



Effect of different abiotic factors on symptom expression and severity of bakanae disease of rice (*Oryza sativa*)

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ABSTRACT

The bakanae disease incited by *Fusarium fujikuroi* Nirenberg is becoming a serious threat to cultivation of rice, especially aromatic rice worldwide. The present study was conducted in the year 2016–17 at ICAR- Indian Agricultural Research Institute, New Delhi to study the effect of different abiotic factors (soil moisture, soil pH, soil type, and temperature) individually and in combination in causation of different kind of bakanae disease symptoms and severity. The highest disease severity (93.33%) was observed at 30% soil moisture conditions. Variation in disease severity was significant in interaction of moisture and soil type and temperature and soil type, whereas, it was non-significant in interaction of moisture and temperature and soil type, moisture and temperature. It was observed that elongation symptoms were more prevalent in high moisture conditions, whereas rotting symptoms were more prevalent in low soil moisture.

Key words: Abiotic factors, Bakanae disease, *Fusarium fujikuroi*, Rice

Rice (*Oryza sativa* L.) is the staple food crop for about half of the population of the world. It is a major food source along with wheat and maize throughout the world. Rice is grown all over the globe on around 165 mha of land with a total annual production of around 745 MT (FAO 2014). About 90% of the global rice is produced and consumed in Asia. Basmati rice is characterized by its extra-long slender grains which have more than 3.5 length: breadth ratio and has soft texture and sweetened taste after cooking. India is the largest producer and exporter of Basmati rice in the world by producing around 70% of the total production. During the year 2017–18 India exported 4056758.62 MT of Basmati rice to the world which worth of ₹ 26870.17 crores (APEDA 2018). The rice production is affected by various biotic as well as abiotic stresses, among these bakanae disease caused by *Fusarium fujikuroi* is emerging as a serious threat to Basmati rice cultivation in India (Bashyal and Aggarwal 2013, Bashyal *et al.* 2016a). The disease is known to cause crop losses up to 20– 80% (Ito and Kimura 1931, Ou 1985). In India, the incidence of this disease is increasing steadily on the Basmati cultivars in Haryana, Punjab and Uttar Pradesh, causing severe qualitative and

quantitative losses to rice production (Gupta *et al.* 2014, Bashyal *et al.* 2016a).

F. fujikuroi produces various kinds of symptoms like pre-emergence seedling death, elongation, pale yellow flag leaves and grain discoloration at maturity (Ou 1985). The disease is mainly seed borne but it can also perpetuate in plant debris as well as in soil (Ou 1985). The pathogen is known to produce a vast group of secondary metabolites and mycotoxins which play an important role in virulence of the pathogen (Bashyal *et al.* 2016b). The development of different kind of symptoms by the pathogen depends on the secondary metabolites produced. The various abiotic factors such as soil moisture, temperature, soil pH etc, influence the development of disease symptoms including the type and severity of symptoms produced by the pathogen. Knowledge about the predisposing factors helps in identifying conditions favorable for the disease and designing a suitable management strategy for the management. The effect of different abiotic factors on bakanae disease severity and its various kind of symptoms is poorly understood. Therefore, the present work was undertaken to study the effect of different abiotic factors on bakanae disease severity and symptoms.

MATERIALS AND METHODS

Inoculum preparation and seed inoculation: The experiments were conducted in the year 2016–17. Virulent isolate of *Fusarium fujikuroi* F250 and susceptible rice genotype Pusa Basmati 1121 (PB1121) were used for evaluating the effect of different factors on bakanae

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disease development. The fungal isolate was multiplied and inoculated as described by Bashyal *et al.* (2016a). The surface sterilized seeds were dipped in the pathogen inoculum in a beaker and were kept overnight at 25°C before sowing. The seeds dipped only in sterilized distilled water were used as control. The experiments were conducted under glasshouse conditions in plastic pots of four inch diameter. The pots were filled with 450–500 g of sterilized soil in each pot.

Effect of soil moisture on disease development: Rice plants were grown under different levels of soil moisture as described by Gill *et al.* (2001). The moisture level of pots was maintained by watering daily twice and adding the water to maintain the initial weight of each pot. Germination was recorded after 7 days and the disease scoring was done on weekly interval. The plant samples were collected after 15 days, 21 days and 30 days of inoculation. All the experiment were conducted under randomized block design (RBD).

Effect of pH on growth of pathogen and disease development : The F250 isolate of *F. fujikuroi* was grown under different pH in Potato Dextrose Agar (PDA) media. The pH of media was adjusted using either 0.1N HCL or 0.1N NaOH (Wang *et al.* 2013) 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 experiment was conducted in completely randomized design (CRD) with three replicates per treatment. After 7 days of incubation at 25°C in B.O.D. incubator the parameters like radial growth, colony colour, sporulation and spore size were measured.

After checking the effect of pH on *in vitro* growth of *F. fujikuroi* under *in vitro* conditions, selected pH was used to evaluate the bakanae disease severity and symptomatology under glass house conditions. The IARI field soil (Sandy Loam soil) with pH of 8.44 was used as control and in others pH was adjusted to different levels, viz. 6.0, 6.5, 7.0, 7.5 and 8.0 by adding lime or gypsum. The pre-inoculated seeds were sown in the pots and were kept in phytotron at 35°C temperature. Experiment was conducted in randomized block design (RBD) with three replications per treatment.

Effect of temperature on growth of pathogen and symptom development: The F250 isolate of the pathogen *F. fujikuroi* was inoculated in PDA media and was incubated for seven days at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C in BOD incubator. After seven days of incubation the radial growth was measured and Red, Green, Blue values were calculated (Bashyal *et al.* 2010). Sporulation was measured with the help of haemocytometer and spore size was also measured using the ocular and stage micrometer. Each treatment was replicated thrice under completely randomized design (CRD).

Selected temperatures (25°C, 30°C and 35°C) were used to evaluate the bakanae disease severity and symptomatology under glasshouse conditions. The pre-inoculated seeds were sown in the pots and were kept in phytotron at different temperatures (25°C, 30°C and 35°C) with fixed relative humidity of 80%. The plants were watered daily. The experiment was conducted in randomized block design

(RBD) with three replications per treatment and repeated twice.

Effect of different soil types on symptom expression and disease severity: Five different types of soils were collected from fields of Indian Agricultural Research Institute, New Delhi, Bundi (Rajasthan), Main Block 14B IARI New Delhi and Hapur (Uttar Pradesh). The samples were collected by digging a V shape cut into the soil upto the depth of 15cm with the help of khurpi or spade and taking 2–3 cm thick vertical slice along the depth. All the samples from a field were mixed to make final sample (Rao 1995). All the collected samples were sent to central soil testing laboratory, Division of Soil Science and Agricultural Chemistry, ICAR-IARI, New Delhi for testing soil type and other parameters. Seeds of rice variety Pusa Basmati 1121 were inoculated as described above and sown in pots under glasshouse conditions with a day temperature of 32–35°C and a night temperature of 23–28°C. All the treatments were replicated three times under RBD experimental design.

Effect of interaction of different abiotic factors on disease severity: To study the effect of interaction between different abiotic factors on bakanae disease severity three different soil moisture levels (saturation or 100%, 75% of saturation and 50% of saturation), three soil type with different soil pH i.e. sandy loam soil (pH 8.44), silty clay loam soil (pH 6.87) and clay loam soil (pH 8.02), and two temperature levels (32°C and 35°C) were selected. Pre-inoculated seeds of Pusa Basmati 1121 were sown as described above. The experiment was conducted in split plot design where moisture levels were used as main plot and soil type and temperature were used as sub plots with three replications and the experiment was repeated twice.

Statistical analysis: The data were statistically analyzed using the online software OPSTAT (Sheoran *et al.* 1998). Least significant difference was calculated after analysis of variance (ANOVA) by Duncan test or LSD at P=0.05.

RESULTS AND DISCUSSION

Effect of soil moisture on disease development: Maximum disease severity (93.33%) was observed at 30% moisture level of saturation followed by 50% soil moisture of saturation (91.00%) and 75% moisture (51.66%) (Fig 1). Lowest disease was recorded under submergence soil moisture conditions (Table 1). The disease severity was negatively correlated with soil moisture. Maximum germination (93.33%) was observed in inoculated plants under 75% moisture of saturation. Plant weight and root length was reduced in inoculated plants as compared to control plants. Elongation symptoms were produced by the pathogen under high moisture conditions and seedling rotting was visible under low moisture conditions. Only rotting of seedlings was observed under 75%, 50% and 30% soil moisture conditions. Similarly, *F. culmorum* activity was also observed to increase under soil conditions. Less soil moisture influence the soil aeration and concentration of salts in soil as well as it also affect availability of oxygen to microorganisms (Mehrotra 1980).

Table 1 Effect of soil moisture and soil type on severity and symptomatology of bakanae disease of rice

Treatment	Soil moisture						Soil type					
	Germination (%)	Disease severity (%)	Plant height (cm)	Root length (cm)	Plant weight (g)	Symptoms	Germination (%)	Disease severity (%)	Plant height (cm)	Root length (cm)	Plant weight (g)	Symptoms
T1	68.33 (56.12)	20.00 (4.56)	29.80	8.00	0.198	E	70.00 (57.09)	7.00 (2.79)	20.80	9.60	0.172	R
T2	76.66 (61.43)	16.66 (4.16)	32.30	8.30	0.295	E+R	51.00 (45.55)	0.00 (1.00)	33.90	9.70	0.237	-
T3	93.33 (78.07)	51.66 (7.22)	28.10	6.30	0.132	R	49.00 (44.40)	2.00 (1.58)	39.10	9.70	0.315	E
T4	88.33 (70.09)	91.00 (9.58)	21.80	4.40	0.092	R	81.00 (64.15)	42.00 (6.54)	36.10	7.40	0.212	E + R
T5	85.00 (67.38)	93.33 (9.71)	18.60	4.40	0.072	R	54.00 (47.37)	2.00 (1.58)	22.80	6.80	0.162	R
C1	70.00 (56.82)	0.00 (1.00)	34.80	9.10	0.326	-	72.00 (58.17)	0.00 (1.00)	21.30	9.60	0.187	-
C2	78.33 (62.26)	0.00 (1.00)	35.60	9.10	0.335	-	53.00 (46.70)	0.00 (1.00)	34.20	9.80	0.242	-
C3	96.66 (83.84)	0.00 (1.00)	30.20	8.60	0.196	-	52.00 (46.14)	0.00 (1.00)	40.20	9.80	0.389	-
C4	96.66 (81.36)	0.00 (1.00)	28.80	7.70	0.19	-	83.00 (65.67)	0.00 (1.00)	44.60	9.80	0.512	-
C5	98.33 (85.68)	0.00 (1.00)	27.50	7.50	0.192	-	57.00 (49.03)	0.00 (1.00)	23.10	7.00	0.168	-
SE(m)	4.46	0.23	0.53	0.16	0.002	-	2.33	0.19	0.19	0.13	0.002	-
SE(d)	6.30	0.33	0.74	0.22	0.003	-	3.29	0.26	0.27	0.18	0.003	-
CD	13.34	0.70	1.58	0.47	0.007	-	6.70	0.54	0.58	0.38	0.006	-

For soil moisture: T1, C1 = Submergence conditions, T2, C2 = 100% soil moisture (fully saturated), T3, C3 = 75% moisture of saturation, T4, C4 = 50% moisture of saturation and T5, C5 = 30% soil moisture of saturation. For soil type: T1, C1 = sandy loam soil, T2, C2 = clay soil, T3, C3 = clay loam soil, T4 and C4 = silty clay loam soil and T5 and C5 = Sandy soil (sand). The values in parenthesis are square root transformed values. E = elongation and R = rotting. T = inoculated and C = control, - = no symptom

Effect of pH on growth of pathogen and disease development: The maximum growth (6.8 cm) of F250 isolate of *F. 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). The spore size was measured and it was found to be range from 10 μ m to 13 μ m but no significant difference in spore size between different pH levels was found. The suitable pH for the mycelial growth of *F. fujikuroi* was 7.0 and abundant spores were also produced at this pH level.

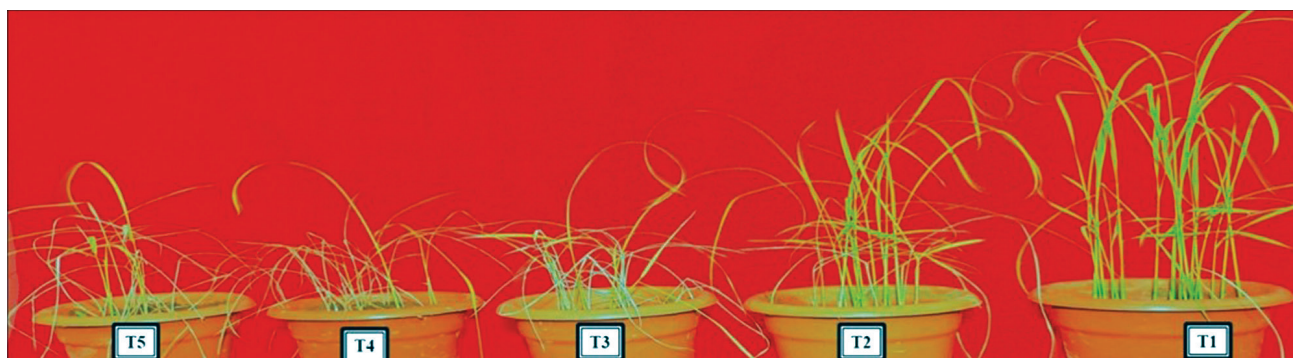


Fig 1 Effect of different soil moisture conditions on bakanae disease severity and symptomatology. Where, T1 = Submergence conditions, T2 = 100% soil moisture (fully saturated), T3 = 75% moisture of saturation, T4 = 50% moisture of saturation and T5 = 30% soil moisture of saturation.

Table 2 Effect of soil pH on disease severity and symptomatology of bakanae disease of rice

Treatment	Germination (%)	Disease severity (%)	Plant height (cm)	Root length (cm)	Plant weight (g)	Symptoms
T1	76.66 (61.19)	16.66 (4.19)	24.40	7.60	0.140	E +R
T2	66.66 (54.72)	21.66 (4.75)	22.50	7.50	0.135	R
T3	70.00 (56.97)	18.33 (4.38)	19.60	5.70	0.126	R
T4	75.00 (60.05)	38.33 (6.26)	20.90	6.10	0.121	R
T5	70.00 (56.81)	25.00 (5.08)	23.40	6.40	0.115	E + R
T6	65.00 (53.74)	16.66 (4.19)	18.40	5.40	0.108	R
C1	80.00 (63.52)	0.00 (1.00)	26.50	7.70	0.152	-
C2	70.00 (56.81)	0.00 (1.00)	25.50	7.80	0.158	-
C3	73.33 (59.03)	0.00 (1.00)	21.50	6.20	0.150	-
C4	76.66 (61.43)	0.00 (1.00)	24.50	6.60	0.163	-
C5	75.00 (60.05)	0.00 (1.00)	24.10	6.70	0.124	-
C6	68.33 (55.74)	0.00 (1.00)	19.10	5.80	0.121	-
SE(m)	2.423	0.14	0.21	0.12	0.002	-
SE(d)	3.427	0.20	0.29	0.17	0.003	-
C D	N/A	0.43	0.62	0.35	0.006	-

Where, T1, C1= control (pH 8.44), T2, C2= pH 6.0, T3, C3= pH 6.5, T4, C4= pH 7.0, T5, C5= pH 7.5 and T6, C6= pH 8.0. The values in parenthesis are square root transformed values. E= elongation and R= rotting. T= inoculated and C= control.

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 (Sunder 1995).

When evaluated under glasshouse conditions highest disease severity (38.33%) was recorded at soil pH 7.0 followed by pH 7.5 (25%) and pH 6.0 (21.66%). Best plant growth was also recorded at soil pH 7.0. The type of symptoms produced varied under different soil pH levels. Average root length and average weight of plants was decreased in inoculated plants as compared to controlled plants. Germination was reduced in inoculated pots as compared to their respective control (Table 2).

Effect of temperature on growth of pathogen and disease

development: 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 2). No growth of pathogen was observed at 40°C. The number of spores were found maximum at 25°C (19.92×10⁶/ ml) followed by 30°C (9.17×10⁶/ ml) and 20°C (9.07×10⁶/ 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. The fungus attained the maximum growth at 25°C. These studies are in confirmation with Anjaneya Reddy (2002) and Ecang (1980), who reported that growth of the *F. udum* differed in their temperature requirement which varied from 20–35°C.

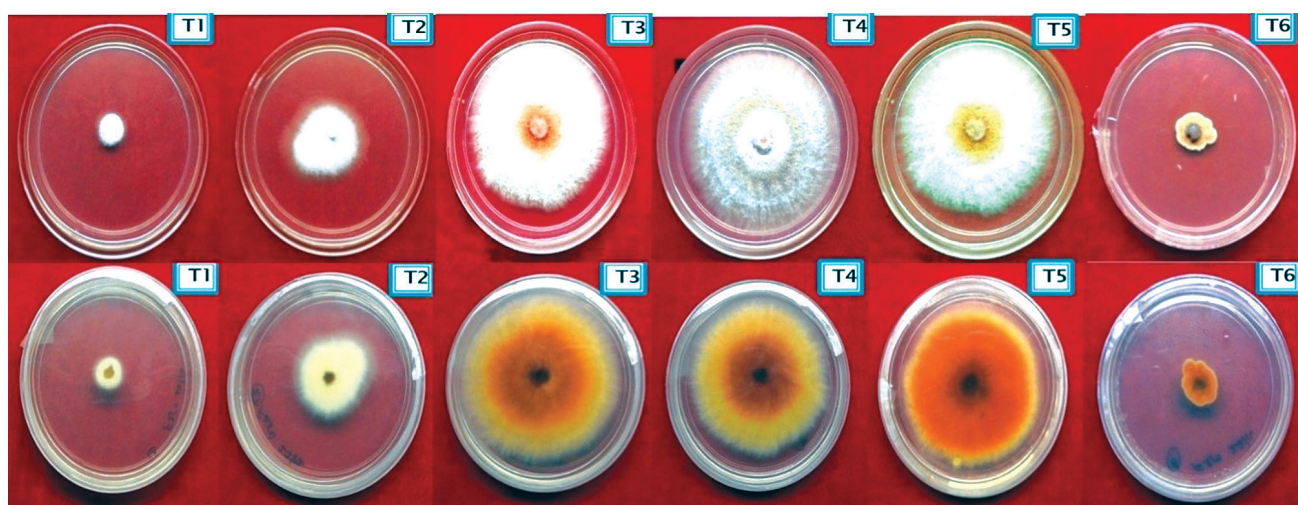


Fig 2 Effect of temperature on the growth of *F. fujikuroi*. Where, T1= 10°C, T2= 15°C, T3= 20°C, T4= 25°C, T5= 30°C, T6= 35°C

Table 3 ANOVA showing interaction effect of different abiotic factors on bakanae disease severity

Source	DF	Sum of squares	Mean square	F-ratio	p-value	Significant
Rep	2	111.37	55.69	2.38	0.2086	NS
M	2	8868.59	4434.30	189.44	0.0001	*
Error(a)	4	93.63	23.41	-	-	
P	2	11071.37	5535.69	197.51	<.0001	*
M*P	4	375.63	93.91	3.35	0.0463	*
Error(b)	12	336.33	28.03	-	-	
T	1	522.66	522.67	96.00	<.0001	*
M*T	2	7.11	3.56	0.65	0.5323	NS
P*T	2	75.44	37.72	6.93	0.0059	*
M*P*T	4	53.77	13.44	2.47	0.0817	NS
Error(c)	18	98.00	5.44	-	-	
Total	53	21613.93	-	-	-	

* - Significant at 5% (level of significance opted by user), NS - Non Significant p-Value < 0.05 - Significant at 5%, p-Value < 0.01 - Significant at 1%. Where, M= main plot, P= sub plot, T= sub-sub plot. Moisture levels were used as main plot, MP1= 100%, MP2= 75% and MP3= 50%. Soil type with different soil pH were used as sub plot, SP1= sandy loam soil (pH 8.44), SP2= silty clay loam soil (pH 6.87) and SP3= clay loam soil (pH 8.02). Temperature was used as sub-sub plot, SSP1= 32°C and SSP2= 35°C.

Highest disease severity was observed under temperature 35°C (55%) followed by 30°C (38.33%). Very less severity (3.33%) of the disease was recorded at 25°C. The growth of plants was also maximum at 35°C and very poor germination (%) was recorded at 25°C temperature under control conditions also. At 35°C only rotting type of symptoms were observed while both elongation and rotting symptoms were present at 30°C. At 25°C only rotting was observed. *Fusarium* wilt/ rot diseases are widely known as warm temperature diseases. This was corroborated in our studies, where disease was most severe at 35°C, whereas disease was observed at 25°C also.

Effect of different soil types on symptom expression and disease severity: Five different kind of soils were used to evaluate the severity and symptomatology of bakanae disease of rice. Maximum disease severity (42.0%) was recorded in silty clay loam soil collected from Hapur (UP), followed by sandy loam soil (7.0%). No disease was observed in clay soil collected from Bundi (Raj) and very low disease severity (2.0%) was recorded in clay loam soil (MB14B, IARI) and sandy soil. The best plant growth was also observed under silty clay loam soil (average plant weight 0.512 g). In clay loam soil, only elongation was observed while both elongation and rotting were found in silty clay loam soil. In sandy and sandy loam soils, only rotting was present (Table 1). Maximum seedling mortality was recorded in silty clay loam soil followed by sandy loam soil. Clay soil did not support disease development. *Fusarium* survives and grows in soils of all types (Amir and Alabouvette1993). Degree of porosity influence growth and parasitism of the pathogens. Sandy loam soil is better drained, holds less water dry faster and may favor multiplication and survival of *Fusarium* spp. (Boyette et al. 1984, Saxena and Khare 1988). Sandy soils were

observed to be more preferred by *Fusarium* spp. (Amir and Alabouvette1993; Larkin et al., 1996).

Effect of interaction of different abiotic factors on disease severity: Highest disease severity (72.33%) was observed in combination of 50% soil moisture, silty clay loam soil with pH 6.87 and 35°C temperature followed by combination of 50% soil moisture, silty clay loam soil with pH 6.87 and 32°C temperature and 75% soil moisture, silty clay loam soil with pH 6.87 and 35°C temperature. Significant difference in disease severity was observed under different moisture conditions, different soil type with different soil pH and different temperature conditions. Significant variation in disease severity was observed in interaction of moisture, soil pH, soil type and in interaction of soil type, soil pH and temperature (Table 3).

Soil moisture, temperature, soil pH and soil type significantly affected the bakanae disease development with significant interactions. Bakanae disease was negatively correlated with soil moisture and positively correlated with temperature. Elongation kind of symptoms were more prevalent in high moisture conditions, whereas rotting symptoms were more prevalent in low soil moisture.

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