EPIDEMIOLOGY AND MANAGEMENT OF FALSE SMUT OF RICE (*Oryza sativa* L.) IN KERALA

by

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DECLARATION

I hereby declare that this thesis entitled **'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any university or society.

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Certified that this thesis entitled **'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala'** is a record of research work done independently by Ms. Rashmi C. R. (2010-21-111) under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associate ship to her.

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EXTERNAL EXAMINER

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Rashmi C. R.

DEDICATED TO MY PARENTS

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µm	Micro meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
nm	Nano meter
DAS	Days after sowing
DAT	Days after transplanting
et al.	And other co workers
Fig.	Figure
g	Gram
ha	Hectares
h.	Hours
g-1	Per gram
i.e.	that is
kg.	Kilogram
t/ha.	Tons per hectare
l.	Litre
m	Meter
mm	Milli meter
mg	Milli gram
ml	Milli litre
rpm	Rotations per minute
sec	Seconds
PDI	Percentage of disease index
SE	Standard error

sp. or spp	Species (Singular and plural)
var	Variety
viz.	Namely
dia.	Diameter
MT	Metric tonnes
AGR	Abortive Grain Rate
cv.	Cultivar
SW	South West
kb	Kilobases
Mb	Megabases
RAPD	Random Amplified Polymorphic DNA
AFLP	Amplified Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
max.	Maximum
d	Days
ITS	Internal Transcribed Spacer
DI	Disease Incidence
DS	Disease Severity
PSB	Potato Sucrose Broth
RH	Relative Humidity
RFS	Rice False smut
p.p.m	Parts per million
min	Minutes
vars.	Varieties
N	Nitrogen

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Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and is the staple food for more than half of the world population. Feeding the ever increasing population is the major challenge faced by the world countries. We have almost reached a stagnant level in production, where, there could no longer be any considerable increase in production level with the limited resources available. Thus, with the limitations with the crop improvement in terms of yield increase, the only available solution for increasing crop production is to reduce the losses in terms of pests, diseases, weeds etc. to the maximum extent possible.

False smut disease of rice caused by *Ustilaginoidea virens* (Cke.) Tak. is an important constraint affecting the rice yield and quality in Asia (Zhou *et al*., 2008), and is an emerging disease world wide (Brooks *et al*., 2009) and apart from yield reduction causes qualitative losses and health problems (Seshavatarm, 1965; Nakamura *et al.*,1994 and Parsons *et al.*, 2001).

The disease, once considered a minor one, has become a serious problem in recent years due to high input cultivation, adoption of high yielding varieties, climate change etc. (Anders *et al*., 2008; Lu *et al*., 2009).

In India, false smut is reported to cause substantial quantitative and qualitative losses under favourable environmental conditions (Baruah *et al*., 1992; Chib *et al*., 1992). False smut had become a serious disease in Tamil Nadu and also the other rice growing states of India and the prominent high yielding rice varieties such as CO 43, CR 1009, ADT 38, ADT 39 and BPT 5204 were found affected by the disease (Anonymous, 2008).

Surveys conducted by the Directorate of Rice Research, India indicated that false smut was found to be emerging as a serious problem in India in states like

Haryana, Uttar Pradesh, Tamil Nadu and Karnataka and many of the hybrids and varieties were heavily infected with false smut (DRR, 2010).

Ladhalakshmi *et al.* (2012 c) reported that false smut disease had been observed in severe form since 2001 in India due to wide spread cultivation of high fertilizer-responsive cultivars and hybrids, extensive application of nitrogenous fertilizer, along with the change in climate. Likewise, this disease has emerged as the most devastating grain disease in the majority of the rice-growing areas of the world.

Not much work has been done so far in the State as well as in the country on the etiology of the disease, its epidemiological aspects and management strategies. Thus the present work, "Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala", was undertaken to investigate the epidemiology of false smut disease of rice and to evolve strategies for effective management of the disease.

The following items of work were undertaken:

- Surveys on the incidence and severity of the disease
- Study on the symptomatology of the disease
- Studies on the cultural and physiological characteristics of the pathogen
- Studies on the epidemiology of the disease
- Screening of rice varieties against false smut disease
- Isolation and *in vitro* testing of biocontrol agents
- *In vitro* evaluation of chemical fungicides
- Field evaluation of bio agents and fungicides
- Biochemical studies

Review of literature

2. REVIEW OF LITERATURE

Rice is the staple food for a large part of the world's population especially in Asia and is the second highest produced food grain in the world. It is the most important food crop of India in terms of area, production and consumer preference (Anonymous, 2013 a).

Rice production in India crossed the mark of 100 million MT (104.32) in 2011-12 accounting for 22.81 % of global rice production in that year. The productivity of rice in India has increased from 1984 kg hectare^{-1} in 2004-05 to 2372 kg hectare^{-1} in 2011-12 (Anonymous, 2013 a). In Kerala, the crop is cultivated in an area of 0.21 million hectares with a production of 0.47 million MT and a productivity of 2668 MT hectare^{-1} (Anonymous, 2013 a).

Historical Aspects

False smut or green smut of rice caused by the fungal pathogen, *Ustilaginoidea virens* (Cke.) Tak., was first reported from Thirunelveli in Tamil Nadu State of India by Cooke in 1878 and was named as *Ustilago virens*. Takahashi named it as *Ustilaginoidea virens* (Cke.) Tak and Hashioka provided the name, *Claviceps oryzae-sativae* for the teleomorph. The fungus was described very early in Chinese literature, but without a scientific name and the presence of the disease was believed to be an indication of a year of good crop (Ou, 1972).

Disease outbreaks and Losses

Vincens (1921) reported *U. virens* as a curious parasite of rice, which develops at the expense of the grain, in place of which appears an orange or olive-green mass, separating the glumes and protruding between them and regarded it as one of the (relatively uncommon) stages of a very widespread parasite, which in other stages frequently caused partial sterility of the spikelets in which case it was of considerable importance.

False smut disease was reported from Ceylon by Gadd (1926). Rhind, (1924) reported that rice was attacked by false smut, but was not found to cause appreciable damage in Burma.

In 1925 Haskell and Diehl (1929) observed that some maize tassels from Louisiana were found bearing excrescences which superficially resembled those of *Ustilago zeae* but on closer inspection were found to be the sclerotia of an *Ustilaginoidea*, and in 1926 similar material was received from the Panama Canal Zone.

Chevalier (1931) reported that rice cultivated in Liberia had been observed to be severely attacked by a smut pathogen which has been identified as *U. virens*, a new record from West Africa. Reyes (1939) described *U. virens* as a minor disease of rice at Philippines.

Mundkub (1940) observed *U. virens* on rice in the summer of 1939 at Afghanistan. Seth (1945) reported that false smut of rice, ordinarily of minor importance in Burma, appeared in epidemic form in 1935 at Hmawbi and the surrounding areas and reappeared in 1936 in a milder form.

False smut was observed in the region of Fenerive, Madagascar by Bouriquet, (1949). *U. virens* occurred sporadically in French Guinea and the Ivory Coast (Mallamaire, 1949).

'False' or 'green' smut of rice was first observed in São Paulo, Brazil, in 1946, noted to be continually extending its range in the State affecting various varieties such as Pratao, Amarelao, 4-month, Cate-tinho, Rabo de Carneiro, Ferrao Preto, Pindorama, Perola, Dourado Agulha, Catetao, Jaguari, and Iguape Agulha (Campacci, 1951). Orejuela (1953) reported that *U. virens* was a principal disease of rice in Columbia, U.S.A.

An account of the diseases of economic plants described from Bihar, India, in 1952-53 by Thirumalachar and Mishra (1953) revealed rice false smut which occurred in Islampur, Purnea, on the border of Bengal, in a severe form.

Petrak (1954) reported *U. virens* from the British North Borneo. In Nigeria, *U. virens* on rice was newly observed during 1951-52 period (Anonymous, 1954). During 1951 and early 1952, false smut occurred on rice in the Cauca Valley, Colombia, with an estimated loss of 20 per cent (Martinez, 1952).

As per the reports by Revilla (1955), false smut of rice, first observed in Peru in 1951, was still confined to the Tumbes Valley but there has been considerable increase in the incidence of the disease.

Important diseases of the chief food crops in Mysore included *U. virens* (Anonymous, 1955). According to Morwood (1956), rice crops suffered a 10 % loss from *U. virens* at Fiji. Alandia and Bell (1957) reported false smut of rice to be found in Santa Cruz of Bolivia.

Jerkes *et al.* (1959) reported the presence of *U. virens* on maize. Ikegami (1959), based on the studies on the occurrence of *U. virens* in rice ears estimated from material collected from 10 fields of upland and lowland rice near Gifu, Japan, found that the weights of the smut balls, the ear excluding smut balls, and thousand kernels decreased with the increase of the number of smut balls/ear.

According to Mouton and Merny (1959) the chief rice diseases in Beteland, Ivory Coast, were *Helminthosporium oryzae* and *U. virens*, of which infection by *U. virens* was the more spectacular and the observations showed that the incidence of infection by *U. virens* was always greater in the lowlands than at higher altitudes.

As reported by Misra (1959), the major diseases of rice in the Andaman included *U. virens*. Toler *et al.* (1959) reported the disease from Panama. De-Mello *et al.* (1962) reported that the wet season of 1960-61 favoured development of false smut disease at Sao Paulo, Brazil.

As per the quarterly report for January-March, 1963 of the Plant Protection Committee for the South East Asia and Pacific Region, *U. virens* was newly recorded on rice from Brunei (Anonymous, 1963). Dunsmore (1970) reported false smut from the upland areas (hill paddy) from Sarawak, Malaysia. False smut disease was first observed in Australia in the Northern Territory by Pitkethley (1970). On the West Coast, Berbice, Guyana, approximately 100 acres of rice was infected by false smut (Anonymous, 1970).

During 1974 false smut disease was observed for the first time at Mauritius on rice (Anonymous, 1974). In 1973, rice false smut appeared as an epidemic in India in Jabalpur (Sharma and Joshi, 1975). During *Kharif* 1975, there was a severe outbreak

of the disease in the rice variety Jaya with 45 % disease incidence and 17 % yield reduction (Tyagi and Sharma, 1976).

Singh and Dube (1976) reported that false smut disease was common in coastal parts of Orissa, Andhra Pradesh, Haryana and Tarai and Hills of Uttar Pradesh and caused considerable loss in grain yield under favourable conditions. With the introduction of new high yielding rice varieties, the disease was on an increase in the Tarai and North West sub-Himalayan range of Uttar Pradesh and up to 44 % yield losses were observed in a variety Ratna due to the disease (Singh and Dube 1978). During 1979, the disease appeared in severe form near Varanasi (Khare and Singh (1979). The disease was reported from the maize crop at Tengnoupal District of Manipur during the crop seasons of 1976 and 1977 (Sharma and Verma 1979). According to Singh (1979), the false smut was a predominant disease of rice grown in non-traditional areas of north-western India.

U. virens had been a major disease of rice in the nontraditional areas of western Uttar Pradesh, Rajasthan, Punjab and Delhi (Chakrabarti, 1979). Roy (1980) reported heavy attack of rice by *U. virens* in Assam in 1965 due to grain infection. Field surveys conducted by Hu (1985) in 1982-83 showed that the damage was mainly due to the diseased grain inhibiting fertilization and development in adjacent spikelets, resulting in a high abortive grain rate (AGR) and in a decrease of 1000-seed wt. When the number of diseased grains on a panicle increased, the AGR increased by 3.46 and 4.95 % for the 2nd and the 1st cropped rice, respectively, and the 1000-seed weight of the first cropped rice decreased by 0.76 g. The diseased grain number, AGR and the decrease of 1000-seed wt. were positively correlated with the yield loss.

Anand *et al.* (1985) recorded the incidence of false smut on some rice cultivars in Jammu and found that the severity of infection was the maximum on cv. PC-19, followed by Jaya and IET-1410. An increase in chaffiness in smutted panicles was also observed in PC-19 and Jaya.

A survey conducted during 1979-81 in Bangladesh revealed the presence of false smut disease in transplanted June-December rice crop (Miah *et al.*, 1985).

Sharma and Chaudhary (1986) reported *U. virens* on maize for the first time from Kashmir.

Eastern Uttar Pradesh had a false smut epidemic during 1984-85 to 1985-86 and the incidence ranged from 10 to 30 %. The infection by the fungus led to reduction in 1000 grain weight, increase in partially filled and unfilled grains and reduction in seed germination (Singh *et al.,* 1987 a). Singh *et al.,* (1987 b) reported that false smut disease, had become very important in wet season crops in this area and the cultivars showing high disease levels included TN1, IR8, Jaya, IR24, Ratna, Prasad, Sona, Bala, Sarjoo 49, Sarjoo 52, Mahsoori, Sita, Saket 4, Narendra 1, Narendra 2 and Cauvery.

In 1986, Pusa-33 rice plants in Iroisemba, Manipur showing yellow dwarf disease symptoms, caused by a mycoplasma-like organism, were also found to be infected by *U. virens*, and in pathogenicity tests, susceptibility to *U. virens* in MLO infected plants was found higher (Singh, 1987 b).

U. virens was reported for the first time from Puerto Rico by Pantoja and Medina-Gaud, (1988) who recorded the occurrence of this disease during March 1986 in a rice farm in Manatí, Puerto Rico. Pathak and Sachan (1991) reported the occurrence of false smut on rice from Uttar Pradesh, India.

During the rainy seasons of 1986 and 1987, false smut of rice was recorded on five rice cultivars (PR 103, PR 106, PR 108, PR 109 and Java) in Gurdaspur, Punjab, India (Dhindsa *et al.*, 1991), where the maximum disease was recorded in PR 109 and the total yield losses were maximum (16.8 %) in PR 109, followed by PR 106 (10.12 %), Jaya (6.64 %), PR 108 (3.47 %) and PR 103 (1.53 %). The disease was recorded from Sikkim by Srivastava *et al.* (1991) in the maize crop.

Chib *et al.,* (1992) reported false smut to be spreading throughout the sub-tropical belt of Jammu and Kashmir in alarming proportion on high yielding cultivars and recorded high incidence of chaffiness due to the disease.

In Assam, severe occurrence of false smut disease during wet season in a large number of rice varieties including the most popular variety Mahsuri had become a cause of serious concern to the rice growers in Assam (Baruah *et al.,* 1992).

During 1991-92, false smut was recorded to adversely affect in epiphytotic proportion many rice cultivars in coastal tracts of Orissa, particularly, on fine grain varieties (Narain, 1992). Singh *et al.* (1992) reported that during 1989, the overall incidence of false smut of rice (*U. virens*) in Haryana, India, was 44.1 %. In two severely infected fields, the number of smut bolls per panicle varied from 1-38, and 1000 grain weight was reduced by 28.5 %. More smut balls were present on the lower third of the panicle (60 %) than on the middle and upper portions (32 and 8 %, respectively).

False smut, which was formerly believed to be an indication of a good cropping year, was shown to be an important disease by the intensive studies that took place after a severe outbreak in Japan in 1988 and the increase in the incidence of rice false smut, had given rise to serious disease problems in Japan (Naito, 1994).

Disease surveys were conducted during the *Kharif* seasons during 1983-90 in Haryana, India, to determine the incidence and distribution of rice diseases revealed false smut to be one of the most important diseases (Dodan *et al.*, 1997).

Yield losses in five intermediate season rice varieties (lines), including three indica rice and two japonica rice varieties, caused by rice false smut, *U. virens*, and the decrease in rate of milled rice were investigated by Ke-Jian *et al.* (1997) and the results showed that the rate of milled rice decreased as the number of infected grains increased. In the five rice varieties tested, the disease severity of indica rice varieties was higher than that of japonica rice varieties.

Lee (1999) reported that false smut caused by *U. virens* (Cooke) Tak., a minor disease which occurred very sporadically in production areas along the U.S. Gulf Coast, was first observed in a few Arkansas production fields during 1997, and during the 1998 growing season the disease became widespread over a significant portion of the Arkansas rice production areas causing great concern in the rice industry.

Rice green smut was reported to be present in the booting stage of rice plants in Jiaxing, China (Ke-Quiang *et al.*, 1999). False smut of rice was reported in 20 counties with the most severe levels in northeast Arkansas and an epidemic was reported at Craighead, U.S. A. (Cartwright *et al.*, 1999).

As per the reports by Rush *et al.* (2000), false smut had been occurring in Louisiana rice since 1906, and an outbreak of false smut in rice, was recorded from Louisiana, USA, in 1997, with 1-15 % of tillers affected in the fields in SW Louisiana.

A total of 609 rice seed samples were collected from various districts of Haryana, India during 1993-97 and were evaluated (Duhan and Jakhar, 2000) for the incidence of false smut (*U. virens*) and it was found that the incidence of false smut across different rice cultivars, districts and years ranged from 0.05 to 0.50 %.

A survey on the disease incidence at rice fields in eight inland provinces carried out by Hong-Sik *et al.* (2001) in 2000 at the Korea Republic, showed that the disease occurred at 104 (7.5 %) out of 1152 rice fields examined, with disease severity values ranging from 1.5 to 13.7 %.

Surveys on the effect of discoloured grains to grain quality of rice cultivars grown in some cultivated areas of the Mekong Delta (Vietnam) showed that there were nine fungal species found on 60 samples of 12 cultivars by 'blotter method' including *U. virens* (Van-Du, *et al.*, 2001)

The yield loss due to false smut disease in five rice cultivars, i.e. NDRSB 9730015, Sita, Jaishree, Shakuntla and Sujata, was 9.8, 7.5, 5.1, 3.50 and 3.0 %, respectively, during *Kharif* 1999-2000 in Patna, Bihar, India. An increase in chaffiness by 25, 18.5, 7.0, 10 and 6 % was also reported in infected panicles compared to healthy panicles in respective cultivars. The difference in 1000-grain weight of healthy and infected panicles was 0.41, 0.35, 0.25, 0.28 and 0.28 g, respectively (Sinha *et al.*, 2003).

Chander *et al.* (2003) observed that false smut was a major disease of rice in Madhya Pradesh and was found to cause substantial damage only after the green revolution. Cartwright *et al.* (2003) reported false smut to be common in northeast Arkansas in the USA.

Bagga and Kaur (2004) observed that false smut disease of rice had become an important disease problem in the Punjab State of northwest India and caused widespread concern in 2001 and 2002 in a commercial cultivar PR 116.

Mandhare *et al.,* 2008, in a study conducted in Maharashtra during 1999 to 2006 to evaluate the incidence of false smut in 630 paddy varieties, observed that the incidence of false smut on commercially cultivated varieties ranged from 0.00 – 72.92 per cent and was highest (72.92 %) on Kundlika variety of paddy.

Anders *et al.*, (2008) observed that in severely affected fields in Arkansas in the USA, a cloud of spores were observed above the combine header during harvest and the dark "balls" up to half inch dia. was found to be getting mixed in the harvested grain. In Myanmar, 12 % false smut incidence was recorded in Kyaukse in Upper Myanmar and 25 % in Kyauktan in Lower during 2002 (Naing *et al.*, 2008)

As per a news report from Tamil Nadu (The Hindu, 2008), false smut of rice, once considered as a minor disease, had become a serious disease in Tamil Nadu and also the other rice growing states of India and the prominent high yielding rice varieties such as CO:43, CR:1009, ADT:38, ADT:39 and BPT:5204 were found to be affected by the disease.

Zhou *et al.* (2008) reported that false smut of rice caused by *U. virens* was an important constraint affecting yield and quality in Asia and in China, it was especially severe under the Japonica rice growing areas in the North China.

In China, false smut had become one of the most important fungal diseases in rice since the 1980s and it affected approximately 200,000 to 330,000 ha in Liaoning from 1984 to 1996 (Lu *et al.,* 2009). In a survey carried out in October 2007 in Chhattisgarh to assess the extent of false smut infestation, Singh and Pophaly (2010) found that more than 600 ha were severely affected by false smut. Late-sown rice varieties such as Swarna, Tapaswani, Komal, and Culture 64 grown in lowland areas showed high levels of false smut injury. The observed epidemic was found devastating and the percentage of infected panicles ranged from 16.8 to 40.7, each usually bearing 5 to 12 smut balls.

False smut was found to be emerging as a serious problem in India in states like Haryana, Uttar Pradesh, Tamil Nadu and Karnataka. Many of the hybrids in Haryana, varieties BPT 5204 and CR 1009 in Tamil Nadu and varieties like PR-116 in Punjab were heavily infected with false smut (DRR, 2010).

False smut disease causes both quantitative and qualitative losses and the yield losses in different states of the country have been estimated to vary between 0.2 % to 49 % depending on the disease intensity and rice varieties grown (Ladhalakshmi *et al.*, 2012 a).

False smut disease had been observed in severe form since 2001 in India due to widespread cultivation of high fertilizer-responsive cultivars and hybrids, heavy application of nitrogenous fertilizer, and an apparent change in climate and had emerged as the most devastating grain disease in the majority of the rice-growing areas of the world (Ladhalakshmi *et al.*, 2012 c).

Li-Min *et al.* (2012), upon designing of yield loss models of Japonica rice, glutinous rice and hybrid indica rice for analyzing the relationships between disease incidence and yield loss by means of randomized block design, moderate controlling disease gradient, grading by number of diseased grains in each diseased ear, and large sampling, showed that disease severity of rice false smut was positively correlated to yield loss of rice.

According to Singh *et al.* (2012), false smut of rice, once considered as a minor disease, had become a serious disease in rice growing area of M. P. and the disease incidence had been reported at many places in an alarming proportion. During 2009, about 800 ha area of paddy fields at Datia district was severely affected due to this disease. The highest disease incidence (61.20 %) and yield loss (14.18 %) was observed in late transplanted paddy fields.

Incidence of sheath blight, false smut and kernel smut disease was recorded on 25 rice varieties and 7 rice hybrids grown in *Kharif* 2008 at Jabalpur by Kapse *et al.* (2012) and the incidence of false smut disease was found to be ranging from 3.0 to 12.0 per cent.

Qualitative losses

Seshavatarm (1965) studied the concentration of spores liberated during threshing of rice so as to determine the spore content of the dust which was responsible for many respiratory troubles, especially in farm workers and reported that respiratory troubles particularly increased when the crop was infected with *U. virens*.

The effect of harvesting operations on the incidence of 13 fungal spore types over a rice crop at Bhimavaram, India, was studied and it was found that the concentration of spores of *U. virens,* was greatly elevated on the day of harvesting compared with two days previously, when the crop was still standing and it was suggested that repeated exposure of farm workers to unusual heavy spore concentration during harvesting may be of considerable risk to their health (Atluri *et al.*, 1988).

Water extracts of false smut diseased grains have been reported to poison animals (Nakamura *et al.* 1994). The smut balls formed by the pathogen contained antimitotic peptides called ustiloxins, which had inhibitory effects on the polymerization of microtubules proteins and could cause acute necrosis of isolated hepatocites and renal tubular cells followed by mitotic arrest and abnormal mitosis resembling that caused by colchicine.

Parsons *et al.* (2001) reviewed that the presence of false smut balls in harvested grain had been a concern to rice mills and buyers in Arkansas, USA.

At Srikakulam, located in North Coastal Andhra Pradesh, India, a major study on the airspora of Srikakulam, the effect of rice and sugarcane harvesting on aerial spore concentrations was studied through rotorod trappings. The results showed an enormous increase in the spore load not only of the fungi pathogenic to the crop but also of saprophytic fungi while the crop was harvested. The increase in the pathogenic fungal spores was high, as evidenced by the increase in rice crop pathogens, such as *Trichoconis padwickii, U. virens, Cochilobolus oryzae* etc. suggesting that the farm workers were likely to be exposed repeatedly to high doses of fungal spore allergens from crop harvesting operations.(Atluri and Murthy, 2002).

Symptomatology

Two different types of rice infection by *U. virens* were observed by Raychaudhuri (1946) at the Central Experiment Station, Dacca, Bengal, one marked by the presence on the panicle of a few sclerotioid structures in place of grains and mostly empty glumes, and the other by a paucity of sclerotia, most of the glumes being normally filled although the hairs are sometimes infected by the greenish-brown spores of the smut.

According to Mulder and Holliday, (1971), false smut of rice appears as olive-green, velvety, globose masses, up to 1 cm diameter in some of the ears of the inflorescence. The spore ball, beneath the dark layer of mature spores, is orange-yellow, paling inwards until it is almost white, and as it ages it becomes almost black. The glumes are closely applied to the lower part of the spore ball which is at first covered with a membrane.

As per the symptom description by Ou (1972), the fungus transforms the individual grains of the panicle into greenish spore balls of a velvety appearance. The spore balls are small at first and are visible in between the glumes, growing gradually to each one cm or more in diameter, and enclosing the floral parts. They are slightly flattened, smooth, and yellow and are covered by a membrane. The membrane bursts as a result of further growth and the colour of the ball becomes orange and later yellowish-green or greenish-black. At this stage, the surface of the ball cracks. When cut open, it is white in the centre, and consists of tightly woven mycelium together with the glumes and other tissues of the host. There are three outer layers, each at different stage of development. The innermost layer is yellowish, with radiating mycelium and spores in the process of formation. The next layer is orange-coloured and is of mycelium and spores. The outermost layer is green, and consists of mature spores together with remaining fragments of mycelium. The surface is covered with powdery dark green spores.

The symptom becomes discernible after flowering, when the ovary of the infected kernel is transformed into a large, velvety yellow to orange pulverulent mass (pseudomorph), changing to olive green in colour (Singh and Dube, 1976), which leads to decrease in grain weight (Singh and Dube, 1978) increased sterility of spikelets (Chib *et al.,* 1992). Rush *et al.,* (2000) observed the disease to be occurring at hard dough to mature stages of the crop, converting the spikelets to velvety spore balls, 2-5 cm in dia. covered by a thin orange membrane which burst open and release powdery dark green spores. Anders *et al.*, 2008 described the disease as appearance of ‘yellow or orange balls’ found on the panicle when the grain begins to fill, and which later turned olive, brown or black as they mature.

The false smut pathogen *U. virens* converted the rice grain into ball of mycelial mat covered with powdery mass of pathogen spores (Ladhalakshmi *et al.*, 2012 a).

Pathogen

The false smut pathogen *U. virens* (Cooke) Takahashi,is an ascomyceteous fungus, the perfect stage being *Villosiclava virens* (Nakata) E. Tanaka & C. Tanaka (Tanaka and Tanaka, 2008).

The pathogen, during various phases of its growth in the host or in the soil, was found to produce different developmental forms and propagules like the smut balls or pseudosclerotia, the chlamydospores, the true sclerotia, stroma, perithecia, asci, ascospores and secondary conidia, the morphology of which has been reviewed by different investigators (Takahashi, 1896; Singh and Dube, 1976, and Tanaka and Tanaka, 2008).

Takahashi (1896) described the characters of the Genus '*Ustilaginoidea*'as forming "fructifications in the ovaries of individual grains of grasses, transforming them into large, very dark olive green or sometimes orange velvety masses: when cut open the inner part is seen to be bright orange towards the surface, almost white in the centre. Mycelium partly superficial, partly immersed. Stroma always present, prosenchymatous. Setae and hyphopodia absent. Conidiophores micronematous, mononematous, sparingly branched, flexuous, intertwined, hyaline, smooth. Conidiogenous cells polyblastic, integrated, intercalary, determinate, cylindrical, denticulate; denticles short, cylindrical. Conidia solitary, dry, pleurogenous, simple, spherical or subspherical, pale to dark olive green, verruculose, 0-septate", with the type species *U. virens* (Cooke) Takahashi.

The morphology of sexual stroma and different types of propagules of *U. virens* were studied in detail by Jun-Cheng *et al.*, (2003). It was observed that annulous and monolayer perithecia were embedded in the stroma rind. The apex of the perithecia that grew out of the surface of the stroma and the papillae was formed on the surface of the stroma. When the asci became fully developed, the apical wall of the perithecia disappeared, and asci emerged from the perithecia. Ascospores were hyaline, unicellular, and filiform. Chlamydospores were spherical or elliptical,

yellowish-brown to black-brown, with thick and compact walls. The surface of the chlamydospore had many verrucae. Ascospores and chlamydospores formed secondary conidia when they germinated. The secondary conidia, with similar morphological characteristics as the submerged conidia obtained from liquid culture, were perhaps thin-walled conidia. The thin-walled conidia, oval or oblong, 2.6-8 x 2-5 micro meters, hyaline, and with smooth surfaces, produced the next generation of the thin-walled conidia upon germination and that type of reproduction was found repetitive.

Bright-field light microscopy showed that conidia of *U. virens* were round to elliptical and warty on the surface with diameters approximately ranging from 3 to 5 mm. Scanning electron microscopy revealed the globose to irregularly rounded and ornamented conidia with prominent spines. The spines were pointed at the apex or irregularly curved, and approximately 200–500 nm long. Hyphae of the fungus had concentric bodies that showed an electron-transparent core surrounded by an electron-dense layer (Kim and Park, 2007).

For the pathogen *Villosiclava virens* (Y. Sakurai ex Nakata) E. Tanak,species description by Nakata in 1934 was emended by Tanaka and Tanaka, (2008) as “sclerotia flat, botuliform, reniform, horseshoe-shaped, or differently shaped, one to several, usually two, protruding from pseudosclerotia (false smut balls) on spikelet of rice, usually 5 - 13 mm long, 2-5 mm wide, 1 - 2 mm thick. Ascomata stipitate and capitate, 1 - 9 arising from the sclerotia. Stipes 5 - 15 mm long, cylindrical, shaggy, phototropic, initially yellow then becoming dark-coloured. Capitula globose, 1 - 3 mm in diameter, papillate when mature. Perithecia ovate to pyriform, 150-350 µm long, 70 - 150 µm wide, embedded in the surface of capitula, ostioles evidently erumpent. Asci cylindrical, hyaline, 130 - 300 µm long, 4 - 7 µm wide with thickened apical cap. Ascospores filiform, hyaline, eight per ascus, septate, 140 - 230 µm long, 1.3 - 1.8 µm wide, disarticulating at septa to form four part-spores. Part-spores aseptate, 30 - 60 µm long”.

The chlamydospores produced in culture medium are found prone to germinate in distilled water and produces secondary spores. The conidia are holoblastically and sympodially produced at the apex of each chlamydospore which are hyaline and subglobose (Fu *et al.*, 2012).

Rong-Tao *et al.* (2012) observed the nuclear numbers of both the conidiospores and hyphae of *V. virens* using 4',6-diamidino-2-phenylindole (DAPI) staining. Because the fungus has small chromosomes and the numbers were not previously known, electrophoretic karyotype was analyzed using a pulsed field gel electrophoresis (PFGE) technique. The results showed that *V. virens* has at least 10 chromosomes ranging in size from 0.6 kb to 6 Mb and the *V. virens* genome size was estimated to be 23 Mb.

Taxonomy and Nomenclature

The pathogen was first described by Cooke in 1878 was named as *Ustilago virens* based on a specimen from India. Later in 1887, Patouillard gave the name *Tilletia oryzae* based on a material from Japan. The name was changed to *Ustilaginoidea oryzae* (Pat.) Brefeld, in 1895 by Brefeld. Takahashi studied the fungus and called it *Ustilaginoidea virens* (Cooke) Tak. Omori, still considering as a true smut, called it as *Sphacelotheca virens*. Sakurai discovered the teleomorph and wrongly recombined the anamorphic epithet *virens* with the teleomorphic genus *Claviceps* as *C. virens* (Cke.) Sakurai. Since that name was not nomenclaturally acceptable, Hashioka provided the name *Claviceps oryzae-sativae* for the teleomorph (Ou, 1972).

Bischoff *et al.* (2004) up on phylogenetic analysis using sequences of the large subunit of the ribosomal RNA gene, suggested that members of Ustilaginoideae were distinct from teleomorphic genera of Clavicepitaceae and that Ustilaginoideae should be recognized as a monophyletic group within Hypocreales and confirmed that the *Ustilaginoidea* anamorph was distinct from *Claviceps*.

Tanaka and Tanaka (2008) conducted phylogenetic study of clavicipitaceous fungi using acetaldehyde dehydrogenase gene sequences and found out that the position of *U. virens* was intermediate between the *Cordyceps* species and the other grass-associated clavicipitaceous species, and proposed that as *U. virens* had no affinity to the genus *Claviceps,* the teleomorph of *U. virens* should be transferred from *Claviceps* to another teleomorphic genus in the family Clavicipitaceae.

The teleomorphic state was introduced in 1934 and was allied with the family *Clavicipitaceae,* and earlier, *Claviceps virens* was applied as the teleomorph name of

this fungus. But the morphological and biological characteristics of the teleomorph were distinct from those of the genus *Claviceps,* and in particular, the *Ustilaginoidea* anamorph differed from the *Sphacelia* anamorph that characterises the genus *Claviceps.* On comparison of the rice false smut fungus with *Claviceps,* it was found inappropriate to any existing clavicipitaceous genera. Thus a new genus *Villosiclava,* a new combination *U. virens* and an emended description for the teleomorph of rice false smut fungus were proposed (Tanaka *et al.*, 2008) and was accepted.

Thus, as per the current classification, the pathogen *U. virens*, the perfect stage being *Villosiclava virens* (Nakata) E. Tanaka & C. Tanaka and is coming under Kingdom Fungi, Division Ascomycota, Subdivision Pezizomycotina, Class Sordariomycetes, Subclass Hypocreomycetidae, Order Hypocreales, Family Clavicipitaceae, Genus *Villosiclava* and species *Villosiclava virens* (Anonymous, 2013b).

Diversity of the pathogen

The pathogenic strains showed high variability both at cultural and field conditions (Ya-Jiao *et al.* 2007 and Lu *et al.* 2009).

Eight isolates from seven localities were classified by Verma and Singh (1978) into two forms on the basis of symptoms produced. One form, consisting of a single isolate, represented the Pantnagar white variant, producing a white pseudomorph; isolates belonging to the second form produced olive-green pseudomorphs.

According to geographical origin the pathogen isolates showed morphological variation in terms of mycelial growth, size of conidia, spore ball echinulation, sclerotial production etc. Verma and Singh (1988) collected samples of rice infected by *U. virens* from Almora, Cuttack, Karnal, Lakhimpur, Meerut, Pantnagar and Varanasi, India and studied the differences in symptoms, conidial morphology, cultural characters and conditions of sporulation for the isolates and accordingly classified the isolates into 4 groups: Pantnagar albino; Almora and Cuttack; Lakhimpur and Meerut; and Karnal, Pantnagar and Varanasi.

Electrophoresis for esterase-isoenzyme and DNA's RAPD gave very different bands between the new strains of white false smut and false smut of rice (Wang *et al.*, 1997).

RAPD analysis conducted by Yong-Li *et al.* (2004) for the genetic diversity and population structure of 56 strains of *U. virens* collected from different rice growing areas in China in 1992, 1996 and 2001 revealed amplification of 223 bands with 32 primers selected from 160 random primers and the results indicated that strains from northern, southern and central rice-growing areas were not tightly clustered based on location and sampling time and thus the preliminary analysis showed that the genetic diversity of *U. virens* in China was not significant.

The genetic diversity of population of *U. virens* collected from a rice field in Changping, Beijing (China) was assessed by Ya-Jiao *et al.* (2006) using amplified fragment length polymorphism and the polymorphisms of 40 strains isolated from different rice cultivars and breeding materials were amplified with 30 primer pairs selected from 256 primer combinations of EcoR I and Mse I and the results showed similarity coefficients above 0.72 among all of the strains and there was no specific interaction observed between rice cultivars and *U. virens*.

The genomic fingerprints of 53 strains of *U. virens* from different counties (districts) in Hunan, China, were analysed using 10 RAPD primers (Xiao-Ping *et al.*, 2008). The strains were classified into 5 genetic lineages (I, II, III, IV and V) at 0.78 level of genetic similarity through cluster analysis based on UPGMA where lineage I was found to be the dominant group and the results indicated that the *U. virens* strains in Hunan were not genetically diverse.

Ya-Jiao *et al.* (2007) upon AFLP analysis of the genetic diversity among 110 strains of *U. virens* from Liaoning and Beijing, found 0.92 and 0.55 coefficients of variation among the strains from Liaoning and Beijing based on which the strains from Beijing were divided into two distinct groups. AFLP analysis of 110 *U. virens* isolates sampled from Liaoning and Beijing of North China showed an extremely high level of genetic differentiation among the isolates from Beijing (Zhou *et al.*, 2008).

Lu *et al.* (2009) studied the variability of fifty nine isolates of *U. virens*, from 46 rice hybrids in 14 counties in Sichuan, China during a survey conducted in 2006 based on pathogenicity analysis found that disease indexes were significantly different both among pathogen isolates and rice hybrids, and there was a significant interaction between isolates and hybrids. There were also significant differences among the 59 isolates in terms of sporulation. The genetic diversity among 60 *U. virens* isolates, collected from six *indica* rice production regions in Sichuan Province (China), was investigated with ERIC-PCR (enterobacterial repetitive intergenic consensus-polymerase chain reaction) and polymorphic bands ranging from 5 to 20 were detected from each isolate using the ERIC primers and it was revealed that the *U. virens* isolates collected from similar regions possessed considerable stability in DNA composition, while the isolates which were collected from the different regions showed some regionalism in different degrees (Min *et al.*, 2009).

Molecular Detection studies

Li (2004) sequenced a 624-625 bp fragment of ITS and 58S r DNA region of the strains of *U. virens* in order to design PCR primer for its detection.

Disease cycle

Several investigations were carried out by different workers throwing light in to the disease cycle of the pathogen (Ou, 1972; Yashoda and Anahosur 2000)

Survival of the pathogen

Several modes of survival of the pathogen, *U. virens* as dormant structures such as sporeballs, chlamydospores, conidia, sclerotia etc. in soil, stubbles of the crop and in collateral hosts had been a subject of intensive research (Singh and Dube 1976; Yashoda and Anahosur, 2000).

Survival in the soil as dormant structures

Studies conducted by Hashioka *et al.* (1951) showed that after four months of incubation all the sclerotia kept under laboratory conditions were still viable, while in upland soil, alternate lowland and upland, and in irrigated lowland soil, respectively, the survival rate decreased to 80, 50, and 30 per cent.

In temperate regions, the pathogen survives in winter by means of sclerotia and the primary infection is initiated by ascospores produced from sclerotia (Ou, 1972).

Singh and Dube (1976), based on extensive surveys at Uttar Pradesh and Haryana, and careful examination of several specimens received from Andhra Pradesh, Orissa and Bihar, observed that true sclerotia of the fungus are not produced in the plains and reported production of sclerotia by the fungus at an altitude of 1200 m and above in Kumaon region of Uttar Pradesh. It was also observed that after the dispersal of conidia, true sclerotia remained loosely attached on the pseudomorphs and with a slight disturbance might be falling in the field.

Propagules multiplied when the fungus was inoculated in flasks containing sterilized pieces of rice stumps, roots and pond water and masses of spores were formed on the bases of the stumps. The findings indicate that the fungus may survive in nature in propagules in pond water (Sharma, 1977).

Singh *et al.* (1985) reported that the fungus survived upto 11 months both under room temperature and under field conditions.

The fungus overcomes adverse conditions by producing sclerotia and hardened spore balls that can survive in the field for several months. Spore balls can survive up to four months in soil and it is presumed that the sclerotia can survive much longer (Lee and Gunnell, 1992).

Sclerotia of *U. virens* were reported by Rathaiah and Bhattacharyya (1993) in the plains region (Jorhat) of Assam (altitude 91 m) for the first time in late November 1991. Less than 1 % of the spore balls of the pathogen found in a rainfed lowland winter (Jul.-Dec.) rice crop had sclerotia and sclerotia (usually two) were formed on the surface of spore balls, never within, and measured 5x3 mm to14x7 mm. The sclerotia were found to be shed easily and were found in the ground near rice hills.

Wang *et al.* (1998) studied the *in vitro* survival ability of the chlamydospores and found that the yellow chlamydospores when preserved at 4℃ and 25℃ retained their germinative ability up to one year and 80 days respectively.

Yashoda and Anahosur (2000) conducted *in vitro* trials on the survival of chlamydospores and pseudosclerotia of the pathogen and found that chlamydospores remained viable for upto four months when stored at room temperature (25-35 °C) and in paddy straw at 25-44 °C while those stored in the refrigerator (4-6 °C) retained their viability for upto seven months. Pseudosclerotia retained their viability upto seven months at room temperature (25-35 °C) and in paddy straw at 25-40 °C and for nine months in the refrigerator (4-6 °C). The presence of true sclerotia and their germination through stromatic head bearing ascospores were observed for the first time in Karnataka, India.

Rush *et al.,* (2000) reported that spore balls of smutted grains would get converted into one or more sclerotia which would act as overwintering structure of the pathogen.

Germination examination studies conducted by Rong-Hui *et al.* (2010) indicated that the germination ability of mature chlamydospores reduced rapidly with prolongation of storage period.

To reveal the relationship between endogenous dormancy mechanism of chlamydospores of *U. virens* and their walls, Na *et al.* (2012) extracted the polysaccharide from cell walls of the dormant (black) and non-dormant (yellow) chlamydospores by complex enzyme-hot water extraction, and its structure and composition were analyzed by using ultraviolet absorption spectrum (UV spectrum)-infrared absorption spectrum (IR spectrum), Congo red test, and gas chromatography (GC). The results showed that polysaccharide in the black chlamydospores wall was composed of glucose, galactose, mannose while the yellow chlamydospore wall was composed of glucose, galactose, mannose and xylose. It was inferred that the composition and composition ratios of monosaccharides in polysaccharide from the dormant and non-dormant chlamydospore walls may be closely related to chlamydospore dormancy.

Survival in seed

Seed-borne nature of the fungus is still in debate. When seed from rice plants infected with *U. virens* was grown in pots of normal soil (Galloway, 1936) no disease resulted, confirming the view that *U. virens* is not seed-borne.

According to the studies by Seth (1945), seed dusted with spores produced healthy plants. Mulder and Holliday, (1971) reported that there was no evidence for seed transmission of the pathogen.

Kulkarni and Moniz (1975), surface sterilized the seeds of paddy, smeared with the chlamydospores of *U. virens* and used such seeds for sowing in sterilized soil and did not find any infection and thus inferred that the disease is not seed borne. Li *et al.* (1986) reported that the source of the initial infection was most probably seed-borne.

Singh and Pophaly, (2010) stated that the disease is not usually considered seed-borne. The fungus is reported to survive in contaminated rice grain as spore balls produced on mature panicles.

Careful observation of rice panicles naturally infected by false smut revealed that healthy seeds adjacent to spore balls were often heavily infested on the surface by spores of false smut in the field and thus the fungus could be considered seed-borne and it is also possible that the disease may be dispersed from field to field by planting infected and/or infested seed (Schroud and TeBeest 2005).

Data from field experiments by Schroud and TeBeest (2005) and Ditmore and TeBeest (2006) on root or seed inoculation of *U. virens* and detection of the pathogen by PCR analysis implied that the false smut fungus is both seed-borne and seed-transmitted.

Survival in collateral hosts

Haskell and Diel (1929) reported that a species of *Ustilaginoidea* on maize tassels from Lousiana and Panama, similar morphologically to *U. virens* on rice. Rao and Reddy (1955) reported false smut disease on *Oryza officinalis* from India.

Infection by *U. virens* producing similar symptoms, has been reported on the male inflorescence of *Zea mays* and on wild species of *Oryza* (Mulder and Holliday, 1971).

Shetty and Shetty (1985) reported *Digitaria marginata* L. as the collateral host of *U. virens* from Dakshina Kannada district of Karnataka.

False smut of rice caused by *U. virens* was found on the grass, *Panicum trypheron*, a common weed around paddy fields. Cross inoculation studies revealed chlamydospores from *P. trypheron* infected rice and *vice versa* (Shetty and Shetty 1987).

During surveys in 2000 in Stoneville, Mississippi, USA, 32 (2.5 %) of 1280 commercial and inbred maize hybrids had false smut (caused by *U.virens*) on the tassel (Abbas *et al.*, 2002).

During a survey conducted (Atia, 2004), *U. virens* was found to infect barnyard grass (*Echinochloa crus-galli*), a common rice weed and congo grass (*Imperata cylindrica*), a common weed on irrigation canals in Egypt

U. virens has been reported on *Oryza officinalis*, *Chionachne koenigi*, some wild species of *Oryza*, weed species *viz*., *Echinochloa crus-galli*, *Digitaria marginata*, *Imperata cylindrica*, and *Panicum trypheron* (Ladhalakshmi *et al.*, 2012c).

Primary inoculum

Chlamydospores in paddy soils are the primary source of infection as reported by Ikegami (1960). According to Ou, (1972), the pathogen survives in winter by means of sclerotia and the primary infection is initiated by ascospores produced from sclerotia.

Kulkarni and Moniz (1975) suggested that primary infection may originate from the chlamydospore balls hibernating in the soil, which under favourable conditions of environment may germinate producing large quantities of secondary conidia that can be carried by wind and rain and initiate infection.

Narain (1992) opined that in South India, where two or three rice crops are cultivated, the pseudosclerotia could produce mycelium and conidia which apparently serve as a source of primary inoculum for subsequent crop.

Fan *et al.*, (1996) conducted studies on the infection cycle of rice false smut and found that conidiospores were the main sources of infection**.**

Spores found in the spore balls can produce one or more secondary spores after germination. Spores, secondary spores, and ascospores are all reportedly capable

of infection (Schroud and TeBeest, 2005).According to Guang-hui *et al.* (2005), panicles were getting infected by the conidia directly. As per the results of studies conducted in China by Jian-ping *et al.* (2009), infected seeds and the pathogens for hibernation in soil were the initial infection sources for rice false smut, and the former was more important.

In hilly areas, presence of sclerotia can serve as primary inoculum whereas in plain regions, chlamydospores act as primary inoculum where occurrence of sclerotia is not common. The infection may start with the over wintered sclerotia where it germinate and produce ascospores, the formation of which coincide with the anthesis of the crop, and such ascospores lodge on the floral parts and initiate infection. The hibernating chlamydospores from the soil, under favourable conditions of environment may germinate; produce large quantities of conidia, which infect the panicles of paddy (Ladhalakshmi *et al.*, 2012c).

Invasion and infection

Hisada (1936), from field observations, suspected that infections occur when rice plants are at booting stage. Inoculation of flowers and of grains in the milk stage failed to produce infection (Seth, 1945).

As a result of histological studies, Raychaudhuri (1946) described two types of infection. One type takes place at very early stage of flowering, when the ovary is destroyed, but the style, stigmas, and anther lobes remain intact and are buried in the spore mass. The second type takes place when the grain is mature. The epidermal and mesocarp cells were found to be the most susceptible to infection, undergoing disorganization at a very early stage. The cross cells are the next to be invaded, while the tube cells of the inner epidermis are still intact. As soon as the mycelium reaches the endosperm its growth is strongly accelerated, and eventually it replaces the entire grain. The spore ball continues to swell and the gap between the two paleae widens, so that yet more spores gain ingress. A green velvety mass is rapidly formed and the spores produced by the mycelium are extruded.

Hashioka *et al.* (1951) observed that 99.6 per cent of the smut balls contained intact anthers, indicating that most infections take place just before flowering.

Ikegami (1960) successfully inoculated rice plants with chlamydospores and ascospores by injecting a spore suspension in to the leaf sheath enclosing the young panicle and inferred that the infection occurs during the rather short period just before heading. It was also found that natural infection by *U. virens* was considered to be caused primarily by ascospores and secondarily by chlamydospores. As a result of inoculations it was concluded that infection occurred just before heading.

In studies at Gifu University (Ikegami, 1962), it was found that the infection by *U. virens* occurred most readily on 1-10 mm tall seedlings inoculated with yellow spores (max. 400 spores/optical field at 25°C); no infection occurred on seedlings 12 mm. or more. Greenish-yellow spores attacked coleoptiles 3-6 mm. in height (but not those of 8 mm. or more) and the infection percentage was much higher with very early inoculation. Yellowish-green spores were most pathogenic, greenish-yellow and yellow spores less so, and green spores least.

Successful inoculation was reported by Kulkarni and Moniz (1975) by applying chlamydospore suspensions with a camel hair brush both to fertilized and unfertilized ovaries, but smearing the seeds with chlamydospores did not lead to infection.

Singh and Gangopadhyay (1981) inoculated rice flowers in the field using chlamydospores produced in culture by opening the flowers with sterile forceps before bursting and placing the chlamydospores on the stigmas. Inoculation of rice plants at various stages with *U. virens* in a field experiment (Li *et al.*, 1986) in 1984-85 showed that infection occurred at the later phase of booting and the latent period was 14-18 days on this 1st rice crop and 18-39 d on the 2nd. A suspension of conidia produced by *U. virens* on potato sucrose agar was injected into the leaf sheath at the booting stage. The inoculated plants were placed in an air conditioned room for 2 d at 15°C, incubated in a moist chamber for 5 d at 26° and then kept in a greenhouse (25-35°) until smut balls appeared (Fujita *et al.*, 1989).

In a study conducted by Yong-Jian *et al.* (1995), overwintered chlamydospores of *U. virens* were inoculated to rice plants by injecting boots, brushing boots, dressing roots and foliar spray to study their infectivity and it was

found that the chlamydospores could infect rice seeds, shoots, seedlings and roots with an incidence of 2.04 - 33.3 %. It is suggested that the booting stage is the sensitive infective stage and that conidiospores are the main sources of infection (Fan *et al.*, 1996).

Artificial inoculation techniques for false smut of rice were studied under field conditions by Jun-Cheng *et al.* (2004) in China. Inoculation with three inocula indicated that chlamydospores collected and kept at -20°C last year could not cause false smut; thin-wall conidia prepared from liquid culture caused the disease, and higher concentration of conidia resulted in higher percentage of infected panicles. The most severe disease occurrence was caused by a mixture of hypha-fragment and thin-wall conidia. Among three inoculation stages of rice, that inoculated 6-9 days before emergence of panicle resulted in the optimum infection and among three inoculation times in one day, inoculation at 16.00-18.00 h induced the most severe disease. Potato juice could increase the percentage of infected panicles when it was added to the inoculum.

Ashizawa and Kataoka(2005) reported that the fungus was present in panicles at the booting stage as the nested-PCRs targeting the species-specific ITS region of ribosomal DNA have confirmed the presence of the fungus in whole panicles before rice heading. The results of the histological observations on the infection of various rice cultivars by *U. virens* during the heading stage done by Guang-Hui *et al.* (2005) showed that the conidia could germinate and form mycelia on the surface of glume and further extend inside the glume hull, which may provide evidence of infecting panicle by conidia directly.

Histological examinations showed that spores placed on roots germinated asynchronously over time and that all of the inoculated roots of rice plants were infected when inoculated in this manner (Schroud and TeBeest, 2005) and thus roots can be the port of entry of the pathogen and the spores coming into contact with rice roots may be a mechanism through which rice is infected in the field. Histological examinations show that roots of rice seedlings were infected by germinating spores of *U. virens* within a few hours after inoculation. It was not clarified whether root

infection can lead to infection of panicles and production of spore balls on plants grown from infested seeds.

In an experiment conducted at the Agricultural Experiment Station at Fayetteville by Ditmore and TeBeest (2006), paddy seeds were inoculated by vacuum infiltration in suspensions containing one million spores per ml and were planted the data were collected at maturity on the number of panicles set per plant per treatment and the amount of chaffiness found on the panicles. The results showed that inoculation of seeds by infiltration reduced the number of panicles produced and infection of rice plants can occur through planting of infested seeds.

Zhen *et al.* (2010), upon pathological and anatomical observations of the grains infected by *U. virens* from the field, observed the integrated floral organ including stamen and pistil within the infected grains without forming yellow chlamydospore. The integrated ovaries, which were green and not obviously enlarged because of grain filling, were found in case of typical infected grains with lots of yellow chlamydospore. According to Jian-Ping *et al.* (2009), the middle and the late booting stages were the favorable infecting period of the pathogens of *U. virens.*

A methodology for the artificial induction of false smut of rice under field conditions was evaluated by Pannu *et al.* (2010) in Punjab, India, during 2006 and 2007. Rice (cv. PR-116) plants at the booting stage were inoculated with *U. virens* using spore suspension with four treatments, i.e. inoculation and maintenance of artificial humidity (MAH), inoculation without MAH, without inoculation but with MAH, and without inoculation and MAH. In 2005 and 2006, the disease incidence (DI) reached 80.65 and 86.85 %, and the disease severity (DS) reached 3.24 and 4.80 %, respectively, under inoculation + MAH. Under inoculation without MAH, DI values were 5.65 and 10.30, whereas DS values were 0.73 and 1.30 %. Plants that were not inoculated but exposed to MAH recorded DI values of 2.67 and 4.56 %, and DS values of 0.05 and 0.10 %. Under no inoculation and MAH, DI values were 1.10 and 1.50 %, and DS values were 0.12 and 0.20 %.

To increase disease effect and stability of artificial inoculation of *U. virens*, the inoculation effects caused by different inocula, culture time, concentrations of

pathogen and inoculation periods of rice were studied by Xiu-Juan *et al.* (2011b) by using methods of injection during booting stage of rice in greenhouse. The results indicated that the inoculation effects of pathogen in PSB culture liquid was better than that in rice bran culture liquid with 100 % and 23.33 % of diseased panicles, respectively. The pathogen in PSB culture liquid cultured for 5-7 d had a better inoculation effect, and the effects were decreased upon prolongation of culture days. When rice variety "Liangyoupeijiu" inoculated with pathogen at a concentration of 4×10^6 conidia/mL in PSB culture liquid in the middle and later stages of booting, the percentage of diseased panicles were 100 %, and the average and the highest numbers of diseased grains were 35.1 and 87 respectively.

Li-Mei *et al.* (2012) the main infection period of pathogen was at early booting and mid-booting of rice.

To clarify how the fungus invades spikelets during the booting stage Ashizava *et al.* (2012) developed a fungal strain that expresses a green fluorescent protein gene, and injected conidia from this strain into rice sheaths. Observations at 48 h post-inoculation showed many conidia were present on spikelet surfaces, and the conidia had germinated and the hyphae have gradually grown by 120 h post-inoculation. By 144 hours, hyphae had invaded spikelets through their apices, via the small gap between the lemma and palea and had reached all floral organs.

As reviewed by Dong-Wei and Wang-Shu (2012), the pathogen could invade rice coleoptiles and roots at the young seedling stage and the filaments of stamen at the earlier booting stage. The pathogen does not form typical appressorial structure, and the hyphae invade and extend intercellularly and the fungal hyphae do not penetrate through the host cell wall. At rice booting stage, the pathogen attacks exclusively rice filaments, and the secondary hyphae can infect the outer layers of cells on lodicules and stigmas occasionally. The pathogen cannot infect the ovary and anthers.

Tang *et al.* (2013), upon examination of serial semithin and ultrathin sections of infected spikelets, showed that he primary infection sites for the pathogen were the upper parts of the three stamen filaments located between the ovary and the lodicules. The stigma and lodicules were also occasionally infected to a limited extent. The

pathogen infected the filaments intercellularly and extended intercellularly along the filament base and the host cells were degraded gradually. The pathogen did not penetrate host cell walls directly and did not form haustoria.

Spread

According to a study conducted by Sreeramulu and Vittal (1966) for estimating the diurnal and seasonal periodicities in the air-borne spores of *U. virens*, by sampling air at 1 m above ground level with the help of a Hirst trap, maximum numbers of spores in the air are observed at the time of heading of the plants. On normal days, a peak diurnal periodicity occurred at 22.00 hours.

The air-borne conidia have a diurnal periodicity with peak at 22.00 hr, numbers being very low between 04.00 and 16.00 h. The ascospores of the reported perfect state may also be air-dispersed (Mulder and Holliday, 1971).

As reported by Ou (1972), chlamydospores play an important role in secondary infection, which is a major part of the disease. The chlamydospores are airborne, but do not free easily from the smut balls because of the presence of a sticky material.

Aerobiological studies carried out by Kulkarni and Moniz (1975) during the rice growing season (July-September) revealed that chlamydospores of *U. virens* could be trapped about one week before the appearance of infection, and the concentration of such spores trapped increased progressively at the approach of the flowering period. Histopathology of the various parts bearing diseased grains such as stem, stalk and inflorescence revealed that the infection is not systemic and all these observations indicated that false smut of rice is an airborne disease.

As per the studies conducted at Andhra Pradesh on the periodicities of the airborne spores over the rice crop by Atluri *et al.* (1988), the spores of *U. virens* sp. showed the peak mean concentration (PMC) at 13:00, increasing in concentrations and decreasing gradually. The highest hourly concentration occurred at 14:00 h and the spore concentrations showed mid day pattern of circadian periodicity.

Monitoring of airborne *U. virens* in case of *U. virens* spores over the rice field in Thoubal District, Manipur, India, was undertaken by Devi and Singh, (2007) during

the crop seasons of 2003 and 2004 by using the Rotorod air sampler and the disease was found to be appearing in the field 17-18 days after the first appearance of the spores of the pathogen in the air.

Ladhalakshmi *et al.* (2012c) reviewed that chamydospores play an important role in the secondary infection and spread of the disease of the disease in the field.

Favourable conditions

The disease is found to be affected by different climatic, nutritional and other conditions.

Favourable weather parameters

Hashioka *et al.* (1951) reviewed that outbreaks of false smut appear to depend on the amount of rain, especially from late July to August, the development of perithecial stromata requiring high air humidity and soil moisture.

As per the observations by Mouton and Merny (1959), no correlation was established between the severity of false smut infection and the annual rainfall but the number of rain days seemed to be of greater importance. Certain microclimatic factors (damp or sheltered situations) were also found to favour the disease. It was shown by Rao (1964) that the environment, especially weather at flowering time and nutritional conditions, influenced outbreaks of false smut. Observations from the field experiments conducted by Singh (1974) during 1969-71 indicated that the disease was favoured by the prevalence of lower minimum and maximum temperatures; high RH (92 % and above) before and during early flowering and lower RH later in the flowering period. Precipitation and cloudy days influence temperature and RH, which determine incidence.

Singh *et al.*, (1987 b) reported that relatively lower temperature (20 ^{0}C) and high humidity coupled with well distributed moderate rainfall during flowering, favoured the disease. According to Li *et al.* (1986) rainy weather at heading was found to be favourable for infection by false smut.

According to Fujita *et al.*, (1989), the disease was favored by low temperature and high RH (100 %) for infection, and by a relatively high temp. for the appearance of symptoms.

Bhardwaj (1990) could not find any correlation of disease severity with the days to 50 % flowering or with the environmental conditions during the flowering period.

Narain (1992) reported heavy infection of false smut in several coastal districts of Orissa, and inferred that such heavy infestation unrecorded earlier was as a consequence of intermittent showers and cloudy days during flowering susceptible rice varieties.

Bhagath *et al.*, (1993) observed greater infection by *U. virens* at comparatively lower day and night temperature around 31 °C and 25 °C respectively, with high precipitation resulting in high RH of 90 %. The number of cloudy hours during the day was positively correlated with disease development.

The maximum and minimum temperature of the week during which sclerotia developed, ranged from 20 to 26 °C, respectively (Rathaiah and Bhattacharyya, 1993). According to Naito (1994) low temperature and high humidity are suitable conditions for infection, and disease development is favored by high temperature.

Dodan and Singh (1996) reviewed that false smut of rice, caused by *U. virens*, caused substantial quantitative and qualitative losses under favourable environmental conditions.

Results of the studies conducted at Karnataka by Yashoda *et al.* (2000c), indicate that the weather parameters during 50 % flowering had a significant effect on false smut disease development in rice. Low maximum temperature, (< 31 °C), low rainfall, high minimum temperature (19 °C) and high relative humidity (>90 %) during this stage were favourable for disease development.

The incidence of RFS was significantly and positively correlated with average temperature within 15 days after heading. The temperature was the primary climatic factor influencing the incidence of RFS, whereas the humidity was the secondary climatic factor (Lu *et al.*, 2003).

Devi and Singh (2007) observed that the highest spore catch of *U. virens* coincided with the maximum and minimum temperatures of 29.0 °C and 13.0 °C and relative humidity of 89 % as well as nil rainfall in 2003 and maximum and minimum

temperatures of 27.0 °C and 12.0 °C and optimum relative humidity of 95 % as well as nil rainfall during the year 2004.

The rainy days and rainfall from panicle emerging stage to full heading stage were favorable conditions for outbreak of rice false smut in medium and late rice (Jian-Ping *et al.*, 2009).

Singh and Pophaly (2010) found intermittent rains at flowering stage and associated humidity favouring the disease. Li-Mei *et al.* (2012) reported that the spore release of *U. virens* was influenced by climatic conditions, mainly at night in the sunny days and all day in the rainy days.

Favourable nutritional and cultural conditions

Ikegami (1960) found that plants grown under conditions of high fertility, favourable for the vegetative growth of rice, were more susceptible to the disease.

Rao (1964) observed that rice varieties of medium duration were most affected by false smut, while those of shorter and longer duration were comparatively less affected. According to Ou (1972), high moisture favoured the development of false smut disease.

According to Li *et al.* (1986), high levels of fertilizers favoured the infection. Increase in application of fertilizers and late sowing was found to favour false smut (Singh *et al.*, 1987 a; Singh *et al.*, 1987 b). Infection with *U. virens* was found to be favoured by late sowing and overdose of fertilizers especially N fertilizers (Patel *et al.*, 1992; Ahonsi *et al.*, 2000).

Atia (2004) found that nitrogen fertilization, planting method, type of soil, and the type of cultivar planted played an important role in the infection and development of RFS in rice by the trials conducted at Egypt. High disease incidence and number of infected grains were recorded at high N levels. Rice plants cultivated directly in the field showed higher RFS disease incidence and number of smutted balls compared with the transplantation method which showed lower infection. Percentage of infected tillers and number of infected balls were lower in clay soil (compact soil) than in semi-sand soil (light soil) which showed higher disease incidence and number of infected grains during the two seasons.

False smut disease severity was positively affected by N fertility (Brooks *et al.*, 2009). Field experiments conducted by Shu *et al.* (2010) revealed that false smut of rice occurred more heavily when sowing and planting periods were late.

In vitro Studies

1. Isolation of the pathogen

Sharma and Joshi (1975) isolated the pathogen *U. virens* by dusting the spore suspension from the smut balls collected from the field over a sterilized coverslip placed over the solidified surface of YPPDA medium and pure-culturing by hyphal tip isolation method from the growth obtained by germination of the chlamydospores.

It was reported that (Yong-li and Qi, 1999) XBZ agar + chloromycetin (50 µg/mL) was an ideal medium for isolation of *U. virens* and the method of chlamydosporal suspension was ideal for rapid isolation of the fungus.

Different methods of isolation of *U. virens* were compared by Yong-Qiang *et al.* (2010), and it was inferred that isolation from sclerotium was ideal but the central thick mycelium of the smut balls was found very difficult to grow in to colonies. Hai-Hai-Yong *et al.* (2011) reported that PSA was the most effective medium to isolate single spores from yellow chlamydospores of rice *U. virens*.

2. Culturing of the pathogen

i. Studies on the media for culturing and sporulation

Thomas (1940) reported that the comparative cultural studies on *U. virens* showed the best growth of *U. virens* on Quaker oats.

Hashioka *et al.* (1951) found that the chlamydospores were produced after 20 to 40 days on Saito's soy agar, unhulled and hulled rice decoction agar, and steamed rice. Mycelial development on potato decoction agar was improved by adding one to three per cent glucose or two to five per cent sucrose.

Results of the *in vitro* studies by Lepori (1951) showed that better growth of *U. virens* occurred on malt agar and pepton-glucose broth than on potato agar. Singh and Gangopadhyay (1981) cultured the pathogen for inoculation purpose on YPPD

agar (yeast 100 mg, peptone 100 mg, potato 200 mg, dextrose 20 g, agar 20 g/l water) covered with butter paper and a 1 ml drop of 100 p.p.m. kinetin solution.

The cultural characters of *U. virens* were studied by Yashoda *et al.* (2000e) with reference to colony character, colony diameter and sporulation on six solid media *viz*., Czapeck's, host extract dextrose, rice yeast dextrose, Sabouraud's and yeast peptone potato dextrose agar, and it was found that the highest radial growth was observed in rice yeast dextrose agar (90 mm) and in yeast peptone potato dextrose agar (87.75 mm) on the 20th day and sporulation was observed only in yeast peptone potato dextrose agar and rice yeast dextrose agar.

Li-Yang *et al.* (2008) tested different solid and liquid media for radial growth and sporulation of *U. virens* and it was found that among the solid media tested, PSA supported the fastest mycelial growth at 2.54 mm per day. In liquid culture, dry weight of mycelia was highest in PDB at 24.5 mg/ml. The number of conidia was the highest in the rice extract medium and PSB medium with $2.63x10^7$spores ml^{-1} and $2.28x10^7$ spores ml^{-1}, respectively.

As per the studies conducted by (Wang-Shu *et al.*, 2008) potato sucrose broth was found the optimal media to promote conidial production of *U. albicans*, increasing the concentration to up to $4.8x10^8$ spores/ml. PSA (potato sucrose agar) was suitable for pathogen growth and there were highest dry weight of mycelia (16.48 mg mL^{-1}) and number of conidia (4.19×10^6 conidia mL^{-1}) after pathogen cultured in liquid culture of PSB (potato sucrose broth) for 13 days and 9 days respectively (Xiu-Juan *et al.*, 2011a).

Laboratory studies (Yan-Hui *et al.*, 2011) on culture conditions of *U. virens* showed that the tested strain of the fungus grew normally on PDA culture medium but it grew the fastest on XBZA among the different solid culture media tested. Hai-Yong *et al.* (2011) reported that the optimum culture condition included PSB or PDB medium.

According to the study conducted by Li-Mei *et al.* (2012), there was only a few of chlamydospores and conidia formed by the fungus *U. virens* in potato saccharose (PS) liquid medium whereas the colonies on potato saccharose agar (PSA) medium could produce abundant chlamydospores. The growth of the fungus in the

cooked unhushed barley and rice grains was much slower than that in the cooked wheat grains and milled rice grains.

Fu *et al.* (2013) compared the radial growth of *U. virens* on different media and found that Potato sucrose agar (PSA) was found to be the best medium for fast mycelial growth, and XBZ agar and potato dextrose agar also favored mycelial growth, whereas Czapek agar was not suitable.

ii. Studies on the carbon and nitrogen sources for culturing of the pathogen

Several C-sources and N-sources increased colony growth on solid or liquid media, and the hyphae grew best with 2 % sucrose as the C-source (Wang *et al.*, 1997).

Sucrose and Ca $(NO_3)_2$ were separately the best carbon and nitrogen source for the mycelium growth among the tested different carbon and nitrogen sources for culturing of *U. virens* (Yan-Hui *et al.*, 2011).

Fu *et al.* (2013) found that sucrose (2.6 mm d^{-1}) and starch (2.2 mm d^{-1}) were the best carbon sources, and ammonium nitrate (2.1 mm d^{-1}), ammonium sulfate (2.2 mm d^{-1}) and ammonium chloride (2.2 mm d^{-1}) were the most suitable nitrogen sources for the mycelial growth of *U. virens*.

iii. Studies on the temperature conditions for culturing of the pathogen

In vitro experiments by Seth (1945) showed that the optimum temperature for growth of the pathogen was found to be 26°C. The fungus failed to grow at 34° C. and prolonged incubation at this temperature was lethal.

Hashioka *et al.* (1951) reported that the optimum temperature for mycelial growth of *U. virens* was 28°C.

The *in vitro* experiments by Chen *et al.* (1994) showed that the optimum temperature for mycelial growth of *U. virens* on PDA was found to be 28 °C and the growth was very slow at 13 °C and ceased at 35 °C.

According to Fan *et al.* (1996), the optimum temperature for mycelial growth ranged from 25-28 °C.

Yan-Hui *et al.*, 2011 reported that the temperature range for the growth of *U. virens* was between 15°C to 35°C with the optimal growth temperature 28°C. Low or high temperature was found to completely inhibit the mycelial growth. Reports by Hai-Yong *et al.* (2011) showed that the optimum culture condition for *U. virens* was 22-29°C or 28°C. Effects of environmental conditions on the mycelial growth, of *U. virens* were studied by Li-Mei *et al.* (2012) and the results showed that temperature ranging from 10°C to 30°C was available for the mycelial growth, with the optimum of 28°C.

The fungus was able to grow *in vitro* at temperatures from 12 to 32°C, with the optimal mycelial growth occurring between 28 and 30°C (Fu *et al.*, 2013).

iv. Studies on the pH conditions for culturing of the pathogen

Hashioka *et al.* (1951) conducted *in vitro* studies on the physiology of the fungus *U. virens*, the results of which showed that optimum growth occurred at pH 6.02 to 6.74. Fan *et al.* (1996) reported that the optimum pH for culturing *U. virens* was found to be ranging from 4.92 to 6.98, whereas according to Wang *et al.* (1997) the optimum pH value for colony growth was pH 6.

As per the report by Yan-Hui *et al.*, 2011, the range of pH for the pathogen growth was 4-10, and the optimal pH was 5-8. The pathogen possessed the ability to grow from pH 3 to pH 10, while the optimum was pH 6-pH 7 (Li-Mei *et al.*, 2012).

Active mycelial growth of *U. virens* was observed at pH between 4.5 and 11, with the optimal growth observed at pH 7 to 8 (Fu *et al.*, 2013).

v. Studies on the light conditions for culturing of the pathogen

Hai-Yong *et al.* (2011) reported that the optimum culture condition for *U. virens* was natural light conditions. Fu *et al.* (2013) reported that the mycelial growth rate of *U. virens* was significantly higher in the dark than in 12 h alternating cycles of dark and fluorescent light, and fluorescent light inhibited mycelial growth.

3. Studies on the sequence of sporulation

As per the experiments by Seth (1945) on germination of spores from fresh sclerotial bodies, after 24 hours it showed about 10 per cent spore germination each

spore producing a short, unbranched germ-tube. After another 48 hours the germ-tubes were found profusely branched and septate, bearing clusters of small pear-shaped, hyaline conidia formed both terminally and laterally at the hyphal tips. Transfers of the branched germ-tubes and secondary conidia to a Quaker oats medium developed a white, fluffy, felt-like mycelium which after three weeks produced many white, compact, almost round sclerotial bodies. These turned orange-yellow and finally olive-green, becoming slightly powdery in appearance and corresponding closely with those on the host plant. On plain agar the secondary conidia germinated similarly to the parent spore. They became slightly swollen at first, and then produced branched and septate germ-tubes bearing clusters of small, piriform, hyaline conidia at the tips. Compared with the parent spores these tertiary conidia were slightly smaller and their germ-tubes narrower and sparsely septate.

In vitro studies on the sequence of sporulation (Rong-Hui *et al.*, 2010) revealed that in the early 20 days, the fungus produced mainly conidia on the specialized hyphal structures, and the hyphal binds and knots. A few conidia also appeared on sparse hyphae. In the later stage, the fungus produced great amount of chlamydospores in the yellow fruiting bodies. The quantity of chlamydospores was much more than that of conidia indicating that conidia are formed mainly in the early stage and chlamydospores in the later stage.

4. Studies on the conditions for spore germination

Hashioka *et al.*(1951) observed that in water, the young spore of *U. virens* produces a fine germ-tube bearing one to three conidia at and near the tip; in a sugar solution one or two wide germ-tubes elongate to several times the diameter of the spore without branching and produce up to several conidia; in sugared potato decoction one to four wide germ-tubes branch vigorously, producing several conidia at the tip of each or elongating further without sporulation; and very rarely short, wide germ-tubes may be produced, bearing a few appressoria-like conidia. The conidia germinate well in a nutrient solution but seldom in water.

Tsai *et al.* (1990) reported that germination of chlamydospores on sterilized water and water agar was best at 25 °C, followed by 30 °C and 20 °C and the opt. pH

range was 5-8. Germination of yellow chlamydospores (93 %) was much better than that of black chlamydospores (6 %).

Dave and Sharma (1992), upon study of the germination characteristics of *U. virens*, found that better germination of spores was observed after storage at 30±2 ºC and 60 % RH compared with fluctuating temperature and RH. Germination was most promoted by sucrose, compared with tap water, rain water and a soil extract.

In a study conducted by Chen *et al.* (1994) on the chlamydospore germination of *U. virens*, it was found that the germination rate after 72 h was approximately 30 %.

The results of the *in vitro* studies conducted by Fan *et al.* (1996) showed that the optimum temperature for chlamydospore germination was 28 °C and the optimum pH ranged from 5.8 to 6.3. Sugar and light was found to promote chlamydospore germination.

The study on the biology *U. virens* conidia (Jun-Cheng *et al.*, 2003) indicated that nutrient in media had an important effect on conidia germination. Pure water was not good for germination and PSA was the optimum medium for germination. Glucose strongly inhibited germination whereas potato juice could counteract the inhibition of glucose and stimulate germination. The germination rate was higher in solid agar medium than in liquid medium.

Li-Yang *et al.* (2008) studied the conditions for germination of the chlamydospores of the pathogen and found that the conidia did not germinate after 3 h in distilled water or 2 % sucrose solution, and primary conidia and chlamydospores germinated and produced secondary conidia and conidia by 6 h. it was also observed that the chlamydospores had higher germination rate in 2 % sucrose solution than that in distilled water. The suitable temperature for germination was 22-31 °C (optimum 28 °C) and the pH 6-7 was optimum for germination.

Studies on germination of chlamydospores (Wei-Ping *et al.*, 2008) showed that chlamydospores germinated at the temperature of 10-35°C and pH 2-12. The optimum temperature for germination was 25-30°C and the optimum pH value was 6-8. By detecting results of spore germination for different culture time on 25°C, it was

found that chlamydospores began to germinate for about 12 h after culture, and germination rate reached 50 % at 24 h after culture.

Effect of ten kinds of sugars (xylose, sorbose, ribose, fructose, mannose, galactose, maltose, glucose, lactose and sucrose) on germination of thin-wall conidia of *U. virens* was tested by Jun-Cheng *et al.* (2009) with agar plate. All the sugars exhibited inhibition to the conidia germination in varying degree at 1 % concentration, except for sucrose. Inhibition of xylose, fructose, sorbose and ribose was very strong. Mixture of several sugars also showed inhibition to the conidia whereas mixture of each sugar and potato juice gave stimulation to the conidia germination.

Xiu-Juan *et al.* (2011a) found that the range of temperature for conidia germination was 15-30°C, with the optimum of 28°C. The favorable pH was 4-11, with the optimum of pH 6. The conidia germination rate were 81.79 % and 44.20 % when exposed to temperature range of 15-33°C and 13-23°C for 48 h under natural condition, respectively, but the conidia germination rate decreased 43.38 % during 28°C for 48 h after they exposed to 4°Cfor 168 h, and no conidia germination was found after they exposed to 40°C for 6 h.

According to Li-Mei *et al.* (2012), the suitable temperature for spore germination of *U. virens* was 28°C; the best inorganic source was the combination of disodium hydrogen phosphate and magnesium sulfate.

As per the results of the studies conducted by Fu *et al.* (2013),**c**onidial germination of *U. virens* occurred at temperatures from 12 to 34°C, and the optimal temperature for germination appeared to be 28 and 30°C for which a minimum wetness period of 2 h was required. The lethal temperature of the conidia was 50°C.

In vitro Management Studies

1. Biological control *in vitro*

Eighteen strains of *Bacillus* bacteria with biocontrol effects against *U. virens* were screened and their antagonistic activity to *U. virens in vitro*. The antagonistic effects of strains A and H-51 were high. The inhibitory rate of strains A and H-51 were 62.66 and 62.87 % on hyphal growth and 77.37 and 59.74 % on spore

germination, respectively. The strain A decreased the thickness of hyphae and lengthened nodes between hyphae, and condensed the protoplasts (Shi-Le *et al.*, 2004).

Activities of *B. subtilis* strain Bs-916 and its extract when tested against *U. virens in vitro*, gave 100 % inhibition to the growth of *U. virens* (Yong-Feng *et al.*, 2007).

Ya *et al.* (2010) screened the microbial flora from duck manure against different rice pathogens including *U. virens*, and found that A168, a strain of antagonistic bacterium identified as *Bacillus cereus* had strong inhibition against *U. virens*.

In a study (Zhi-Huai *et al.*, 2010) conducted to genetically improve the biocontrol *Trichoderma* strains by U.V. irradiation for fungicide tolerance, thirteen mutation strains with higher tolerance against myclobutanil were obtained and among them, strain TUV-13 showed highest antagonistic activity against *U. virens*.

The biocontrol potential of *Trichoderma virens* strain TY009 was assessed by Liu *et al.* (2010) for its antagonism against *U. virens*, including the effect of gliotoxin, seperated from liquid culture of *Trichoderma virens* strain TY009 and purified by chromatographic methods and it was observed that, at 1.0µg ml^{-1}concentration of gliotoxin there was no conidial germination and secondary spore formation of *U. virens.*

Xiao-Le *et al.* (2011) evaluated antagonistic bacteria (1815 bacterial strains) against rice false smut, from 10 soil samples in Yunnan and Jangsu provinces of which two bacterium strains, SF-62 and SF-3-38 (identified as *Bacillus subtilis*), were found to have very high ability in inhibiting *U. virens*, with the inhibition rates being 97.2 % and 85.9 %.

2. Chemical control *in vitro*

Under *in vitro* experiments (Ikegami, 1958), chlamydospores and ascospores of *U. virens* were immersed for 24 hours in Cu fungicides, cupro-mercurials, and mercurials at concentrations 10^{-4}-10^{-9} Hg or Cu g./L, at 28° C and the numbers of conidia produced by chlamydospores and of spherical bodies at the tips of ascospores

after treatment were counted in order to determine the efficacy of different fungicides against *U. virens.* Mercurials proved to be the most effective, causing 100 % inhibition of chlamydospore germination at 10^{-6}-5 x 10^{-7}, and of ascospores at 10^{-5}-10^{-6}.

According to Verma and Singh (1987), the most effective treatments against *U. virens* of 21 compounds tested in both spore germination and poisoned food tests were Du-Ter [fentin hydroxide], TCMTB, Brestanol [fentin chloride], Aureofungin and Difolatan [captafol].

Chen *et al.* (1994) screened 12 compounds *in vitro* for inhibiting mycelial growth of *U. virens* and the most effective were carbendazol [carbendazim] and triadimefon.

The systemic fungicides carbendazim and tricyclazole, and the non-systemic fungicides copper oxychloride, captan, chlorothalonil, wettable sulfur, mancozeb and carbendazim + mancozeb were evaluated for their efficiency against *U. virens* in the laboratory. Among the systemic fungicides, carbendazim resulted in 100 % inhibition of radial mycelial growth at all concentrations. Mancozeb at 0.3 % inhibited mycelial growth completely, while the other non-systemic fungicides were not effective in inhibiting growth. Carbendazim + mancozeb also showed 100 % inhibition of mycelial growth at 0.1 % concentration. All fungicides successfully inhibited chlamydospore germination (Yashodha *et al.*, 2000a).

In a laboratory conducted during 1994 by Ahonsi and Adeoti (2003), Benomyl, Copper Oxychloride, Iprodione, Thiabendazole (TBZ) and Mancozeb were evaluated against *U. virens* at three concentration levels, X, 2X, and 0.5X, where X is the recommended field rate. Copper Oxychloride, Benomyl, and Thiabendazole were most effective, completely inhibiting the mycelial growth of *U. virens* even at 0.5X concentration (5 g/l,1.5g/l, and 0.3 ml/l, respectively).

Laboratory experiments were carried out to evaluate the efficacy of the fungicides, Del-cup 6 % (copper sulfate), copper oxychloride 50 %, Topsin M 70 % (thiophanate-methyl), Beam 75 % (tricyclazole), Plantvax 20 % EC (oxycarboxin), Sumi-8 5 % (diniconazole), and Bremis 2.5 % (triticonazole), against rice false smut at five different concentrations (12, 25, 50, 100 and 200 ppm) for evaluating the spore

germination inhibition of *U. virens* and the results showed that Sumi-8 gave the highest efficiency at both low and high concentrations followed by Del-cup, copper oxychloride, Beam and Bremis (Sehly *et al.*, 2004).

Chen *et al.* (2013) evaluated different fungicides *in vitro* against *U. virens* and found that the EC_{50} ranges of values for prochloraz, difenoconazole, propiconazole and tebuconazole inhibiting mycelial growth of the 102 *U. virens* isolates were 0.04–0.75, 0.04–1.08, 0.04–0.38 and 0.03–0.57 $\mu g\,ml^{-1}$, with the average EC_{50} values of 0.32 ± 0.08, 0.45 ± 0.08, 0.19 ± 0.03 and $0.21 \pm 0.06\ \mu g\,ml^{-1}$, respectively and suggested that the tested *U. virens* isolates were very sensitive to these fungicides.

Field level management studies

1. Physical methods

Use disease free seeds for sowing, removal and destruction of diseased panicles in field and hot water treatment of seeds at 52°C for 10 min. could be used for management of the disease (Anonymous, 2009).

2. Varietal resistance

Varietal differences in susceptibility were observed by Rao (1964) and of 297 varieties examined, 186 remained free from infection despite conditions favouring it.

Mouton and Merny (1959) reported that varieties with hairy glumes were found to be more susceptible to false smut disease than glabrous ones. The incidence of false smut disease was found to be less on rice var. Taichung No. 65 than on Tainan No. 3, both vars. being of the *japónica* type (Chou, 1967).

Singh (1974) evaluated 19 varieties of rice against false smut disease during 1969-1971, and found that the varieties Cauvery, Kanchi, IR20, Jagannath and Pankaj were resistant to the disease in the field.

Ashrafuzzaman (1974) conducted varietal screening under natural conditions against false smut of rice and graded the varieties as resistant (1 to 25 per cent hill infection), moderately resistant (26 to 75 per cent hill infection) and susceptible (above 75 per cent hill infection), where, 91 selections were found resistant to the disease.

Shrivastava (1976) reported that the variety Kranti (R2022), derived from C116 (Bhondu X Parewa) X IR8, was resistant to *U. virens*.

Agrawal and Verma (1978) upon evaluation of the field reaction of 1034 varieties to *U. virens*, found that the total loss in infected panicles varied with varieties from 7.67 to 75.4 % and inferred that resistance was more frequent than susceptibility.

F3 progeny of the rice cross W1263 X W12787 were resistant to *U. virens* and those of Jaya X W1263 and Sona X W1263 showed intermediate resistance as per the results of the studies by Naidu *et al.* (1979).

Singh and Gangopadhyay (1981), with artificial inoculation studies conducted at field level, found that the variety Zenith showed the least infection and smallest smut balls while Jagannath had the highest infection.

Liu *et al.* (1983) reported that the variety Xianghu 25, selected from Xianggen 8 X Nonghu 6, showed resistance *U. virens.*

Rice cultivars played an important role in the degree of rice false smut infection (Singh *et al.,* 1987a). Singh (1987a) reported Paicos 1, derived from Phouren X RP8-9, a semi-dwarf variety to be resistant to *Pyricularia oryzae* and *U. virens.*

Ansari *et al.* (1988) found that, of 22 cultivars grown under natural infection by *Claviceps oryzae-sativae* in the Andaman Islands, India, the most resistant were CR155-5029-216 (0.04 %), CN758-1-1-1 (0.1 %), TNAU (BSR16) (0.23 %) and RP1854-566-1-1-1 (0.3 %).

During 1986-87 and 1987-88 wet seasons 231 rice genotypes when tested for false smut resistance, it was found that early maturing rice genotypes escaped from rice false smut infection, while the late maturing genotypes did not (Singh and Khan 1989).

Singh *et al.* (1989) reported that the variety TCA80-4 (IET7970), released as Rajshree, a photoperiod-insensitive mutant from a land race collection from Bhagalpur was resistant to false smut.

Among 33 advanced lines screened during the rainy season, 1988, in South Andaman, Savithri and IET-9710 showed multiple resistance to bacterial leaf blight, neck and leaf blast and false smut (Ram and Ansari, 1989).

In field trials (Bhardwaj, 1990) with 32 cultivars grown under upland conditions during the 1987 dry season, seven (B3719C-TB-8-1-4, China 988, HPU2202, HPU5101, Nag 1-38, VL 501 and VRS1) were free from infection by *U. virens.* Disease severity on the remaining susceptible cultivars ranged from one to 17.9 % and short cultivars were found more vulnerable than tall ones.

Twenty cultivars were screened under natural infection during the period 1985-89 by Patil and Moghe, (1990) and the high-yielding cultivars SKL6, Sye 75, RP4-14 and SKL6-1-23 were found resistant to *U. virens.*

Shen (1991) reported that rice cultivar Liaoyan 2, a japonica rice, was highly salt tolerant, and moderately resistant to *U. virens*.

In a study of resistance to *U. virens* under natural and artificial infection (Cheng *et al.*, 1992) in 510 accessions, there were significant differences between the varieties (lines), with 7 varieties combining high resistance with good scores for agronomic traits. Early types were found to be more resistant than late ones.

Bhagath (1993) evaluated 42 varieties against false smut of rice and reported that the cultivars SBIR 32-137-1, RAUPT 579-14-1, RAU 77-1, IET 7592, BIET 1165 and IET 6545 were free from disease. Seven cultivars were resistant, 17 moderately resistant and the remainder susceptible or highly susceptible. The reactions of 36 rice genotypes when evaluated (Sugha *et al.*, 1993) against false smut pathogen under rainfed conditions, it was found that none were immune but three were highly resistant.

Twenty local rice varieties selected from wet season stock and 2 widely grown controls (Manoharsali and Jaya) were evaluated by Kalita *et al.* (1993) for 12 agronomic traits and disease reaction under rainfed lowland conditions during the 1989-90 wet season and the results indicated that the variety Bogabordhan outyielded both controls and its field resistance to false smut was found adequate to moderate and better than the variety Jaya.

Bhagat and Prasad (1996) evaluated different rice varieties against false smut of rice and found that the variety Sita was the most susceptible, followed by Mahsuri, and Pankaj was the least susceptible. From a field experiment conducted in Orissa, India, to study the nature and damage caused by false smut in semi deep water rice Dhal and Mohanty (1996) reported that an improved variety Panidhan and a local variety Mayurkantha were free from infection.

Urmila and Dhua (1999) conducted field evaluation of 160 accessions of the Assam Rice Collection against false smut disease under severe natural epiphytotic conditions and reported 18 accessions to be free from the disease and, ARC 11868, with medium slender grain, to possess higher grain density and resistance to the disease.

A new classification standard for *U. virens* was established by Chun-Sheng *et al.* (2000) based on the number of spore balls per panicle, 0-no symptom observed, I-1 spore ball, II-2 spore balls, III-3-5 spore balls, IV-6-9 spore balls, and V-more than 10 spore balls.

The results of the trials conducted by Ahonsi *et al.* (2000) showed that ITA 316 and Ex-China showed some resistance (8.6 and 2.3 %, respectively) to false smut whereas the variety IRAT 170 was completely free from infection.

In a *Kharif* season field trial conducted at West Bengal, India, during *Kharif* 1999, for assessing the field reaction of 41 rice hybrids, eight (MTUHR-2048, PERH-1031, PERH-1037, HKRH-1005, UPHR-1107, NDRH-11, IAHS-200-005 and IAHR-200-006) were found free from the disease (Biswas, 2001).

Niu *et al.* (2001) reported that Jingyou-6 hybrid of rice had stable and high yield (higher than that of the control Quiguang by 14.5-14.9 %) and resistance to green smut.

According to Parsons *et al.* (2001), earlier maturing rice cultivars like Jefferson and Kaybonnet were the least susceptible to false smut disease.

In a survey conducted at the Korea republic during 2000, eight cultivars were reported to be resistant including the variety Heukjinjubyeo which were not damaged by the disease (Hong-Sik *et al.*, 2001).

Use of early maturing varieties could be a good method of escaping the disease (Ahonsi and Adeoti, 2002). Atia (2004) also advocated the use of early maturing varieties in managing the false smut disease and reported the variety Sakha 102 to be highly resistant..

Histochemical observation on the resistant and susceptible varieties showed a higher level of lignin content in the resistant cultivar Shuijing 3 than in the susceptible cultivar 9522 (Guang-Hui *et al.*, 2005).

Resistance evaluation of 11 rice (*O. sativa*) cultivars to *U. virens* was carried out in the farm of Qibao campus of Shanghai Jiaotong University and Shanghai Minhang region (China) by Su-Xin *et al.* (2005). Crystal 3 was resistant, 9522 was susceptible and Dongfan 1 was moderately resistant. The observation from the field showed that the early-maturing cultivars were more resistant than the late-maturing cultivars and the disease was more severe at the edge than that at the centre of the paddy field.

Singh and Singh (2005) screened 98 rice cultivars for their resistance to false smut disease and found 27 varieties to be highly resistant, showing only <1 % disease. They also made correlation studies on morphological characters of rice with false smut incidence and, out of the 26 characters studied, only one trait, flag leaf length to breadth ratio, exhibited highly significant positive correlation with false smut disease.

Da-Dong *et al.* (2006) reported that IIyou 131, a new rice hybrid combination derived from the cross II-32A × Quanhui 131 in Fujian, China was resistant to false smut disease. As per the reports by (Ingale *et al.*, 2007), Sahyadri-2 rice hybrid (a cross combination of female line IR-58025A and the male KJTR-2) showed resistance to false smut.

An experiment was conducted by Yu-Sheng *et al.* (2008) to estimate the genetic model and genetic parameters of rice false smut resistance using artificial inoculation technique to induce the rice false smut efficiently using 157 recombinant inbred lines (RILs) population of F_{10} derived from a cross of IR28 (*indica*) × Daguandao (*japonica*) (parents exhibited resistance and susceptibility to rice false smut, respectively). It was found that, the rice false smut resistance was controlled by two major genes plus polygene mixed genetic model (model E-1-3), and two major genes had equivalent additive effect of 11.41, their heritability was approximately

76.67 %, while the heritability of polygene was approximately 22.86 % and thus it is implied that, the resistance to rice false smut had significant major gene effect, and not only major gene's effect but also polygene's effect should be considered in breeding for resistance to rice false smut.

Nine hrf1 transgenic lines and untransgenic cultivar R109 were tested by Shao-Min *et al.* (2008) for resistance to *U. virens* by inoculating with conidia. Injection inoculation in lab showed that transgenic lines B12-2m, B12, HTRP2 and NJH12 were more resistant. Field tests in Nanjing of Jiangsu Province and Qianshan of Anhui Province confirmed that NJH12 was resistant to *U. virens* in the field with control effect 65 %.

The resistance of 56 indica hybrid rice varieties to rice false smut was monitored by Dai-Hua *et al.* (2008) and the relationships among the rice heading period, morphological characters and the resistance of varieties were studied. The results showed that there was significant difference between the different varieties and the morphological characters revealed that there was a positive relationship between the plant height and the disease index

Lu *et al.*, (2009) reported a linkage between pathogenicity of isolates and resistance of rice hybrids. Studies conducted at the frequent-occurring areas of rice false smut in Jinggangshan and Nanchang regions of Jiangxi Province, to identify resistance to rice false smut in the regional trial varieties showed that, early-maturing varieties had higher resistance than medium-late maturing varieties, Indica varieties had higher resistance than Japonica varieties and, the three-line combinations had higher resistance than two-line combinations (Rui-Rong *et al.*, 2010).

In a study by Yong - Li *et al.* (2010), early- and late-maturity rice cultivars were grown in the fields to investigate the effect of maturity group of cultivar on the severity of rice false smut. From the results, there was significant correlation between the disease severity and cultivar maturity, late-maturity cultivars being significantly more susceptible than early-maturity cultivars and thus, it was suggested to decrease the severity of false smut by planting the early maturing group cultivars.

Mohiddin *et al.* (2012) evaluated four rice genotypes *viz.,* HRI 119, HRI 129, VRH 704 and *Jaya* against the false smut of rice. Among different genotypes HRI 119 was found resistant to false smut with a minimum of 4 and 1 % disease incidence on tillers and grains, respectively.

Information compiled from the Production Oriented Survey (POS) reports for 2005 - 2010 inferred that across the locations, the disease intensity was high in hybrids as well as in inbreds (Ladhalakshmi *et al.,* 2012 a).

Cultural

1. Adjusting the time of planting

As per the report by Gowda (1980) the disease could sometimes be avoided by early sowing, and thus, varieties showing a high disease incidence can be sown early in order to have early flowering and thus avoiding the disease.

Singh *et al.* (1987 b) observed that false smut disease was found to be greatly influenced by the time of transplanting. The crop transplanted early (mid of June) displayed 47 per cent incidence on plant basis, as compared to 6.5 and 1.28 percent on the middle (5 July) and late transplantation (25 July) respectively. The disease was much less on the crop transplanted late.

Dodan and Singh (1995) studied the effect of planting time on the incidence of blast and false smut of rice in Haryana and found that false smut was the maximum on plants planted on Jun. 10 followed by Jun. 25. In field experiments conducted in Karnataka, India, during the *Kharif* seasons of 1996-97 and 1997-98, with the rice cultivars HR-12 and Amrut sown at 10-day intervals starting from 13 May during 1996 and 30 May during 1997, Yashoda *et al.* (2000b) observed that false smut incidence was highest in crops sown on 10 June, while crops sown after this date were not infected.

Ahonsi *et al.* (2000) observed that rice sown with early rains between 2 April and 3 May was free from false smut or showed low incidence (Mean Disease Incidence range = 0 to 3.1 %) whereas rice sown between 16 May and 3 June was highly infected (48.5 to 51.6 % MDI range).

Ahonsi and Adeoti (2002), with the studies conducted at Egypt during 1992 found that planting time could be manipulated to control false smut disease. Early transplanting at beginning of June significantly reduced the disease (Atia, 2004). Shu *et al.* (2010) found that false smut incidence was more with the late planting of rice.

2. Effect of soil type

Percentage of infected tillers and number of infected balls were found to be lower in clay soil (compact soil) than in semi-sand soil (light soil) which showed higher disease incidence (47 and 14.33 %, respectively) and number of infected grains (6.33 and 5, respectively) during the two seasons at Egypt (Atia, 2004).

3. Effect of fertilizer application

Singh *et al.* (1987 b) studied the effect of nitrogen fertilization on false smut disease and found that the incidence of the disease increased with each increase of nitrogen level. On the plant basis, the incidence was the lowest in zero N level treatment and increased to maximum (four fold), at the highest dose of nitrogen (240 kg N/ha.

In field trials conducted during 1987 by Bhardwaj *et al.* (1989), rice cv. Himalaya 741 was exposed to 2 sources of N, 2 N levels and 3 application schedules and it was observed that higher N levels in split applications gave a higher number of smutted florets/ m^2.

In a field trial over 2 seasons with the susceptible cv. GR.11, Patel *et al.* (1992) found that the incidence of disease caused by *U. virens* was lower in plots receiving 100 kg N/ha than in those with 150 or 200 kg N, suggesting that 100 kg N is the best for disease management.

Low disease incidence and number of infected grains were observed at low N amount during the two seasons (Atia, 2004).

Cheng-Wu *et al.* (2005) studied the effect of fertilizer application on disease incidence at Wenzhou area, Zhejiang Province, China. Results showed that the head and grain disease incidence increased with the increase in fertilizer application rate during the growth stage up to 17.92 and 14.19 %, respectively, at 15 kg/667 m^2 top application plus 10 kg/667 m^2 heading fertilizer application rate. The effect of

heading application on the disease incidence was significantly higher than that of top application. The rates of head disease incidence and grain disease incidence were reduced by 7.16 and 6.85 %, respectively, when complex fertilizer (N14 %, P_2O_5 12 %, K_2O 14 %) was applied at the rate of 40 kg/667 m^2 instead of the normal rate.

According to Rautary (2007), and Singh *et al.* (2008), incidence of false smut was found to increase with the incremental dose of fertilizers.

Reducing fertilizer applications resulted in a 33 % reduction in FS (Anders *et al.*, 2008). Singh *et al.* (2008) from a study conducted in Uttar Pradesh, India, to determine the effect of nitrogen rates (40, 80, 120, 160 kg N/ha) on the incidence of false smut (*U. virens*) in rice during the *Kharif* seasons of 2002 and 2003, observed that the disease infected panicles increased with increase in nitrogen levels.

With the studies conducted during 2007-08, in the United States, Brooks *et al.* (2009) observed that moderate nitrogen fertility rates had led to34 % reductions in false smut disease. Increasing N was found to cause increased incidence of false smut disease (Shu *et al.*, 2010).

Biocontrol

Sinha and Sinha (1992) reported a *Fusarium* sp. associated with pseudosclerotia of *U. virens* to be colonizing 33.3-66.7 % of false smut infected florets and thereby reducing the development of the pathogen and advocated the use of the same as a potent biocontrol agent against false smut disease. Kalisena foliar spray @ 2g/litre at the time of grain discolouration could be used for managing false smut disease (Singh and Sasmal 2004). Mei-Liang *et al* (2005) conducted field trials to determine the optimum time of spraying 12.5 % Wenmeiqing spray, containing validamycin and *Bacillus cereus*. The first spray was started when the flag leaf was half of its mature length (20-07-2002). Other sprays were applied in a 2-day interval and the last spray was on 07-08-2002. The best control of false smut was achieved when the spraying was carried out on 24-07 and 26-07, which was about 5 days before the tip of panicle was above the collar of the flag leaf. Different spray combinations were also tested in rice at various growth stages. It was found that the first spray should be applied 5 days after the

pulvinus of the flag leaf became level with the pulvinus of the second last leaf, and a second spray should follow 5 days after.

In a study by Mei-Qin *et al.* (2006), a harpin protein from *Xanthomonas oryzae* pv. *oryzicola* was industrially developed into soluble granule, commercially termed Yilite as a bio-fungicide, containing 1 % of harpin and was sprayed twice during rice growth stage. It was showed that harpin induced SAR in rice with control efficiencies equivalent to those obtained by application of tricyclazole and Jingangmycin, respectively on *U. virens*.

Chu-Ping *et al.* (2007) reported that Strain B-916 of *B. subtilis* had good efficacy against and rice false smut.

According to Hua-Xian *et al.* (2008), in field plots in China, fermentation liquid of *B.subtilis* (Xi-55) markedly controlled the incidence of rice false smut (*U. virens*).

Jeyalakshmi *et al.* (2010) found that the combination of seed treatment, soil application and foliar spray with *P. fluorescens* recorded the minimum disease incidence of bacterial leaf blight with maximum yield in comparison with the chemical treatment and control.

Xiao-Le *et al.* (2011) found that, in the field plots, the control efficacy of liquid fermentation of two *B. subtilis* strains, SF-62 and SF-3-38 on rice diseased kernel infected by *U. virens* were 47.88 % and 43.12 %, respectively.

Use of Antibiotics

In a field trial, application of the antibiotic Jinggangmycin, 15 % Bayleton (triadimefon) or 40 % carbendazim at the bracteal opening stage, or once at bracteal leaf opening and again at full heading gave good control of rice false smut when compared, one or two sprays of Jinggangmycin were found to give 84.38 and 96.88 % control, respectively in comparison with triadimefon giving 79.69 and 87.5 % control and carbendazim with 73.44 and 85.94 % disease control (Mao, 1992).

Bagga and Kaur (2006) in a study conducted at Punjab, evaluated the field level efficacy of Kasugamycin (0.1 %) against false smut disease as two foliar sprays

(the first spray applied during the boot stage and the second 15 days later), and found that the compound was more effective when applied at heading stage.

Chemical Management

Kannaiyan and Rao (1976) evaluated different chemical fungicides on false smut disease of rice and found copper oxychloride to be effective in reducing the disease.

Li *et al.* (1986) reported that Bordeaux mixture or the antibiotic Jingangmycin, sprayed at late booting, gave 78.7-92.6 % control of the disease. Bayleton [triadimefon] was found most effect against *U. virens* when applied at early heading at 105 g a.i./ha (Anonymous, 1986).

Ray (1987) reported that Incidence of *U. virens* was significantly reduced by copper oxychloride and thiophanate-benzyl.

Field tests conducted by Tsai *et al.* (1990) at Taiwan with 8 fungicides revealed that Daconil WP [chlorothalonil] and 58 % Ridomil-MZ WP [metalaxyl] gave the best disease control.

In a field trial conducted by Mao (1992), one or two sprays (at the bracteal opening stage, or once at bracteal leaf opening and again at full heading) of triadimefon gave 79.69 and 87.5 % and carbendazim gave73.44 and 85.94 % control of false smut disease.

Seven fungicides, copper oxychloride, carbendazim, tricyclazole, mancozeb, edifenphos, IBP [iprobenfos] and propiconazole were evaluated against false smut of rice (cv. CR 333-6-1) at the Chaudhary Charan Singh Haryana Agricultural University Rice Research Station, Kaul, Haryana, during *Kharif* 1992 and 1994 and, Copper oxychloride proved most effective, decreasing disease incidence by 95.5 and 96.1 % in terms of infected tillers and grains, respectively, with a corresponding increase of 7.2 % in grain yield. One application of copper oxychloride and propiconazole at 50 % panicle emergence was as effective as one spray at 7 days after 50 % panicle emergence, or two sprays at 50 % panicle emergence and 7 days later, indicating that the disease can be managed by a single application of fungicide (Dodan *et al*, 1997).

Yashoda *et al.*, (2000 a) evaluated efficiency of the systemic fungicides carbendazim and tricyclazole, and the non-systemic fungicides copper oxychloride, captan, chlorothalonil, wettable sulfur, mancozeb and carbendazim + mancozeb against *U. virens* in the field and the results indicated that false smut incidence could be reduced and yield can be increased significantly with one spray of carbendazim at 0.1 % during panicle emergence stage. In a study on the control of false smut, 20 % fentin acetate when applied to rice cv. II You 6216 in plots of 31.12 m^2 resulted in 72.43 and 84.48 % control, which was 13.82 and 25.87 % higher than that of 5 % Jinggangmycin (Hua *et al.*, 2000).

Fungicide trials conducted by Parsons *et al.* (2001) at Arkansas during 1998 indicated that Tilt [propiconazole] provided substantial control of false smut if applied at late boot (just prior to head emergence). Hong-Sik *et al.* (2001) tested different chemicals for the control of the rice false smut and, tebuconazole showed the highest control efficacy of 83-88 % and other chemicals such as azoxystrobin WP and ferimzone WP also effectively suppressed disease development in the field trials.

The fungitoxicants, propineb (Antracol 70 WP; 3 g/litre), prochloraz (Octave 50 WP; 1 g/litre), copper hydroxide (Copper hydroxide 77 WP; 3 g/litre), epoxiconazole (Opus 12.5 SC; 2 ml/litre), chlorothalonil (Chlorothalonil 40 SC; 2 ml/litre) and copper oxychloride (Blue Copper 50 WP; 4 g/litre) when sprayed twice at the time of emergence of panicle tip out of boot leaf sheath and complete panicle emergence, it was found that, copper hydroxide and copper oxychloride were found significantly superior to the other fungitoxicants in reducing the infected panicles and grains. Copper hydroxide decreased the infected panicles and grains by 59.8 and 57.1 %, respectively, along with an increase of 8.2 % in grain yield (Singh *et al.*, 2002).

According to Wei *et al.* (2002), the optimum control period of false smut is from the end of the boot stage to the rupturing stage of rice and twice spraying was the most suitable control method for susceptible or very susceptible crosses.

Ahonsi and Adeoti (2003) used benomyl, copper oxychloride, iprodione, thiabendazole and mancozeb against the disease and found four sprays of any of the five fungicides at an interval of seven days, starting at the sighting of first booting

reduced disease incidence and severity with the best results from copper oxychloride. Spraying rice with copper oxychloride (at 4kg i.e./ha) resulted in 72 % reduction in false smut severity resulting in significant increase of 39 % in grain yield compared with the control treatment.

Spraying rice plants with Topsin-M, Ridomil MZ or copper oxychloride at the beginning of booting stage three times at two weeks interval prevented the disease (Atia, 2004).

Singh and Sasmal (2004) recommended foliar spray of Dithane-M-45 (1 %) at the time of grain discolouration for the control of false smut.

Bagga and Kaur (2004) during a study conducted at Punjab, evaluated nine fungicides against false smut disease out of which, Tilt (Propiconazole) and Contaf (Hexaconazole) at 0.1 % each and Blitox (Copper oxychloride) at 0.3 %, when applied during the boot stage, significantly controlled false smut and improved grain quality.

Field experiments conducted to evaluate the efficacy of the fungicides, Del-cup 6 % (copper sulfate), copper oxychloride 50 %, Topsin M 70 % (thiophanate-methyl), Beam 75 % (tricyclazole), Plantvax 20 % EC (oxycarboxin), Sumi-8 5 % (diniconazole), and Bremis 2.5 % (triticonazole), against rice false smut showed that copper oxychloride showed the highest efficiency (75 %) against the fungus followed by Bremis (64 %), Del-cup (60.7 %) and Topsin M (60 %) (Sehly *et al.*, 2004). Cheng-Wu *et al.* (2005) evaluated the control efficacy of 30 % cupric sulfate and found that the chemical, when applied 14 days before heading with the rate of 400 g/667 m^2, gave 74.75 % control, and when applied for twice (14 and 7 days before heading) gave 85.36 % control, both being significantly higher than that of the fungicide Triadimefon.

Upon evaluation of nine fungicides, *i.e*., Fujione 40E (isoprothiolane), Tilt 25 EC (propiconazole), Contaf 5EC (hexaconazole), Folicur 250 EC (tebuconazole), Hinosan 50 EC (edifenphos), Bayleton 25 WP (triadimefon), Benomyl 50 WP (benlate), Bavistin 50WP (carbendazim), Blitox 50 WP (copper oxychloride), Kasu-B (kasugamycin) and Sixer (carbendazim + mancozeb), given as two foliar sprays (the first spray applied at mid-late boot stage and the second 15 days later) to control false

smut of rice during 2001-03, in Punjab, it was observed that, in 2001, Blitox and Tilt were found most effective whereas in 2002 and 2003, Tilt, closely followed by Contaf, consistently reduced false smut incidence and severity in both years and increased grain yields (Bagga and Kaur 2006).

Application of the fungicide Simeconazole granules at 2–5 weeks before heading was found highly effective against false smut, with treatment 3 weeks before heading being the most effective (Tsuda *et al.*, 2006). Spraying of copper oxychloride at 2.5 g/litre or Propiconazole at 1.0 ml/litre at boot leaf and milky stages would be more useful to prevent the fungal infection (Anonymous, 2008).

Seed treatment with carbendazim 2.0 g/kg of seed have been reported to be useful for management of the disease (Anonymous, 2009).

Pannu *et al.* (2010) evaluated different fungicides [Kocide 3000 [copper hydroxide] (0.1 or 0.2 %), Blitox 50 WP [copper oxychloride] (0.2 5 %)/Tilt 25 EC [propiconazole] (0.1 %), Score 25 EC [difenoconazole] (0.1 %) and Contaf Plus 5 EC [hexaconazole] (0.2 %)] against false smut of rice given as two sprays, one at the booting or heading stage and the second spray given after 10 days. Blitox 50 WP sprayed at the booting stage followed by Tilt 25 EC sprayed after 10 days resulted in the lowest DI (4.00 and 0.00 %) and DS (0.02 and 0.00 %) values. The fungicides were most effective when initially given at the booting stage. The experiments were conducted during *Kharif* of 2005-06, 2006-07 and 2007-08 (Barnwal *et al.*, 2010) revealed maximum disease control over check in plots in which two sprays of propiconazole (0.1 %) were applied followed by single spray of hexaconazole (0.1 %). Out of two new formulations, kresoxim methyl 40 % + hexaconazole 8 % WG (RIL-068/F148WG) was highly significant (3.0 %) in reducing the false smut severity and was on par with check fungicides like propiconazole (3.1 %) and carbendazim (12 %) + mancozeb (63 %) (Saaf) (5.4 %) (DRR, 2010).

In a study conducted at Arkansas, Jecman and TeBeest, (2012) tested demethylation inhibitors (DMI), Quinone outside Inhibitors (QoI) and Cu in *in-vitro* petri-plate growth assays, greenhouse studies and in field studies to identify effective materials and concentrations of those materials and from the preliminary data of the

study, it was suggested that an effective disease management strategy may include using seed treatments or seed treatments followed by foliar applications under prevailing field conditions.

The combination of Prochloraz + Carbendazim decreased the disease incidence of false smut by 90 and 65 % both on tillers and grains and subsequently increased the grain yield by 44 % (Mohiddin *et al.*, 2012).

Based on multilocational data on chemical control of the disease, it was found that application of propiconazole and combination of trifloxystrobin 25 %+ tebuconazole 50 % (Nativo75WG) at 50 % PE (Panicle Emergence) stage was most effective in managing the disease. (Ladhalakshmi *et al.*, 2012 a).

Chen *et al.* (2013) in the field studies conducted at China, found that two sprays of 50 % propiconazole EC at 300 g a.i. ha^{-1} gave the best control of rice false smut during the two consecutive years, 2010 and 2011, with the control efficacy ranging from 71.5 to 74.3 %.

Integrated Management

Revilla (1955) recommended seed treatment with compounds such as Arasan or Granosan M, crop rotation, and replacement of the susceptible Radin China by resistant varieties for management of false smut disease. Parsons *et al.* (2001) recommended the control of false smut in Arkansas through cultural practices including: (1) planting before the first part of May; (2) using no more than the recommended rate of nitrogen (N) fertilizer; (3) seeding and managing the field to encourage uniform development and maturity; and (4) spreading the seeding dates to avoid all fields heading at the same time.

Biochemical studies

The peroxidase and polyphenol oxidase activities in the stems of healthy and *Macrophomina* infected jute plants were studied by Mukhopadhyay and Nandi (1976), and the results showed that in the infected stems the peroxidase and polyphenol oxidase activities were much higher than that of healthy ones and increased gradually with the progress of the disease. Micales, *et al.* (1986) reported that the differences in the isozyme binding pattern are due to variation in the amino

acid content of the molecule, which in turn is dependent on the sequence of nucleotides in DNA. Asiedu, (1992) reported isozyme analysis to be a powerful tool for estimating genetic variability identifying cultivars and germplasm accessions.

Electrophoresis for esterase-isoenzyme gave very different bands between the new strains of white false smut and false smut of rice (Wang *et al.*, 1997).

Tyagi *et al.* (2000) observed increased peroxidase and PPO activity in wheat cultivar resistant to *Alternaria triticina* compared to susceptible ones and suggested that these enzymes had an active role in the defence mechanism in wheat plants.

Histochemical observation showed a higher level of lignin content in the resistant cultivar Shuijing 3 (R) than in the susceptible cultivar 9522 (S). Abundant polyphenolic compounds in the epidermis and endosperm of the grain were detected in rice varieties resistant to false smut, but not in susceptible (Guang-Hui *et al.*, 2005).

Native Polyacrylamide Gel Electrophoresis for isozyme analysis carried out using healthy as well as BBrmv infected sample for peroxidase (PO) and Poly Phenol Oxidase (PPO) enzyme by Dhanya *et al.* (2006) where, a single definite band of PO (Rm 0.63) was seen only in infected sample whereas thickness of theband with Rm 0.65 showed intensified production of PPO in BBrMV infected plants.

Materials and methods

3. MATERIALS AND METHODS

The present study entitled 'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala' was conducted during the period 2010-2013 at College of Agriculture, Vellayani, Thiruvananthapurum and the field experiments were carried out in two research stations; 1) Regional Agricultural Research Station, Pattambi, Palakkad and, 2) Rice Research Station, Moncompu, Alappuzha, and at Farmers fields at Vilayur (Palakkad) and Champakkulam (Alappuzha). The methods employed in the present study such as preparation of culture media, maintenance of cultures etc. were adopted as per standard procedures (Dhingra and Sinclair, 1993). The materials used and methods followed are described below.

3.1. SURVEY ON THE INCIDENCE AND SEVERITY OF THE DISEASE

A total of four surveys were conducted during 2011-2013, two at Palakkad district during the *Kharif* and two at Alappuzha district during *Rabi* season. During each season, thirty affected fields were surveyed for assessing the per cent infected tillers and the disease severity of false smut disease of rice. Alappuzha district was surveyed on geographical basis as Upper Kuttanad, Lower Kuttanad and Kayal areas.

The following calculations were made

a) Per cent infected tillers (Mandhare *et al*., 2008),

$$\text{Per cent infected tillers} = \frac{\text{Total number of tillers infected/m}^2}{\text{Total number of tillers/m}^2} \text{ X } 100$$

b) Per cent infected grains (Singh and Dube, 1978),

$$\text{Per cent grains infected} = \frac{\text{Total number of infected grains/panicle}}{\text{Total number of grains/panicle}} \text{ X } 100$$

Per cent grains infected per panicle was calculated as an average of 20 smutted panicles.

c) Disease severity (Singh and Dube, 1978),

Disease severity = Per cent infected tillers X Per cent grains infected per panicle

3.1.1. Development of a new scoring system for assessing the disease severity of false smut disease of rice

Based on the extent of damage caused by the pathogen in the field, a new scoring system (Table 1, Plate 1) was developed for assessing the disease severity of the false smut disease of rice, taking in to consideration, the chaffiness and blackening symptoms which were also found to be produced by the pathogen in the field.

Score ‘zero’ was given for the completely healthy panicles, and the panicles containing one to two smut balls were given a score of ‘one’. The scores ‘two’ and ‘three’ were given for the panicles with the number of smut balls ranging from, 3 - 5 and 6 - 10 respectively.

All the panicles with the scores 4, 5 and 6 contained >10 smut balls per panicle but varied in the percentage of chaffiness and blackening. The panicles with score ‘four’ had <10 % chaffiness and blackening, whereas those with the scores ‘five’ and ‘six’ had 10-50 % and >50 % chaffiness respectively.

3.2. STUDY ON THE SYMPTOMATOLOGY OF THE DISEASE

Detailed studies on the symptomatology of false smut disease were carried out including the stages of disease development.

3.3. ISOLATION OF THE PATHOGEN

Smut balls collected during surveys were brought to laboratory and different isolation methods were attempted to obtain pure cultures of the

Table 1. Score chart for false smut disease of rice

Score	Number or Per cent of spikelets affected
0	No smut balls
1	1-2 smut balls per panicle
2	3-5 smut balls per panicle
3	6-10 smut balls per panicle
4	>10 smut balls per panicle with <10 per cent chaffiness
5	>10 smut balls per panicle with 10-50 per cent chaffiness and blackening
6	>10 smut balls per panicle with >50 per cent chaffiness and blackening

Plate 1. A new score chart developed for assessing the severity of false smut disease of rice

pathogen. Different procedures undertaken for isolation are detailed below:

3.3.1. Isolation from the whole smutted grains (Singh *et al.* 1985)

Infected grains were carefully washed with tap water to remove dust particles, dried, surface sterilized by immersing in 0.1 % mercuric chloride solution for 1–2 min. The rice grains were then washed several times in sterile distilled water, dried between two sterile filter papers and transferred to Potato Dextrose Agar (PDA, Appendix I) or Rice Yeast Dextrose Agar (RYDA) media and were incubated at 25–28 °C and later the cultures were brought in to pure culture.

3.3.2. Isolation from the pieces of smut balls (Fang, 1996)

Smutballs were sterilized with 75 % ethanol for 2 to 3 min followed by 0.1% mercuric chloride for one to three min. and then washed three times with sterile distilled water. The innermost layer of these sterilized smut balls was cut into pieces (2 - 3 mm) and 5 pieces each were transferred to XBZ agar (Appendix I) and incubated at 28°C until the appearance of mycelium and conidial production. Colonies with characteristics of *U. virens* were then examined under the light microscope, and were transferred to XBZ agar slants in a test tube.

3.3.3. Isolation from the chlamydospore suspensions of (Yong-Li and Qi, 1999)

Smut balls were sterilized with 75 % ethanol for 2 to 3 min followed by 0.1% mercuric chloride for 1 to 3 minand then washed three times with sterile distilled water. Chlamydospore suspensions prepared using sterile water from these surface sterilized smut balls were plated on Yeast Peptone Potato Dextrose Agar (YPPDA, Appendix I) medium.

3.3.4. Isolation by dusting the chlamydospores from the smut balls

Apart from the above mentioned methods, a new method was devised for rapid isolation of the fungus as follows:

Smut balls were surface sterilized under aseptic conditions with 0.1% mercuric chloride for 3 to 3 ½ min followed by three washings with sterile distilled water and were allowed to dry for two min. Chlamydospores from the inner layers were dusted thinly over the solidified YPPDA or PSA plates with the help of a forceps and the plates were incubated at 28 ± 2ºC. After 24 hours the plates were observed under low power objective (Plate 2 A) and the germinating chlamydospores were marked (Plate 2 B) and were transferred aseptically to fresh plates or slants of YPPDA for getting pure culture.

The cultures were serially numbered and were maintained by periodical sub culturing in YPPDA slants and when sufficient growth was attained, were stored in a refrigerator. The cultures were also preserved under mineral oil.

3.4. CULTURAL CHARACTERISTICS

Different cultural characteristics of the pathogen isolates, *viz*., the nature of growth on the solid and liquid media, colour and texture of the colony, colony diameter, sporulation and mycelial dry weight of the seven selected isolates of *U. virens* were studied.

In order to have a detailed study on the cultural characteristics of the pathogen, 4 mm mycelial discs cut from ten days old culture of *U. virens* were inoculated at the center of YPPDA plates and the plates were incubated at room temperature. Type of growth of the pathogen on the solid media, colony colour and sporulation were observed 30 days after inoculation. Colony diameter was measured at five days interval.

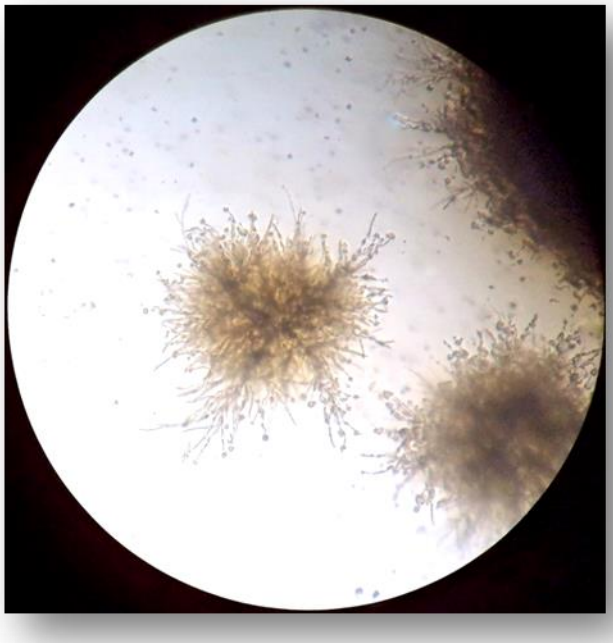


Plate 2 A. Typical colonies of *U. virens* developing on the isolation plate observed under a light microscope under low power

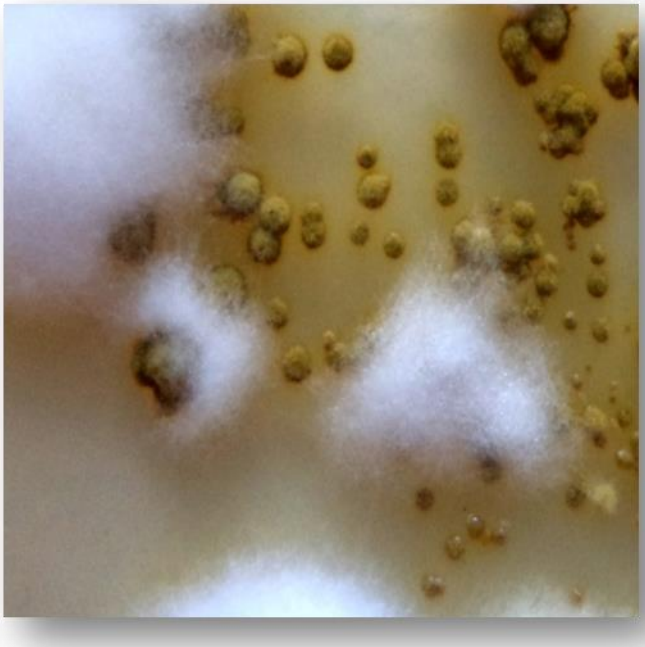


Plate 2 B. Typical colonies of *U. virens* developing on the isolation plate

Mycelial dry weight of each isolate was also observed by culturing the fungus on Potato Sucrose broth and 100 ml of broth was poured in 250 ml conical flasks and sterilized by autoclaving at 1.1 kg/ cm^2 for 20 min. Five mycelial discs of 4 mm of each isolates were inoculated in the broth and the flasks were incubated at room temperature for 20 days. Mycelium was filtered on Whatman filter paper No.1 and dried in the oven at 60 °C and weighed on the next day onwards till constant results were obtained.

3.5. PATHOGENICITY TEST

The pathogenicity was proved following Koch's postulates. Disinfected viable seeds of the rice variety Uma were sown in sterilized paddy soil taken in plastic pots of 8 cm dia. and one to two plants were maintained per pot. The plants were maintained under controlled temperature conditions just before flowering. Inoculations were done using the chlamydospores collected from the infected smut balls and also with secondary conidia obtained four days after inoculating potato sucrose broth with the pathogen mycelial discs. Three methods of inoculations were attempted.

1) Injection method (Ashizava *et al.*, 2010): The panicles while still inside the boot leaf were injected with 2 ml of spore suspension in to the flag leaf at pre-emergence stage.
2) Spraying method (Yashoda *et al.*, 2000): The panicles just after emergence were sprayed with the spore suspension using an atomizer at the stage when 50% of the flowers were open.
3) Deposition method (Singh and Gangopadhyay 1981): Here, the open flowers were deposited with the spore suspension of the pathogen at the stigmatic surface.

The inoculated panicles were labeled and covered with polythene covers and the plants inoculated were first maintained at 15ºC for 24 hours after which the temperature was increased to 25ºC and thereafter the plants were maintained at that temperature.

3.6. IDENTIFICATION OF THE PATHOGEN

The isolates of the pathogen (Uv 1 - Pattambi, Uv 2 - Vilayur, Uv 3 - Koppam, Uv 4 - Kodumpu, Uv 5 - Kadampazhippuram, Uv 6 - Alappuzha and Uv 7 - Trivandrum) obtained from different locations were identified based on the cultural characteristics, spore morphology and by characterizationon the basis of the ITS sequences of the isolates.

3.6.1. Colony Morphology of the pathogen *U. virens*

The morphological characteristics of the pathogen were studied under solid and liquid culture.

3.6.2. Conidia and conidial ontogeny of *U. virens* (Riddel, 1974)

Twenty ml of molten plain agar medium was poured in to sterile petri plates and 6 mm block was cut with a sterile needle and transferred to the centre of a sterile microscopic slide and all the four sides were inoculated with small bits of fungal culture under aseptic condition. On the top of the agar block, a fresh cover slip was placed and the slide was kept in a moist chamber and incubated for 48 h at 28 ± 1^0C. After the incubation period, the cover slip was gently lifted, a drop of 15 per cent alcohol was placed at the centre, and before drying, the cover slip was mounted on lactophenol cotton blue (Appendix II) and were then examined under a light microscope at a magnification of 400 x.

Measurements of spores were taken and the average size of the spore was determined and shapes of spores were also recorded, the stages of conidial ontogeny were examined, and the photographs were taken.

3.6.3. Molecular characterization of the pathogen isolates by DNA sequencing using universal primers of ITS

The isolates of the pathogen obtained from different locations were characterized on molecular basis by comparison of the ITS sequences of the isolates. The procedure for molecular characterization was as follows:

3.6.3.1. DNA isolation using GenElute Plant Genomic DNA Miniprep Kit (Sigma)

The tissue/mycelium (about 50 mg) was transferred to a micro-centrifuge tube and ground in 350 µl of lysis solution A and 50 µl of lysis solution B using a micro pestle. The mixture was incubated at 65°C for 10 min with occasional inversion. Precipitation solution (130 µl) was added to the mixture, mixed completely by inversion and the sample was placed on ice for 5 min.

The sample was centrifuged at 14,000 rpm (Eppendorf Centrifuge 5804 R) for 5 min. to pellet the cellular debris, proteins, and polysaccharides. The supernatant was transferred to the GenElute filtration column tube and centrifuged at 14,000 rpm for 1 min. This removed any cellular debris not removed in the previous step. The filtration column was discarded and 700 µl of binding solution was added directly to the flow through liquid and mixed thoroughly by inversion. 700µl of this mixture was added into GenElute nucleic acid binding column and centrifuged at 14,000 rpm for one min.

The flow through liquid was discarded and the collection tube was retained. The column was returned to the collection tube and the remaining sample was applied to the column. Centrifugation was repeated as above and the flow through liquid and the collection tube were discarded. The binding column was placed into a fresh 2 ml collection tube. 500 µl ethanol-added wash solution was added to the binding column and centrifuged at 14,000 rpm for one min. The flow through liquid was discarded and the collection tube was retained. The wash was repeated once more.

The binding column was transferred to a new collection tube. 30 µl of elution solution (pre-warmed to 65°C) was added to the binding column and centrifuged at 14,000 rpm for one min. The stock DNA was properly labelled and stored at 4 °C.

3.6.3.2. Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6 X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5 µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5 X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.6.3.3. PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1 X PCR buffer (100 mM TrisHCl, pH-8.3; 500 mM KCl), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 20 ng DNA, one unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg/ml BSA and 4 % DMSO, 5 pM of forward and reverse primers.

3.7.3.1. Primers used

Target	**Primer Name**	**Direction**	**Sequence (5' → 3')**	**Reference /Remarks**
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG	White *et al.*, 1990
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC	

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

3.6.3.4. PCR amplification profile

ITS & LSU

95 °C	-	5.00 min	
95 °C	-	0.30 min	} 40 cycles
58 °C	-	0.40 min	
72 °C	-	1.00 min	
72 °C	-	5.00 min	
4 °C	-	∞	

3.6.3.5. Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad)

3.6.3.6. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consisted of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five µl of PCR product was mixed with 2 µl of ExoSAP-IT and incubated at 37^{o}C for 15 min followed by enzyme inactivation at 80^{o}C for 15 min.

3.6.3.7. Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96^{o}C for two min followed by 30 cycles at 96^{o}C for 30 sec, 50^{o}C for 40 sec and 60^{o}C for four min for all the primers.

3.6.3.8. Post Sequencing PCR Clean up

1. Mastermix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction were made.
2. 12 µl of master mix I was added to each reaction containing 10 µl of reaction contents and were properly mixed.
3. Mastermix II of 2 µl of 3 M sodium acetate pH 4.6 and 50 µl of ethanol per reaction were made.
4. 52 µl of master mix II was added to each reaction.

5. Contents were mixed by inverting.
6. Incubated at room temperature for 30 min
7. This was centrifuged at 14,000 rpm for 30 min
8. The supernatant was decanted and added 100 µl of 70% ethanol
9. This was centrifuged at 14,000 rpm for 20 min.
10. The supernatant was decanted and 70 % ethanol wash was repeated
11. The supernatant was decanted and the pellet was air-dried.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.6.3.9. Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010).

Based on the observations obtained in the cultural studies, the isolate Uv 7, which was fast growing with maximum mycelial dry weight, was selected for further studies.

3.7. EVALATION OF THE SUITABLE NUTRIENT MEDIA FOR CULTURING OF THE PATHOGEN

The isolate Uv 7 was used for evaluating the suitable medium for culturing and sporulation of the pathogen. Studies on sporulation were done under broth culture.

3.7.1. Growth on different solid media

The growth of the pathogen, *U. virens* was compared on different solid media. Different solid media used were Potato Sucrose Agar (PSA), Potato Dextose Agar (PDA), Czapek Dox Agar (CDA), XBZ Agar

(XBZA), Rice Yeast Dextrose Agar (RYDA), Yeast Potato Dextrose Agar (YPPDA) and Rice Extract Sucrose Agar (RESA) (Appendix I).

Each medium was added with the antibiotic streptomycin @ 100 ppm before dispensing to plates to avoid contamination from bacteria. Fifteen to twenty ml of each medium was poured into sterilized Petri dishes. Culture disc of four mm was taken from periphery of 20 days old culture of the pathogen. Three replications were maintained for each treatment. The plates were incubated at 28± 1°C. Observation on colony growth was taken when the maximum growth was attained in any one of the media tested.

3.7.2 Growth on different liquid media

The liquid media used were Potato Sucrose Broth (PSB), Potato Dextrose Broth (PDB), Czapek Dox Broth (CDB), XBZ Broth (XBZB), Rice Yeast Dextrose Broth (RYDB), Yeast Potato Dextrose Broth (YPPDB) and Rice Extract Sucrose Broth (RESB) (Appendix I). Hundred ml of broth was poured in 250 ml conical flasks and sterilized by autoclaving at 1.1 kg/ cm^2 for 20 min. Five mycelial discs of 4 mm dia., cut from the active growing margins of 20 days old culture of the pathogen were inoculated in different liquid media and the flasks were incubated at room temperature for 20 days. Mycelium was filtered on Whatman filter paper No.1 and dried in the oven at 60°C and weighed on the next day onwards till constant results were obtained.

3.7.3 Sporulation and pigment production on different liquid media

Sporulation of the pathogen in different liquid media was also studied. The pathogen was inoculated in to different liquid media such as Potato Sucrose Broth (PSB), Potato Dextrose Broth (PDB), Czapek Dox Broth (CDB), XBZ Broth (XBZB), Rice Yeast Dextrose Broth (RYDB),

Yeast Potato Dextrose Broth (YPPDB) and Rice Extract Sucrose Broth (RESB) and incubated as per the procedure described in 3.7.2.

Five day old culture of *U. virens* in different liquid broth was stirred well to bring the secondary conidia in to suspension. From the suspension, 0.1 ml was kept over a haemacytometer, covered with the cover glass and was observed under a microscope and the number of secondary conidia per ml of the media was calculated and sporulation was expressed as very high, high, moderate and low. Pigmentation by the pathogen on different broth was also recorded.

3.8. PHYSIOLOGICAL CHARACTERISTICS

The isolate Uv 7 found fast growing under *in vitro* conditions was selected to study the physiological characteristics of the pathogen

3.8.1. Effect of temperature (Fu *et al*., 2012)

The pathogen was grown in different temperature levels so as to determine the optimum temperature for culturing of the pathogen. For this, mycelial discs of four mm size were cut from twenty days old culture of pathogen and were inoculated on the centre of the solidified YPPDA plates and the plates were incubated at different temperature levels *viz*., 4, 24, 28 and 32°C, and also at room temperature. Four replications were maintained for each temperature level. Colony diameter of the pathogen grown under different temperature levels was measured at 30 days after incubation.

3.8.2. Effect of pH (Fu *et al*., 2012)

For determination of the optimum pH levels for culturing the pathogen *U. virens*, YPPDA media was prepared, the pH was adjusted to 5.5, 6.5, 7.5, and 8.5 by a pH meter by adding 0.1 N alkali (NaOH) or acid (HCl), and sterilized in an autoclave at 1.1 kg/ cm^2 for 20 min. The media were melted and poured in to petri plates @ 20 ml per plate and inoculated with four mm mycelial discs of the pathogen in the center and incubated at

28± 1°C for 30 days.Four replications were maintained for each pH values. The diameter of the colonies of the pathogen was recorded on 30 days after incubation.

3.8.4. Effect of light (Fu *et al*., 2012)

The pathogen was subjected to different light conditions so as to determine the optimum light conditions for the growth of the pathogen. Different periods of exposure to light and darkness were tried; 24 h light, 24 h darkness, intermittent light i.e., 12 h fluorescent light (500 lux) followed by 12 h darkness and the ambient light conditions (c 400 lux during daytime and darkness during night time). The pathogen inoculated on YPPDA plates were incubated at the above mentioned light conditions and the growth in terms of colony diameter was compared 30 days after incubation to find out the optimum light requirement by the pathogen.

3.9. STUDIES ON THE EPIDEMIOLOGY OF THE DISEASE

3.9.1. Survival of the pathogen *U. virens*

Survival of the pathogen on seeds, stubbles, soil and collateral hosts was studied at field level and at laboratory conditions.

3.9.1.1. Laboratory studies on the survival of the pathogen under seeds, stubbles, soil and collateral hosts

In vitro studies were conducted on the survival of the pathogen on seeds, stubbles, soil and collateral hosts.

3.9.1.1.i. *Survival of the smut balls*:

a. Survival on stubbles (Yashoda and Anahosur, 2000): In order to study the survival of the smut balls on stubbles, the smut balls of the pathogen were incubated within the stubbles or the crop residue of rice crop under laboratory conditions and germination of the smut balls were tested at monthly intervals by making isolations from the stored smut balls on YPPDA medium. Isolations were

made from twenty five smut balls each month on YPPDA and the per cent viability was assessed.

b. Survival in soil (Singh *et al*., 1985): For studying the survival rate of the smut balls in soil, smut balls of the pathogen were stored in small packets of muslin cloth at the rate of 25 smut balls per packet and these packets were buried at 5 cm depth in pots filled with clayey soil. Viability of the stored smut balls were tested at monthly intervals by making isolations from 25 smut balls each month on YPPDA medium.

3.9.1.1.ii. *Survival of the chlamydospores*:

a. Survival on stubbles (Yashoda and Anahosur, 2000): the smut balls of the pathogen were incubated within the stubbles or the crop residue of rice crop under laboratory conditions.The survival of the chlamydospores was studied at monthly intervals by checking the germination of the chlamydospores adhering to the stored straw or panicles by hanging drop technique using 2 % sucrose solution, within cavity slides.

For assessing the germination of the chlamydospores, the cavity slides with the chlamydospore suspension were incubated within petri plates lined with moist filter paper for 24 h, after which, observed under a light microscope for counting the number of spores germinated. A chlamydospore was considered to be germinated when the length of the germ tube was more than or equal to that of its diameter. The per cent germination of chlamydospores was found as an average of germination from five microscopic fields.

b. Survival in soil (Singh *et al*., 1985): Smut balls of the pathogen were stored within small packets of muslin cloth at the rate of 25 smut balls per packet and these packets were buried at 5 cm depth in pots filled with clayey soil. Germination of the chlamydospores

from such stored smut balls within the cloth packets were studied at monthly intervals on cavity slides by hanging drop method.

c. Survival on the seed: The seeds of the rice variety Uma were smeared with the chlamydospores of the pathogen and were stored under laboratory conditions. At monthly intervals, the chlamydospores adhering to the seeds were tested for their germination *in vitro* by hanging drop technique on cavity slides.

3.9.1.1.iii. *In vitro* survival on collateral hosts

In vitro inoculation studies were conducted on the survival of the pathogen on collateral hosts. Inoculation was carried out as per the methods described in 3.5.

3.9.1.2. Study of survival of the pathogen in the field:

The survival of the pathogen under field conditions on seeds, stubbles, soil and collateral hosts under field conditions were studied during the conduct of surveys at Palakkad and Alappuzha.

3.9.2. Study on the onset of false smut disease in the field at different cropping seasons of the year

The onset of false smut disease in the field at different cropping seasons was observed at two locations, Palakkad and Alappuzha in the medium duration susceptible variety Uma (120-135 days duration).

3.9.3. Correlation studies on the influence of various weather parameters on the incidence and intensity of false smut disease of rice

The weather parameters prevailing in the field at the time of various field trials were correlated with the per cent infected tillers and disease severity so as to study the influence of weather parameters on the development of false smut disease of rice. The various parameters studied include Minimum temperature, Maximum temperature, Relative Humidity.

(Morning), Relative Humidity (Evening), Relative Humidity (Average) and Total Rainfall.

3.10. SCREENING OF RICE VARIETIES AGAINST FALSE SMUT DISEASE

Field trials were conducted to study the variability of the disease in terms of incidence and intensity with respect to different cultivars of rice at two locations i.e., RRS Moncompu and RARS Pattambi. Twenty varieties of rice were used for the trials and were replicated thrice. The design followed was RBD.

Observations were made on the per cent infected tillers and disease severity in each variety and the varieties were categorized on the basis of the disease reaction as highly resistant, resistant, moderately resistant, susceptible and highly susceptible (Singh and Singh, 2005).

The resistant and the highly susceptible varieties were used further for the molecular level studies.

3.11. DETECTION OF MOLECULAR MARKERS FOR FALSE SMUT RESISTANCE

Four highly resistant and four highly susceptible varieties selected were used for detection of molecular markers of resistance by RAPD profiling in order to check for the possible resistant gene sources against false smut disease.

RAPD profiling was carried out with the pooled genomic DNA isolated from four highly resistant and four highly susceptible varieties. Ten RAPD primers *viz*., OPK-19, OPG-18, OPC-15, OPB-10, OPH-19, OPD-3, OPB-5, OPK-14, OPF-13 and OPD-18 were used to locate molecular markers specific for the resistance against false smut. To make the results more specific, RAPD profiling was repeated for comparing the individual resistant varieties with that of the susceptible varieties using two random primers *viz*., OPB-10 and OPK-14.

The procedure for isolation of DNA and PCR are elaborated below:

3.11.1. Isolation of Genomic DNA

Isolation of genomic DNA from the selected varieties of rice was carried out as per the method by Regowsky *et al*. (1991) with required modifications. The procedure is as follows:

- Approximately 0.1 g of tender leaf sample was weighed and taken in a clean autoclaved mortar and was crushed by freezing in liquid nitrogen.
- The powder was transferred to 2 ml Eppendorf tube and 1 ml of extraction buffer [1.00 g SDS (0.1%), 1.576 g Tris HCl (100 mM), 0.584 g Sodium chloride (100 mM), 0.37224 g EDTA (10 mM), volume made up to 100 ml with distilled water] was added.
- The tubes were then placed in a water bath with the temperature maintained at 60ºC for 30 min after homogenization
- The mixture was then centrifuged at 10,000 rpm at 4ºC for 10 min.
- The aqueous phase was collected and 400 µl of phenol:chloroform (25:24) was added and again centrifuged at 10,000 rpm at 4ºC for 10 min.
- The supernatant was collected to which 200 µl of chloroform iso-amylalcohol (24:1) was added and was centrifuged at 10,000 rpm at 4ºC for 10 min.
- The aqueous phase was collected and 200 µl of chloroform iso-amylalcohol (24:1) was added and was centrifuged at 10,000 rpm at 4ºC for 5 min.
- The supernatant was collected and 60 µl of 3 M sodium acetate and 600 µl of ice cold iso-propanol were added and was kept overnight at -20ºC for precipitation.

- The solution was centrifuged after about 16 h at 12,000 rpm for 10 min and the supernatant was discarded without dislodging the pellet.
- The precipitate was then washed twice using 70 % ethanol and dried.
- After drying, the precipitate was dissolved in 100 µl 0.1 x TE buffer [Tris buffer 0.12 g (10 mM), EDTA (0.037 g)] and stored at -20ºC.

3.11.2. Agarose gel electrophoresis

For carrying out the agarose gel electrophoresis, in a horizontal gel electrophoresis unit was used. Agarose (0.8%) was weighed and melted in 1x TE buffer. When the solution was cooled to 42-45ºC, ethidiun bromide was added at the rate of 12 µl per 100 ml and the solution was poured to a preset, sealed gel casting tray to a height of 3mm-5mm after fixing up of the comb in position.

The assembly was then kept undisturbed for 15-20 min for solidification that is, gelling of the solution. The adhesive tapes and the comb were then removed and the tray was then submerged in the electrophoresis tank filled with 1x TE buffer in such a way that the buffer covered the gel to a height of 1 mm.

The required volume of the sample (DNA) and the loading dye [Glycerol (30%)+ bromophenol blue] were mixed at a ratio of 5:1 and were loaded in to the slots of gel near the negative terminal with the help of a micropipette. The cathode and anode of the electrophoresis unit were connected to the power supply with constant voltage of 60 volts. The power supply was turned out when the loading dye moved about $3/4^{th}$ of the gel. The gel was then documented with the help of a gel documentation system (BIORAD).

3.12. Quantification of DNA

The quality and quantity of DNA samples was measured by using a spectrophotometer. 5 µl of DNA in 1x TE buffer was added to 3 ml of distilled water and absorbance at 260 nm and 280 nm was read against distilled water as blank, using UV spectrometer. The concentration of DNA was calculated using the formula,

$$\text{Amount of DNA (µg/ml)} = \frac{A260X50XDilution\ factor}{1000}$$

Where, A_{260} = absorbance at 260 nm

The quality of DNA was determined from the ratio of absorbance values at 260 and 280 nm. A ratio of 1.8 - 2.0 indicated best quality of DNA.

3.11.4. Random Amplified Polymorphic DNA

For amplification of DNA, the procedure by Williams *et al.* (1990) was followed. The amplification was done using ten reported arbitrarily designed primers, which included primers from different Operon primer series viz., OPK-19, OPG-18, OPC-15,OPB-10, OPH-19, OPD-3, OPB-5, OPK-14, OPF-13 and OPD-18 (Reshmi, 2006).

The reaction was performed in 25 µl reaction mixture containing 20 ng template DNA, 2.5 µl of 1X PCR buffer, 2 µl of 2.5 mM $MgCl_2$, 1µl of 10 mM DNTP mix, 1 unit of Taq DNA polymerase and 1µl of 10 pmoles primer. Amplifiication was done in a programmable thermocycler (BIORAD) that was programmed as follows:

An initial denaturation of 94ºC for five min followed by 40 cycles of denaturation of 94ºC for one min, annealing at 36ºC for one min and extension at 92ºC for 90 sec. The synthesis step of final cycle was extended further by seven min. amplified products were separated by

agarose gel electrophoresis using 1.4 per cent gel as detailed earlier which was photographed using gel documentation system.

3.12. ISOLATION AND TESTING OF BIOCONTROL AGENTS

3.12.1. *Isolation of antagonists*

Fungal and bacterial antagonists were isolated from the spermosphere and rhizosphere of the disease free rice plants among the false smut infected rice plants in the field.

3.12.1.a. Isolation of microflora from rhizosphere region of rice

Dilution plate technique was done for the isolation of microflora from the rhizosphere region of rice. For isolating the microflora, one gram rhizosphere soil along with roots was collected from the disease free plants in the false smut infected paddy fields. One gram of rhizosphere soil was transferred to 100 ml sterile distilled water in 250 ml conical flasks and shaken for 20 min in a rotary shaker. From this, dilutions of 10^4 to 10^6were prepared following the dilution plate technique (Poovendran *et al.*, 2011).

One ml of 10^4 solution was plated using pour plate method on Martin's Rose Bengal Agar medium (Appendix I) for isolating fungi in the rhizosphere. The plates were incubated at room temperature for 48-72 h. After the incubation period fungal colonies were examined and transferred to PDA plates and subsequently purified by hyphal tip culture method. The purified cultures were then stored under refrigerated conditions for identification and subsequent studies on antagonism.

For isolating bacteria from the rhizosphere areas, one ml of 10^6solution was plated using pour plate method on nutrient agar medium and the plates were incubated at room temperature for 24-48 h. After the incubation period, the bacterial colonies were examined and transferred to nutrient agar plates and subsequently purified by streak plate method and were transferred to nutrient agar slants. The purified cultures were then

stored under refrigerated conditions for identification and subsequent studies for antagonism.

3.12.1.b. Isolation of microflora from the spermosphere region of rice

For isolating various microflora associated with the spermosphere region of rice plant, dilution plate technique was followed. For this, one gram of rice grains collected from disease free panicles from a false smut affected field was brought to laboratory conditions and was transferred to 100 ml sterile distilled water in 250 ml conical flasks and shaken for 20 min in a rotary shaker. From this, dilutions of 10^4 to 10^6were prepared following the dilution plate technique. Plating and pure culturing of the fungi and bacteria were done as per the method described in 3.12.1.a.The purified cultures were then stored under refrigerated conditions for identification and subsequent studies on antagonism.

3.12.2. *In vitro screening of the antagonists*

The extent of growth of the fungal and bacterial isolates obtained by the dilution plate technique was measured and the fast growing isolates were selected for *in vitro* screening for their antagonistic effect against the false smut pathogen, *U. virens*.

3.12.2.1. In vitro screening of the fungal antagonists

3.12.2.1.a. In vitro studies for evaluating the antagonistic efficacy of the fungal isolates against *U. virens* in dual culture

The fungal isolates obtained by serial dilution techniques were initially screened based on their growth on PDA and 10 *Trichoderma* isolates selected likewise were evaluated for their antagonistic potential against *U. virens* by dual culture technique. The pathogen *U. virens* being a slow grower was grown alone for 10 days on dual culture plates before inoculation of *Trichoderma*.

Mycelial discs of 4 mm diameter taken from the actively growing margins of ten days old culture of the pathogen *U. virens* were inoculated on YPPDA plates at a distance of 1 cm from the periphery of nine cm plates @ one disc per plate and the plates were incubated at 28 ± 1^0C.

On the eleventh day after incubation four mm mycelial discs cut from the actively growing margins of two days old cultures of the antagonists were placed opposite to the colony of the pathogen at a distance of one cm from the periphery of the 9 cm dia. culture plate containing the *U. virens* colony, maintaining seven cm distance between the discs of the pathogen and the antagonist. Control plates were also maintained with only *Trichoderma* or *U. virens*. The plates were kept for incubation at 28± 1^0C. Observations were recorded on radial growth, overgrowth, lysis and inhibition zone.

Per cent inhibition of the pathogen over control was calculated by adopting the formula (Mishra, 2010),

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

I --- Per cent growth inhibition

C --- Growth in control

T --- Growth in treatment

The most efficient *Trichoderma* isolate from dual culture experiment was selected and used for subsequent studies on depiction of the mechanisms of biological control.

3.12.2.1.b. Study of mechanisms of antagonism of the effective Trichoderma isolate against U. virens

3.12.2.1.b (i). Mycoparasitism

Two methods were used. Cellophane disc method and slide culture method.

a. Cellophane disc method: **(Dennis and Webster, 1971)**

YPPDA after melting was poured in nine cm sterile Petri plates and was allowed to solidify. Sterilized cellophane discs of cut to the size of the Petri plate bottom were placed over the solidified surface, with the help of sterile forceps. Agar discs of 4 mm diameter each of the pathogen and the antagonist *Trichoderma* were placed 3 cm apart at the centre of the cellophane surface. The plates were incubated at room temperature for 48 h. Microscopic observations for hyphal interactions were made by cutting out $1cm^2$ portions of cellophane containing intermingling hyphal growth of the pathogen and the antagonist and mounting in cotton blue lactophenol.

b. Slide culture method: Discs of the pathogen and the antagonist were cut from the actively growing culture and placed two cm apart on a clean microscopic slide and kept in moist chamber and incubated (Rashmi, 2010). When the growth from the two discs met, the slides were observed under microscope.

3.12.2.1.b.(ii). Production of non-volatile compounds by *Trichoderma* (Mohamed *et al*., 2010)

The *Trichoderma* isolate found effective under dual culture was grown on YPPD Broth for seven days at 28± 1^0C. The fungal mat was discarded and the culture filtrate was collected by filtering. The culture filtrate was made cell free by passing through G-3 filter.

The filtrate was diluted to different concentrations (10%, 20 %, 30%, 40%, 50%, 60% and 70%) using YPPD agar medium and was poured in petri plates. The disc cut from actively growing margin of the pathogen culture was inoculated in the centre of each plate. Control plates were maintained without addition of culture filtrate. Four replications for each concentration were maintained. Plates were incubated at 28± 1^0C and daily observations were taken on radial growth of the pathogen.

3.12.2.1.b.(iii). Effect of volatile compounds of *Trichoderma* on *U. virens in vitro*

Effect of *Trichoderma* volatiles on *U. virens* was assessed by following paired plate technique (Dennis and Webster, 1971). Four mm discs of *Trichoderma* and *U. virens* cut from actively growing cultures were separately inoculated on PDA at the centre of 9 cm diameter Petri dishes. The bottom plates with the cultures of *Trichoderma* and *U. virens* were joined and sealed together with gum tape and incubated at 28± 1^0C. Observations were recorded on radial growth of *U. virens.* Check paired plates were maintained with one of the paired plates inoculated with *U. virens* and the other remaining uninoculated.

3.12.2.2. In vitro* studies for evaluating the antagonistic efficacy of the bacterial isolates against *U. virens

Isolates of the bacterial antagonists obtained from spermosphere and rhizosphere of rice plants were tested for antagonistic property against the false smut pathogen, by Kirby-Bauer test (*Bauer* et al., 1966) with slight modifications. Culture vials of 15 ml volume containing nutrient broth was inoculated with a loop-full of inoculum taken from fresh cultures of bacteria and was incubated for 24 h. Sterile plates poured with YPPDA medium and was allowed to solidify. A sterile swab was dipped into the incubated one day old broth culture of the bacteria and excess moisture was removed by pressing the swab against the side of the tube. The surface of the agar was swabbed completely with the inoculum. The surface was allowed to dry for about five min. Mycelial discs of 4 mm size of the pathogen cut from the actively growing margins of 10 days old culture of the pathogen was inoculated opposite to each other over the swabbed surface @ four per plate maintaining a distance of five cm between the opposite discs. Control plates of *U. virens* were also maintained without swabbing of bacteria. The plates were then incubated

for 10 days at 28± 1^0C and measurements were taken on the dia. of the colonies of *U. virens* and were compared with that in the control plates.

Per cent inhibition of the pathogen in presence of the antagonistic bacteria over control was calculated by adopting the formula;

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

I --- Per cent growth inhibition

C --- Growth in control

T --- Growth in treatment

Based on the inhibition, the best bacterial antagonist was selected and was maintained in nutrient agar slants at 24 ±1 °C.

3.13. *IN VITRO* EVALUATION OF CHEMICAL FUNGICIDES AGAINST *U. virens*

The *in vitro* chemical management of *U. virens* was done by making use of two methods 1) Poisoned food technique and 2) Spore germination inhibition studies. Eleven commercially available chemicals were used for the same.

3.13.1. In vitro evaluation of fungicides by poisoned food technique

Eleven selected commercial fungicides (Appendix III) were evaluated *in vitro* against *U. virens*, by poisoned food technique (Nene and Thapliyal, 1993). Ten ml stock solution of 1,00,000 ppm concentration was prepared in sterilized distilled water. To obtain the desired concentration of fungicide (Table 2) in the medium, the required amount of stock solution was added in 50 ml sterilized distilled water (to get the double strength) and then mixed with 50 ml molten double strength YPPDA to get desired concentration. There after 20 ml of the poisoned medium was poured in to sterilized petriplate (9.0 cm diameter) under aseptic conditions in Laminar Air flow inoculation chamber and allowed to solidify.

Table 2. Different concentrations of fungicides tested against *U. virens* (*in vitro*)

Sl. No.	Fungicides	Concentrations (%)
1	Chlorothalonil 75 % WP	0.02
		0.0375
		0.0185
2	Thiophanate Methyl 70 % WP	0.125
		0.025
		0.0125
3	Copper Hydroxide 77 % WP	0.2
		0.0375
		0.01875
4	Mancozeb 75 % WP	0.2
		0.0375
		0.01875
5	Propiconazole 25 % EC	0.1
		0.0125,
		0.0625
6	Tebuconazole 25.9 % m/m EC	0.15
		0.01875
		0.0093

7	Iprodione + Carbendazim 50 % WP	0.1
		0.0125
		0.0625
8	Carboxin 37.5% +Thiram 37.5% 70%WS	0.2
		0.025
		0.0125
9	Pencycuron 22.9 % EC	0.1
		0.025
		0.0125
10	Azoxystrobin 23% SC	0.1
		0.0125
		0.0625
11	Bitertanol 25 %WP	0.125
		0.025
		0.0125

Each plate was inoculated in the centre with 3mm diameter disc cut from the periphery of actively growing one week old test *U. virens* culture

individually under aseptic conditions and incubated at 28±1^{0}C in a BOD incubator.

Unamended YPPDA plates inoculated with *U. virens* served as checks. Radial growth of the test isolates was recorded after 24 h and 48 h of incubation. Per cent inhibition of growth over control was calculated using the formula (Vincent, 1927)

Where, $$I = \frac{C - T}{C} X\ 100$$

I = per cent inhibition.

C = growth of *U. virens* in unamended medium.

T = growth of *U. virens* in amended medium.

3.13.2. Spore germination inhibition studies

The spore germination inhibition studies were conducted by following the method by Peterson (1941). The spore suspension of *U. virens* was prepared by washing the affected smut balls collected from the field with sterilized water. Required concentration of test chemical was prepared by using sterile distilled water and 0.1 ml of the solution was placed at the centre of a clean and sterilized glass slide and allowed it to dry at room temperature (30-35^{0}C). 0.1 ml of spore suspension prepared in YPPDB was placed on the same spot where fungicidal suspension was placed.

Later slides were placed in moist chambers prepared using wet blotting papers placed in petriplate. Entire experimental procedure was followed under aseptic conditions in a laminar air flow chamber. The moist chambers with slides in it were incubated at 28±1^{0}C in a BOD

incubator. Observations on number of spores germinated were recorded 12 h after incubation under high power (40 X objective) of the microscope.

Four replications were maintained for each chemical. From each replication five microscopic fields were observed (Yashoda, 1998) for averaging number of spores germinated per microscopic field.

Per cent spore germination inhibition was calculated by using the following formula (Verma and Singh, 1987),

Where,

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

I = per cent inhibition

C = number of spores germinated in control.

T = number of spores germinated in treatment.

3.14. FIELD EVALUATION OF BIO AGENTS AND FUNGICIDES

Five treatments selected from *in vitro* chemical management experiment, along with talc based formulations of one bacterial and one fungal biocontrol agent (total of seven treatments) were tested in the field (Table 3) during Kharif 2012 at Palakkad and Rabi 2012-13 at Alappuzha.

The susceptible rice variety Uma was used for the field level evaluation of the fungicides and biocontrol agents. Transplanting was done at 25 days after sowing and a spacing of 15X15 cm was maintained at the main field.The design followed was RBD and three blocks were maintained. The treatments were given as foliar sprays at 50 % flowering stage. All the agronomic practices were carried out, in time, as per the Package of Practices Recommendations by the Kerala Agricultural University (POP, 2011). Periodical observations were made for the development of symptoms on grains at the flowering period and thereafter. The per cent infected tillers, per cent infected grains per tiller and disease.

Table 3. Bio agents and fungicides tested against false smut disease of rice in the field

Treatment number	Treatments	Recommended Dosage	Quantity applied (%)
T1	Copper Hydroxide 77 % WP	(1000 g a.i./750 L)	0.2
T2	Chlorothalonil 75 % WP	(1000 g a.i./750 L)	0.2
T3	Iprodione+Carbendazim 50 % WP	(250 g a.i./750 L)	0.1
T4	Propiconazole 25 % EC	(125 g a.i./750 L)	0.1
T5	Tebuconazole 25.9 % m/m EC	(0.1875 kg a.i./500 L)	0.15
T6	Talc based formulation of *Trichoderma harzianum*	20 g/ L	2
T7	Talc based formulation of *Pseudomonas fluorescens*	20 g/ L	2
T8	Control (without any treatment)		--

severity were recorded. Biometric observations such as height of the plant, number of total tillers, grain yield and straw yield were also recorded.

3.15. BIOCHEMICAL STUDIES

3.15.1. SDS- PAGE

Electrophoretic separation of soluble protein from the infected and healthy grains of the susceptible rice variety Uma was carried out as per the procedure described by Laemelli (1970).

1 g each of healthy and infected grain samples were homogenized in 1 ml of cold denaturing solution/ phosphate buffer at 4°C. The extract was centrifuged at 5000 rpm for 15 min. The supernatant was mixed with chilled acetone in the ratio 1:1 and the protein was allowed to precipitate by keeping the mixture at 4°C for 30 min. The sample was centrifuged at 5000 rpm for 15 min at 4°C. Acetone was removed and the pellet was resuspended in 50 µl of denaturing solution and vortexed. The homogenate was centrifuged at 5000 rpm for 15 min. The supernatant was mixed with 10 µl of sample buffer and kept in a boiling water bath for 3 min. These samples were used for running PAGE. The protein concentration was adjusted in each sample to strength of 100 µg of protein following Bradford method (Bradford, 1976). Standard was prepared using known molecular weight marker, 10 µl added with 10 µl sample buffer.

3.15.1.1. Reagents

a) Acrylamide stock (30 %)

Acrylamide – 29.2 g

Bis-acrylamide – 0.8 g

Double distilled water – 100.0 ml

b) Separating (resolving) gel buffer stock (1.5 M Tris-HCl, pH 8.8)

Tris base (18.15 g) was dissolved in approximately 50 ml of double

distilled water. The pH was adjusted to 8.8 with 6 N HCl and made up the volume to 100 ml with double distilled water and stored at 4°C.

c) Stacking gel buffer stock (0.5 M Tris-HCl pH 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH to 6.8 with 6 N HCl and the volume was made upto 100 ml with double distilled water and stored at 4°C.

d) Polymerising agents

Ammonium persulphate (APS) 10 per cent prepared freshly before use.

TEMED – Fresh from refrigeration.

e) Electrode buffer pH 8.3

Tris base - 6.0 g

Glycine - 28.8 g

SDS - 2.0 g

Double distilled water - 2 litre

f) Sample buffer

Double distilled water - 2.6 ml

0.5 M TrisHCl pH 6.8 - 1.0 ml

2-mecaptoethanol - 0.8 ml

Glycerol -1.6 ml

SDS 20 % (w/v) - 1.6 ml

0.5 % Bromophenol blue - 0.4 ml

g) Staining solution

Comassie brilliant blue R 250 - 0.1 g

Methanol - 40.0 ml

Glacial acetic acid - 10.0 ml

Double distilled water - 50.0 ml

h) Destaining solution

As above without Coomassie brilliant blue R 250

3.15.1.2. Procedure

Separating gel was first casted followed by stacking gel by mixing the various solutions as indicated below.

a) Preparation of separating gel (12%)

Double distilled water - 6.7 ml

TrisHCl, pH 8.8 - 5.0 ml

SDS 10 % - 0.2 ml

Acrylamide stock - 8.0 ml

The above solution was mixed well and degassed for three min and then the following were added immediately.

10% Ammonium persulphate (APS)
Freshly prepared - 0.10 ml

Tetra methyl ethylene diamine (TEMED) - 0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerizing solution to quicken the polymerization process.

b) Preparation of stacking gel

Double distilled water - 6.1 ml

TrisHCl, pH 6.8 - 2.5 ml

SDS 10 % - 0.2 ml

Acrylamide stock - 1.3 ml

The solution was mixed well, degassed and the following were added.

APS 10 % - 0.05 ml

TEMED - 0.1 ml

The water layered over the separating gel was removed and washed with a little electrode buffer and then the stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

After polymerization, the samples were loaded into the wells. Standard with known molecular weight is also loaded into one of the wells. The electrophoresis was performed at 100 V till the dye reached the separating gel. Then the voltage was increased to 200 V and continued till the dye reached the bottom of the gel. Immediately after electrophoresis, the gel was removed from the glass plates and incubated in the staining solution for overnight with uniform shaking which afterwards was transferred to the destaining solution. The protein appeared as bands in the gel was photographed after placing on a trans-illuminator (Appligene Model White / UV TMW-20).

3.15.2. Electrophoretic analysis of isozyme

Electrophoresis of protein extracts from plant tissues using different kinds of support media and buffer systems allows separation of the multiple forms of enzymes (isozymes) on the basis of charge and molecular size.

The present work was undertaken to study the enzyme alterations in healthy as well as pathogen infected paddy grains. Discontinuous anionic polyacrylamide gel electrophoresis was conducted under non-dissociating conditions as described by Wagih and Coutte (1982) with slight modification.

3.15.2.1. Polyphenol oxidase isozyme analysis

Soluble and ionically bound enzymes were extracted by grinding the sample under chilled condition in 0.01M potassium phosphate buffer (pH 7.0) containing one per cent Tween 80 in the ratio of 1:1 w/v. The homogenate was centrifuged at 15,000 rpm for 10 min at 4°C. The resulting supernatant was used for isozyme analysis. The protein content was adjusted in each sample to the strength of 100 µg of protein following Bradford method. The PPO isozyme profiles were examined after running the samples on non- denaturing 7.5 per cent polyacrylamide slab gels. The gel was prepared using the stock solution prepared for protein gel electrophoresis without SDS (native gel). Two per cent Triton X-100 was added in the place of SDS. After native PAGE, the gel was equilibriated in a solution of 10 mM L-3, 4 dihydroxyortho phenyl alanine (L-DOPA) in 0.1 M potassium phosphate buffer (pH 7.0) in a plastic tray and kept in a shaker for 30 min. Zones of enzyme activity (Polyphenol oxidase) were observed as grey black bands. The Rm values of the isozyme bands of PPO were also recorded.

3.15.2.2. Peroxidase isozyme analysis

Soluble and ionically bound enzymes were extracted by grinding the sample under chilled condition in 50 mM Tris-Cl (pH 7.6) in the ratio of 1:1 w/v. The homogenate was centrifuged at 15,000 rpm for 10 min at 4°C. The resulting supernatant was used for isozyme analysis. The protein content was adjusted in each sample to the strength of 100 µg of protein following Bradford method. Proteins extracted by 50 mM Tris (pH 7.6) were separated by gel electrophoresis in 7.5 per cent gel. The gel was prepared using the stock solution prepared for protein gel electrophoresis without SDS (native gel). Two percent Triton X-100 was added in the place of SDS.The gel was incubated in 0.6 M sodium acetate buffer (pH 5.4) containing 0.5 percent O– dianisidine HCl for 30 min at room temperature. The gel was transferred to 0.1 M hydrogen peroxide until

visible bands were developed the relative mobilities were calculated.

Reagents

a) Separating gel (7.5 %)

Tris chloride buffer stock solution (pH 8.9)	- 5 ml
Resolving gel acrylamide solution	- 10 ml
Distilled water	- 25 ml
APS	- 300 µl

b) Stacking gel (4%)

Tris chloride buffer stock, pH 6.7	- 2.5 ml
Resolving gel acrylamide solution	- 3.1 ml
Distilled water	- 14.1 ml
APS	- 300 µl

3.16. STATISTICAL ANALYSIS

The data obtained from the experiments were statistically analyzed after appropriate transformation wherever needed using the standard models as described by Gomez and Gomez (1984).

Results

4. RESULTS

The present study on the 'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala' was conducted during the period 2010-2013 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala and at the experimental fields at Regional Agricultural Research Station, Pattambi, Palakkad, Rice Research Station, Moncompu, Alappuzha as well as at the farmers fields at Vilayur (Palakkad) and Champakkulam (Alappuzha). The results obtained from the series of laboratory and field experiments are summarized below:

4.1. SURVEY ON THE INCIDENCE AND SEVERITY OF THE FALSE SMUT DISEASE OF RICE

Field surveys were conducted during 2011-2013, two at Palakkad district during the *Kharif* and two at Alappuzha (Upper Kuttanad, Lower Kuttanad and Kayal areas) district during *Rabi* season for assessing the per cent infected tillers and the disease severity of false smut disease of rice (Plate 3). The results of the surveys are presented in table 4. The results revealed that the average of per cent of infected tillers ranged from 3.3 to 31.1 % during 2011-12 and from 4.3 to 42% during 2012-13. The disease severity index during these periods ranged from 3.3 to 138.8 and 3.9 to 123.9 respectively. Out of the four areas surveyed, Upper Kuttanad recorded maximum percentage of infected tillers (31.1 %) and disease severity (138.8) during 2011-2012. The same trend was observed during 2012-13 with Upper Kuttanad recording the highest per cent infected tillers (42) and disease severity (123.9). During both the years, the lowest disease incidence and severity was observed at Kayal areas with 3.3 percent per cent infected tillers with a disease severity of 3.33 during 2011-12, and 4.3 per cent infected tillers with a disease severity of 3.9 during 2012-13. The disease was found to be more severe at Alappuzha district compared to Palakkad. In Palakkad, the disease was found more prevalent during the Kharif season, whereas it was found more serious during the Rabi season at Alappuzha.

During the surveys it was observed that the disease was found to be more prevalent in the most popular variety of Kerala, Uma, but was present in the other

Kavalam

Kottakkakom

Pulinkunnu

Koppam

Ambalappuzha

Vilayur

Plate 3. Photographs from the surveys on false smut disease of rice

Table 4. Details of surveys during 2011-12 and 2012-13 on the per cent infected tillers and severity of false smut disease of rice

Area Surveyed	2011-12		2012-13	
	% infected Tillers	Disease Severity	% infected Tillers	Disease Severity
Palakkad	6.11	14.5	6.51	12.62
Upper Kuttanad	31.1	138.8	42	123.9
Lower Kuttanad	13.3	26.96	15.3	40.8
Kayal Areas	3.30	3.33	4.3	3.9

commonly cultivated rice varieties like Jyothi, Kanchana and Ponmani (Appendix IV). In those fields having continuous cultivation of the variety Uma, there was recurrent appearance of the disease. In the fields with previous record of false smut over the years, the disease was found more severe.

4.2. STUDY ON THE SYMPTOMATOLOGY OF THE FALSE SMUT DISEASE OF RICE

The pathogen causing false smut disease of rice, *Ustilaginoidea virens* was found to infect the rice plants at the flowering stage of the crop. Due to the infection by the pathogen, the individual spikelets of the panicle were found to get transformed in to yellow to orange coloured ball like structures called smut balls or pseudosclerotia which were almost double the size of the normal rice grains (Plate 4) and are found enlarging forcing open the lemma and palea. The number of smut balls formed ranged from one to fifty per panicle based on the severity of the disease. Usually a few grains in the panicle were found to get affected.

The smut balls, at the initial phases of infection by the pathogen, were found to be covered with a whitish to cream coloured membrane (Plate 5 a). During the later stages, the covering membrane was found to get ruptured exposing the yellow dust like spores (Plate 5 b and 5 c) which are technically the chlamydospores of the pathogen. Upon making a cross section of a smut ball, three zones of development of the pathogen could be observed, an inner core which was white colour containing thick mycelial growth of the pathogen, a surrounding light yellow coloured region having the precursors of chlamydospores which were not perfectly double walled when immature, and an outer yellow to orange coloured region containing the mature chlamydospores. The inner core of the smut ball contained the remnants of the stamens and pistils of the rice flowers.

The chlamydospores were found attached to one another at their initial stages of formation (Plate 5 g) but later were found separated from each other up on maturity of the smut balls and thus became loose (Plate 5 h). In the later stages of the disease, the colour of the smut balls changed to dark and black (Plate 5 d and 5 e) and at that stage the chlamydospores were fully mature, prominently double walled and had highly ornamented outer wall layer (Plate 5 i). At

Plate 4. False smut affected panicle of rice

Plate 5 A. Stages of development of false smut disease of rice in the field

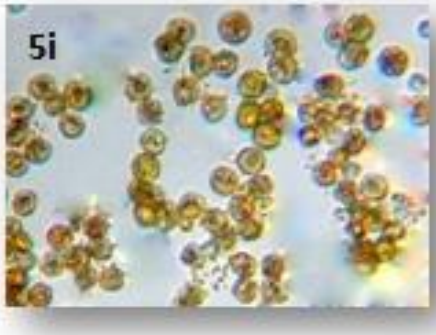


Plate 5 B. Stages of development of false smut disease of rice in the field

that stage the adjacent spikelets of the affected grains (Plate 5f, 5k) and the adjacent leaves were found smeared with the black powdery spore mass (Plate 5j).

The pathogen was also found to cause chaffiness of the panicle and in severe cases completely chaffy panicles with numerous smut balls were observed. Severely affected panicles appeared blackened by the time of harvest due to the presence of the black chlamydospores of the pathogen over them.

4.3. ISOLATION OF THE PATHOGEN

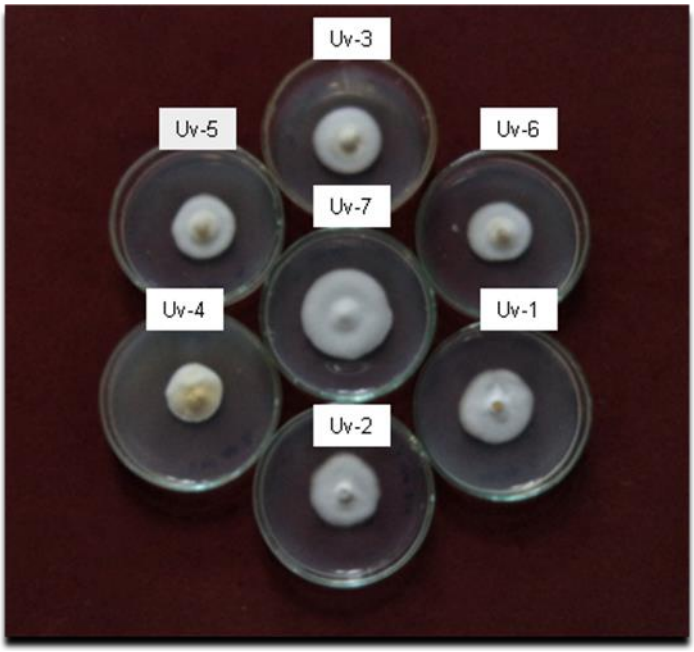
The pathogen causing the false smut disease in rice, *U. virens* was isolated from the infected panicles showing typical symptoms. The samples were collected from different locations during the surveys, and the isolation was done following the procedures as explained in chapter three on yeast peptone potato dextrose agar (YPPDA), potato sucrose agar (PSA) or XBZ Agar medium.

The isolation from the smut balls as such or from the pieces of smut balls or from the chlamydospore suspensions were found less promising with more chances of contamination. Thus a new isolation method was standardized as given at 3.3.4. This method of isolation of the false smut pathogen yielded good results with more number of colonies of the pathogen developing on a single plate making the pure culturing easier. Moreover, observation under light microscope could ensure sub culturing of the typical colonies of the pathogen emerging from the germinating chlamydospores of the pathogen, thus avoiding the chances of error.

Seven isolates were obtained from different locations. The isolates were serially numbered from Uv1 to Uv 7 and were used for further studies. The isolates were subcultured periodically on YPPDA slants and were stored in a refrigerator at 4°C.

4.4 MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF THE ISOLATES OF THE PATHOGEN

The seven isolates obtained were cultured on YPPDA (Plate 6) for their characterization based on morphological and cultural characters. The pathogen was found to be a slow grower under laboratory conditions and thus in all the *in vitro* trials, the observations were taken upto 30 days after inoculation of the plates.



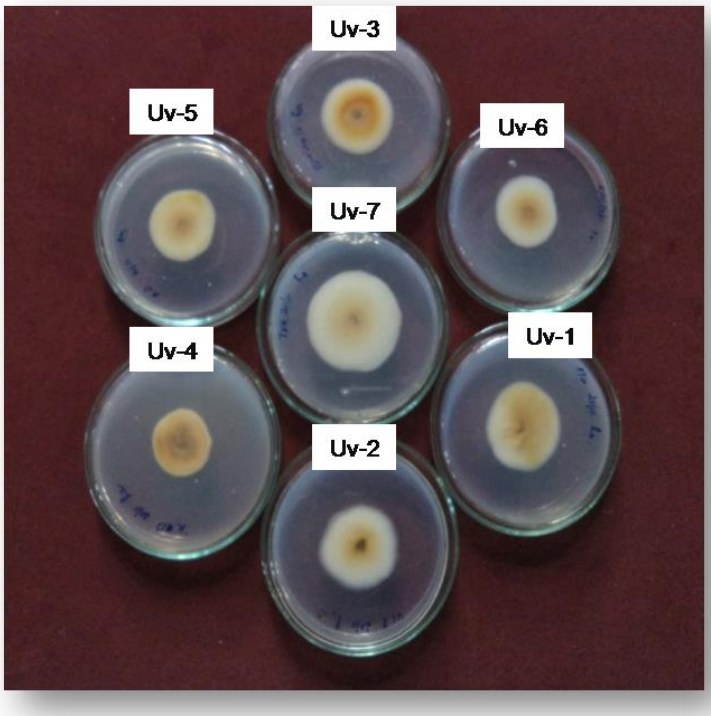


Plate 6. Comparison of growth of different isolates of *U. virens* on YPPDA

The isolates were characterized based on their colony morphology i.e., colony, colour, texture and appearance (Table 5). Up on comparison of the growth of the isolates on YPPDA (Table 6), it was found that the growth of the isolate Uv1 was found to be maximum on 10 DAI but after 20 and 30 DAI, the isolate Uv 7 was found to be showing maximum growth which was significantly different from that of all other isolates.Thus the isolate Uv 7 was selected for further studies on account of its fast growth.

Sporulation of different isolates of the pathogen (Table 7) and the pigment production was studied by growing the pathogen isolates in Potato Sucrose Broth. Production of secondary conidia was observed 4 DAI whereas the pigment production and production of smut balls and chlamydospores were observed on 30 DAI.

The results indicate that production of secondary conidia was found to be very high in case of the isolate Uv 4, whereas the isolate Uv 2 was found to produce maximum smut balls and chlamydospores. Sporulation and smut ball production was very low for the isolate Uv 7 but it gave very fast mycelial growth.

4.5. PROVING OF PATHOGENICITY

The isolate which showed maximum sporulation in broth culture was used for proving the pathogenicity of *U. virens* on the susceptible variety, Uma. Rice plants of variety Uma was grown under four different conditions *viz.*., 1) green house conditions with normal temperature and humidity 2) within an air conditioned room with controlled temperature conditions 3) open terrace conditions 4) green house conditions up to boot leaf stage and controlled temperature conditions within an incubator of 15ºC up to 2 days after inoculation, and thereafter the temperature within the incubator was increased to 25 ºC and the plants were kept within the incubator up to appearance of the symptoms.

In all the conditions, three different methods of inoculation were done. In one method, 2 ml of concentration 10^6spores/ml suspension of the pathogen was injected to the leaf sheath when the panicle is still within the boot leaf. In the second method, the spore suspensions were sprayed on the panicles after panicle emergence. In the third method, spore suspension was deposited within the individual flower by forcing open the lemma and palea.

Table 5. Colony characteristics of isolates of false smut pathogen on YPPDA

Isolates	Type of growth	Colony colour	
		Upper side	Lower side
Uv1	Smooth colony margin, good growth	Pure white	Yellowish center with creamy white margins
Uv2	Slightly wavy colony margin	Pure white	Reddish center with creamy margins
Uv3	Smooth colony margin, slightly raised	Creamy center with pure white periphery	Creamy center with white margins
Uv4	Smooth colony margin, raised colony	Yellowish in the center with creamy periphery	Yellowish throughout
Uv5	Smooth colony margin, slightly raised	Creamy center with pure white periphery	Light yellow in the center with creamy margins
Uv6	Smooth colony margin, slightly raised	Creamy center with pure white periphery	Dark yellow center with white margins
Uv7	Smooth colony margin, good growth	Pure white	Creamy center with white margins

Table 6. Comparison of growth of different isolates of *U. virens* on YPPDA

Treatments	Mycelial growth of *U. virens* (cm)		
	Day 10	**Day 20**	**Day 30**
Uv -1	**1.72^{a}**	3.03^{b}	4.67^{b}
Uv-2	1.42^{b}	2.6^{c}	3.67^{d}
Uv-3	1.32^{c}	2.42^{d}	2.98^{e}
Uv-4	1.17^{d}	1.63^{c}	1.92^{g}
Uv-5	1.37^{bc}	2.28^{f}	2.7^{f}
Uv-6	1.21^{d}	2.95^{b}	4.13^{c}
Uv-7	1.42^{b}	**3.22^{a}**	**4.92^{a}**
SE	0.03	0.04	0.05
CD(0.05)	0.08	0.11	0.13

*Mean of four replications

Table 7. Characteristics of isolates of *U. virens* on YPPD Broth

Isolates	Production of secondary conidia	Production of smut balls and chlamydospores	Pigmentation
Uv1	+	+	+ +
Uv2	+ +	+ + + +	+ +
Uv3	+	+	+
Uv4	+ + + +	+ + +	+ + +
Uv5	+ + +	+ + +	+ + +
Uv6	+ +	+	+ + + +
Uv7	+	+	+

+ + + + : Very High

+ + + : High

+ + :Moderate

+ : Low

The inoculation experiments showed positive results only in case of plants kept in an incubator at controlled temperature conditions with injection method of inoculation. It was observed that the disease appeared in the plants injected with the spore suspensions of the pathogen after panicle emergence at fifteen days after inoculation. A few spikelets of the inoculated panicle got converted in to a smut ball (Plate 16) of enlarged size compared to normal rice grains was observed which contained chlamydospores of the pathogen. The pathogen was reisolated from the smut ball and the culture obtained retained the characters of the original culture of the pathogen.

4.6. IDENTIFICATION OF THE PATHOGEN

The pathogen was identified based on its colony morphology, spore morphology and by DNA barcoding.

4.6.1. Colony Morphology of the pathogen *U. virens*

The pathogen produced pure white coloured with smooth colony margin (Plate 7 a). Up on continuous incubation the colour changed to light yellow (Plate 8 d and 8 e) and in some instances to green. The underside of the plates was initially yellowish in the centre with cream peripheral areas and later changed to reddish to black in the center with yellow periphery (Plate 7 b). The pathogen gave adpressed growth (Plate 8a) in the culture mostly but at times gave puffy growth (Plate 8 b and 8 c) especially under low temperature conditions. Thus the pathogen was found to show considerable variations (Plate 8) in the colony morphology.

In broth culture, the pathogen most of the produced white coloured colonies soon changing to dark green (Plate 8 f). At times yellow coloured mycelial growth was also observed. After one month of incubation the pathogen started production of yellow globular bodies which represent the pseudosclerotia of the fungus (Plate 9 a), which were filled inside with the spores of the fungus (Plate 9 b).

Later, from these globular, light to dark yellow coloured tubular, stalk like structures (Plate 10) produced in groups of five to seven in number from each pseudosclerotia, were found to be formed, the tip of which became bulged on aging

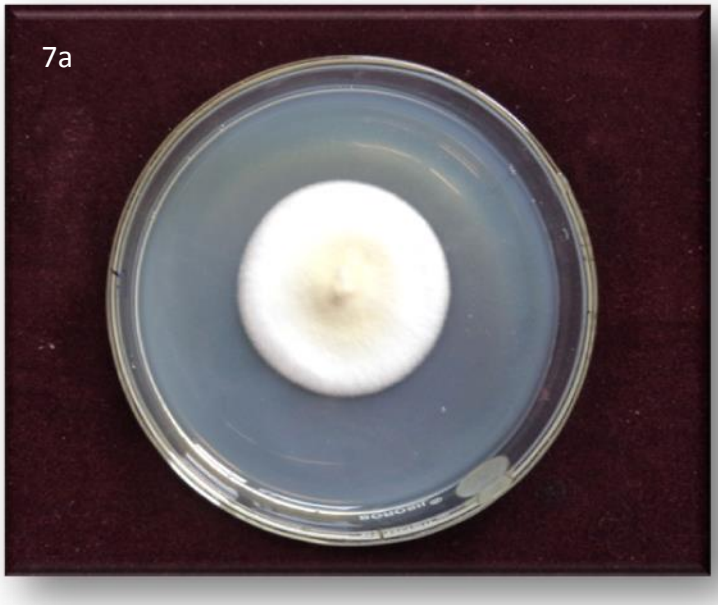


Plate 7. Typical colony of *U. virens* on YPPDA

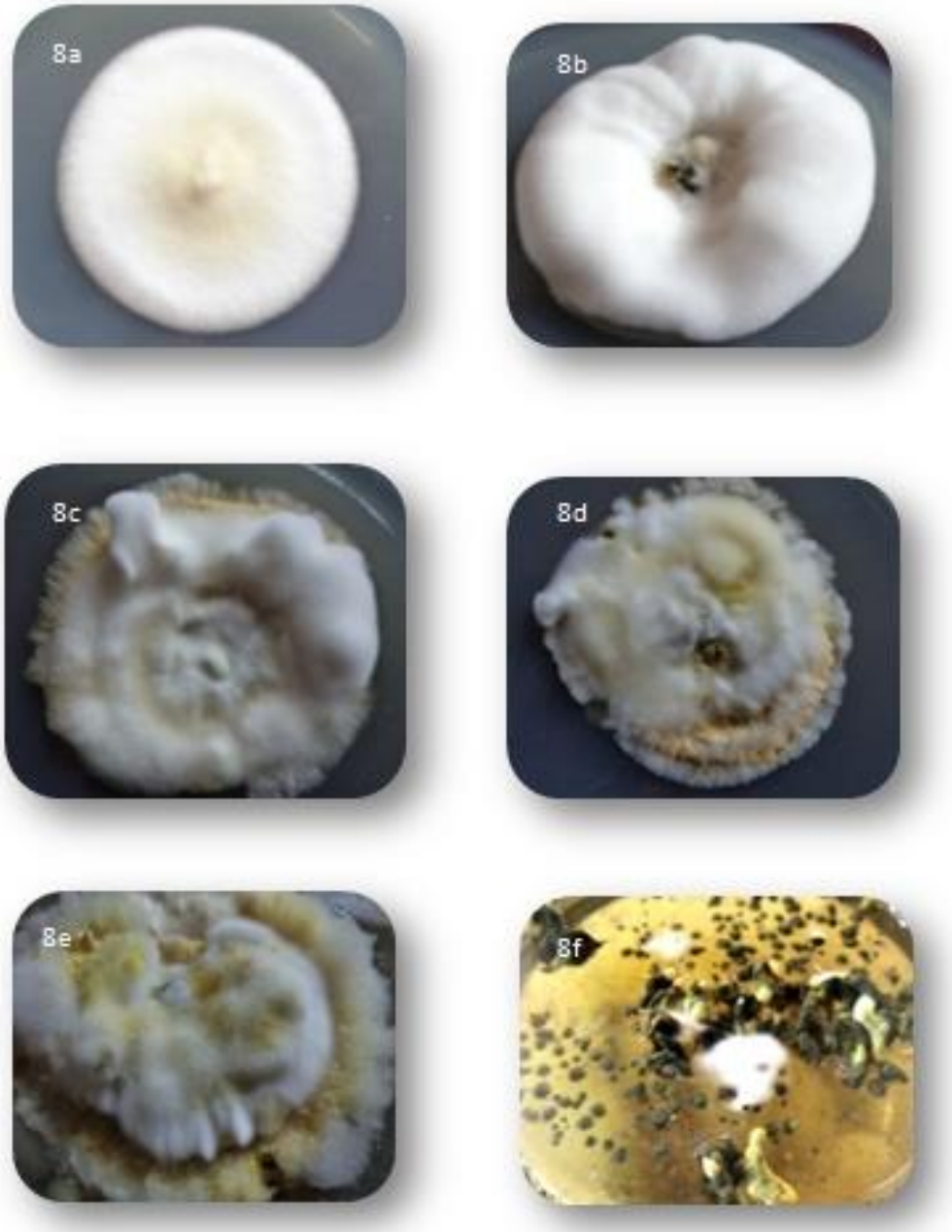


Plate 8. Variations in the colony morphology of *U. virens*

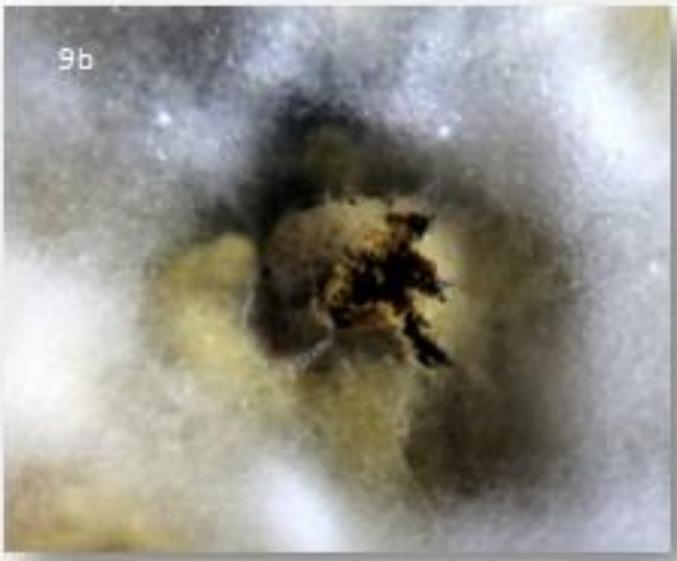


Plate 9. Pseudosclerotia formed in culture

Plate 10. Stromatic heads formed in culture

Plate 16. The symptom obtained under artificial inoculation with *U. virens*

which may represent the stromatic heads produced by the germinating sclerotial bodies of the pathogen in culture. This is the first report on observation of the perfect stage of *U. virens* in culture.

4.6.2. Spore Morphology of the pathogen *U. virens*

The pathogen, *U. virens* produced two types of asexual spores, the secondary conidia and the chlamydospores. Chlamydospores (Plate 11) are the spores produced within the smut balls and these represent the repeating spores of the pathogen which aid in the spread of the pathogen in the field. The pathogen produced similar chlamydospores in culture within the pseudosclerotia. The chlamydospores were yellow coloured round, 4.9 - 7.3µm in diameter, double walled, when mature had highly ornamented wall layers. The chlamydospores produced on YPPPDA medium were consistent with that obtained from the field.

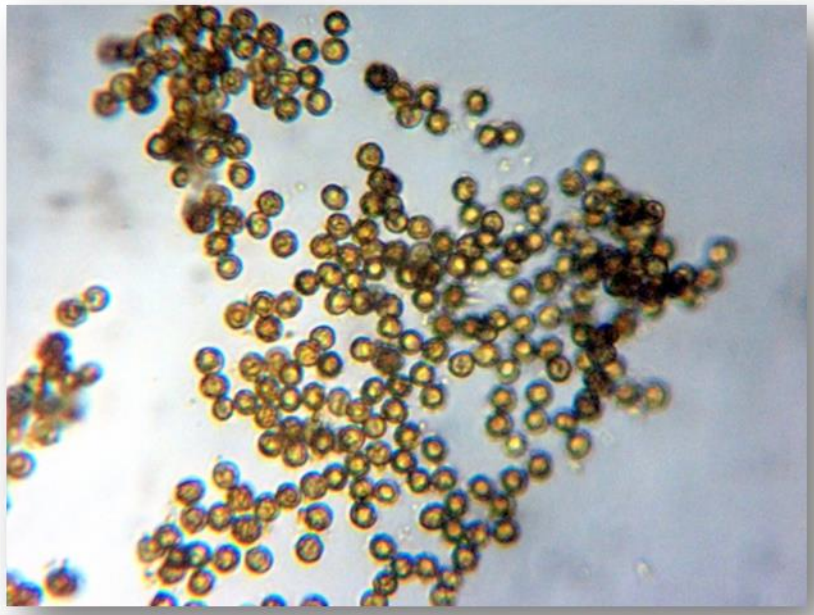
Secondary conidia were produced by the pathogen at three phases *viz*., at the time of germination of the chlamydospores, at the initial phases of colony establishment of the pathogen (Plate 12) and during stress conditions.

The chlamydospores germinated by production of a germ tube at the end of which one to five secondary conidia were produced (Plate 13 a, 13 b and 13 c). The chlamydospores could germinate by production of one or two (Plate 13 d and 13 e) or three germ tubes. The secondary conidia (Plate 14) were oval in shape, were produced on specialized hyphal structures and measured 2.8-4.8 µm in length and 1.5-3.1 µm in breadth.

The pathogen was identified tentatively as *U. virens* based on the colony and spore morphology. For confirmation of the identity of the pathogen, two isolates, one from Pattambi, Palakkad (Uv 1) and another from Moncompu, Alappuzha (Uv 6) were sent to the type culture collection centre at IARI, New Delhi, and were confirmed as *U. virens* with accession numbers ITCC-7335 and ITCC-7334 respectively.

4.6.3. DNA sequencing

The ITS regions of the pathogen were sequenced for the identification and molecular characterization and for studying the variability of the pathogen.



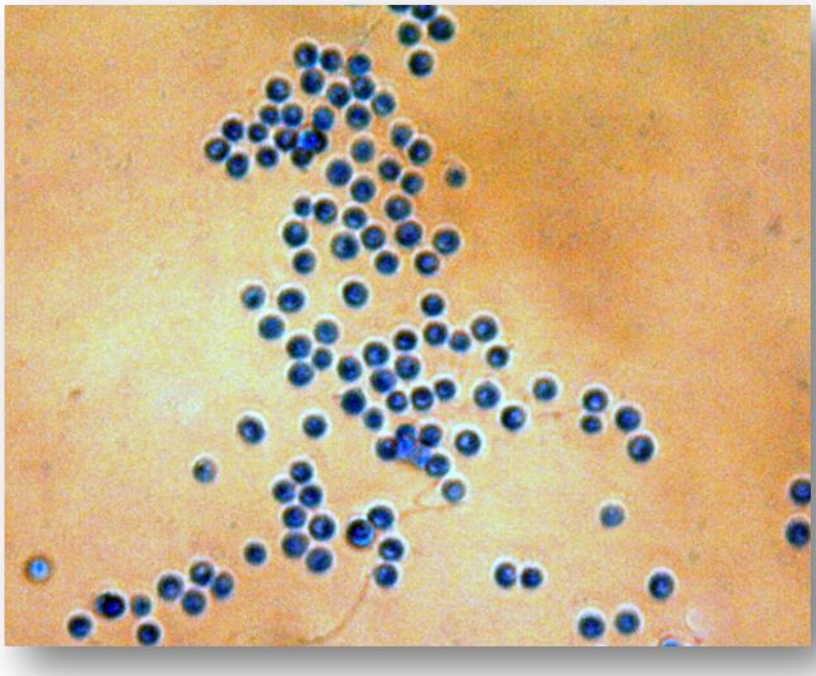


Plate 11. Chlamydospores of *U. virens*

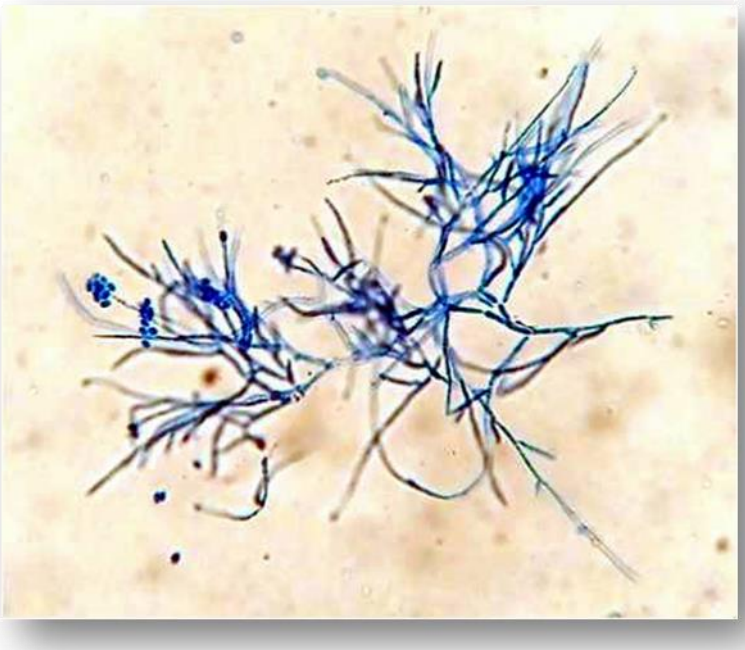


Plate 12. Secondary conidia produced by *U. virens* at the initial phases of colony establishment

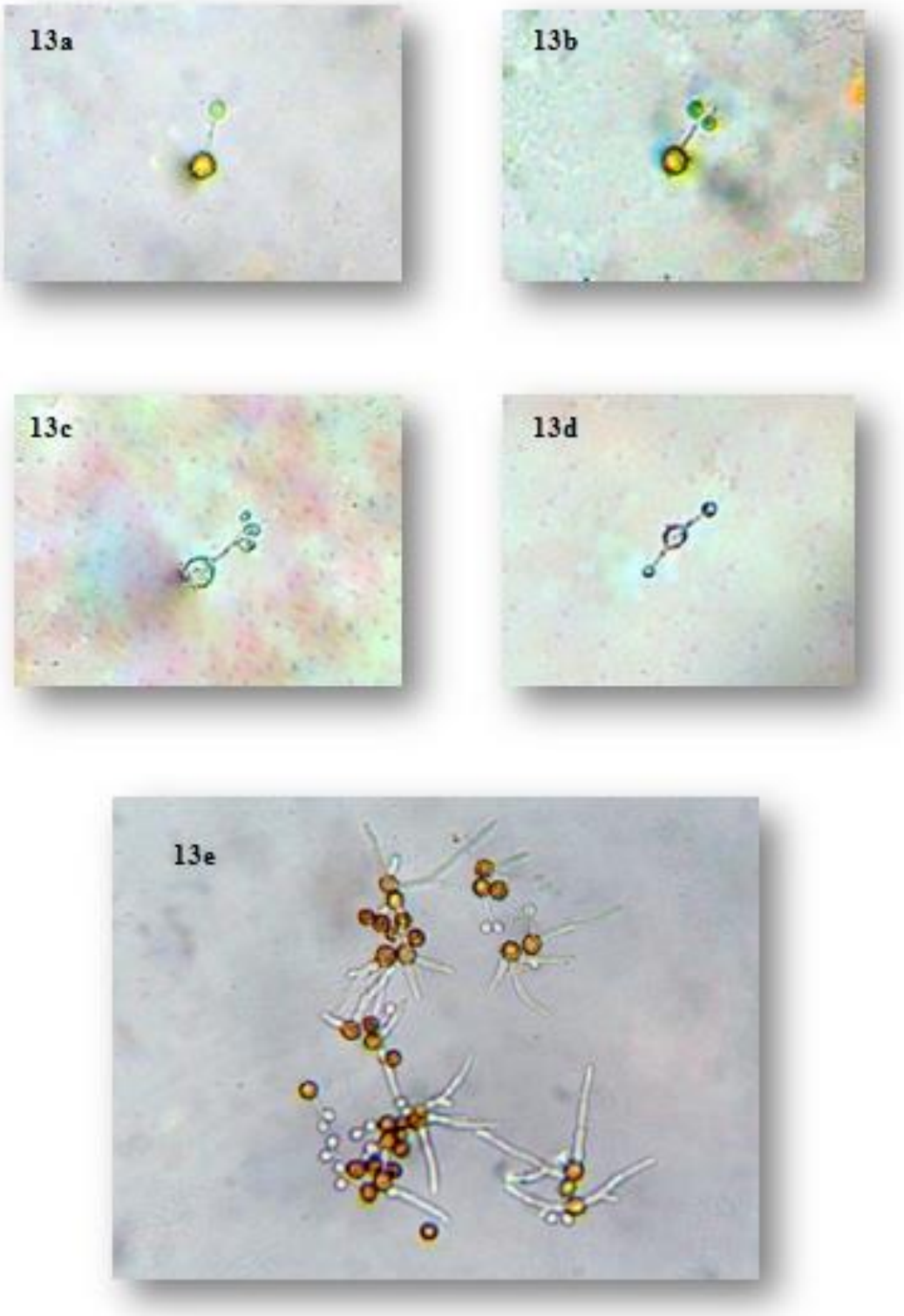


Plate 13. Germination of chlamydospores of *U. vivens*

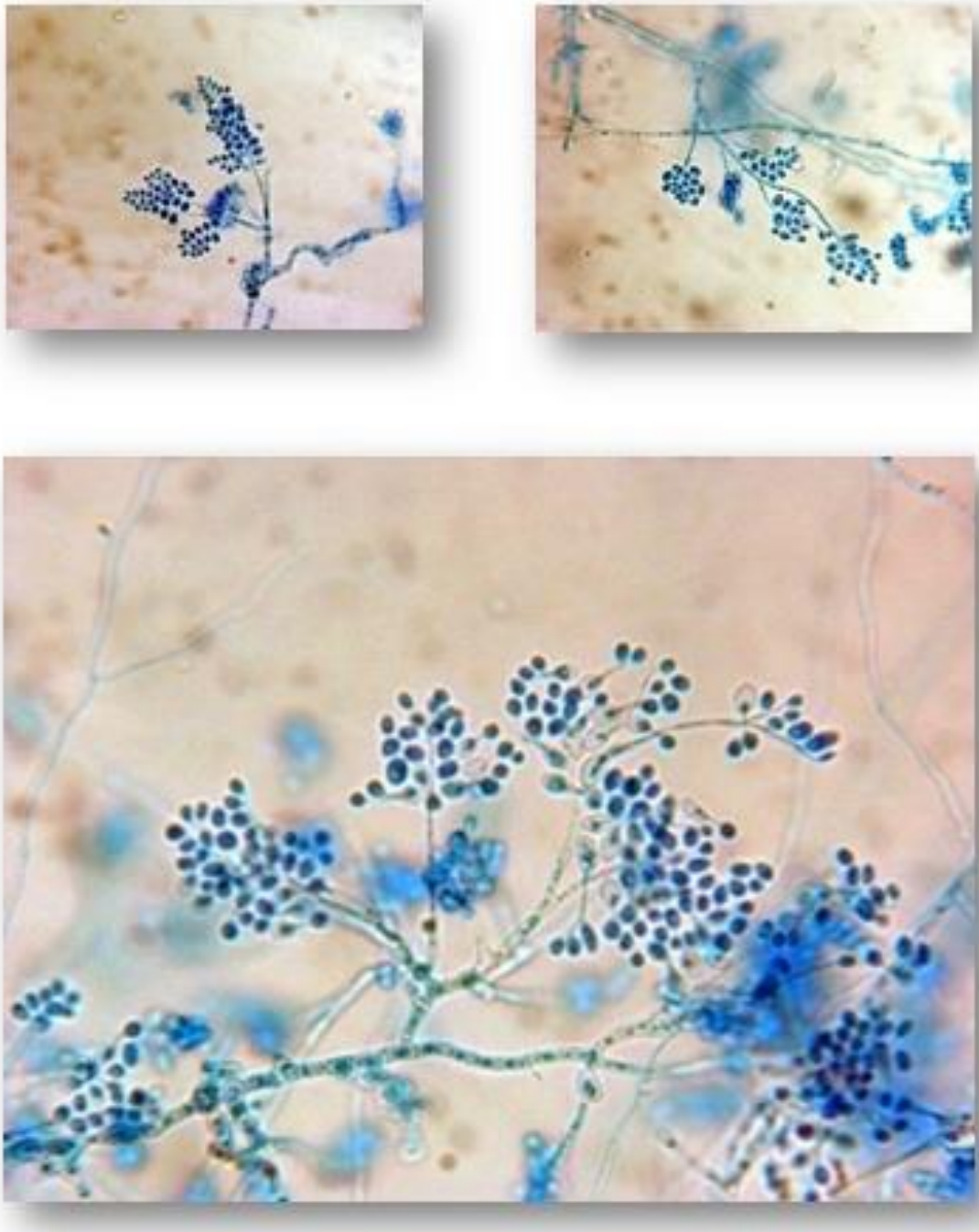


Plate 14, Secondary conidia produced by *U. virens*

Sequencing of eight isolates of the pathogen including an isolate (Uv 8) from a specimen from Rajendranagar, Hyderabad was done using universal primers of ITS (ITS-1F and ITS-4R). later, the PCR amplification was carried out followed by sequencing using the Big-Dye Terminator v3.1 Cycle sequencing Kit. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and editing of the obtained sequences were carried out using Geneious Pro v5.1. The sequences obtained for different isolates were as follows:

>UV1 (from Pattambi, Palakkad, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
AGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGTGT
TTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGATCG
GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV2 (from Vilayur, Palakkad, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
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GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV3 (from Koppam, Palakkad, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCC
GTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTG
GGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGT
GTTTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGG

GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGAT
CGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGC
CTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTA
AAACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV4 (from Kodumpu, Palakkad, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
AGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGTGT
TTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
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GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV5 (from Kadampazhippuram, Palakkad, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
AGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGTGT
TTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGATCG
GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV6 (From Moncompu, Alappuzha, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCC
GTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTG
GGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGT
GTTTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGG
GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGAT
CGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGC
CTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTA

AAACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV7 (from Karamana, Trivandrum, Kerala)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
AGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGTGT
TTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGATCG
GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

>UV8 (from Rajendranagar, Hyderabad, Andhra Pradesh)

AGGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGT
TGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCGCCTGGG
AGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACCAAAAAAACTCTTGTGT
TTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGATCG
GCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCGCAGCCT
CCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGGCCACTGCCCGTAAA
ACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAA

The PCR profile of the amplified regions is given in plate 15. Alignment of the sequence data of the isolates of *U. virens* collected from different geographical locations revealed that the ITS region of this pathogen is highly conserved, with all the samples exhibiting identical sequence and length (900 bp). It was found that ITS sequences of isolates from different parts of Kerala and the isolate from Hyderabad were one and the same indicating that there is no variation present among the isolates from Kerala and Andhra Pradesh.

The sequences were deposited in GenBank and the accession numbers obtained are given in table.8. BLAST analysis was carried out to find out the relationship of the isolates sequenced with the known sequences of *U. virens* from the GenBank (Appendix V).

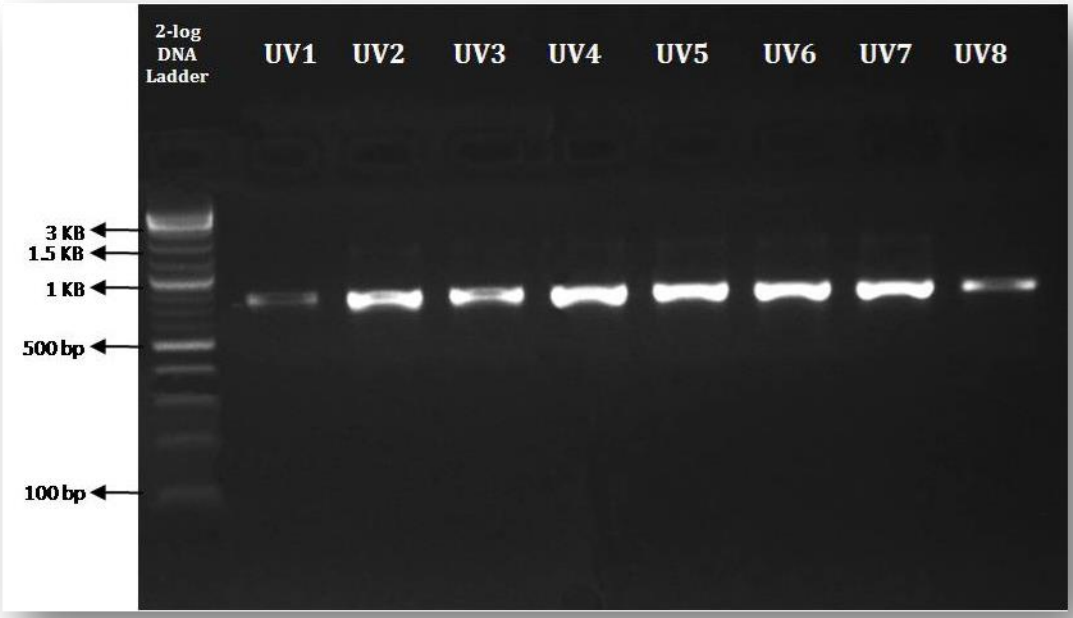


Plate 15. PCR amplification profile of the different *U. virens* isolates with the universal primers, ITS-1F and ITS-4R

Table 8. Accession numbers of the ITS sequences of the isolates of *U. virens*

Isolates	Place of Collection of specimen for isolation	Accession number obtained for the sequences
Uv1	Pattambi, Palakkad, Kerala	KF705026
Uv2	Vilayur, Palakkad, Kerala	KF705025
Uv3	Palakkad, Kerala	KF705024
Uv4	Palakkad, Kerala	KF705023
Uv5	Palakkad, Kerala	KF705022
Uv6	Moncompu, Alappuzha, Kerala	KF705021
Uv7	Karamana, Trivandrum, Kerala	KF705020
Uv8	Rajendranagar, Hyderabad, A. P.	KF705019

The results of BLAST analysis showing the distribution of 100 blast hits on the query sequence of *U. virens* (Fig. 2) showed that the isolates had 100% identity with the known isolates of *Villosiclava virens* or *U. virens* with colour key of red for all the 100 sequences indicating more than 200 alignment scores. The tree view (Fig 3) of the phylogenetic relationship with the isolates of *U. virens* with the known sequences from ten other *U. virens* isolates yielded a dendrogram with three branches with the sequences from the isolates Uv1 – Uv 8 at the same distance showing very close relation with two other known sequences of *U. virens*.

4.7. EVALATION OF THE SUITABLE NUTRIENT MEDIA FOR CULTURING OF THE PATHOGEN

The cultural characteristics of the pathogen in different culture media were studied with the isolate Uv 7 and the optimum medium for culturing of the pathogen was determined. Studies on sporulation were done under broth culture.

4.7.1. Growth of the false smut pathogen on different solid media

The growth of the pathogen, *U. virens* was compared on different solid media. Different solid media used were potato sucrose agar (PSA), potato dextose agar (PDA), czapek dox agar (CDA), XBZ Agar (XBZA), rice yeast dextrose agar (RYDA), yeast potato dextrose agar (YPPDA) and rice extract sucrose agar (RESA).The results (Table 9) showed that 30 days after inoculation, maximum average colony diameter was obtained in YPPDA medium (6.3 mm) which was significantly superior from all other media (Plate 17), followed by PSA (5.86) and RESA (5.67).The pathogen was found to give very scanty growth in case of RYDA and CDA.

4.7.2. Growth of the false smut pathogen on different liquid media

The liquid media used were potato sucrose broth (PSB), potato dextrose broth (PDB), czapek-dox broth (CDB), XBZ broth (XBZB), rice yeast dextrose broth (RYDB), yeast potato dextrose broth (YPPDB) and rice extract sucrose broth (RESB). The results of the study (Table 10, Plate 18) reveal that maximum dry weight of mycelial mat was produced on PSA which was on par with that obtained on YPPDA.

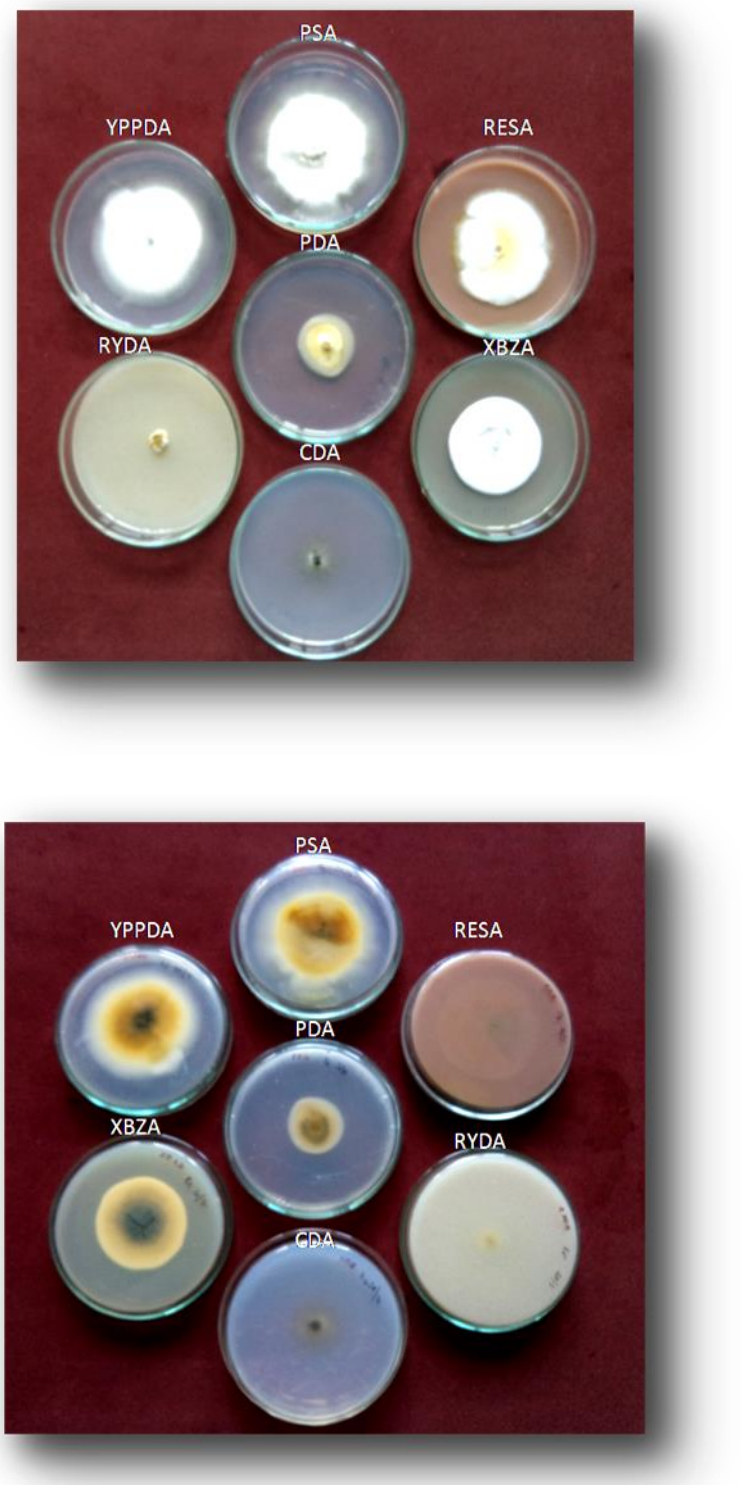


Plate 17. Comparison of growth of *U. virens* on different media

Plate 18. Comparison of growth of *U. virens* on different media broth

Table 9. Colony diameter of *U. virens* on different solid media

Treatments	Mycelial growth of *U. virens* (cm)*		
	Day 10	**Day 20**	**Day 30**
YPPDA	2.76[a]	4.57[a]	6.3[a]
PDA	1.6[c]	2.87[d]	3.3[e]
CDA	0.95[d]	1.93[e]	2.47[f]
PSA	2.5[b]	3.7[b]	5.86[b]
XBZA	1.69[c]	2.8[d]	4.57[d]
RYDA	0.82[d]	0.89[e]	1.55[g]
RESA	1.76[c]	3.56[c]	5.67[c]
SE	0.04	0.04	0.03
CD(0.05)	0.13	0.125	0.10

*Mean of four replications

YPPDA:Yeast Peptone Potato Dextrose Agar PDA:Potato Dextose Agar

CDA :Czapek Dox Agar PSA :Potato Sucrose Agar

XBZA : XBZ Agar RYDA : Rice Yeast Dextrose Agar

RESA : Rice Extract Sucrose Agar

Table 10. Mycelial dry weight of *U. virens* on different liquid media

Sl. No.	Treatments	Mycelial dry weight (g) at 30 DAI
1	YPPDA	1.98
2	PDA	1.91
3	CDA	1.00
4	PSA	2.02
5	XBZA	1.91
6	RYDA	1.57
7	RESA	1.83
	SE	0.02
	CD(0.05)	0.05

*Mean of four replications

4.7.3. Sporulation and pigment production of the false smut pathogen on different liquid media

Sporulation in terms of production of secondary conidia and chlamydospore by the pathogen was studied in all the liquid broth mentioned in 4.7.2. The results of the study indicate that secondary conidia production was found to very high in PSB and RESB and was low in case of CDB (Table 11). The smut ball and chlamydospore production was found to be the highest in PSB, RYDB and RESB. No smut ball production was observed with CDB medium.

The pigment production by *U. virens* in different liquid broth was studied and is presented in table 11. The pathogen produced dark yellow pigmentation in RYDB, and in XBZB reddish yellow pigmentation was produced. Yellow pigmentation was found to be produced in RESB and in PSB, light green pigmentation was observed.

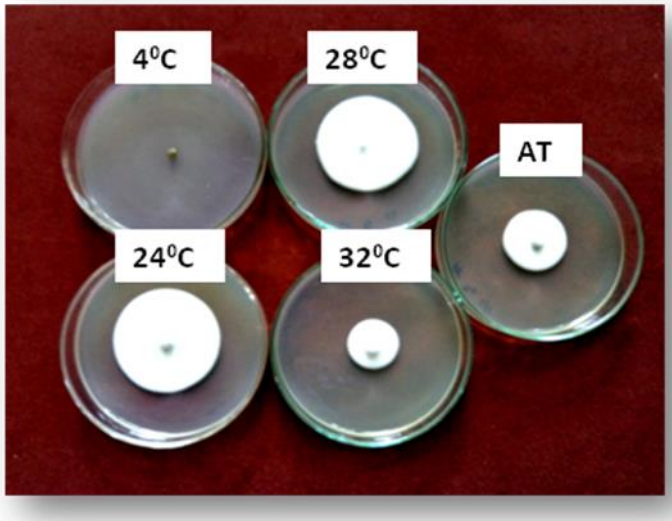
4.8. PHYSIOLOGICAL CHARACTERISTICS OF *U. VIRENS* ON YPPDA

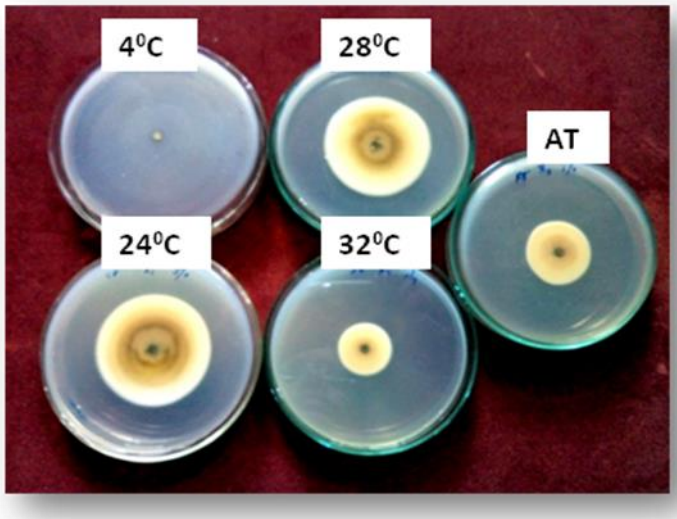
4.8.1. Effect of temperature

The isolate Uv 7 was grown at five different temperature levels *viz.*, 4, 24, 28 and 32°C, and also at room temperature on YPPDA so as to determine the optimum temperature conditions for culturing of the pathogen. Significant difference was observed in growth of the pathogen at different temperature conditions (Table 12, Plate 19). The growth of the pathogen was found to be maximum (5.15 cm) at 28°C which significantly different from the treatment i.e., 24°C which gave a growth of 4.45 cm at 30 days after inoculation which differed from all other treatments. Minimum growth was observed at refrigerated conditions, i.e., 4°C (0.45 cm). From this experiment, it was inferred that the temperature 28°C was found to be optimum for growth of *U. virens*.

4.8.2. Effect of pH

The pathogen was grown at two acidic (5.5 and 6.5) and two alkaline (7.5 and 8.5) pH conditions for determination of the optimum pH levels for culturing the pathogen *U. virens*. Upon comparison of growth at 10, 20 and 30 DAI, it was observed that the pathogen was found to give maximum growth at pH 6.5 (Table 13,





AT: Ambient Temperature

Plate 19. Growth of *U. virens* under different temperature conditions

Table 11. Characteristics of *U. virens* on different media broth

Treatments	Production of secondary conidia	Production of smut balls and chlamydospores	Pigmentation
YPPDB	+ +	+ + +	Yellowish green
PDB	+ +	+ +	Light yellow
CDB	+	+	white
PSB	+ + + +	+ + + +	Light green
XBZB	+ + +	+ + +	Reddish yellow
RYDB	+ +	+ + + +	Dark yellow
RESB	+ + + +	+ + + +	yellow

+ + + + : Very High

+ + + : High

+ + :Moderate

+ : Low

Table 12. Growth of *U. virens* under different temperature conditions

Sl. No.	Treatments	Colony Diameter (cm)
1	4°C	0.45[e]
2	24°C	4.45[b]
3	28°C	5.15[a]
4	32°C	2.31[d]
5	Ambient Temperature	3.2[c]
	SE	0.09
	CD (0.05)	0.28

*Mean of four replications

Table 13. Growth of *U. virens* under different pH conditions

Treatments	Colony Diameter (cm)		
	10 DAI	**20 DAI**	**30 DAI**
pH 5.5	1.65	4.7	6.12
pH 6.5	1.97	5.27	6.9
pH 7.5	1.42	4.61	6.1
pH 8.5	1.32	4.31	5.65
SE	0.07	0.05	0.07
CD (0.05)	0.22	0.16	0.22

*Mean of four replications

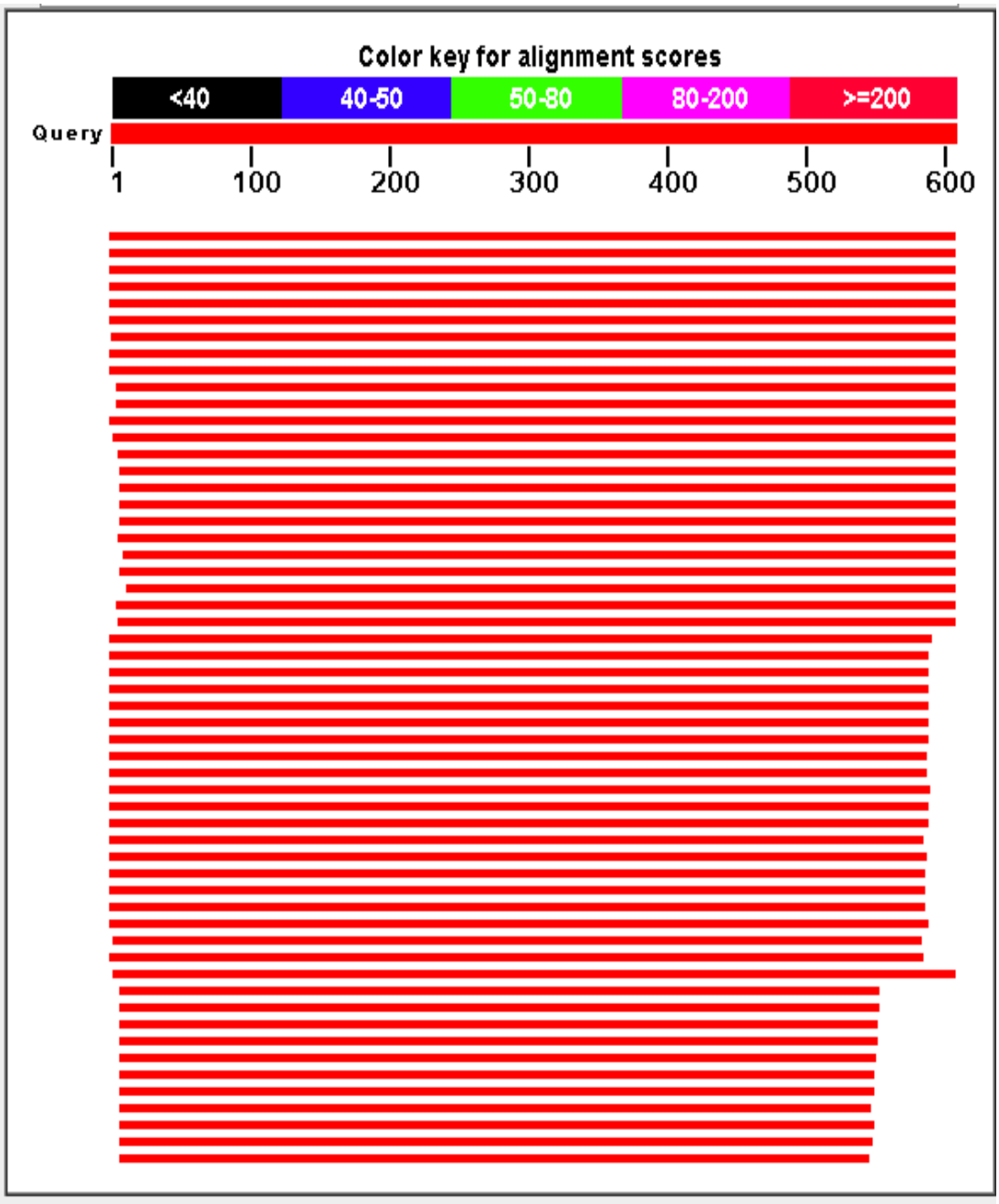


Fig 2. Chromatogram showing distribution of 100 blast hits on the query sequence of *U. virens* (source: BLAST DATA: ALIGNMENT VIEW USING NCBI GENBANK; http://blast.ncbi.nlm.nih.gov/)

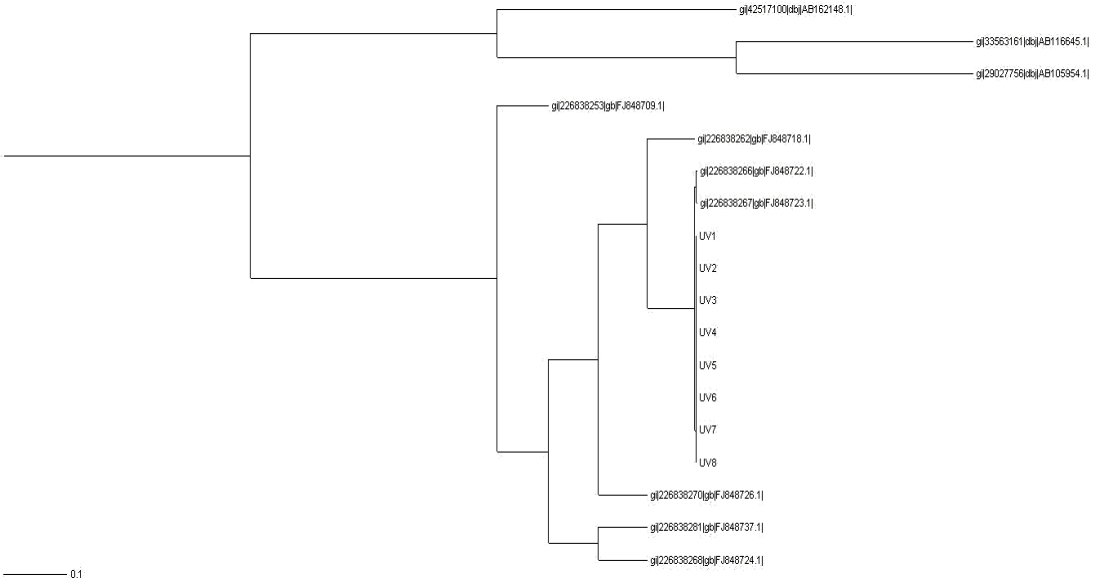


Fig 3. Dendrogram showing the phylogenetic relationship of the sequences of *U. virens* isolates with the already known sequences

Plate 20) which was significantly different from all other treatments. At 30 DAI, the pH level 6.5 gave an average diameter of 6.9 cm, which was found superior in comparison with the growth at all other pH levels, followed by the pH 5.5 giving 6.12 cm dia. which was on par with the growth at pH 7.5 (6.1 cm). Thus, the most ideal pH for growth of the pathogen was identified as 6.5.

4.8.3. Effect of light

The pathogen was subjected to four light conditions *viz*., 24 h light, 24 h darkness, intermittent light i.e., 12 h light followed by 12 h darkness and the ambient light conditions. At 30 DAI, growth in terms of colony diameter on YPPDA was compared (Table 14, Plate 21). The results of the experiments revealed that the pathogen was found to be giving maximum growth at full darkness (4.42 cm) which was significantly different from all other conditions of light. At intermittent light conditions it gave much reduced growth (3.34 cm).

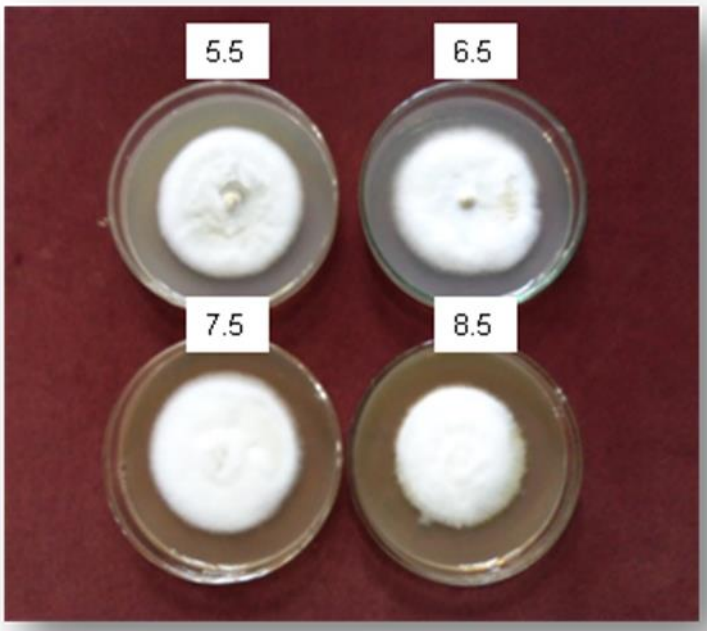
4.9. STUDIES ON THE EPIDEMIOLOGY OF THE DISEASE

4.9.1. Survival of the pathogen

4.9.1.1. *In vitro* survival of the pathogen *U. virens* under seeds, stubbles soil and collateral hosts

4.9.1.1.i. *In vitro* survival of the smut balls of *U. virens*

The smut balls or pseudosclerotia harvested in October 2012 and stored under laboratory conditions within the crop stubbles (Table 15) remained viable up to May 2013 (8 months) even though the viability percentage was found to be very low (4%) in May. Up to four months of storage, the smut balls had >50 % viability, but afterwards the viability percentage decreased abruptly and reached zero at nine months of storage at which time the smut balls appeared almost hollow with the lemma and palea smeared with black dusty particles. The smut balls stored in the clayey soil remained viable only up to three months (from October 2012 to December 2012). The viability percentage at the third month of storage was found to be very low (12 %). Up on continuous storage under the soil, the smut balls were found to get rotten and got converted in to black sticky masses.



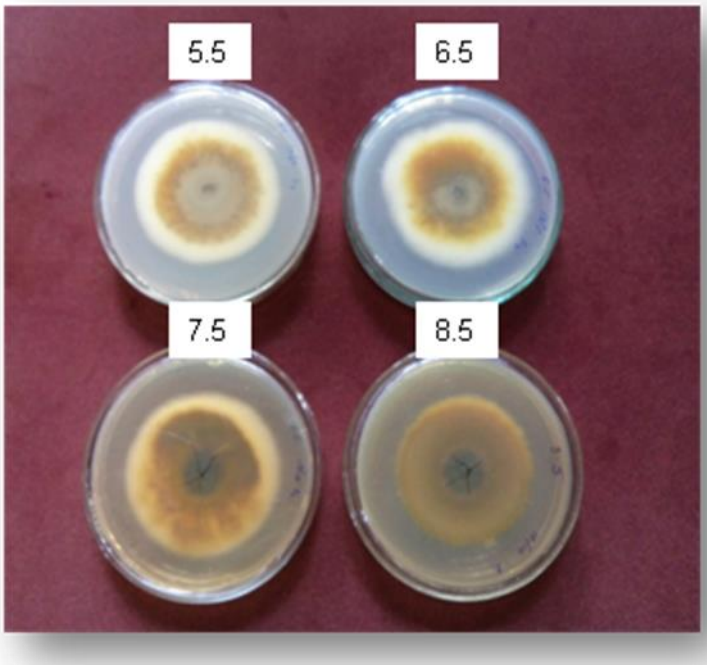
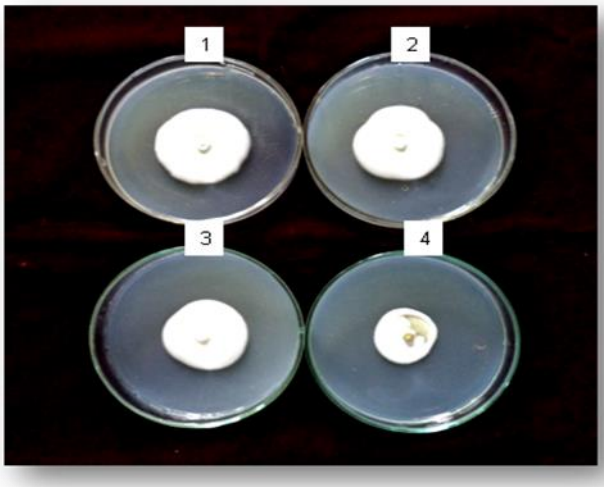
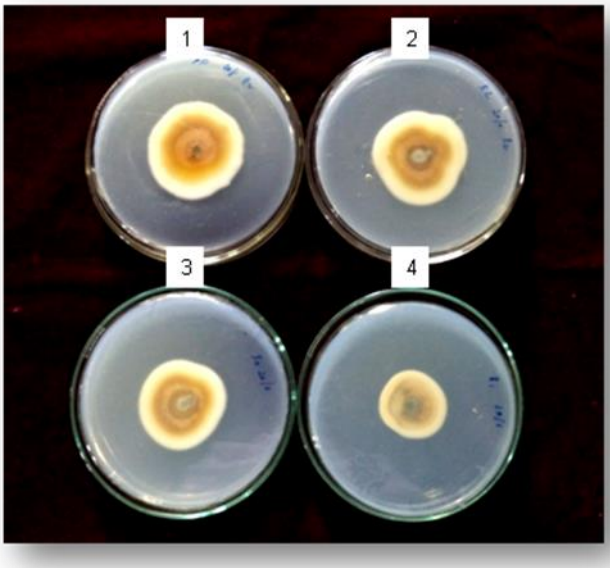


Plate 20.Growth of *U. virens* under different pH conditions





1: Intermittent Light (12 hr light of 500 lux + 12 hr darkness)

2. Full Darkness

3: Ambient Light (400 lux at day time)

4. Intermittent Light (12 hr light of 500 lux + 12 hr darkness)

Plate 21.Growth of *U. virens* under different Light conditions

Table 14. Growth of *U. virens* under different light conditions at 30 DAI

Sl. No.	Treatments	Colony Diameter (cm)
1	Full Light (500 lux)	3.52[c]
2	Ambient Light (400 lux at day time)	4.10[b]
3	Intermittent Light (12 hr light of 500 lux + 12 hr darkness)	3.34[d]
4	Full Darkness	4.42[a]
	SE	0.05
	CD (0.05)	0.15

*Mean of four replications

Table 15. Viability of smut balls of *U. virens* in crop residue/stubbles and soil

MONTHS	VIABILITY OF SMUT BALLS (%)	
	STUBBLES	SOIL
OCTOBER 2012	100	100
NOVEMBER 2012	87	23
DECEMBER 2012	69	12
JANUARY 2013	56	0
FEBRUARY 2013	37	0
MARCH 2013	28	0
APRIL 2013	22	0
MAY 2013	4	0
JUNE 2013	0	0

4.9.1.1.ii. In vitro survival of the chlamydospores of *U. virens*

The viability of the chlamydospores was tested under three conditions *viz*., seed, stubbles and soil. The chlamydospores were able to survive only up to three months (Table 16) within the crop residue and within soil (from October to December 2012), but the per cent viability of the chlamydospores at all the three months was found to be more for the spores stored in the stubbles than in the soil. The chlamydospores in the third month of storage under soil conditions had very low germination percentage (2 %).

The chlamydospores remained viable up to four months on the seeds of paddy, even though the viability percentage was very low at the fourth month (11 %).

4.9.1.1.iii. In vitro survival of the pathogen *U. virens* under collateral hosts

The survival of *U. virens* on different collateral hosts was studied by artificial inoculation studies on common weeds of rice field. Inoculation was done by injection of 2 ml spore suspension of the pathogen in to the leaf sheath covering the panicles of the potted plants. None of the weeds artificially inoculated were found to take up infection (Table 17).

4.1.1.2. Study of survival of the pathogen in the field:

4.9.1.2.i. In vivo survival of the pathogen *U. virens* on stubbles

The survival of the pathogen under the field conditions was observed during the surveys and field experiments. During the survey conducted at Palakkad, at Kizhayur area, the pathogen was found to be surviving on the ratoon rice plants of variety Uma emerged from the stubbles of the previous crop (Plate 22). One to seven grains of the panicle had got converted in to smut balls, and in the whole area, most of the plants were found to be affected.

4.9.1.2.ii. In vivo survival of the pathogen *U. virens* on collateral hosts

The survival of the pathogen under the field conditions on collateral hosts was observed during the surveys. Different graminaceous grasses and sedges in the rice fields were observed for the presence of the pathogen (Table 18). Most of the weed plants observed were free of infection. From Ambalappuzha area of Alappuzha

Plate 22. Presence of the disease on ratoon rice emerged from the stubbles of the previous crop at kizhayur area of Palakkad

Table 16. Germination of the chlamydospores of *U. virens* in seeds, crop residue/stubbles and soil

MONTHS	GERMINATION OF CHLAMYDOSPORES (%)		
	SEEDS	STUBBLES	SOIL
OCTOBER 2012	95	95	95
NOVEMBER 2012	74	70	16
DECEMBER 2012	47	35	2
JANUARY 2013	11	0	0
FEBRUARY 2013	0	0	0

Table 17. Response of common weeds of rice field to artificial inoculation of *U. virens*

Sl No.	Name of the weed	Disease reaction
1	*Echinochloa colona*	-
2	*Echinochloa crussgalli*	-
3	*Digitaria longifolia*	-
4	*Leersia hexandra*	-
5	*Dactyloctenium aegyptiacum*	-
6	*Oryza rufipogon (varinellu)*	-
7	*Cynodon dactylon*	-
8	*Fimbristylis milliaceae*	-
9	*Cyperus rotundus*	-
10	*Cyperus difformis*	-
11	*Cyperus iria*	-

Table 18. Survival of *U. virens* on common weeds of rice field

Sl No.	Name of the weed	Disease reaction
1	*Echinochloa colona*	-
2	*Echinochloa crussgalli*	-
3	*Digitaria longifolia*	-
4	*Leersia hexandra*	-
5	*Dactyloctenium aegyptiacum*	-
6	*Oryza rufipogon* (varinellu)	-
7	***Oryza spontaneum* (varinellu)**	+
8	*Cynodon dactylon*	-
9	*Fimbristylis milliaceae*	-
10	*Cyperus rotundus*	-
11	*Cyperus difformis*	-
12	*Cyperus iria*	-

district, the pathogen was observed on weedy rice, *Oryza spontaneum* (Plate 23). Only one grain in the panicle was found infected.

4.9.2. Study on the onset of false smut disease in the field at different cropping seasons of the year

The onset of false smut disease in the field at different cropping seasons was observed at two locations, Palakkad and Alappuzha in the medium duration susceptible variety Uma (120-135 days duration). The results of the study are presented in table 19.

The results indicated that the disease was spotted in the field with the conspicuous characteristic symptoms when the crop attained an age of 101 to 108 days, that is, at approximately 15 to 25 days after the emergence of the first panicle.

4.9.3. Correlation studies on the influence of various weather parameters on the incidence and intensity of false smut disease of rice

The weather parameters prevailing in the field at the time of various field trials were correlated with the per cent infected tillers and disease severity so as to study the influence of weather parameters on the development of false smut disease of rice. The various parameters studied include Minimum temperature, Maximum temperature, Relative Humidity (Morning), Relative Humidity (Evening), Relative Humidity (Average) and Total Rainfall.

The correlation coefficients are presented in Table 20 to 24. The results revealed that disease severity and % infected tillers were found to be negatively correlated with the minimum temperature at 50% flowering period. Minimum temperature at 15 days, seven days and five days around the day of 50 % flowering and average of seven days after flowering were found to be significantly negatively correlated (Table. 20) with the disease severity and per cent infected tillers with false smut disease.

Maximum temperature during 50 per cent flowering period was found to be non-significantly positively correlated (Table. 21) with the disease severity and % infected tillers.

Relative humidity at morning hours, evening hours and the daily mean relative humidity at seven days after 50 per cent flowering was found to be significantly

Plate 23. Presence of the disease on weedy rice *Oryza spontaneum* from Ambalappuzha area of Alappuzha

Table 19. Onset of false smut disease in the field at different cropping seasons

Sl. No.	Season and the Year	Age of the crop
1	Rabi 2010-11- Alappuzha	105 days
2	Kharif 2011 - Palakkad	101 days
3	Rabi 2011-12- Alappuzha	106 days
4	Kharif 2012 - Palakkad	108 days
5	Rabi 2012-13- Alappuzha	103 days
6	Kharif 2013 - Palakkad	106 days

Table 20. Corrrelation of disease severity and per cent infected tillers with the minimum temperature at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	-0.39	-0.83^{++}	-0.59^{++}	-0.96^{++}	-0.74^{++}	-0.80^{++}
Disease severity	-0.49	-0.90^{++}	-0.69^{++}	-0.96^{++}	-0.84^{++}	-0.87^{++}

1- Minimum temperature on the day of 50% F

2- 15 days average *ie*, 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie*, 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

Table 21. Corrrelation of disease severity and per cent infected tillers with the maximum temperature at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	0.15	0.14	0.34	0.37	0.30	0.18
Disease severity	0.236	0.27	0.44	0.39	0.43	0.29

1- Maximum temperature on the day of 50% F

2- 15 days average *ie,* 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie*, 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

negatively correlated (Table. 22, 23 and 24) with the disease severity and % infected tillers.

There was no significant correlation between the average rainfall during 50 per cent flowering period with the disease severity and % infected tillers (Table. 25).

4.10. SCREENING OF RICE VARIETIES AGAINST FALSE SMUT DISEASE

Twenty red rice varieties were screened for resistance under wet land field conditions against false smut disease of rice caused by *Ustilaginoidea virens* (Cook) Tak. at two locations, Alappuzha and Palakkad during 2012-13. The details of the varieties screened are given in Table 26.

The results of the varietal screening trial conducted during Kharif 2012 at Palakkad indicated that the varieties Harsha and Vaishak were completely free from the disease (Table 27). Disease severity was found to be very low (1.4) in the varieties, Makom and Thekkancheera. The varieties Pa*viz*ham, Karthika and Revathi were also found to have less severity of false smut disease (1.28, 1.28 1nd 1.41 respectively).

During the varietal evaluation trial conducted at Alappuzha during Rabi 2012-13, eight varieties were found completely disease free (Table 28), *viz*., Aruna, Makom, Kanakom, Vytila 6, Thekkancheera, Athira, Harsha and Vaishak. The varieties, Pa*viz*ham, Karthika and Prathyasha recorded very low disease severity (<1).

The varieties screened were grouped based on their resistance reaction in to five groups i.e., highly resistant (completely free from the disease with a disease severity index of 0), resistant (disease severity value < 1), moderately resistant (disease severity value 1-5), susceptible (disease severity value 5-20), and highly susceptible (disease severity value > 20).

Out of the twenty varieties screened, the varieties Harsha and Vaishak released from Regional Agricultural Research Station Pattambi, Palakkad were found highly resistant (completely disease-free, Plate 24) and the varieties, Makom, Thekkancheera, Pa*viz*ham and Karthika were found resistant to the disease (Table 29). The varieties Kanakom, Revathi and Prathyasha showed moderate resistance.

Plate 24. The varieties found resistant against false smut disease

Table 22. Corrrelation of disease severity and per cent infected tillers with the relative humidity (am) at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	-0.03	-0.04	0.26	-0.66^{++}	0.12	0.15
Disease severity	-0.30	-0.20	-0.01	-0.63^{++}	-0.05	0.2

1- Relative Humidity (A.M) on the day of 50% F

2- 15 days average *ie,* 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie,* 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

Table 23. Corrrelation of disease severity and per cent infected tillers with the relative humidity (pm) at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	-0.29	-0.47	-0.08	-0.62^{++}	-0.20	-0.34
Disease severity	-0.49	0.54	-0.22	-0.63^{++}	-0.33	-0.44

1- Relative Humidity (P.M) on the day of 50% F

2- 15 days average *ie*, 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie*, 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

Table 24. Corrrelation of disease severity and per cent infected tillers with the average relative humidity at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	-0.22	-0.42	-0.03	-0.63^{++}	-0.14	-0.27
Disease severity	-0.51	0.53	-0.18	-0.64^{++}	-0.30	-0.40

1- Average Relative Humidity on the day of 50% F

2- 15 days average *ie*, 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie*, 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

Table 25. Corrrelation of disease severity and per cent infected tillers with the average rainfall at 50% flowering period

Parameter	1	2	3	4	5	6
% infected tillers	-0.05	-0.32	-0.39	-0.32	-0.04	-0.20
Disease severity	-0.11	-0.14	-0.46	-0.32	0.24	-0.14

1- Average Rainfall on the day of 50% F

2- 15 days average *ie*, 7 DB50% F+on 50% F +7 DA50% F

3- 5 days average *ie*, 2 DB50% F + on 50% F + 2 DA50% F

4- Average of 7 DB50% F

5- 7 days average *ie*, 3 DB50% F + on 50% F + 3 DA50% F

6- 9 days average *ie*, 4 DB50% F + on 50% F + 4 DA50% F

DB50% F : Days before 50% flowering

DA50% F : Days after 50% flowering

50 % F : 50% flowering

Table 26. Details of the varieties screened against false smut disease of rice

Sl. No	Treatments	Station of release	Duration	Details	Average Yield (t/ha)
1	Bhadra	RRS, Moncompu	120-140	Red, short Bold	4.5-5.5
2	Pavizham	RRS, Moncompu	115-120	Red, short Bold	5-6
3	Karthika	RRS, Moncompu	110-115	Red, Medium Bold	6-6.5
4	Aruna	RRS, Moncompu	100-110	Red, short Bold	5.5-6
5	Makom	RRS, Moncompu	100-110	Red, short Bold	6-6.5
6	Kanakaom	RRS, Moncompu	120-125	Red, short Bold	5.5-6
7	Remanika	RRS, Moncompu	100-105	Red, short Bold	6-6.5
8	Uma	RRS, Moncompu	115-120	Red, Medium Bold	6.5-7
9	Revathi	RRS, Moncompu	105-110	Red, Medium Bold	5.5-6
10	Krishnanjana	RRS, Moncompu	105-110	Red, Medium Bold	5-5.5
11	Gouri	RRS, Moncompu	115-120	Red, Medium Bold	5.8
12	Prathyasha	RRS, Moncompu	100-110	Red, Long Bold	5-5.5
13	Bhagya	RRS, Kayamkulam	95-100	Red, Medium Bold	4.35
14	Vytila	RRS, Vytila	115-120	Red, Medium Bold	4.5-5
15	PTB-10	RARS, Pattambi	90-100	Red	2.5
16	Jyothi	RARS, Pattambi	105-110	Red, Long Bold	6
17	Kanchana	RARS, Pattambi	105-110	Red, Medium Bold	5-5.5
18	Athira	RARS, Pattambi	110-115	Red, short Bold	5-5.5
19	Harsha	RARS, Pattambi	105-110	Red, Long Bold	4-5
20	Vaishak	RARS, Pattambi	117-125	Red, short Bold	2.2-3.9

Table 27. Per cent infected tillers and disease severity of false smut disease during the varietal screening at Palakkad during Kharif 2012

Sl. No.	Varieties	Kharif 2012 Palakkad	
		%infected Tillers	Disease severity
1	Bhadra	4.00(2.23)	10.58(3.4)
2	Pavizham	0.63(1.28)	0.63(1.28)
3	Karthika	0.63(1.28)	0.63(1.28)
4	Aruna	1.64(1.62)	6.54(2.74)
5	Makom	**0.29(1.14)**	**0.29(1.14)**
6	Kanakaom	1.64(1.63)	3.28(2.07)
7	Remanika	7.66(2.94)	16.97(4.24)
8	Uma	11.32(3.51)	31.32(5.68)
9	Revathi	0.99(1.41)	4.00(2.23)
10	Krishnanjana	2.65(1.91)	7.94(2.99)
11	Gouri	5.66(2.58)	9.65(3.26)
12	Pratyasha	1.64(1.63)	3.28(2.07)
13	Bhagya	5.66(2.58)	5.97(4.12)
14	Vytila 6	1.64(1.63)	5.72(3.59)
15	Thekkancheera	**0.29(1.14)**	**0.29(1.14)**
16	Jyothi	4.65(2.38)	15.00(4.00)
17	Kanchana	3.65(2.16)	5.31(2.51)
18	Athira	4.65(2.38)	9.65(3.26)
19	Harsha	**0.00(1.00)**	**0.00(1.00)**
20	Vaishak	**0.00(1.00)**	**0.00(1.00)**
	SE	0.08	0.12
	CD	0.24	0.35

Table 28. Per cent infected tillers and disease severity of false smut disease during the varietal screening at Alappuzha during Rabi 2012 -13

Sl. No.	Varieties	Rabi 2012-13 Alappuzha	
		%infected Tillers	Disease severity
1	Bhadra	2.00(1.73)	3.33(2.08)
2	Pavizham	1.00(1.41)	0.83(1.35)
3	Karthika	1.00(1.41)	0.83(1.35)
4	Aruna	**0.00(1.00)**	**0.00(1.00)**
5	Makom	**0.00(1.00)**	**0.00(1.00)**
6	Kanakaom	**0.00(1.00)**	**0.00(1.00)**
7	Remanika	2.00(1.73)	4.24(2.28)
8	Uma	4.00(2.24)	13.33(3.79)
9	Revathi	1.67(1.63)	1.50(1.58)
10	Krishnanjana	1.00(1.41)	1.00(1.41)
11	Gouri	2.00(1.73)	3.64(2.15)
12	Pratyasha	0.33(1.14)	0.30(1.13)
13	Bhagya	2.33(1.82)	4.23(2.28)
14	Vytila 6	**0.00(1.00)**	**0.00(1.00)**
15	Thekkancheera	**0.00(1.00)**	**0.00(1.00)**
16	Jyothi	1.00(1.41)	1.80(1.67)
17	Kanchana	1.33(1.52)	1.20(1.48)
18	Athira	**0.00(1.00)**	**0.00(1.00)**
19	Harsha	**0.00(1.00)**	**0.00(1.00)**
20	Vaishak	**0.00(1.00)**	**0.00(1.00)**
	SE	0.05	0.06
	CD	0.14	0.15

Table 29. Grouping of twenty varieties of rice screened for resistance against false smut disease of rice

Sl. No.	Range of Disease severity	Category	Name of Varieties
1	0	Highly resistant	Vaishak, Harsha
2	<1	Resistant	Makom, Thekkancheera, Pavizham, Karthika
3	1-5	Moderately resistant	Kanakom, Revathi, Prathyasha
4	5-20	Susceptible	Bhadra, Aruna, Remanika, Krishnanjana, Gouri, Bhagya, Vytila 6, Jyothi, Kanchana, Athira
5	>20	Highly susceptible	Uma

Ten varieties *viz*., Bhadra, Aruna, Remanika, Krishnanjana, Gouri, Bhagya, Vytila 6, Jyothi, Kanchana and Athira were found susceptible to the disease with the corresponding disease severity index. The variety Uma (Plate 25) was found highly susceptible to the disease.

4.11. DETECTION OF MOLECULAR MARKERS FOR FALSE SMUT RESISTANCE

Four highly resistant and four highly susceptible varieties selected were used for detection of molecular markers for false smut resistance by making use of RAPD profiling in order to check for the possible resistant gene sources against false smut disease. RAPD profiling was carried out with the pooled genomic DNA isolated from four highly resistant and four highly susceptible varieties were subjected to PCR using ten RAPD primers *viz*., OPK-19, OPG-18, OPC-15,OPB-10, OPH-19, OPD-3, OPB-5, OPK-14, OPF-13 and OPD-18 to locate molecular markers specific for the resistance against false smut. Out of the ten primers used for RAPD profiling of the pooled DNA, three primers gave products specific to the resistant varieties. The RAPD profile using the pooled DNA from the resistant varieties gave specific products (Plate 26) with respect to the primers OPG-18 (at 300 bp position), OPC-15 (at 400 bp position) and OPD-18 (at two positions *viz*., 1000-1500 bp and 250-500 bp).

To make the results more specific, DNA of the individual resistant varieties were compared with that of the susceptible varieties. RAPD profile of the resistant varieties, Makom, Harsha, Thekkancheera and Vaishak, and the susceptible varieties Bhagya, Gouri, Remanika and Uma, with two random primers *viz*., OPB-10 and OPK-14, showed that, with the primer OPB-10, there was one unique amplicon in case of the variety Harsha, and with the primer OPK-14, one unique amplicon was present in the variety Makom and two in the variety Thekkancheera (Plate 27).

4.12. ISOLATION AND TESTING OF BIOCONTROL AGENTS

4.12.1. *Isolation of antagonists*

Different biocontrol agents both fungi and bacteria were isolated from the spermosphere and rhizosphere of the disease free rice plants among the false smut

Plate 25. False smut affected panicle from the most susceptible variety Uma

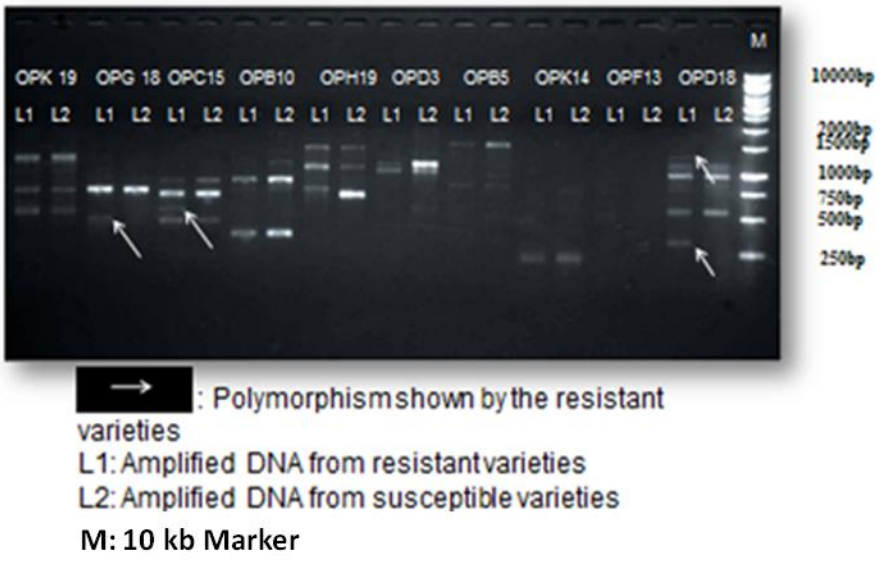


Plate 26. RAPD profile of the pooled resistant and susceptible varieties

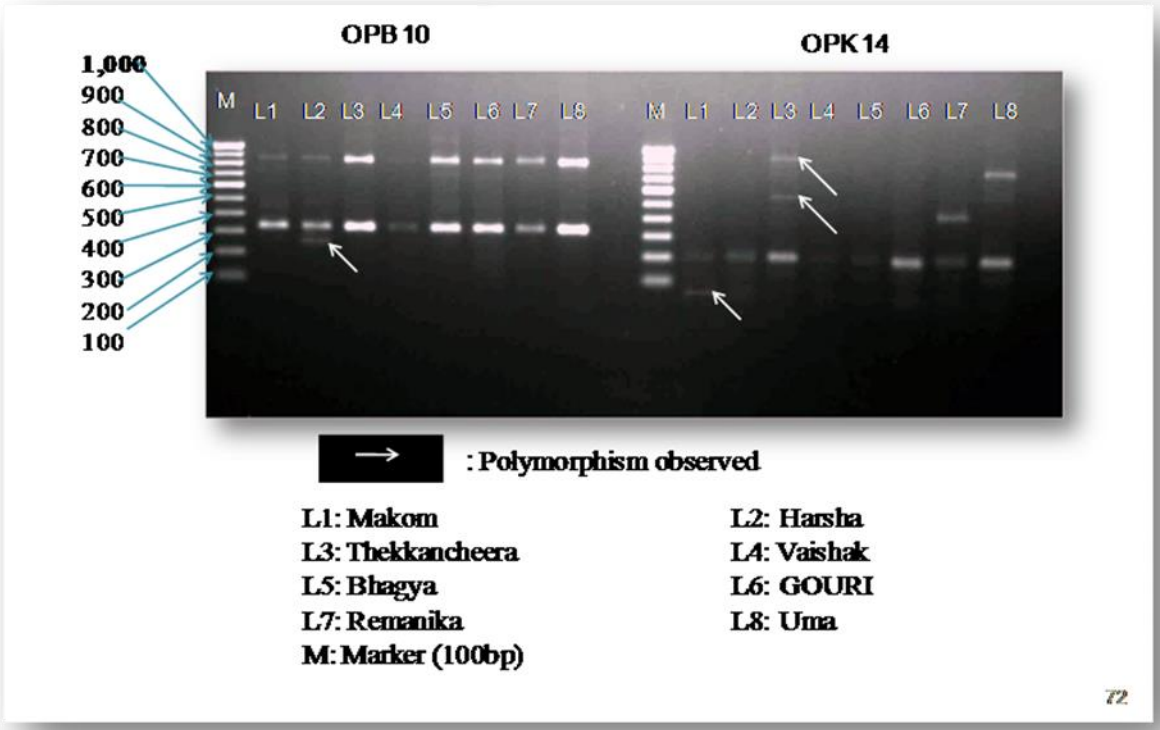


Plate 27. RAPD profile of the resistant and susceptible varieties

infected rice plants in the field. Details of the bio control agents isolated are given in Table 30. The fungal isolates were screened using dual culture technique and 10 isolates of *Trichoderma* were selected and further studies were carried out using these isolates.

4.12.2. *In vitro screening of the antagonists*

4.12.2.1. *In vitro* screening of the fungal antagonists

4.12.2.1.a. *In vitro* studies for evaluating the antagonistic efficacy of the fungal isolates against *U. virens* in dual culture

Ten isolates of *Trichoderma* were screened by dual culture technique for their efficacy against the pathogen *U. virens*. The results of the study (Table 31 and Table 32) revealed that all the isolates of *Trichoderma* were effective in controlling the pathogen but there was no significant difference among the isolates in the per cent inhibition of the pathogen (Plate 28).

The isolate Tri-5 gave the highest inhibition to the mycelial growth of the pathogen (22.28 %) on the third day after inoculation of *Trichoderma*, but did not differ significantly from all other isolates in controlling the pathogen.

All the isolates started overgrowing the pathogen colony from the third day onwards and completely covered the plate the fourth day. Lysis of the pathogen colony was apparent after five days with the isolates Tri-3, Tri-5, Tri-6 and Tri-7 (Table 33).

4.12.2.1.b. Study of mechanisms of antagonism of the effective *Trichoderma* isolate against *U. virens*

4.12.2.1.b.(i). Mycoparasitism

The isolate Tri-5 which gave maximum inhibition of the pathogen growth was used for studying the mycoparasitic effect of *Trichoderma* on *U. virens*. The antagonist *Trichoderma* was found to show various signs of mycoparasitism like, parallel growth, formation of hook like structures, formation of chlamydospores etc (Plate 29). Due to the parasitism by *Trichoderma*, the pathogen hyphae showed granulation of cytoplasm, vesiculation and lysis.

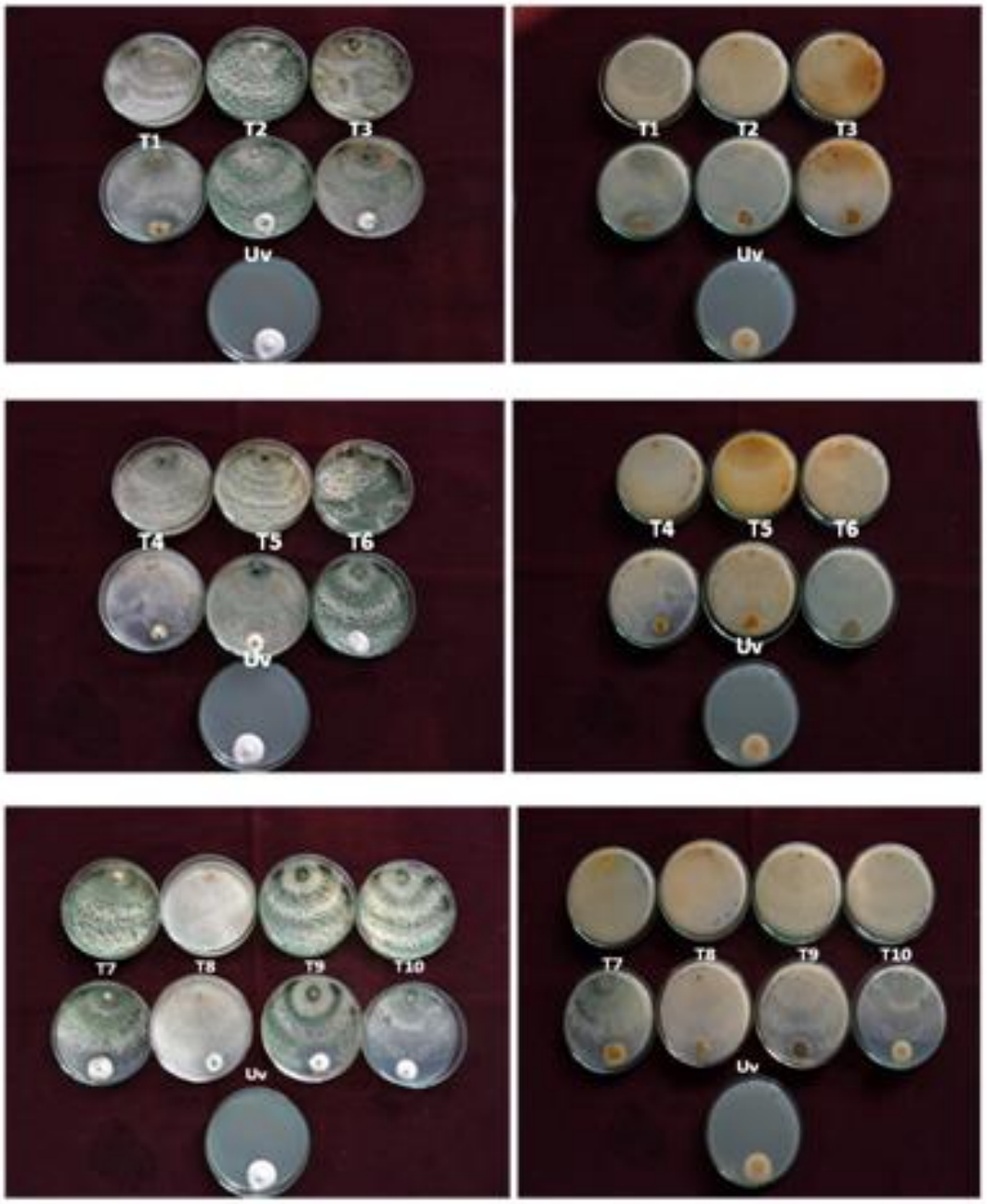


Plate 28. **The antagonistic efficacy of *Trichoderma* isolates against *U. virens* in dual culture**

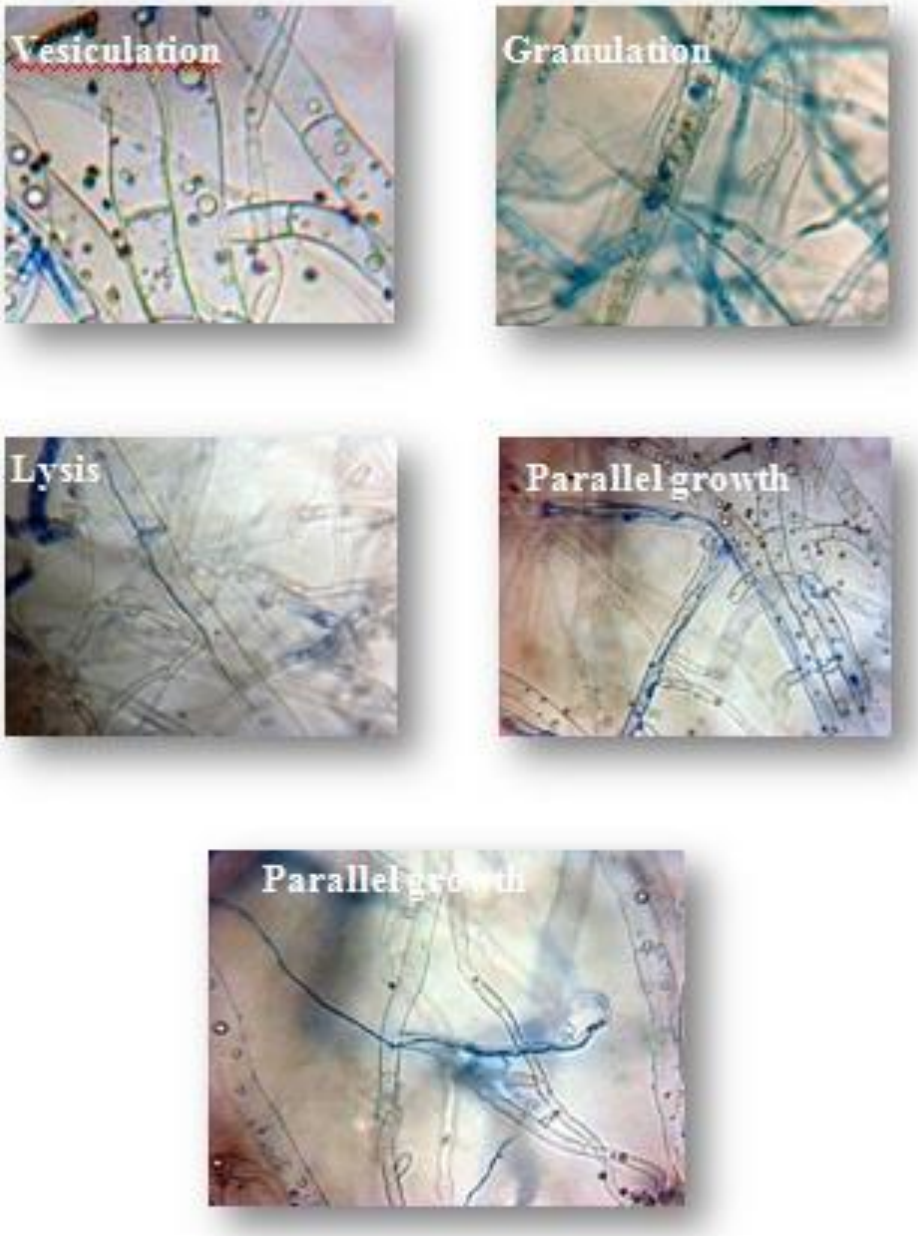


Plate 29. Different signs of mycoparasitism shown by *Trichoderma* on *U. virens*

Table 30. List of microorganisms isolated from the rhizosphere and spermosphere of rice

Sl. No.	Name of Microorganism	No. of Isolates
1	*Aspergillus niger*	3
2	*Aspergillus flavus*	2
3	*Penicillium* spp.	4
4	*Fusarium* spp.	1
5	*Trichoderma harzianum*	8
6	*T. virens*	1
7	*T. viride*	1
8	*Bacillus* spp.	8

Table 31. Radial growth (cm) of *U. virens* on YPPDA in dual culture

Treatments	Day 12	Day 13	Day 14
Uv-Tri 1	1.71^{a}	1.8^{bcde}	0
Uv-Tri 2	1.75^{bg}	1.78^{bcd}	0
Uv-Tri 3	1.76^{bcd}	1.77^{bc}	0
Uv-Tri 4	1.80^{g}	1.83^{bcde}	0
Uv-Tri 5	1.74^{b}	1.67^{a}	0
Uv-Tri 6	1.8^{g}	1.78^{bcd}	0
Uv-Tri 7	1.75^{bc}	1.75^{bc}	0
Uv-Tri 8	1.78^{f}	1.75^{b}	0
Uv-Tri 9	1.77^{bcde}	1.83^{bcde}	0
Uv-Tri 10	1.80^{g}	1.82^{bcde}	0
Uv-check	1.80^{g}	1.95^{f}	2.05
SE	0.008	0.02	0.0001
CD (0.05)	0.02	0.06	0.0005

*Mean of four replications

Table 32. Inhibition of *U. virens* by *Trichoderma* on YPPDA in dual culture

Treatments	Inhibition (%)		
	Day 12	Day 13	Day 14
Uv-Tri 1	6.59 (14.87)	8.87 (17.32)	100.00 (90.00)
Uv-Tri 2	1.92 (7.79)	6.73 (15.03)	100.00 (90.00)
Uv-Tri 3	1.57 (7.19)	7.98 (16.40)	100.00 (90.00)
Uv-Tri 4	0.00 (0.00)	6.9 (15.23)	100.00 (90.00)
Uv-Tri 5	5.31 (13.32)	15.63 (22.28)	100.00 (90.00)
Uv-Tri 6	0.00 (0.00)	8.81 (16.55)	100.00 (90.00)
Uv-Tri 7	5.00 (12.92)	9.53 (17.98)	100.00 (90.00)
Uv-Tri 8	0.56 (4.3)	9.90 (18.33)	100.00 (90.00)
Uv-Tri 9	2.24 (8.61)	7.01 (15.35)	100.00 (90.00)
Uv-Tri 10	0.00 (0.00)	6.42 (14.66)	100.00 (90.00)
SE	-	-	0.00
CD (0.05)	NS	NS	0.00

*Mean of four replications

Table 33. Effect of *Trichoderma* colonization on the growth of the pathogen observed on dual culture plates

Sl. No.	Treatments	Lysis	Overgrowth
1	Uv-Tri 1	-	+
2	Uv-Tri 2	-	+
3	Uv-Tri 3	+	+
4	Uv-Tri 4	-	+
5	Uv-Tri 5	+	+
6	Uv-Tri 6	+	+
7	Uv-Tri 7	+	+
8	Uv-Tri 8	-	+
9	Uv-Tri 9	-	+
10	Uv-Tri 10	-	+

4.12.2.1.b (ii). Production of nonvolatile compounds by *Trichoderma*

Culture filtrate studies conducted on the efficacy of *Trichoderma* culture filtrates on inhibiting pathogen growth (Table 34, Plate 30) revealed that, *Trichoderma* culture filtrate at 70 per cent concentration gave maximum inhibition (81.36) of the radial growth of the pathogen which differed significantly from all other concentrations followed by 60 per cent concentration (71.50).

The culture filtrates of *Trichoderma*, even at the lowest concentration of 10 per cent was found to give considerable inhibition (48.04) to the mycelial growth of the pathogen in comparison with the growth in the control plates. Thus the nonvolatile compounds produced by *Trichoderma* were found effective in checking the growth of the pathogen even at very low concentrations.

4.12.2.1.b (iii). Production of volatile compounds by *Trichoderma*

Studies conducted to determine the efficacy of *Trichoderma* volatiles on the pathogen showed that the growth of the pathogen was found to be getting inhibited by the effect of volatile compounds produced by *Trichoderma*. Even after continued incubation up to 20 days, the growth in the paired plate was only 0.6 cm (Plate 31) in comparison with that in the control plate (3 cm). Per cent inhibition to the growth of the pathogen was 80 per cent. The pathogen was found to increase in radial growth only up to 10 DAI in the paired treatment plates after which it stopped growing due to the effect of volatile compounds produced by *Trichoderma*.

4.12.2.2. *In vitro* studies for evaluating the antagonistic efficacy of the bacterial isolates against *U. virens*

Isolates of the bacterial antagonists obtained from spermosphere and rhizosphere of rice plants were tested for antagonistic property against the blast pathogen, by Kirby-Bauer test.

The results of the study (Table 35, Plate 32) indicate that the bacterial isolates B4, B5 and B6 were found to give maximum inhibition (82.60) of the colony growth of *U. virens* and was found to be on par with B3 (80.48).The isolate B1 gave the lowest inhibition percentage (16.15).

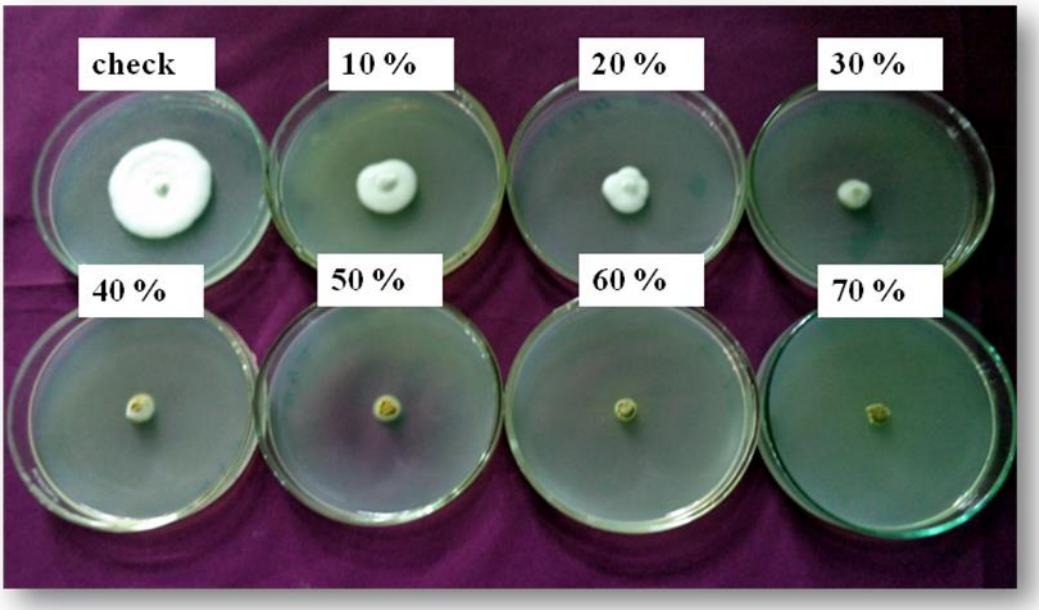
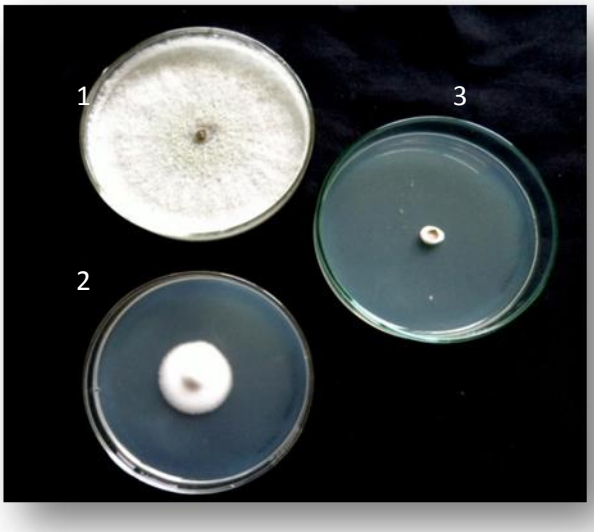


Plate 30. ***In vitro*** **efficacy of the culture filtrates of** ***Trichoderma*** **against** ***U. virens***

Paired plates: 1-*Trichoderma* check, 2-*U. virens* check, 3-*U. virens* (upper plate)+*Trichoderma* (bottom plate)



Open plates: 1-*Trichoderma* check, 2-*U. virens* check, 3-*U. virens* treatment plate

Plate 31. *In vitro* efficacy of volatiles of *Trichoderma* against *U. virens*

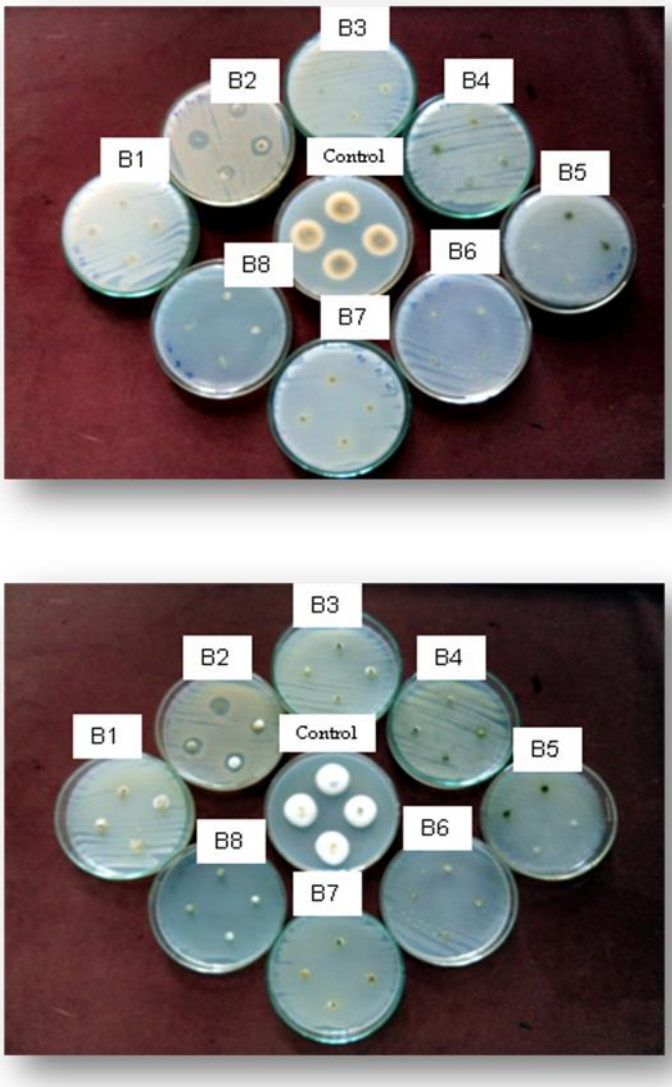


Plate 32. ***In vitro*** **efficacy of the bacterial antagonists against** ***U. virens***

Table 34. Effect of the culture filtrates of *Trichoderma* on the radial growth of the pathogen

Treatments	Colony Diameter(cm)*	Inhibition (%)**
10% cf	1.77	48.04(43.86)[g]
20% cf	1.53	54.90(47.80)[f]
30% cf	1.37	59.81(50.64)[e]
40% cf	1.07	68.58(55.90)[d]
50% cf	0.97	71.58(57.76)[c]
60% cf	0.83	75.50(60.31)[b]
70% cf	0.63	81.36(64.41)[a]
Uv-Check	3.40	-
SE		0.53
CD (0.05)		1.47

*Mean of four replications

** Values in parenthesis are arcsine transformed

Table 35. Evaluation of bacterial biocontrol agents on the radial growth of the pathogen

Treatments	Colony Diameter (cm)*	Per cent inhibition**
Uv-B1	1.92[f]	16.15 (23.69)[e]
Uv-B2	0.6[d]	73.90 (59.30)[c]
Uv-B3	0.45[b]	80.48(63.75)[ab]
Uv-B4	**0.4[a]**	**82.60 (65.32)[a]**
Uv-B5	**0.4[a]**	**82.60(65.32)[a]**
Uv-B6	**0.4[a]**	**82.60(65.32)[a]**
Uv-B7	0.8[e]	65.25(53.86)[d]
Uv-B8	0.5[c]	78.26 (62.18)[b]
Uv-Check	2.3[g]	--
SE	0.027	0.85
CD (0.05)	0.078	2.5

*Mean of four replications

** Values in parenthesis are arcsine transformed

4.13. *IN VITRO* EVALUATION OF CHEMICAL FUNGICIDES AGAINST *U. virens*

The *in vitro* chemical management of *U. virens* was done by making use of two methods 1) Poisoned food technique and 2) spore germination inhibition studies. Eleven commercially available fungicides were used for the *in vitro* studies

4.13.1. In vitro evaluation of fungicides by poisoned food technique

The results (Table 36, Table 37, Plate 33) of the *in vitro* evaluation of fungicides by poisoned food technique revealed that six chemicals *viz*., copper hydroxide, mancozeb, propiconazole, tebuconazole, carboxin and iprodione+ carbendazim gave 100 % inhibition to the growth of the pathogen under *in vitro* culture conditions and differed significantly from all other treatments followed by the chemical chlorothalonil (94.55). The fungicide pencycuron was found ineffective in checking the growth of the pathogen *in vitro*.

Even at the lowest concentration, the fungicides mancozeb, propiconazole, tebuconazole and carboxin gave 100 % inhibition to the pathogen growth.

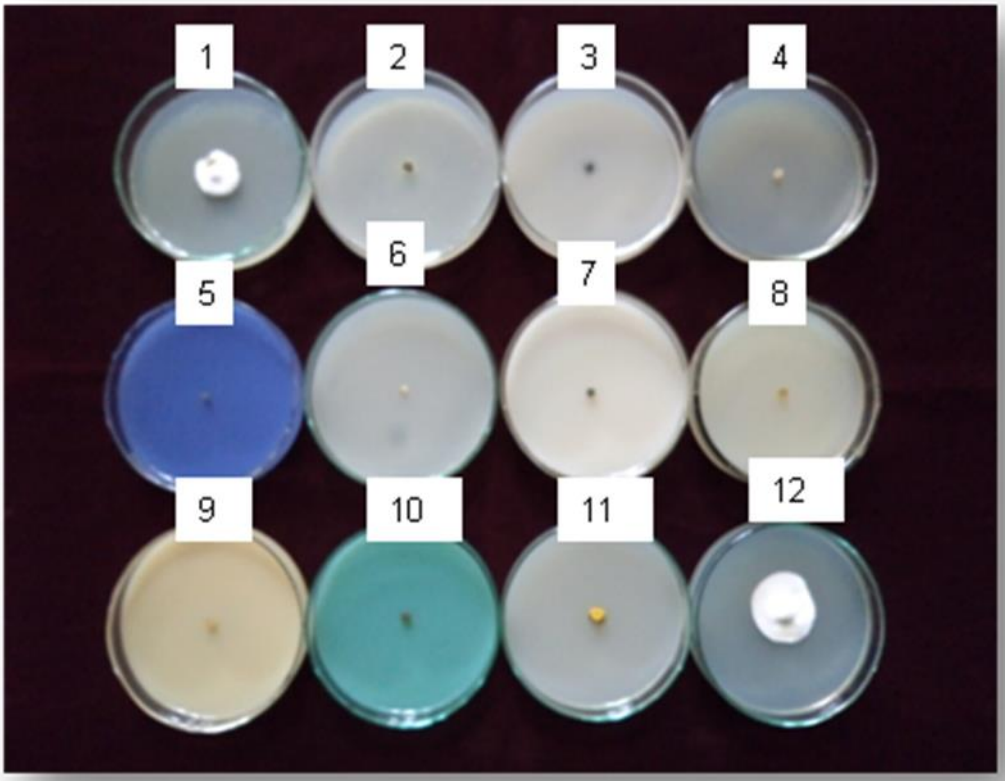
4.13.2. In vitro evaluation of fungicides by spore germination inhibition studies

The results (Table 38) of the *in vitro* evaluation of fungicides by spore germination inhibition studies revealed that all the fungicides at all concentrations gave 100 per cent inhibition to the germination of chlamydospores of *U. virens*.

4.14. FIELD EVALUATION OF BIO AGENTS AND FUNGICIDES

Five treatments selected from *in vitro* chemical management experiment, along with talc based formulations of one bacterial and one fungal biocontrol agent were tested in the field during Kharif 2012 at Palakkad and Rabi 2012-13 at Alappuzha.

The susceptible rice variety Uma was used for the field level evaluation of the fungicides and biocontrol agents (Plate 34). Transplanting was done at 25 days after sowing and a spacing of 15 X 15 cm was maintained at the main field. The design followed was RBD and three blocks were maintained. The treatments were given as foliar sprays at 50 % flowering stage. All the agronomic practices were carried out, in



1-Pencycuron,
2-Azoxystrobin,
3-Tebuconazole,
4-Propiconazole,
5-Carboxin+Thiram
6-Iprodione+Carbendazim,
7-Chlorothalonil,
8-Bitertanol,
9-Mancozeb,
10-Copper Hydroxide,
11-Thiophanate Methyl,
12-Control

Plate 33. ***In vitro*** **efficacy of the chemical fungicides against** ***U. virens***

Plate 34. Field evaluation on the efficacy of the chemical fungicide sand biocontrol agents against *U. virens*

Table 36. *In vitro* evaluation of chemical fungicides on the mycelial growth of *U. virens*

Chemical Fungicides (concentrations)	Colony Diameter of *U. virens* (cm)*		
	C-1	C-2	C-3
Chlorothalonil 75% WP (2.0, 0.375, 0.185 g/l)	0.27 (1.13)	0.46 (1.21)	1.00 (1.41)
Thiophanate methyl 70 % WP (1.25, 0.25, 0.125g/l)	1.02 (1.42)	1.17 (1.47)	1.37 (1.54)
Copper Hydroxide 77% WP (2, 0.375, 0.1875g/l)	0.00 (1.00)	0.50 (1.22)	2.01 (1.74)
Mancozeb 75%WP (2, 0.375, .1875g/l)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Bitertanol 25 %WP (1.25, 0.25, 0.125g/l)	0.55 (1.24)	0.60 (1.27)	0.75 (1.32)
Propiconazole 25 % EC (1,0.125,0.625ml/l)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Tebuconazole% 25.9 % m/m EC (1.5,0.1875,0.93ml/l)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Pencycuron 22.9 % EC (1, 0.25, 0.125g/l)	5.00 (2.45)	5.00 (2.45)	5.00 (2.45)
Carboxin +Thiram 70%WS (2, 0.25, 0.125g/l)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Azoxystrobin 23%SC (1,0.125,0.625ml/l)	0.95 (1.40)	1.01 (1.42)	1.01 (1.42)
Iprodione+ Carbendazim 50 % WP (1,0.125,0.625g/l)	0.00 (1.00)	0.00 (1.00)	0.5 (1.22)
SE	0.006	0.003	0.004
CD (0.05)	0.02	0.01	0.01

*Mean of four replications

** Values in parenthesis are square root transformed

Table 37. *In vitro* evaluation of chemical fungicides on the inhibition of the mycelial growth of *U. virens*

Chemical Fungicides (concentrations)	Colony Diameter of *U. virens* (cm)*		
	C-1	C-2	C-3
Chlorothalonil 75% WP (2.0, 0.375, 0.185 g/l)	94.55 (76.47)	90.75 (72.27)	80.00 (63.41)
Thiophanate methyl 70 % WP (1.25, 0.25, 0.125g/l)	79.51 (63.06)	76.51 (60.98)	72.50 (58.35)
Copper Hydroxide 77% WP (2, 0.375, 0.1875g/l)	100.00 (90.00)	90.00 (71.54)	59.75 (50.60)
Mancozeb 75%WP (2, 0.375, 0.1875g/l)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Bitertanol 25 %WP (1.25, 0.25, 0.125g/l)	89.02 (70.62)	87.88 (69.60)	85.00 (67.19)
Propiconazole 25 % EC (1,0.125,0.625ml/l)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole% 25.9 % m/m EC (1.5,0.1875,0.93ml/l)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Pencycuron 22.9 % EC (1, 0.25, 0.125g/l)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Carboxin +Thiram 70%WS (2, 0.25, 0.125g/l)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Azoxystrobin 23%SC (1,0.125,0.625ml/l)	81.00 (64.14)	79.75 (63.23)	79.75 (63.23)
Iprodione+Carbendazim 50 % WP (1,0.125,0.625g/l)	100.00 (90.00)	100.00 (90.00)	90.00 (71.54)
SE	0.33	0.016	0.15
CD (0.05)	0.94	0.46	0.44

*Mean of four replications

** Values in parenthesis are arcsine transformed

Table 38. *In vitro* evaluation of chemical fungicides on the inhibition of the spore germination of *U. virens*

Chemical Fungicides (concentrations)	Inhibition to spore germination of *U. virens* (%)*		
	C-1	C-2	C-3
Chlorothalonil 75% WP (2.0, 0.375, 0.185 g/l)	100	100	100
Thiophanate methyl 70 % WP (1.25, 0.25, 0.125g/l)	100	100	100
Copper Hydroxide 77% WP (2, 0.375, 0.1875g/l)	100	100	100
Mancozeb 75%WP (2, 0.375, 0.1875g/l)	100	100	100
Bitertanol 25 %WP (1.25, 0.25, 0.125g/l)	100	100	100
Propiconazole 25 % EC (1,0.125,0.625ml/l)	100	100	100
Tebuconazole% 25.9 % m/m EC (1.5,0.1875,0.93ml/l)	100	100	100
Pencycuron 22.9 % EC (1., 0.25, 0.125g/l)	100	100	100
Carboxin + Thiram 70%WS (2, 0.25, 0.125g/l)	100	100	100
Azoxystrobin 23%SC (1,0.125,0.625ml/l)	100	100	100
Iprodione+ Carbendazim 50 % WP (1,0.125,0.625g/l)	100	100	100

*Mean of four replications

time, as per the Package of Practices Recommendations by the Kerala Agricultural University (2011). Periodical observations were made for the development of symptoms on grains at the flowering period and thereafter. Observations were recorded on the per cent infected tillers, per cent infected grains, disease severity and grain yield.

Results of the field experiment conducted at Palakkad revealed that treatment with the fungicide copper hydroxide (0.67) and the bioagent *Pseudomonas* (0.67) followed by treatment with the chemicals propiconazole (0.88) and tebuconazole (0.89) recorded the lowest incidence of the disease in terms of per cent infected tillers (Table.39). The highest percentage of infected tillers was observed in the untreated control (7.56) followed by the treatments chlorothalonil and *Trichoderma*.

The treatments copper hydroxide (0.53) and *Pseudomonas* (0.57) showed lowest percentage of infected grains which differed significantly from all other treatments. The per cent infected grains were found to be the highest in case of untreated control (5.13 %). In case of disease severity, the treatments copper hydroxide (0.36) and *Pseudomonas* (0.38) recorded the lowest disease severities which were on par with the treatment Tebuconazole (1.24). The highest disease severity was recorded for the untreated control followed by the treatment *Trichoderma*.

The yield data obtained from the field experiments at Palakkad (Table.40) showed that treatment with copper hydroxide (4.6) and *Pseudomonas* (4.47) gave the highest grain yield per plot. Grain yield per plot was found to be the minimum for the untreated control (3.60).

Straw yield per plot with different treatments when compared, it was found that highest straw yield was obtained with the treatment copper hydroxide (17.00 t/ha) which was on par with that of chlorothalonil (16.57), *Pseudomonas* (16.50) and tebuconazole (15.67). Minimum straw yield was obtained in plots without any treatment (11.33). There was no significant difference observed in case of plant height and average number of tillers per plant.

Results of the field experiment conducted at Alappuzha revealed that treatment with the fungicide Propiconazole gave lowest incidence of false smut with

Table 39. Evaluation of biocontrol agents and chemical fungicides against false smut disease of rice during Kharif 2012 at Palakkad

Treatments	%infected Panicles*	%infected Grains*	Disease severity
Copper Hydroxide 77 % WP (1000 g a.i./750 L)	0.67(4.70)	0.53(4.18)	0.36
Chlorothalonil 75 % WP (1000 g a.i./750 L)	1.56(7.13)	1.67(7.39)	2.59
Iprodione+Carbendazim 50 % WP (250 g a.i./750 L)	1.33(6.63)	1.78(7.66)	2.86
Propiconazole 25 % EC (125 g a.i./750 L)	0.88(5.36)	1.62(7.30)	1.43
Tebuconazole 25.9 % m/m EC (0.1875 kg a.i./500 L)	0.89(5.33)	1.40(6.72)	1.24
Talc based formulation of *Trichoderma* @ 2 %	1.56(7.13)	2.40(8.89)	3.73
Talc based formulation of *Pseudomonas fluorescens* @ 2 %	0.67(4.70)	0.57(4.31)	0.38
Control	7.56(15.94)	5.13(13.09)	37.63
SE	0.40	0.35	0.3
CD(0.05)	1.22	1.06	0.9

*Values in parenthesis are arcsine transformed

Table 40. Efficacy of different treatments on growth parameters of rice during Kharif 2012 at Palakkad

Treatments	No. of Tillers	Plant Height (cm)	Grain yield (t/ha)	Straw yield (t/ha)
Copper Hydroxide 77 % WP (1000 g a.i./750 L)	11.33	`106.33	4.60	17.00
Chlorothalonil 75 % WP (1000 g a.i./750 L)	10.00	112.33	4.20	16.67
Iprodione+Carbendazim 50 % WP (250 g a.i./750 L)	11.00	109.33	4.30	14.17
Propiconazole 25 % EC (125 g a.i./750 L)	13.33	109.67	4.10	15.33
Tebuconazole 25.9 % m/m EC (0.1875 kg a.i./500 L)	11.00	113.33	4.17	15.67
Talc based formulation of *Trichoderma* @ 2 %	10.67	107.67	4.10	13.67
Talc based formulation of *Pseudomonas fluorescens* @ 2 %	13.33	111.33	4.47	16.50
Control	10.67	106.00	3.60	11.33
SE	NS	NS	0.10	0.49
CD (0.05)			0.29	1.5

only 0.93 per cent of the tillers affected which was found superior to all other treatments followed by treatment with the chemical tebuconazole (1.93 % infected tillers) and the bioagent *Pseudomonas* (2.03 % infected tillers) which also differed significantly from all other treatments (Table. 41). Treatment with the chemical iprodione + carbendazim was found least effective in controlling the disease with 12.80 per cent tillers affected but still significantly different from that of the untreated control (34.67 %).

Observations on the per cent infected grains show that the treatment with propiconazole, tebuconazole and *Pseudomonas* recorded lowest per cent infected grains (1.53, 1.90 and 1.97 respectively) and were on par with all other treatments except iprodione + carbendazim treatment and untreated control.

Disease severity was found to be lowest with the treatment propiconazole (1.43) but was on par with the treatments tebuconazole (3.68) and *Pseudomonas* (4.00) and, all the three treatments differed significantly from all other treatments. Iprodione + Carbendazim treatment recorded the highest disease severity (56.32).

On comparison of the grain yield obtained from each plot (Table. 42), it was found that maximum yield was recorded with the treatment tebuconazole (8.90 t/ha) followed by the treatments *Pseudomonas* (7.18 t/ha) and propiconazole (7.00 t/ha).

Different treatments, when compared based on straw yield per plot; it was found that highest straw yield was obtained with the treatment tebuconazole (20.26 t/ha) which was on par with that of copper hydroxide (19.16), both of which differed significantly from all other treatments. Straw yield was found to be minimum in case of the control plots (11.72). The treatments did not differ significantly with respect to plant height and average number of tillers per plot.

4.15. BIOCHEMICAL STUDIES

4.15.1. SDS- PAGE

Electrophoretic separation of soluble protein the infected and healthy grains of the susceptible rice variety Uma were carried out by SDS-PAGE. The results of the study showed the presence of two extra bands representing the proteins produced by the pathogen of below 3 kb size in case of the protein profile of the diseased samples

Table 41. Evaluation of biocontrol agents and chemical fungicides against false smut disease of rice during Rabi 2012-13 at Alappuzha

Treatments	%infected Panicles*	%infected Grains*	Disease severity
Copper Hydroxide 77 % WP (1000 g a.i./750 L)	4.53(12.29)	2.36(8.82)	10.70
Chlorothalonil 75 % WP (1000 g a.i./750 L)	5.97(14.13)	3.04(10.01)	18.16
Iprodione+Carbendazim 50 % WP (250 g a.i./750 L)	12.80(20.95)	4.40(12.10)	56.32
Propiconazole 25 % EC (125 g a.i./750 L)	0.93(5.52)	1.53(7.04)	1.43
Tebuconazole 25.9 % m/m EC (0.1875 kg a.i./500 L)	1.93(7.99)	1.90(7.78)	3.68
Talc based formulation of *Trichoderma* @ 2 %	6.00(14.17)	3.63(10.98)	21.80
Talc based formulation of *Pseudomonas fluorescens* @ 2 %	2.03(8.19)	1.97(7.99)	4.00
Control	34.67(36.05)	4.53(12.28)	157.16
SE	0.25	0.63	1.22
CD (0.05)	0.76	1.91	3.69

* Values in parenthesis are arcsine transformed

Table 42. Efficacy of different treatments on growth parameters of rice during Rabi 2012-13 at Alappuzha

Treatments	No. of Tillers	Plant Height (cm)	Grain yield (t/ha)	Straw yield (t/ha)
Copper Hydroxide 77 % WP (1000 g a.i./750 L)	12.67	115.33	6.11	19.16
Chlorothalonil 75 % WP (1000 g a.i./750 L)	13.00	109.67	6.96	13.08
Iprodione+Carbendazim 50 % WP (250 g a.i./750 L)	13.33	110.00	6.59	15.20
Propiconazole 25 % EC (125 g a.i./750 L)	12.33	112.67	7.00	14.57
Tebuconazole 25.9 % m/m EC (0.1875 kg a.i./500 L)	13.00	114.33	8.90	20.26
Talc based formulation of *Trichoderma* @ 2 %	11.00	112.00	5.95	12.41
Talc based formulation of *Pseudomonas fluorescens* @ 2 %	13.00	113.00	7.18	15.63
Control	12.00	110.67	5.81	11.72
SE	NS	NS	0.15	0.73
CD(0.05)			0.47	2.22

(Plate 35). In both healthy and diseased samples bands representing rice proteins were present, but in case of diseased samples, the bands of rice proteins were not very clear cut and were light indicating masking of rice proteins by the pathogen proteins.

4.15.2. Electrophoretic analysis of isozyme

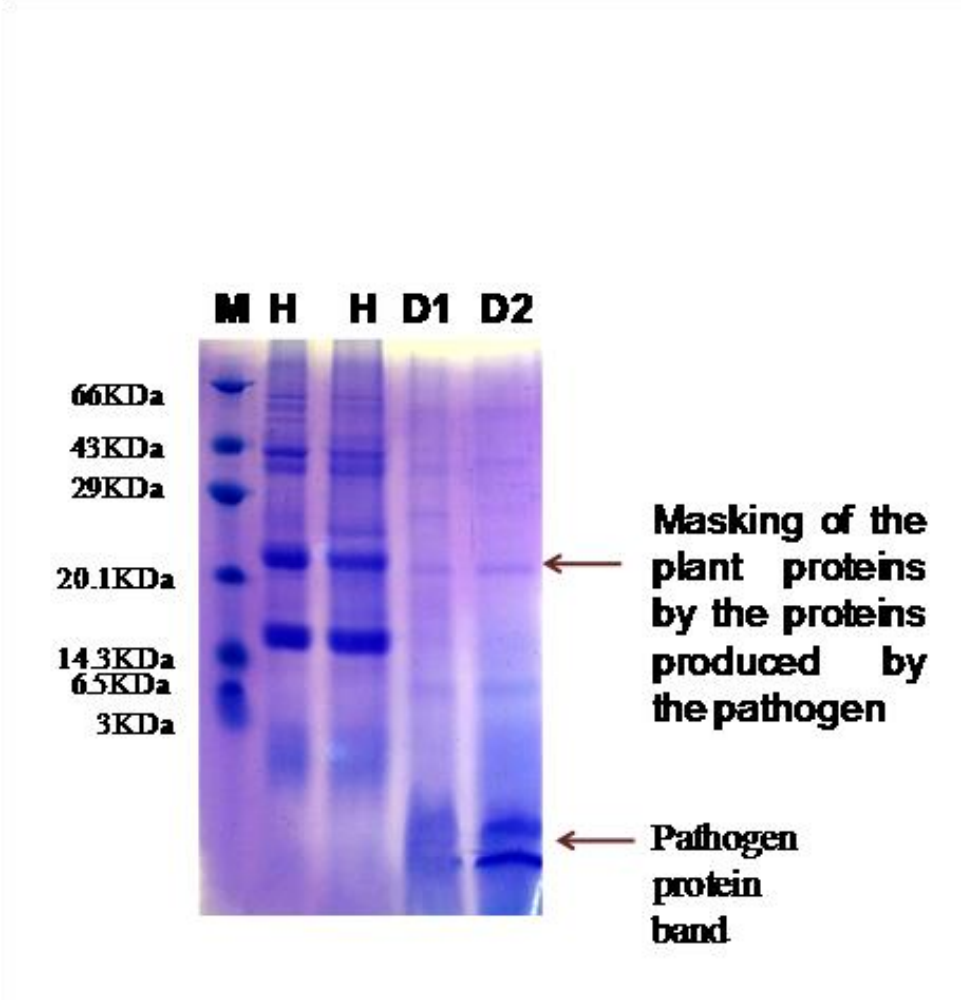
For separation of the multiple forms of enzymes (isozymes) to study the enzyme alterations in healthy as well as pathogen infected paddy grains. Here, discontinuous anionic polyacrylamide gel electrophoresis was conducted under non-dissociating conditions. The relative mobility (Rm) value of each band was calculated and Rm was represented as Zymogram.

4.15.2.1. Polyphenol oxidase isozyme analysis

Results of analysis for expression of polyphenol oxidase isozyme in healthy and diseased grain showed the presence of bands showing the activity of the isozyme polyphenol oxidase only in case of diseased samples (Plate 36 A) whereas there were no bands noticed in case of healthy plant samples. The relative mobility (Rm) value obtained was 0.33.

4.15.2.2. Peroxidase isozyme analysis

Analysis for expression of peroxidase isozymes showed an isozyme profile with bands observed only in case diseased samples (Plate 36 B) with Rm value 0.66. In healthy samples no clear bands were observed indicating the activity of the isozyme peroxidase only in case of diseased grains.



- M: protein marker
- H: protein profile of healthy grains
- D1: protein profile of infected grains at the early stages of infection
- D2: protein profile of completely infected grains

Plate 35. Protein profile of the infected and healthy grains

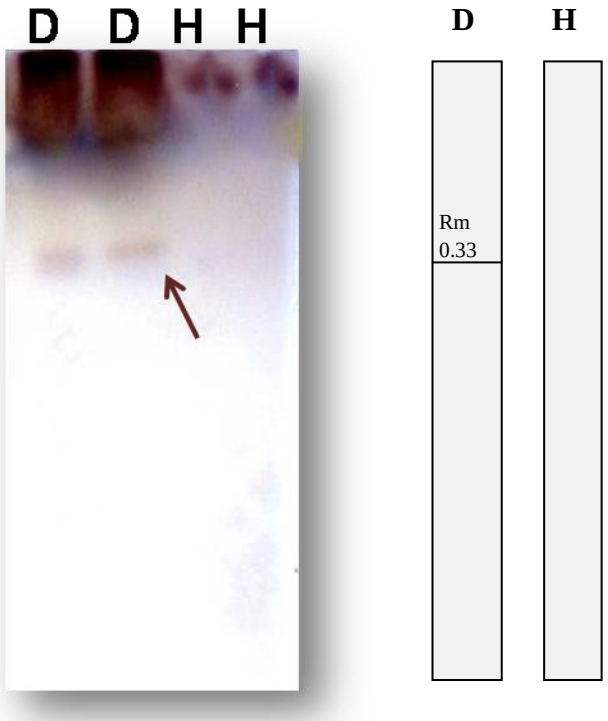


Plate 36 A. Isozyme profile and zymogram of Poly phenol oxidase

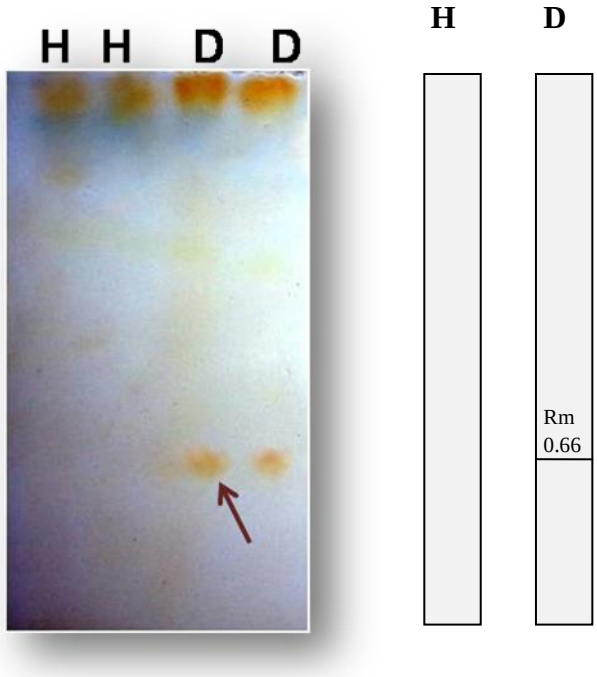


Plate 36 B. Isozyme profile and zymogram of Peroxidase

Discussion

5. DISCUSSION

Diseases are a major constraint in rice production, the management of which is essential for sustained crop production and to realize potential yields. Rice crop is challenged by a number of diseases during the various phases of its life cycle; blast, brown leaf spot, sheath blight and bacterial leaf blight.

Crop protection solely depending on chemical pesticides, with the associated problems of resistance development, ecological risks and health hazards; have given way to the concept of integrated pest management for production improvement in a sustainable manner.

False smut disease of rice, was considered only as a minor disease, without warranting any special methods of disease control, and was even considered in olden times as an indication of prosperity. Changes in production management, in terms of introduction of new high yielding and high fertilizer responsive varieties, and the continuous and extensive cultivation of such susceptible varieties over the years have led to considerable increase in the occurrence and severity of this disease all over the world.

As stated by Brooks *et al.* (2009), false smut disease will not have any visual symptoms until the grain begins to fill, at which time a fungicide application is too late to prevent yield loss and, at growth stages when control with fungicides is achievable, there is no way to determine the degree of disease severity and, thus, to determine whether the application is warranted. Therefore, integration of different management techniques is needed to control this disease at a production scale. Moreover, the knowledge concerning the disease cycle and epidemiology of *U. virens* is minimal and incomplete (Lee and Gunnell, (1992).

It is in this context, that the current study was undertaken, to investigate the epidemiology of false smut disease of rice and to evolve strategies for effective management of the disease.

Results of the field surveys conducted during 2011-2013, showed that the average of per cent infected tillers ranged from 3.3 to 31.1 % during 2011-12 and from 4.3 to 42 % during 2012-13 and the disease severity index during these periods ranged from 3.3 to 138.8 and 3.8 to 123.9 respectively. Out of the four areas surveyed, Upper Kuttanad recorded maximum percentage of infected tillers and disease severity during 2011-2012 and 2012-13. The results of the survey conducted by Ladhalakshmi *et al.* (2012 c) implied that false smut disease is emerging as one of the major diseases of rice in India and the infected tillers were found to vary between 2% and 85% in both the northern and southern parts of India.

The disease was found to be more severe at Alappuzha district compared to Palakkad. The rice growing tracts of Alappuzha (Kuttanad), are low lying areas situated at an altitude 0.6 to 2.2 m below MSL (Sheeja *et al.*, 2013). Mouton and Merny (1959) repoted that the incidence of infection by *U. virens* was always greater in the lowlands than at higher altitudes.

At Palakkad, the disease was found more prevalent during the *Kharif* season, whereas it was found more serious during the *Rabi* season at Alappuzha. Low temperature conditions existing at Alappuzha during *Rabi* season might be the reason for the increased severity of the disease at this area. Fujita *et al.* (1989) reviewed that variation in the disease assessment between different districts as well as between seasons might be referred to one or more of various environmental factors including R.H., duration of plant wetness, temperature, cultivars, differences in sowing dates, soil type, agricultural practices used, fungal virulence and host resistance.

The pathogen was not found to be producing the true sclerotia at the field conditions. Studies conducted by Singh and Dube (1976) revealed that sclerotia are not produced in plains but are found to be produced in high altitude regions.

The disease was found to be more severe in medium duration, high yielding variety, Uma and more prevalent and recurring in fields having continuous cultivation of this variety. Ahonsy *et al.* (2000) reported that the continued cultivation of susceptible varieties leads to a buildup of the disease inoculum in the field. Rao (1964) observed that rice varieties of medium duration were most affected by false smut, while those of shorter and longer duration were comparatively less affected, and he attributed this to higher relative humidity during the period of flowering of the medium duration varieties. Ladhalakshmi *et al.* (2012 a) reported that false smut disease had been observed in severe form since 2001 in India due to widespread cultivation of high fertilizer-responsive cultivars and hybrids.

During the surveys it was observed that in the fields with previous record of false smut over the years, the disease was found more severe. This may be due to the survival of the pathogen in the soil or crop stubbles in the field, initiating infection in the next season. Kulkarni and Moniz (1975) suggested the chlamydospore balls hibernating in the soil may initiate primary infection in the case of false smut.

The studies on the symptomatology of the disease revealed that the pathogen was found to infect the rice plants at the flowering stage of the crop and due to the infection by the pathogen the individual spikelets of the panicle were found to get transformed in to yellow to orange coloured ball like structures called as smut balls or pseudosclerotia having almost double the size of the normal rice grains which contained the chlamydospores of the pathogen.The symptoms observed are similar to the depictions given by Mulder and Holliday, (1971), Ou (1972) and Singh and Dube, (1976).

Based on the extent of damage caused by the pathogen in the field, a new 0-6 scoring system was developed for assessing the disease severity of the false smut disease of rice, taking in to consideration, the chaffiness and blackening symptoms which were also found to be produced by the pathogen in the field. Ahonsi and Adeoti (2002) used a 1-9 scoring system for rating false smut based on the percentage of the panicles infected whereas Lu *et al.* (2009) followed 0-5 scoring system based on the number of smut balls infected per panicle.

The previously reported methods of isolation when found less promising due to increased contamination, a new isolation method was standardized using 0.1% mercuric chloride treatment of the smut balls for 3 to 3 ½ min followed by three washings with sterile distilled water dusting over solidified YPPDA or PSA and pure culturing the next day after observation under a light microscope. This method was useful for consistent and rapid isolation of the pathogen. It was reported by (Yong-li *et al.*, 1999) that the method of chlamydosporal suspension was ideal for rapid isolation of the fungus. Ladhalakshmi *et al.* (2012 a) found a method of streaking of the chlamydospore mass of the pathogen on to potato dextrose agar (PDA) medium to be useful in isolating the pathogen.

Seven isolates of the pathogen were obtained from diseased specimens collected from different parts of the state. The isolates were characterized based on their colony morphology i.e., colony, colour, texture and appearance (Fig 1). Up on comparison of the growth of the isolates on YPPDA, it was found that the isolate Uv 7 was found to be showing maximum growth which was selected for further studies on account of its fast growth. Sporulation of different isolates of the pathogen (Table 8) and the pigment production was studied by growing the pathogen isolates in Potato Sucrose Broth and the results indicated that production of secondary conidia was found to be very high in case of the isolate Uv 4, whereas the isolate Uv 2 was found to produce maximum smut balls and chlamydospores. Verma and Singh (1988) collected samples of rice infected by *U. virens* from Almora, Cuttack, Karnal,

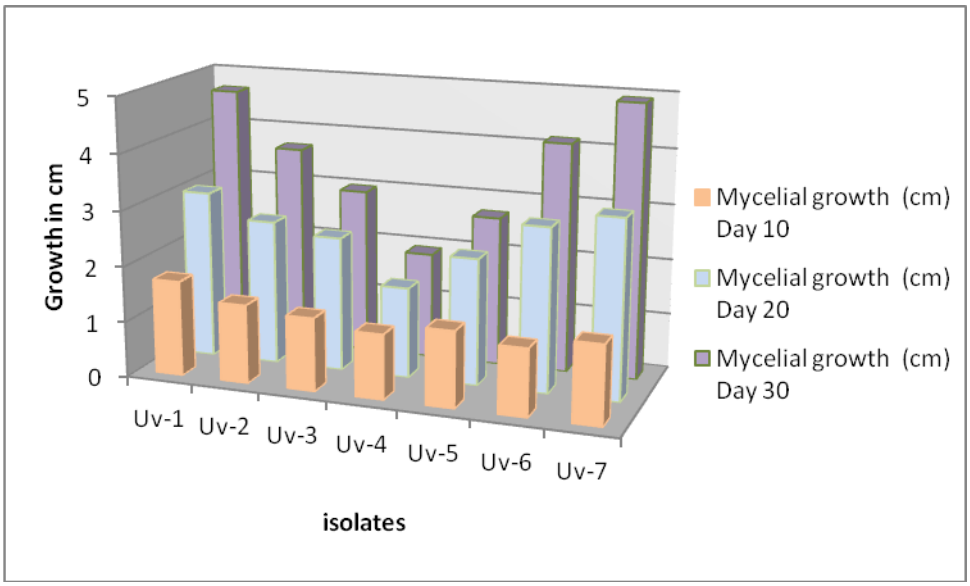


Fig 1. Comparison of growth of different isolates of *U. virens* on YPPDA

Lakhimpur, Meerut, Pantnagar and Varanasi, India and studied the differences in symptoms, conidial morphology, cultural characters and conditions of sporulation for the isolates and accordingly classified the isolates into different groups.

The pathogen was identified based on its colony morphology, spore morphology and by DNA barcoding. The pathogen produced pure white coloured with smooth colony margin and upon continuous incubation the colour changed to light yellow and in some instances to green. The appearance of the culture was similar to that described by Ladhalakshmi *et al.* (2012 b).

The pathogen produced both chlamydospores and secondary conidia in culture. The chlamydospores were yellow coloured round, 4.9-7.3µm in diameter, double walled, when mature, had highly ornamented wall layers.The chlamydospores germinated by production of a germ tube at the end of which one to five primary conidia were produced. The secondary conidia were oval in shape, produced on specialized hyphal structures and measured 2.8-4.8µm in length and 1.5-3.1µm in breadth. Similar morphology for the chlamydospores and secondary conidia were described by Rush *et al.*, (2000), Jun-Cheng *et al.*, (2003) and Ladhalakshmi *et al.* (2012 a).

Secondary conidia were produced by the pathogen at three phases viz., at the time of germination of the chlamydospores, at the initial phases of colony establishment of the pathogen and during stress conditions. *In vitro* studies on the sequence of sporulation (Rong-Hui *et al.*, 2010) reported that in culture, the fungus produced conidia mainly in the early 20 days on the specialized hyphal structures, and in the later stages it produced great amount of chlamydospores in the yellow fruiting bodies.

The ITS regions of the pathogen were sequenced for the identification and molecular characterization and for studying the variability of the pathogen. Sequencing of eight isolates of the pathogen including an isolate (Uv 8) from a

specimen from Rajendranagar, Hyderabad was done using universal primers of ITS and the sequences were deposited in GenBank. BLAST analysis was carried out to find out the relationship of the isolates sequenced with the known sequences of *V. virens* from the GenBank. The results of BLAST analysis (Fig.2, Fig 3, Plate 14, 15, Appendix V) showed that the isolates had 100% identity with the known isolates of *Villosiclava virens.* Ladhalakshmi *et al.* (2012 a) confirmed the identity of the fungus *U. virens* through PCR using specific primers, *viz*., US1-5/US3-3 and US2-5/US4-3.

It was found that ITS sequences of isolates from different parts of Kerala and the isolate from Hyderabad were one and the same indicating that there was no variation present among the isolates from Kerala and Andhra Pradesh. Lu *et al.,* (2009) found variability in *U. virens* isolates to be influenced by the site of isolate origin, the rice hybrid from which the isolate was collected and the parental combinations of the rice hybrids.

The inoculation experiments for proving of pathogenicity showed positive results only in case of plants kept in an incubator at controlled temperature conditions with injection method of inoculation done on leaf sheath of flag leaf. It was observed that the disease appeared in the plants injected with the spore suspensions of the pathogen, after panicle emergence at 15 days after inoculation. A single grain of the inoculated panicle got converted in to a smut ball of enlarged size compared to normal rice grains which contained chlamydospores of the pathogen. Thus it is inferred that the disease can be incited artificially only under controlled temperature and humidity conditions. The results obtained were consistent with that of Ashizava *et al.* (2010) and Xiu-Juan *et al.* (2011b). Ikegami (1960) successfully inoculated rice plants with chlamydospores and ascospores by injecting a spore suspension in to the leaf sheath enclosing the young panicle.

Deposition of spores within the individual spikelets did not yield any results unlike the report Kulkarni and Moniz (1975) who got the rice plants infected with the

pathogen by applying chlamydospore suspensions with a camel hair brush both to fertilized and unfertilized ovaries. Wang-Shu *et al.,* (2008) reported that estimates of mean disease severity were higher for injection than for spraying as assessed by per cent infected panicles and low temperature exposure after inoculation had a strong stimulatory effect on disease development. Hong-Sik *et al.* (2001) could not induce the disease by artificial inoculation of the pathogen.

The optimum conditions for culturing the pathogen were standardized under *in vitro* conditions. Upon evaluation of seven different solid and liquid media, it was found that in case of solid mediaYPPDA was found to be the best (Table 10, Fig 4) for culturing of pathogen followed by PSA. In case of liquid media, PSB (2.02 g) was found to be the best with highest dry weight production but was found to be on par with YPPDB with 1.98 g dry weight. Sharma and Joshi (1975) also got similar results with YPPDA. In several studies on *in vitro* culturing of the pathogen, Potato sucrose agar (PSA) was found to be the best for fast mycelial growth and dry weight (Fu *et al.* 2013, Li-Yang *et al.* 2008 and Xiu-Juan *et al.*, 2011a).

Results of the sporulation studies of the pathogen in terms of production of secondary conidia and chlamydospore by the pathogen indicated that secondary conidia production was found to very high in PSB and RESB (Table 12) whereas the smut ball and chlamydospore production was found to be the highest in PSB, RYDB and RESB thus, potato sucrose broth was found to be supporting production of both chlamydospores and secondary conidia consistent with the results by Wang-Shu *et al.*, (2008) and Hai-Yong *et al.* (2011).

Studies on pigment production by *U. virens* in different liquid broths revealed that the pathogen gave dark yellow pigmentation in RYDB, and in XBZB it gave reddish yellow pigmentation. Hong and Wei (1999) extracted the green pigment from *Ustilaginoidea virens* using 80 % ethanol suggested that this natural pigment could be used as coloring additive in medicine, food stuffs and beverages. Yabuta *et*

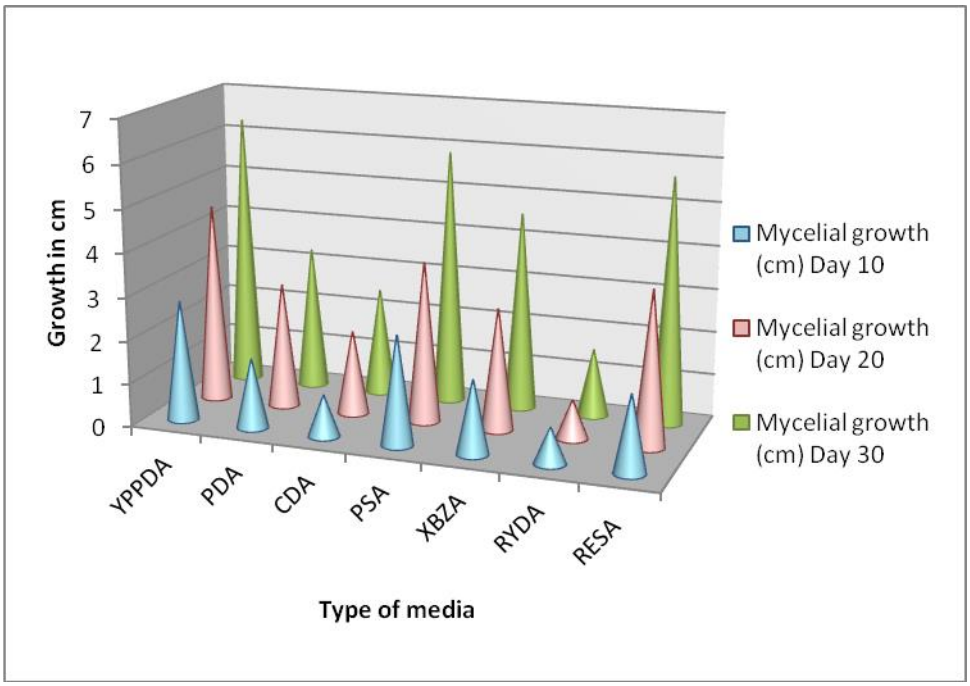


Fig 4. Colony diameter of *U. virens* on different solid media

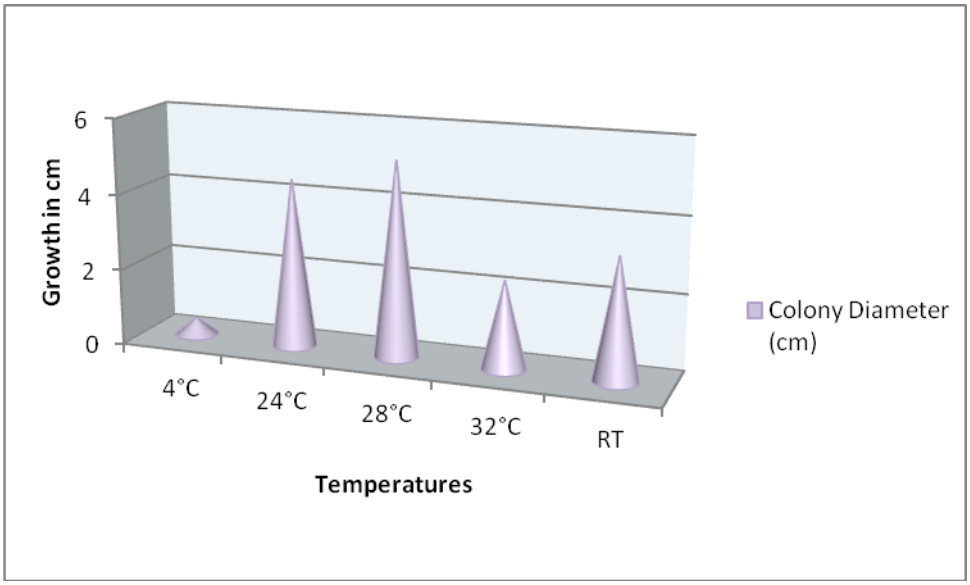


Fig 5. Colony diameter of *U. virens* at different temperature conditions

al. (1933) first isolated a pigment, designated as ustilaginoidin, from the ether extract of the balls which exhibited weak cytotoxicity to human epidermoid carcinoma cells as reported by Koyama *et al.* (1988).

The optimum temperature for the growth of the pathogen when determined under *in vitro* conditions (Fig 5), it was found that the growth was found to be the maximum at 28°C (5.15 cm) which significantly differed from the second best treatment i.e., 24°C. The result is in accordance with that by Hashioka *et al.* (1951), Chen *et al.* (1994) and Yan-Hui *et al.* (2011) and Li-Mei *et al.* (2012).

The results of the *in vitro* studies on the optimum pH conditions for the growth of the pathogen revealed that the pathogen was found to give maximum growth at pH 6.5 (Table 14, Fig 6), consistent with the results of Hashioka *et al.* (1951) and Li-Mei *et al.* (2012) but different from the results by Fu *et al.* (2013) who got the optimal growth observed at pH 7 to 8.

In vitro studies on the optimum light conditions (Table 15, Fig 7) for culturing *U. virens* revealed that the pathogen was found to be giving maximum growth at full darkness (4.42 cm) which was significantly different from all other conditions of light. Fu *et al.* (2013) also got the same result and reported that the mycelial growth rate of *U. virens* was significantly higher in the dark than in 12 h alternating cycles of dark and fluorescent light, and found that fluorescent light inhibited mycelial growth.

The results of the *in vitro* viability studies on the smut balls and the chlamydospore of the pathogen showed that, under soil conditions both the pseudosclerotia and the chlamydospores remained viable up to three months only, but the viability percentage of the smut balls was considerably higher in comparison with that of the chlamydospores. Under crop residue of paddy, the smut balls had very high viability (8 months) compared to the chlamydospores (three months). The smut balls stored in the clayey soil remained viable only up to three months (from October 2012 to December 2012) and the viability percentage at the third month of storage

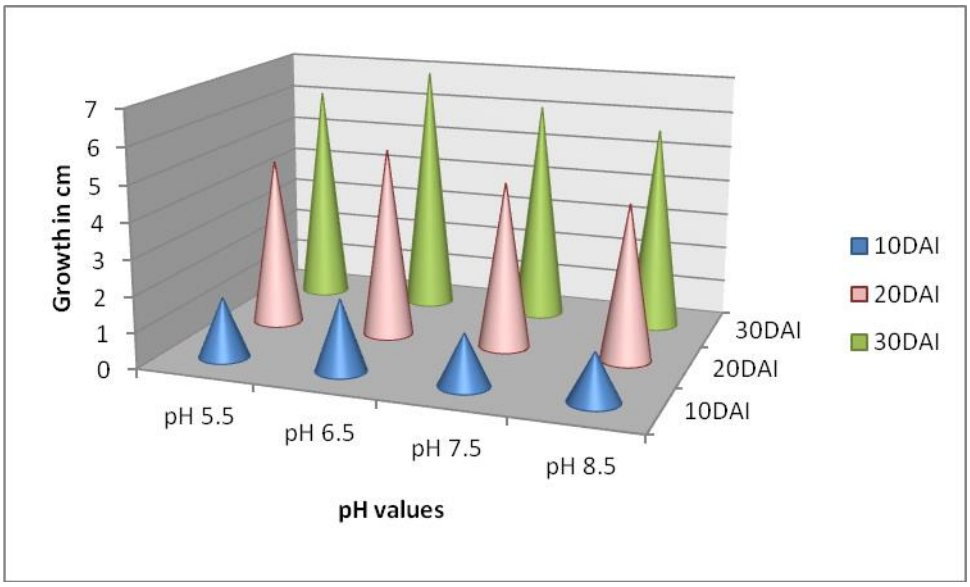


Fig 6. Colony diameter of *U. virens* at different pH conditions

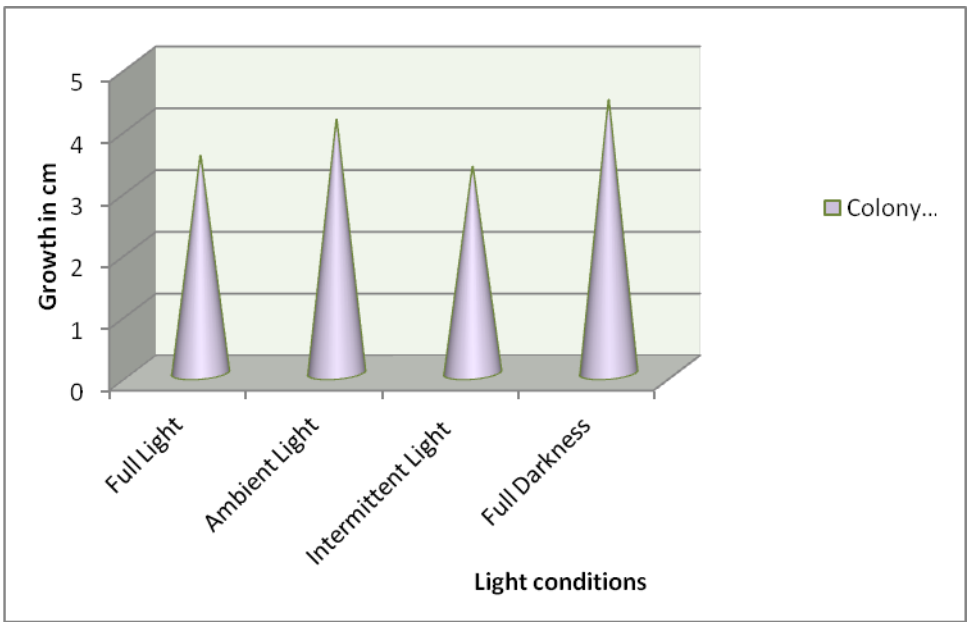


Fig 7. Colony diameter of *U. virens* at different light conditions

was found to be very low (12 %). Up on continuous storage under the soil, the smut balls were found to get rotten and got converted in to black sticky masses similar to the report by Singh *et al.*, (1985).

The chlamydospores were able to survive only up to three months within the crop residue and within soil and were viable only up to four months on the seeds of paddy indicating the very low survival ability of the chlamydospores.

Singh *et al.*, (1985) reported the chlamydospores to be remaining viable only up to three months in room temperature and two months in the soil but the pseudosclerotia to be surviving for six months and for four months respectively, and inferred that the true sclerotia of the pathogen may be the primary source of inoculum for the disease. In Karnataka, the chlamydospores were found to be surviving up to four months and the pseudosclerotia survived up to seven months in paddy straw (Yashoda *et al.*, 2000).

Loss of germination ability of chlamydospores within three months of incubation indicates that the probability of the chlamydospores acting as the primary source of inoculum for the next season is very low and thus these may aid only in the spread of the pathogen during the active crop season. Thus, it is more likely that the smut balls surviving within the crop residue will be acting as the primary inoculum for the onset of the disease in the next season. The low survival ability (three months) of the pseudosclerotia in the soil may be attributed to the activity of soil micro organisms parasitizing the smut balls. Singh *et al.*, (1985) observed that the pseudosclerotia stored under field conditions on continued incubation got rotted up due to the growth of saprophytic fungi, mostly *Aspergillus* and *Penicillium* species and got disintegrated in to black powdery mass.

None of the propagules survived more than eight months in all the cases. In Kerala conditions, where two or three rice crops are raised per year, the pathogen can survive through the seasons with the availability of the crop in the field in most part

of the year. Thus, the chlamydospores produced abundantly from a late sown crop can act as the source of inoculum for the next season in areas raising three crops per year. The pseudosclerotia might be acting as the source of primary inoculum for the disease in Kerala owing to its high survival ability. The availability of the surviving chlamydospores or pseudosclerotia all through the year may be the reason for the recurrent occurrence of the disease in the major rice growing areas of the state.

The survival studies of *U. virens* on different collateral hosts under artificial inoculation, on common weeds of rice field showed that none of the weeds were found to take up infection. This result is in accordance with the report by Yashoda (1998).

The survival of the pathogen under the field conditions was observed during the surveys and field experiments. During the survey conducted at Palakkad, at Kizhayur area, the pathogen was found to be surviving on the ratoon rice plants of variety Uma that emerged from the stubbles of the previous crop. A few grains of the panicle had got converted in to smut balls, and in the whole area, a few plants were found affected. From this observation it is inferred that the pathogen may be surviving in the stubbles of the previous crop and would be able to start fresh infection in the next season crop. This is the first report of false smut disease to be observed on ratoon rice emerging from crop stubbles.

The survival of the pathogen under the field conditions on collateral hosts was observed during the surveys mainly on graminaceous weeds. Most of the weed plants observed were free of infection. But from Ambalappuzha area of Alappuzha district, the pathogen was observed on weedy rice, *Oryza spontaneum* (Plate 23) indicating the survival ability of the pathogen on collateral hosts. Rao and Reddy (1955) reported false smut disease on *Oryza officinalis* Wall from India. Other reported weed hosts include *Digitaria marginata* L., *Panicum trypheron*, *Echinochloa crus-galli*, *Imperata cylindrical* and *Chionachne koenigi* (Shetty and Shetty, 1985; Shetty and Shetty 1987; Atia, 2004 and Ladhalakshmi *et al.*, 2012 c). Shetty and Shetty 1987

suggested that weed hosts like *Digitaria marginata* act as important sources of inoculum between seasons.

The pathogen was observed to be dispersed by means of chlamydospores produced on the smut balls. The chlamydospores, at the initial stages of formation of smut balls, were found to be sticky, not easily detached from the smut balls. In the later stages, upon maturity, these became loosely attached on the smut balls which enabled them to be blown away even with a light breeze and were found to aid in the spread of the pathogen to the adjacent plants or fields. Thus, chlamydospores have an important role within season and spread of the pathogen. Ladhalakshmi *et al.*, (2012c) reported that chlamydospores play an important role in the secondary infection and spread of the disease in the field. It was found that the maximum incidence of spores in the air occurs over a period of about 4 weeks which coincides with the 'heading' phase of the rice plants, indicating that both early and late infections are possible (Sreeramulu and Vittal, 1966).

The weather parameters prevailing in the field at the time of various field trials were correlated with the per cent infected tillers and disease severity so as to study the influence of weather parameters on the development of the disease. The results of this study indicate that disease severity and per cent infected tillers were negatively correlated with the minimum temperature at 50 % flowering period. Minimum temperature at 15 days, seven days and five days around the day of 50 % flowering and average of seven days after flowering were found to be significantly negatively correlated with the disease severity and per cent infected tillers with false smut disease. This result is in accordance with the findings by Singh (1974) stating that the disease was favoured by the prevalence of lower minimum temperatures. Several reports came on low temperature conditions favouring the disease (Singh *et al.*, (1987a), Singh *et al.* (1987 b) and Naito 1994). The result is against the findings by Yashoda *et al.* (2000c) who reported that the disease is favoured by high minimum temperature. Lu-ChuanGen *et al.* (2003) stated that temperature was the primary climatic factor influencing the incidence of rice false smut.

There was no significant correlation observed between the disease and the maximum temperature during 50 per cent flowering period in the current study (Table. 21) unlike the findings by Yashoda *et al.* (2000c), indicating that low maximum temperature, (< 31 °C), was found to favour the disease.

Relative humidity at morning hours, evening hours and the daily mean relative humidity at seven days after 50 per cent flowering was found to be significantly negatively correlated (Table. 22, 23 and 24) with the disease severity and per cent infected tillers. This is in accordance with the observation by Singh (1974) that the disease is favoured by lower relative humidity later in the flowering period.

There was no significant correlation between the average rainfall during 50 per cent flowering period with the disease severity and per cent infected tillers (Table. 25) unlike the reports by Bhagat *et al.*, (1993), Yashoda *et al.* (2000c) and Jian-Ping *et al.*, (2009). As per the report by Bhardwaj (1990) there was no correlation of disease severity with the days to 50 percent flowering or with the environmental conditions during the flowering period.

The results of the varietal screening trial conducted during Kharif 2012 at Palakkad and at Alappuzha during Rabi 2012-13 indicated that out of the twenty varieties screened, the varieties Harsha and Vaishak released from Regional Agricultural Research Station Pattambi, Palakkad were found highly resistant (completely disease-free) and the varieties, Makom, Thekkancheera, Pavizham and Karthika were found resistant (disease severity value < 1) to the disease (Table 29). The varieties Kanakom, Revathi and Prathyasha showed moderate resistance with a disease severity value of 1-5 (Table 2). Ten varieties *viz*., Bhadra, Aruna, Remanika, Krishnanjana, Gouri, Bhagya, Vytila 6, Jyothi, Kanchana and Athira were found susceptible to the disease with 5-20 disease severity index. The variety Uma (Plate) was found highly susceptible (severity value >20) to the disease (Plate 25).

The results indicate that the resistant varieties Harsha, Vaishak Makom, Thekkancheera, Pavizham and Karthika can be used as donor parents in future breeding programmes. The varieties Makom, Pavizham, Karthika and Harsha being comparatively high yielding, can also be recommended for cultivation in disease prone areas.

Resistant varieties reported against false smut include Kranti (Shrivastava 1976), Cauvery, Kanchi, IR20, Jagannath and Pankaj (Singh 1974), Paicos 1 (Singh 1987a), Rajshree (Singh *et al.* (1989), Savithri and IET-9710 (Ram and Ansari, 1989), Panidhan and Mayurkantha (Dhal and Mohanty 1996), and HRI 119 (Mohiddin *et al.* 2012).

Rice cultivars played an important role in the degree of rice false smut infection (Singh *et al.,* 1987a). Lu *et al.*, (2009) reported a linkage between pathogenicity of isolates and resistance of rice hybrids. Pathogen shows high variability with respect to varieties used. The differences between rice cultivars to rice false smut can be attributed to the differences in the genetic makeup of the tested cultivars in addition to the environmental factors that might affect the host-pathogen interactions (Walker, 1975).

Resistant varieties obtained under this trial *viz*., Harsha and Makom, are early maturing which could be a reason for the resistance shown by them. Rao (1964) observed that rice varieties of medium duration were most affected by false smut, while those of shorter and longer duration were comparatively less affected. Singh and Khan (1989) found that early maturing rice genotypes escaped from rice false smut infection. (Parsons *et al.* (2001) and Su-Xin (2005) also reported the same. Rui-Rong *et al.* (2010) reported that early-maturing varieties had higher resistance than medium-late maturing varieties, Indica varieties had higher resistance than Japonica varieties and, the three-line combinations had higher resistance than two-line combinations. Yong - Li *et al.* (2010) also reported significant correlations to be existing between the disease severity and cultivar maturity, late-maturity cultivars

being significantly more susceptible than early-maturity cultivars and suggested planting the early maturing group cultivars to decrease the severity of false smut.

Resistance exhibited by the varieties against the disease can be as a result of their morphological traits, inherent defense mechanisms or due to the presence of resistant genes. Singh and Singh (2005) reported flag leaf length to breadth ratio to have highly significant positive correlation with false smut disease. According to Dai-Hua *et al.* (2008) there was a positive relationship between the plant height and the disease index, against the findings by Bhardwaj (1990), who found short cultivars to be more vulnerable than tall ones. Guang-Hui *et al.* (2005) observed a higher level of lignin content in the resistant cultivars than in the susceptible cultivars.

In order to have an insight in to the molecular basis of the resistance shown by the resistant varieties, RAPD profiling was carried out. RAPD analysis of the resistant varieties, Makom, Harsha, Thekkancheera, Vaishak, and the susceptible varieties Bhagya, Gouri, Remanika and Uma with two random primers, showed that with the primer OPB-10, there was one polymorphic amplification product in case of the variety Harsha, and with the primer OPK-14, one specific amplicon was obtained with the variety Makom and two with the variety Thekkancheera.

RAPD profiliing using the pooled DNA from the resistant and susceptible varieties using ten random primers yielded unique products in case of pooled resistant varieties with respect to the primers OPG-18 (at 300 bp position), OPC-15 (at 400 bp position) and OPD-18 (at two positions *viz*., 1000-1500 bp and 250-500 bp).

Yu-Sheng *et al.* (2008) reported that the resistance to rice false smut had significant major gene effect, and not only major gene's effect but also polygene's effect should be considered in breeding for resistance to rice false smut. Yu-Sheng *et al.* (2011) identified Seven QTLs controlling false smut resistance on chromosomes 1, 2, 4, 8, 10, 11, and 12, respectively and suggested that QTLs *qFsr11* and *qFsr12* can be used in rice breeding for resistance to rice false smut in marker-assisted selection (MAS) program.

The results of the *in vitro* evaluation of fungal antagonists showed (Fig 8) that all the isolates of *Trichoderma* were effective in controlling the pathogen but there was no significant difference among the isolates in the per cent inhibition of the pathogen. All the isolates started overgrowing the pathogen colony from the third day onwards and completely covered the plate the fourth day. Lysis of the pathogen colony was apparent after five days with the isolates Tri-3, Tri-5, Tri-6 and Tri-7. Zhi-Huai *et al.* (2010) and Liu *et al.* (2010) demonstrated the efficacy of *Trichoderma* against *U. virens* under *in vitro* conditions also showed inhibition of conidial germination and secondary spore formation of *U. virens*. Chet *et al.*, (1981) observed direct growth of mycoparasite towards the pathogen, attachment of appressoria-like structures to the host cell wall, parallel growth, coiling of antagonist hyphae around pathogen's hyphae and also production of bulbular or hook like structures and granular cytoplasm of the hyphal cells of the pathogen in the parasitism of *Pythium* and *R. solani* by *Trichoderma hamatum*. The effectiveness of the potential biocontrol agent, *Trichoderma* was reported by many researchers (Gokulapalan, 1989; Mishra, 2010; Yamini, 2011).

Studies on the mycoparasitic effect of *Trichoderma* on *U. virens* showed *Trichoderma* to be exhibiting various signs of mycoparasitism like, parallel growth, formation of hook like structures, formation of chlamydospores etc. Due to the parasitism by *Trichoderma*, the pathogen hyphae showed granulation of cytoplasm, vesiculation and lysis. Coiling and lysis of pathogenic hyphae by the *Trichoderma* spp. were reported by Gokulapalan (1989); Rashmi (2010) and Yamini, (2011).

Culture filtrate studies conducted on the efficacy of *Trichoderma* culture filtrates on inhibiting pathogen growth (Fig 9) revealed that, *Trichoderma* culture filtrate at 70 per cent concentration gave maximum inhibition (81.36) of the radial growth of the pathogen which differed significantly from all other concentrations followed by 60 per cent concentration (71.50). The efficacy of culture filtrates of *Trichoderma* against different pathogens has been demonstrated by Rashmi (2010) and Mishra (2010).

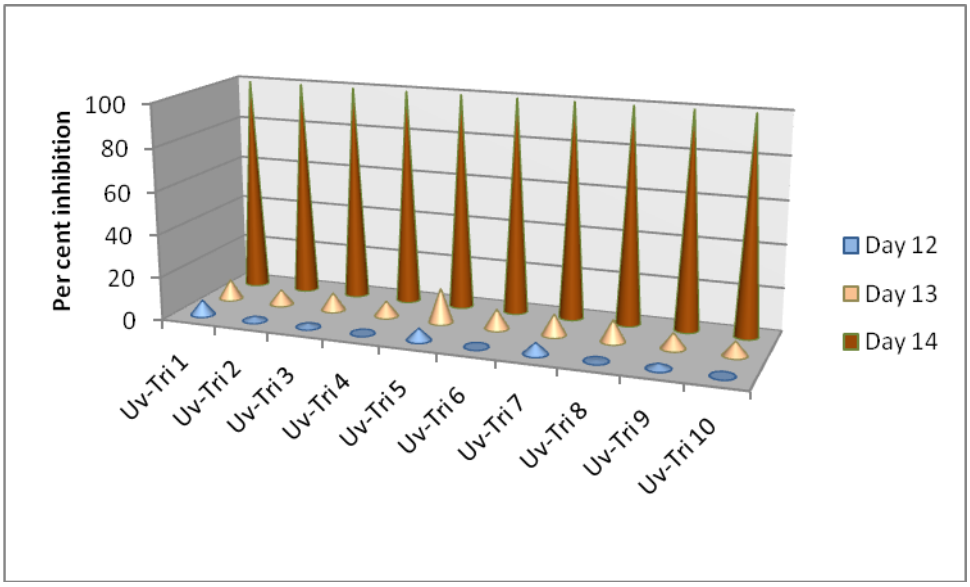


Fig 8. Per cent inhibition of *U. virens* by *Trichoderma* on YPPDA in dual culture

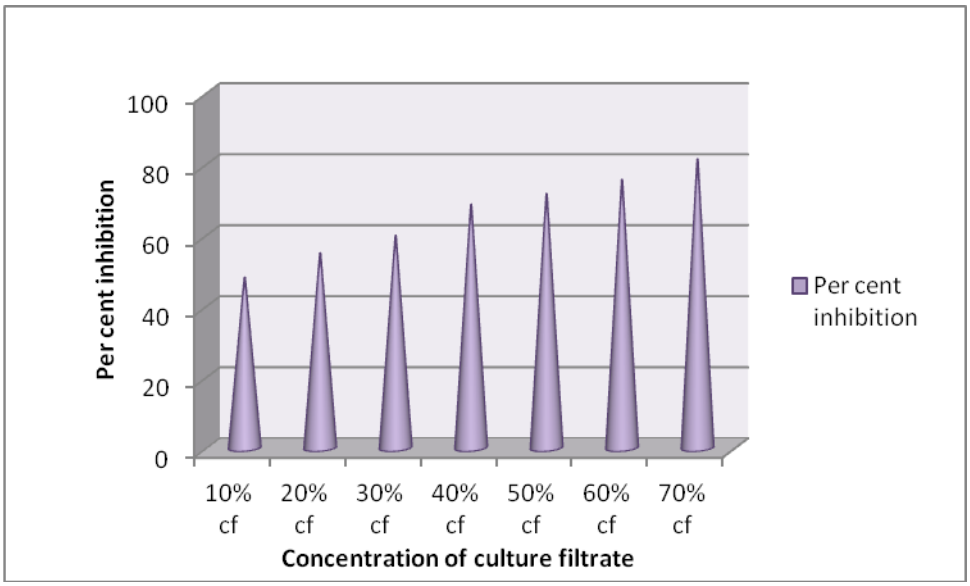


Fig 9. Per cent inhibition of *U. virens* by culture filtrates of *Trichoderma*

Studies conducted to determine the efficacy of *Trichoderma* volatiles on the pathogen showed that the growth of the pathogen was found to be getting inhibited by the effect of volatile compounds produced by *Trichoderma.* Similar results were obtained by Rashmi (2010) with the pathogen *Macrophomina phaseolina*. As per the reports by Eziashi *et al.* (2006), metabolites produced by *Trichoderma* spp. gave 2-64 per cent inhibition by volatiles, 0-74 per cent by non-volatiles and 0-81 per cent from direct diffusible metabolites against *Ceratocystis paradoxa.*

Isolates of the bacterial antagonists obtained from spermosphere and rhizosphere of rice plants were tested for antagonistic property against the blast pathogen,by Kirby-Bauer test. The results of the study (Fig 10) indicate that the bacterial isolates B4, B5 and B6 were found to give maximum inhibition (82.60) of the colony growth of *U. virens* was found to be on par with B3 (80.48). Efficacy of various isolates of *Bacillus* was shown against *U. virens* by many researchers [Shi-Le *et al.* (2004), Yong-Feng *et al.* (2007), Ya *et al.* (2010), and Xiao-Le *et al.* (2011)].

The results of the *in vitro* evaluation of fungicides (Fig 11) revealed that six chemicals viz., copper hydroxide, mancozeb, propiconazole, tebuconazole, carboxin and iprodione+ carbendazim gave 100 per cent inhibition to the growth of the pathogen under *in vitro* culture conditions and differed significantly from all other treatments followed by the chemical chlorothalonil (94.55). Even at the lowest concentration, the fungicides mancozeb, propiconazole, tebuconazole and carboxin gave 100 per cent inhibition to the pathogen growth. Verma and Singh (1987) reported the chemicals Du-Ter [fentin hydroxide], TCMTB, brestanol [fentin chloride], aureofungin and difolatan [captafol] to be effective against *U. virens in vitro.* Yashodha *et al.* (2000) reported carbendazim, mancozeb and carbendazim + mancozeb to be inhibiting mycelial growth completely. Ahonsi and Adeoti (2003) demonstrated the efficacy of copper oxychloride, benomyl, and thiabendazole.

The results of the *in vitro* evaluation of fungicides by spore germination inhibition studies (Table 38) revealed that all the fungicides at all concentrations gave 100 per cent inhibition to the germination of chlamydospores of *U. virens* consistent

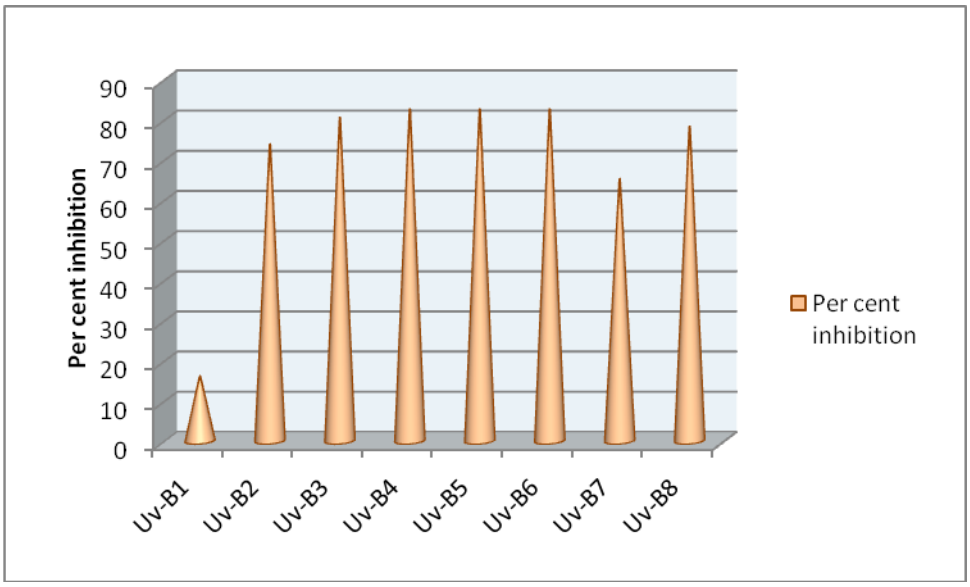


Fig 10. Per cent inhibition of *U. virens* by isolates of bacteria on YPPDA in dual culture

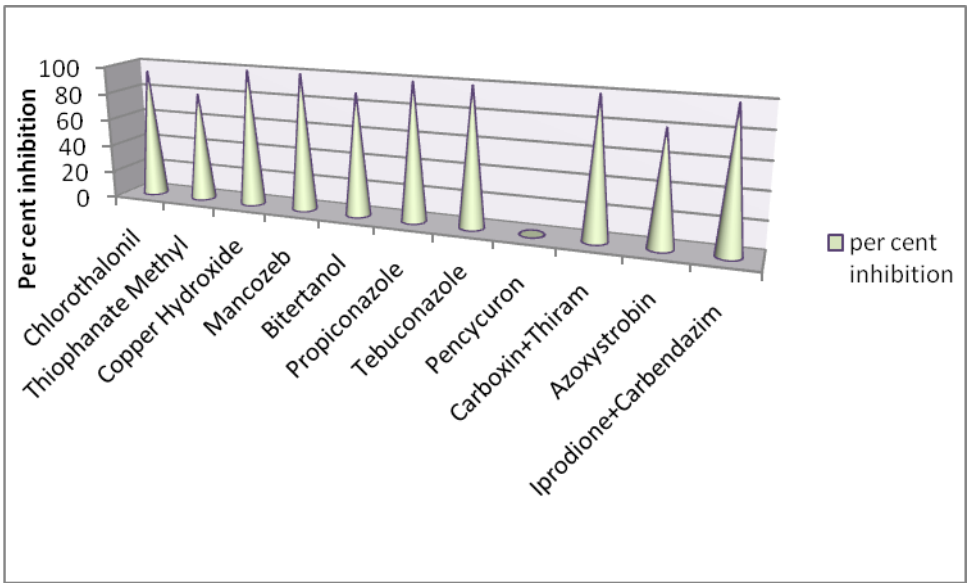


Fig 11. Per cent inhibition of *U. virens* by recommended concentrations of fungicides *in vitro*

with the results by Yashodha *et al.* (2000). Ikegami, (1958) reported the *in vitro* efficacy of Mercurials causing 100 per cner inhibition of chlamydospore germination even at very low concentrations.

Five treatments selected from *in vitro* chemical management experiment, along with talc based formulations of one bacterial and one fungal biocontrol agent were tested in the field during *Kharif* 2012 at Palakkad and *Rabi* 2012-13 at Alappuzha with the susceptible variety Uma.

Results of the field experiment conducted at Palakkad revealed that treatment with the fungicide copper hydroxide (0.67) and the bioagent *Pseudomonas* (0.67) followed by treatment with the chemicals propiconazole (0.88) and tebuconazole (0.89) recorded the lowest incidence of the disease in terms of per cent infected tillers (Fig 12). In case of disease severity, the treatments copper hydroxide (0.36) and *Pseudomonas* (0.38) recorded the lowest disease severities (Fig 13) which were on par with the treatment tebuconazole (1.24). The treatments copper hydroxide (0.53) and *Pseudomonas* (0.57) showed lowest percentage of infected grains which differed significantly from all other treatments consistent with the result by (Singh *et al.*, 2002) where copper hydroxide where highly effective against false smut disease decreasing the infected panicles and grains by 59.8 and 57. Per cent, respectively. Kannaiyan and Rao (1976), Ray (1987), (Dodan and Singh, 1997), (Singh *et al.*, 2002), Ahonsi and Adeoti (2003) and (Bagga and Kaur 2006) reported the efficacy of the copper fungicide copper oxychloride in controlling false smut disease. Chu-Ping *et al.* (2007), Hua-Xian *et al.* (2008) and Xiao-Le *et al.* (2011) reported the efficacy of different species *Bacillus* or its fermentation liquid in controlling false smut disease. Jeyalakshmi *et al.* (2010) found that the combination of seed treatment, soil application and foliar spray with *P. fluorescens* recorded the minimum disease incidence of bacterial leaf blight with maximum yield in comparison with the chemical treatment and control.

The yield data obtained from the field experiments at Palakkad (Table 40, Fig 14) showed that treatment with copper hydroxide (4.6) and *Pseudomonas* (4.47) gave

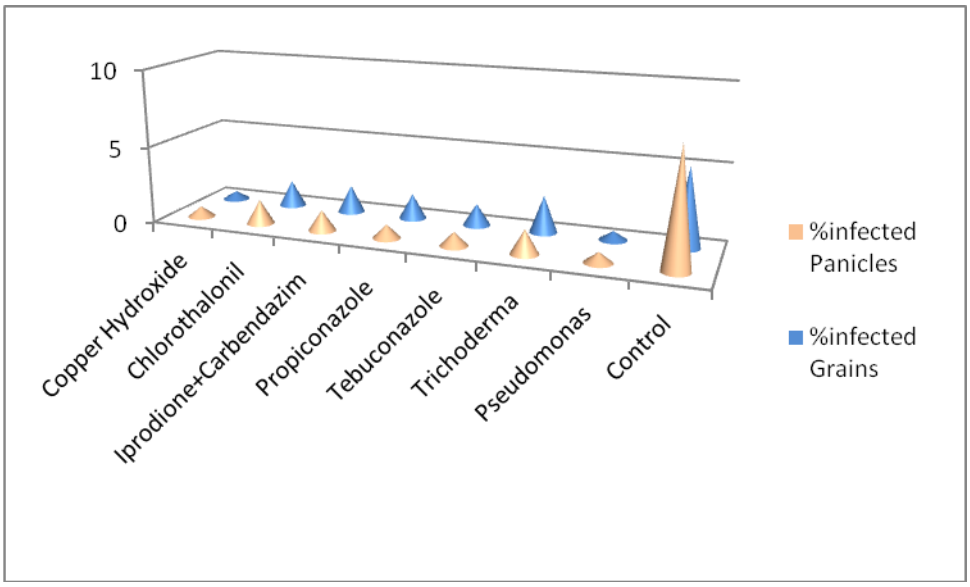


Fig 12. Per cent infected panicles and per cent infected tillers during field evaluation at Palakkad-Kharif 2012

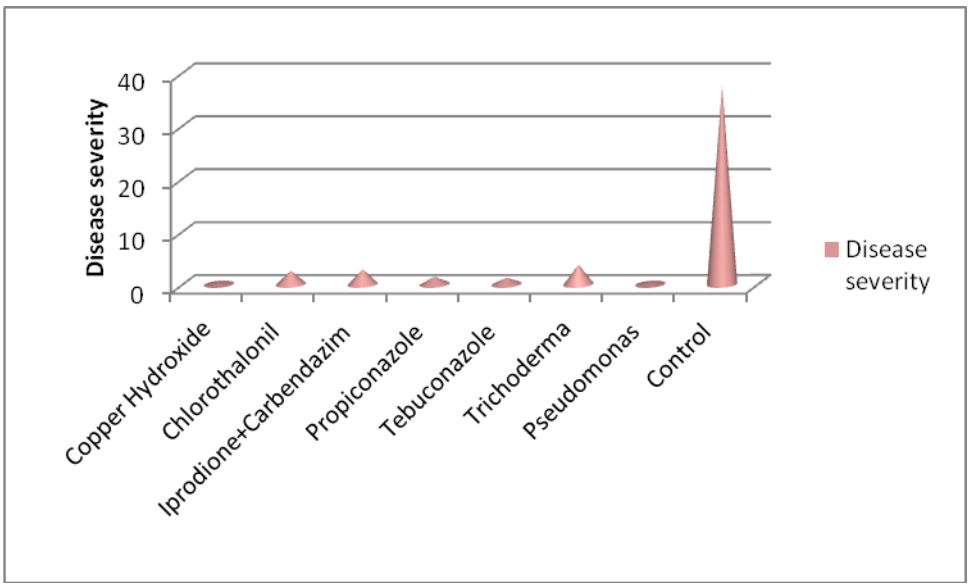


Fig 13. Disease severity of false smut during field evaluation at Palakkad-Kharif 2012

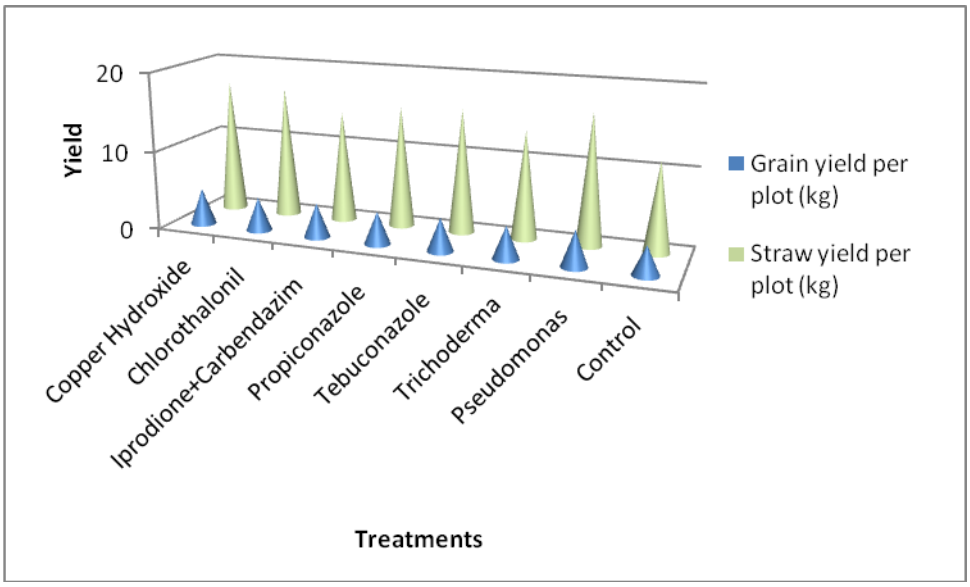


Fig 14. Yield data of field evaluation at Palakkad-Kharif 2012

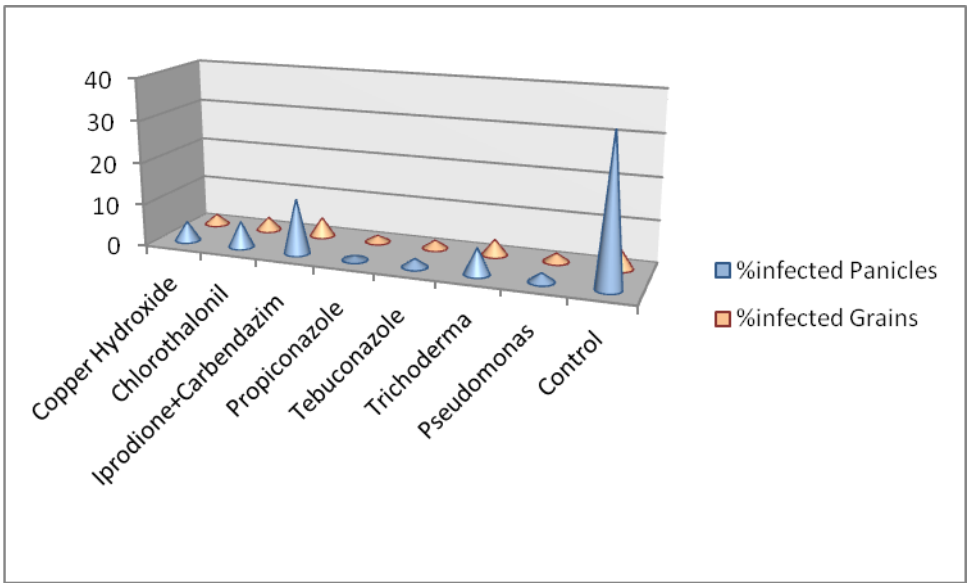


Fig 15. Per cent infected panicles and per cent infected tillers during field evaluation at Alappuzha-Rabi 2012-13

the highest grain yield per plot. The highest straw yield was obtained with the treatment copper hydroxide (17.00 t/ha) which was on par with that of chlorothalonil (16.57), *P. fluorescens* (16.50) and tebuconazole (15.67). The results are in accordance with that by Singh *et al.* (2002) reporting that copper hydroxide decreased the infected panicles and grains along with an increase of 8.2% in grain yield. Ahonsi and Adeoti (2003) found that spraying rice with the copper fungicide copper oxychloride (at 4kg a.i./ha) resulted in 72% reduction in false smut severity resulting in significant increase of 39% in grain yield compared with the control treatment.

Results of the field experiment conducted at Alappuzha revealed that treatment with the fungicide Propiconazole gave lowest incidence of false smut with only 0.93 per cent of the tillers affected which was found superior to all other treatments followed by treatment with the chemical tebuconazole (1.93 % infected tillers) and the bioagent *P. fluorescens* (2.03 % infected tillers) which also differed significantly from all other treatments (Fig 15). Disease severity was found to be lowest with the treatment propiconazole (1.43) but was on par with the treatments tebuconazole (3.68) and *Pseudomonas* (4.00) and, all the three treatments differed significantly from all other treatments (Fig 16). Observations on the per cent infected grains show that the treatment with propiconazole, tebuconazole and *Pseudomonas* recorded lowest per cent infected grains (1.53, 1.90 and 1.97 respectively). The results are in accordance with that by Dodan and Singh, (1997), Bagga and Kaur (2004), Bagga and Kaur (2006), Barnwal *et al.* (2010), Ladhalakshmi *et al.*, 2012 a) and Chen *et al.* (2013) who found Propiconazole to be the most effective chemical against false smut disease under field conditions. Hong-Sik *et al.* (2001) tested different chemicals for the control of the rice false smut and found tebuconazole to be most effective in controlling false smut. Ditmore *et al.* (2006) reported that early detection of the fungus in asymptomatic plants is warranted for timely application of fungicide to minimize yield reduction through chaffing and reduced panicle formation.

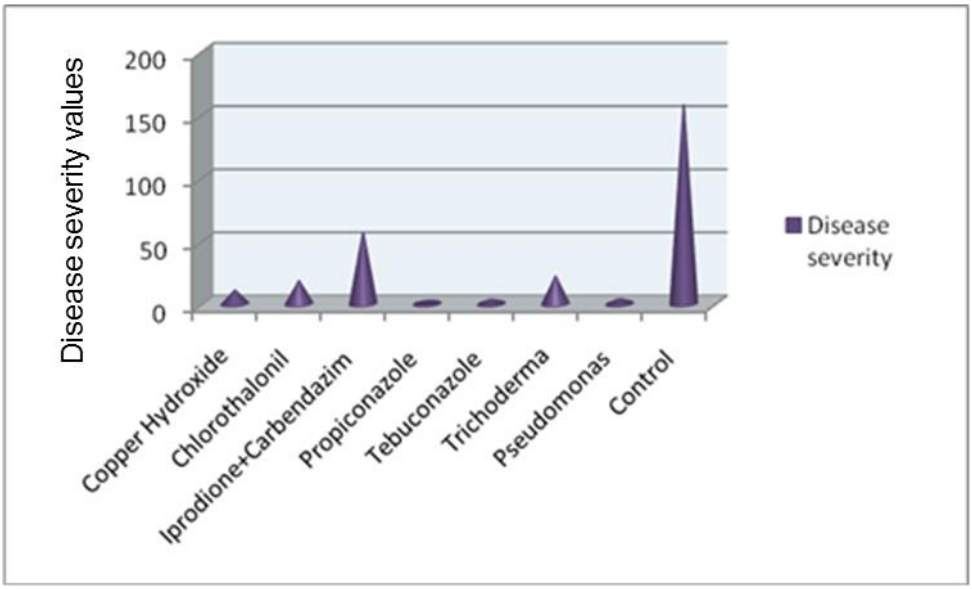


Fig 16. Disease severity of false smut during field evaluation during field evaluation at Alappuzha-Rabi 2012-13

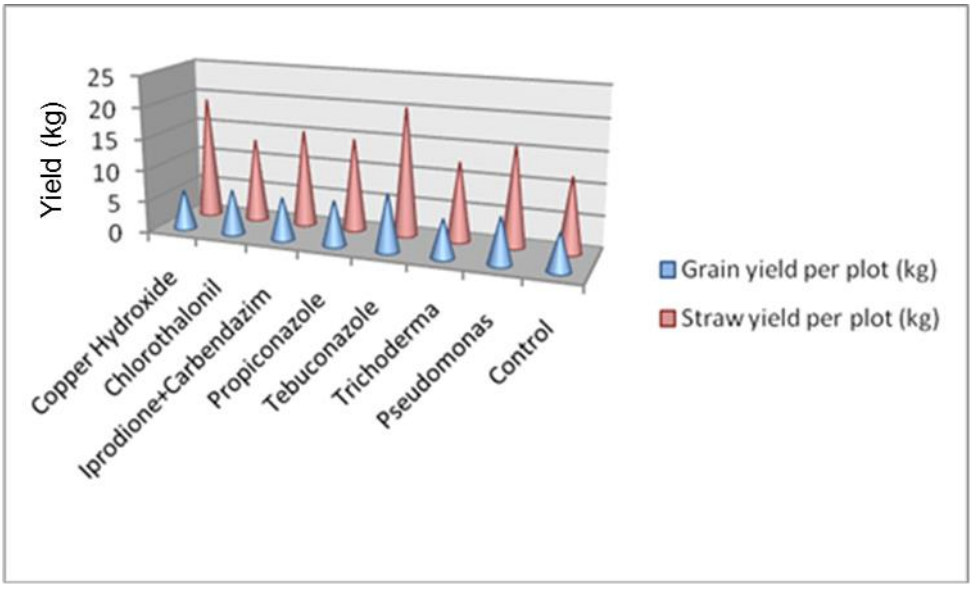


Fig 17. Yield data of field evaluation at Alappuzha-Rabi 2012-13

On comparison of the grain yield obtained from each plot (Fig 17), it was found that maximum yield was recorded with the treatment tebuconazole (8.90 t/ha) followed by the treatments *P. fluorescens* (7.18 t/ha) and propiconazole (7.00 t/ha). Bagga and Kaur (2006) reported that propiconazole closely followed by Contaf, consistently reduced false smut incidence and increased grain yields. Mohiddin *et al.* (2012) reported the combination of prochloraz + carbendazim to be decreasing the disease incidence of false smut and subsequently increasing the grain yield by 44%.

Different treatments, when compared based on straw yield per plot; it was found that highest straw yield was obtained with the treatment tebuconazole (20.26 t/ha) which was on par with that of copper hydroxide (19.16), both of which differed significantly from all other treatments. Straw yield was found to be the minimum in case of the control plots (11.72).

Electrophoretic separation of soluble protein the infected and healthy grains of the susceptible rice variety Uma were carried out by SDS-PAGE. The results of the study showed the presence of two extra bands representing the proteins produced by the pathogen of below 3 kb size in case of the protein profile of the diseased samples. In both healthy and diseased samples bands representing rice proteins were present, but in case of diseased samples, the bands of rice proteins were not very clear cut and were light indicating masking of rice proteins by the pathogen proteins. For separation of the multiple forms of enzymes (isozymes) to study the enzyme alterations in healthy as well as pathogen infected paddy grains. Here, discontinuous anionic polyacrylamide gel electrophoresis was conducted under non-dissociating conditions.

Results of analysis for expression of polyphenol oxidase isozyme in healthy and diseased grain showed the presence of bands showing the activity of the isozyme polyphenol oxidase only in case of diseased samples whereas there were no bands noticed in case of healthy plant samples. Analysis for expression of peroxidase isozymes showed an Isozyme profile with bands observed only in case diseased samples. In healthy samples no clear bands were observed indicating the activity of the Isozyme peroxidase only in case of diseased grains. Difference in the

banding patterrn indicates different electrophoretic mobilities shown by the isozymes, coded by different alleles or separate genetic loci.

The peroxidase and polyphenol oxidase activities in the stems of healthy and *Macrophomina* infected jute plants were studied by Mukhopadhyay and Nandi (1976), and the results showed that in the infected stems the peroxidase and polyphenol oxidase activities were much higher than that of healthy ones and increased gradually with the progress of the disease.

Tyagi *et al.* (2000) observed increased peroxidase and PPO activity in wheat cultivar resistant to *Alternaria triticina* compared to susceptible ones and suggested that these enzymes had an active role in the defence mechanism in wheat plants. As per the results of the *in vitro* experiments conducted by Guang-Hui *et al.* (2005), abundant polyphenolic compounds in epidermis and endosperm of grain were detected in rice varieties resistant to false smut, but not in susceptible. Isozyme analysis carried out using healthy as well as BBrmv infected sample for peroxidase (PO) and Poly Phenol Oxidase (PPO) enzyme was demonstrated by Dhanya *et al.* (2006) where, a single definite band of PO (Rm 0.63) was seen only in infected sample whereas thickness of the band with Rm 0.65 showed intensified production of PPO in BBrMV infected plants suggesting that such studies will be useful in identifying and characterizing resistance against pathogens.

From the above mentioned studies, an integrated package for the management of false smut disease was developed as given in Fig. 18.

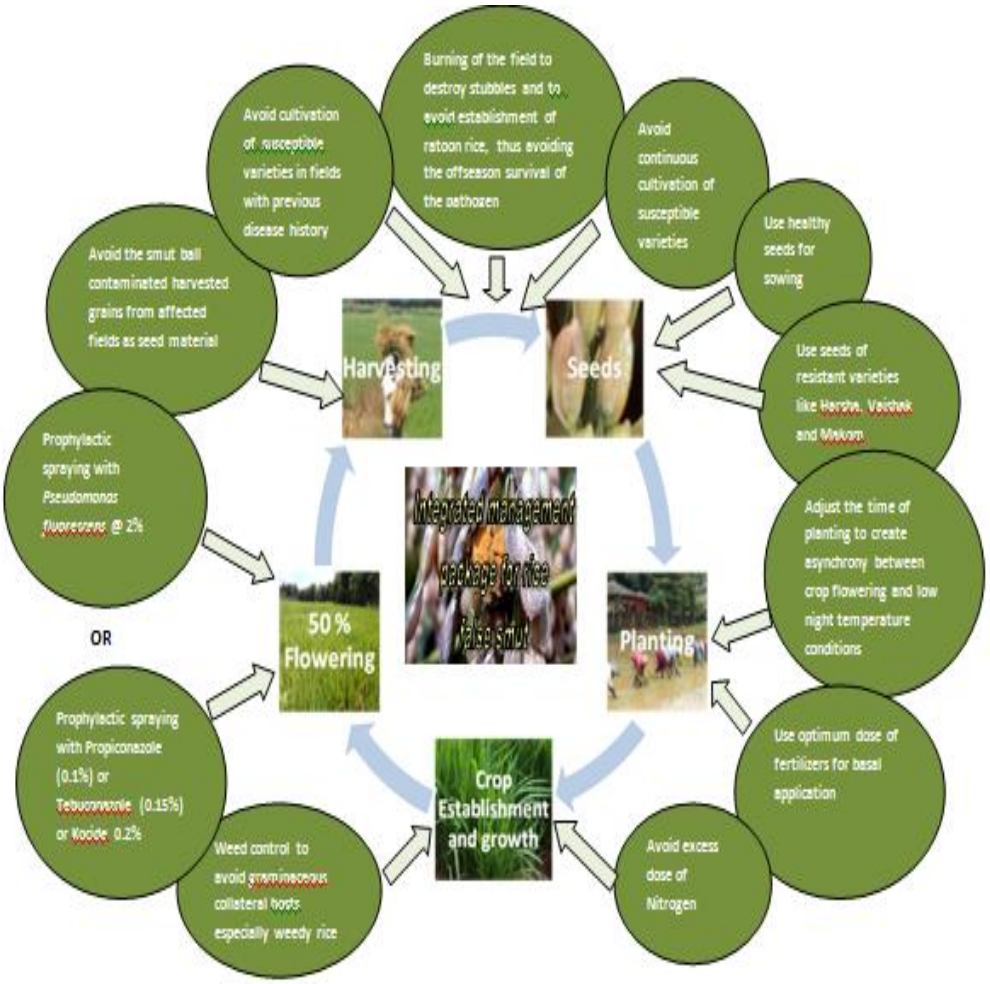


Fig.18. Schematic representation of false smut of rice, its epidemiology and integrated management package

Summary

6. SUMMARY

The present study on the 'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala' was conducted during the period 2010-2013 at the post graduate laboratory, College of Agriculture, Vellayani, Trivandrum and at the experimental fields at Regional Agricultural Research Station, Pattambi, Palakkad, Rice Research Station, Moncompu, Alappuzha and at the Farmers fields at Vilayur (Palakkad) and Champakkulam (Alappuzha).

Field surveys were conducted during 2011-2013, two at Palakkad district during the *Kharif* and two at Alappuzha (Upper Kuttanad, Lower Kuttanad and Kayal areas) district during *Rabi* season revealed that out of the four areas surveyed, Upper Kuttanad recorded maximum percentage of infected tillers (31.1 %) and disease severity (138.8) during 2011-2012. The same trend was observed during 2012-13 with Upper Kuttanad recording the highest per cent infected tillers (42 %) and disease severity (123.9). The disease was found to be more severe at Alappuzha district compared to Palakkad. At Palakkad, the disease was found more prevalent during the Kharif season, whereas it was found more serious during the Rabi season at Alappuzha.

Studies on the symptomatology of false smut disease showed that due to the infection by the pathogen, the individual spikelets of the panicle were found to get transformed in to yellow to orange coloured ball like structures called as smut balls or pseudosclerotia which were almost double the size of the normal rice grains and are found growing out forcing open the lemma and palea. The number of smut balls formed ranged from one to fifty per panicle based on the severity of the disease. Usually a few grains in the panicle were found to get affected. The pathogen was also found to cause chaffiness of the panicle and in severe cases completely chaffy panicles which numerous smut balls were observed.

Based on the extent of damage caused by the pathogen in the field, a new 0-6 scoring system was developed for assessing the disease severity of the false smut disease of rice, taking in to consideration, the chaffiness and blackening symptoms which were also found to be produced by the pathogen in the field.

The pathogen was isolated from the infected panicles showing typical symptoms. A new isolation method was standardized which involves surface sterilization of the smut balls with 0.1% mercuric chloride for 3 to 3 ½ min followed by three washings with sterile distilled water and plating over YPPDA or PSA by dusting the chlamydospores from the inner layers and observation in an inverted position under low power objective after 24 hours and pure culturing. Likewise, seven isolates were obtained from different locations which were serially numbered from Uv1 to Uv 7.

The isolates were characterized based on their colony morphology i.e., colony, colour, texture and appearance and the isolate Uv 7 was found to be showing maximum growth. Production of secondary conidia was found to be very high in case of the isolate Uv 4, whereas the isolate Uv 2 was found to produce maximum smut balls and chlamydospores.

The pathogen produced pure white coloured with smooth colony margin and upon continuous incubation the colour changed to light yellow and in some instances to green. The underside of the plates was initially yellowish in the centre with cream peripheral areas and later changed to reddish to black in the center with yellow periphery. The pathogen was found to show considerable variations in the colony morphology.

After one month of incubation the pathogen started production of yellow globular bodies which represent the pseudosclerotia of the fungus which were filled inside with the spores of the fungus and later, from those pseudosclerotia, light to dark yellow coloured tubular, stalk like structures produced in groups of five to seven

in number from each pseudosclerotia were found to be formed, the tip of which became bulged on aging which may represent the stromatic heads produced by the germinating sclerotial bodies of the pathogen in culture.

The pathogen, *U. virens* produced two types of asexual spores, the secondary conidia and the chlamydospores. The chlamydospores were yellow coloured round, 4.9-7.3 µm in diameter, double walled, when mature, had highly ornamented wall layers. Secondary conidia were oval in shape, were produced on specialized hyphal structures and measured 2.8-4.8 µm in length and 1.5-3.1 µm in breadth.

Two isolates of the pathogen, one from Pattambi, Palakkad (Uv1) and another from Moncompu, Alappuzha (Uv6) were sent to the type culture collection centre at IARI, New Delhi, and were confirmed as *U. virens* with accession numbers ITCC-7335 and ITCC-7334 respectively.

The ITS regions of the pathogen were sequenced for the identification and molecular characterization and for studying the variability of the pathogen. It was found that ITS sequences of isolates from different parts of Kerala and the isolate from Hyderabad were one and the same indicating that there was no variation present among the isolates from Kerala and Andhra Pradesh. The results of BLAST analysis showed that the isolates had 100 per cent identity with the known isolates of *Villosiclava virens* or *U. virens.*

The inoculation experiments showed positive results in case of plants kept in an incubator at controlled temperature conditions with injection method of inoculation. The disease appeared in the plants injected with the spore suspensions of the pathogen after panicle emergence at 15 days after inoculation.

The radial growth of the pathogen, *U. virens* was compared on different solid media and YPPDA medium was found superior followed by PSA and RESA. In case of the media broth, maximum dry weight of mycelial mat was produced on PSB

which was on par with that obtained on YPPDB. Secondary conidia production was found to very high in PSB and RESB and the smut ball and chlamydospore production was found to be the highest in PSB, RYDB and RESB. No smut ball production was observed with CDB medium.

Studies on the optimum conditions for culturing of the pathogen revealed that the pathogen was found to give maximum growth at a temperature of 28°C, at pH 6.5 and at full darkness conditions.

The smut balls or pseudosclerotia stored under laboratory conditions within the crop stubbles remained viable up to eight months whereas those stored in the clayey soil remained viable only up to three months. The viability of the chlamydospores was tested under three conditions *viz*., seed, stubbles and soil. The chlamydospores were able to survive only up to three months within the crop residue and within soil. The chlamydospores remained viable up to four months on the seeds of paddy, even though the viability percentage was very low at the fourth month.

During the survey conducted at Palakkad, at Kizhayur area, the pathogen was found to be surviving on the ratoon rice plants of variety Uma emerged from the stubbles of the previous crop.

The survival studies of *U. virens* on different collateral hosts studied by artificial inoculation studies on common weeds of rice field revealed that none of the weeds artificially inoculated were found to take up infection. Under field conditions, the survival on weeds was observed from Ambalappuzha area of Alappuzha district on weedy rice, *Oryza spontaneum*.

Correlation studies on the influence of various weather parameters on the incidence and intensity of false smut disease of rice revealed that disease severity and per cent infected tillers were found to be negatively correlated with the minimum temperature at 50 per cent flowering period. Relative humidity at morning hours,

evening hours and the daily mean relative humidity at seven days after 50 per cent flowering was also found to be significantly negatively correlated with the disease severity and per cent infected tillers.

Twenty red rice varieties were screened for resistance under wet land field conditions against false smut disease of rice at two locations, Alappuzha and Palakkad during 2012-13. The results of the varietal screening trial conducted during Kharif 2012 at Palakkad indicated that the varieties Harsha and Vaishak were completely free from the disease. Disease severity was found to be very low (1.4) for the varieties, Makom and Thekkacheera. During the varietal evaluation trial conducted at Alappuzha during Rabi 2012-13, eight varieties were found completely disease free (Table 28), *viz*., Aruna, Makom, Kanakom, Vytila 6, Thekkancheera, Athira, Harsha and Vaishak. The varieties, Pavizham, Karthika and Pratyasha recorded very low disease severity (<1). Thus, out of the twenty varieties screened, the varieties Harsha and Vaishak were found highly resistant (completely disease-free, Plate 24) and the varieties, Makom, Thekkancheera, Pavizham and Karthika were found resistant (disease severity value < 1) to the disease. The varieties Kanakom, Revathi and Prathyasha showed moderate resistance with a disease severity value of 1-5.

Out of the ten primers used for RAPD profiling of the pooled DNA, three primers gave products specific to the resistant varieties. The RAPD profile using the pooled DNA from the resistant varieties gave specific products (Plate 26) with respect to the primers OPG-18, OPC-15 and OPD-18. RAPD profile of the resistant varieties, Makom, Harsha, Thekkancheera and Vaishak, and the susceptible varieties Bhagya, Gouri, Remanika and Uma, with two random primers *viz*., OPB-10 and OPK-14, showed that, with the primer OPB-10, there was one unique amplicon in case of the variety Harsha, and with the primer OPK-14, one unique amplicon was present in the variety Makom and two in the variety Thekkancheera.

Ten isolates of *Trichoderma* were screened by dual culture technique for their efficacy against the pathogen *U. virens*. The results revealed that all the isolates of *Trichoderma* were effective in controlling the pathogen but there was no significant difference among the isolates in the per cent inhibition of the pathogen.

Studies on the mycoparasitic effect of *Trichoderma* on *U. virens* showed that *Trichoderma* was found to show various signs of mycoparasitism like, parallel growth, formation of hook like structures, formation of chlamydospores etc. Due to the parasitism by *Trichoderma*, the pathogen hyphae showed granulation of cytoplasm, vesiculation and lysis.

Culture filtrate studies revealed that, *Trichoderma* culture filtrate at 70 per cent concentration gave maximum inhibition (81.36) of the radial growth of the pathogen. Studies conducted to determine the efficacy of *Trichoderma* volatiles on the pathogen showed that the growth of the pathogen was found to be getting inhibited by the effect of volatile compounds produced by *Trichoderma* with an inhibition percentage of 80 per cent. The bacterial isolates B4, B5 and B6 was found to give maximum inhibition (82.60) of the colony growth of *U. virens.*

The results of the *in vitro* chemical management studies by poisoned food technique revealed that six chemicals *viz*., copper hydroxide, mancozeb, propiconazole, tebuconazole, carboxin+thiram and iprodione+ carbendazim gave 100 per cent inhibition to the growth of the pathogen under *in vitro* culture conditions followed by the chemical chlorothalonil (94.55).

The results of the *in vitro* chemical management by spore germination inhibition studies revealed that all the fungicides at all concentrations gave 100 per cent inhibition to the germination of chlamydospores of *U. virens*.

Five treatments selected from in vitro chemical management experiment, along with talc based formulations of one bacterial and one fungal biocontrol agent

were tested in the field during Kharif 2012 at Palakkad and Rabi 2012-13 at Alappuzha using the the susceptible rice variety Uma.

Results of the field experiment conducted at Palakkad revealed that treatment with the fungicide copper hydroxide (0.67) and the bioagent *P. fluorescens* (0.67) followed by treatment with the chemicals Propiconazole (0.88) and tebuconazole (0.89) recorded the lowest incidence of the disease in terms of per cent infected tillers The treatments copper hydroxide (0.53) and *P. fluorescens* (0.57) showed lowest percentage of infected grains. In case of disease severity, the treatments copper hydroxide (0.36) and *P. fluorescens* (0.38) recorded the lowest disease severities which were on par with the treatment tebuconazole (1.24).

The yield data obtained from the field experiments at Palakkad showed that treatment with Copper Hydroxide (4.6) and *P. fluorescens* (4.47) gave the highest grain yield per plot. Grain yield per plot was found to be the minimum for the untreated control (3.60). Straw yield per plot was found to be the highest with the treatment copper hydroxide (17.00 t/ha) which was on par with that of chlorothalonil (16.57), *P. fluorescens* (16.50) and tebuconazole (15.67). There was no significant difference observed in case of plant height and average number of tillers per plant.

Results of the field experiment conducted at Alappuzha revealed that treatment with the fungicide propiconazole gave lowest incidence of false smut with only 0.93 per cent of the tillers affected followed by treatment with the chemical tebuconazole (1.93 %) and the bioagent *P. fluorescens* (2.03 per cent). Observations on the per cent infected grains showed that the treatment with propiconazole, tebuconazole and *P. fluorescens* recorded lowest per cent infected grains (1.53, 1.90 and 1.97 respectively) and were on par with all other treatments except Iprodione+ Carbendazim treatment and untreated control. Disease severity was found to be lowest with the treatment propiconazole (1.43) but was on par with the treatments

tebuconazole (3.68) and *P. fluorescens* (4.00) and, all the three treatments differed significantly from all other treatments.

Maximum yield was recorded with the treatment tebuconazole (8.90 t/ha) followed by the treatments *P. fluorescens* (7.18 t/ha) and propiconazole (7.00 t/ha). Highest straw yield was obtained with the treatment tebuconazole (20.26 t/ha) which was on par with that of copper hydroxide (19.16), both of which differed significantly from all other treatments. The treatments did not differ significantly with respect to plant height and average number of tillers per plot.

The results of the electrophoretic separation studies by SDS-PAGE showed the presence of two extra bands representing the proteins produced by the pathogen of below 3kb size in case of the protein profile of the diseased samples.

Results of analysis for expression of polyphenol oxidase isozyme in healthy and diseased grain showed the presence of bands showing the activity of the isozyme polyphenol oxidase only in case of diseased samples and the relative mobility (Rm) value obtained was 0.33. Analysis for expression of peroxidase isozymes showed an Isozyme profile with bands observed only in case diseased samples with Rm value 0.66.

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Appendices

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes - 200 g

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

Agar-agar - 20 g

Distilled water - 1000 ml

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

2. Potato Sucrose Agar

Peeled and sliced potatoes - 200 g

Sucrose - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

3. XBZ agar medium

Peeled and sliced potatoes - 300 g

Peptone - 5 g

Sugar - 15 g

Ca (NO3)2·4H2O - 0.5 g

Na2HPO4·12H2O - 2 g

Agar-agar - 16 g

Distilled water - 1000 ml

4. Czapek-Dox Agar

$NaNO_3$ - 2 g

K_2HPO_4 - 1 g

$Mg(SO_4).7H_2O$ - 0.5 g

KCl - 0.5 g

$FeSO_4$ - 0.1 g

Sucrose - 30 g

Agar-agar - 20 g

Distilled water - 1000 ml

5. Yeast Peptone Potato Dextrose Agar

Yeast - 100 mg

Peptone - 100 mg

Peeled and sliced potatoes - 200 g

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

Agar-agar - 20 g

Distilled water - 1000 ml

6. Rice Yeast Dextrose Agar

Rice powder - 40 g

Yeast extract - 10 g

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

Agar-agar - 20 g

Distilled water - 1000 ml

7. Host Extract Agar

Powdered rice grains (with husk) - 20 g

Sucrose - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

Rice grains along with the husk were powdered to make a very fine powder. 20 g of the powder was used to prepare the extract. The extract was strained with the help of a strainer and later was heated and was mixed with molten agar and 20 g of sucrose. The volume was made upto 1000 ml with distilled water and medium was sterilized at 15 psi and 121 ºC for 15 min.

8. Nutrient Agar

Beef extract - 3.00 g

Peptone - 5.00 g

Sodium chloride - 1.00 g

Agar Agar - 20.00 g

Water - 1000 ml (to makeup)

pH - 7 (to be adjusted)

APPENDIX - II

COMPOSITION OF STAIN USED

1. Lactophenol –Cotton blue

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

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 3ml glycerol.

APPENDIX - III

Details of the fungicides tested *in vitro* against *U. virens*

Sl. No	Common name	Trade name	Formulation	Source of supply
1.	Chlorothalonil	Kavach	75 % WP	Syngenta India Ltd., Mumbai
2.	Thiophanate methyl	Hexastop	70 % WP	Coromandel International Ltd., Secunderabad
3.	Copper hydroxide	Kocide	77 % WP	E. I. DuPont India, Pvt. Ltd., Haryana
4.	Mancozeb	Indofil M 45	75 % WP	Indofil Chemical Co., Mumbai
5.	Propiconazole	Tilt	25 % EC	Syngenta India Ltd., Mumbai
6.	Tebuconazole	Folicur	25.9 % m/mEC	Bayer Crop Science Ltd., Mumbai
7.	Iprodione+ Carbendazim	Quintal	50 % WP	Bayer Crop Science Ltd., Mumbai
8.	Carboxin 37.5% +Thiram 37.5%	Vitavax Power	70%WS	DhanukaAgritech Ltd., Ahemedabad
9.	Pencycuron	Monceren	22.9 % EC	Bayer Crop Science Ltd., Mumbai
10.	Azoxystrobin	Amistar	23%SC	Syngenta India Ltd., Mumbai
11.	Bitertanol	Baycor	25 %WP	Bayer Crop Science Ltd., Mumbai

Appendix – IV: Details of the surveys conducted during the study

Rabi 2011-12: Survey Alappuzha 1					
Locations			**Variety**	**Infected tillers(%)**	**Disease severity**
Kayal area					
1	Location 1 Kayal	Irupathinalayiram Kayal	Uma	13.8	10.488
		Nalayiram Kayal	Uma	0.8	0.504
		Vadakku Aarayiram - Oonappuram	Uma	1	0.64
2	Location 2 Pulinkunnu	Puthanpurakkal Kayal	Uma	0.8	0.496
		Kannadi-Vadakke mathikayal-pulinkunnu	Uma	0.5	0.38
		Kayalppuram, Mathikayal -pulinkunnu	Uma	0.9	0.72
3	Location 3 Mathikayal	Mathikayal Thazhcha	Uma	2.34	6.856
		Vadakke Mathikayal	Uma	7	5.25
		Thekke Mathikayal	Uma	2.6	4.628
Lower Kuttanad					
4	Location 4 lower Kuttanad	Koolipurackal Padam	Uma	7.5	4.5
		Karalakom Padam	Uma	11.1	8.214
		Kainakari	Uma	51.42	118.27
		Venattukari	Uma	1.25	0.83
5	Location 5 Ambalappuzha	Ambalappuzha-Nalupadam	Uma	0.9	1.089
		Ambalappuzha-Kakkazham padam	Kanchana	0.8	1.12
		Ambalappuzha-Ambathin padam	Uma	0.9	0.747
		Punnapra-Koppamveli	Kanchana	1.14	0.319
6	Location 6 Nedumudi	Nedumudi- Pullupadam	Uma	51.5	128.75
		Nedumudi - Pongayil Padam	Uma	6.1	4.697
		Ponga	Uma	1.6	1.376
7	Location 7 Kavalam	Kizhakkumpuram-Chennankari (LK)	Uma	16.6	13.778
		Kavalam	Uma	55	143
8	Location 8 Moncompu	Moncompu – Ponga moola	Prathyasha	1	0.83
		Moncompu – Ponga thekku	Uma	5	3.55
9	Location 9 Chambakkulam	chambakkulam	Uma	0.39	0.3159
Upper Kuttanad					
10	Location 10 Upper Kuttanad	Manikkathadi-Koorachal-	Uma	1	0.98
		Nedumpuram	Uma	4	5.96
		Parathikkal punja (UK)	Uma	42	123.9
		Peringara-Kottakakom	Uma	34.6	184.418
		Idasseri Thekku	Uma	74	378.88

Rabi 2012-13 Survey - Alappuzha 2					
Locations			**Variety**	**Infected tillers (%)**	**Disease severity**
Kayal area					
1	Location 1 Kayal	Irupathinalayiram Kayal	Uma	12.2	32.574
		Nalayiram Kayal	Uma	4.6	9.2
		Vadakku Aarayiram - oonappuram	Uma	3.3	2.0625
		vadakke thollayiram	Uma	1.9	3.42
		thekke thollayiram	Uma	3.5	8.4
2	Location 2 Pulinkunnu	Puthanpurakkal Kayal	Uma	1.09	0.9701
		Kannadi-Vadakke mathikayal-pulinkunnu	Uma	0.96	2.016
		Kayalppuram, Mathikayal -pulinkunnu	Uma	0.71	0.9443
3	Location 3 Mathikayal	Mathikayal Thazhcha	Uma	1.75	1.4525
		Vadakke Mathikayal	Uma	1.3	1.95
		Thekke Mathikayal	Uma	3.65	5.767
Lower Kuttanad					
4	Location 4 Lower Kuttanad	Kainakari 1	Uma	10.52	29.456
		Kainakari 2	Uma	6.38	14.355
5	Location 5 Ambalappuzha	Ambalappuzha-Ambathin padam	Uma	1.76	2.7808
6	Location 6 Nedumudi	Ponga	Uma	5.8	11.6
7	Location 7 Kavalam	Kavalam	Uma	17.76	54.7008
8	Location 8 Moncompu	Moncompu 1	Uma	0.98	1.6268
		Moncompu2	Uma	1.7	2.04
		pongapra thekku 1	Uma	3.79	9.475
		pongapra thekku 2	Uma	6.16	9.856
		pongapra moola 1	Uma	2.27	2.8375
		pongapra moola 2	Uma	4.13	8.8382
		pongapra vadakku 1	Uma	8.69	16.6848
		pongapra vadakku 2	Uma	7.5	10.65
9	Location 9 chambakkulam	kattakkuzhi padam	Uma	35.29	100.9294
		pallippadam	Uma	15.88	42.3996
		padachal	Uma	12.22	35.3158
		kachampadam	Uma	11.6	16.124
		chempady padam	Uma	6.4	10.432
Upper Kuttanad					
10	Location 10 Upper Kuttanad	Nedumpuram	Uma	42	123.9

Kharif 2011 Survey Palakkad 1			Variety	Infected tillers (%)	Disease severity
Locations					
1	location 1 Pattambi	Pattambi 1	Uma	10.3	21.63
		Pattambi 2	Uma	5	7.05
		Pattambi 3	Jyothi	2	3.6
		Pattambi 4	Jyothi	5.1	4.998
		Pattambi 5	Jyothi	3	2.43
2	location 2 Vilayur	Vilayur 1	Uma	5.17	4.9632
		Vilayur 2	Uma	3.14	6.28
		Vilayur 3	Uma	1.83	1.3359
		Vilayur 4	Ponmani	1.5	1.89
		Vilayur 5	Uma	2.27	4.4038
3	location 3 Kodumpu	Kodumpu	Uma	5	10.05
4	location 4 Koppam	Koppam 1	Uma	1.76	1.7072
		Koppam 2	Uma	6.6	13.2
		Koppam 3	Uma	11.25	33.075
		Koppam 4	Uma	2.16	6.156
5	location 5 Kadavu road	kadavu road lift irrigation padasekharam 1	Uma	19.5	94.575
		kadavu road lift irrigation padasekharam 2	Uma	15.83	56.5131
6	location 6 Kadampazhippuram	Kadampazhippuram	Uma	6	7.68
		Kadampazhippuram	Uma	14.56	31.1584
		Kadampazhippuram	Uma	3.3	6.6
		Kadampazhippuram	Samyuktha	5.83	13.2341
		Kadampazhippuram	Samyuktha	2.7	7.209
		Kadampazhippuram	Kanchana	6.99	10.2753
		Kadampazhippuram	Kanchana	3.5	6.475
7	location 7 Kizhayur	kizhayur 1	Uma	15	37.95
		kizhayur 2	Uma	3.3	7.656
8	location 8 Kannadi	Kannadi	Uma	4.75	10.2125
9	location 9 Peradiyur	peradiyur 1	Uma	5.1	8.262
		Peradiyur 2	Uma	5	5.5
10	location 10 Namparam	Namparam	Uma	5.95	9.401

Kharif 2012 SurveyPalakkad 2			Variety	Infected tillers (%)	Disease severity
Locations					
1	Uma	Pattambi 1	Uma	6.1	12.2
		Pattambi 2	Uma	4.2	7.854
		Pattambi 3	Uma	12.6	46.62
		Pattambi 4	Uma	5.8	11.426
2	location 2 Vilayur	Pattambi 5	Uma	1.3	0.923
		Vilayur 1	Uma	2.6	2.158
		Vilayur 2	Uma	8.91	7.2171
		Vilayur 3	Uma	0.67	0.8375
		Vilayur 4	Ponmani	1.33	1.8886
		Vilayur 5	Ponmani	2.15	3.311
3	location 3 Kodumpu	Kodumpu	Uma	3.45	4.209
4	location 4 Koppam	Koppam 1	Uma	2.7	5.4
		Koppam 2	Uma	7.6	19.456
		Koppam 3	Uma	4.28	9.0308
		Koppam 4	Uma	0.8	0.488
5	location 5 Kadavu road	Kadavu road lift irrigation padasekharam 1	Uma	18.7	70.686
		Kadavu road lift irrigation padasekharam 2	Uma	22.25	78.32
		Kadavu road lift irrigation padasekharam 3	Uma	10.83	25.7754
		Kadavu road lift irrigation padasekharam 4	Uma	5.3	10.971
		Kadavu road lift irrigation padasekharam 5	Uma	15.29	35.4728
6	location 6 Kadampazhippuram	Kadampazhippuram 1	Uma	0.4	0.256
		Kadampazhippuram 2	Uma	1.28	1.5104
		Kadampazhippuram 3	Uma	1.32	2.772
7	location 7 Kizhayur	Kizhayur 1	Uma	9.6	22.272
		Kizhayur 2	Uma	9.81	14.5188
8	location 8 Kannadi	Kannadi	Uma	1.92	0.1152
		Peradiyur 1	Uma	4.4	6.468
9	location 9 Peradiyur	Peradiyur 2	Uma	6.67	10.4052
		Peradiyur 3	Uma	3.9	3.237
10	location 10 Namparam	Namparam	Uma	5.7	7.467

APPENDIX - V

Sequences of the *U. virens* from GenBank used for comparison with the sequences generated in the study

>gi|42517100|dbj|AB162148.1| Villosiclava virens gene for 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, 28S ribosomal RNA, partial and complete cds

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>gi|33563161|dbj|AB116645.1| Villosiclava virens gene for 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, 28S ribosomal RNA, partial and complete sequence

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>gi|29027756|dbj|AB105954.1| Villosiclava virens genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence

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ATCAATAAGCGGAGGAAAAGAAACCAA

>gi|226838253|gb|FJ848709.1| Ustilaginoidea virens isolate GX0202 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CAGCGGGAAGGGATCATTTACCGAAGTTTTTACGCTCCAAACCCCATGTGAA
CCTATACCTACGCCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTC
CCCCCCGACGCCCTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGC
CCGCCGGAGGATACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGA
GTGGATTTTTGCAAATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGG
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCT
TGGTGTTGGGGATCGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAAT
GAATCGGCGGTCTCGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGC
ACTTGGAGCGCGGCGCGGCCACTGCCCGTAAAACGCCCAACTTCTCAAGAGT
TGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATTAA

>gi|226838262|gb|FJ848718.1| Ustilaginoidea virens isolate SX0103 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GGGATCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACG
CCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCCGACGCC
CTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGAT
ACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATTTTTGCA
AATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGAT
CGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCT

CGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGG
CGCGGCCACTGCCCGTAAAACGCCCAACTTCTCA

AGAGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCT

>gi|226838281|gb|FJ848737.1| Ustilaginoidea virens isolate ZJ0202 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GCGCAGAGTCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACC
TACGCCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGA
CGCCCTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGA
GGATACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATTTT
TGCAAATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGG
GGATCGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCG
GTCTCGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGC
GCGGCGCGGCCACTGCCCGTAAAACGCCCAACTTCTCAAGAGTTGACCTCGA
ATCAGGTAGGAATACCCGCTGAACTTAAGCATATCATA

>gi|226838266|gb|FJ848722.1| Ustilaginoidea virens isolate SX0204 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CAGAGTCATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTAC
GCCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGC
CCTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGA
TACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATTTTTGC
AAATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGG
ATCGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGT

CTCGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGC
GGCGCGGCCACTGCCCGTAAAACGCCCAACTTCTCAAGAGTTGACCTCGAAT
CAGGTAGGAATACCCGCTGAACTTAAGCATATCAAA

>gi|226838268|gb|FJ848724.1| Ustilaginoidea virens isolate SX0206 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CAGCGAAGGACATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATA
CCTACGCCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCC
GACGCCCTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCG
GAGGATACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATT
TTTGCAAATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTT
GGGGATCGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGG
CGGTCTCGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGA
GCGCGGCGCGGCCACTGCCCGTAAAACGCCCAACTTCTCAAGAGTTGACCTC
GAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCTAAGGCCGGAAAAA

>gi|226838267|gb|FJ848723.1| Ustilaginoidea virens isolate SX0205 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CAGAGACATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTAC
GCCGTTGCTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGC
CCTCGCGCCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGA
TACAACCAAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATTTTTGC
AAATCAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGG
ATCGGCCCTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGT
CTCGTCGCAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGC

GGCGCGGCCACTGCCCGTAAAACGCCCAACTTCTCAAGAGTTGACCTCGAAT
CAGGTAGGAATACCCGCTGAACTTAAGCATATCAAAAGGGGGAGAAA

>gi|226838270|gb|FJ848726.1| Ustilaginoidea virens isolate SX0208 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

ATTACCGAGTTTTTACGCTCCAAACCCCATGTGAACCTATACCTACGCCGTTG
CTTCGGCGGGCTTTCAAGCCCCGGGCAGCCGCCTCCCCCCCGACGCCCTCGCG
CCTGGGAGGGGGAGGGCACCCGGAACCAGGCGCCCGCCGGAGGATACAACC
AAAAAAACTCTTGTGTTTTCCAATGCATGTCTGAGTGGATTTTTGCAAATCAA
AATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCG
AGCGTCATTTCAACCCTCAAGCTCTGTCTTGCGCTTGGTGTTGGGGATCGGCC
CTGCCCGCCAGCCCGGGCGGGCCGCCCCCGAAATGAATCGGCGGTCTCGTCG
CAGCCTCCTCTGCGTAGTAATTCAGTTATCCTCGCACTTGGAGCGCGGCGCGG
CCACTGCCCGTAAAACGCCCAACTTCTCAAGAGTTGACCTCGAATCAGGTAG
GAATACCCGCTGAACTTAAGCATATCA

EPIDEMIOLOGY AND MANAGEMENT OF FALSE SMUT OF RICE (*Oryza sativa* L.) IN KERALA

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Abstract of the thesis

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ABSTRACT

The study entitled 'Epidemiology and management of false smut of rice (*Oryza sativa* L.) in Kerala' was conducted during the period 2010-2013 at College of Agriculture, Vellayani, Trivandrum and the field experiments and varietal evaluation trials were carried out at the Regional Agricultural Research Station, Pattambi, Palakkad, Rice Research Station, Moncompu, Alappuzha and at Farmers fields at Vilayur (Palakkad) and Chambakkulam (Alappuzha).

The results of the surveys conducted at Alappuzha and Palakkad indicated that maximum disease was observed at the upper Kuttanad area of Alappuzha. A new collateral host for the pathogen, *Oryza spontaneum* was observed at Alappuzha during the survey. Isolation of the pathogen was made from the samples collected during the survey. The method of isolation of the pathogen was standardized. Surface sterilization of smut balls with 0.1 % $HgCl_2$ for 3.5-4 minutes followed by three serial washings with sterile water and dusting of the chlamydospores over YPPDA, and pure culturing after observation under a light microscope after 24 hrs was the method devised for isolation. Ideal conditions for culturing of the fungus under *in vitro* conditions were found to be the use of YPPDA medium of pH 6.5 at 28ºC under full darkness. An indigenous medium *viz.*, rice extract sucrose agar was developed for the culturing of the pathogen.

The pathogen, *Ustilaginoidea virens* was observed to be producing smut balls and chlamydospores in culture similar to that produced under field conditions. Stromatic heads of the fungus was observed under in vitro conditions for the first time in India. Sequencing of the ITS region of pathogen gave 100% similarity with the known sequences of *U. virens* on BLAST analysis and thus the pathogen was conformed to be *U. virens*. The pathogen isolates were identified and deposited at ITCC, New Delhi, and the accession numbers ITCC-7335 (Pattambi, Palakkad) and ITCC-7334 (Moncompu, Alappuzha) were obtained for the isolates.

Laboratory trials revealed the efficacy of five chemical fungicides, different species of *Trichoderma* and three bacterial strains for suppression of the pathogen *in vitro*.

Correlation studies on the influence of weather parameters on the development of the disease revealed that the disease was negatively correlated with the minimum temperature during 50 % flowering. Results of the survival studies of the pathogen revealed that the smut balls of the pathogen can survive for eight months at room temperature. At field level, the fungus was found to be surviving on the ratoon rice emerged from the stubbles of the previous crop.

Under the varietal evaluation trials conducted at Alappuzha and Palakkad, the varieties Vaishak and Harsha were found resistant to the disease and the varieties PTB 10 and Makom were moderately resistant. The varieties, Uma, Remanika, Gouri and Bhagya were the most susceptible ones. RAPD profiling of the resistant and susceptible varieties with 10 random primers showed polymorphism with five primers OPK-14, OPG-18, OPC-15, OPB-10 and OPD-18.

Field trials conducted at Alappuzha and Palakkad showed that spraying of Propiconazole125g a.i./750 L or Tebuconazole0.1875 kg a.i./500 L or Copper Hydroxide1000 g a.i./750 L or *Pseudomonas flourescens* @ 2% of the talc based formulationat 50% flowering could control the disease effectively. Tebuconazole 0.1875 kg a.i./500 L gave significant yield increase over the control.

Biochemical studies like SDS PAGE and NATIVE PAGE were conducted. SDS PAGE showed conspicuous bands of the pathogen proteins and NATIVE PAGE revealed increased activity of peroxidase and polyphenol oxidase on the diseased grains.

Based on the research results, a management package was developed for effective control of the disease at the field level as follows: 1) avoiding use of paddy grains harvested from severely affected fields as seed material 2) avoiding continuous

cultivation of the most susceptible rice varieties like, Uma, Remanika, Gouri and Bhagya 3) use of resistant varieties like Harsha, Vaishak or Makom 4) Weed management to control the collateral hosts like wild rice 5) biological control by spraying of *Pseudomonas flourescens* @ 2% of the talc based formulation at 50% flowering 6) spraying of Propiconazole125 g a.i. / 750 L or Tebuconazole 0.1875 kg a.i./500 L or Copper Hydroxide 1000 g a.i./750 L at 50% flowering.