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Biochemical evidences of induced resistance in tomato plant against Fusarium with through inorganic chemicals

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Prior to pathogenic inoculation with inorganic chemicals provided protection of tomato plant against challenge infection of *Fusarium oxysporium* f.sp. *lycopersici*, resulting reduction of wilt incidence from 12.4 to 59.21 per cent. The biochemical analyses of tomato leaves under different treatments revealed increased content of soluble protein and total phenol. The maximum protein content with 35.83, 36.23 and 35.43 mg/g of leaf at 72, 144 and 216 hrs of inoculation was recorded in calcium chloride treated leaves which was 66.56% and 85.21% increased level at 216 hrs of inoculation over healthy and diseased plant respectively. Similarly, maximum amount of total phenols in treated leaves with calcium chloride (2.24 mg/g) was more than healthy (1.58 mg/g leaf) and diseased (1.69 mg/g leaf) leaves at 72 hrs of inoculation. By and large the concentrations of both phenolic compounds and soluble protein in various treatments were more at 144 hrs but after that both of them decreased. Co-relation co-efficient analysis revealed that there was negative correlation r = -0.4312 (72 hrs), -0.4694 (144 hrs) and -0.4589 (216 hrs) between disease severity and soluble protein content after different hours of treatment. Similarly, total phenol content was also showed negative correlation r = -0.3441 (72hrs). -0.3835 (144 hrs) and -0.3264 (216 hrs) with disease incidence.

Key words : Tomato, induced resistance, inorganic chemicals, Fusarium wilt, biochemical analyses, soluble protein, total phenol

INTRODUCTION

Tomato (*Lycopersicon esculentum* (L.) Krust) is one of the most important vegetable crops in the world and considered as a protective food because of its special nutritive value. It is a rich source of vitamine C, vitamine B, vitamine A, calcium and iron. It also contains some amount of phosphorous, sulfur and potassium. Tomato crop is attacked by a large number of pathogens belonging to fungi, bacteria, virus and nematodes. *Fusarium* wilt caused by *Fusarium oxysporium* f.sp. *lycopersici* is one of the important fungal disease of tomato, causing considerable losses in India. The wilt incidence in tomato ranges from 3.58 to 20.63 per cent in India (Sharma *et al.*,1983). The effective management of the disease could be done through cultural, chemical, biological and use of resistant varieties. But most of the conventional, chemical, biological and use of resistant varieties are tend towards direct control of pests and diseases by their elimination. However, in case of monoculture crop, these practices raise problem due to development of resistance strain among the pathogens. Therefore, induced resistance would be one of the new and novel approaches of disease management in future by supplementing all traditional methods. It has been reported by several workers that pre-inoculation with avirulent or virulent pathogen and seed treatment with non-conventional chemical provided induced resistance in plants against several pathogens (Ouchi *et al.*, 1974, Tuzun and Kuc, 1985). The pre-application of some biotics and abiotics inducers also provides induced resistance in plants against many pathogens (Kuc,I995; Biswas *et al.*,2003, van Loon 1983; Kessmal *et al.*, 1990). Several inorganic compound, like phosphate, salts (dipotassium/ sodium or tripotassium), silicon, SiO_2 have been reported as potential activators of resistance (Gottistein and Kuc, 1987). Biochemical changes associated with induced resistance in crop plant against pathogens by biotics and abiotics agents have been reported by several workers (Kuc, 1995; Chowdhury, 1995; Olga and Ozerestksorskay, 1995;

Biswas *et al.*, 2003; Mondal and Sinha, 1992). Therefore, the present investigation has been undertaken on this aspect.

MATERIALS AND METHODS

Isolation, identification and maintenance of Fusarium oxysporium f.sp. lycopersici

The pathogen was isolated from disease plant collected from Vegetable Research Farm, C.S.A. University of Agriculture & Technology, Kalyanpur (Kanpur). Infected plant showing typical symptom was selected for isolation of pathogen. Initially the diseased part was washed thoroughly with distilled water to remove dust particles. Then diseased portion of stem were cut out into small pieces by a sterilized scalpel and each piece had small bits of diseased and healthy tissues. These pieces were dipped in 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds and then thoroughly washed thrice in distilled water to remove the traces amount of $HgCl_2$ solution. Excess moisture was removed by putting these pieces between two folds of sterilized blotting paper under aseptic conditions. The pieces then transferred to sterilized Petri plates containing 2% potato dextrose agar (PDA) medium in inoculation chamber with the help of sterilized forceps. Two pieces were placed aseptically in each Petri plates and incubated at room temperature (20±1°C). On appreance of the colony around the piece, the pathogen was purified by the transfer of hyphal tip in Petri plates which was previously poured with sterilized PDA in aseptic condition. The purified culture was then maintained at 25°±1°C in refrigerator. On appearance of the colony in Petri plate, the pathogen was examined under compound microscope and identified on the basis of its morphological and cultural characteristics, as described by Synder and Hansen (1940).

Preparation of different concentrations of inducer

For preparation of different concentrations of inducers (as given in Table I), the required quantity of inducing agents likes Salicylic acid (SA), Calcium chloride (CC), Di-Ammonium Potassium hydro-phosphate (DPHP), Hydrogen peroxide (HP), Ferric chloride (FC), Indole-3 acetic acid (IAA) and SAFF were weighed separately and required amount of sterilized water was added to each conical flasks to prepare 10 ppm, l0 ppm, l0 ppm, 1.0%, 5 ppm, 0.2% and 0.2% solution respectively. The water containing chemicals were shaken until become dissolved. The solutions are then used for present investigation.

Preparation of spore suspension of F. o. f. sp. lycopersici

The homogenous spore suspension of *F. o. f. sp. lycopersici* was prepared from 7 days old culture in sterile water. The suspension containing conidia and mycelial bit was churned in a warring blender and strained through cheese cloth. The test inoculums of the pathogen containing approximately @ 10^4 conidia/ml was used in the present investigation.

Measurement of disease incidence

The experiment was conducted in the glasshouse complex, Department of Plant Pathology, C.S.A. University of Agriculture & Technology, Kanpur. The seeds of tomato variety 'Azad-T-6' were sown in 30 cm earthen pots, which was previously filled with a mixture of sandy loam and farm yard manure in the ratio of 2 : 1. In each pot, 20 properly spaced seeds were sown and watered regularly. At 27 days age of seedling, tomato plants were sprayed with different concentrations of inducers separately and after two days the treated plants were inoculated with spore suspension of *F. o.* f. sp. *lycopersici.* Three replications per treatment and two controls were kept, in one case, plants were inoculated with water (Check I) and in second cases, and plants were inoculated with spore suspension of pathogen (Check II). The disease incidence was recorded by counting of diseased plant after 15 days of inoculation and disease incidence was calculated by using the following formula :- Disease incidence = No. of wilted plant / Total no. of plant × 100

Biochemical analyses for resistance

Analyses of biochemical changes in tomato plants due to pre-inoculation with different inducers was conducted. Tomato leaves were collected from different treatments and changes in the contents of soluble protein and phenol in the leaves were estimated.

Protein estimation

The method developed by Lowry *et al.* (1951) was used with slight modification to determine the soluble protein content. Tomato leaves from different

treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quantity of 1.0 g of each sample was cut into small pieces and grounded in pastles and mortar using 1:5 leaves :: extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4 °C. The supernatant was collected. The working standard solution was pipetted out as 0.2, 0.4, 0.6, 0.8 and 1.0 ml and was put into a series of test tubes. A quantity of 0.2, 0.4, 0.6, 0.8 and 1.0 of the sample extract was also pipetted out and kept into other test tubes separately. Then volumes in all the test tube were made up to 1 ml with distilled water. A tube with 1 ml water served as blank. Later on, 5 ml of solution of solution C was mixed well and incubated at room temperature for 10 minutes. Thereafter, 0.5 ml of FCR was mixed well immediately and incubated at room temperature for 30 min at dark place. The absorbance at 660 nm against the blank was read and standard graph was drawn to calculate the amount of soluble protein in sample and represented as mg/g of fresh sample.

Phenol estimation

The accumulation of phenols in tomato plants after treatment with different inducers followed by inoculation of pathogen was estimated following procedure developed by Bray and Thorpe (1954) with slight modification. 1.0 g of leaf sample of tomato was grounded in a pestle and mortar in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 30 minutes at room temperature. Supernatant was separated and re-extraction for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots 0.2, 0.6, 0.8, 1.0 and 1.5 ml were pipetted out into test tube and the volume in each tube was made to 3 ml with water. Subsequently. 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2Co_3 solution was added in each tube and thoroughly mixed. The tubes were then placed in boiling water for 1 minute and then cooled. Then absorbance at 650 nm against black was measured using UV-VIS. Spectrophotometer.

Correlation coefficients (r) of disease incidence with soluble protein and total phenol

The biochemical analyses of tomato leaves under different treatments and disease incidence of the corresponding treatments under pot experiment showed that reduced per cent of disease incidence was associated with increase in soluble protein and total phenol contents. Correlation coefficients (r) between soluble protein and disease severity; and between total phenol and disease severity were calculated by standard statistical calculation. Simple regression equations (Y = a + bx) were also developed for both the variables (protein and phenol) separately to understand their relation with disease severity.

RESULTS AND DISCUSSION

Effect of inducers as foliar spray on disease development

Pre-application of inorganic chemicals on tomato plants revealed that there was a drastic decline in wilt incidence under glasshouse condition (Table 1). The susceptible tomato variety *Azad T-6* developed on an average 12.40 per cent disease incidence, where plants were sprayed with calcium chloride.

Table 1: Effect of foliar spray with inducers on wilt incidence of tomato

Treatment	Disease severity (%)
IAA	29.52
SAAF	21.20
DPHP	21.04
HP	14.20
CC	12.40
SA	19.47
FC	27.95
Check-I	—
Check-II	59.21
CD (P = 0.05)	1.72

Table 2: Effect of foliar spray with inducers on total soluble protein content of tomato leaves after 72, 144 and 216 hrs of pathogen inoculation

Treatment	Total phenol content (mg/g fresh leaves)			(%) Increase in total phenol contents	
	72 hrs	144 hrs	216 hrs	Over Check-I	Over Check-II
IAA	28.1	28.6	27.5	22.8	30.5
SAAF	31.7	32.1	31.6	32.6	39.4
DPHP	33.0	33.6	32.8	35.1	41.6
HP	34.7	35.3	34.1	37.7	43.9
CC	35.8	36.2	35.4	39.9	46.0
SA	28.5	29.4	28.2	24.7	32.3
FC	33.9	34.1	33.6	36.8	43.1
Check-I	21.3	22.5	21.3	—	10.1
Check-II	17.9	19.9	19.1	-11.2	—
CD (P = 0.05)	0.83	0.91	0.99		

But in diseased plants, it was 59.21 per cent. The hydrogen peroxide treated plant was equally effective with calcium chloride and developed only 14.2 per cent disease incidence. The plant treated with SAAF and DPHP were statistically at per, showing 21.2 and 21.04 % disease incidence respectively. From the table, it was also clear that wilt incidence was reduced significantly in all the treatments, showing 59.21% to 12.40% wilt incidence. Thus, the protection provided by inoculation with inorganic chemicals as foliar sprays indicated the resistance, which was expressed by decrease in the disease incidence. Bloter *et al.* (1993) reported that root dipping of tomato and melon in dinitroaniline solution induced disease resistance to Fusarium wilt.

Biochemical changes

Biochemical changes associated with induced resistance in tomato might be due to synthesis of one or more compound(s). The observation on this aspect was taken after 72, 144 and 216 hrs of pathogen inoculation.

Soluble protein

The data presented in the Table 2 showed that plant treated with inorganic chemicals increased the amount of soluble protein in plant significantly. The content of soluble protein in healthy (check-I) and pathogen treated (check-II) plants was 21.25 and 17.94 mg/g of leaf at 72 hrs, 22.46 and 19.96 mg/g of leaf at 144 hrs and 21.27 and 19.13 mg/g leaf at 216 hrs of treatment. The protein content in respect of calcium chloride treated leaves was observed as 35.83, 36.23 and 35.43 mg/g of leaf at 72, 144 and 216 hrs of inoculation respectively which was highest among all the treatment. The per cent increase in protein content due to calcium chloride over healthy plants was 66.56% and over diseased plants was 85.21% at 216 hrs of treatment. Other treatments also revealed the increase content of soluble protein over healthy as well as infected plants but their effect was not superior as calcium chloride treatment. Moreover, inoculation of pathogen indicated the decreasing trend of protein contents showing -11.19% over healthy plants. Soluble protein content in terms of negative values may be due to utilization of some protein by pathogen. The induced resistance in cucumber against *C.lagerarium* by Ciba-Geigy compound (CGA-41396) was attributed to the major accumulations of protein including chitinase and chitinase mRNA (Matraux *et al.*, 1990).Lorenzo *et al.* (1987) also reported that when wheat leaves were treated with kinetin, the synthesis of protein was higher in treated leaves than untreated leaves.

Table 3: Effect of foliar spray with inducers on total phenol content of tomato leaves after 72, 144 and 216 hrs of pathogen inoculation

Treatment	Total soluble protein content (mg/g fresh leaves)			Increase in Protein contents (%)	
	72 hrs	144 hrs	216 hrs	Over Check-I	Over Check-II
IAA	2.11	2.39	2.09	16.75	23.92
SAAF	2.23	2.47	2.24	22.32	29.02
DPHP	2.26	2.37	2.28	23.00	29.65
HP	2.23	2.39	2.37	26.58	32.91
CC	2.24	2.45	2.42	28.10	34.30
SA	2.04	2.35	2.25	23.68	30.26
FC	2.02	2.37	2.27	23.35	29.96
Check-I	1.58	1.67	1.74	—	8.62
Check-II	1.69	1.59	1.43	-9.43	—
CD (P = 0.05)	0.29	0.36	0.34		

Table 4: Correlation of disease severity with soluble protein and total phenol content

Variable	Hrs	Correlation coefficient (r) With disease severity	Regression equation
Soluble protein	72	–0.4312	Y=60.92–1.27x
	144	–0.4694	Y=63.03–1.35x
	216	–0.4589	y=61.57–1.29x
Total Phenol	72	–0.3441	Y=43.03–8.42x
	144	–0.3835	Y=46.25–9.83x
	216	–0.3264	y=46.25–9.56x

Total Phenol

The result presented in Table 3 indicated that the total phenol content was maximum in the leaves treated with calcium chloride. The maximum amount of total phenols in treated leaves with calcium chloride (2.24 mg/g) was more than healthy (1.58 mg/g leaf) and diseased (1.69 mg/g leaf) leaves at 72 hrs. of inoculation. The concentration further increased from 3.24 to 3.45 mg/g leaf in the same treatment at 144 hrs of treatment, but decreased the concentration to 3.12 mg/g of fresh leaf at 216 hrs of treatment. The per cent increase in total phenol in respect to calcium chloride treated leaves over healthy and diseased plants was 28.1% and 34.3% at 216 hrs. of treatment. From the table, it is also clear that there was no appreciable difference in phenolic contents in other treatments, although

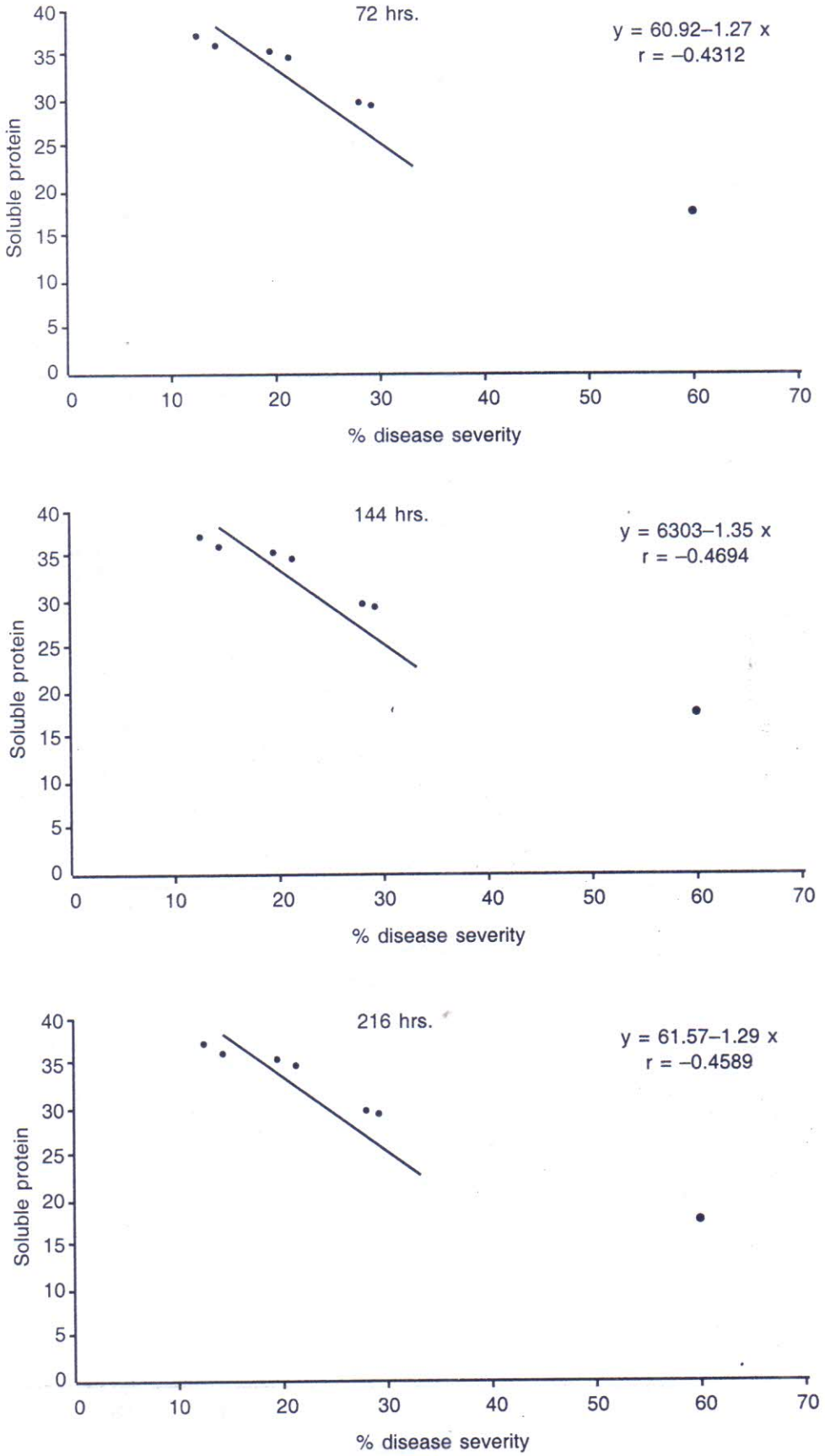

Fig 1. Correlation between disease severity and soluble protein due to pre-application of inducers at 72, 144 and 216 hrs

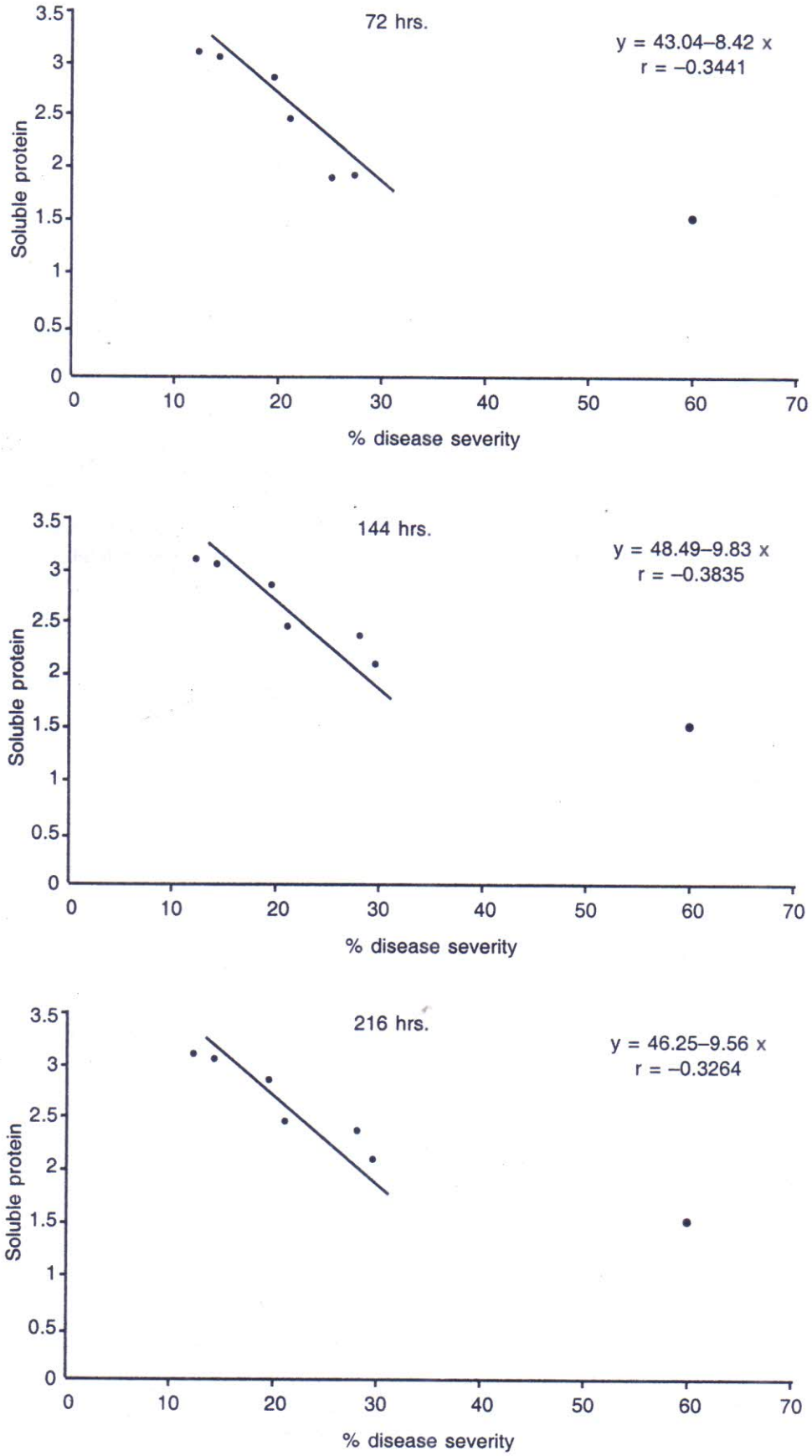

Fig 2. Correlation between disease severity and total phenol due to pre-application of inducers at 72, 144 and 216 hrs

quantitative variation existed among different treatments. By and large the contents of both phenolic compounds and soluble protein in various treatments were more at 144 hrs but after that it decreased. This indicated that both the compounds are produced in plant up to certain period of time. Chowdhury (1995) showed that biochemical resistance in ground nut plants to *Puccinia arachidis* by seed treatment with non-conventional chemicals. Seed treatment with cyclohexamide, cupric chloride, DL-phenylalanine and IAA provided induced resistance in ground nut and recorded 34% to 67% increased in total phenol over diseased plants and 11% to 55% higher as compared to untreated healthy plants. Similarly, total phenol, OD (ortho-dihydric) phenol was increased in ground nut due to infection of *Cercospora arachidicola* (Sindhnan and Prashar, 1996). Cruickshank and Perrin (1964) reported that the metabolic changes occurring in diseased plants, frequently lead to accumulation of aromatics, especially phenolic compounds. From this observation it was apparent that pre-application of chemicals accelerated the accumulation of total phenol in treated plants thereby imparted resistance in tomato against pathogenic infection.

Correlation coefficient and Regression equation

The leaves treated with inducers showed decrease disease incidence with increased level of soluble protein (Table 2). There was negative correlation r = -0.4312 (72 hrs), -0.4694 (144 hrs) and -0.4589 (216 hrs) between disease severity and soluble protein content after different hours of treatment. Similarly, disease incidence was decreased with increased level of total phenol. There was also negative correlation r = -0.3441 (72 hrs). -0.3835 (144 hrs) and -0.3264 (216 hrs) between disease incidence and total phenol after different hours of treatment.

The result summarized that the pre-application of inorganic chemicals sensitized tomato plant to increase elevated level of soluble protein and total phenol content up to certain period of time resulted immunized tomato plant against Fusarium wilt.

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