Oryza Vol. 55 No. 1, 2018 (214-223)
DOI 10.5958/2249-5266.2018.00025.5

Occurance, pathogenicity, characterization of *Fusarium fujikuroi* causing rice bakanae disease from Odisha and *in vitro* management

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Received : 19 February 2018 Accepted : 17 March 2018 Published : 21 March 2018

ABSTRACT

The present investigation was carried out to asses the incidence of bakanae disease in Odisha, characterization of the isolates and its management with fungicides. The disease incidence ranging from 1-25.50 percent on different rice varieties was recorded in five districts mainly, Cuttack, Sambalpur, Bargarh, Ganjam and Jajpur. These regions were identified as new hot spots for the disease. The disease was observed to a significant extent in most of the commercially growing varieties with maximum disease incidence on Pooja (19.0 % and 24.5% respectively for two seasons). A significant variation was observed among the isolates with respect to cultural, morphological characters and degree of pathogenicity. All the ten isolates were grouped into highly virulent group. The amplification of tef-1alpha gene generated approx 700 bp bands. In phylogenetic analysis, based on tef-1alpha gene region, overall two major groups were formed. The F.fujikuroi isolates FJ1 (Kisannagar), FJ2 (Khurda), FJ3 (Tangi-Chodwar) and FJ9 (Chandikole) belonged to same group whereas FJ4 (Cuttack), FJ5 (Jajpur), FJ6 (Kisannagar-2), FJ7 (Sambalpur), FJ8 (Bargarh) and FJ10 (Ganjam) are in a separate group with other world isolates. Among the ten systemic fungicides tested, all found effective with 100 percent reduction in mycelial growth. All the three tested Trichoderma spp. were found effective under in vitro with complete inhibition and lyses of the pathogen mycelium. Seed treatment with Carbendazim 50 % WP @1 g/kg of seeds found effective with maximum germination, vigor index and disease reduction.

Key words: *Bakanae, Fusarium fujikuroi, fungicides, management*

INTRODUCTION

Rice is the most important staple food for the world population living in rural and urban area of humid and sub-humid area of Asia (Panda et al., 2017). The world population is predicted to exceed 8 billion people by 2025 and to meet this growing demand; we need to produce 50% more food grain (Yadav et al., 2017). However, biotic stress has caused a rigorous yield loss that affected the rice production worldwide. Among the biotic stresses, bakanae or foolish seedling (foot rot) is one of the most important emerging seed and soil borne disease caused by the fungal pathogen *Fusarium fujikuroi* Nirenberg [teleomorph *Gibberella fujikuroi* (Sawada) Ito & Kimura] (Carter et al., 2008). With the extension of hybrid rice and the use of new methods for seedling rising, especially the increased application of dry seeded raising for hybrid rice, bakanae disease has become more and more serious (Yang et al., 2003). In India the disease has emerged as a major problem in major growing areas of North Indian states particularly on basmati rice cultivars (Bashyal et al., 2014; Gupta et al., 2014). The pathogen causes various symptoms like elongated seedlings which may be stunted and yellow with crown rot, foot rot and production of un-fillled gains (Hsieh et al., 1977). Reduced availability of effective and ecofriendly pesticides for seed dressing in recent years led to increased disease incidence in several countries, possessing a serious threat for rice cultivation. The disease at a later crop stage usually causes a yield loss of 10 to 20%, and under high severity yield losses could be as high as 70% (Ito and Kimura,

1931; Ou, 1985; Rood 2004). In India, the yield losses ranging from 15-25% have been reported from Uttar Pradesh, Assam, Andhra Pradesh, Tamilnadu, Haryana and Punjab (Pannu et al., 2012; Sunder et al., 2014). Apart from yield loss, the pathogen was also associated in highest percentage (1-24%) in seeds of different basmati rice cultivars (Butt et al., 2011; Bashyal and Aggarwal, 2013) and showed the profound effect on seed quality.

The genetic diversity is measured as the sum of genetic and phenotypic variation and can differ with time and space since these populations can adapt or evolve in response to prevailing environmental conditions (McDonald and Linde, 2002), it is important to study the genetic variation in the population of plant pathogen to understand the co-evolution in the plant pathosystem. Eastern states of India are the traditional rice growing areas where rice is cultivated extensivelly as a monocrop or in rotation with other pulses and vegetables in both rainfed and irrigated ecologies. Due to climate change and improper crop management practices, diseases like bakanae are emerging and causing severe yield loss and there by reduced farm income. Therefore, at this point, the studies on diversity of *F. fujikuroi* using morphological and molecular approaches presume much greater significance. The present study was undertaken to study the existence of variation morphological characters, pathogenecity and genetic variations in diversity of *F. fujikuroi* isolates collected from different regions of Odisha and management of the disease under *in vitro* using fungicides which will help us to develop suitable management strategies for in a particular location. Consequently the long term goal of this study is to devise strategies for developing resistant cultivars and to evolve new molecules for bakanae management.

MATERIAL AND METHODS

Survey and collection of isolates, identification and long term storage

A roving survey was conducted during *kharif* 2016 and 2017 in different villages of five districts of Odisha, namely Cuttack, Jajpur, Sambalpur, Bargarh and Ganjam to study the disease incidence and loss estimation. The disease incidence was recorded at two crop stages; a) tillering & stem elongation and b) heading/panicle initiation. In each block, 2 to 5 villages were randomly selected and 1 m2 area in each field was marked to take the observations. Samplings of diseased plants were made randomly as per the method given by Madden and Hughes (1999). Observations were taken for disease incidence, symptoms and varieties infected. The disease incidence (%) was calculated by the following formula (Zainudin et al., 2008).

$$\text{Per cent disease incidence(PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

During survey, the plants showing characteristic bakanae symptoms such as abnormal elongation, yellowing and drying of the plants, growth of white fungal mycelium on the base of the stem were observed. The infected plants were uprooted and brought to the laboratory for isolation of the pathogen. The pathogen was isolated on Potato Dextrose Agar (potato 200 g, dextrose 20 g, agar 20 g and water 1 lit) medium. The plates were incubated at 25±1°C for 8 days under alternate light and dark conditions. Single spore isolation and hyphal tip method was used to maintain the pure cultures (Leslie and Summerell, 2006).

Morphological characterization and pathogenicity of *F. fujikuroi*

Morphological identification of single spore isolates of the *Fusarium fujikuroi* isolates was made by following the criteria of Nelson et al. (1983) and Booth (1971). The pathogen was sub-cultured on PDA slants and allowed to grow at 25±1°C for one week and later the culture was stored in refrigerator at 4oC for further studies. The morphology of macroconidia, microconidia, presence or absence of chalamydospores, pigmentation, growth rate of the isolates, and appearance were recorded. Then conidia were harvested from profusely growing fungal plates and spore concentration was adjusted to 5x105 spores per ml and used for the pathogenicity assay by seed inoculation method. Seeds of Pooja and PB-1121 (susceptible) were sown in plastic trays. Inoculation was done by soaking the hot water treated seeds in microconidial suspension for 24 hr. then seeds were sown in plastic trays. The observations for the disease symptoms were recorded immediately after germination.

DNA isolation and quantification

The pure cultures of isolates of *Fusarium fujikuroi* maintained in PDA slants were cultured on 30 ml of potato dextrose broth (PDB) and incubated at 27±1°C for 7 days. After incubation, the mycelial mats were harvested by filtration and stored at -80°C for genomic DNA extraction by following the protocol of Murray and Thompson (1980) with slight modifications. Filtered mycelium (3 g) was dried between sterilized blotting paper to remove the excess moisture. The mycelium was ground to a fine powder in liquid nitrogen, transferred to 1 ml of CTAB extraction buffer (0.1M Tris, 1.5 M NaCl, 0.01M EDTA and 2 % CTAB) in a 2-ml tube and kept in waterbath at 65°C for 90 minutes with occasional stirring. An equal volume of chloroform: isoamyl alcohol (24:1) was added to all the tubes and the centrifuged at 10,000 rpm for 20 min at 4 °C. The upper supernitant was pippetted out and transferred to a fresh 1.5 ml tube and mixed with 100 µl of sodium acetate (3 M) and 600µl of ice-cold isopropanol and kept overnight. Next day, the mixture was centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was washed with 70 % ethanol, dried under aceptic conditions of laminar air flow (LAF) hood and finally dissolved in 50 µl of T10E1 buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA) for further use. The DNA was quantified using spectrophotometer and quality analysis was done on 1.0 % agarose gels and Nanodrop Spectrophotometer. After quantification, the DNA was diluted to make final concentration of 30-50 ng/µl for PCR amplification.

Primers and PCR conditions

Isolates morphologically identified as *F. fujikuroi* were further confirmed by polymerase chain reaction using specific primer sets by amplifying the terminal elongation factor (tef-1α) genes specific to *Fusarium* spp. Primer sequences used in this study are EF1 (5' ATGGGTAAGGAAGACAEAGAC 3') and EF 3 (5' GGAGGTACCAGTGATCATGTT 3') reported by O'Donnell and Cijelnik (1997). The primers were synthesized by Integrated DNA Technologies, New Delhi and supplied as lyophilized products of desalted oligos. PCR amplification was carried out in a 20 µl reaction volume containing 20 ng of template DNA, 0.5 µM of primers, 0.2 µM of each of dNTP, , 1.5 mM $MgCl_2$, 1X Taq buffer and 1U of Taq DNA polymerase (DreamTaq, Thermo Scientific, USA). The PCR was performed as follow: initial denaturation of 4 min at 94°C; followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 45 sec at 52°C, and extension for 2 min at 72°C, and final extension for 10 min at 72°C. The amplified PCR products were analyzed using 100 bp DNA ladder (BR Biochem Life Sciences, India) in 1.5%% agarose gels. After electrophoresis, the gels were documented under UV using gel documentation system (AlphaImager, USA). The desired band was cut from the gel clean sterile scalped blade and DNA was eluted as per standard protocol.

TEF Sequencing and phylogenetic relationships

The amplified TEF region of the selected isolates were purified using QIAquick PCR purification kit (QIAGEN, Germany), according to the manufacturer's instructions. The PCR product was sequenced at one direction using forward primers at Eurofins genomics Pvt Ltd., Bangalore. The sequences were compared to those in GenBank (http://www.ncbi.nlm.nih.gov/). The multiple sequence alignment and pair wise alignment were made using ClustalX version 1.81 (Thompson et al., 1997). The phylogenetic relationship was constructed by neighbour-joining method using software Mega 6 (Tamura et al., 2011). Corresponding sequences of *F. fujikuroi* isolates were used for sequence analysis and comparison.

In vitro evaluation of different fungicides and biocontrol agents against the pathogen

The efficacy of four non-systemic fungicides (0.1, 0.2 and 0.3 per cent) and ten systemic fungicides including combination products (0.05, 0.075 and 0.1 per cent) were assayed by 'poisoned food technique'. The required concentration of chemical was prepared and added into sterilized, cooled potato dextrose agar and mixed properly. Twenty ml of cooled medium was poured into 90 mm sterilized petri dishes and allowed for solidification. All the plates were inoculated with actively growing five mm mycelial disc of pathogen separately. The plates were incubated at 25±1^{0}C. The observations were recorded immediately when the growth of the pathogen reached 90 mm in the control plates. Per cent inhibition of mycelial growth over control was calculated by using the formula of Vincent (1947) as follows.

$$I = \frac{CT}{C} \times 100$$

I = Per cent inhibition of mycelial growth.
C= Growth of mycelium in control.
T = Growth of mycelium in treatment.

Statistical analysis

Experiments were performed in triplicate, analyzed as mean ± standard error subjected to two way analysis of variance (ANOVA). Means are separated by Duncan multiple range tests (DMRT) when ANOVA was significant ($p<0.01$). The analysis of data was performed with the SAS version 9.3 (SAS Institute, Cary, North Carolina, USA) software.

RESULTS AND DISCUSSION

In the present study, bakanae disease surveys were conducted during *Kharif* 2016 and 2017 in five districts of Odisha to assess the incidence of bakanae disease. The bakanae disease was present every district surveyed with a disease incidence of 1-25.5% in different locations. Significant disease incidence were observed in rice cultivars; Pooja, Naveen, Abhishek, Pratiksha, Swarna, Swarna sub-1 and Rajalaxmi. The maximum disease incidence was noticed from tillering to heading stage. The highest and lowest disease incidence was observed in Cuttack and Sambalpur district, respectively (Table 1). Interestingly, disease incidence increased significantly from 2016 to 2017 in all the surveyed locations (Fig. 1). This may be due to presence of favorable environmental conditions. Among the six major varieties observed for the disease incidence, Pooja recorded maximum disease incidence while minimum disease incidence was recorded in Rajalaxmi (Fig. 2). The increased disease intensity in Odisha might be attributed to a significant increase in area under cultivation of highly susceptible varieties, occurrence of congenial environmental conditions, dry nursery practices along with previous year infected seeds without any seed treatment. These factors might be important factor in aggravating the bakanae disease in rice growing areas of Odisha. Similarly, other workers have also reported increased in diseases incidence that due to cultivation of rice hybrids, different methods of seedling raising lead to the increased incidence (Li and Luo, 1997; Yang et al., 2003; Kim et al., 2014). In India, the disease was reported in moderate to severe form in different basmati growing areas of north India (Bashyal et al., 2014).

Table 1. Average disease incidence at different districts of Odisha for two years.

Districts	Percent disease incidence (2016-17)	Percent disease incidence (2017-18)
Cuttack	9.35	15.50
Jajpur	6.70	12.50
Sambalpur	3.20	6.00
Bargarh	5.50	8.50
Ganjam	4.50	6.50
Mean PDI	**5.85**	**9.80**

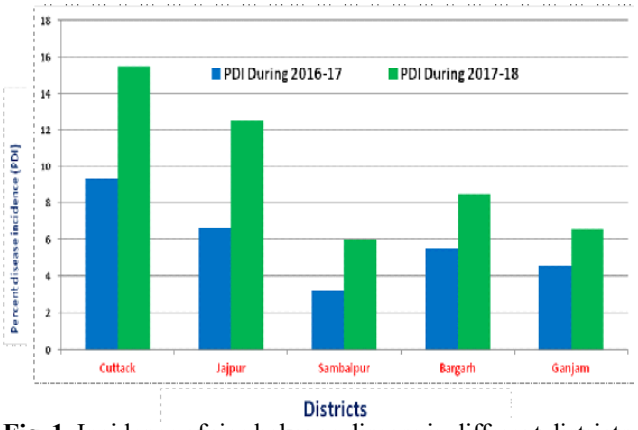

Fig. 1. Incidence of rice bakanae disease in different districts of Odisha.

Isolation, Identification and morphological characterization of *Fusarium fujikuroi*

A total of ten isolates were isolated from infected samples collected from each location and identified through morphological characters. The isolates

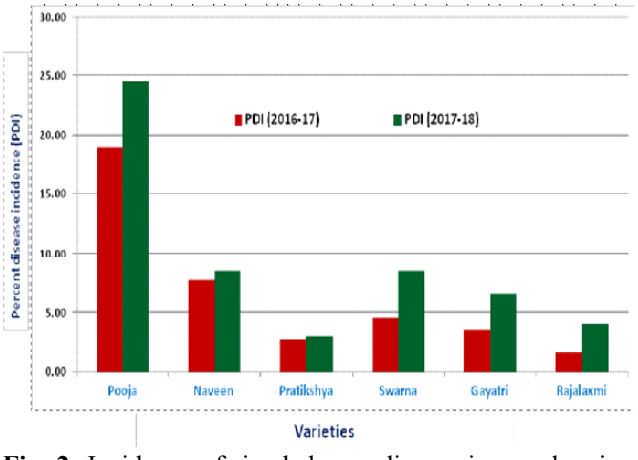

Fig. 2. Incidence of rice bakanae disease in popular rice varieties growing in Odisha.

produced pink pigmentation and 80-100 microconidia (5 x 3.25-8.75 µm) and 10-50 macroconidia (30.25 X 7.00-10 µm) per microscopic field (Table. 2). One isolate F-9 was further tested for its growth and physiological characters. Nine different solid and liquid media were evaluated for the growth and morphological characteristics under in vitro condition. Among the solid media tested, the maximum mycelial growth (90 mm) was observed in Potato Dextrose Agar (PDA), followed by Carrot Agar, Host (Rice) leaf extract agar and Potato carrot agar (PCA) after seven days of inoculation at 25±1°C, whereas, least growth was observed in Elliet's agar medium (46.1 mm). Similar results were found by Wollenweber and Reinking (1935) who reported that pathogen produced different sized macroconidia based on the septations. They also reported that, size of the macroconidia varies with the number of septa present. Colony colour in all the media tested was grayish red to white cottony. Maximum sporulation was observed in Fusarium specific (NSM) medium and least sporulation on elliet's medium. The virulence of different isolates of *F. fujikuroi* was assessed on susceptible variety pooja and different symptoms like abnormal elongation, foot rot, stuting and death were recorded. On the basis of pathogenicity, the isolates were grouped into three categories: highly virulent, moderately virulent and slightly virulent. The results indicated that isolate F-9 was found highly virulent and the same was used for further studies. Zainudin et al. (2008) also reported species such as *F. fujikuroi, F. proliferatum, F. verticillioides, F. sacchari* and *F. subglutinans* associated with the disease based on based on their morphological and pathogenic characteristics. Among all species only *F. fujikuroi* produced characteristics symptoms like abnormal elongation due to the production of gibberellic acid by the pathogen.

Pathogenicity assay

Pathogenicity studies on highly susceptible variety Pusa Basmati 1121 and Pooja as seed inoculation assays resulted that all the 10 isolates varied in pathogenicity and produced elongation and rotting symptoms. Isolates collected from typical elongation symptoms produced foot rot and death of the plants in glasshouse conditions. All the isolates grouped in highly virulent category (Table 2). Present study is in corroborated with the finding of Bhashyal et al. (2016) who categorized the F.fujikuroi isolates into three groups based on their pathogenicity.

TEF sequencing and phylogenetic analysis

To investigate the genetic diversity among the isolates of *F. fujikuroi*, TEF gene (Translation Elongation Factor) were amplified using two primers ef1 and ef2. Tef-1alpha gene is most commonly used for inferring phylogenetic relationships among fungi species. The

Table 2. Details of *F. fujikuroi* isolates used in the present study.

Sl.No	Isolate name	Variety	Location	Size of microconidia (µm)	Size of macroconidia(µm)	Virulent category
1.	FJ1	Pooja	Kisannagar	5.0 x 3.25-8.75	30.25 X 7.00-10	Highly virulent
2.	FJ2	Pooja	Khurda	5.3 x 3.50-7.75	30.25 X 8.00-10	Highly virulent
3.	FJ3	Swarna	Tangi-chowdwar	5.5 x 3.25-8.75	31.25 X 8.50-10	Highly virulent
4.	FJ4	Gayathri	Cuttack	5.7 x 4.25-7.75	30.25 X 7.50-10	Highly virulent
5.	FJ5	Rajalaxmi	Jajpur	5.2 x 3.25-6.75	30.25 X 6.50-10	Highly virulent
6.	FJ6	Naveen	Kisannagar-2	5.5 x 3.25-9.00	32.25 X 8.00-10	Highly virulent
7.	FJ7	Pooja	Sambalpur	5.2 x 3.25-8.75	30.25 X 7.00-10	Highly virulent
8.	FJ8	Pooja	Bargarh	5.0 x 3.25-8.75	30.25 X 8.00-10	Highly virulent
9.	FJ9	Swarna	Chandikole	5.0 x 3.25-8.75	30.25 X 8.50-10	Highly virulent
10.	FJ10	Pratisksya	Ganjam	5.75 x 3.2-9.00	30.25 X 7.00-10	Highly virulent
11.	FJ11	KM586385	India	-	-	-
12.	FJ12	MF356522	China	-	-	-
13.	FJ13	FN252398	Denmark	-	-	-
14.	FJ14	KP009958	India	-	-	-
15.	FJ15	KC121068	Italy	-	-	-
16.	FJ16	LC009439	Japan	-	-	-
17.	FJ17	KX681488	USA	-	-	-
18.	FJ18	JF699615	Spain	-	-	-

Table 3. Sequence identity matrix of *Fusarium fujikuroi* isolates based on terminal elongation factor 1-alpha gene.

Isolate	FJ1	FJ2	FJ3	FJ4	FJ5	FJ6	FJ7	FJ8	FJ9	FJ10	FJ11	FJ12	FJ13	FJ14	FJ15	FJ16	FJ17	FJ18
FJ 1	0																	
FJ 2	0.95	0																
FJ 3	0.96	0.97	0															
FJ 4	0.92	0.92	0.92	0														
FJ 5	0.91	0.92	0.92	0.95	0													
FJ 6	0.92	0.93	0.92	0.96	0.95	0												
FJ 7	0.92	0.91	0.92	0.96	0.95	0.95	0											
FJ 8	0.91	0.92	0.91	0.95	0.95	0.94	0.94	0										
FJ 9	0.96	0.97	0.96	0.92	0.92	0.92	0.91	0.92	0									
FJ 10	0.91	0.91	0.91	0.94	0.96	0.96	0.95	0.95	0.92	0								
FJ 11	0.91	0.91	0.91	0.94	0.95	0.94	0.93	0.93	0.91	0.95	0							
FJ 12	0.87	0.88	0.88	0.90	0.91	0.91	0.90	0.90	0.88	0.91	0.93	0						
FJ 13	0.89	0.91	0.90	0.93	0.95	0.93	0.92	0.93	0.90	0.94	0.97	0.92	0					
FJ 14	0.89	0.91	0.90	0.93	0.94	0.93	0.92	0.92	0.90	0.94	0.97	0.92	0.98	0				
FJ 15	0.89	0.90	0.89	0.92	0.94	0.93	0.91	0.92	0.89	0.93	0.96	0.92	0.97	0.99	0			
FJ 16	0.88	0.90	0.89	0.92	0.92	0.92	0.91	0.91	0.89	0.92	0.95	0.94	0.94	0.95	0.95	0		
FJ 17	0.88	0.90	0.89	0.92	0.93	0.92	0.91	0.91	0.89	0.92	0.95	0.92	0.94	0.96	0.96	0.98	0	
FJ 18	0.86	0.88	0.87	0.90	0.91	0.90	0.89	0.89	0.87	0.91	0.93	0.95	0.92	0.93	0.93	0.97	0.97	0

amplification of tef-1alpha gene generated approx 700 bp bands. Sequence similarities between Fusarium fujikuroi isolate ranged from 86 to 98%, and it showed the greatest sequence similarity of 98% between FJ13 (FN252398-Denmark) with FJ 4 (Cuttack isolate) and FJ16 (LC009439-Japan) with FJ17 (KX681488-USA) isolate, and minimum sequence similarity of 88% was observed between FJ3 (Tangi-Chodwar) and FJ18 (JF699615-Spain), similarly, FJ9 (Chandikole) with FJ18 (JF699615-Spain), isolates (Table 3). In phylogenetic analysis, based on tef-1alpha gene region, overall two major groups were formed. The bakanae isolates FJ1 (Kisannagar), FJ2 (Khurda), FJ3 (Tangi-Chodwar) and FJ9 (Chandikole) isolates belonged to same group whereas FJ4 (Cuttack), FJ5 (Jajpur), FJ6 (Kisannagar-2), FJ7 (Sambalpur), FJ8 (Bargarh) and FJ10 (Ganjam) are in a separate group with other world isolates (Figure-3). Similar results were obtained by O'Donnell and colleagues (2000) who confirmed phylogenetic relationships among Gibberella fujikuroi by using DNA sequence amplified by PCR from four regions (28S rDNA, ITS, mitSS rDNA and β-tubulin) which are associated with two genes encoding proteins of the core of EF-1? and calmodolin and their combination genes. Gene TEF (Translation Elongation Factor) which is a protein that is translated into an essential part of the encoding gene has high phylogenetic application because: 1 a high level of specificity to Fusarium species. 2- No non-identical copies of (non-orthologous) genes are made of this genus (Geiser et al., 2004). This gene first was used as a marker for species identification and phylogenetic relationship of the genus night blades Order Lepidoptera by Cho and colleagues (Cho et al., 1995). Single copies of this gene are found in the genus Fusarium and demonstrated high levels of sequence polymorphism and even in comparison with protein coding genes such as calmodulin, β-tubolin and histone H3 in the species that are relatively close (Geiser et al., 2004) and were selected as a tool for determination of TEF (single-locus) in the genus Fusarium. Present study demonstrated the high degree of variation among *F. fujikuroi* isolates collected from different rice growing region of Odisha.

Effect of fungicides and biocontrol agents on the growth of pathogen

So far, chemical control is the only viable option

Table 4. Effect of different systemic and combination fungicides on the growth of Fusarium fujikuroi under *in-vitro*.

Sl. No	Name of the Chemical	Inhibition of mycelial growth (%) at concentrations			
		0.050	0.075	0.10	Mean
1	Merger	18.52 (25.49) *	54.44 (47.55)	100.00 (90.00)	57.65 (49.40) d
2	Companion	81.11 (64.24)	100.00 (90.00)	100.00 (90.00)	93.70 (75.47) c
3	Vitavax Power	88.15 (69.86)	100.00 (90.00)	100.00 (90.00)	96.05 (78.54) b
4	SAAF	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
5	Nativo	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
6	ICF-110	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
7	Folicure	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
8	Amister	13.70 (21.73)	27.78 (31.81)	27.78 (31.81)	23.09 (28.72) e
9	Tilt	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
10	Bavistin	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00) a
Mean		80.15 (63.54)	88.22 (69.93)	92.78 (74.41)	87.05 (68.91)
Sources		S. Em.±		C.D. at 0.01	
Fungicides (F)		0.53		1.60	
Concentration (C)		0.29		0.87	
Interaction (FxC)		0.92		2.77	

* Values in the paranthesis are the arc sine transformed values of the data. Figures with the same alphabet number was not varied significantly under duncan multiple range test (DMRT).

available for management of this emerging disease. A significant reduction in growth and sporulation of the pathogen was observed in treated plates as compared to control. Significantly, maximum reduction in the mycelial growth and sporulation of the pathogen was recorded in treatments; Carbendazim 12% + mancozeb 63% (SAAF), Trifloxistrobin + Tebuconazole (Nativo), ICF-110 and Tebuzonazole (Folicure), Propiconazole 25% EC (Tilt), Carbendazim 50% WP (Bavistin) with 100% reduction at 0.05% (500 ppm) concentration. All the fungicides were found to be effective at 0.075% (750 ppm) concentration except Tricyclazole 18 %+ Mancozeb 62% (merger) and azoxistrobin (Amister) which showed least reduction in the mycelial growth (Table 4&5)Among the non0systemic fungicides, only Dithane M-45 found effective at 0.3 % (300 ppm) compared to other fungicides (Table 3). This may be the non-efficay of these fungicides to control F. fujikuroi. Three *Trichoderma* spp. (T-1, T-2 & T-3) were tested against the pathogen by dual culture assay. All the three strains were found to be effective in inhibiting the growth by 75-90%. Carbendazim is the best and widely used chemical for seed treatment and foliar application because of its efficacy in controlling various diseases (Pankajkumar et al., 2016). This chemical was further evaluated at pot experiments under net house condition. The results showed sigificantly higher germination, vigour index and less disease incidence, when the seeds were treated with Carbendazime 50% WP @ 0.1% (1000 ppm) as compared to untreated control. Javaid and Ilyas (1995) found that carbendazim even at 5 ppm completely inhibited the mycelial growth and provided effective disease control. This variation may be due to

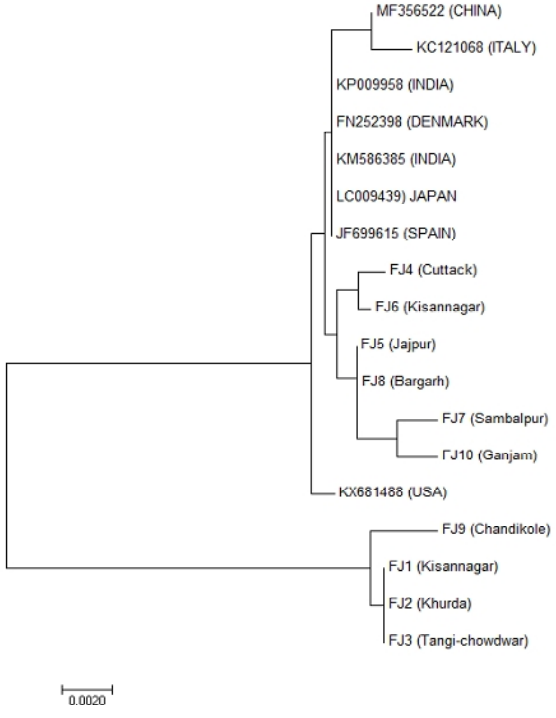

Fig. 3. Phylogenetic relationship among *Fusarium fujikuroi* isolates based on terminal elongation factor 1-alpha gene.

Table 5 . Effect of different contact fungicides on the growth of Fusarium fujikuroi under *in vitro*.

Sl. No	Name of the Chemical	Inhibition of mycelial growth (%) at concentrations			
		0.10	0.20	0.30	Mean
1	Captan	45.19 (42.24) *	59.26 (50.34)	61.11 (51.42)	55.19 (47.98) b
2	DM-45	9.26 (17.72)	41.11 (39.88)	100.00 (90.00)	50.12 (45.07) c
3	Thiram	48.15 (43.94)	62.96 (52.51)	75.93 (60.62)	62.35 (51.15) a
4	Copper oxychloride	3.70 (11.09)	18.52 (25.49)	25.93 (30.61)	16.05 (23.62) d
Mean		26.58 (31.03)	45.46 (42.40)	65.74 (54.18)	45.93 (42.66)
Sources		S. Em.±		C.D. at 0.01	
Fungicides (F)		1.03		3.47	
Concentration (C)		1.00		3.01	
Interaction (FxC)		2.00		6.02	

* values in the paranthesis are the arc sine transformed values of the data. Figures with the same alphabet number was not varied significantly under duncan multiple range test (DMRT).

presence of virulent isolates and development of resistance to fungicide at lower concentration. There is still very less information available on the availability of the durable resistant sources for the disease along with integrated management practices. Though efforts have been made to screen numerous germplasm lines for resistance to bakanae disease, to date no cultivar has shown a complete resistance (Bagga and Kumar., 2000; Desjardins et al., 2000; Ghazanfar et al., 2013; Jeong et al., 2013; Wiemann et al., 2013). Despite the advancements in studying the genetic diversity and elucidating the *F. fujikuroi* genome, limited knowledge is available on the mechanisms of rice resistance to bakanae, which is crucial for the development of appropriate control strategies (Matic et al., 2016).

The studies will helped to demonstrate how widespread occurrence of disease in major rice growing areas of Odisha and the level of damage caused by it. In all the rice-growing areas surveyed in Odisha, there is a need to develop disease resistant cultivars, and integrated disease-management practices. The further studies on development of an effective and less time consuming screening protocol and its utilization in screening for resistance are in progress which leads to come up with some promising resistant sources.

ACKNOWLEDGEMENTS

The authors are extremely grateful to the Director, ICAR-National Rice Research Institute, Cuttack, India for his support and facilitation for carrying out the research work successfully. We do not have any conflict of interest to declare.

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