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Protection of soybean seedling from *Sclerotium*-rot by seed treatment with alternative chemical agents

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Some selected non-coventional chemicals like cupric chloride, ferric chloride, lithium sulphate, manganese sulphate and sodium molybdate used as seed treatment at dilute concentrations (10^{-3}M) were tested in soybean plants against *Sclerotium* rot caused by *Sclerotium rolfsii* Sacc. Ferric chloride and manganese sulphate were the most effective and sodium molybdate, the least effective of the chemicals tested. In the effective treatments, the symptoms production were inhibited from 47%-69% and also brought down the mortality percentage significantly as compared to the untreated plants. In such effective treatments, the infected host tissue recorded marked reductions in polygalacturonase actively and oxalic acid contents and also moderate increases in calcium and magnesium levels.

Key words : Soybean, *Sclerotium rolfsii*, control, non-conventional chemicals, biochemical changes

INTRODUCTION

Sclerotium rolfsii Sacc., a soil-borne pathogen of very aggressive nature causes considerable damages to soybean. Source of resistance to *S.rolfsii* is rarely known and conventional chemical control with some non-conventional chemicals with no or little toxicity can provide substantial protection to many plants from their pathogen (Langcake, 1981; Lazarovits, 1988; Sinha, 1990; Purkayastha, 1994). Extensive studies in this area have been carried out at plant pathology laboratory, Bidhan Chandra Krishi Viswavidylaya, where it has been shown that many chemicals in the nature of metal salts, amino-acids, plant growth regulators and miscellaneous organic compounds are effective inducing high level of resistance in rice plants against brown spot (Sinha and Hait, 1982) and blast (Sinha and Sengupta, 1986), in tomato against *Fusarium* wilt (Mandal and Sinha, 1992), and in groundnut against *Sclerotium* infection (Chowdhury and Sinha, 1997) and rust (Chowdhury, 1995). These and other studies have established that many such compounds can provide effective systemic protection to the crop from their pathogens and that such effect often persists over fairly long periods. In that context, the possibility of controlling *Sclerotium* rot of soybean by the use of select group of chemicals, found effective earlier against different crop diseases, were investigated.

MATERIALS AND METHODS

Soybean cultivar Macs-58, susceptible to collar rot was used as test plant. Test chemicals included cupric chloride, ferric chloride, lithium sulphate, manganese sulphate and sodium molybdate. The selection of chemicals and their concentrations was based on the effectiveness in previous studies made in this Department on groundnut against stem rot (Chowdhury, 1991) and tomato against *Fusarium* wilt (Mandal and Sinha, 1992). Chemicals used were of pure grade.

Seeds were given pre-sowing wet seed treatment by soaking for 24 h in chemical solution (10^{-3} M) or in water for the control. Seeds were sown in 18 cm earthen pots containing field soil mixed with F.Y.M. in 3 : 1 proportion. Pot-grown plants were artificially inoculated with *Sclerotium rolfsii* Sacc. after 2 weeks by adding to each pot 100 g mixture of inoculum grown on sand-maize medium and sterilized soil and mixing it with top soil. There were six replications for each treatment. The symptoms were assessed 2 weeks after inoculation on a 0-4 scale based on the extent of tissue rotting at the stem base and damage to the leaves and expressed plantwise.

For biochemical analyses, 2-3 cm collar region were collected. While collecting tissue samples from inoculated plants, care was taken to keep the unaffected green portion to a minimum.

(a) Polygalacturonase activity : The enzyme activity was measured following the method of Miller (1972) and the results were described as amount of galacturonic acid released in mg per g fresh weight of tissue per hour.

(b) Calcium and magnesium content : Ca and Mg content in plant tissue was determined following the method of Black (1965). The result was given as percentage of the element per g dry weight of tissue.

(c) Oxalic acid content : Its extractions and subsequent estimations were carried out using the methods of Andrews and Viser (1951) as modified by Faboya *et al.* (1983). The amount of oxalate present was calculated as per cent oxalic acid.

RESULTS

Results given in Table 1 show that seed treatment with ferric chloride and manganese sulphate provided soybean plants with high levels of protection against collar rot pathogen as becomes evident from decrease in symptoms and disease incidence from 91% in control to 41% to 48% and plant mortality from 69% to 21-29% in some effective treatments. Best result was achieved with manganese sulphate at 10^{-3} M. The plants with cupric chloride and sodium molybdate showed only minor effect on the rot pathogen.

As *S.rolfsii* is known to be an aggressive pathogen causing rot with strong pectolytic enzyme activity, it is quite possible that in susceptible plants acquiring strong resistance, formation of only limited size lesions may be linked with reduced activity of such enzyme. Polygalacturonase activity in both untreated and treated plants infected with the pathogen was measured at 7 and 14 days after inoculation. It was observed that the amount of D-galacturonic acid released by PG activity was quite high in the untreated plants, 7 days after inoculations (Table 2). However, its activity was 19% to 26% less in effective treatments like ferric chloride, magnesium sulphate and upric chloride. Seven days later, when most of the untreated plants were either dead or heavily damaged, enzyme activity

Table 1. Effect of seed treatment with non-conventional chemicals on symptom expression in pot-grown soybean plants (cv. Macs-58) inoculated with *Sclerotium rolfsii* Sacc.

Treatment	Concentration (M)	Disease incidence (%)	Plant mortality (%)	Mean disease index/plant (0-4 scale)*
Water (control)		91.0	69.0	3.6
Cupric chloride	10^{-3}	67.0	51.0	2.7 (-25.0)[1]
Lithium sulphate	10^{-3}	69.0	52.0	2.8 (-22.2)
Ferric chloride	10^{-3}	48.0	29.0	1.9 (-47.2)
Manganese sulphate	10^{-3}	41.0	21.0	1.1 (-69.4)
Sodium molybdate	10^{-3}	92.0	71.0	3.7 (+2.7)
C.D. (P = 0.01)		17.5	19.7	0.92

*Symptoms were assessed 2 weeks after inoculation; result based on averages of 30-35 plants per treatment.

1 Values in parenthesis percentage reduction (–) or increase (+) in terms of control.

Table 2. Effect of seed treatment on polygalacturonase activity in disease affected soybean (cv. Macs-58) stem tissue in various treatments after inoculation with *Sclerotium rolfsii*

Treatment	Amount of D-galacturonic acid released (mg g^{-1} fresh wt. of tissue / hr)	
	7 days	14 days
Water (control)	1.42	0.75
Cupric chloride	1.15	0.58
Lithium sulphate	1.25	0.67
Ferric chloride	1.05	0.52
Manganese sulphate	1.09	0.55
Sodium molybdate	1.41	0.78

them was much reduced as expected. Even at this stage, enzyme activity was 22% to 30% less in various effective treatments than in the untreated plants.

Since suceptible soybean plants, following treatments, showed strong resistance to *S.rolfsii* infection, it became of some interest to explore the status of calcium and magnesium in plants. Observations recorded in this respect 14 days after inoculation showed that treatment caused only marginal or no change in calcium and magnesium contents while infection resulted in mild increase in all cases, 5% and 8% to 17% respectively in the untreated and effective treatments for calcium and 2% and 4% to 8% for

Table 3. Effect of seed treatment with chemicals on calcium and magnesium content in soybean plants (cv. Macs-58) artificially inoculated with *S.rolfsii* recorded 7 days after inoculation

Treatment	Concentration of calcium and magnesium (on g[1] dry wt. basis)[1]			
	Ca (%)		Mg (%)	
	H	Ino	H	Ino
Water (control)	1.21	1.27	0.49	0.50
Cupric chloride	1.22	1.31	0.49	0.52
Lithium sulphate	1.21	1.30	0.50	0.51
Ferric chloride	1.22	1.40	0.49	0.53
Manganese sulphate	1.22	1.42	0.49	0.51
Sodium molybdate	1.20	1.21	0.48	0.49

1 Mean of three replications; H–Healthy; Ino-Inoculated

Table 4. Effect of seed treatment with non-conventional chemicals on xxalic acid concentration in soybean plants (cv. Macs-58) inoculated with *S.rolfsii* recorded 7 days after inoculation

Treatment	Oxalic acid (g) dry wt. basis (%)*	
	H	Ino
Water (control)	0.03	0.28
Cupric chloride	0.04	0.17
Lithium sulphate	0.04	0.19
Ferric chloride	0.04	0.11
Manganese sulphate	0.05	0.12
Sodium molybdate	0.04	0.27

* Mean of three replications. H–Healthy ; Ino-Inoculated

magnesium content (Table 3).

Results given in Table 4 show the difference between the oxalic acid contents in diseased and healthy plants in untreated and treated plants. There is no significant difference in oxalic acid content of healthy untreated and treated plants. The infection resulted significantly higher increase in the content in untreated than the treated plants.

DISCUSSION

Results show that seed treated with non-conventional chemicals particularly ferric chloride and manganese sulphate can induce considerable resistance in soybean plants against attack by *S.rolfsii.* The inhibitory effect was most striking in reducing disease incidence and plant mortality. In the effective treatment, the infected host tissue recorded marked reductions in specific polygalacturonase activity and increase in calcium and magnesium levels. Such accumulations lead to transformation of enzyme-sensitive cell wall pectic components at and around the lesion site to less soluble enzyme tolerant Ca-and Mg-pectates and this acts as a dynamic resistance factor (Bateman, 1964). The reduced oxalic acid contents in the effective treatments may be a contributory factor in induced resistance as oxalic acid lowers the pH of the cell wall which is favourable for the cell wall pectic components at and around then lesion site to less, enzyme tolarent Ca- and degrading enzymes (mainly PG) to hydrolyze the pectates (Faboya *et al.*, 1983). So, the non-conventional chemicals used in the present study for pre-sowing wet seed treatment at micro doses showed very high potential for controlling *Sclerotium* infection of soybean.

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