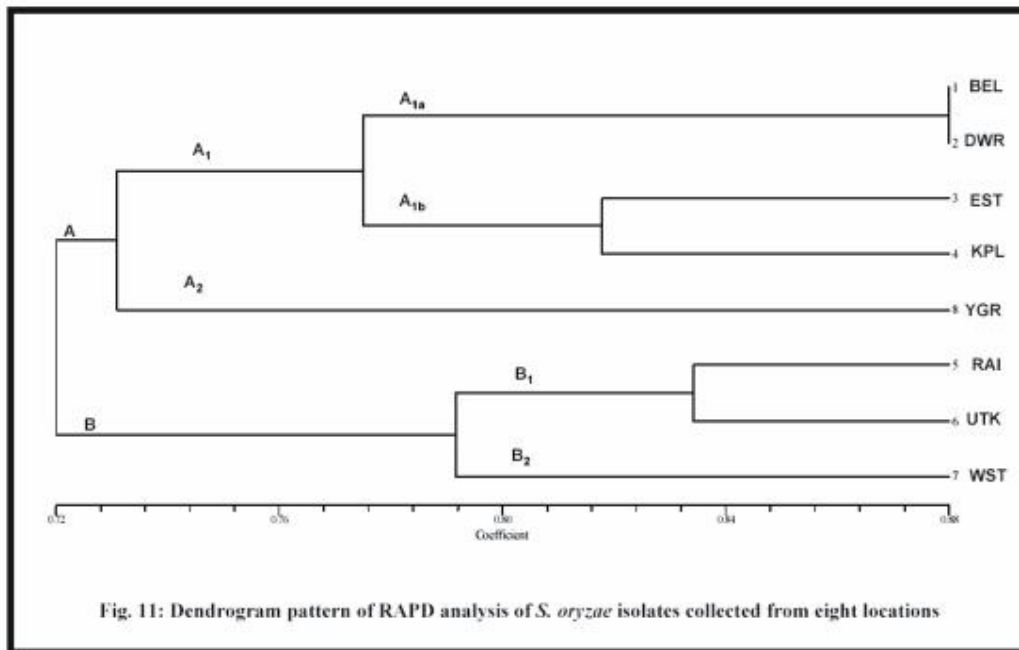
**Plate 10. Morphological variability of eight isolates of *S. oryzae* on potato dextrose broth**

**Table 10a: RAPD banding profile of different primers used for eight isolates of *S. oryzae***

Primer	Total bands	Polymorphic bands	Polymorphism (%)
OPA 1	8	6	75
OPA 5	7	0	0
OPA 7	12	11	91.67
OPA 10	6	6	100
OPA 11	8	0	0
OPA 16	6	5	83.33
OPA 17	10	8	80
OPB 11	7	6	85.71
OPB 13	8	6	75
OPB 18	9	7	77.77

**Table 10b: Similarity coefficient values of eight isolates of *S. oryzae***

Isolates	BGM	DWD	EGV	KPL	RCR	UTK	WGV	YDG
BGM	1							
DWD	0.88	1						
EGV	0.79	0.83	1					
KPL	0.70	0.79	0.82	1				
RCR	0.65	0.65	0.75	0.81	1			
UTK	0.70	0.70	0.61	0.77	0.84	1		
WGV	0.80	0.73	0.78	0.70	0.81	0.78	1	
YDG	0.76	0.74	0.68	0.75	0.70	0.72	0.73	1



**Fig. 11: Dendrogram pattern of RAPD analysis of *S. oryzae* isolates collected from eight locations**

**Fig 11. Molecular variability of eight isolates of *S. oryzae* through RAPD**

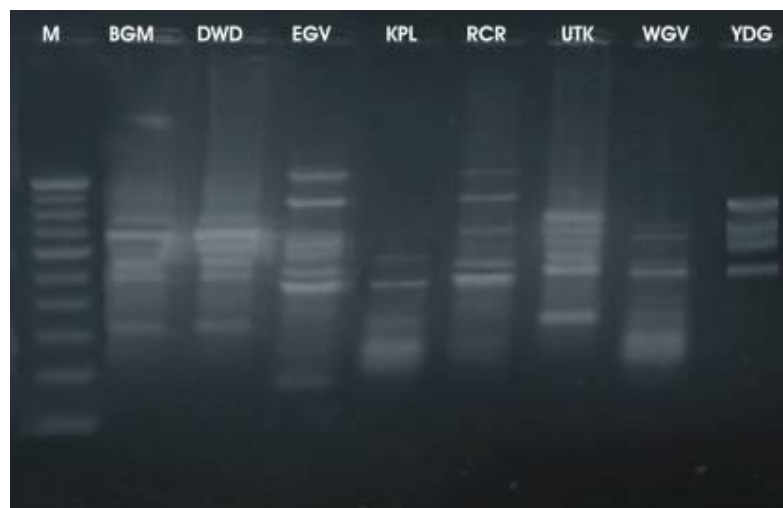
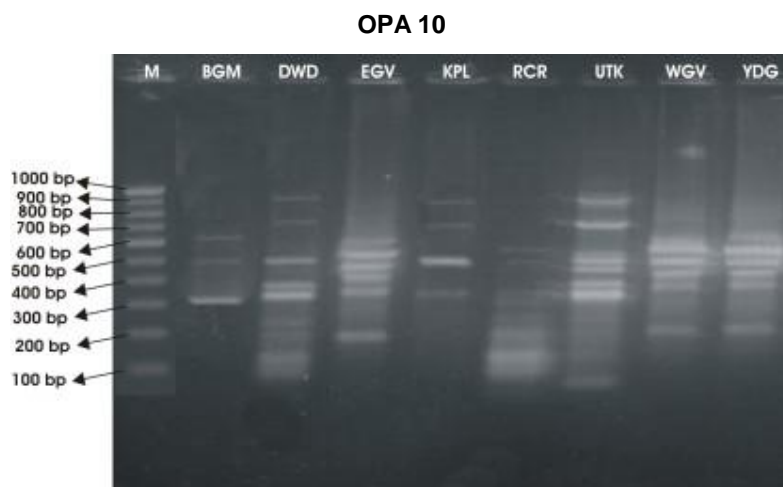


Plate 11. Molecular variability of eight isolates of *S. oryzae* through RAPD

**Table 11a: Evaluation of rice varieties against sheath rot caused by *S. oryzae***

Variety	Sheathrot grade	Reaction
Bili Kalavi	3	R
Tirlu huegge	3	R
Dodda bangar kaddi	3	R
M-181	3	R
Alur sanna	3	R
Bilidadi moratiga	3	R
Holesalu Chipiga	3	R
Y-4 (bangarkaddi)	7	MS
Rare seed	1	HR
Bilidadi goratiga	1	HR
Bili nellu	3	R
HY-26-1	3	R
Kari kalavi	3	R
Dodda valya	5	MR
Hakkala batta	5	MR
HY-449-1	7	MS
Maisali	3	R
HY-246-13	5	MR
Madras sanna	5	MR
Salivahana	7	MS
Valya	9	S
MTU-1001	5	MR
Rajamandri	3	R
Abhilash	5	MR
PR-106	5	MR
Sona Mahasuri	7	MS
Lunishree	3	R
Aditya	9	S
Pusa-682	7	MS
IR-9716-R	9	S
Rasi	3	R
ADT-36	5	MR
IR-39357-71-1-1-2-2	5	MR
Tellahamsa	7	MS
Pusa-677	9	S
IR-19743-46-2-33-2-2	5	MR
IR-64	3	R
Prasanna	3	R
BPT-5204	5	MR
Vajran	7	MS
Kagisali	5	MR
Ware Sanna	5	MR
Mote Bangar Kaddi	5	MR
IET 19396 (check)	9	S

**Table 11b: Evaluation of rice genotypes against sheath rot caused by *S. oryzae***

<b>Reaction</b>	<b>Genotypes</b>	<b>Total no. of genotypes</b>
Highly resistant	Rare seed, Bilidadi goratiga	2
Resistant	Bili kalavi, Tirlu hegge, Dodda bangar kaddi, M-181, Alur sanna, Bilidadi moratiga, Holesalu chipiga, Bili nellu, HY-26-1, Kari kalavi, Maisali, Rajamandi, Lunishree, Rasi, IR-64, Prasanna	16
Moderatly resistant	Dodda valya, Hakkala batta, HY-246-13, Madras sanna, MTU-1001, Abhilash, PR-106, ADT-36, IR-39357-71-1-1-2-2, IR-19743-46-2-33-2-2, BPT-5204, Kagisali, Ware sanna, Mote bangar kaddi	14
Moderatly susceptible	Y-4(Bangarkaddi), HY-449-1, Salivahana, Sona Mahasuri, Pusa-682, Tellahamsa, Vajran	7
Susceptible	Valya, Aditya, IR-9716-R, Pusa-677, IET 19396	5



**Plate 12. Disease scoring using 0-9 scale in field**



**Billidadi goratiga**



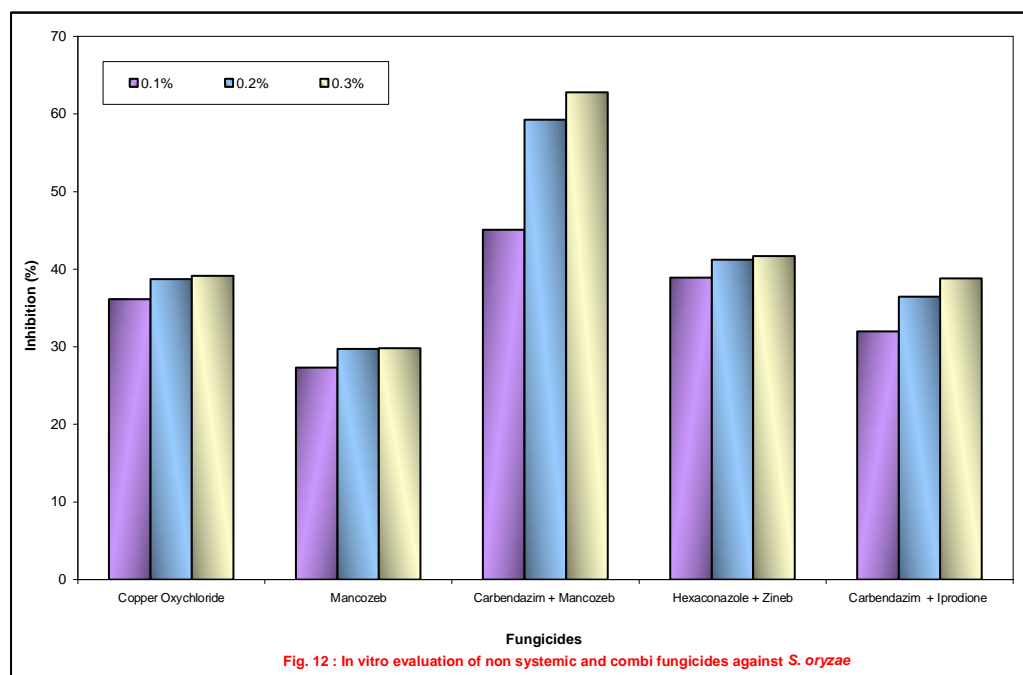
**IET - 19396**

**Plate 13. Screening of different rice genotypes against sheath rot**

**Table 12a: *In vitro* evaluation of non systemic and combi fungicides against *S. oryzae***

Fungicides		Inhibition (%)			
		Concentrations (%)			
Common name	Trade name	0.1	0.2	0.3	Mean
Copper Oxychloride	Blue copper-50%WP	36.15 (36.98)*	38.74 (38.51)	39.15 (38.75)	38.01 (38.08)
Mancozeb	Dithane M- 45 75 %WP	27.34 (31.54)	29.72 (33.05)	29.83 (33.12)	28.96 (32.57)
Carbendazim 12%+ Mancozeb 63%	SAAF 75 %WP	45.09 (42.20)	59.26 (50.36)	62.81 (52.45)	55.72 (48.34)
Hexaconazole 4%+ Zineb 64%	Avtar	38.92 (38.62)	41.23 (39.97)	41.69 (40.23)	40.61 (39.61)
Carbendazim 25% + Iprodione 25%	Quintal	31.97 (34.45)	36.44 (37.15)	38.83 (38.56)	35.75 (36.72)
Mean		35.90 (36.76)	41.08 (39.81)	42.46 (40.62)	39.81 (39.06)
Control – Dry weight of mycelium		295 mg			
		Fungicides (F)	Concentrations (C)	(F)X(C)	
S.Em.±		0.12	0.09	0.21	
CD at 1%		0.49	0.37	0.84	

\*Arcsine values

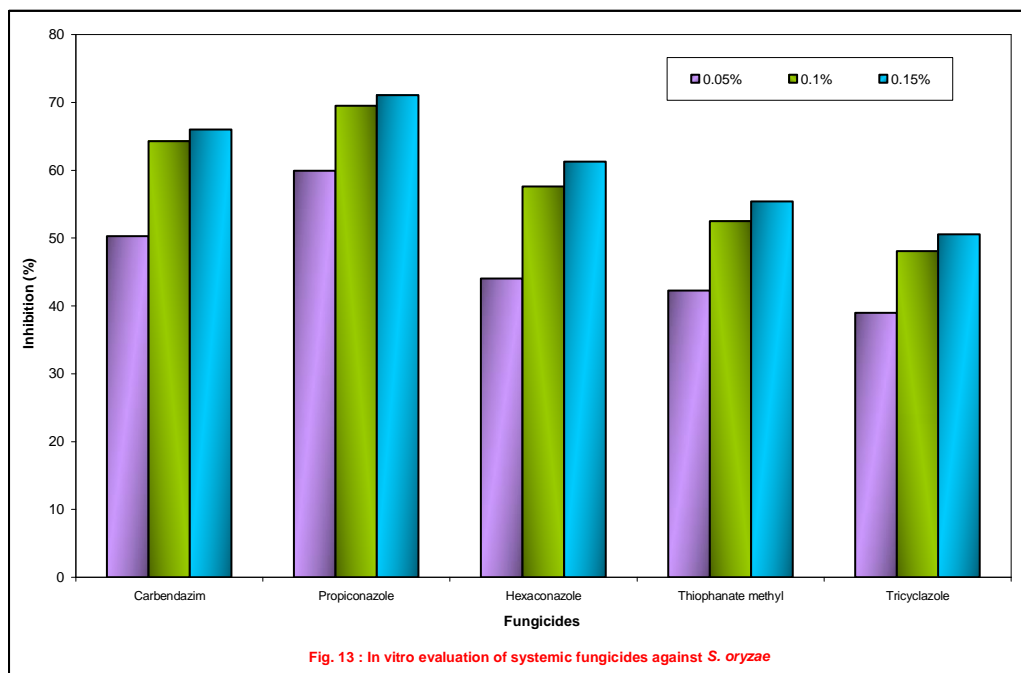


**Fig. 12 : In vitro evaluation of non systemic and combi fungicides against *S. oryzae***

**Table 12b: *In vitro* evaluation of systemic fungicides against *S. oryzae***

Fungicides		Inhibition (%)			
		Concentration (%)			
Common name	Trade name	0.05	0.1	0.15	Mean
Carbendazim	Bavistin 50% WP	50.28 (45.13)*	64.29 (53.33)	65.98 (54.35)	60.18 (50.94)
Propiconazole	Tilt 25% EC	59.94 (50.76)	69.49 (56.50)	71.07 (57.49)	66.83 (54.91)
Hexaconazole	Contaf 5% EC	44.06 (41.61)	57.62 (49.41)	61.24 (51.52)	54.31 (47.51)
Thiophanate methyl	Topsin M 70 WP	42.25 (40.56)	52.48 (46.44)	55.40 (48.12)	50.04 (45.04)
Tricyclazole	Beam 75 WP	38.98 (38.65)	48.07 (43.92)	50.56 (45.34)	45.87 (42.64)
Mean		47.10 (43.34)	58.39 (49.92)	60.85 (51.36)	55.45 (48.21)
Control – Dry weight of mycelium		295 mg			
		Fungicides (F)	Concentration (C)	FXC	
S.Em.±		0.77	0.59	1.34	
CD at 1%		3.02	2.34	5.23	

\*Arcsine values



**Fig. 13 : *In vitro* evaluation of systemic fungicides against *S. oryzae***

Information on banding pattern for all the primers was used to determine the genetic distance between the isolates and to construct a dendrogram.

Based on similarity coefficient, a genetic similarity was constructed to assess the genetic relatedness among the isolates of *S.oryzae*. Genetic similarity coefficient ranged from 0.61 to 0.88 (Table10b). Highest similarity observed between Belgaum-Dharwad whereas least similarity observed between East Godavari- Uttara kannada isolates.

The dendrogram constructed from the pooled data clearly showed, two major clusters like A and B represented in Fig.11. Cluster B formed Raichur, Uttara kannada, west Godavari isolates. In group A, it is divided into several sub-sub groups were observed. Belgaum and Dharwad formed one group (A<sub>1a</sub>), East Godavari and Koppal formed another cluster (A<sub>1b</sub>) whereas Yadgiri isolate remained distinct (A<sub>2</sub>).

## 4.8 Screening of different rice genotypes against sheath rot of rice

Forty four genotypes were screened in the field at Agriculture Research Station, Mugad during *khariif* 2011 as described in materials and methods. Reactions of these genotypes along with severity grade as per international standard are tabulated in Tables 11a and 11b. Disease scoring by 0-9 scale represented in Plate 12 and screening results showed in Plate 13.

The genotypes were further classified into immune (grade 0), highly resistant (grade 1), resistant (grade 3), moderately resistant (grade 5), moderately susceptible (grade 7), susceptible (grade 9) based on their reaction to sheath rot. From Tables 11a and 11b it is revealed that, none of the variety was found immune. Out of forty four genotypes, two genotypes *viz.*, Rere Seed, Bilidadi Goratiga showed highly resistant reaction. Sixteen genotypes showed resistant reaction; fourteen genotypes showed moderately resistant reaction, seven genotypes showed moderately susceptible reaction and about five genotypes showed susceptible reaction.

## 4.9 Management studies

### 4.9.1 *In vitro* evaluation of fungicides against *S. oryzae*

Screening of fungicides was done against *S. oryzae* under laboratory condition by following poisoned food technique as described in "Material and Methods".

Five each of systemic and non-systemic fungicides (three combi products) were evaluated against *S. oryzae* in laboratory at three concentrations by poisoned food technique in potato dextrose broth and incubated for 24 days.

#### 4.9.1a *In vitro* evaluation of non systemic and combi fungicides against *S. oryzae*

Data with respect to inhibition of mycelial growth of *S. oryzae* at three concentrations of two non-systemic fungicides and three combi product was recorded and presented in Table12a and Fig. 12.

Data from Table 12a revealed that, the efficacy of different non-systemic fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *S. oryzae* differed significantly and the mycelia growth in control was 295.00 mg

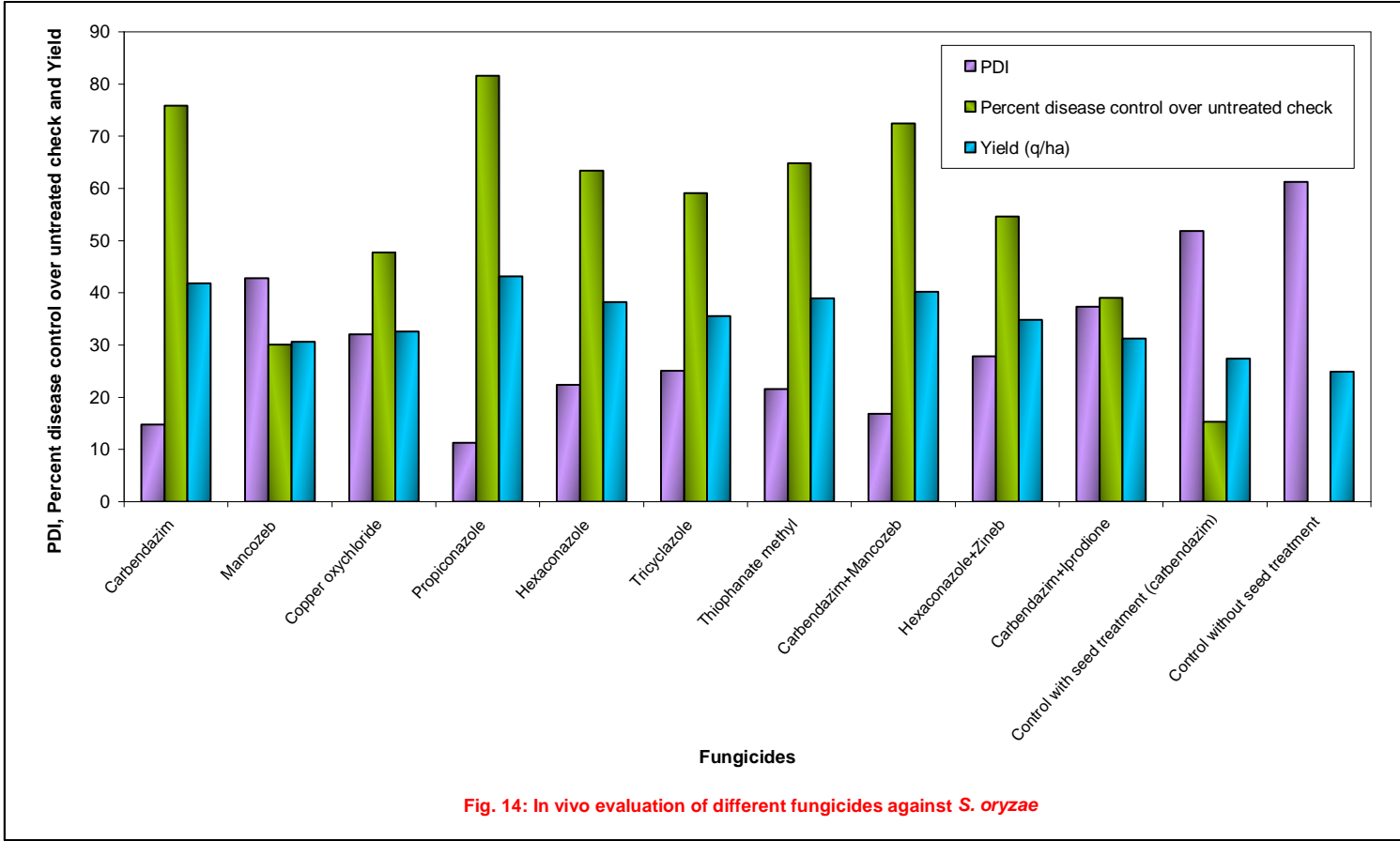
Maximum percent inhibition (55.72%) of *S. oryzae* was recorded in combi product carbendazim + mancozeb which was significantly superior over all other fungicides followed by hexaconazole + zineb (combi product) (40.61%), copper oxychloride (38.01%) and carbendazim + mancozeb (35.75%). Least per cent inhibition was noticed in mancozeb (28.96%). However the maximum per cent inhibition of mycelial growth was at 0.3 per cent concentration irrespective of fungicides.

At 0.3 per cent concentration combi product carbendazim + mancozeb recorded highest per cent inhibition of mycelial growth (62.81%) of fungus which was significantly superior over other fungicides followed by hexaconazole + zineb (41.69%). The least inhibition of fungus was recorded in mancozeb (29.83%).

**Table 13: *In vivo* evaluation of different fungicides against *S. oryzae***

Tr. No.	Fungicides	Dosage (%)	PDI	Percent disease control over untreated check	Yield (q/ha)
T <sub>1</sub>	Carbendazim	0.1	14.79 (22.59)*	75.85	41.81
T <sub>2</sub>	Mancozeb	0.25	42.80 (40.87)	30.12	30.67
T <sub>3</sub>	Copper oxychloride	0.25	32.03 (34.48)	47.70	32.60
T <sub>4</sub>	Propiconazole	0.1	11.30 (19.57)	81.55	43.14
T <sub>5</sub>	Hexaconazole	0.1	22.43 (28.26)	63.37	38.24
T <sub>6</sub>	Tricyclazole	0.1	25.07 (30.06)	59.06	35.55
T <sub>7</sub>	Thiophanate methyl	0.1	21.54 (27.65)	64.83	38.92
T <sub>8</sub>	Carbendazim+Mancozeb	0.2	16.87 (24.24)	72.45	40.25
T <sub>9</sub>	Hexaconazole+Zineb	0.2	27.81 (31.84)	54.59	34.88
T <sub>10</sub>	Carbendazim+Iprodione	0.2	37.32 (37.67)	39.06	31.28
T <sub>11</sub>	Control with seed treatment (carbendazim)	0.2	51.88 (46.10)	15.28	27.43
T <sub>12</sub>	Control without seed treatment	-	61.24 (51.52)	-	24.93
S.Em±			0.74	-	0.72
CD at 5%			2.17	-	2.11

\*Arcsine values



**Fig. 14: In vivo evaluation of different fungicides against *S. oryzae***



**Propiconazole 0.1%**



**Control**

**Plate 14. In vivo evaluation of different fungicides against sheath rot of rice**

At 0.1 per cent concentration maximum per cent inhibition of mycelial growth (45.09%) of the fungus was recorded in carbendazim + mancozeb. Further 0.1 per cent hexaconazole + zineb (38.92%) and 0.2 per cent of copper oxy chloride (38.74%) remain on par with each other. The least per cent inhibition (27.34%) of fungus was in mancozeb. At all the tested concentrations among nonsystemic and combi fungicides, carbendazim + mancozeb recorded highest inhibition at all the three concentrations.

#### 4.9.1b *In vitro* evaluation of systemic fungicides against *S. oryzae*

Data with respect to inhibition of mycelial growth of *S. oryzae* at three concentrations of five systemic fungicides were recorded and per cent inhibition is presented in Table 12b and Fig. 13. It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *S. oryzae*. Among five systemic fungicides, maximum per cent inhibition of growth of *S. oryzae* was observed in propiconazole (66.83%) which was significantly superior to all other fungicides followed by carbendazim (60.18%), hexaconazole (54.31%), thiophanate methyl (50.04%). The least per cent inhibition of fungus was recorded in tricyclazole (45.87%). Among the tested three concentrations, 0.15 per cent concentration of all fungicides was significantly found superior to 0.1 and 0.05 per cent. Maximum per cent inhibition of mycelial growth (71.07%) of the fungus was recorded in propiconazole followed by carbendazim (65.98%) and hexaconazole (61.24%) which are on par with each other.

At 0.1 per cent concentration, maximum per cent inhibition of mycelial growth (69.49%) of the fungus was recorded in propiconazole and (64.29%) in carbendazim which are on par with each other. Least percent inhibition was by tricyclazole (48.07%). At 0.05 per cent maximum inhibition was observed in propiconazole (59.94%) and the least per cent inhibition of mycelia growth was recorded in tricyclazole (38.98%). Further 0.05 per cent propiconazole (59.94%), hexaconazole (57.62%) and 0.15 per cent of thiophanate methyl (55.40%) remained on par with each other.

#### 4.9.2 *In vivo* evaluation of fungicides against sheath rot of rice during *kharif* 2011

This study was undertaken to evaluate the relative efficacy of different fungicides for management of sheath rot of rice during *kharif* 2011. The experiment was conducted in Agriculture Research Station, Mugad with eleven treatments and one untreated control as described in "Material and Methods". Totally two sprays were given at an interval of 10 days. The observations on sheath rot of rice were recorded converted into per cent disease index (PDI) using formula given by Wheeler (1969). The per cent disease reduction over control was worked. Statistically analyzed data are presented in the Table 13, Fig. 14 and Plate 14. From the Table 13 maximum PDI was noticed in untreated control (61.24) and minimum in plots treated with propiconazole at 0.1 percent (11.30) followed by carbendazim (14.79) at 0.1 percent concentration and carbendazim + mancozeb (16.87) at 0.2 per cent concentration. Thiophanate methyl (21.54) at 0.1 per cent concentration and hexaconazole (22.43) at 0.1 per cent were on par with each other. Similarly plots treated with tricyclazole (25.07) and hexaconazole + zineb (27.81) were on par with each other. This was followed by copper oxy chloride (32.03), carbendazim + iprodione (37.32), mancozeb (42.80), control with seed treatment of carbendazim at 0.2 per cent (51.88) which are significantly different with each other. Further per cent disease control over untreated check (PDC) was calculated for all treatments. Among eleven treatments highest PDC of 81.55 was calculated in propiconazole at 0.1 per cent and 75.85 in carbendazim at 0.1 per cent. Similarly minimum PDC 15.28 was observed in control with seed treatment (carbendazim at 0.2 per cent).

#### Yield as influenced by fungicides

Yield obtained in fungicides treated plots indicated that, highest and significant yield of 43.14 quintal per ha was recorded in propiconazole followed by carbendazim at 0.1 per cent (41.81 q/ha) and carbendazim + mancozeb at 0.2 per cent (40.25 q/ha) concentration treated plots, which were on par with each other. hexaconazole (38.24 q/ha) at 0.1 per cent and thiophanate methyl (38.92 q/ha) at 0.1 per cent remain on par with each other.

The next on par yield levels were obtained in tricyclazole (0.1 %), hexaconazole + zineb (0.2%), copper oxychloride (0.25%), carbendazim+iprodione (0.2%), mancozeb (0.25%) and control with seed treatment (carbendazim at 0.2%) treated plots. The lowest yield of 24.93 q/ha was obtained in untreated control plot.

## 5. DISCUSSION

Rice (*Oryza sativa* L) is the important cereal crop grown throughout the world. It is a staple food crop of 60 percent of world's population. Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by the humans. Its biological value is as high as egg protein, due to high content of essential amino acids. Number of diseases is becoming major limiting factors in attaining yield.

Among various fungal diseases, sheath rot caused by *Sarocladium oryzae* (Sawada) Gams and Hawksworth has become potentially destructive disease. Prabhakaran *et al.* (1973) reported 83 per cent yield loss due to sheath rot disease in Tamil Nadu.

In recent years the disease has assumed to be serious problem in Karnataka. Therefore, investigations were carried out on various aspects of the pathogen with respect to symptomatology, severity of disease in different locations, morphological, cultural, physiological aspects of pathogen, morphological and molecular variability among isolates of that pathogen, Screening of varieties for host plant resistance and evaluation of fungicides against the disease in both laboratory and field. The results obtained are discussed here under.

### 5.1 Disease survey and surveillance

Sudarshan Rao (1975) stated that, survey and surveillance form the basis for any successful plant protection that depends on early detection of disease followed by timely adoption of control measures. Hence, the present investigation, roving survey was undertaken in *khari* season in major rice growing areas of Karnataka and Andhra Pradesh. Survey on the incidence and severity of sheath rot of rice reveals the magnitude of the problem on hand and serves as a precursor for evolving the management strategies.

It also gives clue about survival of pathogen during off season on weeds and collateral hosts. The work was initiated on survey to know the severity and distribution of the disease in major rice growing areas of Belgaum, Dharwad, Uttar Kannada, Raichur, Yadgir and Koppal districts of Karnataka and East Godavari, West Godavari districts of Andhra Pradesh.

The survey also supplements the information about intensity and existence of biotypes of in particular geographical locations. The sheath rot of rice expressed in per cent incidence and per cent index. The survey also revealed that severity of sheath rot of rice varied from location to location, type of sowing and varietal performance may be due to various factors like temperature, relative humidity, patterns of rainfall and even it could also be attributed to existence of variability in pathogen.

Among all districts, the maximum disease severity was recorded in Dharwad which may be due to susceptibility of cultivars, type of sowing, closer spacing and favorable environmental conditions *viz.*, optimum temperature and relative humidity, moisture conditions that must have favored to build up of inoculum and subsequently showing increase in disease severity, whereas in East Godavari and West Godavari districts the disease was recorded less compared to other areas. This may be due to unfavorable environmental conditions which reduced the build up of inoculums thus reduced the disease.

From the results of survey, it was observed that, per cent incidence ranged from 8.5 to 35.59 with highest per cent incidence (35.39) recorded in Khanapur (Belgaum) followed by 34.88 incidence in Kustagi (Koppal) whereas least per cent disease incidence in Samalkot (East Godavari). The per cent disease index ranged from 6.67 to 37.78. Arabhavi (Belgaum) recorded as highest PDI (37.78) and least in Veeravalli (West Godavari).

The highest per cent disease incidence 27.89 and per cent disease index 22.42 recorded in Dharwad district and lowest incidence 13.51 in East Godavari. Lowest per cent disease index recorded in West Godavari (14.22) highest in black soils compared to other soils. Drill sown condition showed highest per cent incidence 27.29 and per cent index 21.21.

Shahjahan *et al.* (1977) conducted survey in five major rice growing areas of south eastern Loughani. He observed sheath rot of rice was widely distributed in all the five rice growing areas of Loughani. Of the tiller examined, 11.4 per cent showed symptoms of sheath rot. Srinivasan (1981) reported that heavy infection of *Sarocladium oryzae* resulted in greater yield loss in Thanjavur delta of Tamil Nadu.

Girish (1999) conducted survey in different locations of Karnataka and he identified sheath rot incidence ranged from 26.4 to 44.5 per cent in Bangalore area, and observed the maximum incidence in Tumkur 47.3 per cent. The present findings are also in conformity with the work of Girish (1999) who reported the sheath rot incidence ranged from 26.4 to 44.5 per cent in Bangalore area.

## 5.2 Symptomatology

Sheath rot caused by *S.oryzae* is becoming the major disease of rice in recent days. Temperature and relative humidity requirement is going to decide the development of disease. The disease appeared on the boot leaf sheath as minute speck with brown colour with yellow halo. The spots enlarge by time, coalesce and cover the most of the leaf sheath. In severe conditions the panicle remained inside the boot leaf sheath, discolouration of grains and chaffyness was observed. Similar description of the symptoms of sheath rot of rice were given by previous workers (Ou, 1972; Agnihotrudu, 1973; Amin, 1974; Lakshmanan, 1991 and Narain, 1992).

### 5.2.1 Collection and isolation:

The field survey of disease conducted during 2011-2012 in major rice growing districts of northern Karnataka and Andhra Pradesh showed diverse incidence of sheath rot on rice in different locations. Affected disease samples, showing typical sheath rot symptoms were collected from different locations during survey. Upon tissue isolation the pathogen from the locality was brought into pure culture and identified as *S. oryzae* (Sawada) Gams and Hawksworth based on morphological, cultural characters and pathogenicity in accordance to the description given by Lakshmanan *et al.*, (1992), Sakthivel *et al.* (2002) and Vivekanathan and Rabindran (2008).

### 5.2.2 Pathogenicity

Pathogenicity test was proved by employing single grain insertion method with rice grains inoculated with fungal culture as described by earlier workers Deka Phookan (1997) Hazarika and Phookan(1998).

### 5.2.3 Identification

The study on morphological characters of *S.oryzae* indicated, septate hyphae, separately branched, conidiophores are once or twice branched each time having 3-4 branches in a whorl. Conidia are single celled, smooth, hyaline and cylindrical. Conidia measured 2.13 to 7.14  $\mu\text{m}$  in length and 1.0 to 3.1  $\mu\text{m}$  breadth. The findings were similar to the findings of Ahmed *et al.*, (1974), Shahjahan *et al.*, (1977), Ou (1972), Vivekanathan and Rabindran (2008). Based on the mycelia and spore morphology, the fungus under study was identified as *S. oryzae* (Sawada) Gams and Hawksworth.

## 5.3 Growth phase

Maximum dry mycelia weight of *S. oryzae* was attained on 24<sup>th</sup> day (294.27 mg) potato dextrose broth considered as optimum period for growth of fungus for further studies. The study indicated that further increase in the incubation period resulted in decrease in the dry mycelia weight of the fungus, which might have been due to autolysis of the mycelium, accumulation of toxins and exhaustion of nutrients in the medium after incubation for optimum number of days.

### 5.3.1 Growth and sporulation of *S. oryzae* on different solid and liquid media

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory it is necessary to furnish those essential elements and compounds in the medium for their growth and other life process.

Neither all media are equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow. So, different media including non or semi synthetic were tried for *S. oryzae*.

Among the different non or semi synthetic and solid media maximum radial growth was observed in oat meal agar (8.97 cm) and least was observed in Sabouraud dextrose agar (4.93 cm). Mycelium color varied from white to dark orange and on potato dextrose agar and oat meal agar light orange colonies. Excellent sporulation was recorded in potato sucrose agar. The present studies are in accordance with findings of Mohan and Subramanian (1978) observed the fungus grew and sporulate well in potato dextrose agar and oat meal agar media. Among the four liquid media selected, maximum growth and sporulation were in Czepek's medium. Boa and Brady (1987) reported *S. oryzae* produces whitish pale pink or saffron colonies on oat meal agar at 21°C day light, reverse pink to orange ochre.

Among the non or semi synthetic and synthetic broths used for growth of *S. oryzae*, maximum dry mycelia weight was recorded in oat meal broth (361.67 mg) and least dry mycelia weight (147.23 mg) was recorded in Sabouraud dextrose broth after twenty four days of incubation.

## 5.4 Physiological characters of *S. oryzae*

Temperature is an important factor that governing distribution, growth, reproduction and survival of the fungus. Temperature affects almost every function of fungi including the growth. Temperature has profound effect on the vegetative and reproductive activity of the fungi. Effect of temperature on dry mycelial weight revealed that maximum growth (304 mg) was obtained at 30°C. Increase in temperature to 35°C decrease in mycelial growth was observed and least mycelial growth (88.02 mg) was recorded at 40°C. However the temperature 20-30°C was found to be optimum for growth of *S. oryzae*. The present findings are in agreement with the observations made by Kawamura (1940), Tasugi and Ikeda (1956), Mohan and Subramanian (1978) and Mithrasena and Wijesundera (1992).

Among the external factors, pH of the medium also have profound effect on the growth of the fungus. Although in general fungi prefers acidic pH but most of them varied in their pH requirement with others. The effect of pH on mycelia dry weight revealed that, maximum dry weight was noticed at pH 7.0 and 6.5 (303.67 mg) and least was observed at 9.0 (218.33 mg) when incubated for 24 days in potato dextrose broth. These findings were in confirmity with the findings of Tasuki and Ikeda (1956), Cochrane (1958), Mohan and Subramanian (1978) and Shahjahan *et al.* (1977).

Sugars have important role in fungal growth. The presence of sugars increases the spore germination. Different fungi prefer different sugars for growth.

Among different sugars tested sucrose at 2.0 per cent gave 90.13 per cent germination followed by dextrose at 80.23 per cent. These findings were in accordance with the findings of Mohan and Subramanian (1978), who reported sucrose and starch recorded maximum growth and sporulation of *S. oryzae* followed by glucose and maltose in Czepek (Dox) medium.

## 5.5 Toxin studies

A preliminary indication of toxin production by any fungus *in vitro* has been provided by a number of bioassay methods *viz.*, seed germination bioassay, root and shoot elongation bioassay (Anahosur, (1976); Velazhahan, (1991); Reddy *et al.*, (2000)).

The effect of culture filtrate was studied on seed germination, shoot and root length on sorghum seedlings. Maximum inhibition of sorghum seed germination and also root, shoot length was noticed. There was an inhibition of 9.62 per cent seed germination in 24 and 27 old culture filtrates as the toxin concentration was high. The germination per cent decreased to 8.24 in 30 days old culture filtrate which might be due to reduction in toxicity of the toxins produced.

The maximum inhibition of sorghum shoot and root length was 37.60 and 45.45 per cent in 27 days old culture filtrate with least vigor index. Seed vigor index was maximum in control with sterile distilled water. The present findings clearly indicating that, active metabolites released by the fungus in culture were toxic to seed germination of sorghum.

Current findings are in agreement with Velazhahan (1991) who reported the culture filtrate of *S. oryzae* showed pronounced inhibitory effect on seed germination, shoot length and root length in all varieties. Sakthivel *et al.* (2002) who detected the secondary metabolites; helovioic acid and cerulenin were detected in liquid culture of *S. oryzae*, causal agent of sheath rot of rice and also in diseased sheath tissues. Ghosh *et al.* (2002) detected heloviolic acid and cerulenin in infected rice grains by *S. oryzae* and these metabolites induced chlorosis and reduced the seed viability and seedling health of rice.

## 5.6 Variability studies

### 5.6.1 Morphological variability of different isolates of *S. oryzae*

Variation is the rule in most of the fungi. The variation may arise following change in crop cultivation, genetic modification of hosts, environment or accidental induction of new genetic material into a region or local gene pool. It may also be a way of survival of the pathogen under adverse conditions.

Different isolates *viz.*, Belgaum, Dharwad, East Godavari, Koppal, Raichur, West Godavari, Uttar Kannada, Yadgir showed significant difference in oat meal agar and potato dextrose broth. Accordingly they were classified into different groups. In accordance with solid media the groups were; Group I consists of two isolates Koppal and East Godavari ranging from 6.97cm to 6.8cm. Group II consists of four isolates Belgaum, West Godavari, Uttar Kannada and Dharwad ranging from 6.53 cm to 6.23 cm and group III consists of two isolates Raichur and Yadgiri ranging from 5.9 cm to 5.2 cm of radial growth of pathogen.

As per the growth on liquid media and based on mycelia dry weight three groups were made; Group I consisted of two isolates Koppal and East Godavari ranging from 330.33 mg to 326.33 mg. Group II consisted of Belgaum, West Godavari and Uttar Kannada ranging from 316.67 mg to 309.00 mg. Group III consisted of three isolates Dharwad, Raichur and Yadgiri ranged from 296.3 mg to 280.00 mg.

Isolates exhibited four different colony colors *viz.*, pale orange color, whitish mycelium with pale orange and light pink tinge, white mycelium with light grayish tinge, white mycelium with pale orange and grayish tinge. The variation among the sizes of conidiophores and conidia were not considered, as there was significant variation in sizes within each isolate. The similar observations were made by Phookan and Hazarika (1994) who reported that the morphometric variation among the isolates collected from North East India was insignificant. Reddy *et al.* collected nine isolates of *S. oryzae* from different locations and categorized into five groups based on conidiophores morphology, physiological requirements, virulence and protein and ester banding patterns.

### 5.6.2 Molecular variability of different isolates of *S. oryzae*

It is difficult to distinguish the different isolates using traditional morphological differences. To understand the existence of genetic variation among the isolates of *S. oryzae*, PCR based technique *i.e.*, RAPD was used in the present investigation. RAPD studies also help to know the specific markers for the identification of particular race or detection of pathogen.

Genetic variation detected among all eight isolates of *S. oryzae* using RAPD markers. Ten primers were used for amplification. Among ten primers OPA 10 showed 100 per cent polymorphism. OPA 7, OPB 11, OPA 16, OPA17, OPB 18, OPB 13, OPA 1 showed 91.67 per cent, 85.71 per cent, 83.33 per cent, 80 per cent, 77.77 per cent, 75 per cent, 75 per cent respectively.

Based on similarity coefficient, a genetic similarity was constructed to assess the genetic relatedness among the isolates of *S. oryzae*. Genetic similarity coefficient ranged from 0.61 to 0.88. Highest similarity observed between Belgaum and Dharwad whereas least similarity observed between East Godavari and Uttara Kannada isolates. This reveals that isolates from nearby geographical area will show the maximum similarity coefficient.

The results were in agreement with the findings of Niraikulam *et al.* (2005) who detected the variability in pathogenicity, phytotoxic metabolite production and DNA polymorphs among the *S. oryzae* isolates. According to the level of similarity, *S. oryzae* isolates from north east and south India were grouped separately into two major clusters and 13 genotypes. A high level of genetic variability within *S. oryzae* isolates was identified.

## 5.7 Screening of genotypes against sheath rot of rice caused by *S. oryzae*

Identification of resistant source or sources against any disease is of primary importance. In present study, forty four rice genotypes were screened under artificial conditions against sheath rot during *kharif* 2011, Agricultural Research Station, Mugad, Dharwad. Out of Forty four genotypes two genotypes Rere seed, Bilidadi goratiga showed highly resistant reaction. Sixteen genotypes showed resistant reaction; fourteen genotypes showed moderately resistant reaction, seven genotypes showed moderately susceptible reaction and about five genotypes showed susceptible reaction. None of the varieties were immune.

The present results were in agreement with findings of Singh and Raju (1981) who reported that among different varieties screened, five varieties *viz.*, Basumati-370, Chinsurah, Boro-II, homthong, sigadis and TKM-6 were resistant to sheath rot disease on artificial inoculation. Sahu and Parida (1997) studied the response of 60 rice breeding lines against sheath rot. Out of 60 lines tested, three lines were highly resistant, 18 were resistant and 33 were moderately resistant to the disease.

## 5.8 Disease management

### 5.8.1 *In vitro* evaluation of fungicides

In the absence of resistant cultivars, use of fungicides to manage the disease is an old-age practice. When there is outbreak of epidemic for any reason perhaps use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions.

*In vitro* evaluation of new synthetic molecules of fungicides is very much necessary before they are tried under field condition. Among the non-systemic fungicides at 0.3 per cent concentration carbendazim + mancozeb showed 62.81 per cent inhibition of mycelial growth of fungus followed by hexaconazole + zineb (41.69%). The least inhibition of fungus was recorded in mancozeb (29.83%). Reddy *et al.* (1985) reported that bavistin proved as the most effective fungicide, followed by blue copper and antracol among 11 fungicides evaluated against *S. oryzae*.

Among the systemic fungicides, maximum per cent inhibition of growth of *S. oryzae* was observed in propiconazole (66.83%) which was significantly superior to all other fungicides followed by carbendazim (60.18%) and hexaconazole (54.31%). The least per cent inhibition of fungus was recorded in tricyclazole (45.87%). The effectiveness of the triazole fungicides like propiconazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence cause irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of propiconazole which inhibit the mycelia growth and spore germination of the fungi (Pandiarajakumar *et al.* 1998).

### 5.8.4 *In vivo* evaluation of fungicides against sheath rot of rice during *Kharif* 2011

Field experiment was planned and executed during *kharif* 2011, Agricultural Research Station, Mugad, Dharwad. Eleven different treatments (two non-systemic, three combi products and five systemic), one control with seed treatment by carbendazim at 0.2 per cent with untreated control were evaluated for their efficacy disease control.

The results after two sprays revealed that, propiconazole at 0.1 per cent concentration was significantly superior over other fungicides, followed by carbendazim (0.1%) and carbendazim + mancozeb (0.2%). Yield obtained in fungicides treated plots indicated that, highest and significant yield of 43.14 q/ha was recorded in propiconazole followed by Carbendazim at 0.1% (41.81 q/ ha) and carbendazim + mancozeb at 0.2 % (40.25 q/ ha) concentration treated plots, which were on par with each other. The results are in agreement with findings of several workers (Lakshmanan, 1984; Dodan *et al.*, 1996; Thapak *et al.*, 2003).

## Future line of work

The present investigations on physiological requirements, morphological and molecular variability of sheath rot pathogen gave an insight about the existence of variability in the fungus. This information may be further extended to identify the;

1. Survey for the disease in other important rice growing areas of Karnataka and Andhra Pradesh.
2. Identification of variability in relation to bio chemical components, by using specific markers in ITS, RFLP in different isolates.
3. To study the epidemiology of the disease.
4. To develop alternate methods of disease control.

## 6. SUMMARY AND CONCLUSIONS

An investigation of sheath rot of rice caused by *S. oryzae* was carried out with reference to survey and surveillance of disease; cultural, morphological, physiological aspects of pathogen; morphological and molecular variability of the pathogen; screening of rice genotypes against disease; management of disease by fungicides the results obtained are summarized here under,

An extensive roving survey was carried out in northern Karnataka and Andhra Pradesh to assess the severity of sheath rot of rice. This study revealed that highest per cent disease incidence (27.89) and per cent disease index (22.42) of disease was recorded in Dharwad district. Correspondingly, the lowest disease incidence (13.51) was recorded in East Godavari and lowest PDI (14.22) recorded in West Godavari.

. Among the varieties, Sona Masuri was found more susceptible and recorded highest PDI. The maximum disease incidence and severity were observed in drill sowing paddy.

The colony morphology in general indicated that fungus on potato dextrose agar produced white to pale orange mycelium, verticillate conidiophores, with conidia single celled hyaline, straight cylindrical. On the basis of cultural and morphological characters, the fungus was identified as *S. oryzae*. Pathogenicity of the fungus was done with single grain insertion method with rice seeds incubated in fungal culture.

The maximum dry mycelial weight of *S. oryzae* was observed after 24 days of incubation on potato dextrose broth (294.27 mg) and this period was considered as optimum period for fungal growth.

The fungus *S. oryzae* exhibited diversified cultural characters, on both synthetic and semi/non-synthetic liquid and solid media. In case of solid media maximum radial growth of the fungus (8.97 cm) was recorded on oat meal agar whereas least on Sabouraud dextrose agar (4.93 cm). However, among the liquid media maximum dry mycelial weight (361.67mg) was recorded on oat meal broth and least weight (147.23mg) on Sabouraud dextrose broth.

The pH of 6.5 and 7.0 were found to be significantly superior by recording the maximum dry mycelial weight (303.67 mg) in potato dextrose broth and least dry mycelial weight (218.33 mg) at pH 9.0. Temperature of 30° C was found to favor the growth of the fungus in liquid media, which recorded the highest radial dry mycelial wight (304.00 mg) where as the least dry mycelial weight (88.02 mg) recorded at 40° C. Among different sugars tested to know the effect on germination the sucrose at 2 per cent enhanced the germination up to 90.13 percent followed by dextrose 80.23 percent.

The different days of incubation of culture filtrates of *S. oryzae* differed in their action to inhibit the sorghum seed germination, root and shoot length. The maximum inhibition of seed germination, root and shoot length, least vigor index of sorghum was noticed within 27 days old culture filtrate and least inhibition and highest vigor index was in three days old culture filtrate.

The eight isolates from different places were studied to know the variability. According to the colony diameter they were made into three groups. Group I consists of two isolates Koppal and East Godavari ranging from 6.97cm to 6.8cm. Group II consists of four isolates Belgaum, West Godavari, Uttar Kannada and Dharwad ranging from 6.53 cm to 6.23 cm and group III consists of two isolates Raichur and Yadgiri ranging from 5.9 cm to 5.2 cm. The variation among the sizes of conidiophores and conidia were not significant. Genetic variation detected among all eight isolates of *S. oryzae* using RAPD markers. Highest similarity observed between Belgaum and Dharwad whereas least similarity observed between East Godavari and Uttara kannada isolates.

Out of forty four genotypes used for screening two genotypes Rere seed, Bilidadi goratiga showed highly resistant reaction. Sixteen genotypes showed resistant reaction; fourteen genotypes showed moderately resistant reaction, seven genotypes showed moderately susceptible reaction and about five genotypes showed susceptible reaction.

Among the non-systemic (one combi product) and systemic fungicides evaluated *in vitro*, carbendazim + mancozeb recorded highest per cent inhibition of mycelial growth (62.81). However least per cent inhibition of mycelium was noticed in mancozeb (28.96). In case of systemic fungicides, propiconazole at 0.15 per cent recorded highest per cent inhibition of mycelium growth (71.07) least per cent inhibition of mycelia growth was recorded in tricyclazole (38.98%). Propiconazole and carbendazim were very effective in inhibiting the mycelial growth of the fungus at 0.1 per cent concentrations.

The management of sheath rot using different fungicides *in vivo* indicated that the spraying of propiconazole at 0.1 per cent was significantly most effective and recorded higher yield and on par with carbendazim at 0.1 per cent. mancozeb (0.25%) were found to be least effective.

#### Conclusions

- \* Maximum mean disease incidence and index were recorded in Dharwad district followed by Belgaum and Uttar Kannada districts.
- \* Maximum mean disease index on was recorded sona masuri variety.
- \* Maximum disease index was recorded drill sown conditions.
- \* The fungus was white with pale orange tinge on potato dextrose agar and produced septate mycelium. Conidia were hyaline, unicellular and cylindrical and borne on verticillate conidiophores.
- \* Pathogenecity of sheath rot of rice was proved by single grain insertion method in artificial condition.
- \* Maximum growth of *S. oryzae* was attained on 24 days after incubation (294.27mg).
- \* Maximum dry mycelial weight of *S. oryzae* was recorded in oat meal broth.
- \* On non-synthetic media maximum radial growth of *S. oryzae* was recorded on oat meal agar (8.97 cm).
- \* Maximum dry mycelia weight was recorded at pH of 6.5 and 7.0 and temperature 30° C.
- \* Sucrose at 2% enhances the spore germination.
- \* Among the culture filtrates tested maximum inhibition of seed germination, root and shoot length, least vigour index of sorghum was recorded in 27 days old culture filtrate. The least inhibition of seed germination, root and shoot length and highest vigour index was recorded in three days old culture filtrate.
- \* Morphological variability among the isolates was based on their radial growth and in molecular level highest similarity observed between Belgaum and Dharwad isolates whereas least similarity observed between East Godavari and Uttara Kannada isolates.
- \* Out of forty four genotypes two genotypes Rere Seed, Bilidadi Goratiga showed highly resistant reaction and no one of them were immune.
- \* Among the non-systemic fungicides, carbendazim + mancozeb (combi-product) recorded highest inhibition of mycelial growth at 0.3 per cent concentration under laboratory condition.
- \* Among four systemic fungicides, maximum inhibition of growth was recorded in propiconazole at 0.15 per cent concentration.
- \* Propiconazole at 0.1 per cent managed the sheath rot disease very effectively with high yields.

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# STUDIES ON SHEATH ROT OF RICE CAUSED BY *Sarocladium oryzae* (Sawada) Gams and Hawksworth

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## ABSTRACT

Rice (*Oryza sativa* L.) an important cereal crop belongs to family Poaceae commonly known as Asian rice, is a staple food crop for sixty per cent of world's population. Sheath rot of rice is of common occurrence in all rice growing countries and more prevalent in recent decades. Sheath rot was observed in all the locations surveyed with range of 6.67 to 37.78 per cent disease index. Maximum disease severity was noticed in Dharwad district (22.42) followed by Belgaum (21.52) and the least was observed in West Godavari (14.22).

Maximum dry mycelial weight was recorded on 24<sup>th</sup> day of inoculation in potato dextrose broth and Richard's broth. Maximum mycelial weight was observed on Oat meal broth and radial growth on oat meal agar. The temperature of 30°C and the pH of 6.5 to 7.0 found best for mycelial growth. Sucrose at two per cent concentration enhanced the conidial germination (90.13%). Least vigor index of sorghum was noticed when soaked in 27 days old culture filtrate, indicating maximum toxin production. Among different isolates tested maximum growth was observed in Koppal isolate on oat meal agar, PDB and based on growth of isolates three groups were made. The variation among sizes of conidia and conidiophore was insignificant. When RAPD markers used higher genetic similarity was observed between Belgaum and Dharwad isolates and the least similarity was observed between East Godavari and Uttara Kannada isolates.

Out of forty four isolates screened under artificial conditions none of them were immune and two genotypes (Rare Seed and Bilidadi Goratiga) showed highly resistant reaction. Under *In vitro* conditions Carbendazim + Mancozeb (62.81%) and Propiconazole (71.07%) showed the highest per cent inhibition of mycelial growth. In field conditions propiconazole at 0.1 per cent was the most effective in managing the disease and recorded highest yield.