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Effect of fly ash as a source of silicon and potassium on the disease incidence of Brown leaf spot in rice under different abiotic stress condition

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Abstract
Experiments were carried out in two field conditions (induced drought and flood stress) to analyse the effect of fly ash which is a source of silicon on incidence of brown leaf spot disease (*Helminthosporium oryzae*) and grain yield of rice. The treatments include fly ash, Silicate Solubilizing Bacteria (SSB), Farm yard manure (FYM), and graded levels of soil test based potassium. The results revealed that the combination of Fly ash, SSB, FYM, with graded level of soil test based potassium significantly reduced the incidence of Brown leaf spot disease as compared to untreated plots. The silicon content was analysed in the leaf blade and it was negatively correlated with the brown leaf spot disease. As the age of the plant increased, silicon content proportionately increased in the plants at different growth stages. Further, the incidence of brown leaf spot was negatively correlated with yield. Application of fly ash as a source of silica and potassium with SSB, FYM with STBK reduced the incidence of Brown leaf spot and increased the yield in rice.

Keywords: Fly ash, silica, brown leaf spot, incidence

Introduction

Fly ash is a major industrial waste in India. It is a by-product of thermal power station where electricity is produced by burning finely powdered coal. In India around 12.21 M t of fly ash is produced every year and for storing one tonne of fly ash, 0.35 m^2 area is required. This huge quantity of fly ash produced is dumped in ash disposal areas which are posing a great threat to the environment. fly ash contains 0.2 to 0.3 per cent potassium and 15 to 60 per cent SiO_2, In an attempt to effectively solve the disposal problem of the enormous solid industrial waste, some efforts have been made to use it as an amendment to improve soil and its fertility and for crop production.

Rice is considered to be a Si accumulator and tends to actively accumulate Si to tissue concentrations of 5% or higher (Epstein, 1994; Miyake and Takahashi, 1983) [8, 20]. Relatively large amounts of plant available Si appear to be very important for both robust growth and fungal disease resistance of rice (Winslow, 1992; Datnoff *et al*., 1997) [24, 6]. Although many rice-growing soils initially contain significant quantities of Si, repeated rice cropping can reduce Si levels to the point that Si fertilization becomes beneficial for growth and disease resistance (Datnoff *et al*., 1997) [6]. Rice is a silicicolous plant that absorbs Si in the form of monosilicic acid (H_4SiO_4) through active aerobic respiration. Silicon is the second most abundant element in the earth's crust with soils containing approximately 32 per cent Si by weight (Lindsay, 1979) [15]. Silicon deposited in the walls of epidermal cells after absorption by plants contributes considerably to stem strength. The epidermal cell walls become effective barriers against both fungal infections and water loss by cuticular transpiration when impregnated with a firm Si layer (Jones and Handreck, 1967) [10].

The protective role of K in plants suffering from drought stress by maintenance of high pH in stroma and against the photo-oxidative damage to chloroplast was also reported by Cakmak (1997) [2]. Silicon can reduce the severity of several important diseases of rice including blast, brown spot, sheath blight, leaf scald and grain discoloration and of sugarcane including leaf freckling and ring spot (Datnoff and Snyder, 2001; Savant *et al*., 1997; Korndorfer *et al*., 1999) [4, 22, 12]. Hence the application of fly ash as a source of silicon and potassium had a significant effect on the incidence of Brown leaf spot (*Helminthosporium oryzae* Breda de Haan) under abiotic conditions.

Materials and Methods

Field experiment was conducted under induced drought and flood condition in split plot design with two replications. The plot size was 5x4 m^2 with 4 main plot treatments and 5 sub plot treatments. The main plot treatments, M1 – Control (0 fly ash), M2 – fly ash @ 25 t ha^{-1} + silicate solubilizing bacteria (SSB) @ 2 kg ha^{-1}, M_3 - fly ash @ 25 t ha^{-1} + Farm yard manure (FYM) @ 12.5 t ha^{-1}, M_4 - fly ash @ 25 t ha^{-1} + (SSB) @ 2 kg ha^{-1} + FYM @ 12.5 t ha^{-1} were followed and subplot treatments were graded level of soil test based Potassium (STBK), S_1 - Control, S_2 - 25% STBK, S_3 - 50% STBK, S_4 - 75% STBK, S_5 - 100% STBK. The fly ash was applied before transplanting followed by incorporation of silicate solubilizing bacteria and farm yard manure. The rice variety BPT 5204 was selected for field experiment. The incidence of Brown leaf spot was observed at flowering stage under natural condition using Image Analysis System of Delta- T device, U.K and it was compared with conventional method using Disease incidence scoring chart. The silicon content was analysed in leaf blade at flowering stage.

Estimation of Silicon in plant samples

The powdered samples of different parts of rice plant viz., leaf blade, leaf sheath cum stem, ear head, were dried in an oven at 70 °C for 2-3 hrs prior to analysis. A 0.1 g sample was digested in a mixture of 7 ml of HNO_3 (62 per cent), 2 ml of hydrogen peroxide (H_2O_2) (30 per cent) and 1ml of hydrofluoric acid (46 per cent) kept in for 10-15 min for pre-digestion. The samples were digested using microwave digester (Microwave reaction system Antonpaar Multiwave 3000 solv) with following program 500 watt for 17 minutes with a ramp at 10 ° C per minute to reach the temperature of 150 ° C, 500 watt for 10 minutes for holding the temperature of 150 °C and venting for 10 minutes. The digested samples were diluted to 50ml with 4% boric acid (Ma *et al.*, 2002) [16].

The Si concentration in the digested solution was determined by transferring 0.1 ml of digested aliquot to a plastic centrifuge tube, added with 3.75 ml of 0.2 N HCl, 0.5 ml of 10 per cent ammonium molybdate and 0.5 ml of 20 per cent tartaric acid and 0.5 ml of reducing agent 1- amino -2- napthol- 4- sulfonic acid (ANSA) and the volume was made up to 12.5 ml with distilled water and kept it for one hour. After one hour, the absorbance was measured at 600 nm with a UV- Visible spectrophotometer. Similarly, standards (0, 0.2, 0.4, 0.8, and 1.2 ppm) were prepared by using 1000 ppm, by following the same procedure. The stock standard of silica was obtained from Merk.

The data obtained from field experiments were subjected to statistical scrutiny (Snedecor and Cochran, 1967) and the analysis was carried out in Agres Agdata. Square root transformation was followed for converting the population numbers. The treatment means were compared by Duncan's Multiple Range Test (DMRT) at p=0.05 for their significance (Gomez and Gomez, 1985).

Results (Tables 1 & 2):

The brown leaf spot disease incidence was observed at flowering stage. The treatments comprise of fly ash decrease the incidence over control. Among the different main plot treatments, application of FA + SSB + FYM recorded the mean incidence of 49.3 per cent followed by FA + FYM (55.7%), FA + SSB (60.5%) under drought stress whereas under flood stress the main plot treatments showed significant difference due to the application of fly ash with SSB and FYM and recorded the least brown leaf spot incidence of 40.85 per cent followed by fly ash with FYM (52.72%), fly ash with SSB (68.57%) whereas control recorded the highest mean incidence of 66.1 per cent under drought stress and 75.79 per cent under flood stress. In drought stress condition the application of graded levels of STBK showed progressive reduction of disease incidence with increased K level. However, application of 100 per cent STBK recorded the lowest mean incidence of 55.4 per cent which was on par with 75 per cent STBK. The control recorded the highest mean incidence of 61.7 per cent. Under flood stress condition, the graded level of STBK showed significant difference and recorded the disease incidence of 55.4 per cent due to the application of 100 per cent STBK over control (63.58%). The disease reduction of 12.75 per cent was recorded due to the application of 100 per cent STBK. The interaction effect of treatments showed significant difference, the addition of fly ash with SSB and FYM with graded levels of 100 per cent STBK recorded brown leaf spot incidence of 37.6 per cent over control. It was observed that the percentage of disease reduction was 51.5 per cent due to the application of fly ash with SSB and FYM. The brown leaf spot disease incidence was negatively correlated with content of Si.

Table 1: Effect of fly ash with SSB + FYM with soil test based K on incidence of brown leaf spot disease in leaf blade at flowering stage of rice under flood stress

Treatments	Brown leaf spot disease under drought stress						Brown leaf spot disease under flood stress					
Main/Sub	Soil Test Based K (%) (S)					Mean	Soil Test Based K (%) (S)					Mean
	0	25	50	75	100		0	25	50	75	100	
M_1	67.6	66.0	66.4	65.7	65.1	66.1	77.60	76.35	75.45	75	74.55	75.79
M_2	63.9	62.0	59.0	58.9	58.6	60.5	73.7	70.3	70.1	66.6	62.15	68.57
M_3	60.8	57.5	59.8	53.1	51.6	55.7	57.6	55.2	54	49.20	47.60	52.72
M_4	54.7	51.2	49.3	45.4	46.3	49.3	45.4	42.7	40.1	38.4	37.6	40.85
Mean	61.7	59.1	57.6	55.7	55.4	-	63.58	61.15	59.92	57.3	55.47	-
	SEd		CD(P=0.05)				SEd			CD(P=0.05)		
M	0.48		1.54				1.0			3.2		
S	0.72		1.52				0.3			0.8		
M at S	1.37		NS				1.2			3.4		
S at M	1.44		NS				0.7			1.6		

Table 2: Effect of fly ash with SSB + FYM with soil test based K on total phenol content (mg g^{-1}) at flowering stage of rice* under drought and flood stress

Treatments	Total phenol content in leaf blade at flowering stage under drought stress						Total phenol content in leaf blade at flowering stage under flood stress					
Main/Sub	Soil Test Based K (%) (S)					Mean	Soil Test Based K (%) (S)					Mean
	0	25	50	75	100		0	25	50	75	100	
M_1	1.66	1.72	1.78	1.84	1.87	1.73	1.80	1.82	1.82	1.83	1.83	1.82
M_2	1.74	1.80	1.86	1.92	1.95	1.85	1.85	1.88	1.88	1.89	1.89	1.88
M_3	1.80	1.89	1.94	1.98	2.07	1.91	1.90	1.93	1.94	1.94	1.95	1.93
M_4	1.86	1.99	2.08	2.19	2.25	2.07	1.92	1.95	1.98	1.99	1.99	1.96
Mean	1.71	1.8	1.90	1.94	2.05	-	1.86	1.90	1.91	1.91	1.92	-
	SEd			CD(P=0.05)			SEd			CD(P=0.05)		
M	0.014			0.044			0.006			0.01		
S	0.019			0.041			0.003			0.008		
M at S	0.037			0.085			0.009			0.023		
S at M	0.039			0.082			0.007			0.016		

Silicon content in rice

Under drought stress, the Si content in the flowering stage was increased due to the addition of fly ash with SSB and FYM and showed significant difference which recorded the highest mean Si content of 3.65 per cent in leaf blade, 2.99 per cent in leaf sheath cum stem and 2.03 per cent in ear head. The control recorded the Si content of 3.51, 2.85, 1.87 per cent in leaf blade, leaf sheath cum stem, and ear head, respectively. The graded level of STBK showed significant difference and recorded the highest mean silicon content of 3.59 per cent in leaf blade and 1.96 per cent in ear head due to the addition of 100 per cent K. However the graded levels of STBK did not show significant difference in the silicon content in leaf sheath cum stem. The interaction effect of treatment did not show significant difference. Under flood stress, in the flowering stage the Si content was increased due to the addition of fly ash with SSB and FYM and showed significant difference which recorded the highest mean Si content of 3.36 per cent in leaf blade, 2.79 per cent in leaf sheath cum stem, 1.79 per cent in ear head whereas control recorded 3.18 per cent in leaf blade, 2.62 per cent in leaf sheath cum stem and 1.65 per cent in ear head. Among the graded levels of STBK, the mean Si content of 3.2 per cent in leaf blade, 2.7 per cent in leaf sheath cum stem and 1.74 per cent in ear head were recorded due to the addition of 100 per cent STBK which was on par with 75 per cent. The interaction effect of treatment did not show any significant difference.

Discussion (Fig.1 and 2)

Under both drought and flood condition, the brown leaf spot disease incidence was less due to the application of fly ash with SSB and FYM. The soluble Si taken up by plants can produce phenolic and phytoalexin in response to infection by pathogen thereby silicon nutrition suppress brown spot. This might be due to the mechanism (i) Si acting as a physical barrier and (ii) soluble Si acting as a modulator of host resistance to pathogen, (iii) Si is deposited beneath the cuticle to form a cuticle – Si double layer which mechanically impede penetration of fungi and thus disrupt the infection process (Datnoff and Rodrigues, 2005) [3]. The phenols also play a role in conferring resistance to pathogen. This might be due to (i) direct toxicity to the pathogen (ii) The oxidation products of phenols being more toxic might inhibit the pathogen (iii) It might interfere with electron transport system leading to a blockage in the energy release. (iv) Binding with enzymes and inactivating them. (Williams, 1996) [23].

The large amount of phenol was observed in infected resistant rice varieties than in the susceptible rice varieties. This might be due to the rapid accumulation of phenolic compounds in incompatible host pathogen interaction. (Prasad *et al.* 1972) [21]. The application of 100 per cent STBK registered low incidence of brown spot. This might be due to the fact that deficiency of potassium reduces Si accumulation in the epidermal cells of the leaf blades leading to increased susceptibility to diseases. There is an inverse relationship between the K supply and brown leaf spot disease in rice. These results are in line with the findings of Haerdter (1997), Mallika *et al.* (2000) [9, 17]. Hence the application of fly ash with SSB and FYM significantly increased the silicon content at different growth stages and deposition of silicon in the leaf reduced the incidence of brown leaf spot thereby help to overcome abiotic and biotic stresses. Although silicon is not considered to be an essential nutrient for most terrestrial plants, it is beneficial to many plants. Si has the potential to significantly decrease the susceptibility of certain plants to both biotic and abiotic stresses. Furthermore, in plants such as rice, Si fertilization may even increase growth and yield in addition to reducing disease severity.

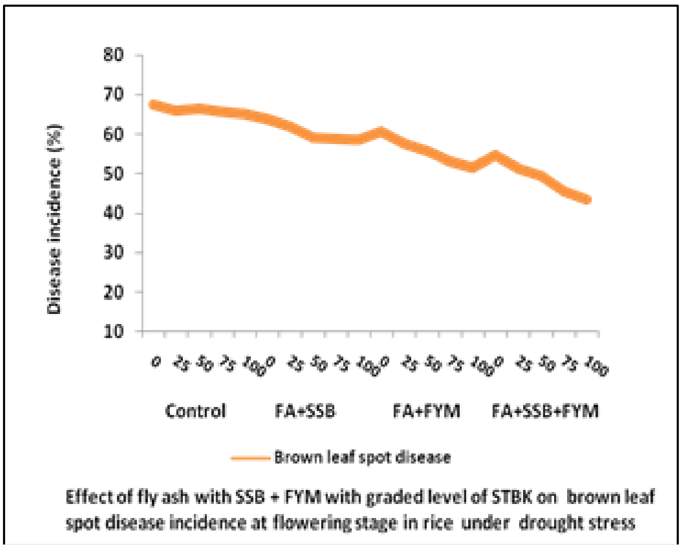

Fig 1a

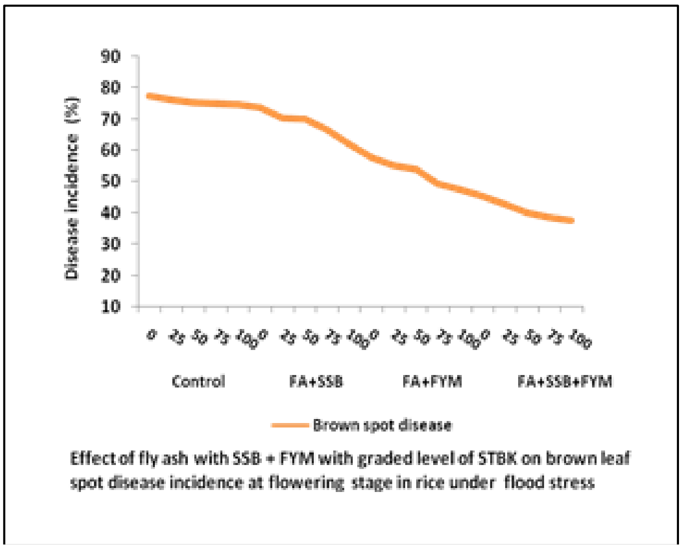

Fig 1b

Fig 1a & b: Effect of fly ash with SSB+FYM with graded levels of STBK on brown leaf spot disease incidence at flowering in rice under drought and flood stress

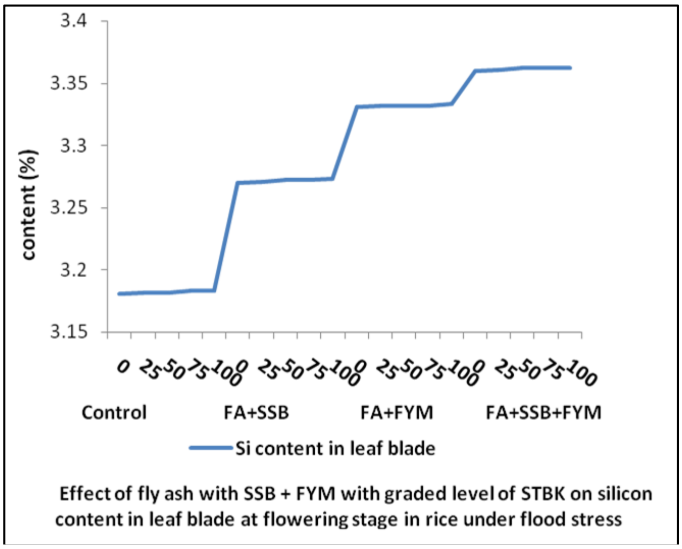

Fig 2a

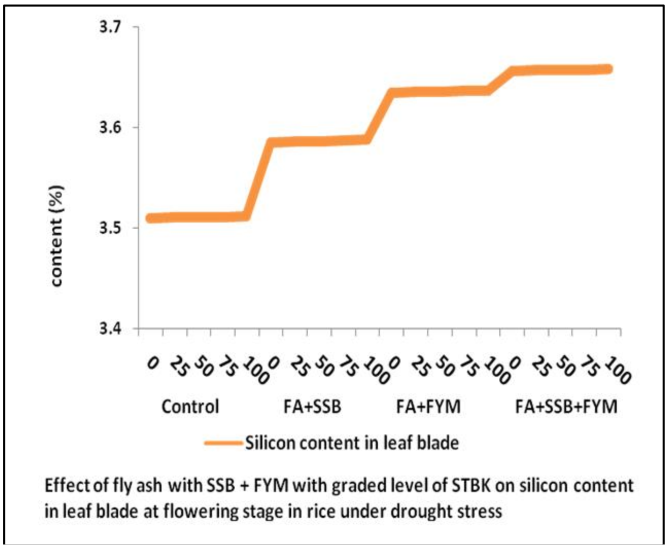

Fig 2b

Fig 2a & b: Effect of fly ash with SSB+FYM with graded levels of STBK on silicon content in leaf blade at flowering in rice under drought and flood stress

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