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Evaluation of fungicides and rice genotypes for the management of Bakanae

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ABSTRACT

Bakanae disease of rice caused by Fusarium moniliforme is emerging as one of the major biotic challenge to rice production under temperate agro-ecosystem . The disease is an important limiting factor for rice production in higher altitudes of Kashmir valley. Six different fungicides were evaluated both in vitro and as seed dressing Carbendazim + mancozeb was found most efficient in reducing the disease severity. The resistant sources for the disease identified in the study are GSL-5, GSL-9, GSL-12, GSL-44, GSL-60, GSL-66, GSL-67, GSL 36 and GSL-68

*Key **words:** bakanae, rice, genetic resistance, chemical control*

Rice crop plays significant role in livelihood of people of Jammu and Kashmir state. Although area under rice is about 0.27 million ha, it plays an important role in the state economy (Gupta *et. al.*). The rice grown area of the Kashmir valley is divided in three agroecological zones. The indica rices are in vogue in the plain belt of valley covering an altitude range of 1450-1650 m MSL, whereas1850-2250 m MSL covers higher belts of the valley with preponderance of japonica rices. The intermediate zone or transitional zone covers an altitude range of 1650-1850 m MSL. However, cultivation of amalgam of both sub species can be observed with preponderance of indica from lower side and japonica rices from higher side of the intermediate zone. Rice crop is frequently challenged by various biotic and abiotic stresses particularly rice blast, sheath blight, grain discoloration etc. Bakanae disease is emerging as one of the potential threats particularly against cultivated japonica rice under high altitude conditions of Kashmir. The rice genotypes of sub species japonica carrying semi-dwarf (*sd-1*) gene are highly susceptible to such disease (Zheng *et al,* 1993; Khokar and Jaffery,2002).

Bakanae is one of the most important diseases of rice widespread in many rice growing areas, both tropical and temperate, and, if no control measures are taken, it may be a factor limiting rice production. The yield loss estimated ranges from 10-50 percent (Khokhar and Jaffrey, 2002). The disease is seed as well as soil borne (Ahmad and Raza, 1991). The types of symptoms produced by an infected plant may depend upon the strain of the fungus and nutritional conditions. The most visually striking symptoms of the disease are chlorotic, elongated, thin seedlings that are often several inches taller than healthy seedlings. Infected seedling may also be stunted and chlorotic, exhibiting a rot and produce foul smell. Severely infected seedlings die before transplanting, and those that survive may die after transplanting. The anamorph form of the pathogen produces gibberellin and fusaric acid. Biological studies of the two substances showed that fusaric acid causes stunting and gibberellin causes elongation of rice plants (Nyvall,1999).

Severe incidence of Bakanae disease during 2009-2011was observed at the Rice Research Sub Station, Larnoo of SKUAST-K located at 2280 m MSL. Some of the varieties were severely affected by the disease both at farmer's fields and the Research Station. The fields were looking shabby and grain quality was greatly reduced. Hence to combat the emerging challenge both short term and long term objectives were

devised at the station. Chemicals due to seed treatment is the safest means to control seed-borne fungal diseases and to prevent fungal bio-deterioration of grains (Bagga and Sharma, 2006). Among long term objectives breeding for resistant varieties after through screening of rice germplasm and identifying donors for resistance is economically and environmentally a very good option. Resistant breeding has been reported from other part of the world (Zheng *et al.*, 1993: Liangyong *et al*, 2008). Therefore, the present investigation was undertaken to study the efficacy of fungicides for the management of bakanae and identification of rice genotypes for resistance.

MATERIALS AND METHODS

Infected plants were collected from rice fields of Kashmir valley during wet seasons of, 2010 and 2011. Infected plants showing white heads and stem elongation collected during survey were attempted for the isolation of associated pathogens. The infected portions of selected plants were washed in running tap water. From each selected plant, a single segment of 5 mm in length was cut at the zone of advancing decay. Three to four of these segments were used immediately for isolation of associated fungal pathogen. The bits were surface sterilized and the bits were aseptically transferred to potato dextrose agar (PDA) medium in sterile petri-plates and incubated at 25±2°C. The outgrowing mycelia were subcultured immediately. The cultures were purified by hyphal tip method (Dasgupta, 1988). Various cultural and morphological characteristics of isolated fungus were recorded by making visual observations and microscopic examinations and were compared with standard descriptions given by Nelson *et al.* (1983) and Ilija *et al*. (2009).

The pathogenicity test of isolated fungus was first carried out on japonica rice varieties viz. K-332 and Kohsar, released and recommended for higher altitudes of Kashmir valley. The rice field soil was sterilized by autoclaving for 1 hour at 1.05 kg cm^{-2} for 3 consecutive days and was put in earthen pots of 12 x 14 inches. Fungal inoculum, multiplied on sand-maize medium (Riker and Riker, 1936), was added @ 50 g kg^{-1} of pot soil and was allowed to grow for seven days for maximum infestation. The inoculum density of the fungi used was 5 x 10^4 cfu per gram. Viable paddy seeds were surface sterilized and after germination, 20 seeds were sown in each pot with triplicate and arranged in a completely randomized design. The experiment was carried out during the month of June in the open environment and severity was recorded after 75 days of sowing. The causal pathogen was reisolated and compared with mother isolate. The pathogenesis by the pathogen was thus confirmed by following the Koch's postulates.

Six fungicides viz., Captan, Carbendazim, Mancozeb, Hexaconazole, Tricyclozole, Carbendazim + Mancozeb and Captan+ Hexaconazole were evaluated for toxicity against the mycelial growth of *Fusarium moniliforme* by food poisoning technique. The fungicides were mixed into potato dextrose agar medium @ 50, 100, 200 and 300 ppm. The plates were inoculated at the centre with a 5mm disc of seven day old culture of test pathogen and incubated at 25±2°C. Observations on radial growth of pathogen was recorded on 7th day of incubation and mycelial growth inhibition of the pathogen over control was calculated using the formula given by Vincent (1947).

Seeds infected with *Fusarium moniliforme* were given total of seven treatments comprising few fungicides and their combinations namely, Captan, Carbendazim, Mancozeb, Hexaconazole, Tricyclozole, Carbendazim + Mancozeb and Captan + Hexaconazole by soaking method (Gangopadhyay and Kapoor,1977) at a dose of 0.3%, 0.1%, 0.3%, 0.06%, 0.06%, 0.2% and 0.2% respectively. The seeds were germinated and sown in plots of 3 rows of 1m length. The inter and intra row spacing was 20 and 15cm, respectively The experiment was laid out under Completely Randomized Block Design with three replications per treatment at Mountain Crop Research Station Larnoo of SKUAST of Kashmir. The data for disease severity were recorded at one month interval.

Seventy six rice genotypes along with two checks comprising of indigenous and exotic collections from different sources were screened for resistance against bakanae disease of rice during wet seasons of 2010 and 2011 at the Mountain Crop Research Station, Larnoo. The seeds of each entry were separately inoculated with the *Fusarium moniliforme* spore suspension with inoculum load of 10^7 spores ml^{-1} suspension. Each entry was given 1.5m row length and sown in two rows at inter and intra row spacing of 20 cm and 15 cm respectively. The entries were grown in

augmented field design and both checks were replicated five times. The disease intensity was recorded by 0-9 rating scale following IRRI Standards of Evaluation System for rice (Anonymous, 1996) and the entries were graded in different categories of resistance and susceptibility as follows:

RESULTS AND DISCUSSION

In the present study the frequent isolation of *Fusarium moniliforme* from diseased rice plants collected from the surveyed areas envisaged its possible role in development of bakanae symptoms. Attempts made to prove the Koch's postulates for the isolated fungus revealed that fungus was pathogenic to rice plants and produced characteristic bakanae symptoms. The symptoms characterized were chlorotic, elongated and thin seedlings that were often several inches taller than healthy seedlings. Infected seedling also showed chlorotic and stunted growth and exhibited rotting symptoms with powdery growth of conidiophores on the lower parts. The infected plants produced adventitious roots at lower nodes and when the stem was split vertically white growth of fungal mycelium was observed at the point of nodes. The abnormal growth of seedlings was probably due to the production of gibberillic acid (GA_3), a growth promoting hormone by the pathogen (Sun and Synader, 1981). GA3 is a simple growth hormone promoting the elongation of plant cells (Johnson and Coolbaugh,1990). The pathogen also produces some secondary metabolites which affect the growth of rice plants such as carotenoids, fusarin, fumonisin, moniliformin and fusaric acid as shown by various researchers (Saremi *et al*., 2004., Mohd Zainudin *et al*., 2008).

Result of fungicides treated against the pathogen revealed fact that *in vitro* fungicidal effect at different concentrations had significant differences in controlling mycelia growth of fungus (Table 1). Further significant differences for fungitoxic effect within different concentrations were noticed for different concentrations. Mycelial growth of the fungus was restricted proportionally with the increase in fungicidal concentration with highest reduction noticed at concentration of 300ppm followed by 200 ppm. Similarly fungitoxic effect was observed to be highest for fungicide Carbendazim 12% + Mancozeb 63%WP followed by Captan 70% +Hexaconazole 5%WP and Carbendazim 50%WP with non significant difference at all concentrations, while the lowest impact in inhibiting the mycelia growth of the fungus was demonstrated by fungicide Mancozeb 75%WP. Bhali *et al,*.2001, tested *in vitro* application of eight fungicides against *Fusarium moniliforme* and found Benlate and Derosal the most effective fungicides which completely inhibited the growth of the test fungus at 100ppm. However, Gour and Chakrabarty (2009) found Captan and Carbendazim to be most effective in inhibiting the mycelia growth. Banlate, Carbendazim and Topsin-M belong to same Bezimidazole group with similarity in fungitoxic spectrum and mode of action. They are

Table 1. *In vitro* evaluation of fungicides at different concentrations against *F. moniliforme*.

S. No.	Treatment	Mycelial growth (mm) at various fungicidal concentrations				
		0 ppm(C 1)	50 ppm(C 2)	100 ppm(C 3)	200ppm(C 4)	300ppm(C 5)
T1	Captan 50%WP	90	58.5	36.8	16.4	3.6
T2	Carbendazim 50%WP	90	9.2	4.6	0.0	0.0
T3	Mancozeb 75%WP	90	62.5	42.5	21.8	5.7
T4	Tricyclozole 75%Wp	90	47.3	23.9	10.2	0.0
T5	Hexaconazole 5% EC	90	26.8	15.3	6.5	0.0
T6	Carbendazim 12% + Mancozeb 63% WP	90	5.8	0.0	0.0	0.0
T7	Captan 70% + Hexaconazole 5% WP	90	7.2	0.0	0.0	0.0
	Mean	90	31.04	17.58	7.84	1.32
	CD		7.75	5.45	3.12	1.86

T statistic: $t_{1.2}$(6.23*), $t_{1.3}$(5.24*), $t_{1.4}$(4.28*), $t_{1.5}$(4.27*), $t_{2.3}$(6.53*), $t_{2.4}$(9.79*), $t_{2.5}$(10.53*), $t_{3.4}$(7.48*), $t_{3.5}$(6.97*), $t_{4.5}$(8.10*) $t_{1.2=}$ t value for group comparison of two concentration C1 and C2 and similarly so on. * = significance of t values for group comparisons.

systemic and are readily absorbed to reach the target tissue. Iqbal *et al*., (2011) also reported that no fungal mycelia growth was observed in case of Derosol, Daconil and Topsin-M treatment at concentration of 0.25%.

Data on disease intensity for two genotypes demonstrates that the varieties respond differently with respect to different fungicides (Table 2). The effect of fungicides was more pronounced on variety Kohsar as compared to K-332. It was further observed that different fungicidal treatments within the group also behaved significantly while restricting the disease development. The impact of fungicidal treatment

Seventy eight genotypes including two popular varieties as checks viz.K-332 and Kohsar were screened against the bakanae disease of rice after inoculating each genotype. Some of the genotypes were indigenous collections, whileas others were received from exotic sources. The mean disease scoring for each genotype (plot basis) was observed at maximum tillering stage. It was observed that most of the genotypes were categorized under moderate resistant group followed by the moderately susceptible group (Table 3). Only nine genotypes namely GSL-5, GSL-9, GSL-12, GSL-44, GSL-60, GSL-66, GSL-67, GSL 36 and GSL-68 could be classified as resistant group on the basis of lowest disease incidence (less than 1%). The check

Table 2. Efficacy of different fungicides for two rice genotypes against bakanae disease of rice

S. No	Treatments	Dosage(g)/ liter of water	Disease intensity (%)		Disease control (%)	
			K 332	Kohsar	K 332	Kohsar
T1	Captan 50%WP (T1)	3	14.3 (22.2)	9.0 (17.4)	43.7	53.8
T2	Carbendazim 50%WP (T2)	1	9.2 (17.6)	6.3 (14.5)	63.7	67.6
T3	Mancozeb 75%WP (T3)	3	18.8 (25.7)	14.3 (22.2)	25.9	26.6
T4	Tricyclozole 75%Wp (T4)	0.6	12.0 (20.2)	8.8 (17.2)	52.7	54.8
T5	Hexaconazole 5% EC (T5)	0.5	13.2 (21.3)	7.2 (15.5)	44.9	63.0
T6	Carbendazim 12% + Mancozeb 63%WP (T6)	2.0	6.0 (14.2)	3.0 (10.0)	76.3	84.6
T7	Captan 70% +Hexaconazole 5%WP (T7)	2.0	7.9 (16.3)	4.5 (12.2)	68.8	76.9
T0	Control (T0)	-	25.4 (30.2)	19.5 (26.2)	-	-
Mean						
CD		2.55	1.95			

T statistic t12 (3.52*) t value of group comparison for two genotypes
Figures in parenthesis: Arc sine transformed values; * = significance of t values for group comparison

Carbendazim 12% + Mancozeb 63%WP and Captan 70% +Hexaconazole 5%WP were at par. The reduction in severity of disease was reduced by 76.3% and 68.8 % in K-332 and 84.6 % and 76.9 % in Kohsar against the control. Similar results were reported by Bhali *et al* ., (2001) and it was reported that Derosil was the most effective fungicide followed by Benlate and Topsin-M in reducing the infection of rice seedlings by *F. moniliforme* when used as seed treatment or soil drench. Narmada and King (1992) reported that 0.2% Carbendazim was best of nine sprays used for reducing infection (81.2%) by *F. moniliforme* (*G. fujikuroi*). Ibiam (2006) demonstrated that seed dressing of fungicides *viz.* Benomyl, Carbendazim, and Mancozeb controlled seed borne fungi of rice *F. moniliforme, Bipolaris oryzae* and *F. oxysporum* which causes damage to rice both in storage and field.

genotypes K-332 and Kohsar were observed as moderately resistant and moderately susceptible respectively. Similar efforts have been made to identify and utilize rice germplasm with bakanae resistance. Li *et al*, (1993) found only one variety with high resistance to bakanae and 12 with moderate resistance from a total of 411 rice accessions. Zheng *et al.*, 1993 showed only a few accessions (2.5%) out of the 411 lines with moderate resistance and none with high resistance. Similarly a few accessions were reported to have high resistance to bakanae in other studies (Li *et al*, 1994; Khokar and Jaffery, 2002). Nine of the 78 genotypes were identified highly resistant in the present study. This is a very high percentage possibly because all the tested lines for the disease belong to japonica sub species and *sd1* gene which makes the rice variety susceptible to the diseases is present in half of the japonica varieties

Table 3. Evaluation of different japonica rice genotypes against bakanae disease of rice under high altitude conditions of Kashmir valley

S. No	Entries	Disease intensity (%)	Disease Grade (0-9)	S. No	Entries	Disease intensity (%)	Disease Grade (0-9)
1	GSL1	21.8	5	40	GSL 44	1.0	1
2	GSL2	18.7	5	41	GSL 59	5.3	3
3	GSL 3	12.9	5	42	GSL 60	0.7	1
4	GSL 4	5.0	3	43	GSL 61	13.0	5
5	GSL 5	0.8	1	44	GSL 66	0.3	1
6	GSL 6	2.0	3	45	GSL 67	0.6	1
7	GSL 7	10.2	5	46	GSL 68	0.9	1
8	GSL 8	4.9	3	47	GSL 69	5.3	3
9	GSL 9	1.0	1	48	GSL 70	17.4	5
10	GSL 10	3.6	3	49	GSL 71	28.5	7
11	GSL 11	2.5	3	50	GSL 72	32.7	7
12	GSL 12	1.0	1	51	GSL 73	35.6	7
13	GSL 13	5.0	3	52	GSL 74	8.0	5
14	GSL 14	8.7	5	53	GSL 75	5.1	3
15	GSL 15	20.0	5	54	GSL 76	29.5	7
16	GSL 16	18.6	5	55	GSL 77	22.3	5
17	GSL 17	19.2	5	56	GSL 78	28.9	7
18	GSL 18	15.4	5	57	GSL 79	12.0	5
19	GSL 19	19.8	5	58	GSL 80	10.7	5
20	GSL 20	14.3	5	59	GSL 81	12.4	5
21	GSL 23	4.9	3	60	GSL 82	4.6	3
22	GSL 25	18.9	5	61	GSL 84	21.2	5
23	GSL26	5.0	3	62	GSL 296	5.5	3
24	GSL 27	22.8	5	63	GSL 302	10.0	5
25	GSL 28	4.8	3	64	GSL 318	30.9	7
26	GSL 29	6.3	5	65	GSL 376	24.8	5
27	GSL30	14.8	5	66	IVT 29	8.6	5
28	GSL 31	5.0	3	67	IVT 32	7.8	5
29	GSL 32	15.6	5	68	IVT 33	2.9	3
30	GSL 33	14.0	5	69	IVT 38	5.0	3
31	GSL 34	3.9	3	70	V-40	29.9	7
32	GSL 35	24.7	5	71	IRTON 410	4.6	3
33	GSL 36	0.5	1	72	K332 (Check-1)	23.5	5
34	GSL 37	3.0	3	73	K402 (Check-3)	14.8	5
35	GSL 38	9.4	5	74	Kohsar (Check-2)		3.4
3							
36	GSL 39	15.5	5	75	Kamad	12.6	5
37	GSL 41	6.0	5	76	C. Mochie	5.2	3
38	GSL 42	4.7.	3	77	Setejari	14.6	5
39	GSL 43	5.0	3	78	Chadi China	4.8	3
Mean						11.56	
CD of test genotypes						3.8	

Disease grade: 0= 0, 1= 1%, 3=1-5%, 5=6-25%, 7= 26-50%, 9= 51-100%

(Liangyong *et al*, 2008). For this reason Li *et al*,(1994) also found half of the japonica materials displaying moderate resistance when evaluated during maximum tillering and flowering stage.

The above findings thus revealed that the fungicide treatment with Carbendazim + Mancozeb proved most efficient in controlling the bakanae disease both *in vitro* and in field conditions up on seed dressing. Thus in situations of sudden occurrence of disease use of the same fungicide is suggested as seed dressing for effective and viable solution for suppression of the disease. Similarly resistant lines identified in the study may be used breeding programme for generating the broad spectrum variability for selection of appropriate

segregants, besides the direct utilization as varieties after thorough testing in yield trials.

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