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Increase resistance in chickpea plants to *Fusarium*-wilt following treatment with chitosan

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The performance of chitosan, a deacetylated product of chitin, the natural biopolymer was evaluated against *Fusarium* wilt of chickpea (*Fusarium oxysporum* f. sp. *ciceri*). Chitosan at 0.3% and 1.0% used as seed treatment showed very strong inhibitory effect in respect of both symptom expression and plant mortality. These reduced wilt symptoms by 45% to 59% and prevented plant mortality appreciably. In response to infection, treated susceptible plant recorded higher level of increases in respect of total and ortho-dihydroxyphenol contects and also showed enhanced polyphenoloxidase, peroxidase and phenylalanine ammonia-lyase activities usually associated with the defense responses of plants. The post-infection levels of these parameters were moderataly higher than that of untreated plants and came closer to those of resistant plants.

Key words : Chickpea, *Fusarium*-wilt, chitosan, disease resistance, biochemical changes

INTRODUCTION

Chitosan, a polymer of β-1,4 - linked glucosamine and strong elicitor of phytoalexin provide protection to pea plants from *Fusarium solani* infection (Hadwiger and Beckman, 1980). Chitosan is also antifungal (Hadwiger, 1979). In our laboratory, seed treatment with chitosan has proved to be effective in reducing disease symptoms in peanut against *Sclerotium rolfsii* (Chowdhury and Sinha, 1997) and *Cercospora arachidis* infection (Sinha *et al.* 1995) and in rice against brown spot and blast diseases (Sinha *et al.*, 1995). The present investigations were undertaken to see the effect of chitosan, used as seed treatment in chickpea plants against *Fusarium oxysporum* f. sp. *ciceri*, the wilt pathogen and the possible biochemical changes associated with induced resistance.

MATERIALS AND METHODS

Two cultivars of chickpea viz. ICC-4951 and ICC-11323 with variable disease reaction to *Fusarium oxysporum* f. sp. *ciceri* were used as plant materials. Seed materials were obtained from ICRISAT, Hyderabad.

Seeds taken in a concial flask were treated by adding chitosan solution (2 ml/100 g) drop by drop followed by vigorous shaking and air drying. Chitosan solutions (0.1% - 1.0%) were made by dissolving its flakes in 1% glacial acetic solution and maintaining its pH at 6.0. 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 *Fusarium oxysporum* f. sp. *ciceri* 14 days after sowing by replacing the top soil with 100 g mixture of fungal inoculm made up of culture grown on sand maize meal medium and sterilized soil mixed in 1:1 ratio and injuring a few roots in the process, so that fungal penetration might be facilitated.

The external symptoms of the wilt disease were assessed on 0-5 scale (0 plant is healthy : 5 = complete wilting of the leaves and the plant dead).

For biochemical analysis, the basal region with roots were collected from the inoculated and also from comparable portion of uninoculated plants.

Total phenol

Phenols were extracted from the living tissues following the procedure of Bisnn *et al.* (1968) and phenol content was estimated following the method of Bray and Thorpe (1954) using Folin-ciocalteu reagent.

Ortho-dihydroxphenols

The estimation of orthodihydroxy phenols was done by using Arnow's reagent (Mahadevan, 1966).

Polyphenoloxidase activity

Determination of polyphenoloxidase activity was done following the method of Jennings *et al.* (1969).

Peroxidase activity

Peroxidase activity was measured following the method of Addy and Goodman (1972).

Phenylalanine ammonia-lyase activity (PAL)

The enzyme activity was determined by measuring the production of cinnamic acid from phenylalanine spectrophotometrically (Bhattacharya and Ward, 1987).

RESULTS AND DISCUSSION

The susceptible chickpea plants in different treatments significantly (P-0.05) showed reduction of the symptoms at all stages of sampling as campared to those in the untreated plants (Table 1). As campared to 78% disease incidence recorded for the untreated plants, different treatments showed 44% to 61% disease incidence and at the time of final sampling 18% to 59% symptoms. The plant mortality was reduced from 61% to 20-45%.

Table 1 : Effect of seed treatment with chitosan on symptom expression in chickpea plants artificially inoculated with *Fusarium oxysporun*' f. sp. *ciceri,* recorded at intervales (days) after inoculation

Treatment	Conc.	Mean disease index/plant[1]				Disease	Mortality
	(%)	10	17	24	31	incidence (%)	(%)
ICC-4951 (Susceptible)							
Water (Control)		0.5	2.3	2.7	3.2	78.0	61.0
Chitosan	0.1	0.2	1.1	1.9	2.6 (–18.7)[2]	61.0	45.0
	0.3	0.1	0.7	0.9	1.3 (–59.4)	44.0	20.0
	1.0	0.2	0.9	1.1	1.7 (– 46.8)	49.0	22.0
ICC-11323 (Resistant)							
Water (Control)		0	0.4	0.6	0.9 (–71.8)	29.0	12.0
C.D. (p=0.05)	0.11	0.23	0.35	0.34	15.7	16.8	

1. Results represent the average of 32-35 plants. Data on disease incidence and mortality were those collected on the last sampling data
2. Values in parentheses indicate percentage reductions in terms of control

Chitosan at 0.3 and 0.1% had stronger and lesser inhibitory effects on the pathogen respectively. The most effective treatment to brought down symptoms and mortality is quite close to the levels in resistant plants.

As the seed treatment with chitosan provide

Table 2 : Effect of seed treatment with chitosan on phenol and ortho-dihydroxyphenol contents in healthy and infected chickpea plants, recorded days after inoculation with *Fusarium oxysporium* f. sp. *ciceri*

Treatment	Total phenol				Ortho-dihydroxyphenols			
	10 days		17 days		10 days		17 days	
	H	Ino	H	Ino	H	Ino	H	Ino
ICC-4951								
Water (Control)	1.65	2.27	1.72	1.62	0.11	0.15	0.12	0.10
Chitosan (0.3%)	1.85	3.07	1.97	3.32	0.13	0.20	0.13	0.20
Chitosan (0.1%)	1.87	2.93	1.95	2.70	0.12	0.18	0.13	0.19
ICC-11323								
Water (Control)	1.91	3.47	2.07	3.63	0.13	0.22	0.14	0.21

C.D. for treatment X inoculation at 5% = 0.083 (phenol) and 0.008 (OD – phenols)

, Data are expressed as mg/g fresh weight of tissue

substantial protection in susceptible chickpea plants against the wilt pathogan, basic biochemical changes usually associated with the defense responses of plants like phenolics, polyphenoloxidase (PPO), peroxidases (PO) and phenylalanine ammonia-lyase (Pal) activities in the effective treatments were measured.

In respect of total phenol, the seed treatment with chitosan caused small increases (12-15%) and bringing them closer to the resistant plants (Table 2). Infection resulted moderate increase (37%) in susceptible cultivar recorded mild increases following treatment (Table 3). In the susceptible plants, the initial response to infection was considerable (71% increase) for PPO and only moderate (6.1% increase) for PO activities but with time, such effects weakened. For the resistant plants, initial increases were very high (81% to 104%) for both enzymes and because of only slow weakening of the effect with time, considerable effects persisted till the end. In treated susceptible plants, the enzyme activities following infection showed the same trend as in the resistant plants but were at some what lower level.

phenol level in susceptible plants, 10 days after inoculation but this effect rapidly decreased or disappeared in the next week. Plants in different treatment recorded considerable increase in postinfection phenol levels i.e. 29% to 35% after 10 days and 66% to 104% after 17 days of inoculation than untreated plants but fell slightly short of the levels in comparable resistant plants. The trend of orthodihydroxyphenol content showed the same way as that of the phenol content.

Regarding PPO and PO activities, the

Table 3 : Effects of seed treatment with chitosan on polyphenoloxidase and peroxidase activity in healthy and infected chickpea plants, recorded at days after inoculation with *F. oxysporium* f. sp. *ciceri*

Treatment	Polyphenoloxidase activity[1] (10 mg of extracted tissue)				Peroxidase activity[2] (unit of activity/g. fresh tissue/min)			
	10 days		17 days		10 days		17 days	
	H	Ino	H	Ino	H	Ino	H	Ino
ICC-4951								
Water (Control)	0.07	0.12	0.08	0.09	11.33	18.33	12.33	15.33
Chitosan (0.3%)	0.08	0.17	0.11	0.16	14.33	26.66	14.67	25.67
Chitosan (0.1%)	0.08	0.17	0.10	0.15	15.33	27.33	15.33	24.67
ICC-11323								
Water (Control)	0.11	0.20	0.12	0.21	16.0	32.67	16.67	31.33

C.D. for treatment X inoculation at 5% = 0.009 (PPO) and 0.847 (PO)

[1] Data expressed in terms of change in optical density /0.05 ml of extract after 30 minutes
[2] Data expressed as units of activity (= a change in the absorption by 0.01 per minute)

The PAL activity in susceptible plants following infection had moderate increase (17%) whereas the different treatments showed considerable increases (74% to 86%) and the final post-infectional enzyme activity had 56% to 64% as 10 days and 64% to 75% at 17 days after inoculation, more higher than susceptible plants (Table 4).

The results of the present study demonstrate that the seed treatment of chitosan has the potential for providing high level systemic protection to chickpea plants from wilt pathogen, *Fusarium oxysporum* f. sp. *ciceri*. Significant metabolic changes that occur in treated susceptible plants after infection almost in the line of responses in resistant plants, make the internal conditions more unfavourable to the wilt pathogen and substantially

Table 4 : Effect of chitosan on phenylalanine ammonia-lyase activity in healthy and infected chickpea plants, recorded at days after inoculation with *F. oxysporium* f. sp. *ciceri*

Treatment	Phenylalanine ammonia-lyase activity (µg cinnamic acid released/g/min)			
	10 days		17 days	
	H	Ino	H	Ino
ICC-4951				
Water (Control)	92.5	108.0	90.0	95.5
Chitosan (0.3%)	95.5	178.0	97.0	168.0
Chitosan (0.1%)	97.0	169.0	96.5	157.0
ICC-11323				
Water (Control)	112.5	185.0	110.0	175.5

C.D. for treatment and inoculation at 5% = 12.85

limit its disease causing potential through effective restriction of pathogenic biosynthesis, greater oxidation of phenols and also increased peroxidase and phenylalanine ammonia-lyase activities. Similar observations were recorded in pea against *Fusarium solani* (Hadwiger *et al.*, 1981).

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