



Enhancing Pear Tolerance to Salinity Using Salicylic Acid, Acetylsalicylic Acid and White Willow Extract

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Abstract: Production of pear (*Pyrus* spp.) as one of the most cultivated and nutritionally vital fruit trees in the world is limited by salinity. The present study aims to alleviate the adverse effects of salinity on growth of 'Le-Conte' pear seedlings by using foliar application of salicylic acid (SA), acetylsalicylic acid (ASA) and natural white willow (*Salix alba*) extract. Spraying treatments enhanced photosynthetic pigment contents under salt stress conditions, increased proline contents under severe salinity and stimulated antioxidant system. The expression patterns of PbHB1-22 gene revealed that, it is widely involved in responding to salt-induced stress in pear. Effective *in vitro* inhibition of *Salix alba* leaves extract against *Erwinia amylovora*; the causal bacterial pathogen of fire blight, was found to increase in concentration dependent manner.

Keywords : Pear, Salinity, Salicylic acid, Acetylsalicylic acid, White willow, *Erwinia amylovora*.

Introduction:

Pear (*Pyrus* spp.) which belongs to family Rosaceae, subfamily Pomoideae; the pome fruits¹, is one of the most cultivated and nutritionally vital fruit trees in the world. In 2017 according to FAOSTAT, Egypt has a pears production quantity of 64,680 tonnes and area harvested of 10,188 feddan. However, pear production is limited by salinity, where pear trees are generally classified as salt-sensitive².

Salinity is one of the major constraints in the development of irrigated agriculture in humid, arid and semiarid regions all over the world. Every year about 4×10^4 ha of land becomes unfit for agricultural production because of salinization problem. In addition, about 50% of irrigated area of the world is either salinized or has potential danger at future³. Extensive area of land in the arid regions and, particularly, in Egypt became out of cultivation due to salt accumulation⁴. The increased salinization of soils and ground waters in recent decades is considered to be a major problem of agriculture in Egypt and is thought to be a result of the Nile's weak demineralization of the soil due to the absence of flooding⁵. The injurious effects of salt stress on plant activities are associated with decreased water supply in the soil due to osmotic effects⁶ thereby causing physiological drought, nutritional deficiencies, direct toxicity, or combination of all these factors⁷.

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Salix alba (White Willow) of the family Salicaceae, genus *Salix* is a species of willow with white tone on the underside of the leaves. Ancient people of different civilizations recorded the use of white willow to cure pain and inflammation. The most famous chemical constituents of *Salix alba* leaves are Salicyl alcohol; Linolenic acid; Galactose, 4,6-O-nonylidene; 4-Acetoxy-3-methoxycinnamic acid; Stearic acid; Stearyl aldehyde⁸. Acetylsalicylic acid is one of the most widely used drugs in the world. Its ancestry the salicylates, including salicin and salicylic acid, are found in the bark and leaves of the willow trees^{9,10}.

Salicylic acid (SA; 2-hydroxybenzoic acid) is one of many phenolic compounds (defined as compounds containing a benzene ring bearing one or more hydroxyl groups) that are synthesized by plants. Despite the diversity and ubiquity of plant phenolics, these compounds were traditionally assumed to be rather unimportant, secondary metabolites. However, phenolics were subsequently shown to be involved in many important processes, including lignin and pigment biosynthesis, allelopathy, and the regulation of responses to abiotic and biotic stresses¹¹. SA is a critical hormone that plays direct or indirect roles in regulating many aspects of plant growth and development, as well as thermogenesis and disease resistance¹². Exogenous SA has been shown to affect resistance to biotic (pathogen-associated) stress and tolerance to many abiotic stresses (drought, chilling, heat, heavy metal, UV radiation, and salinity/osmotic stress), as well as multiple aspects of plant growth and development, including seed germination, vegetative growth, flowering, fruit yield, senescence, thermogenesis, stomatal closure, root initiation/growth, photosynthesis, respiration, glycolysis, Krebs cycle, and the alternative respiratory pathway^{13,14}.

The present study aims to alleviate the adverse effects of salinity on growth of 'Le-Conte' pear seedlings by using foliar application of salicylic acid (SA), acetylsalicylic acid (ASA) and natural white willow plant extract.

Experimental

This study was conducted during 2016 and 2017 seasons at the orchard of Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. One-year-old budded seedlings of 'Le-Conte' pear on *Pyrus betulifolia* rootstock were used as plant experimental materials.

White willow aqueous extract (WAE)

One hundred grams of White Willow (*Salix alba*) leaves were washed thoroughly under running tap water followed by distilled water and chopped with 200 ml distilled water (w/v) by using a domestic blender for 1 min at average speed. The mixture was macerated during 24h at +4°C. After that, resulting extract was filtered through double layered Whatman No.4 filter paper. Dilutions were prepared (10 and 20%). The extract was freshly prepared one day before its foliar application (25 ml/plant).

Preliminary phytochemical analysis of *Salix alba* leaves extract⁸ revealed the presence of steroids, alkaloids, phenols, glycosides and tannins.

Forty phytochemical compounds were identified in *Salix alba* leaves extract using GC-MS⁸. The major compounds were Linolenic acid (16.17%), 1,2 Cyclohexanediol (12.89%), Stearic acid (12.66%), Galactose 4,6-O-nonylidene (11.49%) and Salicyl Alcohol (7.19%).

Field experiment

A randomized complete block design was used with three replicates. Each replicate consisted of 5 pots (35 x 50 cm), each containing one seedling. Pots filled with sandy clay soil were planted in February and watered using tap water till the mid of July.

Salinity treatments started July 15th for two months in repetition twice a week as follows:

- 1- 3000 ppm of NaCl
- 2- 1500 ppm of NaCl
- 3- 0 ppm of NaCl

Foliar spraying treatments were done once every week during the experiment period as follows:

- 1- Salicylic acid (SA) at 0.5 mM
- 2- Acetylsalicylic acid (ASA) at 0.5 mM
- 3- White Willow Aqueous Extract (WAE) at 10%
- 4- White Willow Aqueous Extract (WAE) at 20%
- 5- Water treated control.

Tween 20 was added at 0.05% as a wetting agent. Survival percentage, growth parameters; green leaves percentage, number of roots and average length of roots (cm), were measured in September 15th, then samples of leaves and roots were collected to be used in chemical analysis. The content of total chlorophylls (chlorophyll a and chlorophyll b) and total carotenoids were determined by^{15,16}. Proline was assayed according to¹⁷. Total phenolics were analyzed by the method described by¹⁸. The antioxidant activity of plant methanol extracts was determined by the method of¹⁹.

The expression patterns of the PbHB1-22 gene in pear leaves and roots under severe salinity conditions (3000 ppm NaCl) as affected by different foliar applications comparing to control were analyzed by quantitative real-time PCR (qRT-PCR) according to²⁰.

In-vitro screening of antibacterial activity against *Erwinia amylovora*; the causal bacterial pathogen of fire blight, was performed using disc diffusion method²¹. 0.5 mM SA, 0.5 mM ASA, 10 and 20% WAE were evaluated as compared to sterile water control.

Statistical analysis.

Obtained data were statistically analyzed according to²² and significant difference was determined using L.S.D. values at $P = 0.05$.

Results and Discussion

Survival percentage

It's notice that, saline irrigation water up to 3000 ppm NaCl during the experiment period didn't affect survival percentage of pear seedlings regardless of spraying treatments as compared to those irrigated using tap water. Obtained results are in agreement with² who mentioned that, none of the scions grafted onto *Pyrus betulifolia* rootstock died under 50 mM NaCl irrigation up to ten weeks.

Vegetative growth

Percentage of green leaves gradually decreased as affected by increasing NaCl concentration in irrigation water (Figure 1). In the same context, increasing irrigation water salinity level negatively affected leave number of pear seedlings as reported by^{23,24,2}. All spraying treatments enhanced percentage of green leaves under both levels of saline irrigation water as compared to control during both seasons. Control seedlings recorded lowest green leaves percentages in average; 69.88, 70.95% for first and second seasons, respectively.

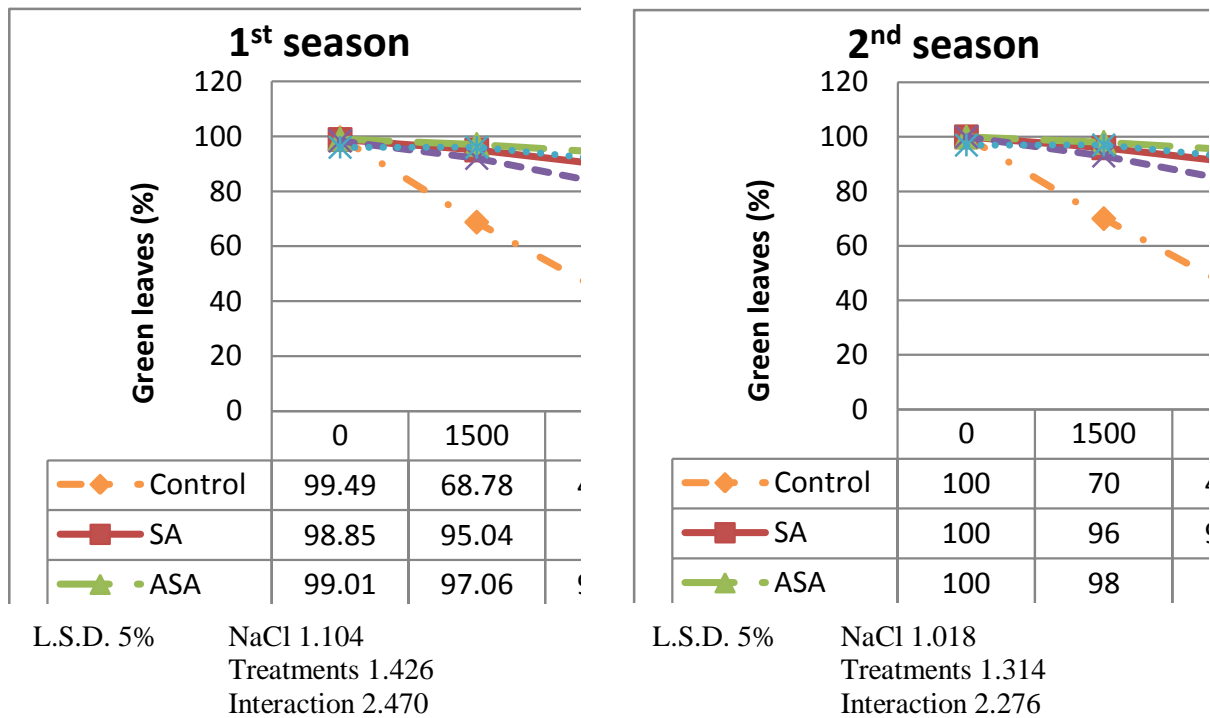


Figure (1). Effect of irrigation with saline water on green leaves percentage of pear seedlings as affected by different foliar spraying treatments.

Total chlorophyll content of leaves gradually decreased with increasing NaCl concentration in irrigation water (Table 1). In *Pyrus calleryana* seedlings, salt stress significantly reduced the chlorophyll contents²⁵. Also, ²⁴revealed that, reduction of Le-Cont pear seedlings growth parameters as affected by higher salinity was accompanied with reduction of leaf chlorophyll content. When plants are subject to salt stress, chlorophyll metabolism is most easily affected, thus chlorophyll content is often regarded as a hallmark of the damage level²⁵. The decrease in chlorophyll concentration in stressed plants could be attributed to the increased activity of the chlorophyll-degrading enzyme, chlorophyllase, or inhibition in chlorophyll biosynthesis²⁶.

Highest total chlorophyll contents under severe salinity (3000 ppm NaCl) were achieved by spraying seedlings with ASA or WAEs at 10 and 20% recording 1.888, 1.821, 1.839 mg/g in first season and 1.955, 1.884, 1.903 mg/g for second season, respectively. On the other hand, lower total chlorophyll contents were observed when seedlings sprayed using tap water and irrigated with 1500 and 3000 ppm NaCl giving 1.285, 1.089 mg/g for first season and 1.316, 1.108 mg/g in second season, respectively. ASA and 20% WAE were superior in this respect giving in average 2.084, 2.203 mg/g in first season and 2.162, 2.288 mg/g for second season, respectively. Previously, ASA positively affected photosynthetic pigments under both salt and drought stresses²⁶ as well as chilling stress²⁷.

Table (1). Total chlorophyll and carotenoid contents (mg/g) in leaves of pear seedlings grown under saline conditions of irrigation as affected by different foliar spraying treatments.

		Total chlorophyll				Carotenoids			
		First season				Second season			
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		1.571	1.285	1.089	1.315	1.285	1.067	0.957	1.103
SA		2.330	1.998	1.547	1.958	1.898	1.668	1.221	1.596
ASA		2.296	2.067	1.888	2.084	1.864	1.661	1.574	1.700
10% WAE		2.094	1.936	1.821	1.950	1.536	1.419	1.559	1.505
20% WAE		2.640	2.129	1.839	2.203	1.937	1.710	1.520	1.722
Mean		2.186	1.883	1.637		1.704	1.505	1.366	
L.S.D. 5%	NaCl	0.096				0.092			
	Treatments	0.124				0.118			
	Interaction	0.215				0.205			
		First season				Second season			
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		1.618	1.316	1.108	1.347	1.387	1.158	1.026	1.190
SA		2.423	2.071	1.593	2.029	2.072	1.808	1.328	1.736
ASA		2.387	2.144	1.955	2.162	2.049	1.808	1.716	1.858
10% WAE		2.173	2.006	1.884	2.021	1.671	1.546	1.699	1.639
20% WAE		2.752	2.210	1.903	2.288	2.119	1.869	1.660	1.883
Mean		2.271	1.949	1.689		1.860	1.638	1.486	
L.S.D. 5%	NaCl	0.106				0.103			
	Treatments	0.137				0.133			
	Interaction	0.237				0.229			

Generally, increasing concentration of NaCl in irrigation water negatively affected leaves carotenoids content (Table 1).²⁶ suggested that, the decrease in carotenoids under stress condition is due to the degradation of β -carotene and formation of zeaxanthins, which are apparently involved in protection against photo inhibition.

All spraying treatments had beneficial effects regarding leaf carotenoids content with relative advantage for both ASA and 20% WAE treatments recording in average 1.700, 1.722 mg/g for first season and 1.858, 1.883 mg/g in second season, respectively. However, lower total carotenoid contents were observed when seedlings sprayed using tap water and irrigated with 1500 and 3000 ppm NaCl giving 1.067, 0.957 mg/g for first season and 1.158, 1.026 mg/g in second season, respectively.

Root system growth

Concerning number of roots, WAE at 10% was the most effective giving the highest means of 10.3 and 10.4 in both seasons, respectively (Table 2).

Table (2). Root system growth of pear seedlings grown under saline conditions of irrigation as affected by different foliar spraying treatments.

		Number of roots				Average length of roots (cm)			
		First season							
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		9.0	8.0	10.0	9.0	40.0	37.0	33.0	36.7
SA		8.0	9.0	11.0	9.3	37.0	42.0	41.0	40.0
ASA		8.0	9.0	9.0	8.7	38.0	41.0	41.0	40.0
10% WAE		10.0	10.0	11.0	10.3	39.0	40.0	39.5	39.5
20% WAE		8.0	9.0	9.0	8.7	37.0	43.0	43.0	41.0
Mean		8.6	9.0	10.0		38.2	40.6	39.5	
L.S.D. 5%	NaCl	1.084				0.876			
	Treatments	1.399				1.130			
	Interaction	2.423				1.958			
		Second season							
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		8.7	8.3	9.0	8.7	40.4	37.8	33.9	37.4
SA		8.7	9.3	9.7	9.2	37.5	42.8	41.8	40.7
ASA		8.3	10.0	9.0	9.1	38.6	41.8	41.8	40.7
10% WAE		10.0	10.3	11.0	10.4	39.4	40.9	40.2	40.2
20% WAE		8.3	8.3	9.3	8.6	37.3	43.9	43.8	41.7
Mean		8.8	9.2	9.6		38.6	41.4	40.3	
L.S.D. 5%	NaCl	0.629				1.309			
	Treatments	0.812				1.690			
	Interaction	1.407				2.928			

Root length gradually decreased as salinity of irrigation water increased in control seedlings (Table 2).²⁴ observed that, salinity significantly minimized Le-Cont pear seedlings root system growth in terms of root length.

Regarding the effect of spraying treatments, foliar applications during both seasons showed significant positive effects in terms of root length as compared to control which gave the lowest values recording in average 36.7, 37.4 cm for first and second seasons, respectively.

Proline content

Generally, proline contents of pear seedlings increased under salt stress conditions (Table 3). In parallel, proline content in pear seedlings gradually increased by increasing irrigation water salinity as reported by^{23,24}. Proline plays a role in the protection against abiotic stress by assisting osmotic regulation, removing hydroxyl free radicals, avoiding the destruction of enzymes and protection of macromolecules as well as DNA structures^{27,28}.

Table (3). Proline content (mg/100g) of pear seedlings grown under saline conditions of irrigation as affected by different foliar spraying treatments.

		Leaves				Roots			
		First season							
NaCl (ppm)		0	1500	3000	Mean	0	1500	3000	Mean
Treatments									
Control		3.660	4.664	2.152	3.492	2.946	2.830	2.284	2.687
SA		3.603	4.389	4.203	4.065	1.930	2.389	2.435	2.251
ASA		3.847	4.340	4.529	4.239	2.225	2.398	2.706	2.443
10% WAE		2.837	4.997	5.745	4.526	1.440	2.511	2.777	2.243
20% WAE		4.703	5.095	4.729	4.842	2.252	3.600	3.745	3.199
Mean		3.730	4.697	4.272		2.159	2.746	2.789	
L.S.D. 5%		NaCl				0.100			
		Treatments				0.129			
		Interaction				0.224			
		Second season							
NaCl (ppm)		0	1500	3000	Mean	0	1500	3000	Mean
Treatments									
Control		3.761	4.805	2.192	3.586	3.214	3.098	2.486	2.933
SA		3.701	4.519	4.325	4.182	2.108	2.602	2.663	2.458
ASA		3.955	4.468	4.664	4.362	2.447	2.619	2.961	2.676
10% WAE		2.905	5.151	5.929	4.662	1.565	2.747	3.039	2.450
20% WAE		4.845	5.253	4.872	4.990	2.466	3.948	4.108	3.507
Mean		3.833	4.839	4.396		2.360	3.003	3.051	
L.S.D. 5%		NaCl				0.103			
		Treatments				0.133			
		Interaction				0.230			

All foliar applications enhanced proline content of leaves and roots under severe salinity conditions (3000 ppm NaCl) of irrigation water compared with control. However, foliar application of 20% WAE recorded the highest averages in both leaves (4.842, 4.990 mg/100g in first and second seasons, respectively), and roots (3.199, 3.507 mg/100g in first and second seasons, respectively).

The exogenous application of ASA significantly increased the accumulation of proline under chilling²⁷ and water stress conditions²⁸. Also, treatment with 0.5 mM SA mitigates heat stress by increasing proline production by increasing γ -glutamyl kinase and decreasing proline oxidase activity, resulting in the promotion of osmotic potential necessary for maintaining crucial physiological processes²⁹.

Total phenols content

As for the effect of spraying treatments, all foliar applications enhanced total phenols of both leaves and roots under salinity conditions of irrigation water compared with control (Table 4). WAEs revealed higher seedlings total phenol contents in average during both seasons.

Table (4). Total phenols content (%) of pear seedlings grown under saline conditions of irrigation as affected by different foliar spraying treatments.

	Leaves				Roots			
	First season							
NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Treatments								
Control	0.540	0.373	0.131	0.348	0.333	0.317	0.325	0.325
SA	0.272	0.379	0.384	0.345	0.468	0.489	0.586	0.514
ASA	0.393	0.388	0.303	0.361	0.364	0.462	0.579	0.468
10% WAE	0.616	0.445	0.374	0.478	0.369	0.530	0.631	0.510
20% WAE	0.522	0.473	0.390	0.462	0.568	0.556	0.599	0.574
Mean	0.469	0.412	0.316		0.420	0.471	0.544	
L.S.D. 5%	NaCl	0.042			0.044			
	Treatments	0.054			0.057			
	Interaction	0.093			0.098			
Second season								
NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Treatments								
Control	0.607	0.412	0.145	0.388	0.373	0.357	0.365	0.365
SA	0.310	0.416	0.441	0.389	0.509	0.530	0.628	0.556
ASA	0.427	0.434	0.333	0.398	0.404	0.503	0.621	0.509
10% WAE	0.649	0.461	0.411	0.507	0.409	0.572	0.674	0.552
20% WAE	0.559	0.515	0.425	0.500	0.610	0.598	0.641	0.616
Mean	0.510	0.448	0.351		0.461	0.512	0.586	
L.S.D. 5%	NaCl	0.043			0.051			
	Treatments	0.055			0.065			
	Interaction	0.096			0.113			

Antioxidant activity

Regarding antioxidant activity of pear seedlings extract (Table 5), foliar applications during both seasons showed significant positive effects for both leaves and roots as compared to control which gave the lowest values recording in average 83.37, 83.96% in the first season and 83.95, 84.79% in the second season, respectively. Antioxidant system stimulation by some applications is reported to alleviate the adverse conditions of salinity^{30,31}. There appears to be a significant correlation between the content of total phenolics and antioxidant activity³².³³ showed that, the phenolic compounds are the major contributors to the antioxidative activities. They form complexes with the metals, which catalyse oxygenation reactions and inhibit activity of oxidizing enzymes³⁴.

Table (5). Antioxidant activity (%) of pear seedlings grown under saline conditions of irrigation as affected by different foliar spraying treatments.

		Leaves				Roots			
		First season							
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		84.41	85.67	80.03	83.37	81.61	83.82	86.46	83.96
SA		95.57	96.32	84.92	92.27	84.31	86.66	95.03	88.67
ASA		91.54	95.01	83.85	90.13	95.28	94.97	95.49	95.25
10% WAE		87.14	91.65	81.69	86.83	93.06	92.70	92.16	92.64
20% WAE		87.47	95.54	84.33	89.11	94.88	93.54	92.95	93.79
Mean		89.23	92.84	82.96		89.83	90.34	92.42	
L.S.D. 5%	NaCl	0.989				0.551			
	Treatments	1.277				0.711			
	Interaction	2.212				1.231			
		Second season							
Treatments	NaCl (ppm)	0	1500	3000	Mean	0	1500	3000	Mean
Control		84.89	86.50	80.47	83.95	82.01	84.76	87.59	84.79
SA		95.83	97.25	85.08	92.72	84.92	87.45	95.92	89.43
ASA		92.08	95.93	83.98	90.66	96.08	95.85	96.38	96.10
10% WAE		87.48	92.31	81.97	87.25	93.59	93.71	93.02	93.44
20% WAE		87.96	96.02	84.63	89.54	95.43	94.55	93.85	94.61
Mean		89.65	93.60	83.23		90.41	91.26	93.35	
L.S.D. 5%	NaCl	1.301				1.296			
	Treatments	1.680				1.674			
	Interaction	2.910				2.899			

Expression of PbHB1-22 gene

The results confirmed that PbHB1-22 displays stress-inducible expression patterns (Figure 2). These results are in agreement with²⁰ who revealed that, the expression level of PbHB22 gene was up-regulated as response to biotic and abiotic stresses in pear.

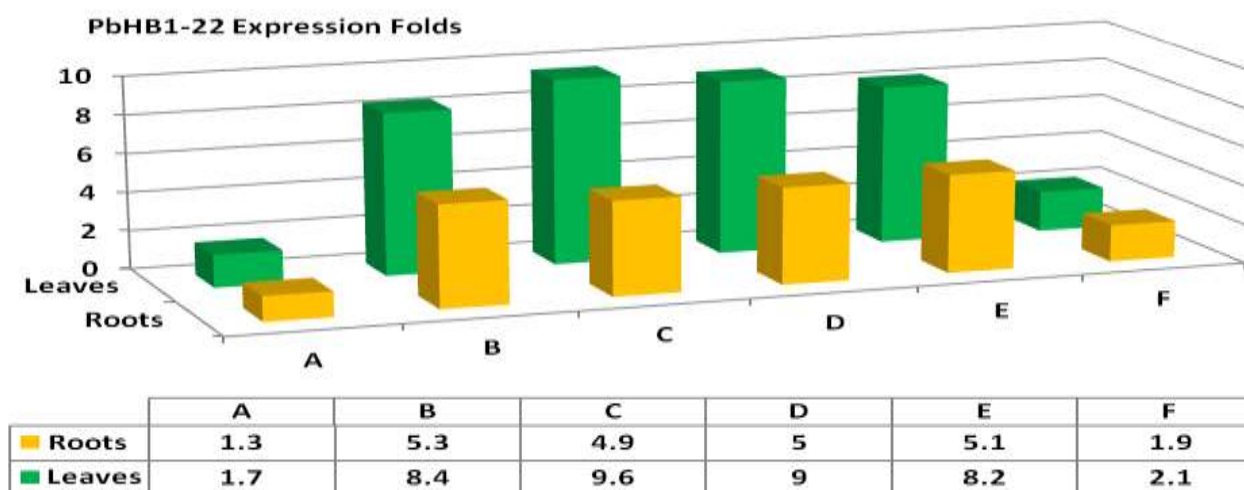


Figure (2). Change in regulation of PbHB1-22 gene in pear leaves and roots under severe salinity conditions (3000 ppm NaCl) as affected by different foliar applications comparing to control. A: tap water irrigated control, B-F: stressed seedlings; B: tap water, C: 0.5 mM salicylic acid, D: 0.5 mM acetylsalicylic acid, E: 10% white willow aqueous extract, F: 20% white willow aqueous extract.

Remarkable reduction in PbHB1-22 expression folds under salt stress was observed when pear seedlings were sprayed using 20% WAE to reach almost normal levels, while slight upregulation in leaves occurred as a response to SA and ASA treatments. Obtained results are partially in agreement with²⁷ who revealed that, ASA significantly upregulated the cold-responsive genes CBF3 and COR47.

Plants launch complex cellular, physiological, and molecular responses during their developmental processes. These responses are usually regulated by transcription factors. The Homeodomain-leucine zipper (HD-Zip) transcription factors are unique to the plant kingdom. Studies show that HD-Zip family genes participate in different biological processes of growth and development of plants as well as adaptation response to environmental stress. Members of HD-Zip subfamilies I and II act as the integrators of development and environmental cues and endogenous hormone signal pathway to regulate targeted gene expression and plant adaptation response³⁵. Previous studies have shown that HD-Zip genes were involved in regulating adaptive response of plants to various stress conditions, such as drought³⁶, salt³⁵ and bacteria³⁷. The expression patterns of PbHB genes revealed that, pear HD-Zip genes were widely involved in responding to drought- and salt-induced stress as well as pathogen infection²⁰. The HD-Zip I Arabidopsis genes ATHB7 and ATHB12 act as negative regulators of growth and development by reducing plant growth under water-deficit conditions³⁸⁻⁴⁰. HAHB10, a member of HD-Zip II subfamily in sunflower, was involved in promoting the accumulation of salicylic acid (SA) upon biotic stresses³⁷.

In-vitro* screening of antibacterial activity against *Erwinia amylovora

Effect of salicylic acid (SA), acetylsalicylic acid (ASA) and white willow extracts (WAEs) as antibacterial agents on *in vitro* growth of *Erwinia amylovora* was studied. Only both WAE concentrations (10 and 20 %) were effective against *E. amylovora* (Figure 3), on the other hand, SA, ASA and control had no effect against the bacteria. The inhibition zone was significantly increased by increasing WAE concentration (10 and 20%) recording 6.7 and 12.3 mm, respectively.

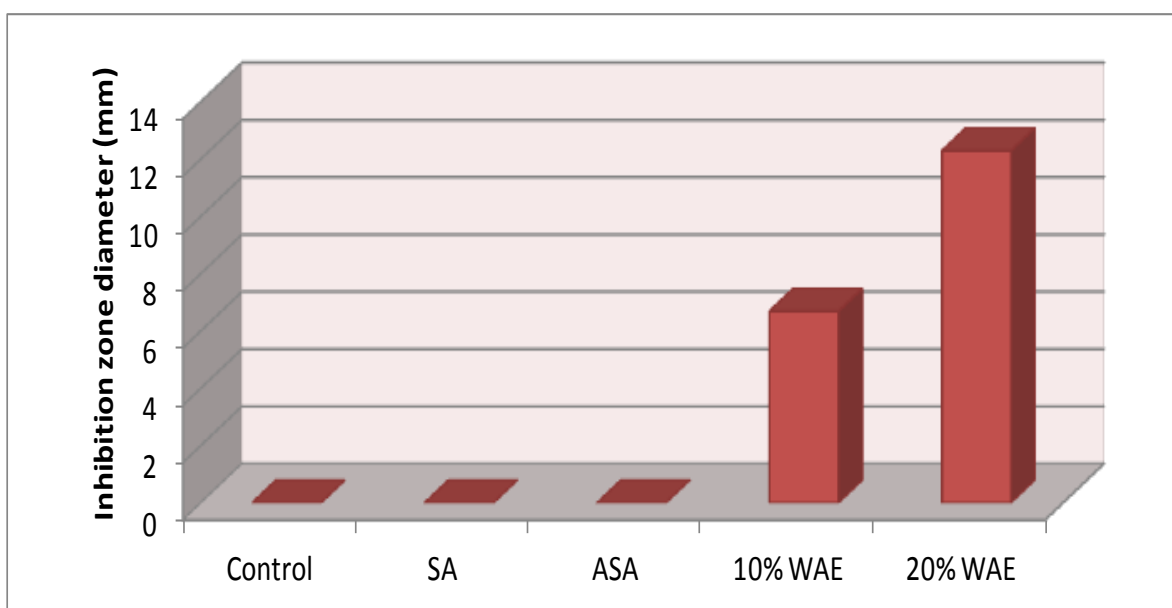


Figure (3). Effect of salicylic acid (SA), acetylsalicylic acid (ASA) and white willow extracts (WAEs) on *in vitro* growth of *Erwinia amylovora* (L.S.D. at 5%= 0.4175).

Salix alba leaves extract at 25% inhibited the *in vitro* growth of *Erwinia carotovora* subsp. *carotovora*²¹. In addition, the crude *Salix alba* leaves extract was found to have minimum inhibitory concentration (the lowest extract concentration that showed significant inhibition of growth compared to the controls) of 800 - 1600 µg/ml against different *Candida* yeast species, effective inhibition found to increase in concentration dependent manner⁸.

Salicylic acid inhibited *in vitro* growth of *Erwinia carotovora*^{41, 42} revealed that, only at higher SA concentrations (over 5 mM) could a clear decrease in the *in vitro* growth rate of *Erwinia carotovora* subsp. *carotovora* be observed. The most likely explanation for SA-induced resistance to *E. c. subsp. carotovora* at lower concentrations (up to 1 mM), where higher SA concentrations negatively affected plant growth, is the elicitation of plant defense. The molecular mechanism of the SA-induced resistance to *E. c. subsp. carotovora* appears to involve inhibition of cell wall-degrading enzymes secreted by the pathogen.

The application of exogenous acetylsalicylic acid (ASA) alleviated the adverse effects of various abiotic stresses; salt, water²⁶, chilling²⁷ and heat⁴³. Pretreatment of ASA appeared to induce pre-adaptive responses to salt and water stresses leading to promote protective reactions²⁶. The exogenous application of ASA most likely significantly increases the activity of the antioxidant system, directly impacting the intensity of photosynthesis²⁷. Also, ⁴⁴reported that, SA-induced salt tolerance due to the improvement in chlorophyll content and the flow of antioxidant enzyme activity that consequently alleviates oxidative stress and enhances the photosynthetic process.

SA or ASA at 0.5 mM were effective against heat, cold and drought stresses, while concentrations over 1 mM ASA or SA appeared to have adverse effects⁴⁵. They suggested that, SA and ASA trigger the expression of the potential to tolerate stress by inducing systemic acquired resistance (SAR) rather than having any direct effect as a protectant.

Acetylsalicylic acid (ASA) or aspirin, a synthetic derivative of salicylic acid (SA), undergoes spontaneous hydrolysis to SA in aqueous solution. Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. SA could be included in the category of phytohormones. Exogenous application of SA may influence a range of diverse processes in plants, including seed germination, stomatal closure, ion uptake and transport, membrane permeability, photosynthetic and growth rate. SA is also known as an important signal molecule for modulating plant responses to environmental stresses. It is now clear that SA provides protection against a number of biotic and abiotic stresses²⁶. SA is involved in the adjustment of vital plant physiochemical activities, such as the light and dark reactions, carbon–nitrogen metabolism, proline metabolism, and ROS scavenger systems, and therefore offers protection against abiotic stresses in plants²⁷.

The beneficial effects of plant's natural extracts on growth, yield and some chemical constituents under abiotic stress conditions has been reported with different plant extracts⁴⁶⁻⁵⁰. Natural resources have provided an unparalleled source of chemical scaffolds with diverse biological activities. Plants containing phytochemicals not only have effect on some particular diseases, but because of their antioxidant or hormone like actions, they also give more beneficial effects. Therefore, the benefits ascribed to *Salix* cannot be unambiguously attributed to a single class of compounds, but rather to the multiple contribution of its different bioactive compounds. The biological properties of *Salix* may attributed to its phytochemical composition rich in phenolics, with positive contribution of flavonoids, as antioxidants as well as salicylates.

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