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ALTERATION IN BIOCHEMICAL RESPONSES IN LEAVES OF POTATO DUE TO COMMON SCAB DISEASE

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ABSTRACT

The current study was designed at the research area of Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan during 2017–2020. Fresh leaves of inoculated and un-inoculated potato varieties highly susceptible (FD 76-36), moderately susceptible (FD 73-110), and resistant (Esmee)/ test lines were collected during 2017-18 and 2018-19, at 35, 65 and 95 days after sowing for biochemical analysis. Superoxide dismutase activity, Catalase activity, Per-oxidase activity, Protein and Total phenolics contents were quantified. FD 76-36 exhibited minimum SOD activity (67.733, 45.637, 24.910) %, followed by FD 73-110 (70.303,57.893, 42.513) % and Esmee (84.567, 65.167, 45.873) % after 35, 65 and 95 days of inoculation respectively as compared to control. FD 76-36 expressed minimum CAT activity (10.990, 7.473, 3.413) %, followed by Esmee (13.537, 9.630, 8.147) % and FD 73-110 (19.277, 8.147, 6.170) % after 35, 65 and 95 days of inoculation respectively as compared to control. FD 76-36 exhibited minimum POD activity (0.2133, 0.2767, 0.3600) %, followed by FD 73-110 (0.2900, 0.5567, 0.6300) % and Esmee (0.8800, 1.2733, 1.5433) % after 35, 65 and 95 days of inoculation respectively as compared to control. FD 76-36 expressed minimum protein contents (3.097, 2.873, 1.260) %, followed by FD 73-110 (7.907, 5.423, 4.267) % and Esmee (12.163, 8.633, 5.127) % after 35, 65 and 95 days of inoculation respectively as compared to control. FD 76-36 expressed minimum TPC (136.31, 115.58, 70.77) %, followed by FD 73-110 (165.85, 136.75, 86.89) % and Esmee (188.42, 158.38, 109.00) % after 35, 65 and 95 days of inoculation respectively as compared to control. The experiment was conducted with a randomized complete block design (RCBD) and three replications. The experimental data were analyzed using Fisher's analysis of variance technique and treatment means were compared by the least significance difference (LSD) test at a 5% probability level.

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INTRODUCTION

Potato is a commonly grown beneficial food crop that brings about more energy and useable protein for consumers. It comprised higher nutritional value along with carbohydrates, proteins, minerals, and vitamins (Reddy *et al.*, 2018). Potato crop is prone to attack by a number of diseases, but common scab is an economically important disease of potato in Pakistan as well as worldwide, causing $1.2 million loss every year in Canada (Al-Mughrabi *et al.*, 2016).

Common scab is caused by *Streptomyces scabies,* is the most ravaging biotic stress to potato crop (Li *et al.*,

2021). The genus *Streptomyces* is the largest genus of the bacterium, present in the soil rhizosphere. Thirty-eight species of *Streptomyces* are pathogenic to plants, out of which twenty-three species are pathogenic to potato. The *Streptomyces scabies* is a gram positive, filamentous, and aerobic prokaryote, belongs to order *Actinomycetale* family *Streptomycetaceae*; genus *Streptomyces* and species *scabies* (Lerat *et al.*, 2009).

Under biotic stress, plants have the potential to resist themselves by activating their defense machinery (Iriti and Varoni, 2015). Antioxidant system is a very significant system evolved by plants to cope up supra-optimally produced superoxide radicals and other organic radicals. Increased activity of antioxidant enzymes restores photosynthetic imbalance caused by the production of bacterial lesions and decreases oxidative damage (Ramzan *et al.*, 2021). Progressive alterations in biochemical affect the physiological process in plant and reduce nutrient uptake, assimilation, absorption, mobility and their consumption (Gomes *et al.*, 2014). Reactive oxygen species (ROS) production is a crucial event in plant pathogen interaction (O'Brien *et al.*, 2012). Pathogen attack, results in elevated levels of ROS induced by signaling molecules like salicylic acid (SA) during hypersensitive response (HR) and programmed cell death (Kumar *et al.*, 2011). Defense mechanism in plant is highly influenced by phenolics and sugar compounds as these substances contribute well to strengthen the cell wall by preventing it from mechanical penetration, reducing susceptibility towards certain cell-wall degrading enzymes and blocking the entrance of toxins (Boriboonkaset *et al.*, 2013). It has been investigated that phenols and their oxidizing products affect plant resistance mechanism against pathogen attack (Lattanzio *et al.*, 2006). Peroxidase plays an important role in non-specific defense for plants under stressful conditions and in the presence of injury up to subcellular levels (Pandey *et al.*, 2017). Biochemical compounds are important factors in plant-disease interactions. Quantifying these alterations helps researchers to develop suitable solution for disease management (Hameed *et al.*, 2021).

MATERIAL AND METHODS

Preparation of Medium

Nutrient agar medium (NA) was used for isolation of bacteria (Carmen, 1995). The beef extract (3.0 g) and peptone (5.0 g) were mixed with 500 mL of sterilized water in a conical flask. Agar (15 g) was added slowly in the medium and the contents were thoroughly stirred. Glucose (2.5 g) was added to the boiled solution and the medium was filtered through cheese cloth and made the volume 1000 mL. The conical flask (1L) was plugged properly and autoclaved the dissolved mixture at 121 ˚C for 20 minutes at 15 Psi. The conical flask and Petri plates were taken out from the autoclave (RTA 85) and allowed to cool at room temperature. Lukewarm medium (20 mL) was poured into 9 Cm diameter sterilized Petri plates. When medium was solidified Petri pates were wrapped with parafilm tape and stored in the refrigerator (PL 6500) at 4 ˚C for further use (Turkensteen, 1986).

Isolation, Identification and Purification of Pathogen (*Streptomyces scabies*) from Tuber Samples

Potato tubers having scab symptoms were washed out with tap water and cut into small pieces (2-3 cm). The infected pieces were surface sterilized by dipping in 1% NaOCl and rinsed with distilled water twice for 1 minute. Then these infected pieces of tubers were dried on a sterilized blotter paper.

Five small, infected pieces were placed on NA medium plates. Then these Petri plates were incubated at 30 $\pm$ 2 ^{o}C (Khan *et al.*, 1999) for 96 hours. Whitish growth of the bacterial colonies appeared on the surface of the medium, were picked with the help of loop of needle, and streaked on other medium containing plates for purification. The pathogen was purified by using streaking method (Goszczynska *et al.*, 2000). Sterilized streaking loop was touched on the *bacterial* colonies and streaked in Petri plates having medium in zigzag way. Loop was kept sterilized after every streaking Pure single colony from the isolated colonies of the bacterium was picked up and transferred to each slant of nutrient agar (NA) medium and incubated at 30 ^{o}C for further use (Carmen, 1995). Through gram staining, slides of pathogen were prepared and examined under the light microscope with magnification 200x (X-2006). Morphological characters of the pathogen (spore shape, spore colour and spore size) were observed and bacterium was identified as *Streptomyces scabies* (Goth *et al.,* 1995).

Extract Preparation for Biochemical Profiling

For analyzing soluble protein and antioxidant enzymes (SOD, POD and CAT) extraction was made as described by Naqvi *et al.* (2011). About 0.5 g of sample was ground with mortars and pestle in 2 mL of 50mM phosphate

buffer and extract was prepared. The extracts were filtered and centrifuged (Centrifuge Machine M-800) at RPM (12000 x g) for 5 minutes. Extracts were separated from residues in Eppendorf tubes (2 mL) for analysis. Due to perishable nature, extracts were stored at -80^{0}C until analysis in a refrigerator (PL 6500).

Soluble Protein Contents

Protein contents were determined by following the method described by Bradford (Bradford, 1976). A mixture of sample (50µL) and Bradford reagent (2 mL) was prepared. Blanks was prepared using Bradford reagent. Absorbance was taken at 595 nm (Stat Fax 4200). Protein content was determined by a standard curve prepared with different concentrations of bovine serum albumin (BSA).

Catalase

Activity of catalase (CAT) was determined by using the method described by Liu *et al.* (2009) with some modifications. For the determination of CAT activity, reaction solution 1.9 mL phosphate buffer (PH 7), 1 mL 5.9 Mm H_2O_2 and 0.1 mL enzyme extract was taken and placed about 200uL of the above solution in a 96-well micro plate and took the absorbance at 240 nm every 20 seconds by using microplate reader. One unit of CAT activity was defined as "the change in absorbance is 0.01 unit/minute". The activity of each enzyme is expressed on a protein basis. By following Bradford (1976) protocol who determined the protein concentration from the crude extract.

Peroxidase Activity (POD)

The determination of peroxidase activity was carried out by adopting the method of Liu *et al*. (2009) with some modifications. The POD reaction solution includes 1.9 mL 50mM phosphate buffer (pH 5), 1 mL 40mM H_2O_2, 0.1 mL 20mM guaiacol and 0.1 mL enzyme extract. The absorbance change of the reaction solution was measured at 470nm. One unit of POD activity is defined as "0.01 unit/minute change in absorbance". The activity of each enzyme is expressed on protein basis. by following Bradford (1976) method.

Superoxide Dismutase Activity (SOD)

Activity of SOD was determined by measuring the inhibitory ability of SOD on the photoreduction of nitro blue tetrazole (NBT) by using Stainer and Popovic protocol. The reaction solution contains 0.222 g methionine (15 mL distilled water), 0.015 g NBT (17.5 mL distilled water), 0.0375 g Triton-X (17.5 mL distilled water), 0.0132 g riboflavin (17.5 mL distilled water) and 0.2 molar buffer. The adequate amounts of following ingredients viz. phosphate buffer 500µL, Methionine 200 µL, NBT 100 µL, Triton X 200 µL, Riboflavin 100 µL, Enzyme extract 200 µL and distilled water 800 µL were mixed and kept under UV radiations for 15 minutes after loading 200 µL solution in each well of ELISA plate and absorbance of the solution was estimated at 560 nm with a spectrophotometer. One unit of SOD activity is defined as "the amount of enzyme that inhibits 50% of the photoreduction of NBT".

Total Phenolics Contents (TPC)

Total phenolic contents of the reaction mixture were measured by mixing 5ml FC reagent with 45mL double distilled water, and 10g sodium carbonate with water. 100 µl leaf extract was taken along with 50 µl FC reagent mixture in eppendorf and shaked them thoroughly 150 µL of the sample was loaded on a micro-plate (UltraCruz® ELISA plate, 96 wells), and absorbance was taken at 765 by using ELIA plate reader (Shahid *et al.*, 2012).

Statistical Analysis

The experimental data was analyzed using Fisher's analysis of variance technique and treatment means were compared by least significance difference (LSD) test at 5% probability level (Steel *et al.*, 1997).

RESULTS

Determination of Protein Contents from Inoculated and Un-Inoculated Potato Plants under Greenhouse Conditions

The amount of protein contents was determined from leaves of three varieties; highly susceptible (FD 76-36), moderately susceptible (FD 73-110), and resistant (Esmee). These varieties were inoculated with *Streptomyces scabies* and same set was chosen as control (un-inoculated). Interaction between treatments (T) and time (t) after inoculum indicated that FD 76-36 expressed minimum protein contents (3.097, 2.873, 1.260)%, followed by FD 73-110 (7.907, 5.423, 4.267)%, and Esmee (12.163, 8.633, 5.127)% after 35, 65 and 95 days of inoculation respectively as compared to control (un-inoculated), FD 76-36 (5.787 , 4.607, 2.270), while FD 73-110 (9.833, 7.813, 5.067) and Esmee (15.177, 12.377, 8.157)% after 35, 65 and 95 days respectively (Table & Figure 1).

Table1. Impact of disease on the protein contents with respect to time on potato plants under greenhouse conditions.

Protein (µg/g)						
Time after inoculation/ un-inoculation	FD 76-36		FD 73-110		Esmee	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
After 35 days	3.097de	5.787e	7.907b	9.833c	12.163a	15.177a
After 65 days	2.873e	4.607f	5.423c	7.813d	8.633b	12.377b
After 95 days	1.260f	2.270g	4.267cd	5.067f	5.127c	8.157d
LSD	Inoculated				1.2116	
	Un-inoculated				0.6069	

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

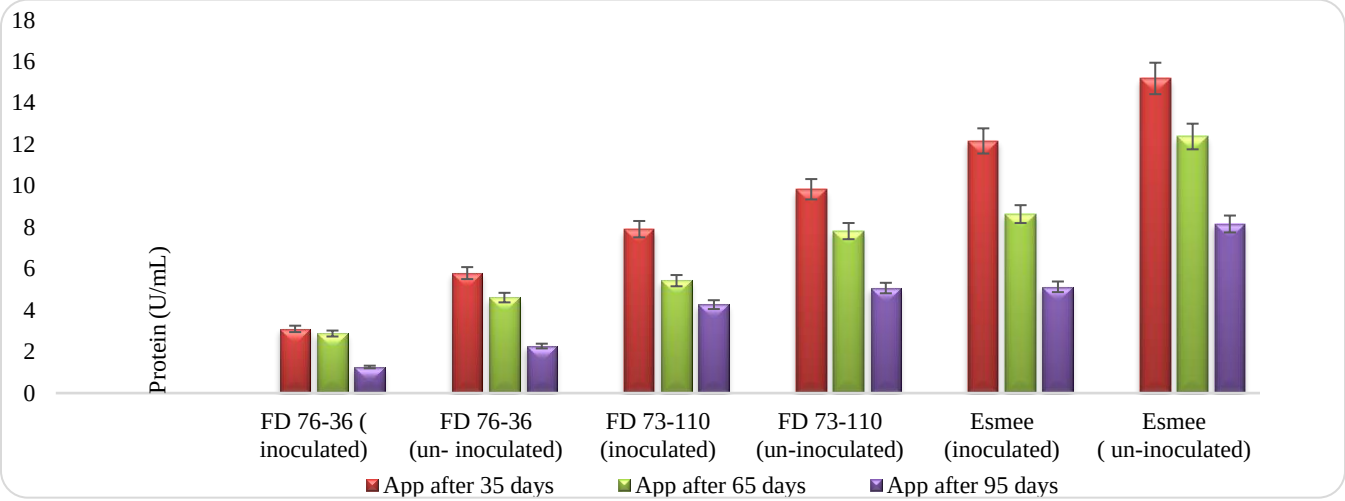

Figure 1. Impact of disease on the protein contents at different time interval on potato plants under greenhouse conditions.

Determination of Peroxidase Activity (POD) from Inoculated and Un-Inoculated Potatoes Plant against Common Scab of Potato under Greenhouse Conditions

The amount of peroxidase activity was determined of three varieties that were found highly susceptible (FD 76-36), moderately susceptible (FD 73-110), and resistant (Esmee). These varieties were inoculated with *Streptomyces scabies* and same set was chosen as control (un-inoculated). Interaction between treatments (T) and time (t) indicated that FD 76-36 exhibited minimum peroxidase activity (0.2133, 0.2767, 0.3600)%, followed by FD 73-110 (0.2900, 0.5567 , 0.6300)% and Esmee (0.8800, 1.2733 , 1.5433)% app after 35, 65 and 95 days after inoculation respectively as compared to control FD 76-36 (0.293, 0.723, 1.470)% , FD 73-110 (0.640, 0.986, 2.770)% and Esmee (0.370, 0.870, 1.483)% app after 35, 65 and 95 days respectively. (Table & Figure 2).

Determination of Superoxide Dismutase Activity (SOD) from Inoculated and Un-Inoculated Potato Plants under Greenhouse Conditions

The amount of superoxide dismutase activity was determined of three varieties that identified as highly susceptible (FD 76-36), moderately susceptible (FD 73-110), and resistant (Esmee). These varieties were inoculated with *Streptomyces scabies* and same set was chosen as control (un-inoculated). Interaction between treatments (T) and time (t) after application indicated that FD 76-36 exhibited minimum superoxide dismutase activity (67.733, 45.637, 24.910)%, followed by FD 73-110 (70.303,57.893, 42.513)% and Esmee (84.567, 65.167, 45.873)% app after 35, 65 and 95 days of inoculation respectively as compared to control ,FD 76-36 (72.797, 51.153, 30.203)% , FD 73-110 (80.743, 58.483, 46.593)% and Esmee (90.253, 71.057, 55.653) % app after 35, 65 and 95 days respectively (Table & Figure 3).

Table2. Impact of disease on the POD at different time interval on potato plants under greenhouse conditions.

Peroxidase activity POD(µg/g)						
Time of App	FD 76-36		FD 73-110		Esmee	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
App after 35 days	0.213f	0.293e	0.290f	0.640cde	0.880c	0.370de
App after 65 days	0.276f	0.723cde	0.556de	0.986bc	1.273b	0.870cd
App after 95 days	0.360ef	1.470b	0.630f	2.770a	1.543a	1.483b
LSD	Inoculated			0.1977		
	Un-inoculated			0.5552		

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

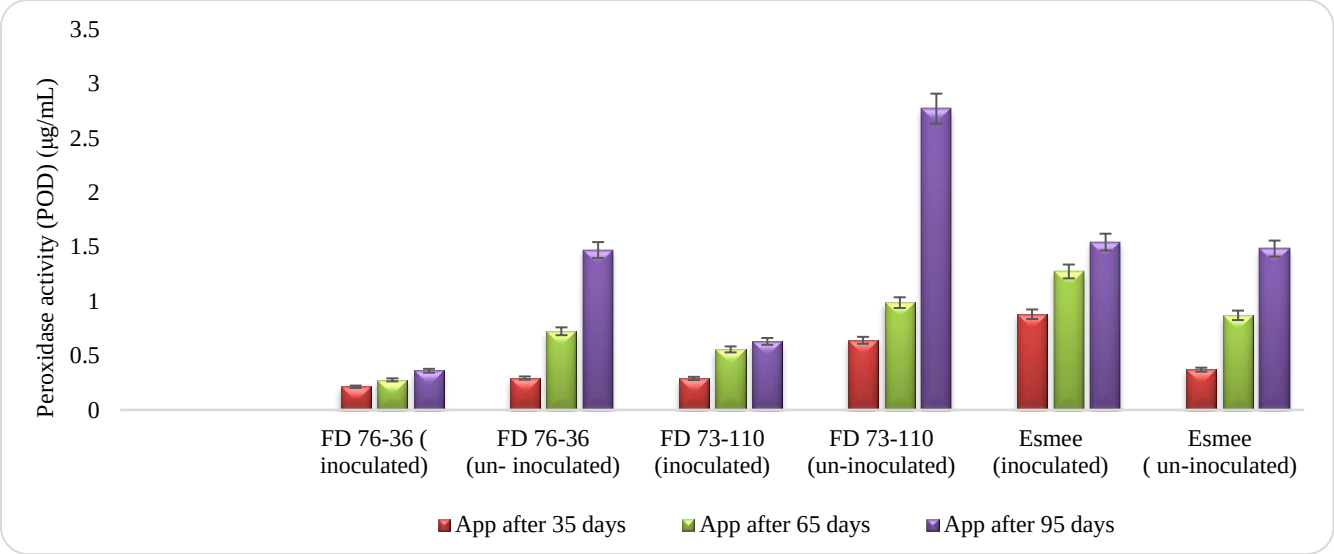

Figure 2. Impact of disease on the POD (µg/g) at different time interval on potato plants under greenhouse conditions.

Table3. Impact of disease on the SOD at different time interval on potato plants under greenhouse conditions.

Superoxide dismutase activity (SOD) (µg/g)						
Time of App	FD 76-36		FD 73-110		Esmee	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
App after 35 days	67.733c	72.797c	70.303b	80.743b	84.567a	90.253a
App after 65 days	45.637f	51.153g	57.893e	58.483e	65.167d	71.057d
App after 95 days	24.91h	30.203i	42.513g	46.593h	45.873f	55.653f
LSD	Inoculated				1.4538	
	Un-inoculated				0.6190	

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

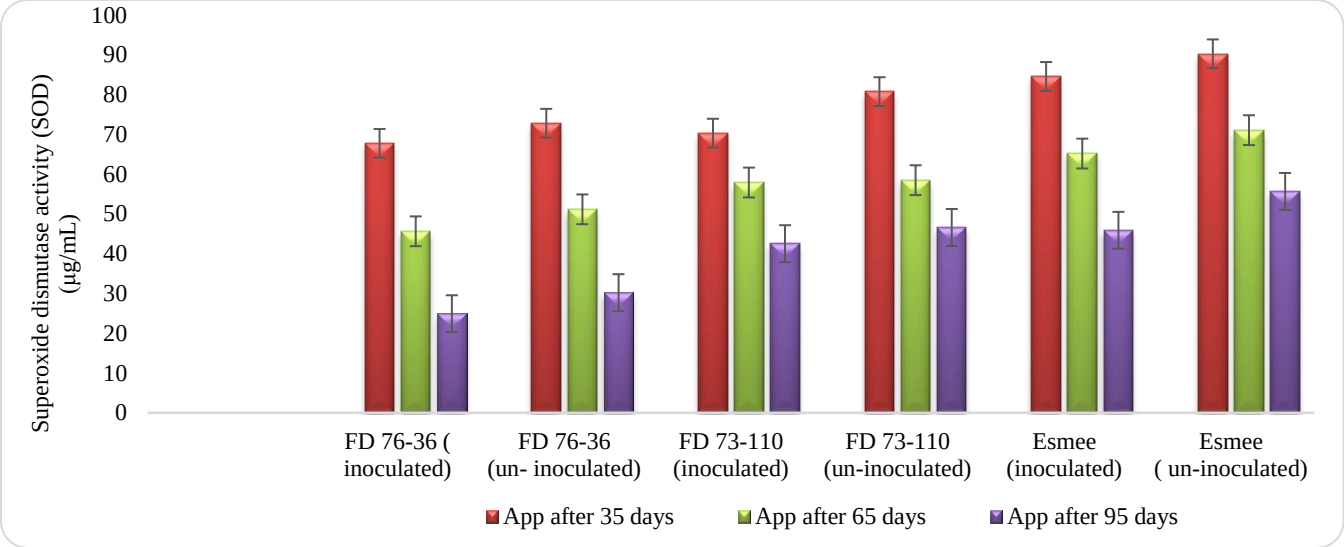

Figure 3. Impact of disease on the SOD at different time interval on potato plants under greenhouse conditions.

Determination of Catalase activity (CAT) from Inoculated and Un-Inoculated Potato Plants under Greenhouse Conditions

The amount of Catalase activity was determined of three varieties that were found highly susceptible (FD 76-36), resistant (Esmee), and moderately susceptible (FD 73-110). These varieties were inoculated with *Streptomyces scabies* and same set was chosen as control (un-inoculated). Interaction between treatments (T) and time (t) indicated that FD 76-36 expressed minimum Catalase activity (10.990 , 7.473, 3.413) %, followed by Esmee (13.537, 9.630, 8.147) % and FD 73-110 (19.277, 8.147, 6.170) % app after 35, 65 and 95 days of inoculation respectively as compared to control , FD 76-36 (9.510, 6.530, 5.57)%, Esmee (10.5, 9.183, 8.313)%, and FD 73-110 (13.847, 11.363, 9.32) % app after 35, 65 and 95 days respectively. (Table & Figure 4).

Determination of Total Phenolics Contents (TPC) from Inoculated and Un-Inoculated Potato Plants under Greenhouse Conditions

The amount of total phenolics contents was determined from three varieties being highly susceptible (FD 76-36), moderately susceptible (FD 73-110), and resistant (Esmee) to bacterium. These varieties were inoculated with *Streptomyces scabies* and same set was chosen as control (un-inoculated). Interaction between treatments (T) and time (t) indicated that FD 76-36 expressed minimum total phenolics contents (136.31, 115.58, 70.77)%, followed by FD 73-110 (165.85, 136.75, 86.89)% and Esmee (188.42, 158.38, 109.00) % app after 35, 65 and 95 days of inoculation respectively as compared to control , FD 76-36 (147.06, 126.29, 78.75) , FD 73-110 (176.05, 147.56, 92.38) and Esmee (208.11, 174.56, 111.29) % app after 35, 65 and 95 days respectively. (Table & Figure 5).

Table4. Impact of disease on the CAT at different time interval on potato plants under greenhouse conditions.

Catalase activity (CAT) ((µg/g))						
Time of App	FD 76-36		Esmee		FD 73-110	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
App after 35 days	10.99	9.51	13.537	10.5	19.277	13.847
App after 65 days	7.473	6.53	9.63	9.183	8.147	11.363
App after 95 days	3.413	5.57	8.147	8.313	6.17	9.32
LSD	Inoculated				1.7571	
	Un-inoculated				1.7478	

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

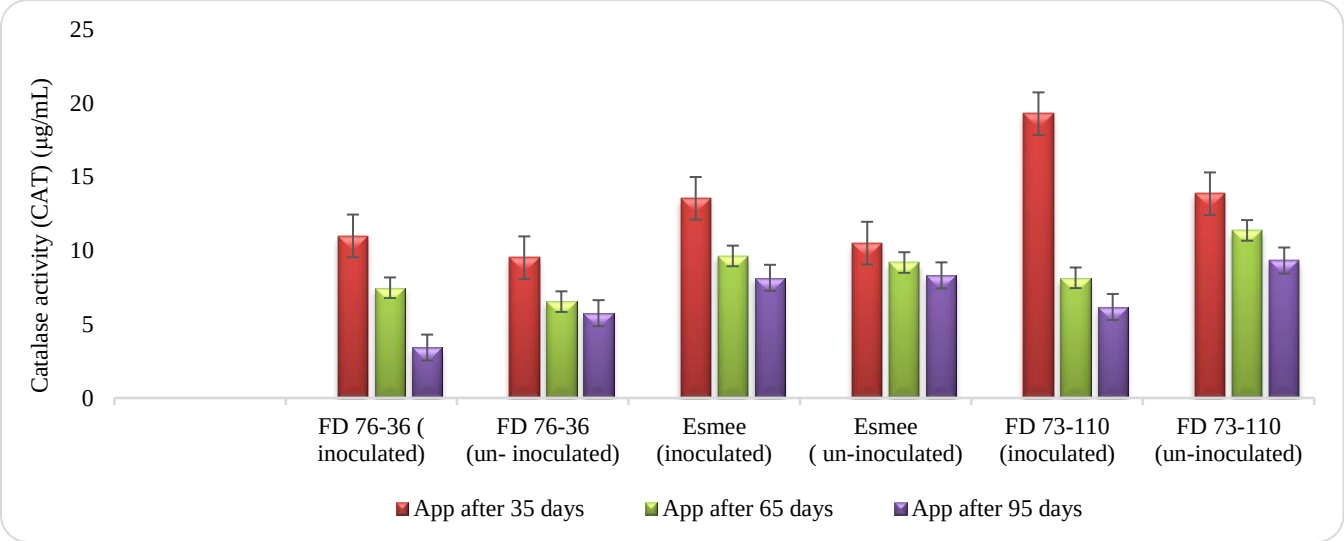

Figure 4. Impact of disease on the CAT with respect to time on potato plants under greenhouse conditions.

Table5. Impact of disease on the TPC (µg/g) at different time interval on potato plants under greenhouse conditions.

Total phenolic contents (TPC) (µg/g)						
Time of App	FD 76-36		FD 73-110		Esmee	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
App after 35 days	136.31d	147.06c	165.85b	176.05b	188.42a	208.11a
App after 65 days	115.58e	126.29d	136.75d	147.56c	158.38c	174.56b
App after 95 days	70.77h	78.75g	86.89g	92.38f	109.00f	111.29e
LSD	Inoculated				2.1974	
	Un-inoculated				1.5994	

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

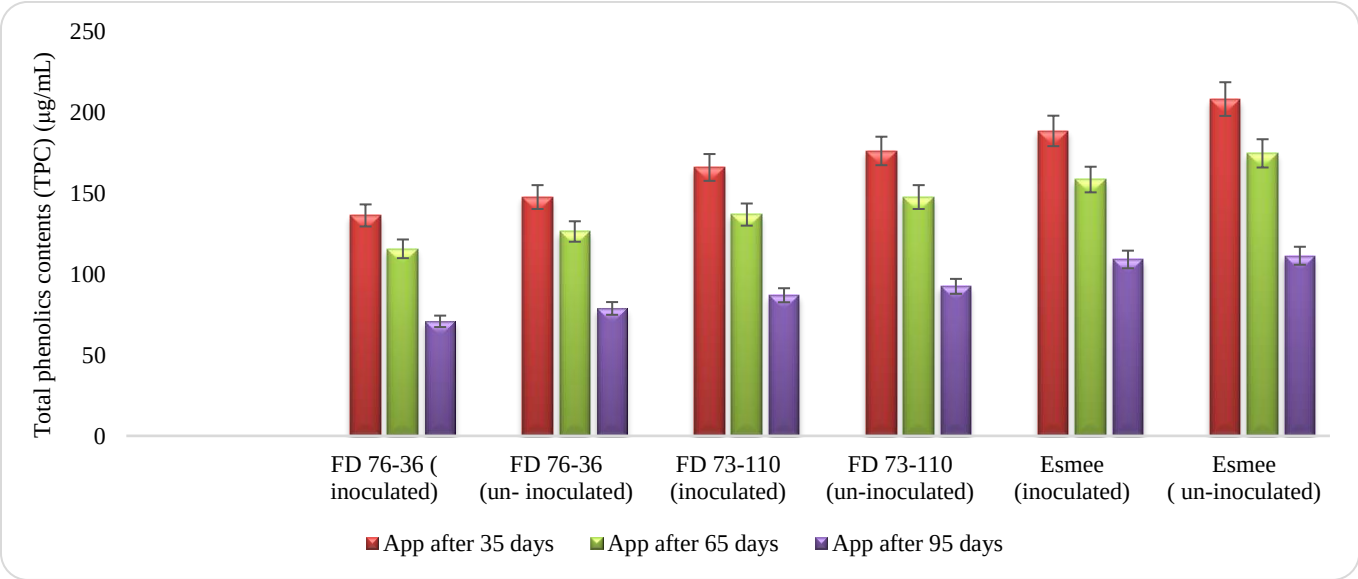

Figure 5. Impact of disease on the TPC at different time interval on potato plants under greenhouse conditions.

DISCUSSION

The present study was designed to quantify the alterations in biochemical compounds (SOD, POD, CAT, TPC, Protein) of both inoculated and uninoculated potato leaves. Biochemicals were significantly varied in inoculated and non-inoculated potato plant. SOD converts super oxides into H_2O_2 and it is involved to increase production of H_2O_2 which is responsible for inhibition of pathogen (Zhang *et al.*, 2009). CAT is involved in scavenging the increased level of H_2O_2 by converting millions of H_2O_2 molecules into water and oxygen. Protein contents were low in infected plant due to host defense and pathogen attack mechanism. Results of the current study exhibited that catalase (CAT), peroxidase (POD), Superoxide dismutase (SOD), protein contents and TPC contents were maximum in non-inoculated resistant variety whereas minimum in inoculated highly susceptible cultivars. Similar performance was given by physiological and biochemical changes in soybean in relation to leaf senescence (Fu *et al.*, 2000), mungbean plant infected by phytoplasma (Hameed *et al.*, 2021), biochemical change in leaves of cotton genotypes infected by CLCuBuV (Siddique *et al.*, 2014). Plant used phenolics for pigmentation, resistance to pathogen, reproduction and many other purposes. The occurrence of phenolic breakdown in plants is significantly affected by the environmental conditions and controlled hereditarily. In present study maximum concentration of TPC was noted in non- inoculated resistant cultivars, however minimum in inoculated highly susceptible cultivars. Changes in phenolic contents caused by raspberry spur and cane blight was reported that healthy plant have maximum total phenolic contents as compared to diseased plants (Mikulic-Petkovsek *et al.*, 2014) while biochemical change in leaves of cotton genotypes infected by CLCuBuV reported that healthy plant has maximum total phenols as compared to diseased plant (Siddique *et al.*, 2014). POD performs different biological functions such as auxins metabolism, biosynthesis of lignin and stiffening of cell wall to provide protection against phytopathogens (Bhardwaj *et al.*, 2014). It also plays vital role in lignification, wound curing as well as in defense against both biotic and abiotic stresses. It was detected that leaves of inoculated citrus plants contained higher amount of Peroxidase while leaves of uninoculated plants have low POD level that was also supported by (Cernadas *et al.*, 2008). The current study is in line with (Hameed *et al.*, 2021). During plant pathogen interaction, antioxidants have a prime role in defense mechanism at the site of pathogen attack. Antioxidant enzymes like CAT, POD and SOD are key components in the production of H2O2 which have an important role in the inhibition of pathogens (Zhang *et al.*, 2009).

CONCLUSION

In present study, progressive alterations in enzymatic and nonenzymatic metabolites of both inoculated and uninoculated potato leaves were quantified. CAT, SOD, Protein and TPC were lower in inoculated plant leaves while higher in un-inoculated plant leaves.

AUTHORS' CONTRIBUTIONS STATEMENT

Kamra Mahmood conduct research trials and wrote manuscript, Prof Dr. Shahbaz Talib Sahi Conceived the idea and supervised research work, Dr. Muhammad Atiq Co-supervised the research and edited the manuscript, Dr. Muhammad Shahid helped in research trials.

CONFLICT OF INTEREST

The authors have no conflict of interest among authors regarding manuscript submission.

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