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Biochemical Evidences of Defence Response in Tomato against *Fusarium* Wilt Induced by Plant Extracts

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Abstract: The potentiality of different plant extracts like bark of *Eucalyptus lanceolotus* and *Terminalia arjuna*, tubers of *Cyperus rotundus*, leaves of *Withania somnifera*, *Azadirachta indica*, *Datura stramonium*, *Acacia arabica*, *Cymbopogon flexuosus* and *Parthenium hysterophorus*, cloves of *Allium sativum,* bulb of *Allium cepa*, fruits of *Emblica officinalis* and rhizome of *Zingiber officinale* as inducers were assessed on physiological and biochemical activities in tomato against fusarim wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and the results showed that pre-application of inducers provided protection to the tomato plant and reduced the disease intensity. The minimum disease intensity (8.93%) was reported from garlic extract treated plant whereas, in case of control-I it was 96.12%. Treatment with plant extracts as inducers prior to challenge inoculation sensitized the seedlings to produce increased levels of soluble protein. The maximum increase in protein content was found in garlic extract treated seedlings (32.62 mg g^{-1}) after 15 days of pathogen inoculation. A high content of phenols which are indicators of first stage of defence mechanism, was also recorded in treated leaves with maximum in garlic extract treatment representing 2.28 mg g^{-1} of fresh leaves against 1.52 mg g^{-1} of fresh leaves in control-II after 15 days of pathogen inoculation. The soluble protein content (r = -0.5995) and total phenol content (r = -0.5313) both showed a negative correlation with disease incidence. Apart from inducing effect in plant defences, plant extracts have also some direct effect on growth and development of the pathogen. Protein profiling by SDS-PAGE revealed that one new protein is synthesized due to effect of inducers that might be responsible for disease.

Key words: Tomato, induced resistance, *Fusarium oxysporum* f. sp. *lycopersici*, plant extracts, biochemical changes

INTRODUCTION

Tomato is one of the most valued vegetable crops of the world. It has a very high nutritive value and also has antioxidant and curative properties. Production of tomato is limited due to various insect pest and diseases. Among these, fusarium wilt is of great economic importance. The conventional method of its control is based on direct elimination of the pathogen but researches are going on in search of non-conventional and eco-friendly management measures that can give good return to growers. In this context, induced resistance as a technique of phyto-immunity has received great attention. Various types of biological agents, virulent or avirulent strains of pathogens, plant extracts, crude extracts of bio-agents and chemicals which are not considered as fungicides are used as inducers for induction of resistance in various crops (Seleim *et al*., 2011; Metraux *et al*., 1990; Cohen, 1994; Van Loon and Antoniew, 1982; Attitalla *et al*., 1998; De Cal and Melgarejo, 2001). The physical and bio-chemical changes associated with induction of resistance was reported by several workers (Kuc, 1995; Benhamou, 1995; Biswas *et al*., 2003; Van Loon, 1983; Kessmann *et al*., 1990). There are also several reports indicating resistance due to activity of plant extracts as well as components of plant extracts, (Doubrava *et al*., 1988; Singh *et al*., 1990; Derbalah *et al*., 2011; Yokoyama *et al*., 1991; Baysal *et al*., 2002; Wurms *et al*., 1999; Daayf *et al*., 2000). The mechanism of resistance might be due to increased levels of p-coumaric, caffeic and ferulic acids and ferulic acid methyl ester in cucumber treated leaves. Akila *et al*. (2011) found that reduction in fusarium wilt of banana by plant products like extracts of *Datura metal*, two botanical fungicides along with biocontrol agents *P. fluorescence* (Pf-1) and *Bacillus subtilis* (TRC-54) was positively correlated with induction of defence related enzymes peroxidase and polyphenol oxidase. Mandal *et al*. (2009)

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also found increased activities of PAL and peroxidases at the time of induction of resistance in tomato against fusarium wilt by exogenous application of salicylic acid. The induction of resistance associated with such biochemical changes in plants were reported by several workers (Retig, 1974; Boller, 1985; Martern and Kneusel, 1988; Hammerschmidt and Kuc, 1995; He *et al*., 2002; Biswas *et al*., 2003; Kumawat *et al*., 2008). Therefore, the present study was under taken to find out the biochemical evidences of defence response in tomato against *Fusarium* wilt induced by plant extracts.

MATERIALS AND METHODS

Collection of diseased plant sample: The present investigation was undertaken during 2008-2010 at Department of Plant Pathology, CSA University of Agriculture and Technology, Kanpur. The pathogen *Fusarium oxysporum* f. sp. *lycopersici* was isolated from a diseased plant showing typical wilt symptoms collected from Vegetable Research Farm, CSAUAT, Kanpur.

Isolation and purification of the pathogen: The diseased plant's stem showing typical wilt symptom was washed thoroughly with distilled water and a part was cut with sterilized knife into small pieces. The chopped pieces were further sanitized by dipping in 0.1% $HgCl_2$ solution and than washed with distilled water thrice. The surface sterilized stem pieces were then placed over PDA which was poured previously in sterilized petri plate. The plates were sealed and incubated at 27±1°C. After growth, the fungus was purified by hyphal tip method.

Identification of the pathogen: The fungus was observed under a compound microscope and identity of the pathogen was established on the basis of morphological and cultural characteristics described by Synder and Hansen (1940). The culture of the pathogen was maintained on PDA at 27±1°C for further investigation.

Preparation of pathogen inoculum: The Petri plate containing 14 days old culture of the pathogen was taken and flooded with sterile water. The mycelia along with spores were scrapped off with the help of a sterile forceps and collected in a beaker. The suspension was then was sieved with the help of a strainer to remove PDA clods. The collected spore suspension was diluted with distilled water and required concentration of spore suspension was measured with the help of a haemocytometer. About 250 μL spore suspension was pipette into the counting chamber. The counting chamber of the haemocytometer was covered with a cover slip. The haemocytometer was further mounted over a compound microscope. Average number of spores per square was counted and the spore suspension was adjusted to 10,00,000 conidia mL^{-1}.

Collection and preparation of plant extract

Collection: The different plant parts (Table 1) were collected from Students Research Farm, C.S. Azad University of Agriculture and Technology, Kanpur and the vicinity area of Kanpur. The extracts of such plants were used as inducers to induce resistance.

Preparation of plant extracts solution: Different plant extracts were prepared by crushing the plant parts in mortar and pestle along with distilled water. The concentration was kept 1:5 (w/v). It was later filtered with muslin cloth and pure extracts were collected for further study. At the time of spraying, the extracts were diluted in distilled water to make final solution of concentration 1:10 (v/v).

Evaluation of plant extracts as inducer in induced resistance: In order to ascertain the activities of different plant extracts as inducers, pot experiments was conducted in glasshouse at Department of Plant Pathology, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. About 30 cm diameter earthen pots were filled with sterilized soil and water was added to bring the soil in a good tilth. Healthy seeds of tomato variety, Azad T-6 were soaked overnight in different plant extracts (1:5 (w/v)). The next day plant extract treated seeds were than sown in earthen pots. After one month, plants were sprayed with different plant extract solutions (1:10 v/v) separately. Two controls were kept, in one case, plants were sprayed with distilled water only serve as control 1 and in another case, plants were inoculated with conidial suspension of fungus serve as control 2. Three replications were kept for each treatment as well as for both the control. After two days of spraying, all the treated plants except Control-1 were inoculated with spore suspension of the pathogen by root inoculation method. About 2 mL of spore suspension of the pathogen was inoculated in the root zone of each plant. Then all the plants were kept on glasshouse bench at 25±1°C.

Measurement of disease intensity: The measurement of disease intensity was taken after 5, 10 and 15 days of pathogen inoculation. The disease severity was recorded by using 0-4 scale as described by Song *et al*. (2004). The 0-4 scale of the disease severity was classified as follows:

0: No severity, 1: Slight severity, where 25% leave become wilted and one or two leaves became yellow, 2: Moderate severity, two or three leaves became yellow, 50% of leaves became wilted, 3: Extensive severity, the all plant leaves became yellow, 75% of leaves become wilted and growth is inhibited and 4: Complete severity, the whole plant leaves become yellow, 100% of leaves become wilted and the plants die.

The percentage of disease intensity was determined using the formulas as given by Song *et al.* (2004).

$$\text{Disease intensity (\%)} = \frac{\Sigma \text{scale} \times \text{No. of plants infected}}{\text{Highest scale} \times \text{Total No. of plants}} \times 100$$

Biochemical changes due to induced resistance: Tomato leaves were collected from plants sprayed with different treatments and the changes in the content of soluble protein and phenols in leaves were estimated at 5, 10 and 15 days after inoculation of the pathogen.

Estimation of total phenols: The accumulation of phenols in tomato plants after treatment with different inducers followed by inoculation of pathogen was estimated following the procedure developed by Bray and Thorpe (1954) with slight modification. In this method, the total phenols estimation was carried out with Folin-Ciocalteu Reagent (FCR) which was measured at 650 nm calorimetrically.

Exactly, 1.0 g of leaf sample of tomato was ground in a pestle and mortar along with 80% ethanol (1:10 w/v). It was then centrifuged at 10,000 rpm for 30 min at room temperature in order to homogenate the suspension. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated near to dryness and residues were dissolved in 5 mL of distilled water. Different aliquots were pipette out into test tubes and the volume in each tube was made to 3 mL with distilled water. A test tube with 3 mL distilled water served as blank. Subsequently 0.5 mL of FCR was added and after 3 min, 2 mL of 20% Na_2CO_3 solution was thoroughly mixed in each tube. After that the tubes were placed in boiling water for 1 min and then cooled at room temperature. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) spectrophotometer and the standard curve using different concentration of catechol was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg g^{-1} of sample materials.

Estimation of total soluble protein

Protein extraction: Tomato leaves were harvested from plants sprayed with different treatments. It was then washed with distilled water several times and blotter dried. A quantity of 1.0 g of each sample was cut into small pieces and ground in pestle and mortar using alkaline copper as extraction buffer. The concentration was kept 1:5 (w/v). Alkaline copper solution was prepared by mixing 20% sodium carbonate in 0.1 N NaOH and 0.5% copper sulphate in sodium potassium tartrate. The suspension was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was collected and used for quantification and profiling of protein.

Quantification of protein: The method developed by Lowry *et al.* (1951) was used with slight modification for quantification of the total soluble protein content. Different aliquots of working standard solution of Bovine Serum Albumin were pipette out into a series of test tubes. Similarly, same volumes of sample extracts were also pipette out and kept in other test tubes separately. Then volume in all the tubes was made up to 1 mL with distilled water. A tube with 1 mL of distilled water served as a blank. Later on, 5 mL of alkaline copper solution was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 mL of FCR was mixed well and incubated at room temperature for 30 min in dark place. The absorbance at 660 nm against the blank was read. The standard graph of BSA was drawn to calculate the amount of soluble protein in different samples. Protein estimated was represented as mg of fresh leaf samples.

Protein profiling: Profiling of soluble proteins was also done in various treatments. Analysis of total soluble proteins through SDS PAGE was carried out to determine when other new protein is synthesized or not due to response of resistance to *Fusarium oxysporum* f. sp.

Table 1: List of plant parts used as inducer

Common name	Botanical name
Bark of eucalyptus	*Eucalyptus lanceolotus*
Bark of arjun	*Terminalia arjuna*
Tubers of motha	*Cyperus rotundus*
Ashwagandha leaves	*Withania somnifera*
Neem leaves	*Azadirachta indica*
Onion bulb	*Allium cepa*
Datura leaves	*Datura stramonium*
Garlic cloves	*Allium sativum*
Babool leaves	*Acacia arabica*
Lemongrass leaves	*Cymbopogon flexuosus*
Aonla fruit	*Emblica officinalis*
Ginger rhizome	*Zingiber officinale*
Parthenium leaves	*Parthenium hysterophorus*

Table 2: Chemical composition for preparation of stacking and resolving gel

	Quantity	
Component	Stacking gel (4%)	Resolving gel (12%)
Acrylamide-bisacrylamide 30%	2.6 mL	20.0 mL
Tris-HCl	5.0 mL (0.5 M, pH 6.8)	12.5 mL (1.5 M, pH 8.8)
SDS (10%)	0.20 mL	0.50 mL
TEMED	10.0 μL	25 μL
APS (10%)	100.00 μL	250 μL
Distilled water	12.1 mL	16.7 mL
Total	20.0 mL	50.0 mL

lycopersici. Soluble protein was electrophoresed by 12% SDS polyacrylamide gel, based on the method of Laemmli (1970).

Gel preparation: In order to prepare stacking and resolving gel, the following quantities of different chemicals were mentioned in Table 2.

All the chemicals required for preparing resolving gel were mixed well and poured into vertical cassette leaving behind 3-4 cm from upper side. Subsequently, stacking gel solution was poured over the resolving gel. A comb was inserted into the gel mould to create wells for sample loading.

Sample loading: Exactly 75 μL of extracted soluble protein were taken in an Eppendorf and mixed with 25 μL of sample buffer and 5 μL of tracking dye (Bromophenol blue). Before loading the sample, it was boiled for 1 min at 100°C in presence of reducing agent 2-mercaptoethanol which further denatures the protein by reducing disulfide linkage, thus overcoming some forms of tertiary and quaternary structures. Exactly 20 μL of sample was poured in each well. Then electrophoresis was carried out in Tris-glycine buffer at 30 mA current in stacking gel and 40 mA in separating gel. The electrophoresis was stopped after the tracking dye reached at the bottom of the gel. The gel was then separated gently from the electrophoresis unit and placed in staining solution of Coomassie Brilliant Blue. After destaining, gel was illuminated with diffused fluorescent light and photographed.

Statistical analysis: All the experiments were conducted in triplicates in along with equal number of appropriate controls. The data obtained was subjected to analysis of variance technique using Completely Randomized Design (CRD) following Gomez and Gomez (1976).

RESULTS

Effect of inducers on development of disease: The effect of pre-inoculation with plant extracts on tomato plants revealed that there was decline in wilt intensity under glasshouse condition (Table 3). The susceptible variety Azad T-6 of tomato showed maximum with 96.12% disease intensity in control-II. On the other hand the minimum wilt intensity was recorded in garlic extract treated plants which was 8.93% followed by neem, ginger, onion and motha treated plants, showing 12.83, 13.01, 13.56 and 16.41%, respectively at 15 days of pathogen inoculation. The decrease in disease intensity may be due to the activities of plant extracts which act as inducers in inducing resistance in plant against *Fusarium oxysporum* f. sp. *lycopersici*.

Table 3: Effect of plant extracts on disease intensity of *Fusarium* wilt of tomato

	Wilt intensity (days)		
Treatment	5	10	15
Eucalyptus lanceolotus	12.75	18.82	21.53
Terminalia arjuna	15.56	20.11	23.81
Cyperus rotundus	9.04	13.04	16.41
Withania somnifera	9.32	13.32	16.92
Azadirachta indica	5.28	9.48	12.83
Allium cepa	6.20	10.31	13.56
Datura stramonium	14.21	19.14	22.23
Allium sativum	1.75	5.62	8.93
Acacia arabica	10.89	15.91	18.31
Cymbopogon flexuosus	10.96	16.04	19.16
Emblica officinale	12.50	18.21	21.32
Zingiber officinale	6.13	10.02	13.01
Parthenium hysterophorus	19.48	23.15	26.51
Control-1 (healthy)	0.00	0.00	0.00
Control-2 (diseased)	62.11	80.92	96.12
SE	6.912807	5.742	6.314
CD (p = 0.05%)	14.115950	13.270	14.004

Biochemical changes associated with induction of resistance by plant extracts as inducers

Total soluble protein: The soluble protein content (Table 4) was found increased in all treatments but maximum increase was noted in case of garlic extract treated plant followed by neem, ginger and onion which are 51.23, 49.51, 46.27 and 42.69% increase over control-1 and 55.44, 53.58, 50.08 and 46.21% over control-2. Other treatments also showed increased amount of the total soluble protein content over both the controls. From the table it is also clear that the total soluble protein content increased from 5 to 10 days of pathogen inoculation but it was again decreased from 10-15th days. These indicated that maximum production of soluble protein take place at 10 days of pathogen inoculation which perhaps provided protection against pathogen infection.

Total phenols: The result of total phenols content (Table 5) shows that there is increased level phenol in plant extract treated plants compared to both controls. The total phenols content is maximum for garlic (2.28 mg g^{-1}), followed by neem (2.24 mg g^{-1}), ginger (2.22 mg g^{-1}), onion (2.19 mg g^{-1}) and motha (2.05 mg g^{-1})

Table 4: Effect of foliar spray with plant extract on total soluble protein content of tomato leaves after 5, 10 and 15 days of pathogen inoculation

	Total soluble protein content mg g^{-1} fresh leaves (day)			Increase (%)	
Treatment	5	10	15	Control-1	Control-2
Eucalyptus lanceolotus	25.85	27.63	26.45	22.62	24.49
Terminalia arjuna	24.14	26.58	24.63	14.19	15.35
Cyperus rotundus	29.49	30.67	29.16	35.19	38.08
Withania somnifera	29.06	29.67	28.95	34.21	37.02
Azadirachta indica	30.39	32.75	32.25	49.51	53.58
Allium cepa	29.67	31.52	30.78	42.69	46.21
Datura stramonium	25.37	26.58	25.57	18.54	20.07
Allium sativum	30.51	33.24	32.62	51.23	55.44
Acacia arabica	28.16	29.42	28.86	33.80	36.58
Cymbopogon flexuosus	27.57	28.76	27.49	27.45	29.70
Emblica officinalis	26.04	28.31	27.33	26.70	28.90
Zingiber officinale	30.19	32.11	31.55	46.27	50.08
Parthenium hysterophorus	23.40	25.40	23.38	8.39	9.08
Control-1 (healthy)	20.94	22.49	21.57		7.60
Control-2 (diseased)	19.89	20.77	19.93	-8.22	
SE	0.16529	0.200726	0.155595		
CD (p = 0.05)	0.33745	0.409883	0.317726		

Table 5: Effect of foliar spray with plant extracts on total phenols content of tomato leaves after 5, 10 and 15 days of pathogen inoculation

	Total phenols content mg g^{-1} fresh leaves (days)			Increase (%)	
Treatment	5	10	15	Control-1	Control-2
Eucalyptus lanceolotus	1.606	1.78	1.75	15.13	16.20
Terminalia arjuna	1.55	1.69	1.63	7.23	7.75
Cyperus rotundus	1.89	2.11	2.05	34.86	37.32
Withania somnifera	1.83	2.08	1.99	30.92	33.09
Azadirachta indica	2.09	2.31	2.24	47.37	50.70
Allium cepa	1.98	2.22	2.19	44.08	47.18
Datura stramonium	1.58	1.74	1.68	10.53	11.27
Allium sativum	2.12	2.37	2.28	50.00	53.52
Acacia arabica	1.78	1.92	1.91	25.66	27.46
Cymbopogon flexuosus	1.75	1.86	1.84	21.05	22.53
Emblica officinalis	1.68	1.84	1.79	17.76	19.01
Zingiber officinale	2.03	2.27	2.22	46.05	49.30
Parthenium hysterophorus	1.52	1.62	1.54	1.32	1.409
Control-1 (healthy)	1.49	1.59	1.52		
Control-2 (diseased)	1.38	1.48	1.42		
SE	0.016724	0.008878	0.010306		
CD (p = 0.05)	0.034151	0.018128	0.021046		

at 15 days of pathogen inoculation. Similarly, total phenols content was also found maximum at 10 days of pathogen inoculation. The disease intensity in these treatments was also less.

Protein profiling: The result of protein profiling showed that there is increase in number of protein bands in various treatments compared to control-1 and control-2 (Fig. 3). The increased number of bands indicates that some new types of proteins are synthesized at the time of induction of resistance.

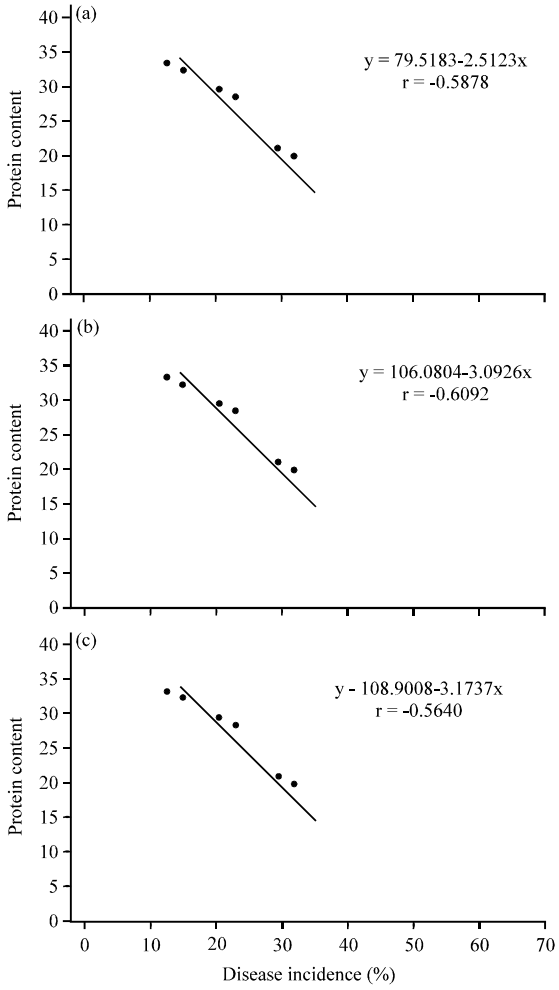

Fig. 1(a-c): Correlation between disease intensity and protein content due to effect of inducers at (a) 5, (b) 10 and (c) 15 days of final inoculation

Correlation coefficient of disease intensity with total soluble protein and total phenol: The leaves treated with plant extracts as inducers of defence response showed that decreased disease intensity with increased level of soluble protein. A negative correlation (r), -0.5878, -0.5934 and -0.6092 was found between disease intensity and soluble protein content (Fig. 1). The reduced disease intensity indicates that some protein must be associated with induction of resistance against the pathogen. Similarly, disease intensity decreased with increased level of total phenols content and there was also a negative correlation (r), -0.5423, -0.5640 and -0.5119 between total phenols content and disease intensity (Fig. 2). The negative correlation coefficient between disease intensity and phenols content indicates the role of phenols in

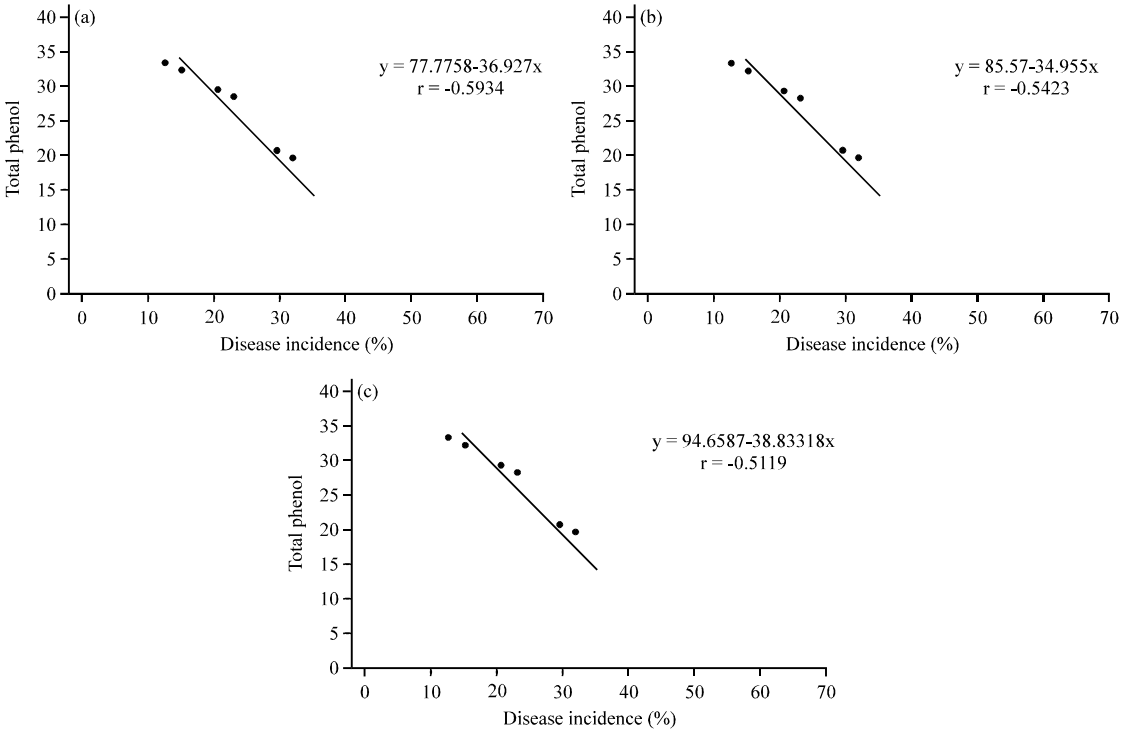

Fig. 2(a-c): Correlation between disease intensity and total phenol due to effect of inducers at (a) 5, (b) 10 and (c) 15 days of final inoculation

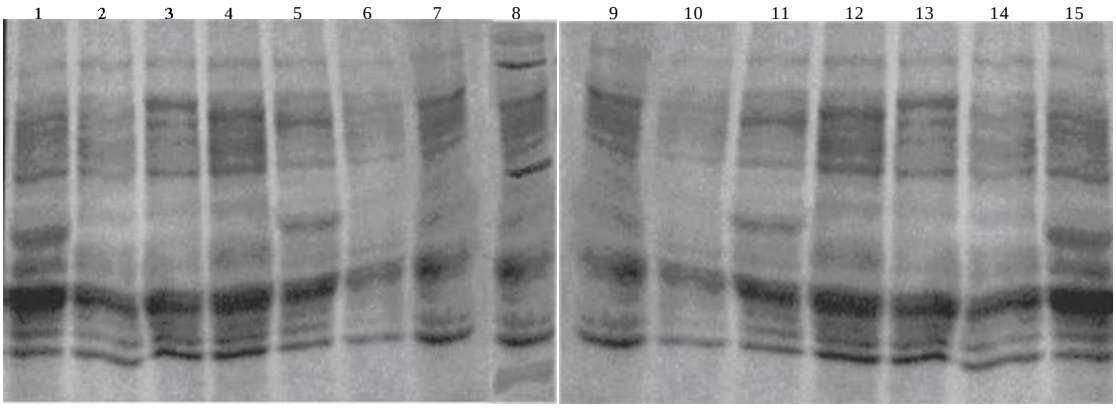

Fig. 3: Banding pattern of soluble protein of different treatment resolved by SDS-PAGE, 1: Garlic, 2: Datura, 3: Babool, 4: Onion, 5: Motha, 6: Parthenium, 7: Arjun, 8: Eucalyptus, 9: Control-1, 10: Control-2, 11: Ginger, 12: Neem, 13: Ashwagandha, 14: Aonla and 15: Lemon grass

inducing resistance. Phenols are well known antifungal, antibacterial and antiviral compounds. The corresponding simple regression equation also showed the negative relation between total soluble protein and disease intensity as well as total phenols and disease intensity.

DISCUSSION

In the present study, the tested plant extracts showed antifungal activity against Fusarium wilt pathogen in tomato. The efficacy of different plant extracts against (*Lycopersicon esculentum* (L.) either

under laboratory or green house condition have been reported (Sallam, 2011; Zaker and Mosallanejad, 2010; Bergaoui *et al*., 2007; Latha *et al*., 2009; Seleim *et al*., 2011). Antoniw *et al*. (1980) considered that Pathogenesis Related proteins (PR protein) are involved in plant defense response against the pathogens. Boller (1985) also opined that proteins are associated with defense of plants against fungi and bacteria by their action on cell walls degrading enzyme. Most of antifungal proteins are in the form of chitinase, peroxidases, β-1, 3 glucanase etc. In the presence of defense response, synthesis of antifungal enzymes are enhanced and accumulation of these antifungal elements causes lysis of the cell wall of pathogens (Biswas *et al*., 2003).

Phytoalexins have their role in defense response involved in disease resistance are phenolic in nature in chemical constitution. Phenols are involved in disease resistance in many ways like hypersensitive cell death or lignifications of cell walls or increased content of phenols (Nicholson and Hammerschmidt, 1992; Kumar, 2008; Kumawat *et al*., 2008). Jabeen *et al*. (2009) found that total phenols, ortho-dihydroxy phenols and enzyme activity were high in wilt resistant chilli cultivars and there was a positive correlation between host resistance and amount of phenols and increased enzymatic activities. Retig (1974) found that enhanced resistance in tomato plant against *Fusarium* wilt by ethophone treatment was associated with enhanced peroxidase and polyphenol oxidase activities. He *et al*. (2002) found that reduced disease severity of *Fusarium oxysporum* f. sp. *asparagi* in *Asparagus officinalis* with non pathogenic isolates of *F. oxysporum* was associated with induction of systemic resistance where activities of peroxidase and Phenylalanine Ammonia Lyase (PAL) and lignin concentration were higher. Patil *et al*. (2011) also found increased concentration of peroxidase and polyphenol oxidase activities at the time of induction of ISR by non-pathogenic strains of *F. solani* and *F. moniliforme* against *F. o*. f. sp. *lycopersici*. Biswas *et al*. (2003) also reported that some new proteins were associated with resistance to *Bipolaris sorokiniana* induced by crude extracts of *Chaetomium globosum*. The banding pattern obtained in protein profiling by SDS-PAGE showed qualitative and quantitative differences on comparing the pattern of soluble proteins with standard among the treatments. They also found that some new proteins of different molecular weight i.e., 110, 105, 38, 35 and 32 kDa were resolved by SDS PAGE analysis which was missing in unchallenged healthy seedlings, diseased seedlings and in seedlings receiving some other treatments. Li *et al*. (2003) also isolated a new 28 kDa protein by western blot using polyclonal antibody against β-1,3-glucanase from *Verticillium dahliae* resistant cotton cultivars. El-Kallal (2007) reported increase in total soluble proteins in both roots and leaves of arbuscular mycorrhiza, JA and SA with treated plants at the time of induction of resistance against fusarium wilt of tomato. The possible role of the new proteins for induction of resistance was speculated. Antoniw *et al*. (1980) considered that PR-proteins are involved with defense in plants against the pathogens. Tuzun *et al*. (1989) reported that the induction of systemic resistance in tobacco after inoculation with *Pseudomonas tabaci* followed an increase in concentration of PR-proteins. A 23 kDa protein was detected in leaves of tobacco which was previously immunized with TMV (Spiegel *et al*., 1989). Mishra *et al*. (2011) reported that biochemical mechanism of resistance to *Alternaria* blight by different varieties of wheat.

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