Interactive effect of salt stress and *Fusarium solani* on dieback in an important timber species *Dalbergia sissoo*

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Abstract

Dalbergia sissoo is a native tree species of the Indo-Pak subcontinent. It is one of the most common timber-producing species used in the agroforestry, fuelwood, and furniture industries. Abiotic stress, such as salinity, is known to affect the establishment of *Dalbergia sissoo* plantations in Pakistan. This research aimed to determine the pathogenicity of *Fusarium solani* on *D. sissoo* under three levels of salinity (EC2, EC4, and EC6 dS m^{-1}). The seedlings were inoculated either simultaneously or predisposed to salt stress for two weeks before being subjected to fungal inoculation. High disease incidence was observed in seedlings predisposed to salinity following fungal inoculations. Controlled seedlings did not show dieback symptoms thus establishing the role of salinity predisposition in causing shisham dieback. Reduced plant growth rate and increased lesion growth were attributed to low leaf osmotic and water potential as a result of combined stresses. Photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, and carotenoids, were negatively affected. A marked increase in CAT and POD was observed which effectively lower H_2O_2 levels in stressed seedlings. It was concluded that abiotic stress may predispose shisham to the fungal pathogen that causes tree dieback. Therefore, it is suggested that abiotic stress remediation or the selection of the genotypes tolerant to abiotic factors would help manage shisham dieback effectively.

Keywords: *Climate change; Salinity; Fungal pathogens; Host response; Shisham dieback; Salinity x pathogen interaction*

Introduction

Climate change affects the relationship of plants with their immediate environment. This transition affects plants around the world and poses serious threats to sustainability. The balance between plants and microflora is also shifting. As a result of this change, many plants are succumbing to new but weak pathogens mainly due to predisposition to different stress.

The most frequently identified interactions are those between abiotic factors and fungal pathogens (Cieślak, 2013; Rasmussen et al., 2013; Sewelam et al., 2014). Our understanding of how abiotic factors can modify plant-pathogen associations is poor, with a few exceptions of drought and pathogen interaction (Choudhury et al., 2017).

Globally, salinity is widespread stress, and plant pathogens provide an excellent example of biotic and abiotic stresses that are occurring together in nature, and their interactions can have a serious effect on the balance of various habitats, such as forests (Munns and Gilliham, 2015). The effect of salinity and pathogenic fungi have been studied extensively however as individual stress (Manandhar and Shreeshta, 2000; Bashyal et al., 2002; Khan et al., 2004; Mukhtar et al., 2014; Naliwajski and Skłodowska, 2014; Forieri et al., 2016). The combined interactive effects on tree species are not well recognized, although evidence suggesting their association continues to grow (Dileo et al.,2010; Nostar et al.,2013; Nejat and Mantri, 2017; Zhang and Sonnewald, 2017).

Shisham is one of Pakistan's most important native species. It is mainly a tropical to subtropical species and has recently been introduced in semi-arid regions of Pakistan (Khan et al., 2004). Shisham populations in the region have been declined sharply due to tree dieback. (Khan et al., 2004; Mukhtar et al., 2014). Most of the newly introduced areas are saline and waterlogged (Khan et al., 2004). Previous research on the combined effects of biotic and abiotic stresses has revealed synergistic effects, indicating that abiotic stress has both positive and negative effects on the host-pathogen relationship (Al-Sadi et al., 2010; Egamberdieva et al., 2011). The majority of studies on tree species subjected to heat/drought stress combined with biotic stress revealed the main role of abiotic factors in developing plant diseases (Luo et al., 2005; Kiraly et al., 2008), particularly those caused by weak facultative parasites (Desprez-Loustau et al., 2006). Periodic abiotic stresses that occur before pathogen infection have also been reported to predispose the host to different pathogens (Bostock et al., 2014). This increased susceptibility of the host to stress may be attributed to a shift in hormonal balance, downregulation of resistant genes, and a down-regulated response to primary metabolism, all of which have been suggested to trigger a general reaction to multiple stresses (Prasch and Sonnewald, 2013). Previous research on shisham dieback emphasized the role of biotic factors i.e. fungi, ignoring the importance of abiotic factors and their synergistic effects on dieback progression (Manandhar and Shreeshta 2000; Bashyal et al., 2002; Khan et al., 2004; Mukhtar et al., 2014). *Fusarium solani* is a genus complex of at least 26 closely related filamentous fungi in the Ascomycota division of the Nectriaceae family (Summerbell 2003). It is a natural soil fungus and causes diseases in many plant species (Summerbell 2003). There have been several reports on the incidence and relationship of *Fusarium solani* with dieback in *D. sissoo* (Bakshi, 1954; Bakshi et al., 1956; Bakshi & Singh, 1959; Javaid et al., 2003; Javaid et al., 2004; Rajput et al., 2012; Mukhtar et al., 2014). *Fusarium solani* is one of the most common species of Nectriaceae found in shisham plantations in Pakistan, both as pathogen and endophyte (Javaid et al., 2004; Rajput et al., 2012; Mukhtar et al., 2014). There is very little information available about the morpho-physiological effects of salinity on shisham infected with *Fusarium solani*. There is still a knowledge gap on salinity x disease interaction in the performance of woody plants, and *D. sissoo* is no exception.

Considering the socio-economic importance of *D. sissoo* and the urgent need to understand tree response to multiple stress, this study was designed to assess the role of salinity in *D. sissoo* when combined with *Fusarium solani* by assessing infection rate, plant growth, biochemical and physiological responses.

Materials and Methods

Experimental Design

Open-pollinated seeds were randomly collected from various mother trees in the Shisham natural range in the foothills of the Himalayan Khyber Pakhtunkhwa (KPK) province of Pakistan. This area is known to be the center of origin of the shisham. Climate is predominantly subtropical, characterized by high rainfall, high temperatures, and humidity. The soil is sandy loam with good drainage.

D. sissoo pods were soaked overnight in water at room temperature and planted in plastic pots containing sandy loam soil. Seedlings were daily watered until they were 6 months old. The experiment included three levels of salinity EC2, EC4, EC6 combined with *F. solani*. Both stresses were either applied simultaneously or predisposed to salinity for two weeks before pathogen inoculation. Electrical conductivity was developed in the pots by using sodium chloride (NaCl) salt. Three levels of EC (EC2, EC4, and EC6) were used, to develop the required levels of EC. Salt concentrations of EC2, EC4, and EC6 were prepared by dissolving (1.55 g, 3.24 g, and 5.1g) of NaCl. In the case of control treatments, seedlings were planted in soil without NaCl and inoculated with PDA. Each treatment was replicated by 10 seedlings and the entire experiment was designed in a fully randomized complete block design. Plants were watered on alternative days to maintain plants at required levels of salinity after the determination of soil electrical conductivity.

Pathogen Inoculation

Fusarium solani previously isolated from dieback infected shisham tree was identified based on ITS region and sequence was submitted in Genbank with accession number MW159862 was used for seedling inoculation. Pure cultures of *F. solani* were used to inoculate the plants. Mycelial plugs (approximately 5 mm diameter) were cut from the margins of 1–week old cultures grown on PDA at 25^{o}C as fungal inocula for seedling inoculation. The stem surface was sterilized with 70% ethanol before seedling inoculation. Using a sterile scalpel, a shallow "I" shaped cut was made at the base of the seedling (5 cm above soil surface) to remove the bark and expose the cambium. According to Jung and Nechwatal's (2008) process, a mycelial plug was inserted in each cut facing the wound. The original cut length was recorded and the inoculation site was sealed with a parafilm to prevent desiccation. Similarly, sterile PDA plugs were inserted into the cut stems of control plants. The fungus was re-isolated from each inoculated plant at the end of the experiment to confirm its association with the host.

Monitoring of Plants

Plants were observed regularly, and data on external symptoms including leaf color changes and wilting was collected. Data on various growth parameters, disease incidence, physiological, and biochemical traits were collected after two months of treatment.

Growth parameters

On the day of inoculation and again on the last day of the trial, plant height was assessed. The difference between the plant's final and original height was used to calculate the plant's growth rate.

Monitoring of infection

Visible external symptoms such as stem lesions, foliar chlorosis, and wilting were reported regularly during the experiment. The growth of stem lesions (cm) was estimated after 2 months. The difference between the final lesion length and the original cut length was the increment in lesion growth (cm).

Osmotic Potential

Fresh leaf tissue was sampled and frozen at O^{o}C for three to four days. For cell sap extrusion from frozen leaves, tissues were thawed at room temperature. In Osmometer, a 50 µl amount of cell sap was used to test the osmotic potential of the leaves (Ball and Oosterhuis, 2005). Leaf water potential was measured at 6:00 a.m. by a pressure chamber (Plant Moisture Stress, PMS Instruments) with five replicates per treatment (Scholander et al., 1965).

Chlorophyll contents

For the determination of chlorophyll, leaf samples were randomly collected and crushed to powder form by taking a 0.5 g leaf mass and 2 ml of 80 percent acetone. The mixture was separated at 15000 rpm for 5 min. The supernatant was used for chlorophyll determination using the NanoDrop Spectrophotometer (NanoDropTM). Chlorophyll *a* was estimated at 663 nm wavelength. The above-mentioned method was repeated for the determination of chlorophyll *b* and carotenoids, and readings were taken at 645 nm and 480 nm absorbance, respectively (Arnon, 1949). Five separate readings were taken of the leaves harvested for each plant and the final reading was the mean value.

Antioxidant enzymes assays (CAT, POD, H_2O_2)

Fresh leaf (0.5 g) crushed to powder and homogenized with 10 ml of sodium phosphate buffer (pH 7.0) comprising 1 mM EDTA, 1% PVP, and 1 m NaCl. The mixture was separated at 10000 rpm for 10 min. The supernatant was used to test the activity of the CAT. The assay mixture contained 50 mM of sodium phosphate buffer pH 7.0, the extract, and 15 mM of H_2O_2. The H_2O_2 decomposition was analyzed at 240 nm using a NanoDrop Spectrophotometer (NanoDrop^{TM}). CAT function was expressed in μmol H_2O_2 min^{-1} mg protein. POD behavior was judged after Fielding and Hall (1978). 0.1 g fresh leaf was ground and peroxidase activity was measured in 2 ml of 0.05 M potassium phosphate buffer containing 1.0 mL of 0.05 M guaiacol and 1.0 mL of 2% H_2O_2. Absorption increases at 470 nm were observed after the addition of 20 percent chloroacetic acid to 2.0 mL. The quantity of H_2O_2 was calculated using the Bernt & Bergmeyer methods (1974). A leaf sample of 0.1 g was crushed to powder form and suspended at a volume of 1.5 mL of 100 mM of potassium phosphate buffer (pH 6.8). The mixture was then isolated by centrifuging at 18000 x g at 4°C for 20 min. H_2O_2 peroxidation was accomplished by taking 0.25 mL of supernatant and 1.25 mL of peroxidase reagent, consisting of 83 mM of potassium phosphate buffer (pH 7.0), 0.005% (w/v) of o-dianizidine and 40 μg of peroxidase/mL incubated at 30°C. After 10 min, the reaction was stopped by adding 0.25 mL of 1 N perchloric acids, and the mixture was centrifuged at 5000 x g for 5 min. Absorption was read at 436 nm, and the quantity of H_2O_2 was measured by using an extinction coefficient of 39.4 $mM^{-1}.cm^{-1}$ (Zhang et al., 2014).

Data Analysis

All treatments were analyzed by analysis of variance (ANOVA) using a package "*agricolae*" incorporated in R (R Core Team 2013). Treatments that appeared significantly different were compared by Duncan Multiple Range Test (DMRT).

Result

Different levels of salinity applied simultaneously or applied after predisposition to salinity in combination with *F. solani* significantly affected increment in plant height, lesion growth, osmotic potential, water potential, chlorophyll *a* & *b*, CAT, POD, and H_2O_2 respectively (Table 1). Similarly, a high rate of mortality was observed with an increase in salinity levels when

Table 1. Two-way ANOVA summary table for height, lesion length, biochemical and physiological traits of *D. sissoo* infected with *Fusarium solani* under different levels of salinity stress (simultaneous and predisposed)

*p≤0.05. **p ≤ 0.01. ***p ≤ 0.001.

Parameters	**Df**	**SS**	**MS**	**F-value**	**P-value**
Height (inch)	6:28	21.522	3.587	13.75	3.15e-07***
Lesion Growth (cm)	6:28	78.77	13.128	10.13	2e-16***
Osmotic Potential (MPa)	6:28	33.82	5.636	1787	<2e-16***
Water Potential (MPa)	6:28	31.89	5.314	3442	<2e-16***
Chlorophyll *a* (µmol/ml)	6:28	0.7272	0.12120	11.05	2.61e-06***
Chlorophyll *b* (µmol/ml)	6:28	0.3480	0.0580	9.406	1.11e-05***
Carotenoid (µmol/ml)	6:28	0.07462	0.01243	2.728	0.0325*
CAT	6:28	6.534	1.0890	9.356	1.05e-09***
POD	6:28	0.7365	0.1228	11.58	1.68e-06***
H_2O_2	6:28	0.6770	0.11284	3.317	0.0136*

1

Table 2. Mean comparison table based on DMR - Test for lesions growth, morphological and physiological parameters.

Traits	**EC2+ *F. solani***	**EC4+ *F. solani***	**EC6+ *F. solani***	**PDA**	**PreDEC2+ *F. solani***	**PreDEC4+ *F. solani***	**PreDEC6+ *F. solani***
Height (inch)	1.12±0.248c	0.84±0.482c	1.10±0.648c	3.14±0.907a	2.24±0.403b	1.44±0.260c	0.92±0.238c
Lesion Growth (cm)	3.46±1.303ab	4.70±0.982a	4.84±2.097a	0.00±0.000c	2.86±0.502b	2.52±0.614b	3.28±1.173ab
Osmotic Potential (MPa)	- 2.847±0.03d	- 2.933±0.02c	- 2.952±0.01c	- 0.392±0.02e	- 3.2155±0.02b	- 3.263±0.03ab	- 3.271±0.09a
Water Potential (MPa)	- 3.288±0.071d	- 3.664±0.052a	- 3.628±0.074ab	- 0.757±0.037e	- 3.374±0.033c	- 3.568±0.065b	- 3.698±0.046a
Chlorophyll a (µmol/ml)	0.4652±0.082bc	0.5538±0.155bc	0.4148±0.055c	0.7292±0.135a	0.6046±0.083ab	0.4826±0.103bc	0.2348±0.082d
Chlorophyll b (µmol/ml)	0.1794±0.042cd	0.3012±0.134b	0.1848±0.043cd	0.4354±0.068a	0.2894±0.056bc	0.2774±0.105bc	0.1046±0.046d
Carotenoid (µmol/ml)	0.1482±0.020b	0.1566±0.034b	0.1332±0.027b	0.2594±0.102a	0.1576±0.043b	0.1850±0.111ab	0.1000±0.068b
CAT	0.9408±0.230c	1.3630±0.497abc	1.8008±0.453a	0.3748±0.179d	0.9286±0.155c	1.2608±0.311bc	1.5168±0.394ab
POD	0.1274±0.089c	0.3368±0.096b	0.4724±0.138a	0.1382±0.102c	0.1256±0.079c	0.3068±0.069b	0.4780±0.126a
H_2O_2	0.3334±0.282b	0.1530±0.087b	0.2624±0.235b	0.6028±0.158a	0.2572±0.142b	0.3390±0.192b	0.1724±0.114b

combined with both pathogens. Predisposed seedlings showed a high mortality rate (28.33%) as compared to seedlings inoculated simultaneously (21.67%).

Lesion length was significantly different in all the treatments (Fig. 1., Table 1). It was ranged from 2.52 to 4.84 cm. Overall seedlings inoculated simultaneously showed more susceptibility to *F. solani* at all salinity levels as compared to those who were predisposed to salinity (Fig. 2 & 3., Table 1). Maximum lesion growth (4.84 cm) was observed at EC_6, followed by EC_4 (4.70 cm), then EC_2 (3.46 cm) among seedlings simultaneously inoculated (Table 2). While no lesion growth was observed in controlled seedlings (Table 2).

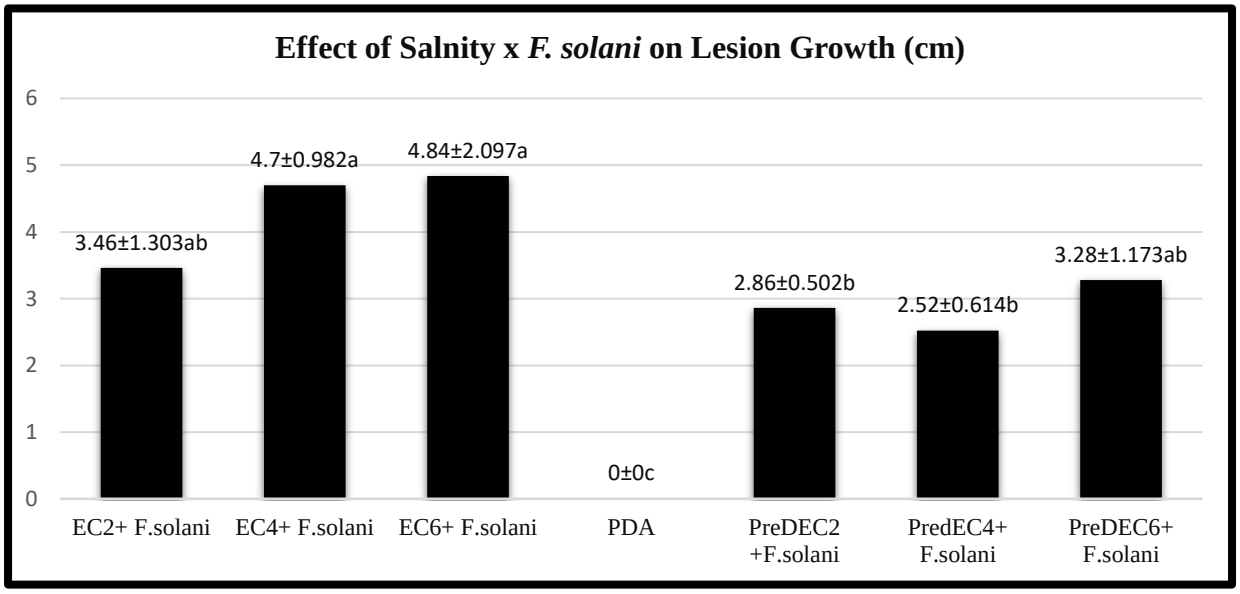


Figure 1. Influence on lesion growth of *D. sissoo* seedlings under the different levels of salinity stress, simultaneous application of salinity stress, pathogen (*Fusarium solani*) inoculation, and after two weeks of salinity stress application inoculation of pathogen *Fusarium solani*) (Predisposed).

EC2 x FS **EC4 x FS** **EC6 x FS**

Figure 2. Effect of simultaneous inoculation of salt x *Fusarium solani* on dieback in *D. sissoo*.

EC2 x FS EC4 x FS EC6 x FS

Figure 3. Effect of predisposition to salt for 2 weeks before inoculation to pathogens on lesion growth. Lesion growth was more pronounced as compared to simultaneous salt x pathogen inoculation. *Fusarium solani* showed more lesion growth as compared to *Ceratocystis fimbriata*.

Growth and plant water status

Before the salinity x pathogen inoculation treatment, similar-sized seedlings were assigned randomly to all the treatments. Height increment was significantly affected by all the treatments (Table 2). The growth rate was high in control seedlings (3.14 inches). Simultaneous inoculation showed a negative effect on plant height as compared to predisposed seedlings (Fig. 4., Table 2). Generally high salinity levels i.e. EC_4 and EC_6 regardless of the timing of stress applied showed a slow growth rate. A minimum plant height was observed in seedlings stressed simultaneously at EC_4 salinity level, followed by predisposed seedlings at EC_6 respectively (Fig. 4).

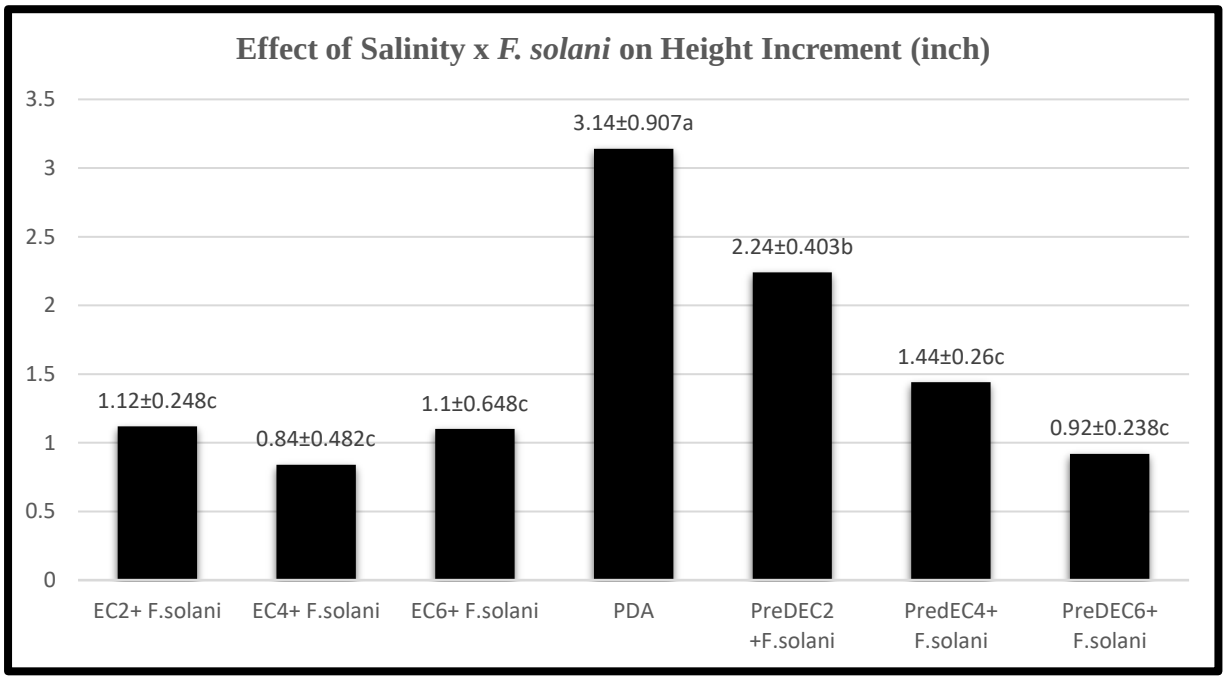


Figure 4. Influence on the height of *D. sissoo* seedlings under the different levels of salinity stress, simultaneous application of salinity stress, pathogen (*Fusarium solani*) inoculation, and after two weeks of salinity stress application inoculation of pathogen *Fusarium solani*) (Predisposed).

Pathogen and salinity stress significantly affected leaf osmotic and water potential (Table 1). Maximum negative water and osmotic potential were observed when seedlings were predisposed to high salt concentration i.e. EC6 followed by those that remained under both stresses for a longer period i.e. EC4 and EC6 simultaneously with *F. solani* inoculation (Fig. 5 & 6; Table 2). Plant and lesion growth was also adversely affected by seedlings at low levels of water and osmotic potential (Table 2). Water potential and osmotic potential were significantly different for control plants than stressed seedlings. Control plants also showed high plant growth and low lesion growth.

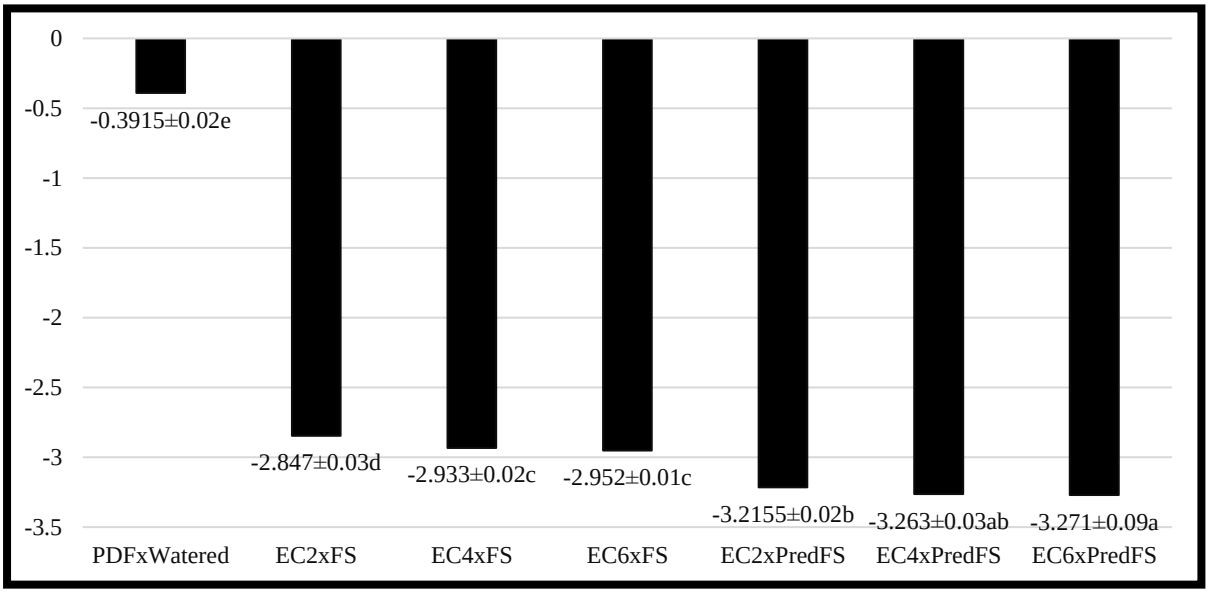


Figure 5. Influence on leaves water potential of *D. sissoo* seedlings under the different levels of salinity stress, simultaneous application of salinity stress, pathogen (*Fusarium solani*) inoculation, and after two weeks of salinity stress application inoculation of pathogen *Fusarium solani*) (Predisposed).

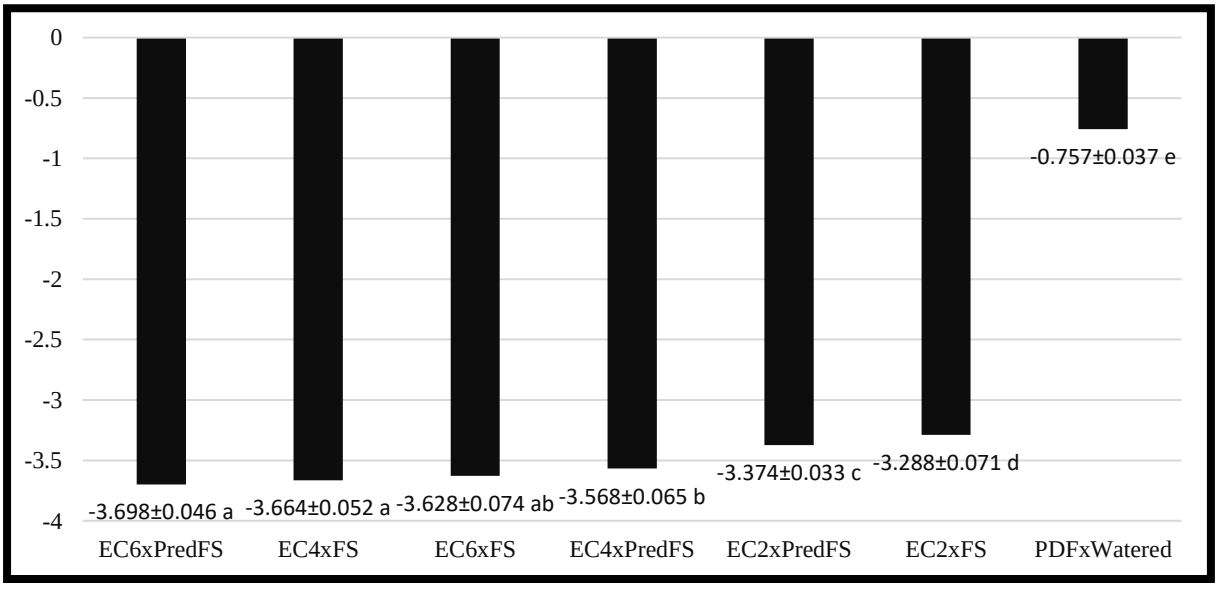
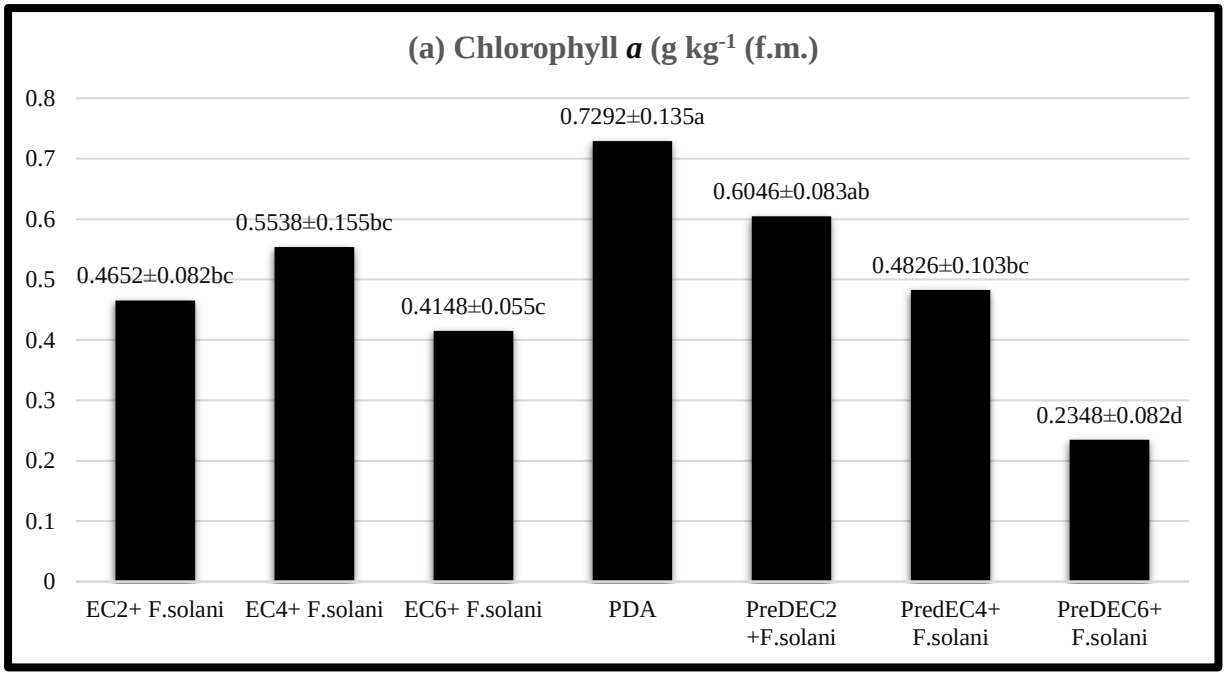


Figure 6. Influence on leaves osmotic potential of *D. sissoo* seedlings under the different levels of salinity stress, simultaneous application of salinity stress, pathogen (*Fusarium solani*) inoculation, and after two weeks of salinity stress application inoculation of pathogen *Fusarium solani*) (Predisposed).

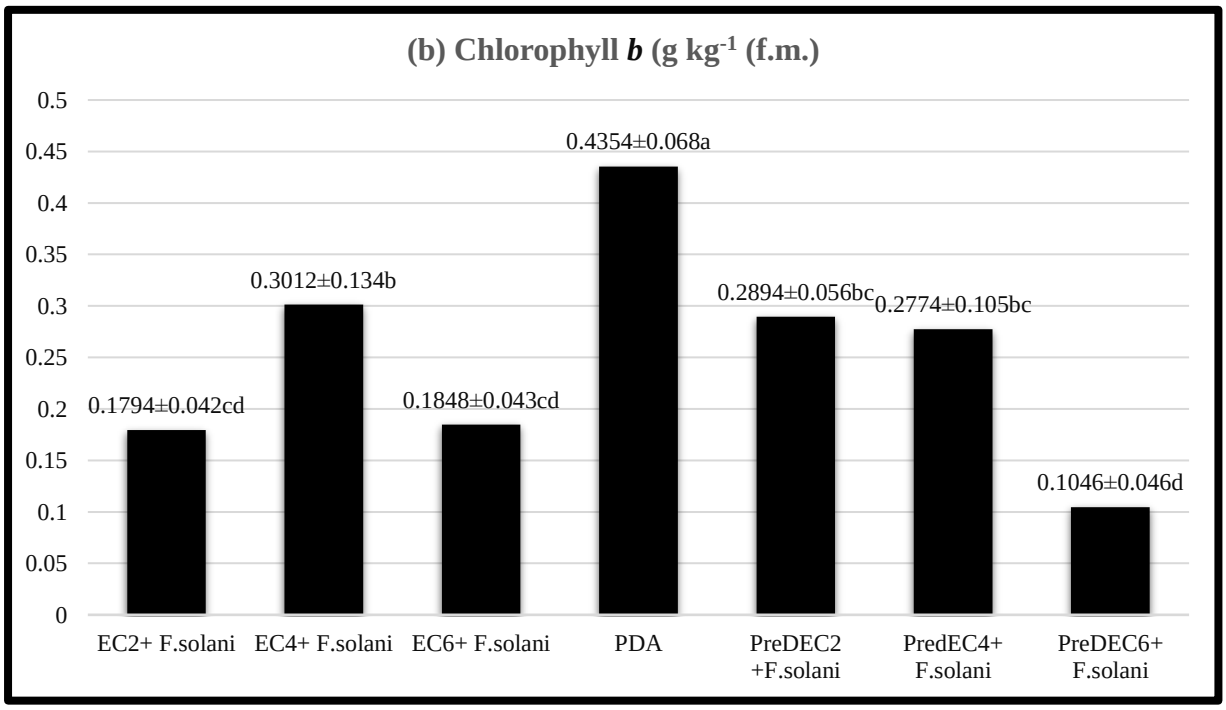
Chlorophyll and Carotenoid

Carotenoid, chlorophyll *a*, and *b* were significantly affected in treated as compared to control seedlings (Table 2). Maximum chlorophyll content both *a* and *b* was observed in control as compared to stressed seedlings. The pigments growth was declined at a high salt concentration of EC4 when seedlings were predisposed to salt alone and then inoculated (Fig. 7). *D. sissoo* leaves turned completely brown at EC6 thus resulting in a decline of pigments and death of seedlings.


(a) Chlorophyll *a* (g kg-1 (f.m.)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.4652±0.082bc
0.5538±0.155bc
0.4148±0.055c
0.7292±0.135a
0.6046±0.083ab
0.4826±0.103bc
0.2348±0.082d
EC2+ F.solani
EC4+ F.solani
EC6+ F.solani
PDA
PreDEC2 +F.solani
PredEC4+ F.solani
PreDEC6+ F.solani

97

98


(b) Chlorophyll *b* (g kg-1 (f.m.)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.1794±0.042cd
0.3012±0.134b
0.1848±0.043cd
0.4354±0.068a
0.2894±0.056bc
0.2774±0.105bc
0.1046±0.046d
EC2+ F.solani
EC4+ F.solani
EC6+ F.solani
PDA
PreDEC2 +F.solani
PredEC4+ F.solani
PreDEC6+ F.solani

99

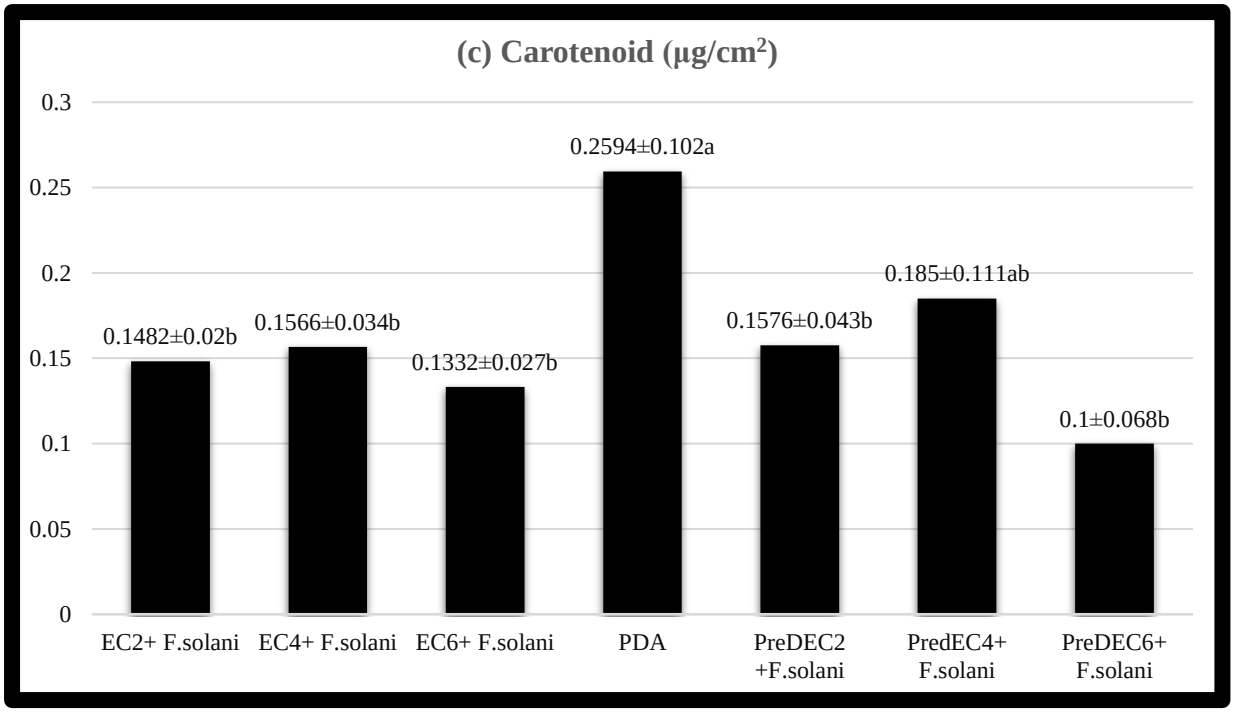
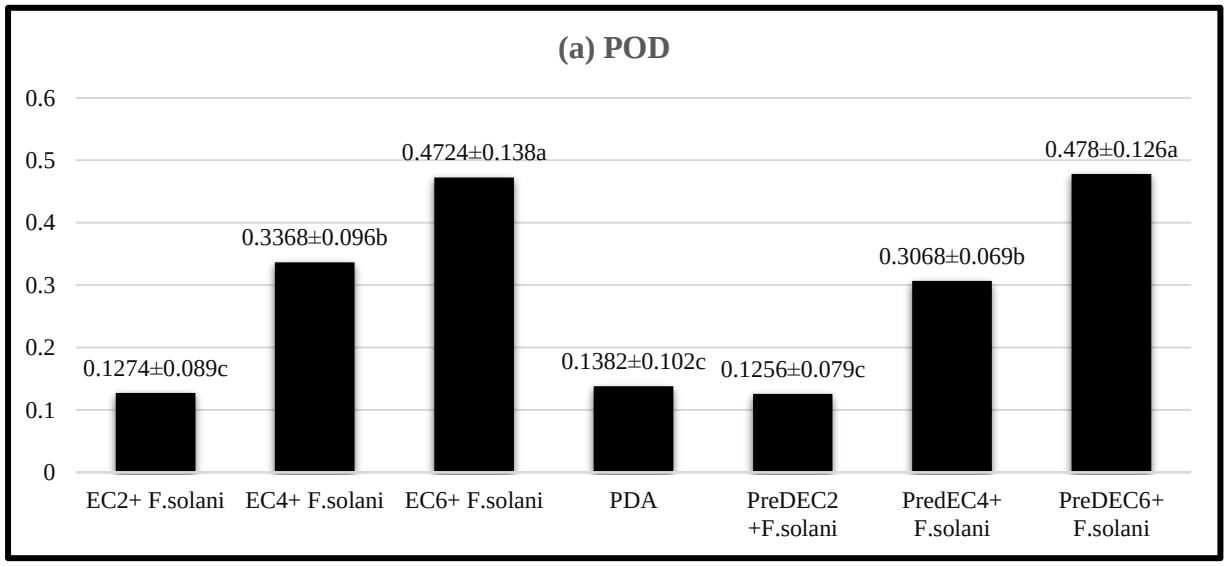
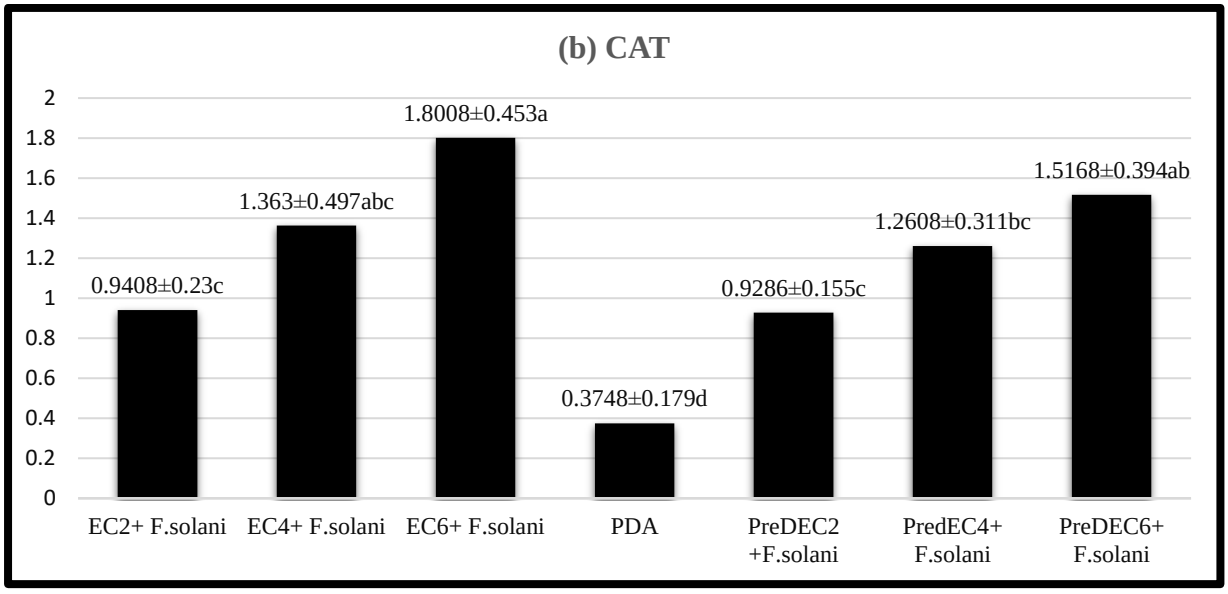


Figure 7 (a-c). Influence on Chlorophyll *a* content of *D. sissoo* seedlings under the different levels of salinity stress, simultaneous application of salinity stress, pathogen (*Fusarium solani*) inoculation, and after two weeks of salinity stress application inoculation of pathogen *Fusarium solani*) (Predisposed).

Antioxidant enzyme assay

Salt x pathogen interaction had significantly enhanced CAT activity (Fig. 8a) as compared to control treatment at EC4 and EC6 however the effect was more pronounced at EC6. POD activity at EC2 in both treatments i.e. simultaneous and predisposition was comparable to control seedlings (Fig. 8b). However, it was significantly high at high salt levels EC6 as compared to EC4. The result indicated a relatively higher H_2O_2 production at a low salt concentration (EC2, EC4) and control as compared to higher (EC6) salt concentration (Fig. 8c).


(a) POD
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1274±0.089c
0.3368±0.096b
0.4724±0.138a
0.1382±0.102c
0.1256±0.079c
0.3068±0.069b
0.478±0.126a
EC2+ F.solani
EC4+ F.solani
EC6+ F.solani
PDA
PreDEC2 +F.solani
PredEC4+ F.solani
PreDEC6+ F.solani


(b) CAT
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.9408±0.23c
1.363±0.497abc
1.8008±0.453a
0.3748±0.179d
0.9286±0.155c
1.2608±0.311bc
1.5168±0.394ab
EC2+ F.solani
EC4+ F.solani
EC6+ F.solani
PDA
PreDEC2 +F.solani
PredEC4+ F.solani
PreDEC6+ F.solani

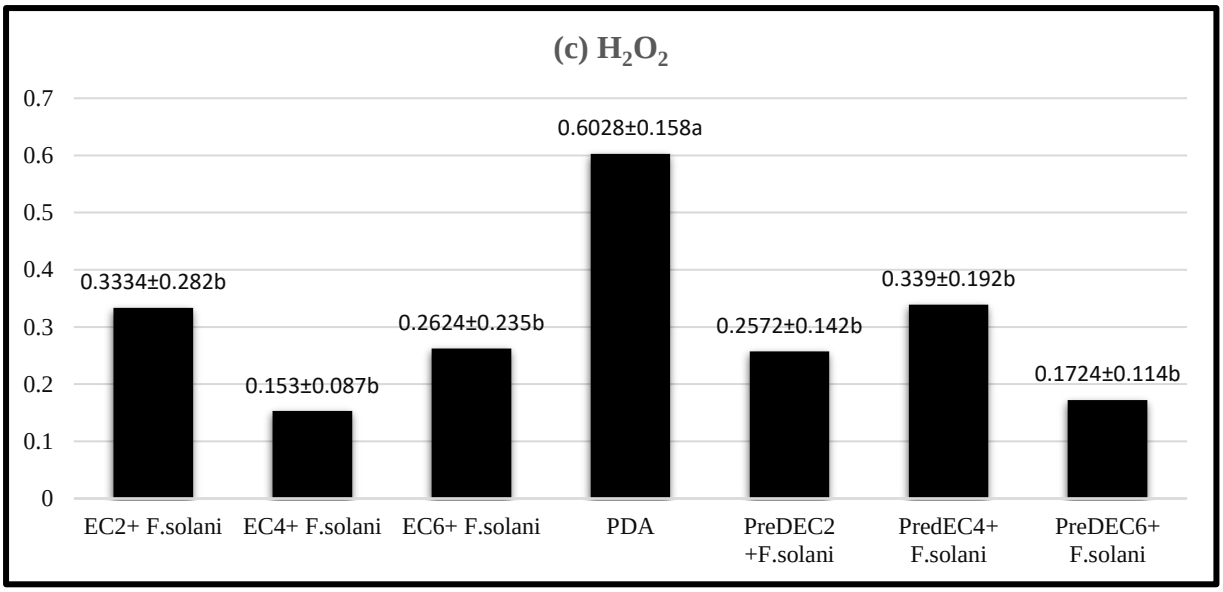


Figure 8 (a-c). Interactive effect of salinity x *F. solani* on POD, CAT, and H_2O_2 activity**.**

Discussion

Salt stress disrupts the balance between the amount of energy produced and the ability to process it in all plants under suboptimal environmental conditions. The main implications of such energy imbalances are the development of reactive oxygen species (ROS), photo-inhibition, and growth inhibition (Parida et al., 2004., Li et al., 2009). The decrease in the plant growth, chlorophyll, carotenoid pigments, and mortality observed with increasing salinity in this study indicated that the growth reduction was due to non-cyclic photosynthetic electron transport or CO_2 assimilation impairment. Salinity adversely affected growth-related parameters such as photosynthetic pigments, water potential, and reactive oxygen species (ROS) and increased susceptibility to *Fusarium solani* in *D. sissoo* in a time and dose-based manner.

A significant decrease in osmotic balance at increasing salinity levels decreased the water potential of shisham plants. This transition was reflected as part of the defensive mechanism by which plants tolerate the deleterious effects of salt build-up inside the cell by an increased water absorption (Cachorro et al., 1995). Low water potential was resulted due to the accumulation of salt and the effects were more pronounced at high salt concentrations. It could be suggested that at EC2, shisham seedlings maintained their osmotic potential at low levels than the osmotic potential of the rhizosphere (Zhu, 2001). Sugar and amino acids that are plasma membrane–compatible solutes could enhance the capacity of a cell for osmotic adjustment and thus enhanced the degree of membrane protection from the effects of dehydration caused by increased salinity. It is suggested based on normal seedlings growth that membrane stability was not affected at 2.0 dS m^{-1}, and it was more pronounced at high salinity levels i.e. at 4 dS m^{-1} and 6 dS m^{-1} indicating shisham seedlings failed to adjust membrane integrity under high saline conditions (Shahid et al., 2012; Roussos et al., 2013; Shoaib et al., 2018). An increased reduction in chlorophyll a & b, and carotenoid content was observed in shisham under combined stresses. Rawat and Banerjee (1998) and Mazher et al., (2006) have reported similar responses to NaCl stress which caused a significant decline in chlorophyll, protein, and carotenoid content with increasing salinity in shisham. Zhen et al., (2006) revealed that under increased salinity an increase in physiological indexes such as chlorophyll content, photosynthetic rate, stomatal conductance, and transpiration rate were observed first that decreased gradually. The ionic imbalance caused due to cellular dehydration resulted in toxicity

that negatively impacted the chlorophyll contents as a result of disruption of the pigment synthesis pathway (Youssef and Awad, 2008).

ROS activation under stress conditions is a defensive mechanism employed by plants. To reduce cellular damage which may occur due to ROS activation is achieved by activation of CAT and POD enzymes. Indirect evidence of ROS generation is provided by the differential expression of CAT and SOD in response to increasing salt stress. In this analysis, comparing CAT and SOD through varying salt concentrations revealed that H_2O_2 accumulation was higher at EC4 and EC6, as demonstrated by increased CAT and SOD activity. At a high salt concentration of 400 mM, a halophyte, *Bruguiera pariflora*, was reported to trigger high SOD activity similar to that of salt-affected areas of Sunderban, West Bengal, India (Jalali-e-Emam et al., 2011). CAT and POD activity was high at a high salt concentration which was similar to *Acacia auriculiformis* to have a similar increase in CAT and POD behavior at a high salt concentration (Gupta et al., 2019). It is suggested that the high expression and the maintenance of CAT and POD activities under saline conditions are the most important adaptive factors triggered by shisham plants under oxidative stress. Salt stress was mediated by the signaling of ROS elements such as CAT, POD, and H_2O_2 that also enhance tolerance pathogen (Tang et al., 2015). Salinity and pathogenic fungi in dieback were previously studied individually, however, their combined effect on shisham dieback, in particular, is not studied, although data confirming their co-occurrence is still growing (Manandhar and Shreeshta, 2000., Bashyal et al., 2002., Khan et al., 2004; Zhang and Sonnewald, 2017). Shisham is one of the most important timbers producing species in the region is commonly planted on farmlands, roadsides and canal banks showed low tolerance to salinity and dieback (Mukhtar et al., 2014; Dharamvir et al., 2018). Dieback symptoms were reported from shisham growing in diverse ecological zones (Mukhtar et al., 2014), including saline and dry areas of Pakistan. The combined stress of salt and *F. solani* increased disease severity. Salinity stress has been proved to increase the frequency and severity of *Fusarium* infection in cotton, tomato, and bean (You et al., 2011). Similarly, in cucumber where 17% of the plants that were planted in saline soils were infected by *Fusarium solani* (Egamberdieva et al., 2011), and with increasing salinity from 0.01 to 5 dS m^{-1} showed an increased *Pythium* damping-off ranging from 40 to 93% (Al-Sadi et al., 2010). Two primary mechanisms have been suggested for microbial resistance against high levels of salts i.e. (1) osmotic effects and (2) osmotic adjustment respectively. Microorganisms tolerate salinity stress by accumulating organic compounds and inorganic osmolytes (Sagot et al., 2010). Production of organic osmolytes required high energy input (Wichern et al., 2006) that can negatively affect microbial growth and activity. Therefore, an increase in fungal growth at EC ranges of 2 – 4 dS m^{-1} might show the accumulation of NaCl necessary for metabolism by counteracting an increase in osmotic pressure. Whereas high salinity levels resulted in a high concentration of solute that spikes negative osmotic potential, that can pull water out of cells and can impact microbes as a result of plasmolysis (Boumaaza et al., 2015).

Periodic abiotic stress such as drought and salinity occurring before pathogen infection was suggested to predispose the host to disease (Bostock et al., 2014). Plants showing tolerance from early abiotic stress may conflict with those for resisting pathogens (Boyer, 1995). Therefore high susceptibility to secondary pathogens under abiotic stress may be linked to the reduced defense genes expression, changed hormonal balance, and to primary metabolism downregulation that was experienced as a broad tolerance response to multiple stress factors (Mohr and Cahill, 2003; Prasch and Sonnewald, 2013). The idea of cross-tolerance between biotic and abiotic factors has also been reported (Sharma et al., 1996; Achuo et al., 2006; Foyer et al., 2016). This concept confirms that biotic and abiotic stresses induced similar signals, reactive genes, and products. However, we have observed a high rate of infection in shisham predisposed to salinity as compared to those which were inoculated simultaneously to saline stress.

Conclusion

It is concluded that combined stress leads to a marked reduction in shisham fitness that exceeds either of the single stresses applied. Shisham adaptation to combined stresses may vary widely on the sequence and intensity of the stresses applied. Although many studies have shown that combined stresses increase specific transcriptomic responses however a very little information is available about the stress combination unique response is shown by the overlapping stressors at the physiological and metabolic levels. It seems at least in the case of shisham dieback the response to combinatorial biotic and abiotic stresses is usually led by abiotic stress, at the cost of resistance to pathogens. However, our current knowledge and understanding of the mechanism, in shisham affected by abiotic stress and subsequently to the pathogen are still limited. The understanding of how different abiotic environmental factors such as drought and salinity influence shisham resistance to different pathogens and of various processes involved in response to combined abiotic and biotic stress has important implications for dieback management, sustainable growth, and in breeding new shisham genotypes against multiple stress tolerance.

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