Experimental

4.1. Pathogenicity test of *Sclerotium rolfsii* on different soybean varieties

Sclerotium blight of soybean [*Glycine max* (L) Merrill] incited by *Sclerotium rolfsii* Sacc.(*Corticum rolfsii* Curzi), a soil borne rotting pathogen of very aggresssive nature cause a considerable damage to one of our major legume crops with hundreds of food,feed and industrial uses. In the first experiment six soybean varieties viz. PK-262, Bragg , NRC-7 , Pusa – 16 , J-80 and Macs–58 were screened in respect of their disease reaction to infection with *Sclerotium rolfsii* . The plants were inoculated at the age of 14 days by adding sand-maize meal grown inoculum to the soil. First symptom of this disease was the sudden wilting of a branch of a plant which was in complete or partial contact with the soil. Leaves turned brown, wilted and remain attached to the plant (Plates 2 & 3) . A whitish growth of the fungal mycelium was seen at the junction of the branch with the stem closed to the soil level, which was the most favoured point of attack. With time, the disease progresses and a white mycelial web spreads over the soil and the basal canopy of the plant followed by the appearance of the Sclerotia of mustard seed size on the infected areas. The entire plant was killed but sometimes only 2-3 branches get affected. In its advance stage infection was very much prominent in the root system also (Plate- 4).

Symptoms were assessed at regular intervals, disease index were computed . Results recorded 14 days after inoculation when the symptoms appeared to be fearly advanced are presented in Table-1, Fig.1. It is evident from Table-1 that all six varieties are susceptible to *Sclerotium rolfsii* pathogen as evident from the data on disease index , percent disease index and mortality percentage , recording 2.8 to 3.2 , 70 to 80% and 71 to 88% respectively. Among the six soybean varieties tested, Macs-58 showed highly susceptible to *S.rolfsii* and all other varieties have more or less similar disease reaction. Hence Macs-58 was selected for further experiments.

S.rolfsii is a facultative parasite that occurs in diverse soils and has a very wide host range .The colonies of *S.rolfsii* were very fast growing on potato dextrose agar media, reaching 9 cm. diameter in 3 days at 28^0C, with white, cottony fan like mycelium and produces an abundance of globular, tan to reddish brown or dark brown sclerotia about the sizes of mustard seeds after 6-7 days (Plates – 5 & 6).

Plate 2 (A & B): Soybean plants (Macs-58) artificially inoculated with *Sclerotium rolfsii* [12 days after inoculation]
(A)Healthy ; (B) Infected

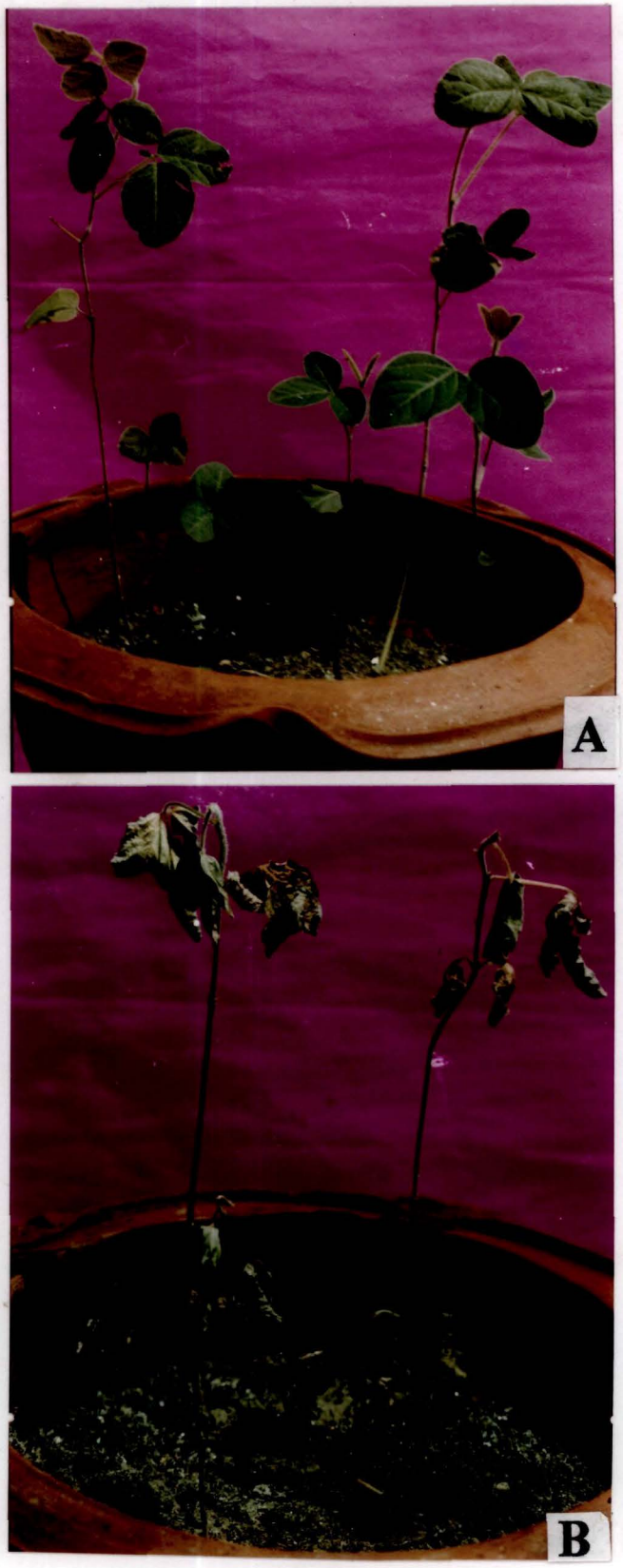


Plate 3 (A & B): Soybean plants (J-80) artificially inoculated with *Sclerotium rolfsii* [14 days after inoculation]
(A) Healthy ; (B) Infected

Plate 4 (A & B): Uprooted soybean plants (PK-262) artificially inoculated with *Sclerotium rolfsii*
(A) Healthy; (B) Infected

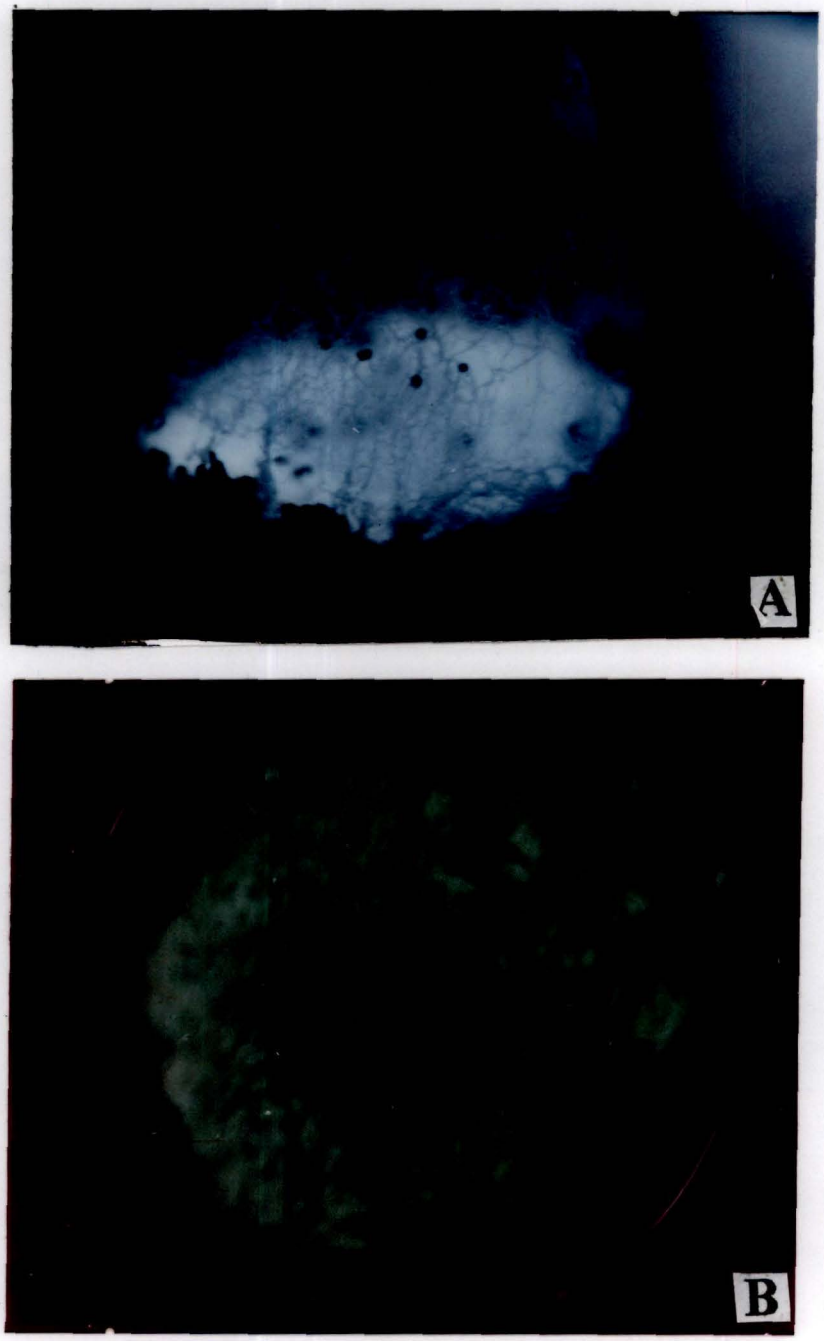


Plate 5 (A & B): *Sclerotium rolfsii* grown in Richard's medium
(A) Hyphal growth and sclerotia formation
(B) Close up view of sclerotia on mycelial mat

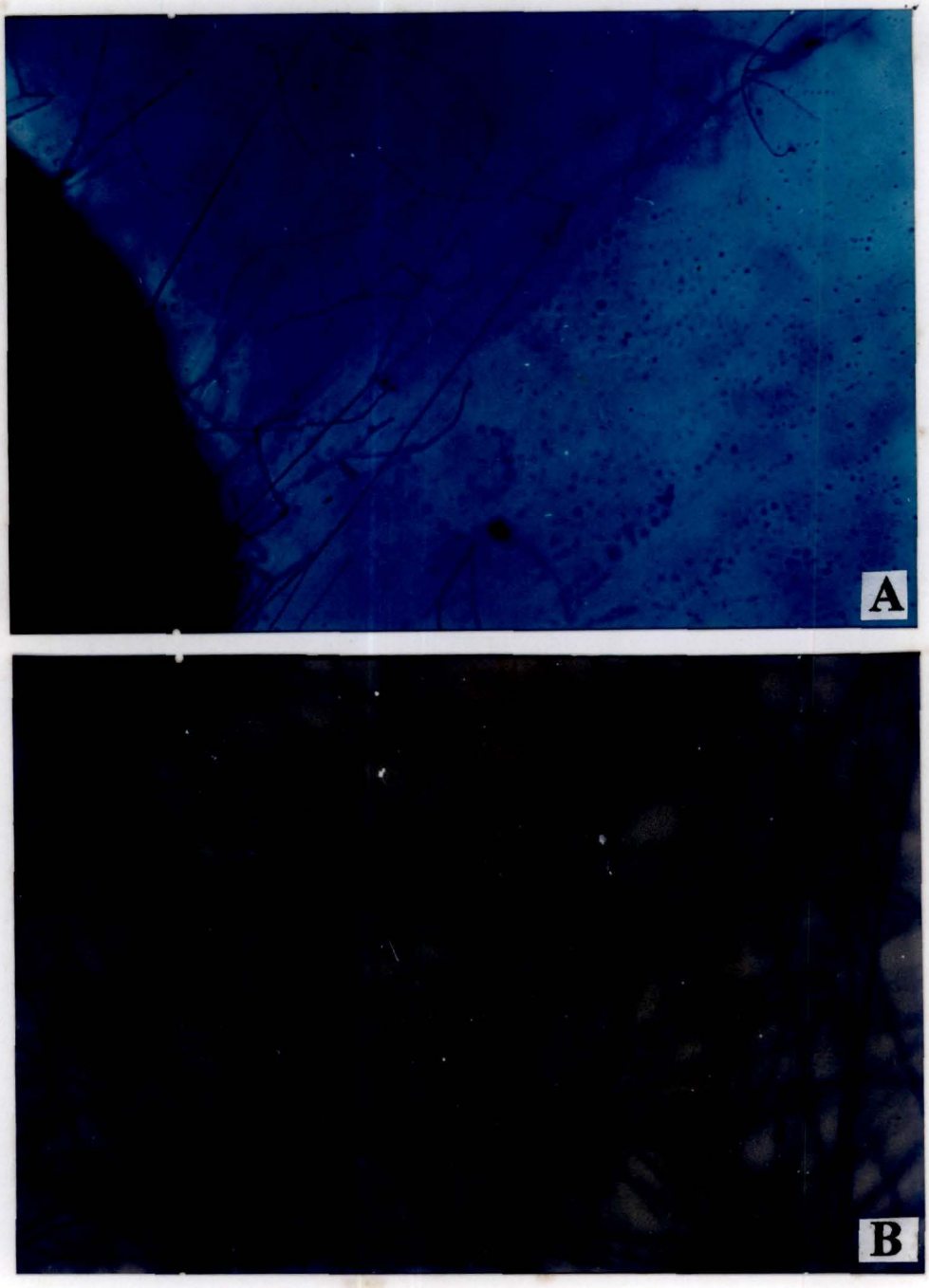


Plate 6 (A & B): Germinated sclerotium of *S.rolfsii* on glass slide
(A) Germination after 24h of incubation
(B) Close up view of mycelia

ıthogenecity test of *Sclerotium rolfsii* on different soybean varieties

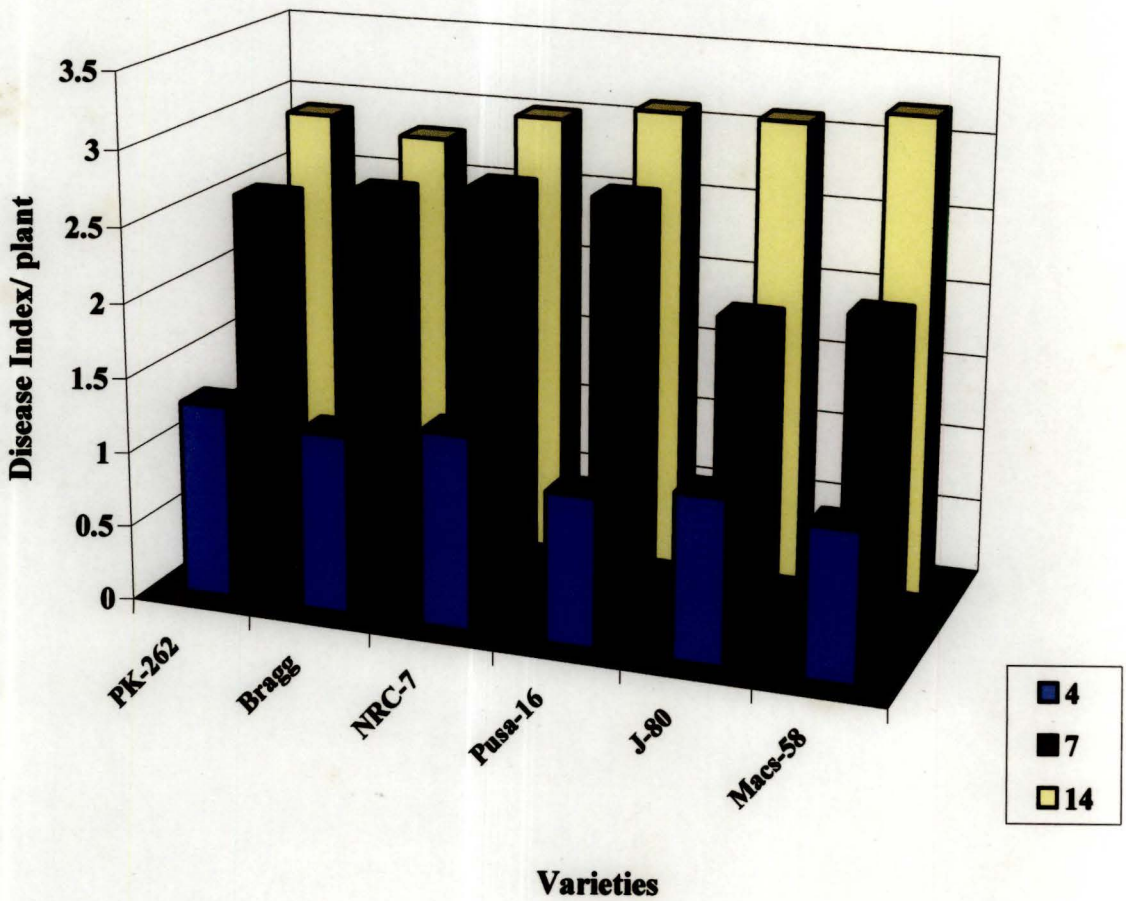


Fig. 1

Table-1 : Varietal reaction of soybean plants against *Sclerotium rolfsii*

Variety	Mean disease index/ plants.	Percent Disease	Plant mortality (%)
PK - 262	2.9	72.5	71.0
Bragg	2.8	70.0	73.0
NRC-7	3.0	75.0	76.0
Pusa-16	3.0	74.5	80.0
J-80	3.1	77.5	79.0
Macs-58	3.9	80.0	90.0

Results represent the averages of 60-70 plants per treatment, recorded at 14 days after inoculation

4.2. Comparison of glyceollin contents of soybean varieties after inoculation with *S.rolfsii*

It is evident from the results in Table-1and Fig.1 that among six soybean varieties (PK-262, Bragg, NRC-7, Pusa-16, J-80 and Macs-58) tested against *S.rolfsii* , Macs-58 was found to be highly susceptible. The differential response of soybean varieties towards *S.rolfsii* may be attributed to differences in their abilities to produce glyceollin (phytoalexin). Disease resistance of several crop plants has been correlated with the rate of production of phytoalexin by a number of previous research workers (Keen, 1981; Bhattacharyya and Ward, 1986; Purkayastha, 1995; Hammerschmidt,1999). In the present study, it was considered worthwhile to compare the accumulation of glyceollin in these varieties after challenge against *S. rolfsii* . Glyceollin was extracted and seperated from the infected roots of soybean. Healthy roots were also used for extraction which was considered as control. In this case, initially the healthy soybean plants were grown in sterilized soil under control

uprooted, washed thoroughly with sterile distilled water and dipped in mycelial and sclerotial suspension of *S. rolfsii* following the "water culture" inoculation technique as described under materials and methods. Yellowing of lower leaves and browning of root system was evident after 96h of inoculation in susceptible soybean varieties Macs-58. Sampling were done after 24 and 48h after inoculation for extraction of glyceollin. In order to detect glyceollin from infected soybean roots as well as to select the best solvent system (for better seperation), initially ethyl acetate fraction of root extracts loaded on TLC plates, developed in three different solvent systems viz. hexane:ethyl acetate: acetic acid (80:20:4); benzene: methanol (95:5) and chloroform: acetone: acetic acid (90:10:0.5) and then sprayed with p-nitroaniline. The Rf value of glyceollin obtained from infected roots (Table 2) were compared after co-chromatography with authentic sample. Finally, the solvent system hexane:ethyl acetate:acetic acid (80:20:4) was chosen.

Table 2 :Comparison of Rf values of authentic glyceollin with the glyceollin extracted from soybean roots inoculated with *S. rolfsii*.

Solvent system [a]	Rf values of glyceollin	
	A	B
Benzene : Methanol (95 : 5)	0.25	0.24
Hexane: Ethyl acetate: Acetic acid (80:20:4)	0.60	0.62
Chloroform : Acetone : Acetic acid (90:10:0.5)	0.48	0.45

A = Authentic glyceollin.

B = Glyceollin obtained from soybean roots inoculated with *S. rolfsii.*

[a] Spray reagent- Diazotised p-nitroaniline.

Methanolic solution of glyceollin when examined in a UV-spectrophotometer, maximum absorption was observed at 286nm. No such absorption was noted for healthy root extraction. After detection of glyceollin, its antifungal activity was tested following TLC plate bioassay and radial mycelial growth assay methods. The TLC plate bioassay using *Curvularia lunata* as a test organism, 10mm diam inhibition zone at Rf 0.60 was observed on the chromatogram after 96h of incubation at 25^{0}C. Silica gel corresponding to the inhibition zone (Rf 0.60) were removed from freshly prepared TLC plates and eluted by spec methanol. Purified eluants obtained after re-chromatography were tested for antifungal activity following sclerotial germination method. The relative antifungal activity of glyceollin was compared against *Sclerotium rolfsii, Fusarium oxysporium, Rhizoctonia solani* and *Macrophomina phaseolina* following petridish bioassay method. Inhibition of radial growth of the fungal species are presented in Table 3.

Table 3 : Inhibition of radial growth of fungi by glyceollin.

Fungi	**Percent inhibition of mycelial growth * (25 µg/ml glyceollin)**
Sclerotium rolfsii	94
Fusarium oxysporum	90
Rhizoctonia solani	92
Macrophomina phaseolina	86

*[Fungi were bioassayed in 2ml of medium in petriplates (35 mm diam.). Measurement were taken when net radial growth in the controls was 30±2 mm. Growth was calculated by measuring two diameters for each of three replicate colonies and substracting the diameter of the mycelial plug (2 mm) used to inoculate the plates.]

Thus, antifungal nature of glyceollin was confirmed. Accumulation of glyceollin from six different soybean varieties were detected after 24 and 48h of inoculation with *S. rolfsii.* It appears from the result that PK-262 and Bragg contained more glyceollin than Macs-58. This experiment was repeated twice and same trend in glyceollin accumulation was noticed in those six varieties. Average glyceollin content of six varieties after 24 and 48h of inoculation with *S. rolfsii* are presented in Table 4. Highest accumulation of glyceollin at 48h interval was noticed. Hence in further experiment this time period was considered for detection of glyceollin accumulation.

Table 4: Comparison ofglyceollin content of soybean roots infected with *S. rolfsii*

Varieties	Glyceollin content [a] (µg/g fresh wt. of tissue)	
	24h.	48h.
PK-262	286.7	392 .0
Bragg	236.8	377.2
NRC-7	168.0	329.4
Pusa-16	155.8	285.5
J-80	137.1	212.0
Macs-58	98.5	189.2

[a] Values shown are the mean from two determinations

4.3 Fungitoxicity assay of various chemicals on *S.rolfsii* and their effects on alteration of disease reaction

4.3.1. Fungitoxicity assay of test chemicals

Chemicals of diverse nature of which eight metal salts viz. cupric chloride , lithium sulphate , ferric chloride , manganese sulphate , sodium molybdate , manganese sulphate , zinc chloride , barium sulphate and three growth regulators viz. indole – 3- acetic acid ,

2,4- dichlorophenoxy acetic acid and 2,4,5 – trichlorophenoxy acetic acid and one biological compound , chitosan were tested against *Sclerotium rolfsii*. All these compounds were screened at a range of 3 concentrations each for their possible fungitoxic effect, if any, on sclerotial germination of pathogen. Both the percentage and nature of sclerotial germination make it clear that most of the test chemicals exhibited no toxic effect on the sclerotial germination at the concentrations screened. Only chitosan at 1.0% and 0.5% completely inhibited *Sclerotium* germination. The same compound at 0.3% and 0.1%, cupric chloride at 10^{-3}M, and ferric chloride at 10^{-3}M caused only mild inhibition (Table-5).

4.3.2. Seed treatment with chemicals and their effect on disease development

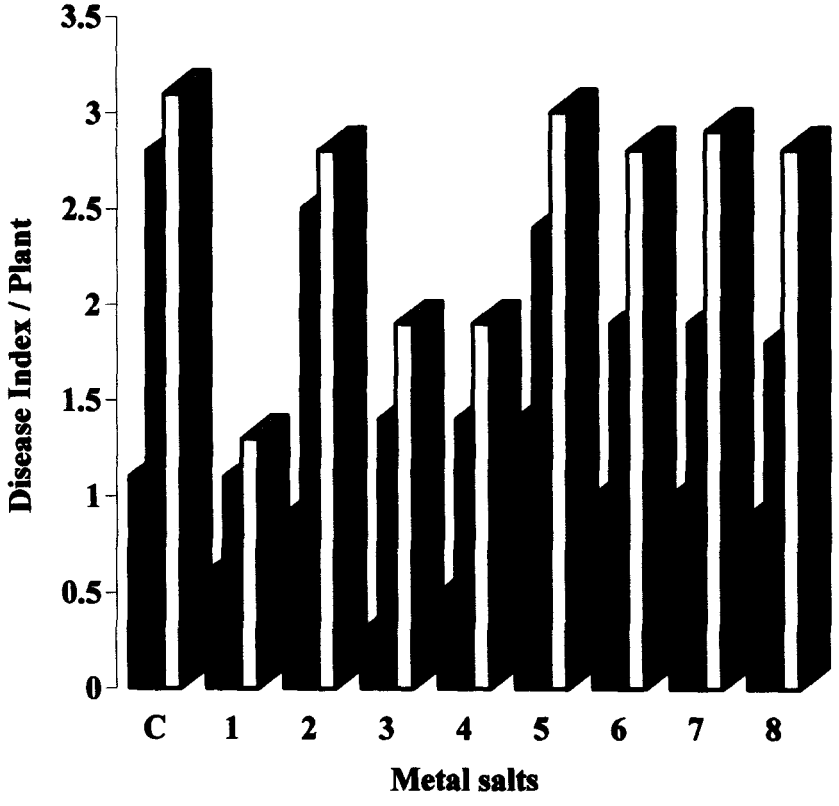
Non-conventional chemicals of diverse nature which includes eight metal salts viz. cupric chloride , lithium sulphate , ferric chloride , manganese sulphate , sodium molybdate, manganese sulphate , zinc chloride , barium sulphate and three growth regulators viz. indole – 3- acetic acid , 2,4- dichlorophenoxy acetic acid and 2,4,5 – trichlorophenoxy acetic acid and one biological compound , chitosan were used as seed treatment and their effect on disease development were studied on pot grown soybean plants. For this purpose, soybean seeds of susceptible variety (Macs-58) were soaked in above chemical solutions of desired concentration for overnight separately. Symptoms were assessed at regular intervals after inoculation of plants. All plants showing even incipent sign of rotting was taken as infected . It appears from the results that plants treated with cupric chloride , ferric chloride and manganese sulphate, provided soybean plants with high levels of protection against the rotting pathogen, the reduction in disease index in these treatments varying between 38% and 58%, being always highly significant (P=0.05). These treatments also checked plant infection by bringing down plant mortality from 73% to 42-53%. Best results were achieved with cupric chloride at 10^{-3} M. Plants treated with IAA, 2,4 -D, and chitosan also showed protective effects against the pathogen. These treatments reduced the disease symptoms by 25% to 57% as compared to the untreated plants at 14 days after inoculation and also brought down plant mortality recording from 70% to 39 in relation to untreated control plants.

Table 5 : Effect of test chemicals on sclerotial germination of *Sclerotium rolfsii in vitro*

Chemical / compound	Concentration	Germination (%)
Water (control)		95
Cupric chloride	10^{-3}M	55
	10^{-4}M	65
	10^{-5}M	76
Ferric chloride	10^{-3}M	60
	10^{-4}M	85
	10^{-5}M	90
Lithium sulphate	10^{-3}M	93
	10^{-4}M	95
	10^{-5}M	95
Sodium molybdate	10^{-3}M	75
	10^{-4}M	95
	10^{-5}M	95
Manganese sulphate	10^{-3}M	85
	10^{-4}M	90
	10^{-5}M	95
Zinc chloride	10^{-3}M	72
	10^{-4}M	90
	10^{-5}M	95
Magnesium sulphate	10^{-3}M	90
	10^{-4}M	95
	10^{-5}M	95
Indole 3-acetic acid	10^{-3}M	100
	10^{-4}M	
	10^{-5}M	95
2,4-dichlorophenoxy-acetic acid	10^{-5}M	95
	10^{-6}M	90
	10^{-7}M	95
	10^{-5}M	96
2,4,5-trichlorophenoxy acetic acid	10^{-6}M	96
	10^{-7}M	Nil
	1.0%	Nil
	0.5%	58
Chitosan	0.3%	60
	0.1%	75
	0.05%	90
	0.01%	

As a follow up experiment all the eight metal salts along with three plant growth regulators and one biological compound were further tested using mostly 3 concentrations ($10^{-3}$and 10^{-5}M) as seed treatment in order to determine the optimum concentration for induction of disease resistance in susceptible variety Macs-58. The results presented in Tables 6-9, confirm the earlier observation. The plants treated with 10^{-3}M cupric chloride and 10^{-3}M ferric chloride significantly (P=0.05) reduced symptoms as compared to the untreated plants as early as 7 days after inoculation. Subsequently, symptom development was distinctly slower in the treated plant and after 14 days these showed very significant differences in symptoms compared to untreated inoculated (control) plants. Regarding the concentration effect the higher concentration caused greater reduction of disease incidence. The plants treated with lithium sulphate had minor effect on the disease reduction. Cupric chloride and ferric chloride at their effective concentration i.e. at 10^{-3} M reduced the symptoms by 40% and 56% as compared to those in control plants, percent disease index from 75% in control plants to 45% and 32.5% while mortality percentage from 67% to 25% and 32% respectively (Table 8, Figs.2 & 3). The plants treated with manganese sulphate only showed strong protective effects at 10^{-4} M concentration against *S.rolfsii* which is evident as 53.3% less disease symptom in comparison to untreated inoculated (control) plants. This treatment also reduced the plant mortality substantially. The other chemicals i.e. sodium molybdate and magnesium sulphate had no effect on the reduction of disease incidence . It appears from Table 6, Fig.4 ; that susceptible plants (Macs58) treated with IAA at 10^{-3} M and 10^{-4} M concentration and 2,4-D at 10^{-6} M concentration significantly (P=0.05) reduced the disease index as compared to the control plants when final sampling was done. The plants treated separately with 3 concentrations (10^{-5}M – 10^{-7}M) of 2,4,5–T had no significant effect on reduction of disease incidence. Six different concetrations (0.01% to 1.0%) of chitosan were used for seed treatment and tested for their induced protective effect in soybean plants against *S.rolfsii.* Result presented in Table 7, Fig.5 indicate a graded concentration effect of chitosan on the reduction of disease index except with 1% chitosan. Plant mortality also gradually decreased with increasing concentration.

Effect of metal salts on disease development



C - Control
1 - Ferric chloride
2 - Cupric chloride
3 - Lithium sulphate
4 - Zinc chloride
5 - Barium sulphate
6 - Manganese sulphate
7 - Sodium molybdate
8 - Magnesium sulphate

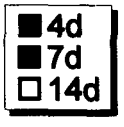


Fig. 2

Table 6 : Effect of wet seed treatment with chemicals at three concentration on disease development in soybean plants artificially inoculated with *Sclerotium rolfsii,* recorded at intervals of 4,7and 14 days

Treatment	Concentration (M)	Mean disease index/ plants.			Percent Disease index [a].	Plant mortality [a] (%)
		4 days	7 days	14 days		
Water (control)		0.9	2.1	2.9	72.5	80.0
I.A.A	10^{-3}	0.5	1.0	1.8(-37.9)[b]	45.0	51.0
	10^{-4}	0.3	0.7	1.0(-65.5)	25.0	35.0
	10^{-5}	0.6	1.2	1.9(-34.5)	47.5	42.0
2,4-D	10^{-5}	0.6	1.5	2.0(-31)	53.0	55.0
	10^{-6}	0.4	1.0	1.8(-37.9)	45.0	49.0
	10^{-7}	0.7	1.6	2.3(-20.6)	57.5	72.0
2,4,5-T	10^{-5}	0.6	1.7	2.4(-17.2)	60.0	77.0
	10^{-6}	0.6	1.4	2.1(-27.6)	52.5	65.0
	10^{-7}	0.5	1.0	2.0(-31)	50.0	53.0
C.D at 5%		0.16	0.73	0.51	15.13	17.25

a Plant Disease Index (PDI) and plant mortality percentage were computed at the last date of sampling.

b Values in the parenthesis indicate percentage reductions in terms of control

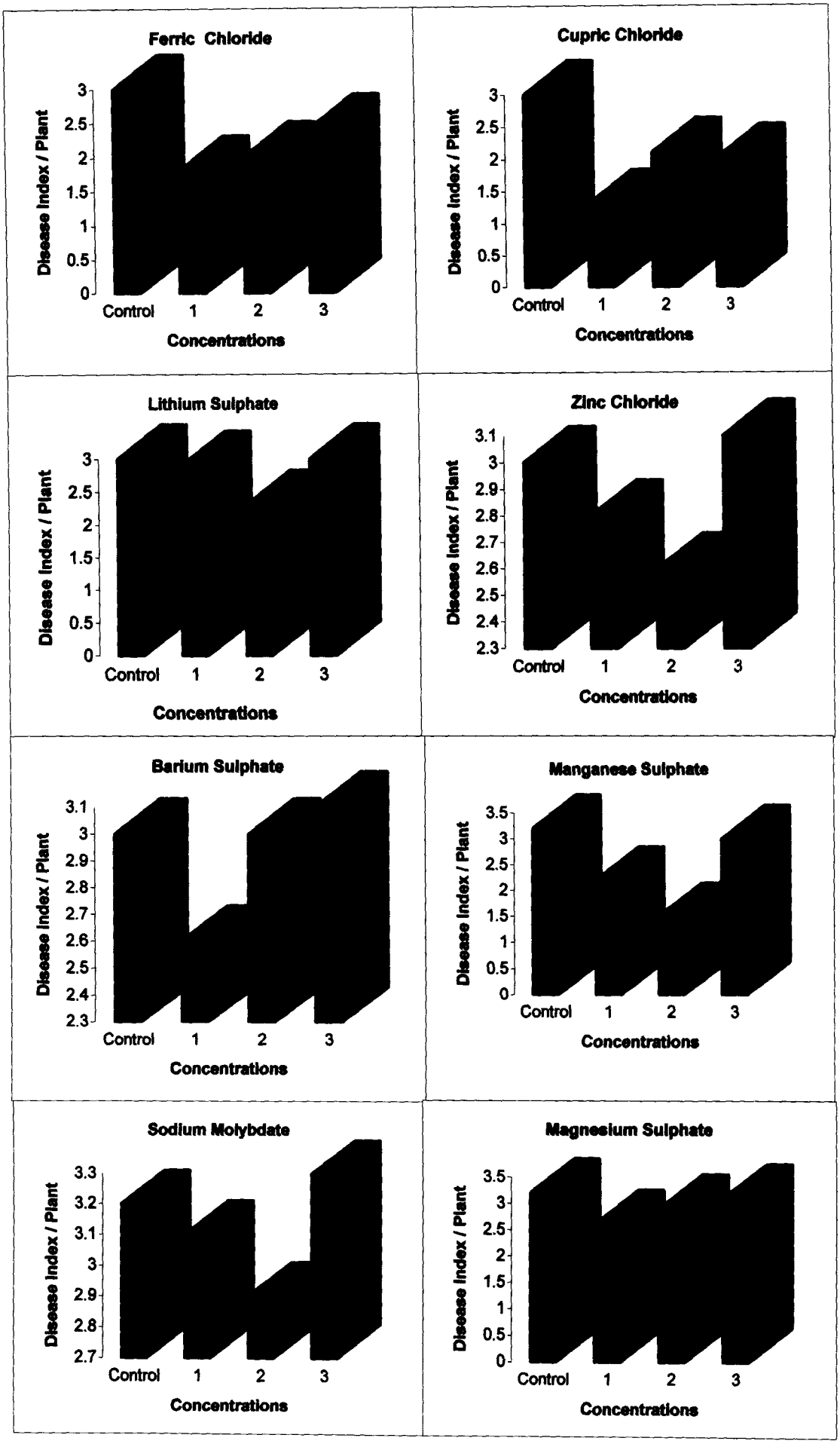
Table 7 : Effect of seed treatment with chitosan at different concentrations on disease development in soybean plants artificially inoculated with *Sclerotium rolfsii* ,recorded at 4,7and 14 days

Treatment	Concentration (%)	Mean disease index/ plants.			Percent Disease index [a].	Plant mortality [a] (%)
		4 days	7 days	14 days		
Water (control)		1.0	2.1	3.2	80.0	88.0
Chitosan	0.01	0.4	1.6	2.8(-12.5)[b]	70.0	78.0
	0.05	0.6	1.7	2.7(-15.6)	67.5	72.0
	0.1	0.5	1.0	1.9(-40.6)	47.5	46.0
	0.3	0.5	1.1	1.8(-43.7)	45.0	40.0
	0.5	0.3	0.9	1.3(-59.4)	32.5	28.0
	1.0	0.4	1.0	1.5(-53.1)	37.5	35.0
C.D at 5%		0.42	0.63	0.95	15.30	16.15

a. PDI and plant mortality percentage were recorded at 14 days after inoculation.

b. Values in the parenthesis indicate percentage disease reduction in terms of control

Effect of metal salts on disease development



(1) 10^{-3}M, (2) 10^{-4}M, (3) 10^{-5}M

Table 8: Effect of seed treatment with metal salts on disease development in soybean plants inoculated with *Sclerotium rolfsii*,[a].

Treatment	Concentration (M)	Mean disease index/ plants.	PercentDisease index [b].	Plant mortality [b] (%)
Water (control)		3.0	75.0	69.0
Cupric chloride	10^{-3}	1.7(-43.3)[c]	42.5	43.0
Ferric chloride	10^{-3}	1.2(-60)	30.0	31.0
Lithium sulphate	10^{-4}	2.4(-20)	60.0	61.0
Manganese sulphate	10^{-4}	1.7(-43.3)	42.5	42.0
Sodium molybdate	10^{-4}	2.8(-6.7)	70.0	67.0
C.D at 5%		0.71	18.2	15.6

a. Data recorded 14 days after inoculation.

b. PDI and plant mortality percentage were computed at the last date of sampling.

c. Values in the parenthesis indicate percentage reductions in terms of control.

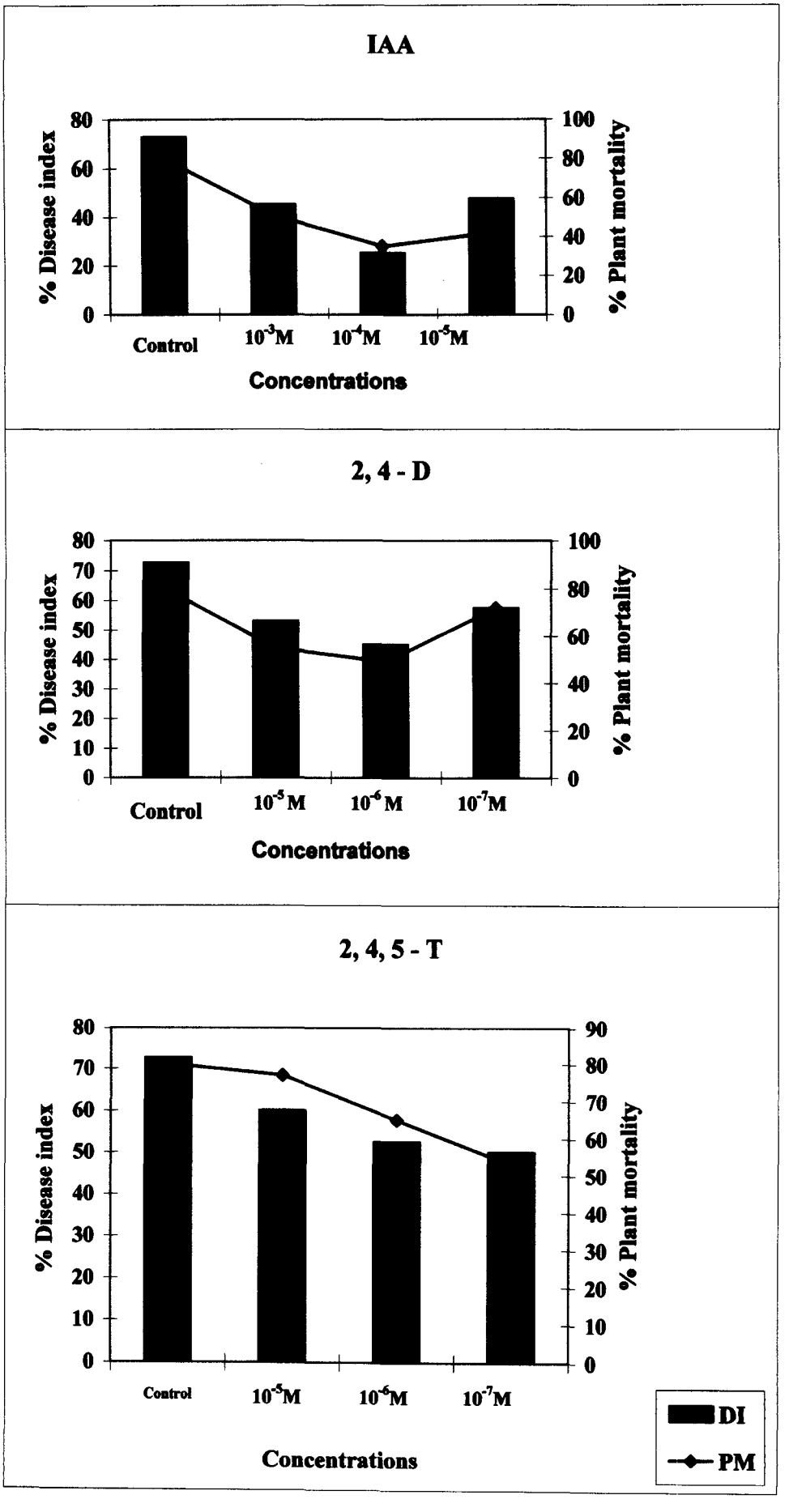

IAA
% Disease index
% Plant mortality
Control
10-3M
10-4M
10-5M
Concentrations
2, 4 - D
% Disease index
% Plant mortality
Control
10-5M
10-6M
10-7M
Concentrations
2, 4, 5 - T
% Disease index
% Plant mortality
Control
10-5M
10-6M
10-7M
Concentrations
DI
PM

Fig. 4

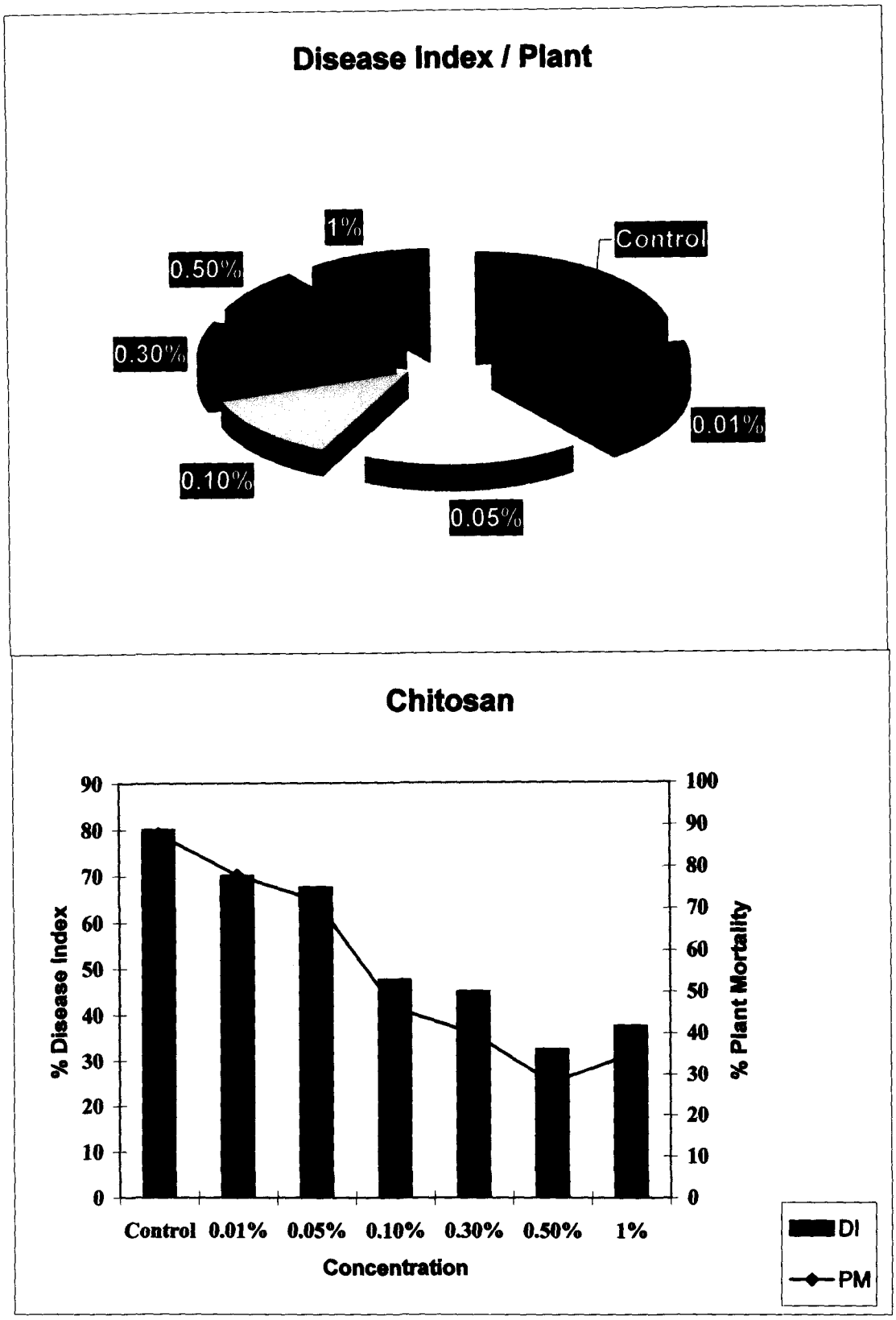

Disease Index / Plant
1%
Control
0.50%
0.30%
0.01%
0.10%
0.05%
Chitosan
% Disease Index
% Plant Mortality
90
80
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
Control
0.01%
0.05%
0.10%
0.30%
0.50%
1%
Concentration
DI
PM

Fig. 5

Table 9: Effect of seed treatment with chemicals on disease devolpment in soybean plants (cv. Macs-58) inoculated with *Sclerotium rolfsii.* [a]

Treatment	Conc.	Mean disease Index / plant.	Percent Disease index.	Plant mortality (%)
Water (control)		3.1	84.4	81.0
1.A.A.	10^{-4}M	0.9(-70.9)b	19.5	25.0
2.4 – D	10^{-6}M	2.5(-19.4)	65.0	60.0
2,4,5 – T	10^{-7}M	2.7(-12.9)	68.3	62.0
Chitosan	0.5%	1.2(-61.3)	30.0	32.0
C.D. at 5%		0.97	17.54	23.63

[s] Data recorded 14 days after inoculation.
[b] Values in parenthesis indicate percentage reductions in terns of contol.

4.4 Biochemical changes associated with induction of resistance in soybean plants

Previous results make it clear that wet seed treatment with many of the test compounds has an inhibitory effect on *Sclerotium* infection of soybean plants. This not only inhibit the initial successful establishment of infection and its subsequent spread but also substantially prevents mortality. Since no direct toxic effect on the pathogen can be envisaged for most of the test compounds, it is felt that these act indirectly through host responses that either (a) inactivate the pathogen, (b) restrict its spread or (c) inactivate or suppressed its mechanism of pathogenesis. To explore these possibilities, studies on changes in biochemical responses associated with chemically induced resistance in susceptible soybean plants were undertaken seperately with two groups of effective

chemicals. Areas investigated includes: total phenol, orthodihydroxyphenol, oxalic acid, calcium, magnesium and lignin contents; polyphenol oxidase, peroxidase, pectolytic enzyme and phenylalanine ammonialyase activities in healthy and infected tissue of untreated and treated plants. Five metal salts such as cupric chloride(10^{-3} M), ferric chloride (10^{-3} M),lithium sulphate(10^{-4} M), manganese sulphate (10^{-4} M) and sodium molybdate(10^{-4} M); three growth regulators such as IAA (10^{-4} M), 2,4 – D (10^{-6} M) , 2,4,5 –T(10^{-7} M) and chitosan (0.5%) were used for seed treatments. Both untreated and treated plants were artificially inoculated at the age of two weeks and the progress of symptom were recorded at intervals of 4,7 and 14 days after inoculation. On each sampling date plant materials were collected for biochemical studies and analysis were made following methods described earlier. Observations on symptom development in plants receiving treatment with the chemicals from which tissue samples were taken for biochemical studies noted for comparison. The plants treated with cupric chloride, ferric chloride and manganese sulphate suppressed *Sclerotium* infection from the earliest stage onwards and 43% to 60% reduction with respect to the control plants were recorded at the final stage of sampling . The chemicals like lithium sulphate and sodium molybdate had no effect on disease reduction. In the effective treatments , progress of disease was strikingly slowed down and as results mortality was brought down from 69%(control plants) to 31% (ferric chloride treated plants) and 43% (cupric chloride treated plants) . Plants treated with IAA and chitosan significantly (P=0.05) reduced the symptoms in soybean plants as compared to the untreated plants . In these treatments the plants had 61% to 70% less symptoms than in the control plants which showed severe symptoms and mortality was also markedly checked and was brought down from 81% in the untreated plants to 25% to 32% . The chemicals like 2,4-D and 2,4,5-T had less pronounced effect on all aspects of disease . IAA at 10^{-4} M concentration had the most marked protective effect in all respects. These results fully confirm the initial observations made with these compounds.

4.4.1. Total phenol

It appears from Tables 10 and 11; Fig.6 that different treatments differently stimulated phenol biosynthesis in soybean plants . Seed treatment caused marginal

increases in phenol level at all stages of sampling. Inoculation resulted in a moderate increase (20%) in phenol level in untreated plants at the early stages of infection , assessed 4 days after inoculation . But this effect rapidly declined with the age , i.e. at 7 and 14 days after inoculation . In the effective treated plants i.e. $CuCl_2$, $FeCl_3$ and $MnSO_4$, however , inoculation resulted in marked(78 - 87 %) and very significant (P = 0.05) increases 4 days after inoculation and the phenol level reaches peak , recording 7 days after inoculation , 83% to 101% higher phenol level but after 14 days , i.e. at the late stage of infection , this effect some what declined but 70% to 77% higher levels could still be noted . The final post- infection phenol levels in different treatments were 48% to 59% higher after 4 days , 68% to 85% higher after 7 days and 73% to 78% higher after 14 days of inoculation as compared to the control treatment . It is evident from the results that plants in different treatment showed no significant (P = 0.05) increases in phenol level over the untreated plant at different stages of sampling , between 4 and 14 days after inoculation . Infection mostly led to an increase in phenol level . Following infection the untreated plants recorded a moderate (25%) increase over healthy plants at the early stage of sampling but later this effect was less pronounced and recorded only marginally higher 3% to 13% levels . On the other hand the treated plants showed very significant (86%) increases quite early following inoculation and the stimulatory effects slightly less pronounced to record 70% higher levels at 7 days and 53% to 56% higher levels at 14 days after inoculation . The plants treated with IAA , the most effective treatment also showed highest stimulation in phenol level among the test compounds . Results clearly showed that susceptible plants in different treatment which displayed effective resistance to *Sclerotium* infection also developed much higher phenol levels when infected . Though any significant difference rarely existed among the responces of plants in different effective treatments , still the relation between their ability to induce resistance in host plants and to stimulate post infection increase in phenol level appears to be good . Maximum increase in phenol level was recorded for cupric chloride , the most effective compound , and minimum increase for sodium molybdate , the compound with least effect in disease suppression .

Table 10 : Effect of seed treatment with metal salts and / or inoculation with *S.rolfsii* on total phenol content in soybean plants, recorded at intervals of 4,7 and 14 days after inoculation .

Treatment	Total phenol (mg /g tissue) [a]					
	4days		7days		14 days	
	H	I	H	I	H	I
Water (Control)	1.74	2.10	1.75	1.91	1.74	1.75
Cupric chloride	1.78	3.34	1.76	3.54	1.76	3.12
Ferric chloride	1.75	3.12	1.75	3.21	1.74	3.03
Lithium sulphate	1.76	2.58	1.75	2.61	1.75	2.12
Manganese sulphate	1.78	3.29	1.79	3.48	1.78	3.03
Sodium molybdate	1.75	2.32	1.76	2.31	1.75	2.02

C.D. at 5%

Days X Treatment = 0.058

Days X Inoculation = 0.037

Treatment X Inoculation = 0.19

[a] =Mean of three replications

H= Healthy; I = Inoculated.

Table 11 : Effect of seed treatment with growth regulators and chitosan and / or inoculation with *S.rolfsii* on total phenol content in soyben plants recorded at intervals of 4,7 and 14 days after inoculation .

Treatment	Total phenol (mg/g tissue)[a]					
	4 days		7 days		14 days	
	H	I	H	I	H	I
Water (control)	1.72	2.15	1.74	1.98	1.74	1.80
1.A.A.	1.76	3.29	1.75	2.99	1.76	2.75
2,4 – D	1.75	2.75	1.76	2.45	1.75	2.25
2,4,5 – T	1.72	2.90	1.74	2.31	1.73	2.01
Chitosan	1.75	3.26	1.77	3.0	1.77	2.72

C.D. at 5%

Days x Treatment = 0.057

Days x Inoculation = 0.074

Treatment x Inoculation = 0.27

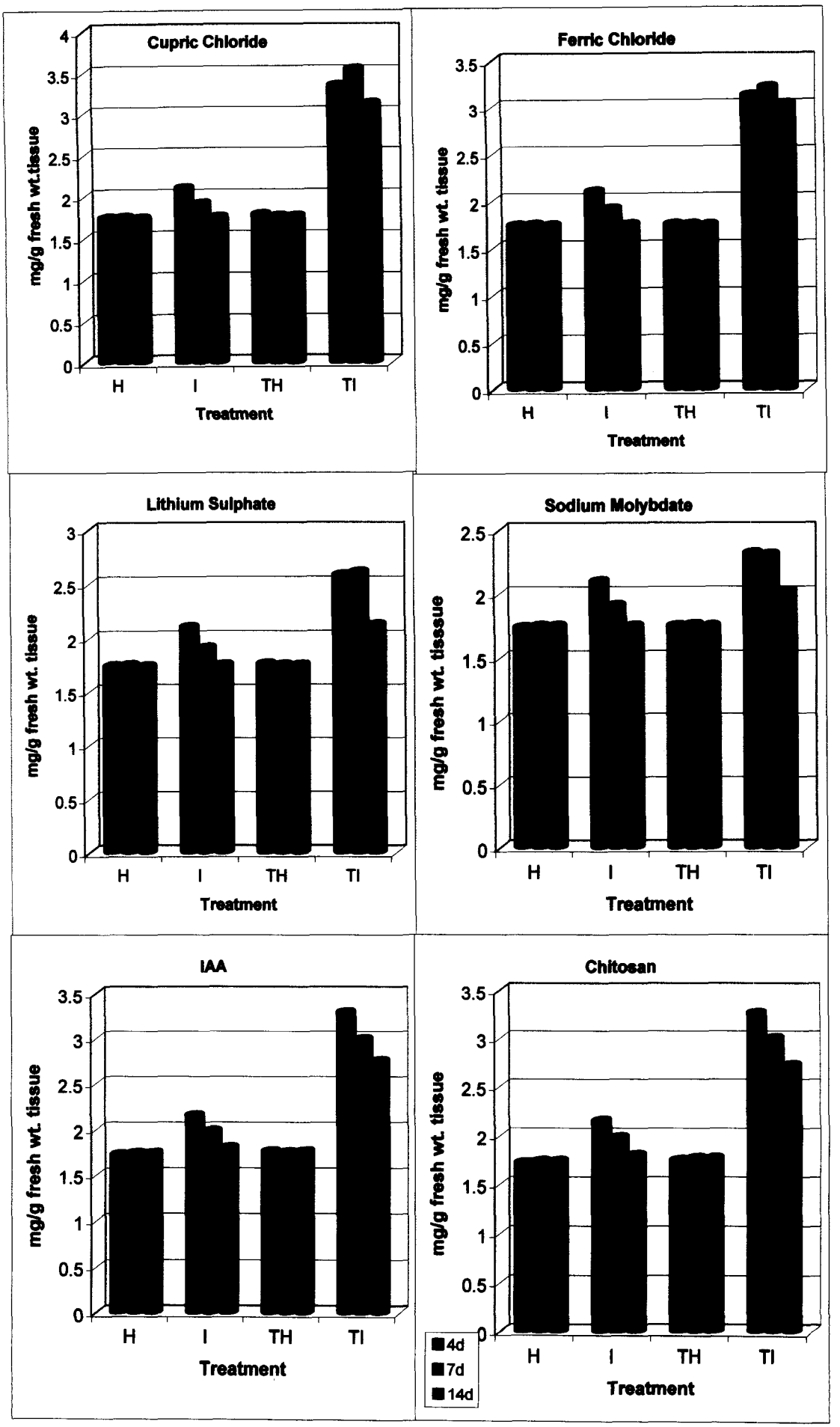
[a] = Mean of three replicarions

H = Healthy

I = Inoculated

NS = Not significant

Total Phenol content in soybean roots after induction of resistance



H- Healthy; I- Inoculated; TH- Treated healthy; TI- Treated inoculated

Fig. 6

4.4.2. Orthodihydroxy phenol

Table12 shows that treatments induced in susceptible plants vary small or no increases in O.D. phenol level over that in the untreated plants at different stages of sampling. Following inoculation, untreated plants recorded marked (70% increase) in O.D. phenol content 4 days after inoculation but this effect was less pronounced within next 3 days and during the next 7 days its content came down to only 8% increase. In three highly effective treatments, very significant increases were noticed after 4 days (81 – 90%) and seven day (84 – 108%) but during the next seven days the stimulatory effect declined so that 46% to 53% higher level was recorded. The plants in the least effective treatment with sodium molybdate recorded lower increases at all stages of sampling. The final post infection level of O.D. phenol in treated plants was significantly higher than that in the untreated plants, particularly in the three highly effective treatments, recording 11% to 17% after 4 days, 84% to 108% after 7 days and 46% to 53% after 14 days of inoculation (Fig. 7)

It is evident from Table 13 that treatment induced in susceptible plants had no significant increases in orthodihydroxyphenol level over that in the untreated plants at different stages of sampling . Following 4 days after inoculation, untreated plants recorded marked increases (100%) in orhtodihydroxy phenol content but these effects was almost disappeared at later stages of infection. In two highly effective treatments, very significant increases were noticed after 4 days (155 – 166 %) and 7 days (81 – 110 %) but during the next 7 days the stimulatory effect was further decline so that 72% higher level was recorded . The plants in the least effective treatment with 2,4,5 – T recorded lower increases at all stages of sampling. The final post infection level of orthodihydroxy phenol in higher effective treatments was significantly higher than that in the untreated plants.

Table 12 : Effect of seed treatment with metal salts and / or inoculation with *S.rolfsii* on ortho di -hydroxy phenol content in soybean plants, recorded at intervals. (at 4,7 and 14 days)

Treatment	Orthodihydroxy phenol content (mg/g tissue) [a]					
	4 days		7days		14 days	
	H	I	H	I	H	I
Water (Control)	0.10	0.17	0.12	0.13	0.12	0.13
Cupric chloride	0.11	0.20	0.12	0.25	0.13	0.19
Ferric chloride	0.10	0.19	0.13	0.24	0.13	0.20
Lithium sulphate	0.11	0.18	0.12	0.19	0.12	0.18
Manganese sulphate	0.10	0.19	0.12	0.24	0.12	0.20
Sodium molybdate	0.10	0.18	0.13	0.18	0.12	0.16

C.D. at 5%

Days X Treatment = NS

Days X Inoculation = 0.032

Treatment X Inoculation = 0.06

[a] =Mean of three replications

H= Healthy ; I= Inoculated.

NS = Not significant

Table 13 : Effect of seed treatment with growth regulators and chitosan and / or inoculation with *S.rolfsii* on orthodihydroxy phenol content in soybean plants, recorded at the intervals of 4,7and14daysafter inoculation

Treatment	Orthodihydroxyphenol (mg /g tissue)[a]					
	4 days		7 days		14 days	
	H	I	H	I	H	I
Water (control)	0.09	0.18	0.10	0.15	0.11	0.12
1.A.A.	0.09	0.24	0.11	0.20	0.11	0.19
2,4 – D	0.08	0.21	0.10	0.19	0.10	0.14
2,4,5 – T	0.10	0.20	0.10	0.16	0.11	0.13
Chitosan	0.09	0.23	0.10	0.21	0.11	0.19

C.D. at 5%

Days x Treatment = N S

Days x Inoculation = 0.03

Treatment x Inoculation = 0.05

[a] = Mean of three replications

H = Healthy

I = Inoculated

NS= Not significant

Ortho-dihydroxyphenol content in soybean roots after induction of resistance with metal salts

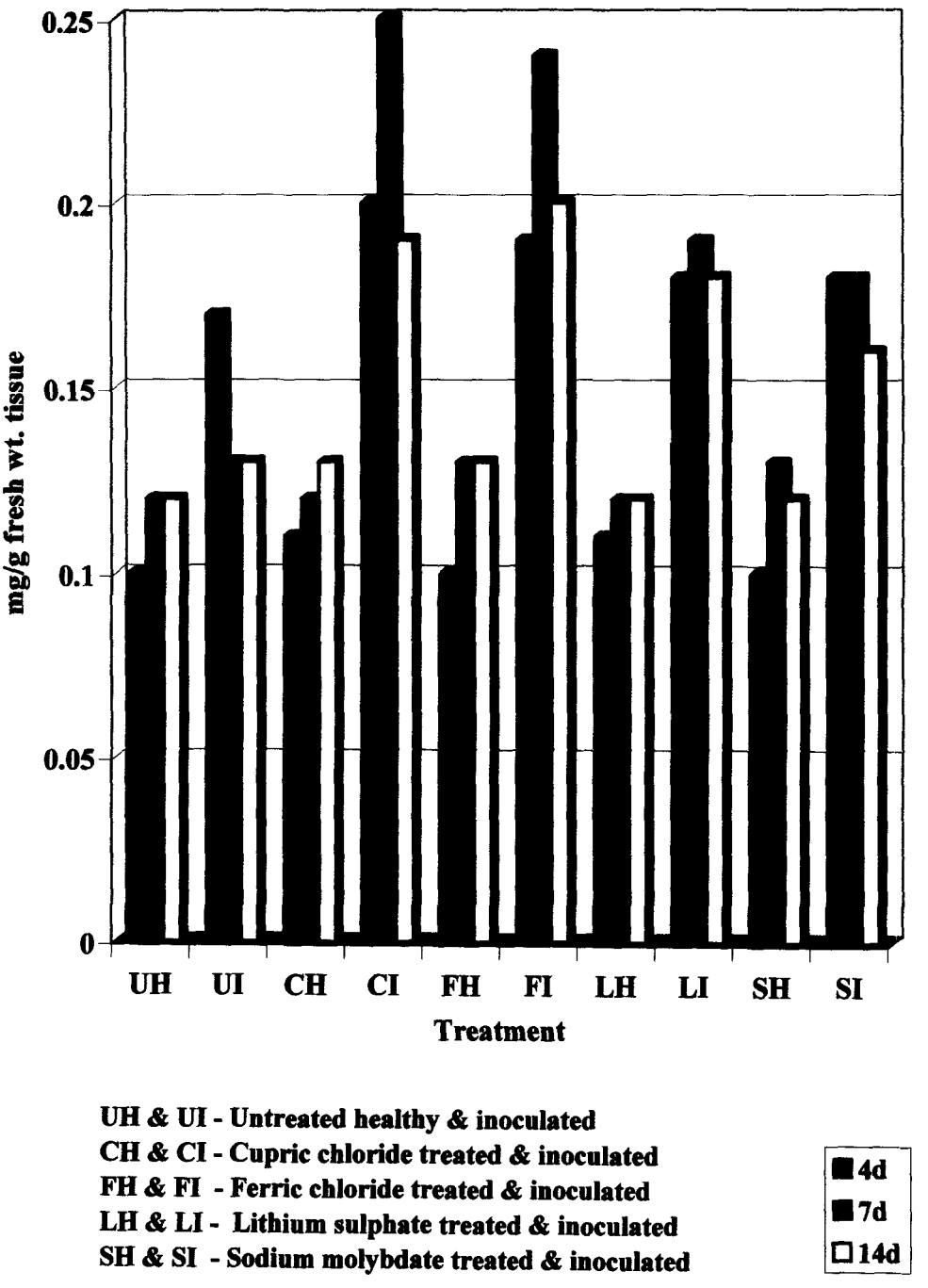


Fig. 7

4.4.3. Polyphenol oxidase

Treated susceptible plants invariably recorded mild to moderate (14 –28%) increase in the level of polyphenol oxidase activity than the untreated plants at different stages of sampling (Table 14). Following inoculation, polyphenol oxidase activity appreciably increased (28%) in susceptible plants after 4 days and further increased to attained 57% higher levels during the next 3 days. However, this effect sharply declined during the last 7 days, to much lower values, only 14 % higher than that in the untreated non-infected plants. At every stage of sampling, susceptible plants in 3 effective treatments responded to inoculation with greater increases of enzyme activity, as much as 112% to 157% after 4 days, 125% to 171% after 7 days and 66% to 100% after 14 days. Their post infection levels were also much higher than in the untreated plants, the differences varying between and 100% after 4, 63 and 72% after 7 and 87 and 112% after 14 days of inoculation. Barring the treatments with sodium molybadate and lithium sulphate which had the lesser protective effect and elicited much reduced responses, the other treatments with substantial protective effect elicited strong responses in all respect. The plants treated with cupric chloride, the most effective compound, elicited the maximum increases in enzyme activity following inoculation (Fig.8)

Treatment with compounds in susceptible soybean plants had no significant increase in the level of polyphenol activity than the untreated plant at different stages of sampling (Table 15). Following inoculation polyphenol oxidase activity appreciably increased (80%) in susceptible plants and further increased to attain 100% in higher levels during the next three days .With time the response become weaker so that after 14 days moderately higher (50 %) levels were recorded . Plants in two highly effective treatments also responded to inoculation with greater than normal increases and this effect persisted till the end with slightly reduced responces , their final post infection levels of polyphenol oxidase activity being always significantly higher than that in untreated plants .

Table 14 : Effect of seed treatment with metal salts and / or inoculation with *Sclerotium rolfsii* in soybean plants (cv. Macs – 58) on polyphenol oxidase activity, recorded at 4,7 and 14 days interval after inoculation.

Treatment	Δ O.D. / 10 mg tissue/min [a]					
	4 days		7days		14 days	
	H	I	H	I	H	I
Water (Control)	0.07	0.09	0.07	0.11	0.07	0.08
Cupric chloride	0.07	0.18	0.08	0.19	0.08	0.16
Ferric chloride	0.08	0.17	0.08	0.18	0.09	0.15
Lithium sulphate	0.08	0.13	0.08	0.13	0.08	0.14
Manganese sulphate	0.07	0.17	0.07	0.19	0.09	0.17
Sodium molybdate	0.08	0.10	0.08	0.12	0.07	0.09

C.D. at 5%

Days X Treatment = N.S.

Days X Inoculation = 0.030

Treatment X Inoculation = 0.04

[a] =Mean of three replications, expressed as the change in optical density / 0.05ml of extract after 30minutes.

H = Healthy

Ino = Inoculated.

NS = Not significant.

Table 15 : Effect of seed treatment with growth regulators and chitosan and / or inoculation with *S.rolfsii* in soybean plants (cv. Macs–58) on polyphenoloxidase activity, recorded at 4,7 and 14 days after inoculation

Treatment	Δ O.D. / 10 mg tissue/min [a]					
	4 days		7 days		14 days	
	H	I	H	I	H	I
Water (control)	0.05	0.09	0.06	0.12	0.06	0.09
1.A.A.	0.07	0.17	0.06	0.21	0.07	0.13
2,4 – D	0.05	0.15	0.07	0.16	0.07	0.10
2,4,5 – T	0.04	0.09	0.06	0.15	0.07	0.09
Chitosan	0.06	0.20	0.07	0.20	0.06	0.14

C.D. at 5%

Days x Treatment = N S

Days x Inoculation = 0.028

Treatment x Inoculation = 0.05

[a] = Mean of three replications and expressed as the changes in optical density / 0.05ml of extract after 30 minutes.

H = Healthy ; I= Inoculated ; NS= Not significant

Polyphenol oxidase activity in soybean roots after induction of resistance

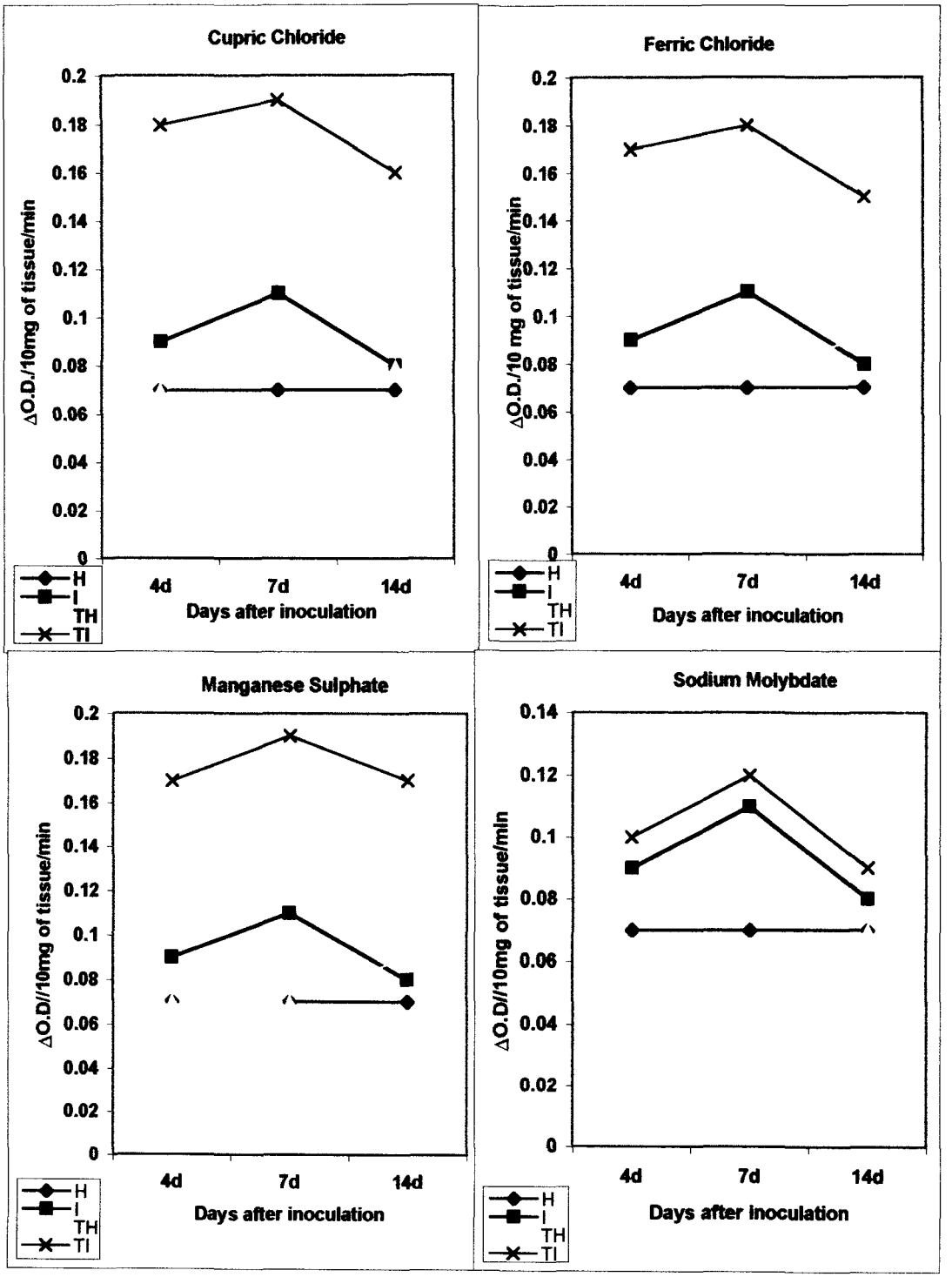


Fig.8

4.4.4. Peroxidase

It is evident from Table 16, Fig 9 that treated plants always had higher (3 – 12%) levels of peroxidase activity than the untreated plants at different stages of sampling. The susceptible plants responded to inoculation with considerable increases (66–80%) in enzyme activity between 4 and 7 days after inoculation, but during the next 7 days the stimulated activity sharply declined to only 32% increases. The plants in three effective treatments responded to inoculation with pronounced increases, 114% to 163% after 4 days and 167% to 207% after 7 days. Though this treatment induced effect declined with time still after 14 days, i.e. at the late stage of infection 81% to 110% higher levels could be noticed. Though all of the treatments recorded higher post infection levels of peroxidase activity, the three more effective treatments recorded much higher levels than the lesser effective treatments like lithium sulphate and sodium molybdate. Though maximum effect of treatments mostly occurred between 4 and 7 days of inoculation, generally the peak period of pathogenic activity, still significant effects persisted even at the late stage of infection.

Treated susceptible plants in two effective treatments viz. IAA and chitosan always had higher (2–15 %) levels of peroxidase activity than the untreated plants at different stages of sampling . The minimum increase always recorded with the least effective 2,4,5 – T treatment. The susceptible plants responded to inoculation with considerable increases (74 – 111 %) in enzyme activity between 4 and 7 days after inoculation , but during the next 7 day the stimulated activity sharply declined recording only 35% higher level of enzyme activity . The plants treated with IAA and chitosan, responded to inoculation with pronounced increases, 187% to 195% after 4 days and 205% to 218% after 7 days and 119% to 186% at the late stage of infection. Though maximum effects of treatments mostly occur between 4 and 7 days of inoculation, generally the pick period for pathogenic activity, still significant effects persist at the late stage of infection (Table 17).

Table 16 : Effect of seed treatment with metal salts and / or inoculation with *S.rolfsii* in soybean plants (cv. Macs – 58) on peroxidase activity recorded 4,7 and 14 days after inoculation

Treatment	Δ O.D. / g tissue/min X 100 [a]					
	4 days		7days		14 days	
	H	I	H	I	H	I
Water (Control)	12.8	21.3	13.0	23.5	13.5	17.9
Cupric chloride	13.5	35.6	13.5	41.5	13.0	27.3
Ferric chloride	14.0	30.0	14.3	38.3	14.6	26.5
Lithium sulphate	13.3	29.8	13.3	28.5	13.0	22.2
Manganese sulphate	14.3	38.8	14.6	41.5	14.6	30.5
Sodium molybdate	13.5	23.8	14.0	25.0	14.0	18.5

C.D. at 5%

Days X Treatment = 1.02

Days X Inoculation = 1.47

Treatment X Inoculation = 2.14

[a] = Mean of three replications; expressed as a change in the absorption by 0.01 per minute as a unit of activity.

H = Healthy, I = Inoculated

Table 17: Effect of seed treatment with growth regulators and chitosan and / or inoculation with *S.rolfsii* in soybean plants (cv. Macs – 58) on peroxidase activity, recorded 4,7 and 14 days after inoculation

Treatment	Δ O.D. / g tissue/min X 100 [a]					
	4 days		7 days		14 days	
	H	I	H	I	H	I
Water (control)	11.5	24.3	12.8	22.3	13.5	18.3
1.A.A.	12.8	37.8	13.5	43.0	13.8	39.5
2,4 – D	11.8	30.0	13.0	31.0	13.0	22.0
2,4,5 – T	11.8	29.8	13.0	24.8	13.5	20.3
Chitosan	13.3	38.3	13.5	41.3	13.8	30.3

C.D. at 5%

Days x Treatment = 1.04

Days x Inoculation = 1.85

Treatment x Inoculation = 3.54

[a] = Mean of three replicarions, expressed as change in the absorption by 0.01 per minute as a unit of activity

H = Healthy

I = Inoculated

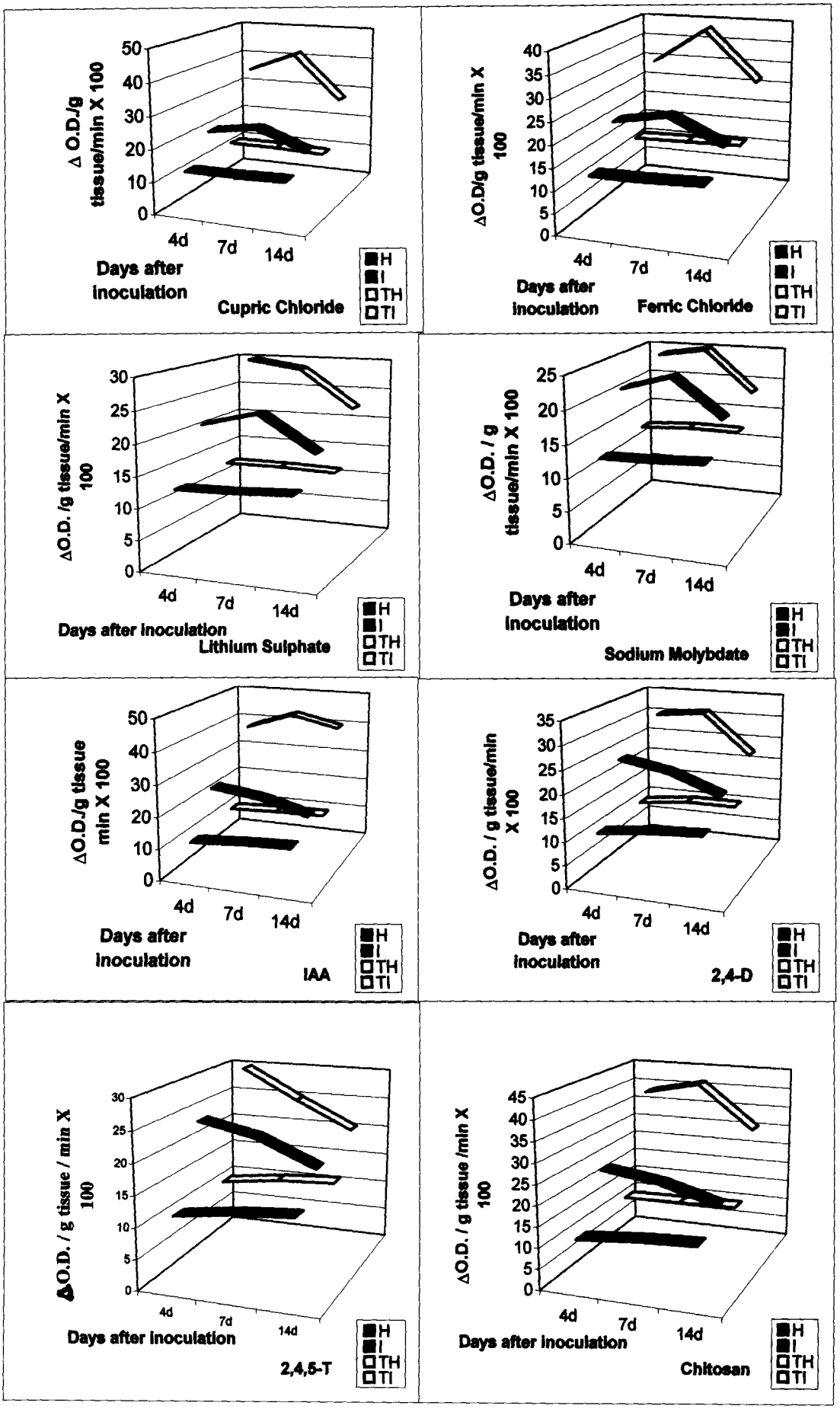

Δ O.D./g tissue/min X 100
Days after inoculation
4d
7d
14d
Cupric Chloride
H
I
TH
TI
ΔO.D/g tissue/min X 100
Ferric Chloride
ΔO.D. /g tissue/min X 100
Lithium Sulphate
ΔO.D. / g tissue/min X 100
Sodium Molybdate
ΔO.D./g tissue min X 100
IAA
ΔO.D. / g tissue/min X 100
2,4-D
ΔO.D. / g tissue / min X 100
2,4,5-T
ΔO.D. / g tissue /min X 100
Chitosan

Fig. 9

4.4.5. Pectolytic enzyme

Since rotting of stem tissue in *S. rolfsii* infected plants is known to result from pectolytic enzyme activity of fungal origin, if there had been any significant change in such enzyme activity following various treatments was investigated . Both polygalacturonase and pectin methyl galacturonase activity in the infected tissue were estimated on the basis of D – galacturonic acid released from their respective pectic substrates. Trends of changes in pectolytic enzyme activity in the infected plants in different teatments appeared to be nearly similar. Among the five different treatments, strong reduction (26 – 40%) in PG and PMG activities were recorded with cupric chloride, ferric chloride and manganese sulphate as compared to that in control plants at 7 days after inoculation, when the first sampling was done (Table 18). Later, the enzyme activities were declined with time both in control and treated plants but still the final levels in effective treatments were lower (21 – 30%) as compared to that in the untreated plants . The lesser effective lithium sulphate and sodium molybdate could reduce pectolytic enzyme activities to a small extent only. A good correlation was found between the supression of pectolytic enzyme activity and lowering of disease index in different treatments.

There was high pectolytic enzyme activity in *Sclerotium* infected tissue of untreated soybean plants 7days after inoculation. At this stage, infected tissue from effective treatments like IAA and chitosan show much reduced (25-36%) enzyme activity (Table 19). During the next seven days, the enzyme activity becomes less pronounced in the control plants as well as in other treatments. However, at this stage, the more effective treatments had 18-23% less activity than the control. Chitosan at 0.5% and 2,4,5-T at 10^{-4}M, the treatments exercising the most and least protective effect against *Sclerotium* infection, as well as suppression of pectolytic enzyme activity respectively (Fig.10)

4.4.6. Oxalic acid

As it is known that oxalic acid content may play a contributory factor in induced resistance by lowering the pH of the cell wall which is favourable for the cell wall degrading enzymes (mainly polygalacturonase type) to hydrolyze the pectates (Faboya *et.al*, 1983), its contents were estimated both in the treated and untreated infected plants. There is no significant difference in oxalic acid content of untreated

and treated healthy plants. The infection resulted significantly higher increase in the oxalic acid content in untreated than the treated plants and the final levels in the effective treatments viz., $CuCl_2$, $FeCl_3$ and $MnSO_4$ had (39 – 60 %) less after 7 days and 47% to 57% less after 14 days of inoculation as compared to that of the untreated infected plants (Table 20) Soybean plants treated with IAA or chitosan has no significant (P = 0.05) effect on changes in oxalic acid content after inoculation with *Sclerotium rolfsii* (Table 21)

Table 18: Effect of seed treatment on pectolytic enzyme activity in disease affected soybean stem tissue in various treatments, 7 and 14 days after inoculation.

Treatment	Amount of D-galacturonic acid released (mg /g fresh weight of tissue/hr)[a]			
	7days		14 days	
	Pectin	Sodium polypectate	Pectin	Sodium polypectate
Water (Control)	1.42	1.47	0.75	0.76
Cupric chloride	0.85	0.89	0.52	0.55
Ferric chloride	0.95	0.93	0.58	0.55
Lithium sulphate	1.25	1.35	0.67	0.69
Manganese sulphate	1.05	0.92	0.59	0.58
Sodium molybdate	1.40	1.43	0.75	0.73
C.D. at 5%	0.21	0.25	0.18	0.17

[a] Mean of three replictions and expressed as D- gulacturonic acid equivalent.

Table 19 : Effect of seed treatment on pectolytic enzyme activity in disease affected soybean stem tissue in various treatments , 7 and 14 days after inoculation .

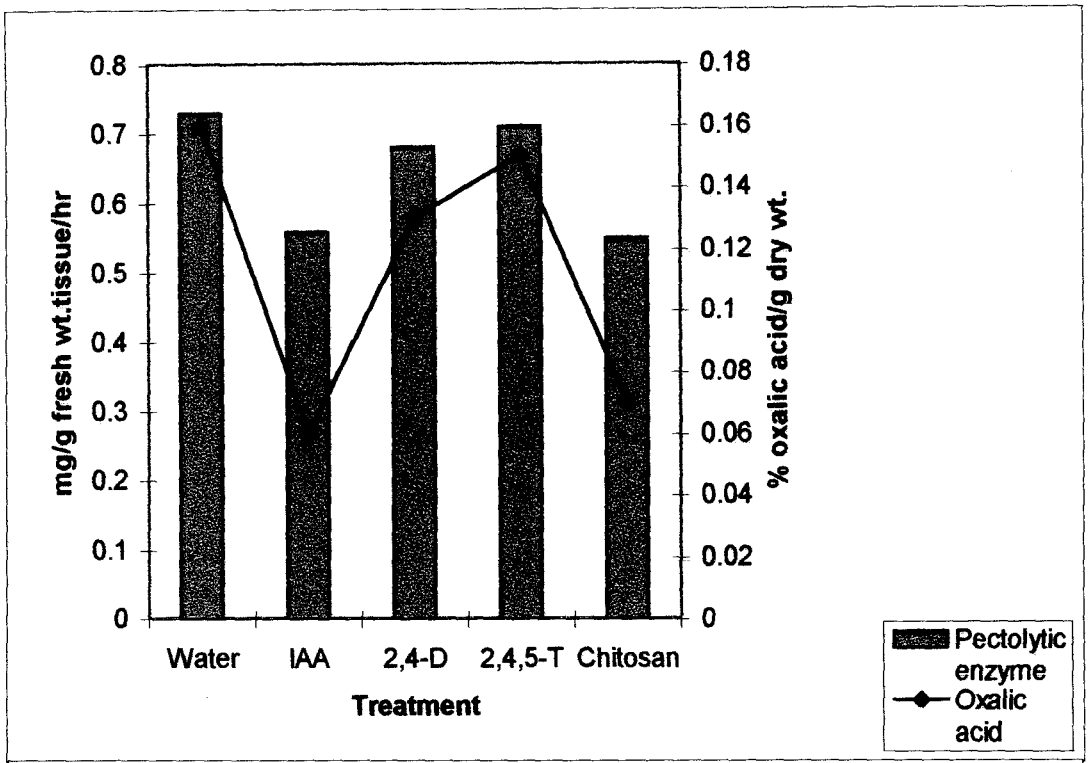
Treatment	Amount of D-galacturonic acid released (mg/ g fresh weight of tissue / hr)[a]			
	7 days		14 days	
	Pectin	Sodium polypectate	Pectin	Sodium polypectate
Water (control)	1.37	1.43	0.73	0.71
I.A.A.	0.89	0.91	0.56	0.58
2,4 – D	1.28	1.35	0.68	0.67
2,4,5 – T	1.30	1.40	0.71	0.68
Chitosan	1.02	0.92	0.55	0.55
C.D. at 5%	0.18	0.23	0.08	0.06

[a] Mean of three replications and expressed as D – galacturonic acid equivalent.

4.4.7. Calcium and magnesium content

It has been demonstrated that the pectolytic enzyme activity was significantly less in the infected stem tissue of the treated plants than in the control plants, and this was correlated with reduced rotting symptoms in the former. Both limited tissue rotting and reduced enzyme activity, have often been linked with greater accumulation of Ca^{++} and Mg^{++} ions at and around the infection side and conversion of pectic compounds to calcium and magnesium pectates that are less amenable to enzyme degredation (Reddy *et al.*, 1988) keeping this possibility in mind the amount of Ca^{++} and Mg^{++} ions in the stem tissue of soybean around the infection site was estimated following the methods describe earlier.

Pectolytic enzyme activity and oxalic acid concentration in soybean roots after induction of resistance



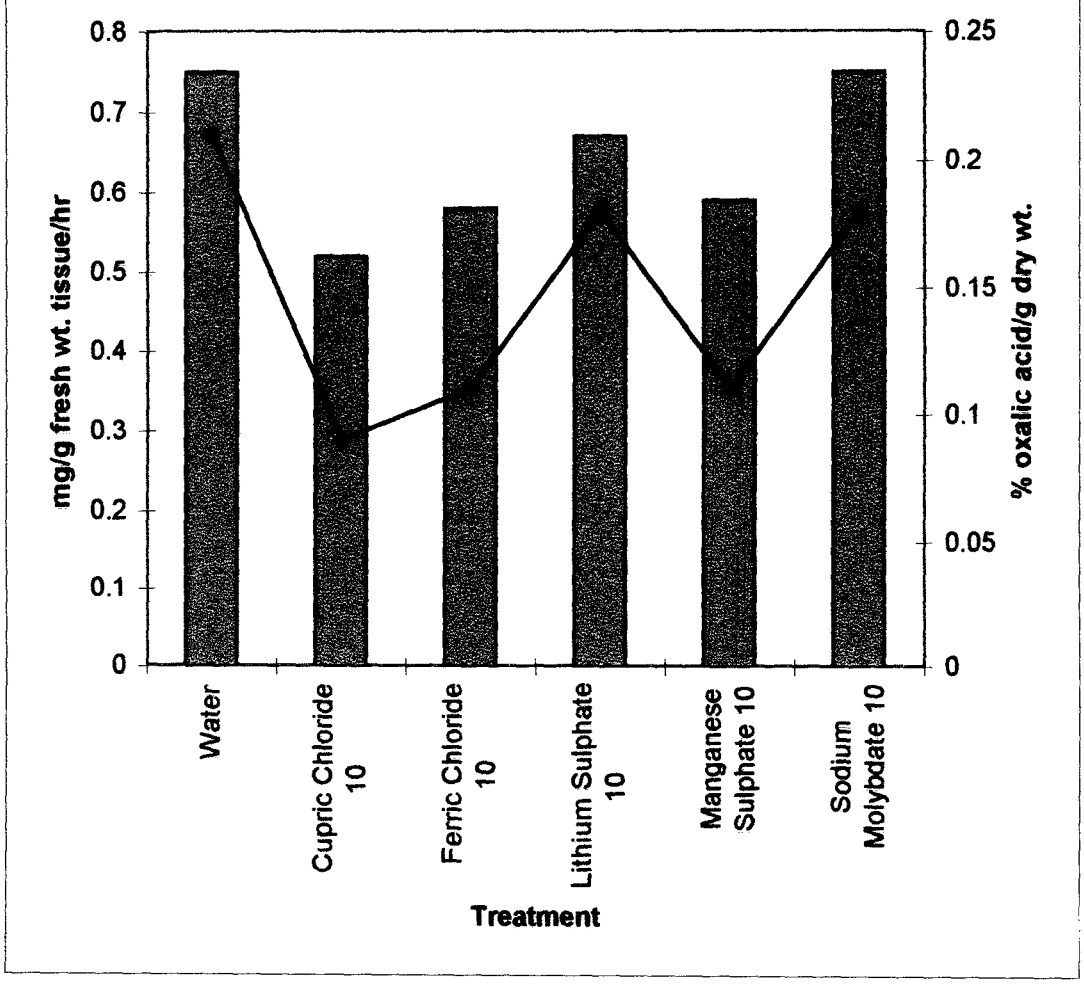


Fig.10

Table 20: Effect of seed treatment with metalic salts on oxalic acid concentration in soybean plants inoculated with *S.rolfsii*, 7 and 14 days after inoculation.

Treatment	% oxalic acid /g dry weight basis			
	7days		14 days	
	H	I	H	I
Water (Control)	0.03	0.28	0.03	0.21
Cupric chloride	0.03	0.11	0.04	0.09
Ferric chloride	0.04	0.17	0.04	0.11
Lithium sulphate	0.04	0.21	0.03	0.18
Manganese sulphate	0.03	0.12	0.04	0.11
Sodium molybdate	0.03	0.27	0.03	0.18
C.D. at 5%	NS	0.07	NS	0.05

[a]=Mean of three replictions .
H=Healthy ; I= Inoculated
NS = Not significant .

It appears from Table 22 that various treatments as such had little or no effect on the calcium content of the stem tissue. Infection resulted in small (4%) increase in the untreated plants but those in different effective like $CuCl_2$, $FeCl_3$ and $MnSo_4$ treatments responded with greater (14 –18 %) increases in calcium content. The final post–infection calcium content in those treated plants were also appreaciably higher (7 – 12%) than that in untreated plants. The metal salts like Li_2 So_4 and Na_2 MoO_4 had no or less effect on calcium content. The trend was almost similar in respect of magnesium content also. Following inoculation the magnesium level increased by 6% over that in noninfected untreated plants but the treated plants in effective treatments recorded 8% to 14% increase in magnesium level in response to inoculation. The final post infectional level of magnesium in these treated plants were also marginally (3 –7 %) higher. In respect of both calcium and magnesium accumulation, the maximum effect was achieved with ferric chloride.

Table 21 : Effect of seed treatment with growth regulators and chitosan on oxalic acid concentration in soybean plants inoculated with *S.rolfsii* 7 and 14 days after inoculation .

Treatment	% oxalic acid/ g dry weight basis [a]			
	7 days		14 days	
	H	I	H	I
Water (control)	0.04	0.22	0.04	0.16
I.A.A.	0.04	0.09	0.03	0.06
2.4 – D	0.03	0.16	0.04	0.13
2,4,5 – T	0.04	0.18	0.03	0.15
Chitosan	0.04	0.09	0.04	0.07
C.D. at 5%	NS	0.06	NS	0.04

[a] = Mean of three replicarions

H = Healthy

Ino = Inoculated

NS = Not significant

The concentration of calcium and magnesium in soybean plants as influenced by seed treatments with IAA, 2,4-D, 2,4,5-T and chitosan are presented in Table 23. Following inoculation, the untreated plants showed 4% increase in calcium content, where as the two effective treatments (IAA and chitosan) caused greater (23 – 25 %) increase. The final post infectional level of calcium in such treated plants were also appreciably (22 – 21 %) higher than that in the untreated infected plants. The trend was almost similar in respect of magnesium content also. In the untreated plants following inoculation, resulted in marginal increase (7%) but plants in two effective treatments showed appreciably higher (15 – 20 %) increase in magnesium content of stem tissue. Both in respect of calcium and magnesium content, the maximum increase was recorded with IAA, the most effective compound in protecting symptom expressing.

Table 22 : Effect of seed treatment with metal salts on calcium and magnesium content in soybean plants inoculated with *S.rolfsii*, recorded 14 days after inoculation.

Treatment	Concentration of calcium and magnesium (on g^{-1} dry weight basis) [a]			
	% of Ca		% of Mg	
	H	I	H	I
Water (Control)	1.21	1.27	0.49	0.52
Cupric chloride	1.22	1.42	0.48	0.55
Ferric chloride	1.21	1.43	0.49	0.56
Lithium sulphate	1.20	1.29	0.50	0.51
Manganese sulphate	1.20	1.37	0.49	0.54
Sodium molybdate	1.22	1.28	0.51	0.52
C.D. at 5%	NS	0.66	NS	0.02

[a]= Mean of three replications ; H= Healthy ; I = Inoculated

4.4.8. Phenylalanine ammonia lyase activity:

It has been clearly established that in the early experiments that wet seed treatment with some metal salts can induce strong protective effects in soybean plants against *Sclerotium rolfsii* infection. Such induced effects are mostly correlated with an increased biosynthesis of phenolics and stimulated oxidase activity at and around the site of host – pathogen interaction. It is well that phenylalanine ammonia lyase (PAL) is the first enzyme of the phenyl propanoid pathway and considered as the key enzyme in the regulation of the flux of the phenyl propanoid compounds such as lignin and their derivatives (Camm and Towers , 1973) and also appeared to be associated with hypersensitive reaction (Novacky and Acedo , 1970) . The PAL activity of soybean plants in different treatments and /or inoculation with *S. rolfsii* was estimated following the method desccribed earlier and the results are presented in Table 24 – 25, and Fig.11

Table 23 :Effect of seed treatment with growth regulators and chitosan on calcium and magnesium content in soybean plants inoculated with *S.rolfsii*, recorded 14 days after inoculation

Treatment	Concentration of calcium and magnesium (on g^{-1} dry weight basis)[a]			
	% of Ca		% of Mg	
	H	I	H	I
Water (control)	1.18	1.23	0.53	0.59
I.A.A.	1.19	1.49	0.53	0.64
2,4 – D	1.19	1.25	0.52	0.59
2,4,5 – T	1.18	1.24	0.53	0.58
Chitosan	1.20	1.48	0.53	0.61
C.D. at 5%	NS	0.45	NS	0.55

[a] = Mean of three replications ; H = Healthy ; I = Inoculated ; NS = Not significant

Treated susceptible plants recorded only very marginal increases in PAL activity over that in the untreated plants at different stages of sampling. The PAL activity in untreated plants following infection had mild increase (16%) after 7 days and the stimulatory effect weakened in them with time and become marginal during the later stage of infection, recording only 6.0% higher level after 14 days of inoculation .The plants in effective treatments responded to inoculation with greater increases in enzyme activity, as much as 73% to 86% after 7 days and 34% to 46% after 14 days. Their post infection were also much higher than in the untreated plants, the differences varying between 49 and 64% after 7 days and 31 and 44% after 14 days. The plants treated with cupric chloride, the most effective treatment, elicited the maximum increases in enzyme activity following inoculation and also led to the highest post-infection level.

Soybean plants treated with growth regulators and chitosan as such had little or no effect on the phenylalanine ammonia lyase activity at different stages of sampling, higher levels were always recorded with IAA and chitosan , the two most effective compounds (Table 25). Following infection the untreated plants had very marginal increases (7–14 %) in enzyme activity but susceptible plants in highly effective treatments recorded moderately higher (95%) increases but the quantum of increase became less pronounced thereafter and varied between 39% to 40% at the late stage . The final post infection levels in these treatments were always much higher than the untreated plants .

Table 24 : Effect of seed treatment with metal salts on phenyalalanine ammonialyase activity in soybean plants (cv. Macs-58) , inoculated with *S.rolfsii.*

Treatment	Phenylalanine ammonia-lyase activity (µg cinnamic acid released / g tissue / min .)[a]			
	7days		14 days	
	H	I	H	I
Water (Control)	92.5	108.0	90.0	95.5
Cupric chloride	95.5	178.0	94.0	138.0
Ferric chloride	93.0	161.0	93.5	125.5
Lithium sulphate	92.0	111.5	91.0	98.5
Manganese sulphate	94.0	169.0	93.5	137.0
Sodium molybdate	93.0	112.0	92.5	98.0

C.D. at 5%

Days X Treatment = N.S.

Days X Inoculation = 15.35

Treatment X Inoculation = 12.85

[a]=Mean of three replications ; H= Healthy, I = Inoculated; NS= Not significant .

PAL activity in soybean roots after induction of resistance

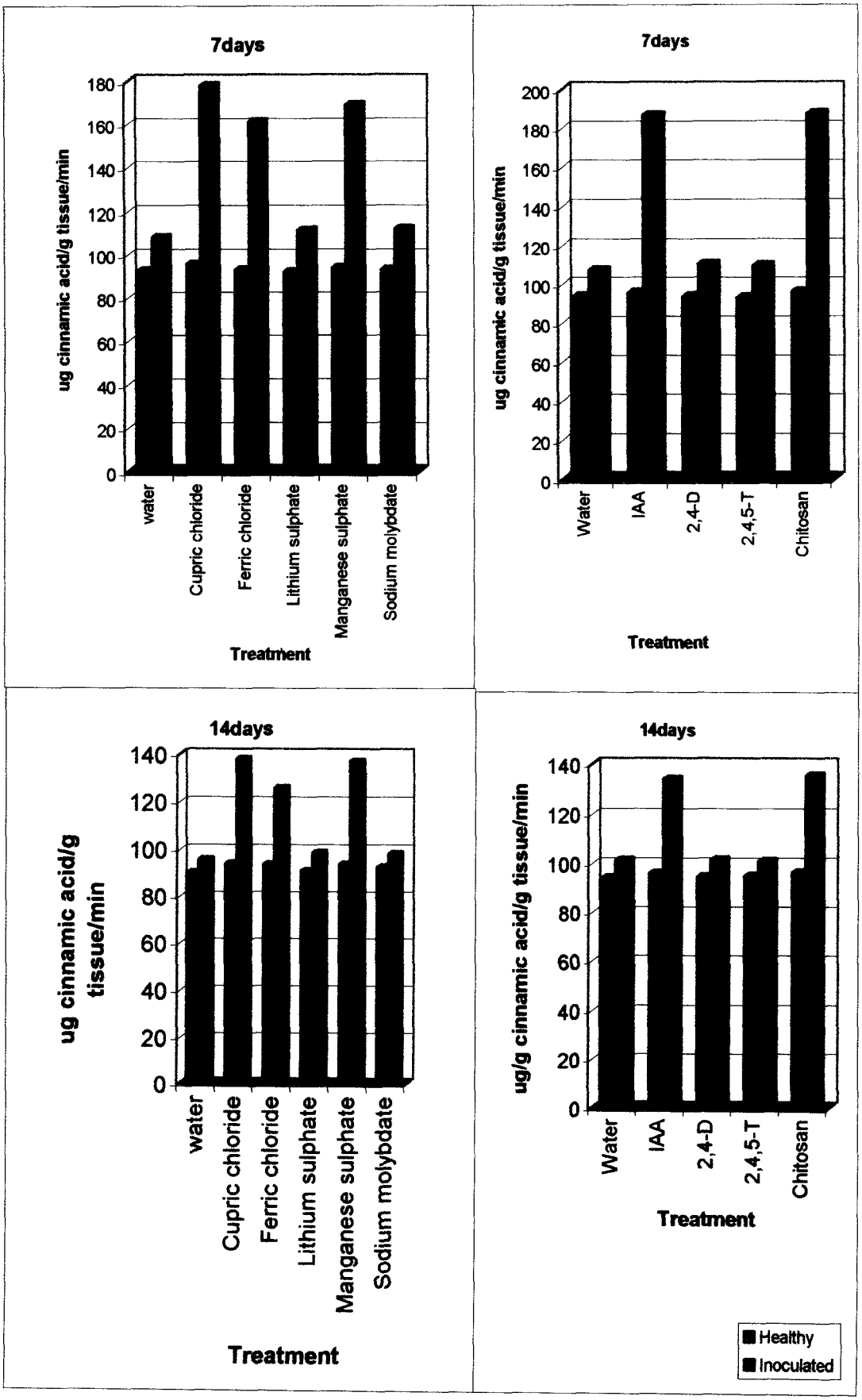


Fig.11

Table 25: Effect of seed treatment with growth regulators and chitosan and / or inoculation with *S.rolfsii* in soyben plants (cv. Macs – 58) on phenylalanine ammonia lyase activity, recorded 4,7 and 14 days after inoculation .

Treatment	Phenylalanine ammonia lyase activity (µg cinnamic acid released / g tissue/ min)[a]			
	7 days		14 days	
	H	I	H	I
Water (control)	93.5	107.0	94.0	101.3
I.A.A.	95.5	186.5	96.0	134.0
2,4 – D	93.5	110.3	94.5	101.5
2,4,5 – T	93.0	109.5	95.0	101.0
Chitosan	96.0	187.3	96.5	135.5

C.D. at 5% Days x Treatment = 1.16
Days x Inoculation = 12.56
Treatment x Inoculation = 15.42

[a] = Mean of three replications ; H = Healthy , I = Inoculated

4.5. Effect of seed treatment on lignification in soybean plants

Results described earlier indicated an association between resistance induced in soybean plants against *S. rolfsii* by chemical treatment and increased biosynthesis of phenolics and greater activity of peroxidase. It is well known that peroxidase is the terminal enzyme in phenyl-propanoid pathway and increased peroxidase activity is presumed to be associated with enhanced lignification, an important mechanism for limiting the spread of the pathogen. This possibility was explored in soybean plants using three highly effective treatments like cupric chloride(10^{-3} M), ferric chloride (10^{-3} M) and chitosan(0.5%). To quantitate lignin, the ionization difference spectra was examined from 230 to 380nm in a Beckman UV-spectrophotometer following the method as described earlier. The extracts of soybean

stem gave two major peaks at approximately 247nm due to simple phenolic groups and at 351nm, presumably due to phenols with conjugated side chains such as lignin. The absorbance difference at 351nm and 247nm of dialysed extracts of soybean stem at 4 days and 7 days after inoculation with *S.rolfsii* are presented in Table 26. The trend was nearly the same for the simple phenolics recorded at 247nm. Following inoculation, the untreated plants showed an increase in the absorbance (43-68%) but the final post-infection levels were higher in the treated plants than in the untreated plants, 73% to 78% after 4 days and 66% to 77% after 7 days, though the differences among the treatments were not significant. Here, plants treated with cupric chloride showed maximum increases in the absorbance.

More conclusive evidence for lignification was provided by estimating phenolic aldehydes resulting from oxidation with alkaline nitrobenzene (Heartley, 1973). Stem material was pre-extracted with organic solvents and cold alkali to remove free and esterified low molecular weight phenols and the residue containing the lignin 'core' was analysed. A significant (P=0.05) increase in all three characteristic aldehydes (vanillin, syringaldehyde and p-hydroxybenzaldehyde) was observed 7 days after inoculation with cupric chloride, ferric chloride and chitosan, but not after inoculating alone (Table 27). The results also indicated that the three treatments induced essentialy the same type of polymer, since lignin induced by the three compounds yielded similar relative proportions of aldehydes.

Table 26: Induced lignification in dialysed extracts of soybean plants in different treatments .

Treatment	ΔE at 351 nm		ΔE at 247 nm	
	4 days	7days	4 days	7days
Healthy, Uninoculated	0.23	0.24	0.16	0.16
Control [a]	0.29	0.35	0.23	0.27
Cupric chloride: Inoculated	0.47	0.58	0.41	0.48
Ferric chloride : Inoculated	0.44	0.56	0.40	0.46
Chitosan : Inoculated	0.48	0.55	0.41	0.45
C.D. (P = 0.05)	0.04	0.06	0.03	0.07

[a] = Inoculated and treated with water

Table 27: Yield of alkaline nitrobenzene oxidation products from soybean plants inoculated with *S.rolfsii*, harvested 7 days after inoculation.

Treatment	Yield [a]		
	Vanillin	Syringaldehyde	p-Hydroxy benzaldelyde
Healthy, Uninoculated	4.64	0.44	0.61
Contral [b]	5.29	0.56	1.03
Cupric chloride: Inoculated	9.58	3.26	6.05
Ferric chloride : Inoculated	8.28	2.65	5.77
Chitosan : Inoculated	9.25	3.20	5.80
C.D. (P = 0.05)	1.10	0.87	2.17

[a] =μ mol g^{-1} alkali extracted, mean of three samples.

[b] = Inoculated and treated with water

4.6 Accumulation of glyceollin in soybean plants before and after alteration of disease reaction by selected chemicals

In order to alter disease reactions in one of the highly susceptible soybean variety (Macs-58), a series of experiments were performed and finally on the basis of the results obtained with special reference to biochemical chages associated with induction of resistance , two chemicals viz. cupric chloride and ferric chloride were selected for further study. It was decided to investigate whether chemically induced resistance in Macs-58 was related to higher production of glyceollin. Since cupric chloride and ferric chloride markedly reduced disease in susceptible soybean variety, accmulation of glyceollin in this variety before and after alteration of disease reaction by the said chemicals were determined. To study the effect of cupric chloride and ferric chloride on the production of glyceollin, soybean seeds (variety- Macs58) were treated with $CuCl_2$ and $FeCl_2$ (10^{-3}M) as described under Materials and Methods, sown in the earthen pots and susequently the plants (15-old-plants) were treated with

said chemical(s) as foliar spray, uprooted and inoculated with *S.rolfsii* following water culture methods. The glyceollin content in soybean roots (cv. Macs-58) – untreated healthy, untreated and inoculated with *S.rolfsii* , treated with $CuCl_2$ and/or $FeCl_2$ as well as treated and inoculated with *S.rolfsii* were estimated after 24, 48 and 72h of inoculation. Results (Table-28) reveals that glyceollin reached a maximal concentration after 48h of inoculation with *S.rolfsii*. It is significant that $CuCl_2$ and $FeCl_2$ induced glyceollin synthesis in uninoculated soybean plants also (Table 28 & 29). Glyceollin accumulation reached maximum following 72h of treatment alone. In this case cupric chloride and ferric chloride act as abiotic elicitors of glyceoolin in soybean roots.

Table 28: Glyceollin accumulation in soybean roots (cv. Macs-58) at verious times after inoculation with *S.rolfsii* or treatment with non-conventional chemicals.

Time after inoculation or treatment (h)	Glyceollin content (µg/g fresh weight of roots)		
		Treatment	
	Inoculated with *S. rolfsii*	$CuCl_2$ (10^{-3}M)	$FeCl_2$ (10^{-3}M)
24	98 ± 2.2	87 ± 1.8	95 ±2.6
48	189 ± 3.6	12 1 ± 4.6	109 ± 3.3
72	125 ± 4.0	166 ± 3.9	135 ±4.5

Values represent the means ± SE from three experiments with three different preparations.

The glyceoolin content of soybean roots (Macs-58), before and after alteration of disease reactions by cupric chloride and ferric chloride treatments, were estimated and cmparisons were made 48h after inoculation as well as 48h after foliar treatment followed by inoculation. The production of glyceollin was maximum when plants were treated with these abiotic elicitors followed by inoculation with *S.rolfsii* (Table 29). The experiments were repeated three times and the results clearly indicate that the accumulation of glyceollin induced by $CuCl_2$ increases in soybean plants following inoculation with *S.rolfsii* than the $FeCl_2$ treatment.

Table 29: Effect of non-conventional chemicals on the accumulation of glyceollin in soybean roots (cv. Macs-58).

Plant treated with	Glyceollin content (µg/g fresh weight of roots)	
	Uninoculated	**Inoculated with *S. rolfsii***
Water (control)	0	190 ± 3.9
Cupric chloride (10^{-3}M)	144 ± 4.8	485 ± 4.2
Ferric chloride (10^{-3}M)	117 ± 5.2	332 ± 3.8

Glyceollin was extracted from soybean roots 48h after inoculation. Values represent the average of three separate experiments ± SE.

4.7. Analysis of host-parasite protein by SDS-PAGE

Plant immunity towards fungal pathogens may also depend on the speed and extent of protein synthesis induced in the host by the pathogen. In case of compatible combination, however, changes in protein configuration in the host may induce host accessibility to the pathogen, which is related to the induced susceptibility. There is also evidence that alteration in the protein sysnthesis in the plants can lead to the development of local resistance for immune layer around infection sites.

In view of these findings, attempts have been made to analyse the protein patterns of root , leaf as well as collar regions of soybean plants following infection alongwith the protein pattern of the fungal pathogen. Total soluble proteins were extracted from (a) roots of healthy and artificially inoculated soybean plants (PK-262, Bragg, NRC-7, PUSA-16, J-80 and Macs-58); (b) different parts – leaf, root as collar regions of healthy and artificially inoculated soybean plants (Macs-58)- 14 days after inoculation; (c) roots of untreated healthy control plants (Macs-58), inoculated with *S.rofsii*, treated with Chitosan (0.3%), IAA (10^{-4}M), $CuCl_2$ ((10^{-3}M), $FeCl_2$ ((10^{-3}M), as well as mycelial proteins of *S.rolfsii* (5-day-old and 12-day-old mycelia) and estimated . Experimental procedure has been described in detail under Materials and Methods. Results are presented in Table 30 and Plate 7 (A& B).

Table 30 : Protein content of soybean roots (Macs-58) treated with chemicals and inoculated with *S.rolfsii*

Treatment	Protein content (mg/g tissue)	
	Uninoculated	Inoculated with *S.rolfsii*
Water control	4.2 ± 0.23	5.4± 0.77
Chitosan (0.3%)	5.3± 0.44	4.0± 0.54
IAA (10^{-4}M)	4.4± 1.12	4.8± 0.50
$CuCl_2$ (10^{-3}M)	5.2± 0.93	4.6± 0.44
$FeCl_3$(10^{-3}M)	4.8± 0.80	4.1± 0.69

Protein content increased in untreated inoculated roots. Protein content in roots of treated plants also increased , however, in treated and inoculated roots protein content decreased. When protein samples of infected root, stem (collar regions) and leaf were compared with healthy control in SDS-PAGE , infected collar region yielded more protein. Soluble mycelial protein from 12-day-old culture of *S.rofsii* yielded maximum number of protein bands than 5-day-old culture (Plate 7 A).

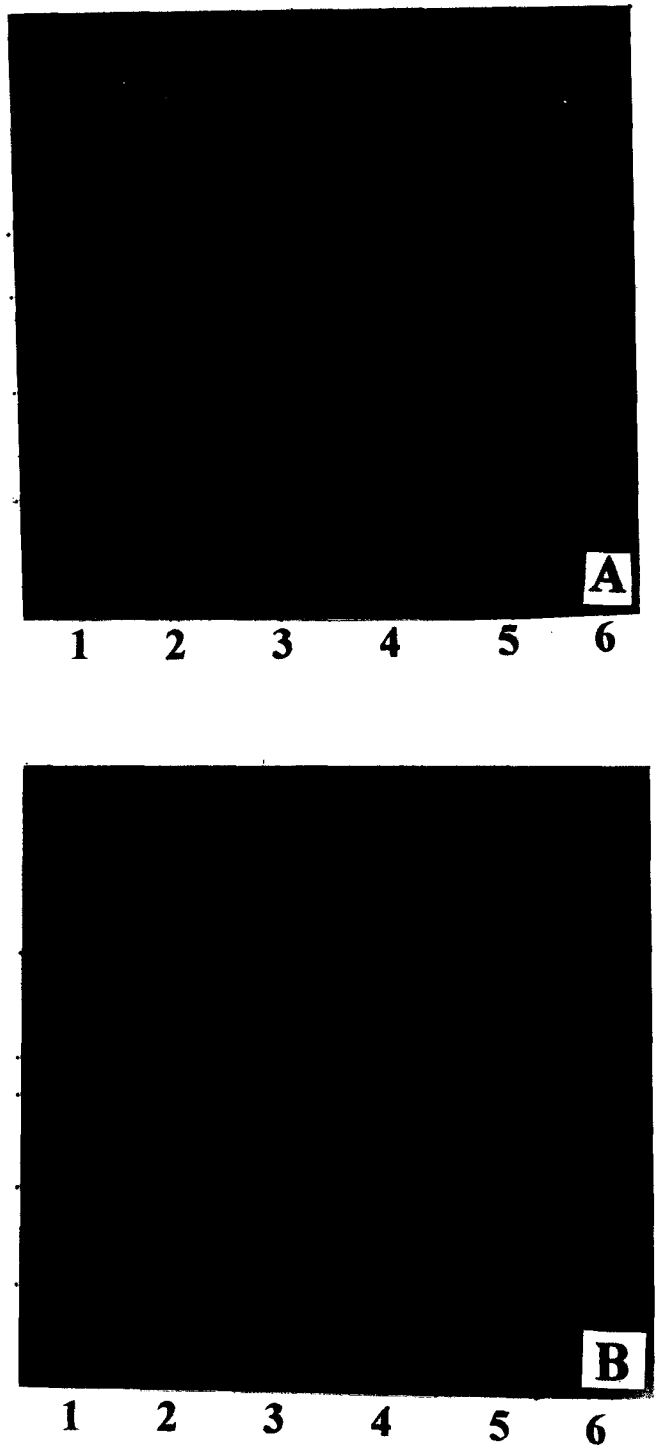


Plate 7 (A & B): SDS-PAGE analysis of soluble proteins
(A) Mycelial proteins of *S.rolfsii*; Lane 1-3: 5-day old;
4-6: 12-day old; (B) Soybean plants Lane 1&2: leaf;
3&4: collar region; 5&6: root
Healthy – 1,3 & 5; Infected – 2,4 & 6

4.8. Detection of cross reactive antigens between *S.rolfsii* and soybean varieties

The presence of cross reactive antigens (CRA)among host and pathogenic organisms is a well documented phenomenon. Existing studies on plant hosts and pathogens suggests that whenever, an intimate and continuing association of host and pathogen occurs, partners of this association have a unique serological resemblance to one or more antigenic determinants. Various methods have been generally used to detect the presence of CRA between host and parasite. In the present investigation, CRA between *S.rolfsii* and soybean varieties have been detected using immunodiffusion, immunoelectrophoresis and enzyme linked immunosorbent assay. Series of experiments performed and results obtained have been presented below.

4.8.1. Immunodiffusion

The effectiveness of antigen preparations from *S.rolfsii* and soybean roots (Macs-58) were checked by homologous cross reactions following agar gel double diffusion tests. Control sets involving normal sera and antigens of soybean roots and pathogen were all negative. Strong precipitin reaction occurred when antiserum raised against mycelia of *S.rolfsii* was reacted with its own antigen (soybean isolate) and the antigens of five more isolates of *S.rolfsii* from cowpea, pea , marigold, tea . (Plate 8 A). When anti *S.rolfsii* antiserum was cross reacted with root antigens prepared from four soybean varieties such as Macs-58, J-80, PK-262 and Bragg , precipitin bands were observed in immunodiffusion tests (Plate 8 C). No such precipitin reactions were observed in case of cross reactions between anti *S.rolfsii* antiserum and antigens preapred from two non pathgens of soybean plants (*Fomes lamaoensis* and *Ustulina zonata*). Reciprocal cross reaction between antiserum raised against soybean root antigen (Macs-58) and antigens of all six isolates of the pathogen (*S.rolfsii*) in agar gel double diffusion test develop one strong precipitin band (Plate 8 B) confirming common antigenic relationships among isolates of the pathogen (*S.rolfsii*) and highly susceptible soybean variety (Macs-58).

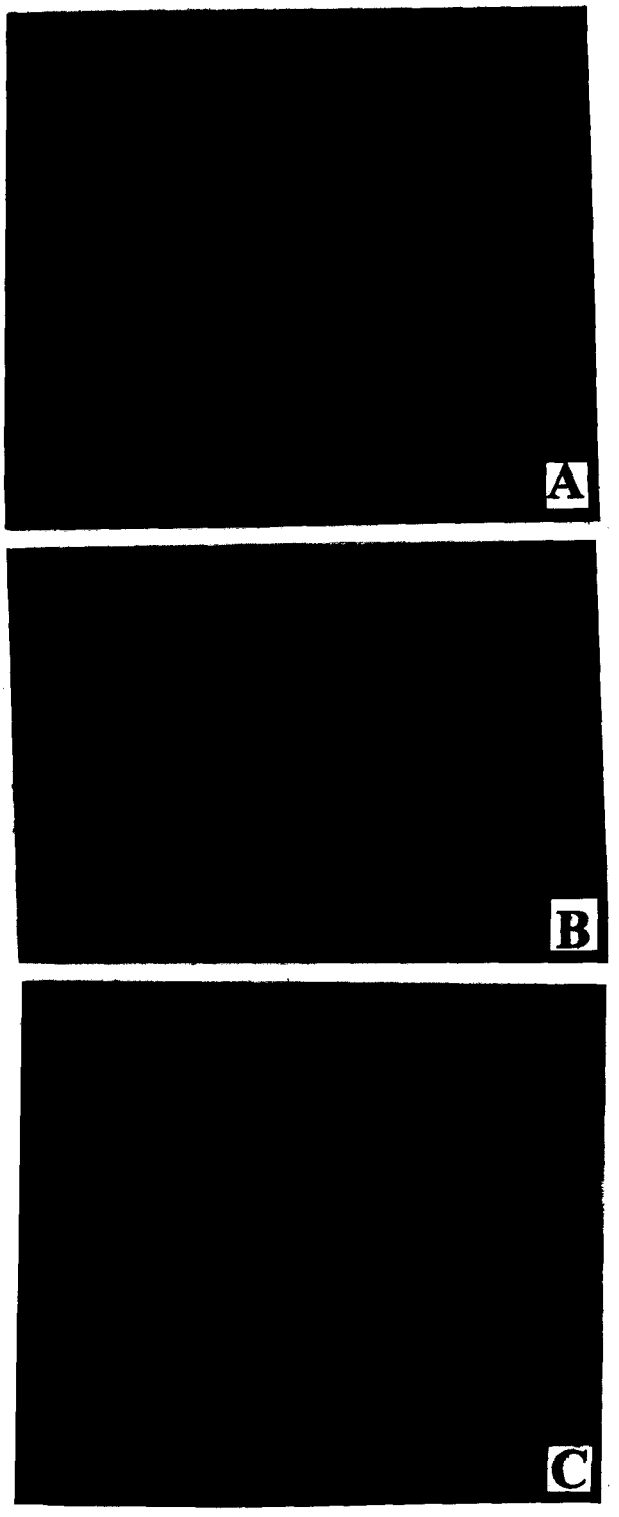


Plate 8 (A-C) : Agar gel double diffusion tests.
Central wells (7) contain antisera of *S.rolfsii* (**A&C**)
and soybean (Macs-58) root (**B**). Peripheral wells (1-6)
contain antigens.**A & B**–*S.rolfsii* isolates (1-6);**C**–*S.rolfsii*(1);
Macs-58 (2,3); J- 80 (4); PK-262 (5); Bragg (6)

4.8.2. Immunoelectrophoresis

The presence or absence of cross reactive antigens between isolates of *S.rolfsii* and soybean varieties were established by immunodiffusion tests. Many of the cross reactions in immunodiffusion tests gave diffused preciptin bands which could not be clearly distinguished. It was not clear, whether the preciptation reaction is due to single or several antigenic substances. In this experiment, antigenic comaprison among six soybean varieties and six isolates of the pathogen (*S.rolfsii*) and two non pathogens of soybean (*Fomes lamaoenis* and *Ustuliza zonata*) using antisera of pathogen (*S.rolfsii*) and host (Macs-58) were done following conventional set up. Results are shown in Plate 9 (A-C) and Table 31.

Table31: Antigenic comparison among soybean varieties, pathogen (*S.rolfsii*) and non pathogens

Antigens of host and parasite	**Total no. of precipitin lines**	
	Antisera of host and parasite	
	Macs-58	***S.rolfsii***
Soybean varieties		
Macs-58	3	2
PK-262	2	1
Bragg	3	1
NRC-7	3	1
J-80	2	2
Pusa 16	2	1
Pathogen (*S. rolfsii*)		
Isolate –1	2	4
Isolate-2	1	2
Isolate-3	2	3
Isolate-4	1	2
Isolate-5	2	2
Isolate-6	1	2
Non-pathogens		
Fomes lamaoensis	0	0
Ustulina zonata	0	0

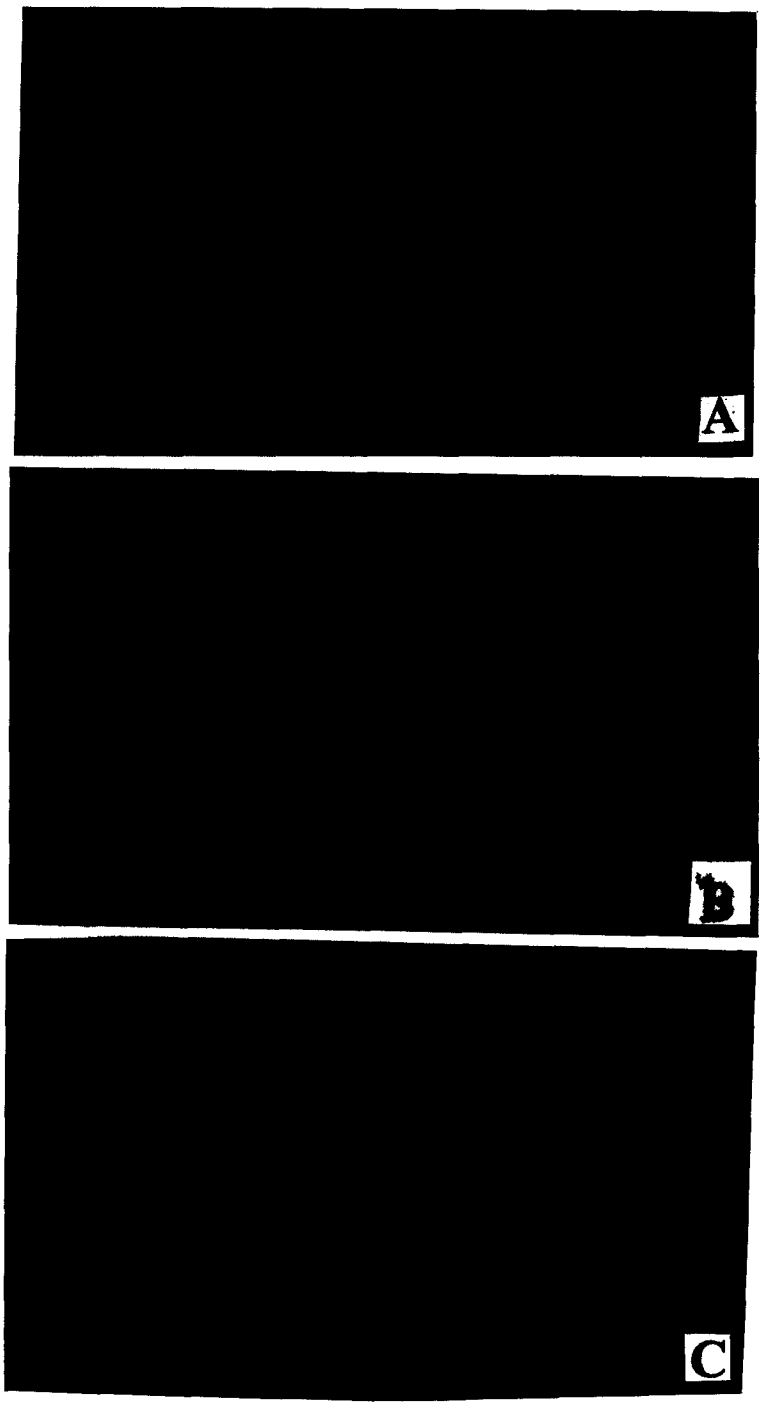


Plate 9 (A-C): Immunoelectrophoretic patterns of the antigens of soybean roots and mycelia of *S.rolfsii*. Central rectangular troughs charged with antiserum of S.rolfsii (**A&B**), soybean root–Macs58 (**C**) and the surrounding wells with antigens of *S.rolfsii* (1 & 2), Macs-58 (3 & 6); *Fomes lamaoensis* (4&5); Macs-58 (6),

4.8.3. Direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA)

The enzyme linked immunsorbent assay is one of the most sensitive serological techniques for detection of CRA between host and pathogen as well as for detection of pathogens in diseased tissue. In indirect or DAC-ELISA, antigens are bound to the microtitre plates after which the antibody is allowed to bind to the antigen. To this antigen antibody complex, the conjugate (an antibdy conjugated to an enzyme) is added. Finally, a non coloured substrate is added which is converted to a coloured end product, which is generally detected by a reader. In the present investigation, DAC ELISA has been used in most of the experiments. Since ELISA depends on a number of factors and these varies from system to system, it was considered essential to optimize the conditions in this particular host-pathogen system.

4.8.3.1. Optimization of ELISA

Optimization of ELISA was done using IgG fraction of antisera raised against mycelial antigens of *S.rolfsii*. Three variables such as dilution of enzyme, dilution of the antiserum and dilution of the antigens were optimized. In all cases, homologous ELISA reaction using antigens of *S.rolfsii* was carried out as described under Materials and Methods.

4.8.3.1.1. Enzyme dilution

In this experiments, keeping the antigen (10μg/ml) and antiserum dilution (1:125) constant, different dilutions of alkaline phosphatase was used. Dilution ranged from 1:10,000 to 1:40,000. On the basis of results 1:10,000 of alkaline phosphatase was used in all further experiments.

4.8.3.1.2. Antiserum dilution

Antiserum dilutions ranging from 1:125 to 1:16,000 were tested against homologous antigen at a concentration of 10μg/ml. Absorbance values in ELISA decreased from the dilution of 1:125 to 1:16,000 (Fig.12). An absorbance value of 1.81 was obtained at 1:125 dilution which was 3 times of that obtained at 1:16,000 dilution (Table 32). 1:250 dilution was selected for further experiments.

Table 32 : ELISA reaction with various dilution of anti-*S. rolfsii* antiserum and homologous antigen

Antiserum dilution	Absorbance at 405 nm
1:125	1.81± 0.032
1:250	1.68± 0.015
1:500	1.65±0.003
1:1000	1.54±0.014
1:2000	1.27±0.002
1:4000	1.17±0.016
1:8000	0.86±0.001
1:16000	0.61±0.002

Mycelial antigen concentration 10 µg/ml.;Enzyme dilution 1:10,000 ; ± Standard error

4.8.3.1.3. Antigen dilution

Doubling dilutions of S.rolfsii mycelial antigen ranging from 8000 to 62.5 ng/ml were tested against two antiserum dilutions (1:125 and 1:250). ELISA values increased with the concomitant increase of antigen concentration (Table 33). Concentrations as low as 62.5ng/ml could be easily detected by ELISA at both antisera dilutions (Fig. 13).

4.8.3.2. Comparison of ELISA reactivity among antigens of different soybean varieties against antiserum of *S.rolfsii*

Among six varieties of soybean tested for varietal resistance tests against *S.rolfsii* , differential responses were obtained. Certain varieties exhibited high susceptibility, others were moderately susceptible. Conventional techniques for determination of host resistance or susceptibility are being replaced by more rapid and sensitive modern serological techniques. It was therefore considered worthwhile to detemine the ELISA reactivity of different soybean varieties against antiserum of the pathogen.

Effect of dilution of anti-*S.rolfsii* antiserum on ELISA reaction with homologous mycelial antigen

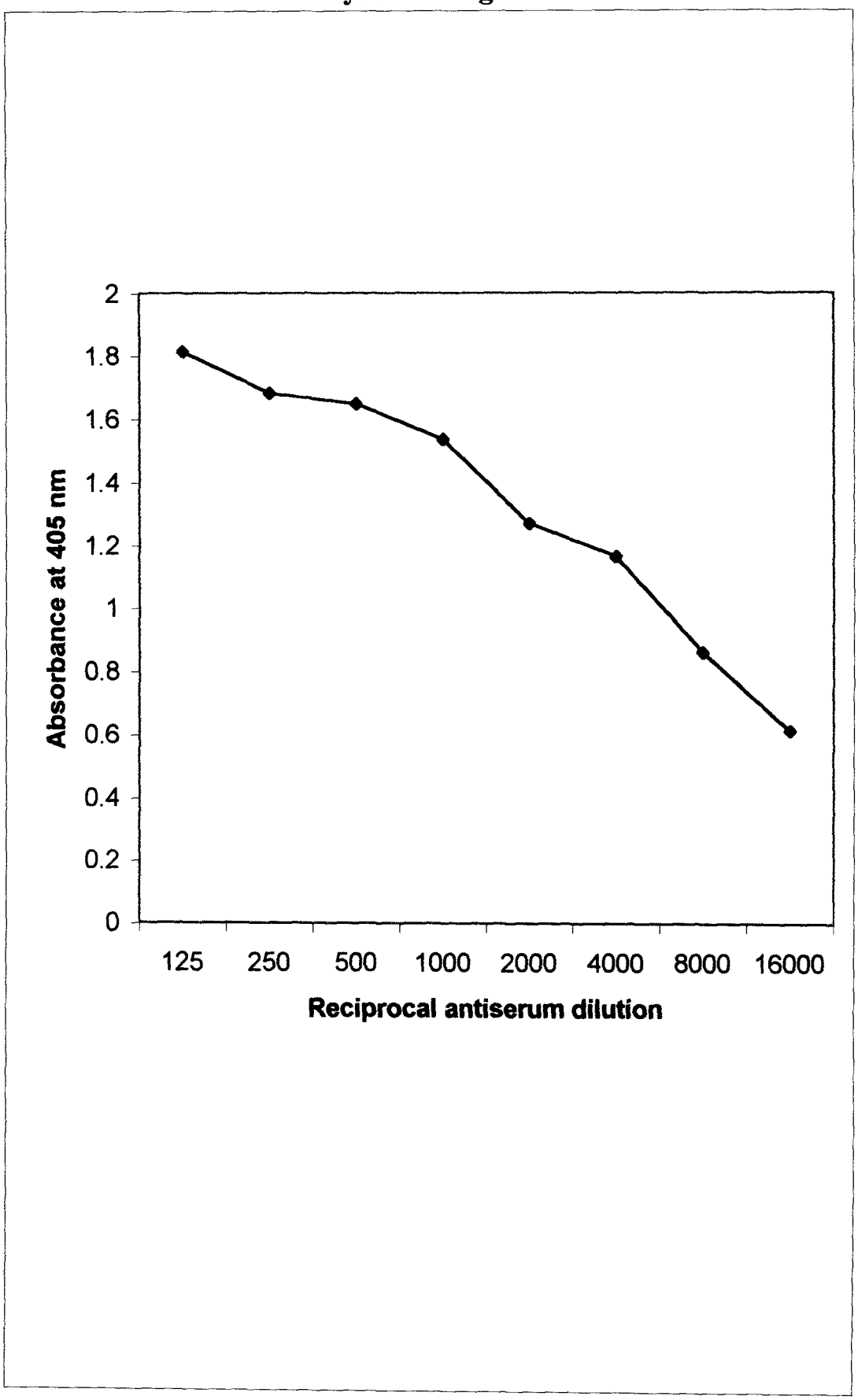


Fig.12

Table 33 : ELISA reaction with various concentration of mycelial antigen of *S. rolfsii* and homologous antiserum

Antigen dilution (ng/ml)	Absorbance at 405 nm	
	Antisera dilution (1:125)	Antisera dilution (1:250)
8000	2.380±0.112	2.803±0.122
4000	2.110±0.010	1.940±0.102
2000	1.985±0.065	1.798±0.0045
1000	1.823±0.026	1.727±0.033
500	1.808±0.015	1.511±0.022
250	1.710±0.032	1.436±.116
125	1.660±0.077	1.310±0.049
62.5	1.591±0.045	1.250±0.551

Anti -*S. rolfsii* antiserum dilution 1:125 and 1:250

Enzyme dilution = 1: 10,000 ; ± = Standard error

Antigens were prepared from soybean roots of six varieties, six isolates of the pathogen (*S.rolfsii*) , as well as two non –pathogens. All these antigens at a concentration of 40 µg/ml were tested by ELISA against purified antiserum of *S.rolfsii,* except antigens of the *S.rolfsii* isolates, which were used at a concentration of 10 µg/ml. In all caes, experiments were repeated under similar concentrations. Results (Table 34) revealed that absorbance values in ELISA varied with the different varieties. The different isolates of the pathogen tested also showed reactivity with the antiserum of the pathogen. Highest absorbance value, however, was obtained in the homologous reaction. Absorbance values for normal serum controls were below the corresponding test values.

Effect of dilution of mycelial antigen of *S.rolfsii* on ELISA reaction with homologous antiserum

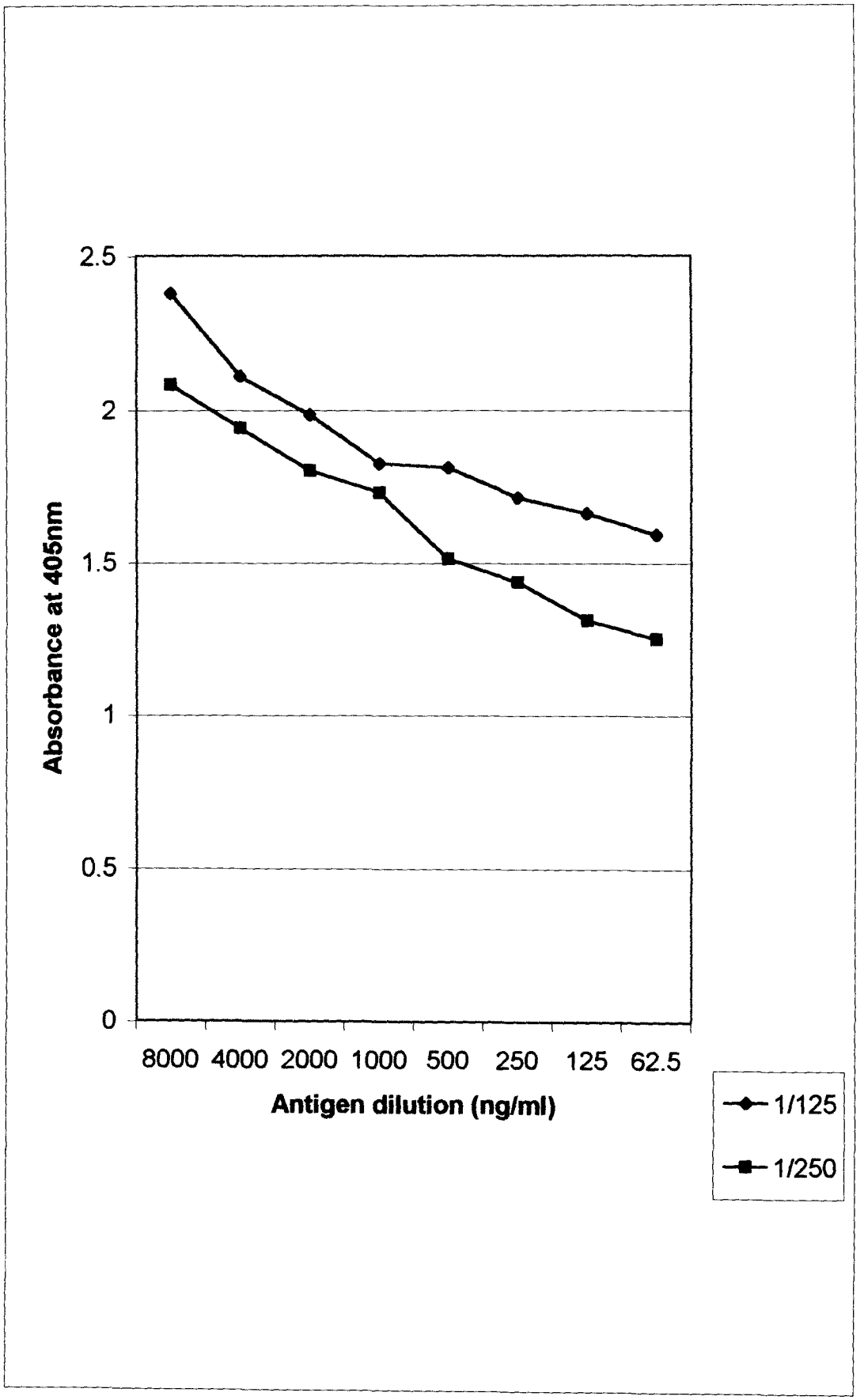


Fig.13

Table 34 : Indirect ELISA values (A 405) of soybean root antigens and mycelial antigen (pathogen and non-pathogen) reacted with antiserum of *S. rolfsii*.

Antigens of host and parasite	Absorbance at 405 nm
Host (soybean root)	
PK-262	0.988±0.021
Bragg	0.922±0.045
NRC-7	0.866±0.049
Pusa-16	0.997±0.027
J-80	1.032±0.031
Macs-58	1.124±0.033
Pathogen (*S. rolfsii*)	
Isolate 1	1.983±0.023
Isolate- 2	1.880±0.015
Isolate –3	1.795±0.026
Isolate – 4	1.696±0.022
Isolate – 5	1.772±0.037
Isolate – 6	1.894±0.028
Non-pathogen	
Fomes lamaoensis	0.542±0.011
Ustulina zonata	0.441±0.016

Anti *S. rolfsii* antiserum 1: 250 dilution.

Antigen concentration 10 μg/ml.

± Standard error

4.9. Detection of *S.rolfsii* in artificially inoculated soybean root tissues by DAC-ELISA

Pathogen detection in host tissues by the use of ELISA with the antiserum raised against the pathogen is an effective method. Difference in ELISA readings between healthy and infected antigen extracts indicates the measure and extent of infection. Initially, in the previos experiments, it was observed that cross reactive antigens are present between *S.rolfsii* and susceptible soybean varieties. In this experiment, six soybean varieties were artificially inoculated with *S.rolfsii.* It has been observed in pathogenicity tests, that well established symptoms of *S.rolfsii* infection appeared on aboveground level within 10-12 days after inoculation. Therefore, root antigens were prepared from healthy and infected (artificially inoculated with *S.rolfsii*) soybean plants (varieties PK262, Bragg, NRC7, Pusa16, J80 and Macs58) , 12 days after inoculation with *S.rolfsii.* Concentration of root antigen and dilution of anti-*S.rolfsii* antiserum were 40 μg/ml and 1:250 respectively. Results are presented in Fig.14. Absorbance values for antigen prepared from inoculated roots of all varieties were higher than their respective healthy root antigens.

4.10. Cellular location of CRA using Immunofluorescence

Fluorescent antibody labellig with fluorescein isothiocyanate (FITC) is known to be one of the powerful techniques to determine the cell or tissue location of major cross reactive antigens (CRA) shared by host and parasite. In the presen study, following immunodiffusion, immunoelectrophoresis, DAC-ELISA, the presence of CRA shared by *Glycine max* and *Sclerotium rolfsii* has been detected. It was decided to determine the tissue and cellular location of CRA in root tissues soybean varieties as well as mycelia and sclerotia of *S.rolfsii.* Detailed methods of antibody staining of root sections and mycelial preparations have already been described in Materials and Methods. Photographs were taken under UV-fluorescence.

ELISA reactions of anti-*S.rolfsii* antisera with healthy and inoculated soybean plants

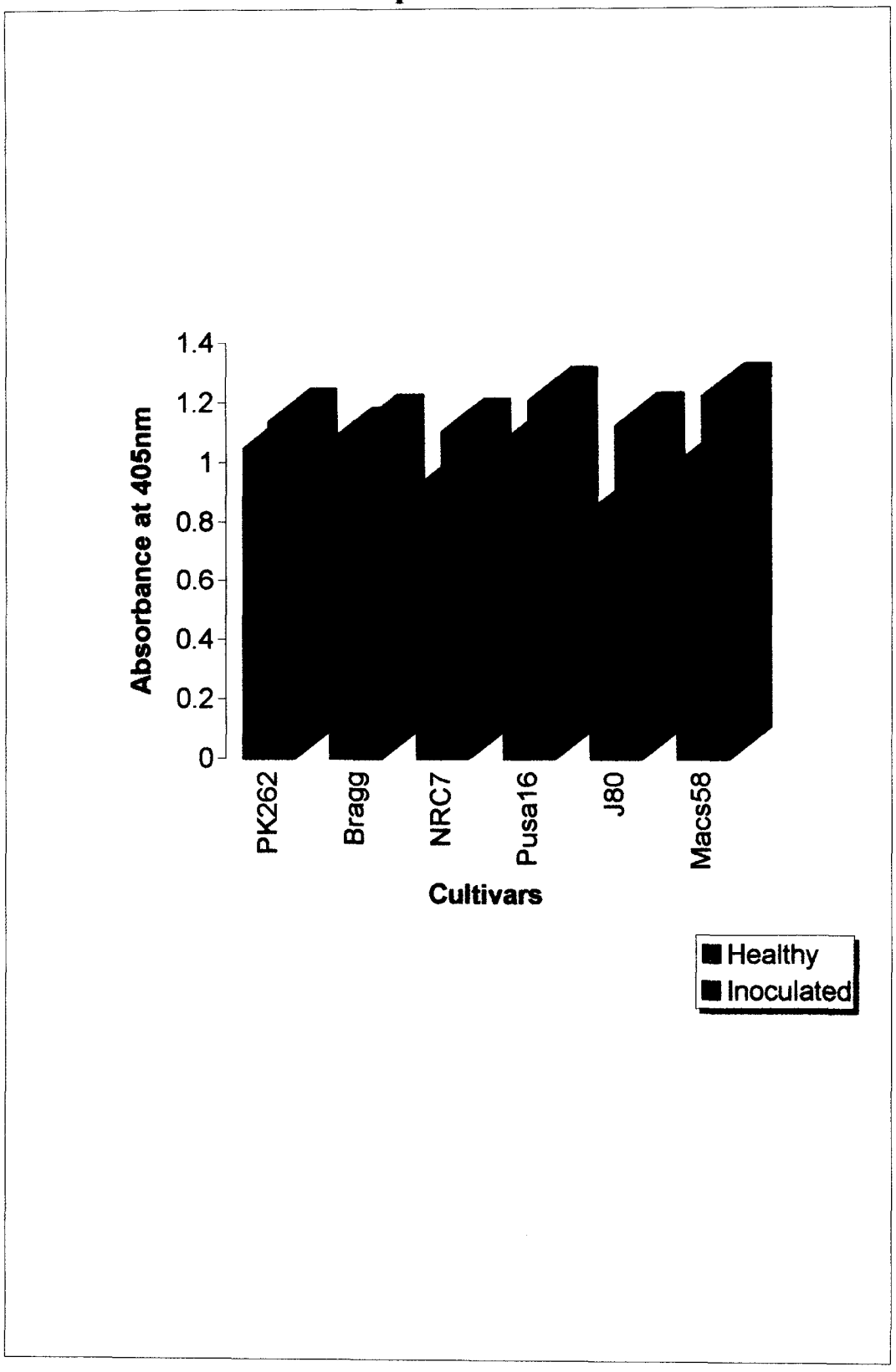


Fig.14

4.10.1 Root tissue

Cross sections of soybean roots (variety- Bragg and Macs-58) were treated separately with normal serum, homologous and pathogen antisera, then reacted with FITC. Root sections exhibited a natural autofluorescene under UV-light on the cuticle. Same observations were noted when the root sections were treated with normal serum and labelled with FITC. Root sections treated with antiserum of Macs-58 and then reacted with FITC, developed bright fluorescence which was distributed throughout the root tissues. Of much significance was the strong reaction of anti-*S.rolfsii* antiserum with root tissues of two soybean varieties (Macs-58 and Bragg). CRA was concentrated mainly around epidermal and cortical cells in case of Bragg (Plate 10 B), whereas in case of Macs-58, CRA was distributed throughout the epidermal, cortical tissues (Plate 11 A & B) and also vascular tissues (Plate 12 A & B).

4.10.2 Mycelia and Sclerotia

Mycelia and sclerotia of *S.rolfsii* were not auto-fluorescent nor did they fluoresce when treated with normal serum followed by FITC. Treatment of mycelia and sclerotia of *S.rolfsii* with homologous antiserum and FITC showed a general flurescence that was more intense on young hyphal tips (Plate 13) and on the germinated sclerotia (Fig.14 A&B).

4.11. Serological changes associated with induction of resistance in soybean plants

In the present investigation attempt was made to induce disease resistance in soybean plants (variety-Macs58) applying twelve chemicals belonging to three separate groups i.e. metal salts, growth regulators and biological compound (chitosan). Among the tested chemicals cupric chloride and ferric chloride were found to be highly effective in reducing disease intensity. Consequent changes in glyceollin level due to induction of resistance were determined. Similarly alteration in antigenic patterns after chemical induction of resistance by cupric chloride were also worked out since both are believed to be associated with plant disease resistance.

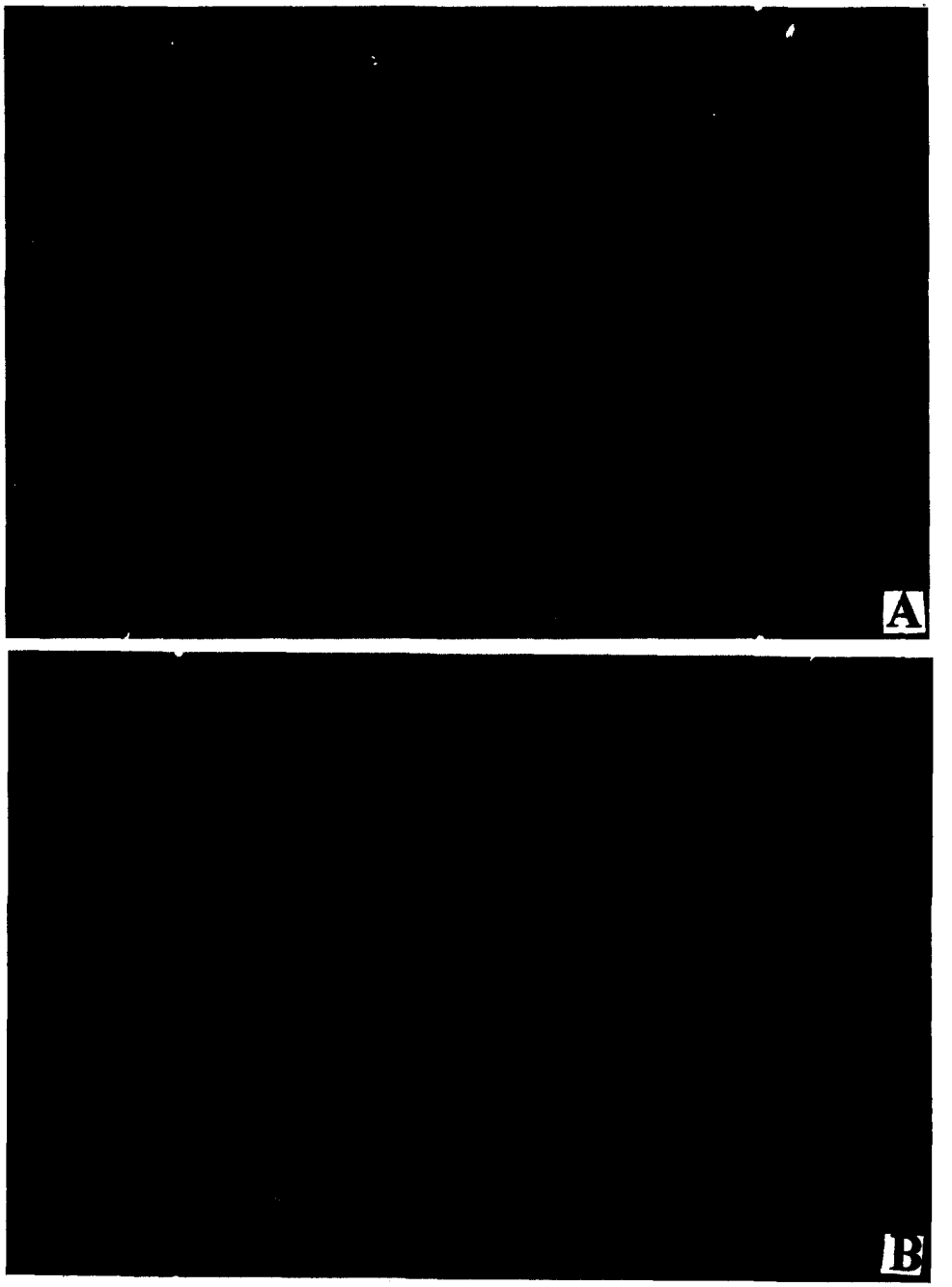


Plate 10 (A & B): T.S. of soybean root (Variety - Bragg)
(A)- Unstained; (B) Treated with antiserum of *S.rolfsii* and FITC antibodies of goat specific for rabbit globulin

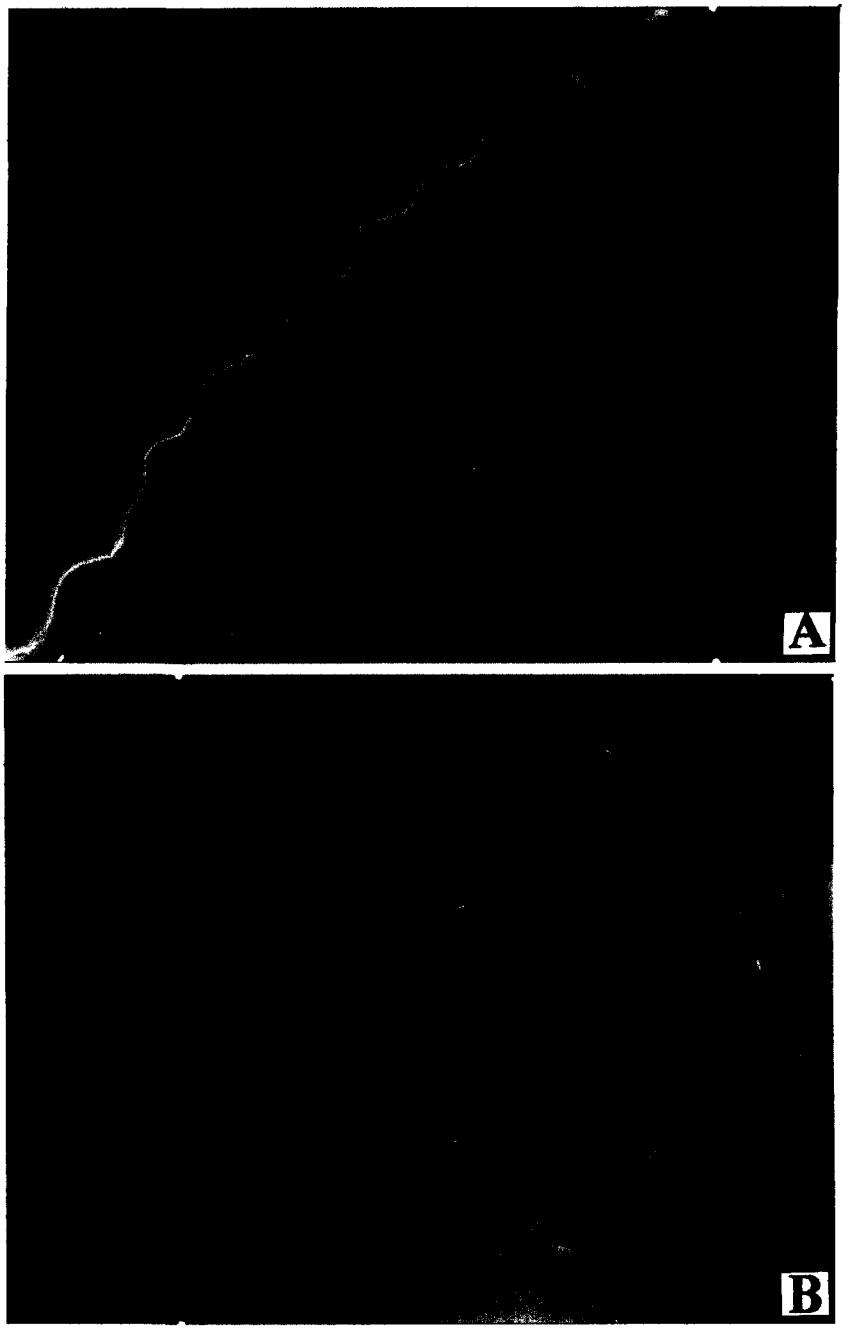


Plate 11 (A & B): Fluorescent antibody staining of soybean root tissues (Variety- Macs58) for cross reactive antigens shared with *S.rolfsii*. Root sections treated with antiserum of *S.rolfsii* and FITC antibodies of goat specific for rabbit globulin

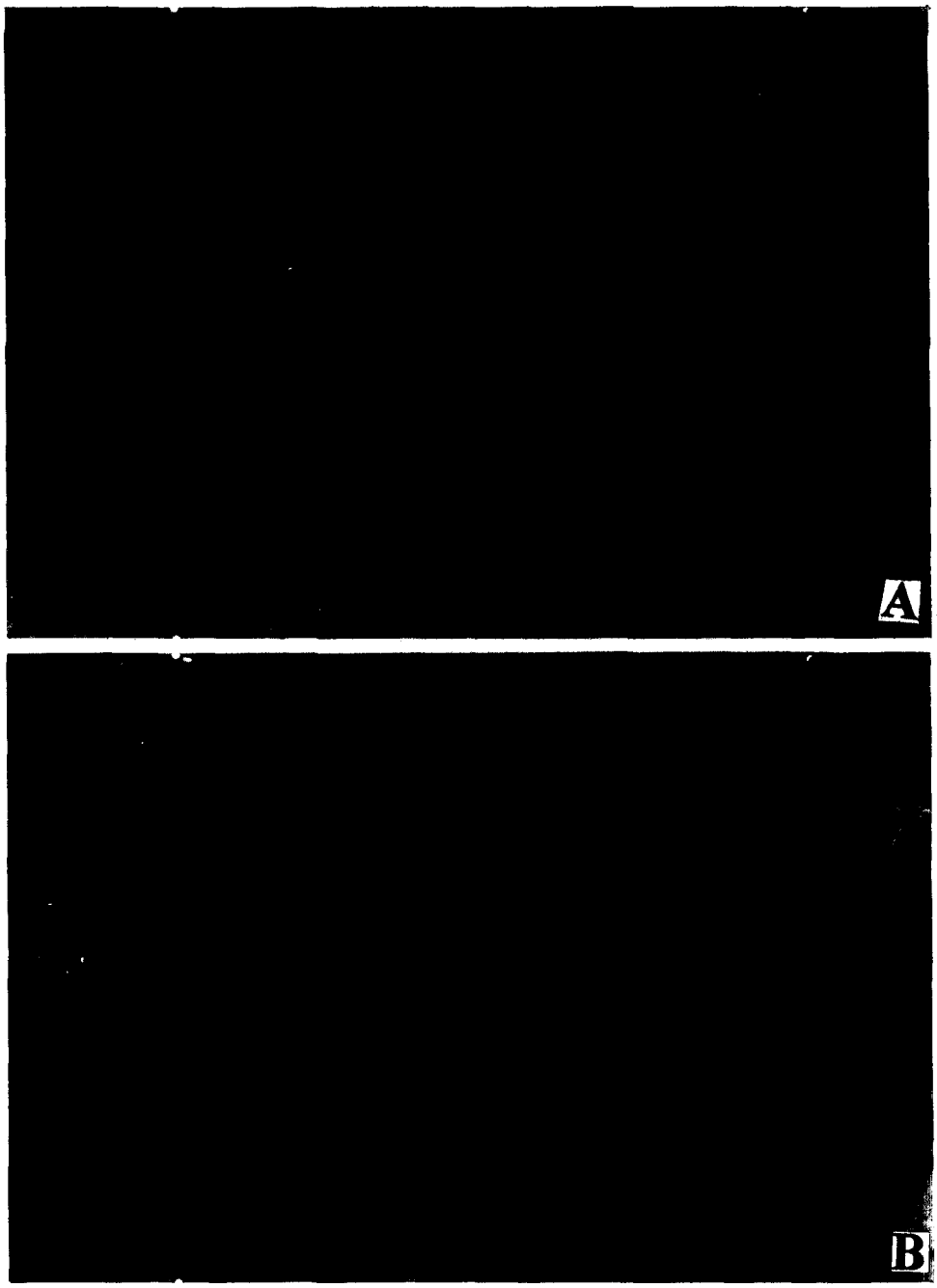


Plate 12 (A & B): T.S. of collar region of soybean plants (Variety- Macs58) treated with antiserum of *S.rolfsii* and FITC antibodies of goat specific for rabbit globulin

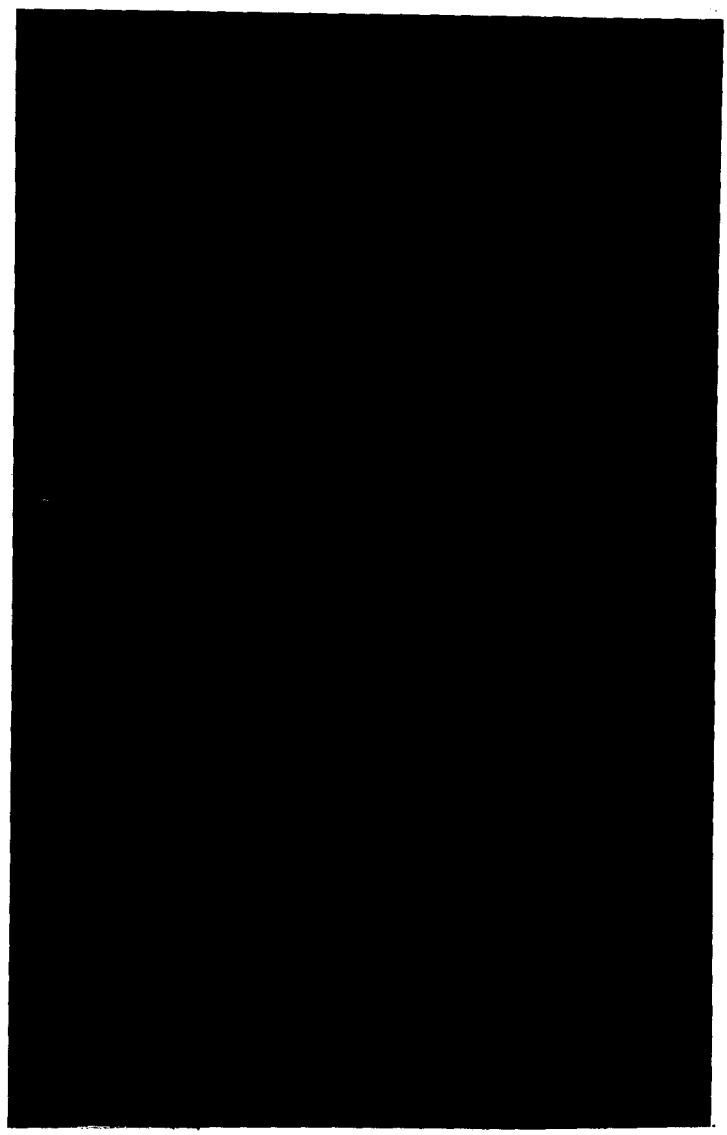


Plate 13: Mycelia of *S.rolfsii* treated with antiserum of *S.rolfsii* and FITC antibodies of goat specific for rabbit globulin

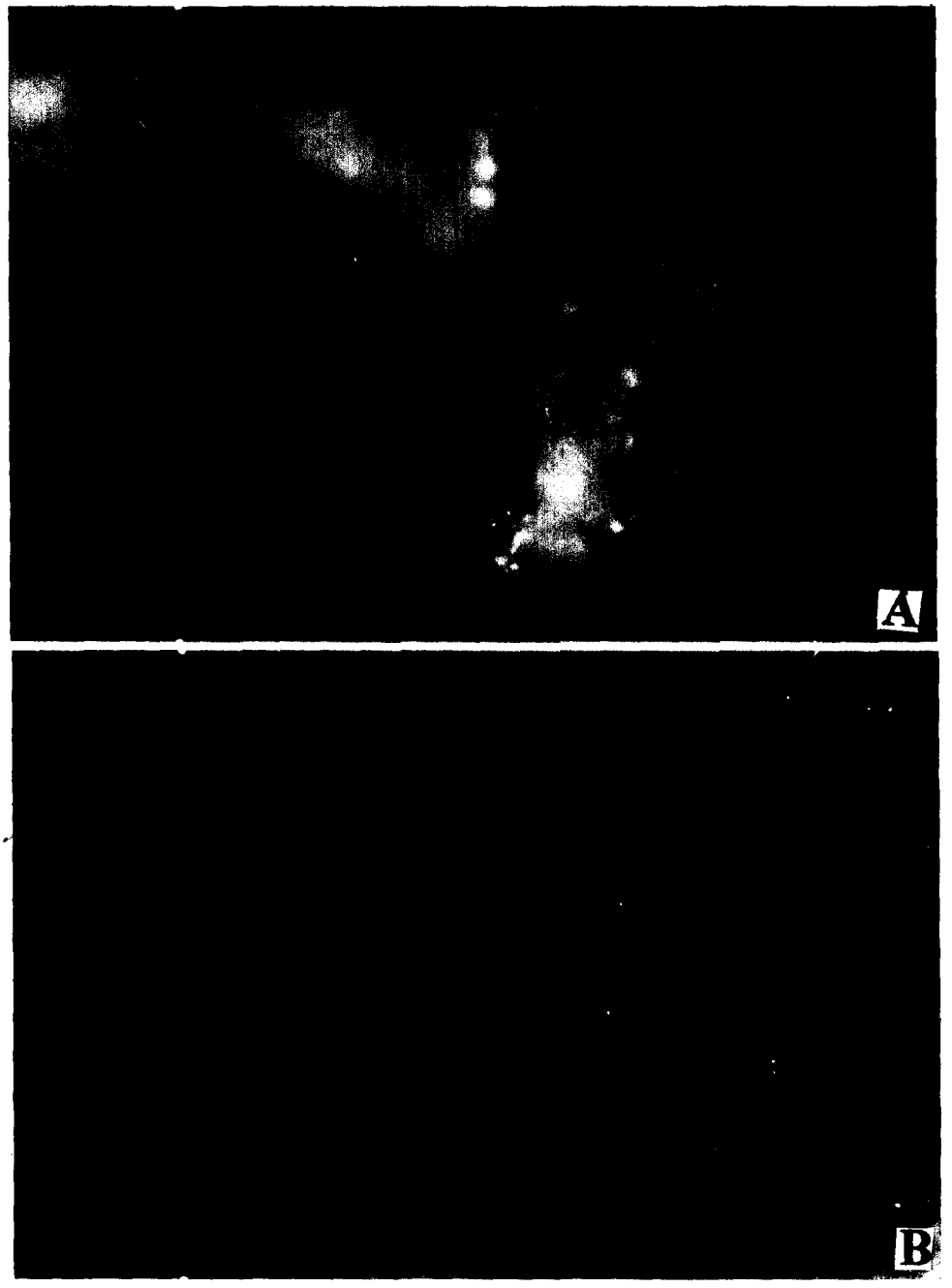


Plate 14 (A & B): Germinated sclerotia treated with antiserum of *S.rolfsii* and FITC antibodies of goat specific for rabbit globulin

To study the consequent changes in antigenic patterns in cupric chloride treated soybean plants (Macs-58), agar gel double diffusion tests were performed with antisera raised against untreated and cupric chloride treated soybean root(Macs-58) antigens as well as antiserum of *S.rolfsii.* against both homologous and heterologous antigens. Results (Table- 35) reveal that strong precipitin reactions occurred when antiserum of *S.rolfsii* was reacted against its own antigens as well as root antigens of untreated susceptible soybean variety- Macs-58. However, antigens of treated soybean roots showed no precipitin reaction. Reciprocal cross reaction between antiserum of treated roots and the pathogen antigens also failed to develop even weak precipitin bands.

Table 35: Immunodiffusion tests of antigens of *S.rolfsii* and soybean root tissues before and after treatment with cupric chloride

Antigens	**Antisera**		
	Macs-58 (Untreated)	**Macs-58 (Treated)***	***S.rolfsii***
Soybean variety (Macs – 58)			
Untreated	+	+	+
Treated*	±	+	-
Pathogen			
S.rolfsii	+	-	+

- *Plants treated with cupric chloride (10^{-3}M)
- Common precipitin band: + = present ; ± weak band ; - = band absent

The presence or absence of common antigens between host and parasite could be confirmed by the immunodiffusion test, but it was not clear whether precipitin reactions in all cases were due to single or several antigenic substances. Therefore, further resolution was attempted by subjecting the antigens to electrophoresis before exposing them to antisera following immunoelectrophoresis.

Table 36 : Immunoelectrophoretic analysis of antigens of *S.rolfsii* and soybean root tissues before and after treatment with cupric chloride

Antigens	No. of precipitin lines with host and parasite antisera		
	Macs-58 (Untreated)	Macs-58 (Treated)*	*S.rolfsii*
Soybean variety (Macs – 58)			
Untreated	3	1	2
Treated*	1	2	0
Pathogen			
S.rolfsii	2	0	4

*Plants treated with cupric chloride (10^{-3}M)

The effectiveness of each antigen extract in raising antibodies was checked by homologous reactions. The homologous patterns formed by antigens and antisera of *S.rolfsii* , untreated soybean roots and treated soybean roots contained four, three and two precipitin lines respectively (Table 36) When antigens of untreated roots and the fungal antigens were cross reacted with antiserum of Macs-58 (untreated), only two precipitin bands were visible for the pathogen as common antigen. However, cupric chloride treated root antigens shared only one precipiin line with untreated root antigens. But no common antigenic substances could established with treated root antigens and pathogen antisera or vice versa . It appears that these observed antigenic changes owing to cupric chloride treatment have some significance in the resistance of soybean to *S.rolfsii.*