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Metal salts reduce sheath blight infection of rice

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Effects of seed treatment of soaking in dilute solutions of seven metal salts on sheath blight infection of rice caused by *Rhizoctonia solani* was investigated in a few pot and field trials. Salts like ferric chloride, cupric chloride, lithium sulphate, barium sulphate and zinc sulphate were found to have moderate to strong effects in different trials. For all of them, the lower concentration (10^{-4} M) had distinctly stronger effect on disease symptoms, reducing them by 41.4 % to 65.5 %. Besides these effects on symptoms, all of them mostly increased panicle length, number of productive tillers per hill, yield, etc. as compared to the untreated susceptible plants. Similar changes also occurred in treated susceptible plants in respect of total phenol and protein and activities of polyphenoloxidase and peroxidase enzymes. They responded with greater increases in these when infected then the untreated susceptible plants and behaved more like resistant plants. In respect of polygalacturonase activity which is critical for this type of pathogen same thing occurred. This enzyme activity decreased in treated plants, often drastically, to levels closer to that in resistant plants. Ferric chloride and cupric chloride were the two most effective salts in all respects. The utility of such chemicals in plant protection has been discussed.

Key words : *Rhizoctonia solani,* rice, metal salts, seed soaking treatment, induced resistance

INTRODUCTION

Besides fungicides, bactericides, etc. there are also some other chemicals which can control disease in plants. These are called systemic compounds. Inorganic salts, particularly the metal salts, are one of them. Wain and Carter (1972), Langcake (1981), Kuc (1987) and Sinha (1995) have provided many such examples. Sheath blight of rice caused by *Rhizoctonia solani* Kuhn is one of the most destructive diseases of this crop causing considerable loss. Its control by the use of fungicides is rather costly and can not be always attempted. Control of the diseases has sometimes been attempted by unconventional chemicals and some of the results are quite interesting. Sarkar and Sinha (1991) have first reported moderate control of sheath blight disease by the use of cycloheximide, sodium selenite and ferric chloride. Bhatacharyya and Roy (1998) also reported the use of unconventional chemicals on this disease. Similar reports on other disease have come from Mandal and Sinha (1992), Sinha and Hait (1982), Sinha and Sengupta (1986) and Sinha (1995). This present paper reports the results of the effects of some metal salts on this disease and biochemical changes associated with it.

MATERIALS AND METHODS

Plant

Four rice cultivars, viz. IET 6141, Indrasail, Swarna masuri and IR 20, the first three susceptible and the last one resistant to *Rhizoctonia solani* — sheath blight, were used in different experiments.

For pot trials, 100 treated or untreated seeds were shown in 17 cm earthen pots filled with garden soil supplemented with farm yard manure and after 21 days seven seedlings were transplanted to larger 21 cm pots, three pots per treatment. These pots were arranged in a randomised block design in open space and watered as required. There were additional pots for biochemical analysis.

For field trials, seedlings were raised in seed beds about 1 m × 1.5 m in size and transplanted when 30 days old into 2.5 m × 3 m main plots. The plots were arranged in a randomized block design, three replications per treatment, unless otherwise mentioned. Fertilizers were used at 100-50-50 kg of NPK/ha as basal and top dressings.

Pathogen

A sclerotium producing isolate of *Rhizoctonia solani* Kuhn [*Corticium sasakii* (Shirai) Matsumoto], perfect stage *Thanatephorus cucumeris* (Frank) Donk, grown on potato dextrose agar (PDA) medium, was used to inoculate the pot-grown plants. The pathogen was grown in 9 cm petridishes at 28 ± 1°C for 14 days to allow the production of enough sclerotia for artificial inoculation. To maintain its pathogenicity, the organism was isolated from artificially inoculated plants at intervals.

Inoculation of plants

For inoculation of pot-grown plants, ten medium sized sclerotia were inserted into leaf sheath of individual plants. To create humid conditions necessary for infection, all seven plants were kept loosely tied and kept as such for 2 days. In field trials, plants were exposed to natural infection.

Assessment of symptoms

In pot trials, disease symptoms were assessed twice, 14 and 21 days after inoculation (DAI). In case of field trials, the assessment was also made twice, 95 and 110 days after sowing (DAS). Disease severity was measured following the International Rice Research Institute approved scale. This was a 0-9 scale, where 1 = lesion limited to lower 1/4th of the leaf sheath area and 9 = lesion reaching the top of the tiller, with severe infection of all the leaves and some plants killed. Taking the numerical values of each tiller of a plant and every plant in a pot, mean disease index of a plant was calculated in the 0-9 scale. Panicle length, number of productive tillers per hill, 1000 grain weight and yield were also determined in different experiments. While the results of pot trial were based on 21 observations, those of field trial were based on 45 observations, 15 plants from each plot collected from selected spots.

Chemicals used

The following metal salts were used : cupric chloride, zinc chloride, barium chloride, barium sulphate, lithium sulphate, manganese sulphate, nickel chloride and ferric chloride. All of them were pure grade chemicals. They all were used at 10^{-4}M aqueous solution and only occasionally at higher concentration.

Treatments

Seeds were first surface sterilized with 1% mercuric chloride solution and washed thoroughly with distilled water. They were then kept dipped in chemical solution of known concentration for 24 hs at room temperature, after which they were directly sown in pots or seed beds. For soaking 5 g of seed, 50 ml of chemical solution was used. For the control treatment water was used instead.

Methods for biochemical analysis

Leaf sheaths collected from both uninoculated and inoculated plants were thoroughly washed in distilled water and then cut into small segments. Such pieces were used for different extraction methods.

Total phenol

Tissue was extracted following the method of Biehn *et al.* (1968) and total phenol was estimated by the method of Bray and Thorpe (1954). Data were expressed as mg g^{-1} fresh weight of tissue.

Total portein

Total protein was estimated following the method of Lowry *et al.* (1951). Result was expressed as mg g^{-1} fresh weight of tissue.

Polyphenoloxidase activity

Polyphenoloxidase activity was determined following the method of Jennings *et al.* (1969) and expressed as activity / 10 mg of extracted tissue.

Peroxidase activity

Activity of peroxidase was measured following the method of Addy and Goodman (1972). Results were expresed as activity U min^{-1} g^{-1} of tissue.

Polygalacturonase activity

Determination of polygalacturonase activity was done viscosimetrically following Bell *et al.* (1955), as modified by Hancock *et al.*(1964). The specific enzyme activity was determined from the formula 1/t x 1000, where t is the time required in minutes for 50% reduction in viscosity.

RESULTS

Screening of varieties

Four varieties were initially tested in pot trial for their responses to infection with *R. solani*. Transplanted seedling in 21 cm pots were artificially inoculated with sclerotia and symptoms asseseed 14 and 21 days after inoculation in 0-9 scale. Results are given in Table 1.

Table 1 : Comparative effect of artificial inoculation of four rice cultivars with *Rhizoctonia solani* on disease expression.

Cultivar	Mean disease index / plant 4 DAI	Mean disease index / plant 21 DAI	Panicle length (cm)	No. of productive tillers/hill
IR 20	1.7	3.0	23.8	17.3
Swarna Masuri	5.5	6.3	21.9	13.6
IET 6141	5.9	7.5	21.7	13.0
Indrasail	5.9	6.9	21.8	12.7
C. D. (P=0.05)	0.28	0.24	0.38	1.79

DAI = Days after inoculation

It was found that while IR-20 developed only mild symptoms indicating resistant reaction, other three, viz. Swarna Masuri, IET 6141 and Indrasail, developed considerable symptoms indicating susceptible reaction. They significantly reduced panicle length and number of productive tillers per hill as compared to that of IR-20. While IET 6141 was exclusively used for pot trials, Swarna Masuri and Indrasail were used for field trials. IR 20 was used as resistant material for comparison of the effects of different treatments.

Effect of chemicals on disease

An exploratory trial was conducted with pot-grown susceptible rice plants (IET 6141) whose seeds were soaked in dilute solutions (10^{-4} M) of the following chemicals : cupric chloride, zinc chloride, barium sulphate, nickel chloride, lithium sulphate, ferric chloride and manganese sulphate. There were control treatment for both susceptible and resistant plants (IR 20) also. Symptoms were assessed both 14 and 21 days after inoculation at the age of 30 days. Panicle length and the number of productive tillers per hill were also noted. Results are shown in Table 2.

Table 2 : Effect of seven metal salts for seed soaking treatment of susceptible rice plants (IET 6141) grown in pots on symptom expression following 21 days after inoculation with *R. solani*

Treatment	Mean disease index / plant	Panicle length (cm)	No. of productive tillers/hill
IET 6141 (Sus.)			
Water (control)	6.3	21.7	9.3
Cupric chloride	4.2 (-33.3)	26.1	12.0
Zinc chloride	4.2 (-33.3)	25.9	15.0
Barium sulphate	4.3 (-31.8)	26.6	17.7
Nickel chloride	5.9 (-6.4)	23.9	13.7
Lithium sulphate	4.3 (-31.8)	25.8	17.0
Ferric chloride	3.9 (-38.1)	26.9	12.3
Manganese sulphate	6.0 (-4.8)	23.1	10.0
IR 20 (Res.)			
Water (control)	3.4	23.0	13.3
C. D. (P=0.05)	0.89	1.19	4.79

Values in parenthesis indicate percentage reduction in terms of susceptible control.

Results did not show any effect for nickel chloride and manganese sulphate. However, other five had moderate but very significant effects. Ferric chloride appeared to be the most effective chemical tested, cupric chloride being a close second. They reduced symptoms by 30.5% to 39.4% after 14 days and by 31.8% to 38.1% after 21 of inoculation. Both panicle length and the number of productive tillers also increased in different treatments.

In an experiment next year, the effective salts were used each at two concentrations, 10^{-3}M and 10^{-4}M, to see if there would be any concentration effect. Zinc sulphate replaced zinc chloride. Infection was heavy. Besides the assessment of symptoms and measurement of yield components, biochemical aspects of the disease i.e. the total phenol and protein contents and the polyphenoloxidase and peroxidase activities were also investigated for each treatment to see if there would be any relation with induced effects on disease symptoms. This was done with tissue collected 21 days after inoculation.

Table 3 : Effect of wet seed treatment with five metal salts, used at two concentrations each, on symptom expression and some yield components in pot-grown susceptible plants (IET 6141) as compared to those in resistant plants:

Treatment	Conc. (M)	Mean disease index / plant 4 DAI	Mean disease index / plant 21 DAI	Panicle length (cm)	No. of productive tillers/hill
IET 6141 (Sus.)					
Water (control)		5.8	7.7	21.6	12.7
Cupric chloride	10^{-3}	4.3(-25.9)	5.9(-23.4)	25.2	15.5
	10^{-4}	2.6(-55.2)	3.7(-52.0)	26.2	16.6
Ferric chloride	10^{-3}	3.9(-32.8)	5.6(-27.3)	25.4	15.7
	10^{-4}	2.0(-65.5)	3.3(-57.1)	26.3	16.9
Barium sulphate	10^{-3}	4.4(-24.1)	6.5(-15.6)	24.5	14.4
	10^{-4}	3.2(-44.8)	4.3(-44.2)	25.9	16.1
Lithium sulphate	10^{-3}	4.5(-22.4)	6.6(-14.3)	23.7	14.2
	10^{-4}	3.4(-41.4)	4.5(-41.6)	25.8	16.0
Zinc sulphate	10^{-3}	4.5(-22.4)	6.3(-10.4)	24.7	14.7
	10^{-4}	2.7(-53.5)	4.1(-46.8)	26.2	16.2
IR 20 (Res.)					
Water (Control)		1.8	3.3	23.9	17.0
C. D. (P=0.05)		0.54	0.46	1.03	0.75

Values in parenthesis indicate percentage reductions or increases in terms of susceptible control.

DAI = Days after inoculation.

It appears that while the higher concentration of the five salts (10^{-3}M) reduced symptoms by 22.4% to 32.1% and 10.4% to 27.3% after 14 after 21 days respectively, the lower concentration (10^{-4}M) caused 41.4% to 65.5% and 25.9% to 57.1% reductions at the same time causing much stronger effect. Lower concentration of different treatments increased panicle length by 26% to 33% and yield by among the test chemicals in the all the above respects. Cupric chloride was a close second.

Biochemical results make interesting reading. Treated susceptible plants showed distinct changes as compared to untreated susceptible plants when infected. These showed increased total phenol (28-76%) and total protein (68-147%) contents and activities of polyphenoloxidase (29-167%) and peroxidase (18-54%) enzyme activities. Specific PG enzyme activity was also drastically reduced (64-85%). In all these respects ferric chloride and cupric chloride had the best results. In these treatments the levels of different components came closer to those in resistant plants.

Field trials

An exploratory field trial was conducted at a farmer's field in Barasat with cv. Indrasail and four salts in treatment at 10^{-4}M. Besides an untreated control, there were two treatments with Emisan-6 and Bavistin both at 1% used for seed treatments. Unfortunately disease appeared in very mild form. The symptoms were assessed 110 days after sowing. The four salts reduced disease but had only mild effect, reduction in disease index being cupric chloride – 17%, barium sulphate – 14.8%, lithium sulphate 15.6% and ferric chlorid 20.4%. In contrast, Emisan-6 and Bavistin had 13.7% and 12.5% less symptoms respectively. Next year, another field trial was conducted at a farmer's field at Barasat with cv. Swarna Masuri treated with five salts at 10^{-4}M. The plants were exposed to natural

Table 4 : Effect of wet seed treatment of pot-grown susceptible rice plants (IET 6141), with five metal salts at 10^{-4}, on biochemical responses as compared to those to control susceptible and resistant plants, 21 days after inoculation

Treatment	Mean disease index / plant	Total phenol (mg g^{-1} fresh wt. of tissue)	Total protein (mg g^{-1} fresh wt. of tissue)	Polyphenoloxidase activity (10 g of extracted tissue)	Peroxidase activity (unit of activity/g fresh tissue/min)	Specific PG enzyme activity
IET 6141 (Sus.)						
Cupric chloride	3.7	4.2	10.3	0.28	51.77	14.92
Ferric chloride	3.3	4.4	10.9	0.29	53.20	14.49
Barium sulphate	4.3	3.6	7.6	0.22	43.61	32.54
Lithium sulphate	4.5	3.2	7.4	0.18	41.79	38.97
Zinc sulphate	4.1	3.9	8.8	0.23	57.13	21.31
Water (Control)	7.7	2.5	4.4	0.14	37.40	107.3
IR 20 (Res.)						
Water (Control)	3.3	4.4	14.5	0.41	57.58	11.37
C. D. (P=0.05)		0.08	0.37	0.040	1.669	3.56

Results of biochemical analysis all represent means of three replications.

Table 5 : Effect of wet seed treatment with five metal salts at 10^{-4}M on field-grown susceptible rice plants (cv. Swarna Masuri), exposed to natural infection of *R. solari* on symptom expression and yield components, recorded 95 and 110 days after sowing.

Treatment	Mean disease index / plant		Panicle length (cm)	No. of productive tillers/hill	1000 grain wt. (g)	Yield (ton/ha)
	95 DAS	110 DAS				
Water (Control)	4.4	5.7	21.6	14.2	17.5	3.25
Cupric chloride	2.6(-40.9)	3.6(-36.8)	23.7	18.8	18.6	4.03(+24.0)
Ferric chloride	2.4(-45.7)	3.5(-38.6)	23.7	19.1	18.6	4.09(+25.8)
Barium sulphate	3.0(-31.8)	4.0(-29.8)	23.1	18.2	18.1	3.87(+19.1)
Zinc sulphate	2.8(-36.4)	3.9(-31.6)	23.1	18.5	18.3	4.0(+23.0)
Lithium sulphate	3.0(-31.8)	4.1(-28.1)	23.0	18.2	18.1	3.87(+19.1)
C. D. (P=0.05)	0.75	0.46	0.34	2.47	0.17	0.17

Results in parenthesis indicate percentage reduction or increase in terms of control
DAS = Days after sowing

Table 6 : Effect of wet seed treatment with five metal salts on field-grown susceptible rice plants (cv. Swarna Masuri), exposed to natural infection, on biochemical changes recorded 95 days after sowing.

Treatment	Mean disease index / plant	Total phenol (mg g^{-1} fresh wt. of tissue)	Total protein (mg g^{-1} fresh wt. of tissue)	Polyphenoloxidase activity (10 g of extracted tissue)	Peroxidase activity (unit of activity/g fresh tissue/min)	Specific PG enzyme activity
Water (Control)	4.4	2.6	5.2	0.17	39.51	98.48
Cupric chloride	2.6(-40.9)	4.1	10.3	0.30	50.43	19.10
Ferric chloride	2.4(-45.5)	4.1	10.7	0.32	51.48	17.70
Barium sulphate	3.0(-31.8)	3.4	8.5	0.23	45.68	37.71
Lithium sulphate	3.0(-31.8)	3.3	7.4	0.22	43.44	41.22
Zinc sulphate	2.8(-36.4)	3.7	8.5	0.26	47.40	29.53
C. D. (P=0.05)		0.40	0.83	0.045	3.624	4.15

Results of biochemical analysis all represent means of three replications.

infection and symptoms were assessed 95 days after sowing for biochemical analysis. Results given in Table 5 shows that infection was good though not extensive. They reduced symptoms by 30% to 46% after 95 days and 28% to 39% after 110 days following infection. Best results were obtained with ferric chloride and cupric chloride, other three salts being slightly less effective. This order was followed by their effects on panicle length, number of productive tillers per hill, yield and 1000 grain weight. The effects were all significant.

Results of biochemical analyses followed the same pattern as noticed in case of pot-grown plants. Treatment with five salts induced significant increases in total phenol (27-58%), total protein (42-106%), polyphenoloxidase activity (29-88%), peroxidase activity (10-30%) and reduction in polygalacturonase (58-82%) activity. These changes were mostly correlated with resistance inducing ability of the chemicals. Maximum effects were obtained with ferric chloride and cupric chloride.

DISCUSSION

Results of the present series of investigations clearly shows that many of the metal salts have the power of inducing appreciable resistance in rice plants to sheath blight infection in rice. This is in line with observations recorded for some other diseases (Chowdhury, 1992 ; Mandal and Sinha, 1992, Sinha, 1995). Different pot and field trials indicated the same pattern. In them, salts like ferric chloride, cupric chloride, barium sulphate, lithium sulphate and zinc sulphate reduced sheath blight symptoms by 22.3% to 65.5% in different experiments, the first two being most effective and distinctly superior to others. These salts reduced other characteristics of this disease by increasing panicle length, number of productive tiller per hill, grain weight and yield significantly. None of these salts had any untoward effect on seed germination and sclerotial germination of the pathogen. That the reduction in symptoms was not due to any direct inhibitory effect on the pathogen of these chemicals becomes evident from the fact that each salt reduced disease more significantly at the lower concentration than at the higher concentration.

Besides their adverse effect on visual aspects of the disease, these salts had profound effects on the biochemical conditions of the plants and induced changes in susceptible plants when infected. The treatments thus brought the levels of total phenol, total protein and both polyphenoloxidase and peroxidase activities close to the levels of resistant plants. This is particularly evident in the treatments with ferric chloride and cupric chloride. Total phenol, total protein and polyphenoloxidase and peroxidase activities often increase in plants in response to inoculation, particularly in resistant plants, and are regarded by many as defence components of the plant. Further, sheath blight infection is caused by *R. solani,* a rotter pathogen, which mainly functions by the action of polygalacturonase which it profusely secretes in a susceptible host but not in a resistant host. Here, in all the treatments, PG production is significantly reduced more so in ferric chloride and cupric chloride treatments. So this will suggest that plant defence is a multicomponent phenomenon. This condition does not functionally exist in susceptible plants and represents a repressed condition. As a result of treatment, this repression is lifted off and the different components starts production, may be to a different extent and different degrees of resistant is induced. These are not the only components of plant resistance, there may be many others too.

Treatment with fungicides, the most common method of plant disease control is very expensive and often needs more than one application. In contrast, the seed treatment by soaking in metal salt solutions at extremely minute doses costs practically nothing and is within the reach of even the poor farmers. Even 30-40% control with these salts may be a boon to them and may provide a healthy crop at practically no expense.

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