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IMPACT OF SALICYLIC ACID PRODUCING BACTERIA ON TOLERANCE OF RICE PLANTS TO SOIL SALINITY AND THEIR *IN VITRO* ASSESSMENT TO SUPPRESS SOME PATHOGENIC FUNGI

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ABSTRACT: Salinity hinders plant growth and decreases the yield of crops. Specific plant growth promoting rhizobacteria produces salicylic acid (SA) in significant amounts. The use of these bacteria is an environmentally friendly technique to counteract salinity stresses and play a key role in pathogens attack. In this study, the efficacy of eight bacterial strains of *Rhizobium leguminosarum* bv. *viciae* (441 & 207), *R. leguminosarum* bv. *trifolii* (100 & 103), *Bacillus polymyxa, Bacillus megaterium, Pseudomonas fluorescens* and *Azotobacter chroococcum* were tested *in vitro* for SA production. Upon this test, *A. chroococcum* and *P. fluorescens* were nominated for further experiments. Both selected strains were tested for SA production as well as their *in vitro* diseases control effectiveness by using the extracts of some waste materials. The results indicated that the extract of onion skin and banana peels produced the maximum amounts of SA in *A. chroococcum* and *P. fluorescens*. Moreover, *P. fluorescens* grown on extracts of onion or banana waste materials *in vitro* restricted the mycelia of *Fusarium solani* and *Sclerotinia sclerotiorum* and exhibited an antagonistic effect with a high PIRG (Percent Inhibition of Radial Growth) of 53.85 and 58.97 % respectively against *F. solani* and 66.5 and 65.52 % respectively in case of *S. sclerotiorum*. Field experiments outcomes revealed that adding of *A. chroococcum* + *P. fluorescens* based on onion skin extract was the most successful treatment in raising some growth and yield parameters in comparison with other rice (*Oriza sativa* cv. Sakha 101) treatments grown under saline soil.

Key words: Salicylic acid, Salinity, Rice, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, Pathogenic Fungi.

INTRODUCTION

Salinity is one of the key environmental factors restricting productivity and plant growth. Around one third of the cultivated areas in the world are estimated to be affected by salinity **(Kaya *et al.*, 2002)**. The detrimental salinity effect on metabolism and plant growth is primarily due to increased absorption of Na^+ ions, that induces an abundance of sodium ions in plant tissues **(Abbas *et al.*, 1991)**. Improving stress tolerance in plants has a significant effect on agriculture **(Senaratna *et al.*, 2000)**. Therefore, it is essential to know the various methods in which plants can resist salinity. In this regard, different compounds such as salicylic acid (SA) have been used to alleviate plant stress. SA considered as plant hormone **(Raskin, 1992)**, that has phenolic properties and involved in regulating several plant growth processes. **Stevens *et al.* (2006)** proved that in many crops, the effects of SA in inducing salt tolerance have been well reported. On the other hand, many studies have displayed that SA is an important factor of plant resistance to pathogens attack and involved in plant response to adverse environmental conditions as indicated by **Ryals *et al.* (1996)**. Also, treatment with exogenous SA can induce pathogenesis-related protein expression **(Malamy *et al.*, 1990)**, and set up systemic acquired resistance **(Beckers and Spoel, 2006)**. **Rajjou *et al.* (2006)** confirmed that under salt stress, SA substantially enhanced germination; also, SA induced resistance in different plant species by exogenous application **(Bakker *et al.*, 2003)**.

SA naturally exist, in plants, in very limited concentrations and involved in controlling different physiological processes like protein synthesis,

nutrient absorption, stomatal closure, transpiration and ethylene biosynthesis inhibition **(Shakirova *et al.*, 2003)**. The amount of SA increases, when plants are infected, to fight the infection. On the other hand, SA production was observed in several bacterial strains as indicated by **Leeman *et al.* (1996)** who confirmed that SA production was recorded in *Pseudomonas fluorescens*. In addition, different studies displayed that bacterial species like *Serrtia macrcescens*, *P. aeruginosa* and *P. fluorescens* produce SA and their colonization of host plants increases the endogenous SA levels, besides enhancing host defenses **(De Meyer and Höfte, 1997; Press *et al*., 1997; An and Mou, 2011)**.

In fact, it is interesting to find a method to use the wastes from vegetable peels in a beneficial way. The management of undesirable agricultural waste in a valuable way is a big challenge **(Saranraj and Jayaprakash, 2018**). **Bedrníček *et al.* (2019)** confirmed that onion skin is a rich natural source of flavonoids and its water extracts could be used as an antioxidant material. **Tartrakoon *et al.* (1999)** reported that banana peels have been shown to contain proteins, carbohydrates, metals and oily substances. **Liguori *et al.* (2017)** identified and quantify seven organic acids in onion samples (succinic, oxalic, tartaric, pyruvic, citric, malic and ascorbic acids). Moreover, banana peels contains succinic, palmitic and malic acids in addition to potassium, phosphorus, magnesium, iron and fiber. In banana peels, fatty acids are responsible for their antimicrobial function as indicated by **Sumathy and Sumathy (2011)**.

Rice (*Oryza sativa*) is the biggest world food crop and one of the main staple diets for around 50 % of the world's population. In Egypt, rice is considered the second important cereal crop, following wheat, as a main food for Egyptian population **(Bastawisi *et al*., 2003)**. Rice is very sensitive to salinity stress and is currently listed as the most salt sensitive cereal crop with a threshold of 3 dSm^{-1} for most cultivated varieties **(USDA, 2016)**. Rice yield in salt-affected land is significantly reduced with an estimation of 30–50 % yield losses annually **(Eynard *et al*., 2005)**. Farmers prefer to add more chemical fertilizers to increase the rice yield, which can be harmful to the environment if applied excessively. Alternatively, using an environmentally friendly biofertilizer, that is also cost-effective, is a relatively safer solution. Therefore, the aim of this study is to evaluate the ability of eight effective strains of bacteria to produce SA in culture media and testing the selected strains for SA production from waste materials and their biocontrol activities against phytopathogenic fungi *in vitro* and investigate the influence of SA producing bacteria in minimizing the damaging effects of salinity stress and environmental conditions on rice plants *in vivo*.

MATERIALS AND METHODS

Microorganisms used

Strains of *Rhizobium leguminosarum* bv. *viciae* (441 & 207), *R. leguminosarum* bv. *trifolii* (100 & 103), *Bacillus polymyxa*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Azotobacter chroococcum* were used in the present study. These strains were kindly provided by Biofertilizers Production Unit, Agricultural Microbiology Research Department at Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt. Also, pathogenic fungi, *Fusarium solani* and *Sclerotinia sclerotiorum*, were used and obtained friendly from the Central Laboratory of Organic Agriculture, Agric. Res. Center, Giza, Egypt.

Salicylic acid production by bacterial strains

The bacterial strains production of SA was calculated in accordance with the procedure of **Meyer *et al.* (1992)**. Bacterial strains were grown for 48 hrs at 28±2˚C in the standard succinate medium (SSM) [SSM: Succinic acid, 4 g; KH_2PO_4, 3 g; K_2HPO_4, 6 g; $(NH4)_2$ SO_4, 1 g; $MgSO_4.7H_2O$, 0.2 g in 1 L. H_2O; (pH 7.0)]. The cells were collected at 6000 rpm for 5 minutes by centrifugation. Cell-free culture filtrate (4 ml) was acidified with 1N HCl to pH 2 and SA was extracted with equal chloroform volume. The pooled chloroform phases were supplemented with 4 ml of water and 0.5 ml of 2M $FeCl_3$. At 527 nm, the absorption of the purple iron SA complex formed in the aqueous phase was read spectrophotometrically. Using SA, a standard curve was prepared and the produced SA amount was expressed as µg/ml.

Production of salicylic acid using waste materials

Plant extracts of onion skin, banana peels and sugar beet root skin were prepared according to **Tripathi and Sirohi (2016)** and **Roy *et al*. (2015)** by washing them several times with tap water to remove the dusts and other pollutants, cutting them into small pieces and adding 20g of each waste plant separately into clean conical flask containing 100 ml of sterile distilled water then were incubated in water bath for 20 minutes at 60°C-100°C to facilitate the formation of aqueous plant extracts. The plant extracts were filtrated by using grade 1 filter paper to remove insoluble fractions and macromolecules. Finally, plant extracts were centrifuged at 10000 rpm for 10 minutes and was stored at 4°C in refrigerator for further experiment**.** In SSM, succinic acid was substituted with these aqueous extracts separately. Modified SSM was prepared and autoclaved at 121°C for 15 min then inoculated with bacterial strains which were grown at 28±2ºC for 48 h.

***In vitro* antagonistic effect of salicylic acid producing bacteria against plant pathogenic fungi**

The selected bacterial strains were examined *in vitro* against plant pathogenic fungi (*Fusarium solani* and *Sclerotinia sclerotiorum*) for their biocontrol activity. All plates were incubated on adequate growth temperature and growth inhibition was recorded. Briefly, by using nutrient agar medium as a suitable media for both bacteria and fungi, fresh fungal disc (5 mm in diameter) was placed at 2 cm from the edge of three replicates of Petri dishes and loop of each bacterial strain (24 h old culture) was then streaked at 2 cm (as a line) from the other edge of the plate. Following incubation at 28±2°C for 5-7 days the data were collected and the percent inhibition of radial growth (PIRG) was reported by using the following formula **(Begum *et al*., 2008)**:

$$PIRG = (R_1-R_2)/R_1\times100$$

Where, R_1 is the radial growth of fungal disc in control plate and R_2 is the radial growth of the fungal disc interacting with antagonistic bacteria in the dual culture plate.

Analysis of the soil

As shown in Table 1, the soil properties of the field experiment were calculated as defined by **Black (1965)** as well as available N was measured by the modified method of Kjeldahl. Available P was colorimetrically calculated in accordance with the technique of Olsen in addition to total soluble salt (EC) and soil pH were determined as in **Jackson (1967)**. The Flame Photometer was used to measure available K according to **Soltanpour and Schwab (1977).**

Table 1. Certain physical and chemical properties of the soil prior to sowing

Particle size distribution (%)				Texture	O.M (%)	$CaCO_3$ (%)
Coarse sand	Fine sand	Silt	Clay			
4.56	25.60	30.81	39.03	Clay	0.58	12.40

pH (1:2.5)	EC (dSm^{-1})	Soluble cations (meq/l)				Soluble anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
8.19	8.50	14.90	22.74	46.59	0.77	7.40	35.93	41.67

Macronutrients (mg/kg)			Micronutrients (mg/kg)		
N	P	K	Fe	Mn	Zn
35.55	3.20	183.10	1.88	4.75	0.55

Field experiment

On salt affected clay soil, a field experiment was carried out at Khaled Ben El-Waled village at the Experiment Station of Sahl El-Hussinia, El-Sharkia Governorate, Egypt during growing season of 2018 to study the efficiency of SA producing bacterial strains with rice (*Oriza sativa* cv. Sakha 101) plants under saline soil conditions.

Rice grains were coated with carrier-based inoculants of SA producing bacteria individually using arabic gum as adhesive agent to form a bio-film of bacteria around grains before planting, this process performed on the day of sowing and dried in shadow before planting. Inoculated plots received a liquid bacterial culture after 40 days of planting. Exogenous SA with rate of 150 µg/ml was sprayed as a foliar application on chemical SA treated rice plants twice at 30 and 40 days from sowing **(El-Hedek, 2013)**. The treatments were organized with three replicates in a randomized complete block design where the treatments were as follows:

1- Control

2- Salicylic acid

3- *Azotobacter chroococcum*

4- *Pseudomonas fluorescens*

5- *Azotobacter chroococcum* based on onion skin extract

6- *Pseudomonas fluorescens* based on onion skin extract

7- *A. chroococcum* + *P. fluorescens*

8- *A. chroococcum* + *P. fluorescens* based on onion skin extract

Mineral nitrogen at a rate of 100 kg N/feddan (1 feddan = 0.42 hectare) was added in the form of urea (46 % N) in three doses during the growing season. At a rate of 30 kg P_2O_5/feddan during soil preparation, calcium super-phosphate (15.5 % P_2O_5) was applied whereas potassium sulphate (48 % K_2O) was added in three doses at a rate of 100 kg K_2O/feddan to conserve it from leaching. The experimental plots received the half dose of NPK except control treatment which received the recommended N, P and K fertilizer doses without any sources of SA.

Content of pigments

After 60 days of planting, rice contents of chlorophyll-a, -b and total chlorophyll were measured in accordance with **Arnon (1949)**. Fresh leaves (200 mg) were homogenized into 8 ml of acetone (80 %). The homogenates were centrifuged at 3000 rpm for 15 min/4°C. For pigments analysis, supernatants were used and absorbances at 645, 652, 663, and 470 nm were measured. Calculations were estimated by using equations of **Lichtenthaler and Wellburn (1983)**.

Leaf relative water content (RWC)

To determine RWC, the leaves were collected in polythene bags and transferred as soon as possible for analysis in the laboratory to reduce water loss due to evaporation. Samples were directly weighted as fresh weight (FW), then cut into pieces of 2 cm and floated for 4 h in distilled H_2O. To eliminate surface water, the turgid leaf discs were quickly blotted and weighted to achieve turgid weight (TW). The leaf discs were dried for 24 h at 60° C in the oven, then dry weight (DW) was measured. By the equation given by **Barrs (1968)**, the RWC was calculated as follow:

$$RWC\ (\%) = [(FW- DW)/(TW-DW)]x100$$

Plant growth parameters

Plants of rice were harvested and divided into straw and grains and the yield was registered as ton/feddan. The selected samples were grinded and then the method defined by **Page *et al.* (1982)** was used to digest 0.5 g of each sample. According to **Cottenie *et al.* (1982)**, the concentrations of NPK and Na in grains and straw were determined.

Statistical analysis

The obtained findings were analyzed statistically using the **SAS (1999)** by general linear model method. The differences were statistically tested using Duncan's multiple range tests to measure the degree of significance.

RESULTS AND DISCUSSION

***In vitro* salicylic acid screening assays**

Salicylic acid (SA), in many plants, is an endogenous regulator of localized and systemic acquired resistance, also was observed in different bacterial strains **(Leeman *et al.*, 1996)**. Among the eight tested bacterial strains, *Pseudomonas fluorescens* recorded maximum SA production (27.9 µg/ml) followed by *Azotobacter chroococcum* (19.5 µg/ml) and *Rhizobium leguminosarum* bv. *viciae* (441) (19.36 µg/ml) while the strain of *Bacillus megaterium* recorded the minimum SA production (5.8 µg/ml) (Table 2).

These results are in line with **Leeman *et al.* (1996)** who stated that the production of SA has been reported in *P. fluorescens*. **Syamala and Sivaji (2017)** verified that the maximum SA production (53.24 µg/ml) was recorded in isolates of *P. fluorescens*. Furthermore, **Bakker *et al.* (2003)** confirmed that SA production was observed in several bacterial strains. **Abdel-Aziez *et al.* (2014)** recorded that the maximum production of SA in *A. chroococcum* being 7.4 µg/ml. **Islam *et al.* (2020)** reported that *Pseudomonas tremae* and *Curtobacterium herbarum* were the two endophytic bacteria producing the highest amounts of SA. Therefore *A. chroococcum* and *P. fluorescens* were selected for further studies

Table 2. Production of salicylic acid by different effective bacterial strains *in vitro*

Bacterial strain	Salicylic acid ($\mu g\ ml^{-1}$)
***Rhizobium leguminosarum* bv. *viciae* (441)**	19.36^{c}
***Rhizobium leguminosarum* bv. *viciae* (207)**	17.6^{d}
***Rhizobium leguminosarum* bv. *trifolii* (100)**	14.09^{f}
***Rhizobium leguminosarum* bv. *trifolii* (103)**	14.49^{e}
Bacillus megaterium	5.8^{g}
Bacillus polymyxa	14.1^{f}
Azotobacter chroococcum	19.5^{b}
Pseudomonas fluorescens	27.9^{a}

According to Duncan's test, means with the same letters in the same column do not differ significantly (P<0.05).

Onion skin, banana peels and sugar beet root skin were supplemented as a substrate substituting succinic acid in standard succinate medium and analyzed for SA production. Among the three waste materials, onion skin produced the maximum SA amount of 26.9 and 43.06 µg/ml in *A. chroococcum* and *P. fluorescens* respectively followed by banana peels (Figure 1). These findings are consistent with **Liguori *et al.* (2017)** who recognized and quantify seven organic acids as succinic acid in onion samples. **Renga Ramanujam *et al.* (2015)** proved that *P. fluorescens* produced maximum of 42 µg/ml of SA from banana skin.

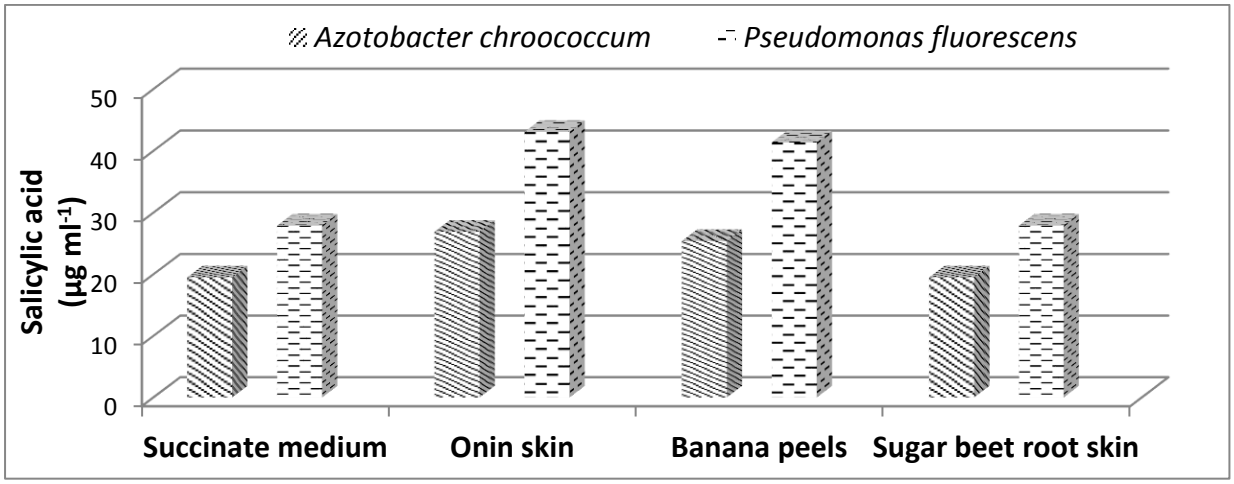


Figure 1. Salicylic acid production by *A. chroococcum* and *P. fluorescens* using waste materials

In vitro* inhibition of *Fusarium solani* and *Sclerotinia sclerotiorum

SAs of microbial origin are being used as an extensive strategy in order to protect the plants from pathogens. In this study, the antagonistic effects of the selected SA producing bacteria grown on succinate medium and different waste materials were evaluated *in vitro* against phytopathogenic fungi (*Fusarium solani* & *Sclerotinia sclerotiorum*) as indicated in Table 3 and Figure 2.

Table 3. Antagonistic effect of salicylic acid producing bacteria against *Fusarium solani* and *Sclerotinia sclerotiorum*

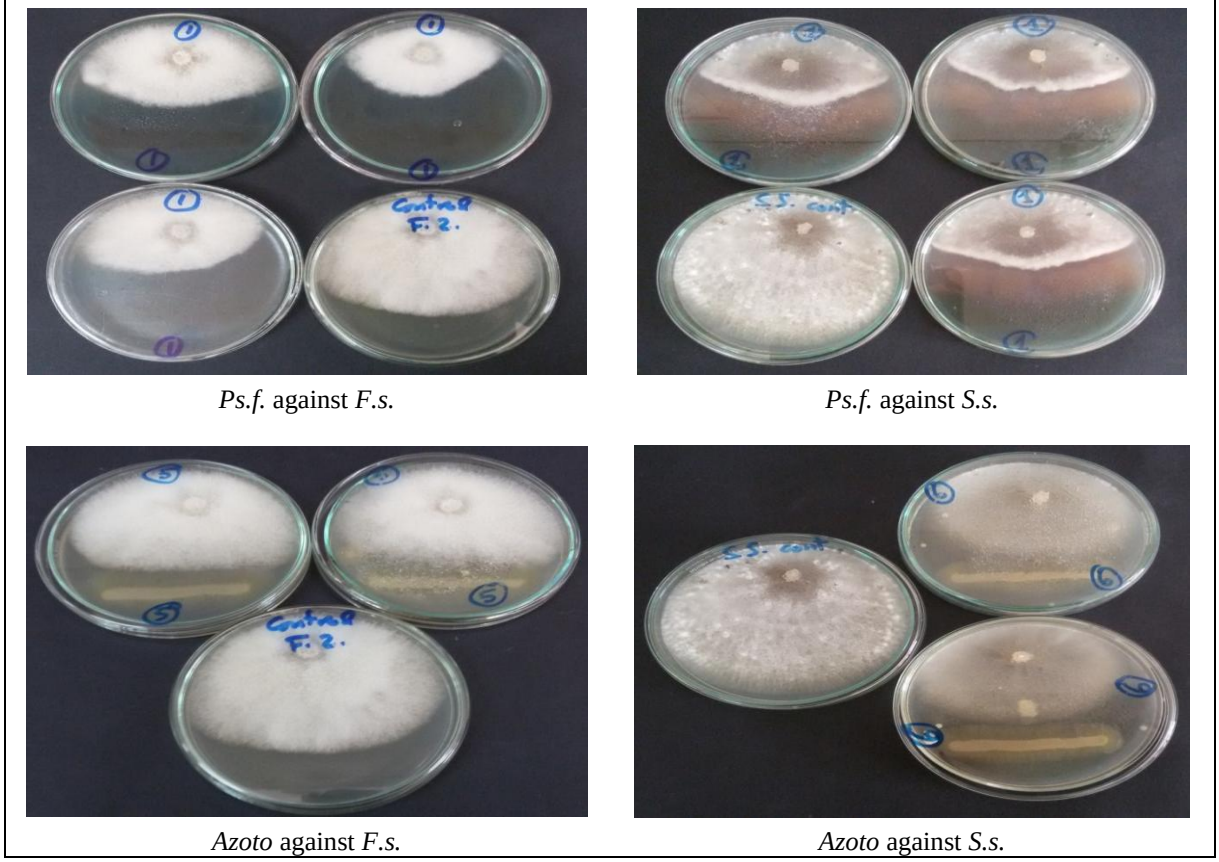
Treatments	PIRG (%)	
	Fusarium solani	***Sclerotinia sclerotiorum***
Ps.f. based on onion skin extract	53.85^{b}	66.50^{a}
Ps.f. based on banana peels extract	58.97^{a}	65.52^{b}
Ps.f. based on sugar beet skin extract	51.28^{c}	64.04^{d}
Ps.f. based on succinate medium	47.44^{d}	65.02^{c}
Azoto. based on onion skin extract	26.28^{f}	30.05^{g}
Azoto. based on banana peels extract	26.92^{e}	31.03^{f}
Azoto. based on sugar beet skin extract	24.36^{g}	41.87^{e}
Azoto. based on succinate medium	23.72^{h}	12.81^{h}

- According to Duncan's test, means with the same letters in the same column do not differ significantly (P<0.05).
- PIRG = Percent Inhibition of Radial Growth; Ps.f. = *Pseudomonas fluorescens*; Azoto = *Azotobacter chroococcum*.

It is cleared that the tested *P. fluorescens* grown on extract of waste materials restricted the mycelia of *Fusarium solani* and *Sclerotinia sclerotiorum* and displayed a highly antagonistic effect with a high PIRG (percent inhibition of radial growth) where *P. fluorescens* with onion skin and banana peels exhibited 53.85 and 58.97 % respectively against *F. solani* and 66.5 and 65.52 % respectively in case of *S. sclerotiorum* (Table 3).

This indicates that SA can provide a considerable level of protection against pathogens as shown by **Van Kan *et al.* (1995)**. The obtained data are in

harmony with **Qi, *et al*. (2012)** who showed that the *Fusarium graminearum* mycelial growth and conidia germination were significantly inhibited in the presence of increasing concentration of SA in both liquid and solid media. Moreover, **Klessig *et al.* (2000)** indicated that after pathogen attack, SA is known to play a crucial signaling role in activating plant defense responses. **Achuo *et al*. (2004)** revealed that SA induced resistance on tobacco and tomato plants to *Botrytis cinerea* (gray mould disease). Also, treated pomegranate arils with SA reduced the total microbial population and decay **(Shaarawi *et al*., 2016)**. Along with prevented the softening during ripening of kiwifruit and banana **(Srivastava and Dwivedi, 2000; Zhang *et al*., 2003)**.



Ps.f. against *F.s.* | *Ps.f.* against *S.s.*

Azoto against *F.s.* | *Azoto* against *S.s.*

Figure 2. Antagonistic effect of salicylic acid producing bacteria against plant pathogenic fungi. (Ps.f.= *Pseudomonas fluorescens*; Azoto= *Azotobacter chroococcum*; F.s. = *Fusarium solani*; S.s.= *Sclerotinia sclerotiorum*)

Plant growth parameters

SA may be involved in reducing the harmful impact of salinity on plants. Data in Table 4 indicate that growth parameters have improved significantly at all treatments of SA producing bacteria where adding of *A. chroococcum* + *P. fluorescens* based on onion skin extract was the best efficient treatment in increasing plant height and shoot dry weight followed by *A. chroococcum* + *P. fluorescens* treated plants in comparison with control. The promotive effect of SA on growth characters could be attributed to its bioregulator effects on physiological and biochemical processes in plants as well as increase the antioxidant capacity of plant as indicated by **EL Tayeb (2005)**. Comparable outcomes were reported by **Cornelia *et al.* (2010)** who observed a great raise in the growth parameters of SA treated plants (e.g. leaf area, plant length and total dry weight) when compared with non-treated ones, they attributed this effect to SA's role in activating cell division and availability and movement of mineral nutrients towards the leaves. **Noreen and Ashraf (2008)** suggested that growth enhancement of treated plants with SA could be due to the influence of SA in inducing changes in photosynthesis and antioxidant capacity. In agree with this result, **Meena *et al.* (2006)** have also proved that the highest percentage of germination and maximum plant height were recorded when the seeds treated with *P. fluorescens* taking in our consideration that production of SA has been reported in *P. fluorescens* as indicated previously by **Leeman *et al.* (1996)**.

Table 4. Influence of salicylic acid producing bacteria on some rice growth parameters under salt soil conditions

Treatments	Plant height	Dry wt. of shoot (g)	Leaf relative water content (RWC%)	Chlorophyll -a (µg/ml)	Chlorophyll -b (µg/ml)	Total chlorophyll (µg/ml)
Control	49^{d}	1.098^{d}	31.6^{h}	0.632^{f}	1.14^{f}	1.88^{f}
Salicylic acid	46^{e}	1.142^{c}	34.7^{g}	0.46^{g}	0.641^{g}	1.27^{g}
Azotobacter chroococcum	46^{e}	1.04^{e}	38.9^{f}	1.297^{c}	2.73^{a}	2.97^{e}
Pseudomonas fluorescens	51^{c}	0.879^{f}	45.3^{e}	1.16^{d}	1.32^{e}	3.14^{d}
***A. chroococcum* based on onion skin extract**	40^{f}	2.64^{a}	49.8^{d}	1.47^{a}	1.69^{d}	5.53^{a}
***P. fluorescens* based on onion skin extract**	53^{b}	1.04^{e}	54.7^{c}	1.405^{ab}	2.19^{c}	3.83^{b}
A. chroococcum* + *P. fluorescens	54^{b}	1.428^{b}	57.6^{b}	0.968^{e}	2.39^{b}	3.47^{c}
***A. chroococcum* + *P. fluorescens* based on onion skin extract**	56^{a}	2.64^{a}	62.6^{a}	1.40^{b}	2.19^{c}	3.83^{b}

According to Duncan's test, means with the same letters in the same column do not differ significantly (P<0.05).

The leaf relative water content (RWC) is a useful measure of the physiological water status of plants **(González and González-Vilar, 2001)**. Water stress often results when plants are subjected to high salt concentrations. Previous studies have indicated that salt stress reduced RWC as in the leaves of cucumber seedlings **(Yildirim *et al.*, 2008)**. Also, SA is reported to increase RWC and water potential tolerance of plants to salt stress **(Tari *et al.*, 2002)**. In this study results indicated that treated plants with *A. chroococcum* + *P. fluorescens* based on onion skin extract were recorded higher moisture content (62.6 %) followed by *A. chroococcum* + *P. fluorescens* that gave 57.6 % of RWC as comparing to control plants that was 31.6 % (Table 4). This is probably due to the fact that SA increases ABA, which ultimately helps in maintaining better water balance in the plants as confirmed by **Sakhabutdinova *et al.* (2003)**. **Fricke *et al.* (2004)** showed that with increasing salinity, leaf RWC decreases. **EL Tayeb (2005)** illustrated that SA plays a significant role in regulating a variety of physiological processes in plants**.**

Photosynthetic characteristics

The obtained data suggested that the adding of SA producing bacteria considerably improved the content of chlorophyll -a, -b and total chlorophyll in comparison with those grown under salinity without SA (Table 4). The content of chlorophyll -a, -b and total chlorophyll were 1.4, 2.19 and 3.83 µg/ml respectively in the plants treated with *A. chroococcum* + *P. fluorescens* based on onion skin extract and 0.968, 2.39 and 3.47 µg/ml respectively in *A. chroococcum* + *P. fluorescens* treatment showing high rate of chlorophyll contents as compared to the control plants. **Gautam and Singh (2009)** stated that chlorophyll content of plant leaves decreased under salt stress because of the adverse effects of ions of various salts in chlorophyll biosynthesis. As well as salinity effect on chlorophyll content by stopping certain enzymes accountable for assembly of green pigments in plants. **Türkyilmaz Ünal *et al.* (2005)** proved that spraying SA on leaves which were grown in saline soil led to increasing the content of chlorophyll in bean plants. **Sudhir and Murthy (2004)** showed that salt stress can limit photosynthesis by reducing green pigments. Also, **Khodary (2004)** reported the favorable effect of SA on the content of chlorophyll in maize leaves.

Nutrients concentration of rice plants

Increasing salinity levels of soils led to increasing concentrations of Na^{+} and Cl^{-} and reducing concentrations of K^{+}, Ca^{2+} and Mg^{2+} in many plant species **(Bayuelo-Jiménez *et al.*, 2003)**. Presented data in Table 5 revealed that using of SA producing bacteria decreased Na concentration and increased NPK contents in straw and grain and *A. chroococcum* + *P. fluorescens* based on onion skin extract was the most efficient treatment followed by *A. chroococcum* + *P. fluorescens* as compared to control plants.

Table 5. Nutrients concentration in straw and grain after harvesting rice plants grown in saline soil

Treatments	Rice Straw Macronutrients concentration (%)				Rice Grains Macronutrients concentration (%)			
	N	P	K	Na	N	P	K	Na
Control	1.09^{f}	0.23^{e}	2.30^{f}	0.45^{b}	1.20^{g}	0.38^{e}	1.95^{h}	0.31^{a}
Salicylic acid	1.13^{e}	0.25^{d}	2.35^{c}	0.42^{c}	1.28^{e}	0.44^{d}	2.04^{f}	0.29^{b}
Azotobacter chroococcum	1.22^{c}	0.26^{d}	2.39^{b}	0.39^{d}	1.32^{c}	0.47^{c}	2.09^{d}	0.28^{b}
Pseudomonas fluorescens	1.13^{e}	0.22^{e}	2.34^{dc}	0.48^{a}	1.30^{d}	0.47^{c}	2.06^{e}	0.22^{d}
***A. chroococcum* based on onion skin extract**	1.18^{d}	0.20^{f}	2.29^{e}	0.36^{e}	1.23^{f}	0.39^{e}	1.97^{g}	0.25^{c}
***P. fluorescens* based on onion skin extract**	1.19^{d}	0.28^{c}	2.33^{d}	0.22^{f}	1.28^{e}	0.48^{c}	2.13^{c}	0.21^{d}
A. chroococcum* + *P. fluorescens	1.24^{b}	0.33^{b}	2.38^{b}	0.19^{g}	1.36^{b}	0.55^{b}	2.17^{b}	0.17^{e}
***A. chroococcum* + *P. fluorescens* based on onion skin extract**	1.29^{a}	0.37^{a}	2.45^{a}	0.16^{h}	1.40^{a}	0.58^{a}	2.22^{a}	0.14^{f}

According to Duncan's test, means with the same letters in the same column do not differ significantly (P<0.05).

This indicated that SA under saline conditions could play a significant role in the selectivity of these ions. The beneficial impact of SA on the absorption of K and the inhibitory effect on Na^{+} may be responsible for plant stress management. These findings are in harmony with those obtained for barley **(Munns, 2005)** and in maize by **Gunes *et al.* (2007)** in which they illustrated that the exogenous SA applications inhibited the accumulation of sodium ions, but stimulated the concentrations of other macro- and micro-nutrients (e.g. N, P, K, Fe, Mg, Cu and Mn). **EL Tayeb (2005)** showed that the increase in concentration of K and Ca in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield**.** Also, **Al-Hakimi (2006)** confirmed that application of SA had an inhibitory effect on the accumulation of Na. **Wahid *et al.* (2004)** showed that the N uptake was often disrupted under salt stress where excess salts could reduce the accumulation of N in plant because the antagonism between Cl^{-} and NO_3^{-}.

Plant yield parameters

Data in Table 6 showed that plants treated with SA producing bacteria were able to rise significantly the grain yield, straw and weight of 100 grain of rice plants comparing to control and *A. chroococcum* + *P. fluorescens* based on onion skin extract was the most successful treatment followed by *A. chroococcum* + *P. fluorescens*. This may be because the exogenous application of SA producing bacteria increased the photosynthetic rate and also preserved membrane stability, thus improving the growth of plants stressed by salinity as indicated by **El Tayeb (2005)**. This confirmed by **Sakhabutdinova *et al.* (2003)** who proved that SA played effective role in saline stress tolerance because it has capability to motivate a defensive impact on plants under salinity condition.

Table 6. Influence of salicylic acid producing bacteria on rice yield components grown in salt soil

Treatments	wt. of grains ton/fed*	wt. of straw ton/fed*	wt. of 100 grain (g)
Control	2.09^{g}	3.330^{g}	2.87^{b}
Salicylic acid	2.315^{e}	4.275^{d}	2.055^{g}
Azotobacter chroococcum	2.25^{f}	3.730^{f}	2.25^{f}
Pseudomonas fluorescens	1.715^{h}	5.835^{a}	1.965^{h}
***A. chroococcum* based on onion skin extract**	2.380^{d}	3.900^{e}	2.35^{d}
***P. fluorescens* based on onion skin extract**	2.565^{c}	2.435^{h}	3.015^{a}
A. chroococcum* + *P. fluorescens	2.66^{b}	5. 5^{b}	2.3^{e}
A. chroococcum* + *P. fluorescens based **on onion skin extract**	2.85^{a}	5.35^{c}	2.6^{c}

According to Duncan's test, means with the same letters in the same column do not differ significantly (P<0.05).

*One feddan = 0.42 hectare.

CONCLUSION

From all findings it is clear that if salicylic acid production is promoted, it will play an important role in triggering the plant immune system that in-turn will pave ways for protection of the host from pathogens like *Fusarium solani* and *Sclerotinia sclerotiorum*. The future application of this hormone is highly promising for providing tolerance to different agricultural crops against salinity stress.

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