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Evaluating the impact of temperature and pH on *in vitro* growth and sporulation of *Fusarium fujikuroi* causing rice bakanae disease

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Abstract
Bakanae disease is a serious threat to basmati rice cultivation. Climatic factors like temperature, pH and humidity influence the growth, survival and infestation of *Fusarium* species. In the present study, the impact of temperature and pH on the growth and sporulation of *Fusarium fujikuroi* was evaluated. Maximum radial growth of *Fusarium fujikuroi* was recorded at 25 °C followed by 30 °C and 20 °C. Very little growth was observed at 10 °C and 35 °C whereas, no growth of pathogen was observed at 40 °C. The number of spores were found maximum at 25 °C followed by 30 °C and 20 °C. The spore size was measured in range of 10 to 13.5 µm but no significant difference in size of spores at different temperatures was observed. The maximum growth *Fusarium fujikuroi* was observed at pH 7.0 followed by pH 7.5 and pH 8.0. No growth of pathogen was observed at pH 4.5. The sporulation of pathogen was observed maximum at pH 7.0 followed by pH 7.5 and pH 8.0. The spore size was measured and it was found to be range from 10 µm to 13 µm but no significant difference in spore size between different pH levels was found.

Keywords: Temperature, pH, PDA, *Fusarium*, radial growth, sporulation

Introduction
Bakanae disease caused by *Fusarium fujikuroi* is emerging as a serious threat to production of rice cultivation in India, Japan, Taiwan and Thailand (Singh and Sunder, 2012; Webster and Gunnell, 1992; Saremi, 2005; Kini *et al.*, 2002) [17, 20, 16, 10]. The pathogen is known to produce both macro as well as micro conidiophores which bears macro and micro conidia, respectively. The microconidia are fusiform oval, 1-2 celled and remain joined or cut off in false heads and agglutinated in chains. The macroconidia are sickle-shaped or almost straight and delicate. They have narrow ends and are bented at the apex into a hook like structure and have foot cell at the base (Leslie and Summerell, 2006) [12]. The pathogen is known to produce a vast group of secondary metabolites and mycotoxins which play an important role in virulence of the pathogen. It produces gibberellic acid (Malonek *et al*., 2005) [13], fusaric acid (Bacon and Hinton, 1996) [2], bikaverin (Lale and Gadre, 2016) [11] and some other mycotoxins such as fumonisin B1 and B2 (Desjardins *et al.*, 1997) [5], moniliformin and fusaproliferin and beauvericin etc. Three different *Fusarium* spp. *viz*. *F. fujikuroi*, *F. verticillioides* and *F. proliferatum* were identified to be associated bakanae disease in rice in India. The distribution profile of bakanae disease of rice but *F. fujikuroi* is the most prominent species associated with bakanae disease. *Fusarium* spp. are widely distributed across all geo-graphic regions including soils, plants and air. But several factors influence the occurrence of *Fusarium* in the soil and the infestation and infection it generates in cereal plants. Geographical factors including climate are important for the occurrence of *Fusarium* and for the pattern of infestation by various *Fusarium* species. Climatic factors like temperature, soil pH and humidity influence the growth, survival, spreading and hence the incidence of *Fusarium* species and the crop damage. The influence of these factors on *Fusarium* disease is complicated by the fact that *Fusarium* species are able to cause the diseases individually or in com-plex (Doohan *et al*., 1998) [7]. The effect of climatic conditions on the incidence of *Fusarium* species is probably both direct (e.g., an effect on mode of re-production) and indirect (e.g., an effect of soil and vegetation type). There are some reports on how the *Fusarium* species differentially respond to different environmental variations, mostly temperature, origin and humidity (Conrath *et al.*, 2002) [4].

These factors are also important in mycotoxicosis epidemiology, because the production of mycotoxins by the different species is differentially affected by some environmental factors such as temperature (Di Menna *et al*., 1991 and Jimeneza *et al*. 1996) [6, 9]. *Fusarium* spp. are known to survive at wide temperature and pH range and its ability to survive in extreme conditions make them as a potent pathogen that can survive in soil for a long time. Environmental factors such as temperature, water activity and pH have a great influence on fungal development (Yadav *et al*., 2014) [21]. So, keeping this in view, in the present study, we investigated the effect of different temperature and pH on the growth and sporulation of *Fusarium fujikuroi* inciting bakanae disease of rice.

Materials and methods

F250, a virulent isolate of *Fusarium fujikuroi* was used to carry out all the experiments of the present study. The pathogen was maintained in PDA plates at 25±2 ºC in the BOD incubator.

Effect of temperature on growth and sporulation

The effect of temperature on colony growth was studied on PDA in 100 × 15 mm diameter Petri plates using F250 isolate. After 3–4 days growth on PDA under fluorescent lights, a 1 × 1 cm block of PDA + mycelium was cut from the margin of colonies and the blocks were placed mycelium side down in the centre PDA plates (one block per Petri plate). The cultures were then sealed with Parafilm and incubated without light at different temperature levels. The pathogen was incubated for at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C in a growth chamber. Temperatures were monitored using Watchdog data loggers with external temperature sensors. During incubation, round colonies developed with the 1 × 1 cm square block of PDA + mycelium in the centre. After 7 days, the diameters of fungal colonies were measured along two perpendicular directions which passed through the centre of the colony. RGB value was calculated using imaging softwares. Sporulation was measured with the help of haemocytometer and spore size was also measured. One block of fungal culture was placed in an eppendorf tube and 1 ml of distilled water and was centrifuged for 5 minutes. Number of spores per ml of suspension were calculated with the haemocytometer. Each treatment was replicated thrice under C.R.D. design.

Effect of pH on growth and sporulation

The effect of temperature on colony growth was studied on PDA in 100 × 15 mm diameter Petri plates using F250 isolate. After 3–4 days growth on PDA under fluorescent lights, a 1 × 1 cm block of PDA + mycelium was cut from the margin of colonies and the blocks were placed mycelium side down in the centre PDA plates (one block per Petri plate) with different pH levels. The pH of media was adjusted to different levels *viz*, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. The normal media with a pH of 5.7 ± 0.2 was used as control. The pH of media was adjusted by using either 0.1N HCL or 0.1N NaOH. The cultures were then sealed with Parafilm and incubated without light at 25±2 ºC in a growth chamber. During incubation, round colonies developed with the 1 × 1 cm square block of PDA + mycelium in the centre. After 7 days, the diameters of fungal colonies were measured along two perpendicular directions which passed through the centre of the colony. RGB value was calculated using imaging softwares. Sporulation was measured with the help of haemocytometer and spore size was also measured. One block of fungal culture was placed in an eppendorf tube and 1 ml of distilled water and was centrifuged for 5 minutes. Number of spores per ml of suspension were calculated with the haemocytometer. Each treatment was replicated thrice under C.R.D. design.

Statistical analysis

Data obtained from growing *Fusarium* strains at different temperatures and pH levels were analyzed by one-way ANOVA using SAS software version 9.2 (SAS Institute, Cary, NC, USA). Duncan's multiple range test was performed for comparing means of different treatments of all experiments at $P < 0.05$ using SAS software.

Results and Discussion

Effect of temperature on growth and sporulation of *Fusarium fujikuroi.*

The radial growth of *Fusarium fujikuroi* isolate F250 was recorded maximum at 25 °C (6.60 cm) followed by 30 °C (6.38 cm) and 20 °C (6.06 cm) (Fig 1). No growth of pathogen was observed at 40 °C. The number of spores were found maximum at 25 °C (19.92 $\times 10^6$/ ml) followed by 30 °C (9.17$\times 10^6$/ ml) and 20 °C (9.07$\times 10^6$/ ml). The spore size was measured in range of 10 to 13.5 µm but no significant difference in size of spores at different temperatures was observed. Significant differences were observed for the colony colour at different temperature (Table 1). The study revealed that the isolate of *F. fujikuroi* grew at the temperature range of 10-35 ºC. However, growth of the fungus drastically reduced below 15 ºC and started to decline above 30 ºC and become zero at 40 ºC, as this temperature did not favour for growth of the fungus. The fungus attained the maximum growth at 25 ºC temperature. These studies are in confirmation with Anjaneya Raddy (2002) [1], who reported that growth of the *F. udum* differed in their temperature requirement which varied from 20-35 ºC. While Ecang (1980) [8] reported that 30 ºC is most favourable for mycelial growth of *F. moniliforme*. Almost similar results were reported by other researchers (Sundar, 1995 and Ou, 1985) [19, 14]. After seven days of incubation highest 19.92 X 10^6 spore/ml were produced at 25 ºC temperature, conversely the sporulation of the fungus declined below or above at 25 ºC temperature. The findings are in accordance with the observation of Sundar (1995) [19], where the maximum sporulation has been reported at 25-30 ºC. The colour of the colony and pigmentation also varied with different temperature regimes. These findings are in agreement with Sood (1964) [18], who have reported that optimum growth of the fungus was at 25 ºC to 30 ºC with good and fluffy growth and very poor growth of the fungus was beyond a temperature of 40 ºC.

Table 1: Effect of temperature on *in vitro* growth and sporulation of *Fusarium fujikuroi*

Temp (°C)	Radial growth (cm)	Sporulation (×10^6 spores/ ml)	RGB front			RGB back		
			R	G	B	R	G	B
T1 (10)	1.11^{e}	2.92^{e}	224.33	213.00	147.67	223.33	222.33	176.33
T2 (15)	3.37^{c}	7.11^{cd}	157.00	73.333	34.00	237.67	232.67	240.33
T3 (20)	6.06b	9.07^{b}	170.33	181.33	182	166.33	173.67	173.67
T4 (25)	6.60^{a}	19.92^{a}	194.33	179.33	173.67	217.33	83.67	35.00
T5 (30)	6.38^{ab}	9.17^{b}	181.67	155.00	126.33	214.67	81.00	30.67
T6 (35)	1.62^{d}	7.08^{cd}	85.00	61.00	35.00	158.67	78.333	44.333
T7 (40)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SE(m)	0.080	0.322	8.65	11.18	14.54	9.64	5.77	4.56
SE(d)	0.112	0.455	12.23	15.82	20.56	13.63	8.16	6.44
C.D.	0.238	1.002	26.94	34.84	45.30	30.02	17.97	14.20

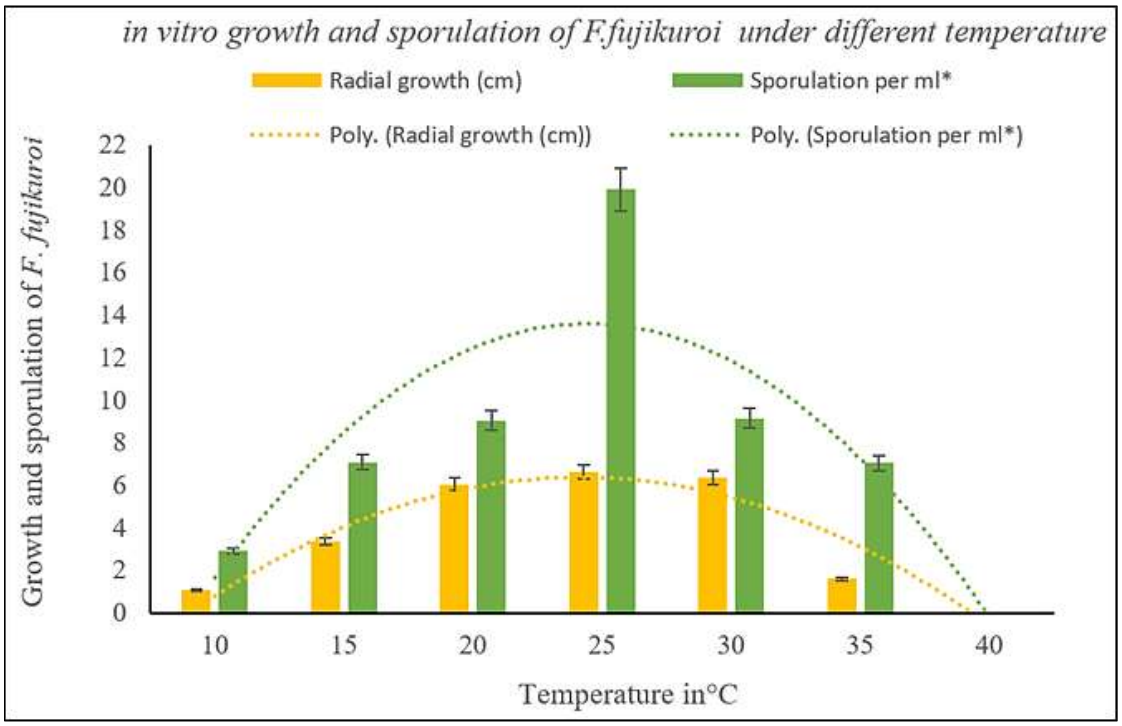


Fig 1: Impact of different temperature levels on growth and sporulation of *F. fujikuroi*

Effect of pH on growth and sporulation of *Fusarium fujikuroi*

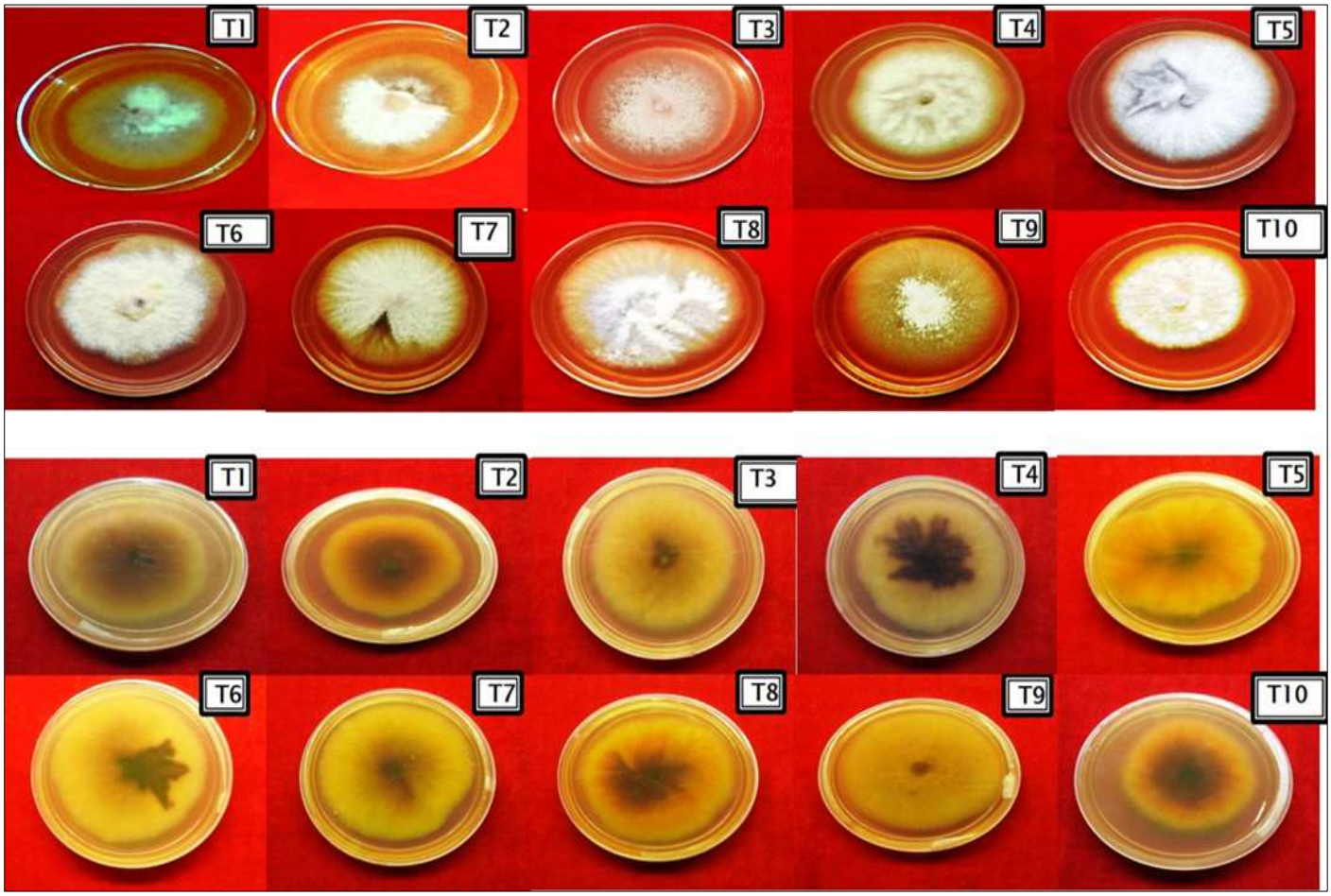
The maximum growth (6.8 cm) of F250 isolate of *Fusarium fujikuroi* was observed at pH 7.0 followed by pH 7.5 (6.63 cm) and pH 8.0 (6.56 cm). No growth of pathogen was observed at pH 4.5. The sporulation of pathogen was observed maximum (18.25×10^6/ ml) at pH 7.0 followed by pH 7.5 (18.18×10^6/ ml) and pH 8.0 (12.47×10^6/ ml) (Table 2). The spore size was measured and it was found to be range from 10 µm to 13 µm but no significant difference in spore size between different pH levels was found. Significant difference was observed for RGB values in different pH conditions (Fig. 4.2). In this study, pH effect on growth and sporulation of pathogen was observed. The suitable pH of mycelial growth of *F. fujikuroi* was 7.0 and abundant spores were also produced at the 7.0 pH level. Further decreasing or increasing of the pH levels from the optimum level, the rate of growth and sporulation gradually decreased. The results are in agreement with the findings of several workers (Sundar, 1995; Yogeswari, 1948) [19, 22]. Chaudhary (1971) [3] and Prasad *et al.* (1992) [15] reported that the pH 6.0 as the best for the growth and sporulation of *F. moniliforme*. Sundar (1995) [19] also reported that *F. moniliforme* grew and sporulated well at pH 6.0.

Table 2: Effect of pH on *in vitro* growth and sporulation of *Fusarium fujikuroi*

pH	Radial growth (cm)	Sporulation (×10^6 spores/ ml)	RGB front			RGB back		
			R	G	B	R	G	B
T1 (5.0)	5.52^{gh}	2.22^{i}	223.00	209.33	191.33	139.33	74.67	23.33
T2 (5.5)	5.92^{gh}	7.76^{gh}	173.67	154.33	83.33	184.33	161.00	69.67
T3 (6.0)	6.15^{def}	9.57^{ef}	198.67	181.00	162.33	219.67	225.67	185.67
T4 (6.5)	6.37^{cde}	13.27^{c}	181.67	184.67	172.00	119.67	79.333	46.33
T5 (7.0)	6.80^{a}	18.25^{ab}	191.67	195.67	177.00	182.67	171.67	51.67
T6 (7.5)	6.63^{ab}	18.18^{ab}	224.67	238.00	246.67	228.67	170.67	32.33
T7 (8.0)	6.56^{abc}	12.47^{d}	152.67	100.67	15.00	225.00	167.67	9.33
T8 (8.5)	6.45^{bcd}	7.20^{gh}	211.33	200.33	187.67	165.33	137.00	29.67

T9 (9.0)	6.3^{de}	2.18^{i}	207.33	210.00	173.33	215.33	148.33	7.67
T10 (C)	6.00^{fg}	9.34^{ef}	203.67	198.67	189.67	191.67	133.00	28.67
T11 (4.5)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SE(m)	0.182	0.482	7.45	11.20	3.23	7.09	6.17	3.34
SE(d)	0.258	0.682	10.53	7.92	4.56	10.03	8.72	4.73
C.D.	0.529	1.433	22.12	23.53	9.59	21.06	18.32	9.93

Where, R: Red, G: Green and B: Blue



Where, Where, T1= pH 5.0, T2= pH 5.5, T3= pH 6.0, T4= pH 6.5, T5= pH 7.0, T6= pH 7.5, T7= pH 8.0, T8= pH 8.5, T9= pH 9.0, T10= Control (pH 5.8)

Fig 2: Effect of pH on *in vitro* growth of *F. fujikuroi*

References

1. Anjaneya Reddy B. Variability of *Fusarium durum* and evaluation of pigeonpea (*Cajanus cajan* (L). Mills) genotypes. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore. 2002, 115p.
2. Bacon CW, Hinton DM. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. Canadian Journal of Botany. 1996;74(8):1195-1202.
3. Chaudhary SK. Studies on the physiology of *the Fusarium oxysporum f. udum* (Butler) Snyder and Hensen (causal organism of wilt of *Cajanus cajan* (Linn.) Millsp.) PhD Thesis, Department of Botany, Ranchi University. Ranchi, 1971, p 297.
4. Conrath U, Pieterse CM, Mauch-Mani B. Priming in plant–pathogen interactions. Trends in plant science. 2002;7(5):210-216.
5. Desjardins AE, Plattner RD, Nelson PE. Production of Fumonisin B (inf1) and Moniliformin by *Gibberella fujikuroi* from Rice from Various Geographic Areas. Applied and Environmental Microbiology. 1997;63(5):1838-1842.
6. Di Menna ME, Lauren DR, Smith WA. Effect of incubation temperature on zearalenone production by strains of Fusarium crookwellense. *Mycopathologia.* 1991;116(2):81-86.
7. Doohan FM, Parry DW, Jenkinson P, Nicholson P. The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat. Plant pathology. 1998;47(2):197-205.
8. Ecang MT. Seed Detection and Control of *Fusarium Moniliforme* Sheldon, Causal Organism of" bakanae" Disease and Foot Rot of Rice. University of the Philippines, 1980.
9. Jimenez M, Manez M, Hernandez E. Influence of water activity and temperature on the production of zearalenone in corn by three Fusarium species. International Journal of Food Microbiology. 1996;29(2-3):417-421.
10. Kini KR, Let V, Mathur SB. Genetic variation in *Fusarium moniliforme* isolated from seeds of different host species from Burkina Faso based on random amplified polymorphic DNA analysis. Journal of Phytopathology. 2002;150:209-212.

11. Lale GJ, Gadre RV. Production of bikaverin by a *Fusarium fujikuroi* mutant in submerged cultures. AMB Express. 2016;6:34. http://doi.org/10.1186/s13568-016-0205-0
12. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. Blackwell Publishing, Oxford, UK. 2006, pp 388.
13. Malonek S, Bömke C, Bornberg-Bauer E, Rojas MC, Hedden P, Hopkins P, *et al*. Distribution of gibberellin biosynthetic genes and gibberellin production in the *Gibberella fujikuroi* species complex. Phytochemistry. 2005;66(11):1296-1311.
14. Ou SH. Bakanae disease and foot rot. In Rice diseases Survey. Kew: Commonwealth Mycological Institute, 1985, pp. 262-272.
15. Prasad A, Chaudhary RS, Chaudhary SK. Effect of different pH levels on growth and sporulation of *Fusarium moniliforme V. subglutinans* Wr. and Rg., the causal organism of wilt of maize. Bio. J. 1992;4(1):75-78.
16. Saremi H. *Fusarium*, biology, ecology and taxonomy. Jihad Daneshgahi, Ferdossy Mashhad University, Iran, 2005, 152.
17. Singh R, Sunder S. Foot rot and bakanae of rice: an overview. Reviews of Plant Pathology. 2012;5:566-604.
18. Sood PN. Investigations on the Foot rot disease of rice caused by Fusarium moniliforme Sheld under the Punjab conditions. M.Sc Thesis, Punjab Agricultural University, Ludhiana, 1964.
19. Sundar S. Survival and variability of Fusarium moniliforme sheld. and management of bakanae disease of rice. PhD. Thesis, CCS, Hariana Agricultural University, Hisar, India, 1995, p.155.
20. Webster Gunnell, Webster RK, Gunnell PS. Compendium of Rice Diseases. The APS Press St. Paul, Minnesota, USA., 1992, pp. 62.
21. Yadav RS, Tyagi S, Javeria S, Gangwar RK. Effect of different cultural condition on the growth of *Fusarium moniliforme* causing Bakanae disease. Eur. J Mol. Biotechnol. 2014;4(2):95-100.
22. Yogeswari L. Trace element nutrition of fungi. 1. The effect of boron, zinc and manganese on Fusarium spp, Proceedings of Indian Academic Science Bulletin. 1948;28:177-201.