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Salicylic Acid is an Effective Eco-Friendly Technique

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ABSTRACT

The rate of salicylic acid (SA) degradation in the experimented peat soil was conducted to determine the degradation by five concentrations of ^{14}C radioactive salicylic acid (i.e. 0.001, 0.01, 0.1, 1.0 and 10 µM) at different time periods. Results showed that the rate of salicylic acid degradation increased as the concentration raised from 0.0001 to 10 mM. Thereafter, the rate of SA degradation increased slightly to reach about 28.7% after one week (168 h). In addition, a pot experiment was carried out at Bilqas city, Dakhalia Governorate during summer season of 2017 to study the effect of salicylic acid spraying on its translocation in lettuce (*Lactuca sativa L.*) tissues, as well as its effect on fresh yield and chemical analysis. Salicylic acid was sprayed at concentrations of 0, 0.0001, 0.001, 0.01, 0.1, 1.0 and 10 mM at pH values of 4 and 7 after one week from cultivation once a week, and stopped two weeks before harvesting. Further, spraying with salicylic acid at concentration of 0.01 mM was the best concentration on enhancing chlorophyll content, fresh weight yield, phosphorus and potassium concentrations, in addition, alleviation of free nitrate accumulation in lettuce tissues. Concerning the effect of pH value, it was noticed that pH value 7 was better than pH value 4 on enhancing chlorophyll content, fresh weight yield, phosphorus and potassium concentrations, while pH value 4 was better than pH 7 on alleviation of free nitrate accumulation in plant tissues.

Keywords: Salicylic acid degradation, Chemical composition, Lettuce plants

INTRODUCTION

It is well known that, 2-hydroxybenzoic acid (Salicylic acid) belongs to an extraordinary diverse group of plant phenolics usually defined as substances that process an aromatic ring bearing a hydroxyl group or its functional derivative (Fig 1).

It was shown that SA synthesized through the isochorismate route has an important role in plant defense against pathogen infection as well UV- or ozone-treated Arabidopsis thaliana L., Nicotiana benthamiana and tomato (Ogawa *et al*. 2005; An and Mou 2011).

Plant phenolics have often been referred to as secondary metabolites, the term "secondary" implied that such compounds were only of minor importance to the plant and could sometimes be equated with waste products.

This notion has been gradually replaced, however, by the view that many phenolic compounds play an essential role in the regulation of plant growth, development, and interaction with other organisms (Harbone 1980)

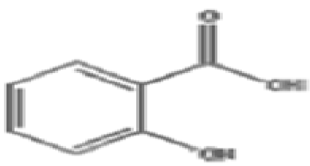

Figure 1. Structure of salicylic acid

SA is generally present in plants in quantities of a few µg/g fresh mass or less, either in a free state or in the form of glycosylated, methylated, glucose-ester or amino acid conjugates (Lee *et al*., 1995)

It is well documented that phenolic compounds exert their influence on physiological and biochemical processes. One, such a natural compound is salicylic acid that may function as plant growth regulator (Arberg, 1981), consequently salisylic acid will accelerate the leaf area and dry mass production (Khan *et al*., 2003).

One of the roles of SA which attributed with increasing photosynthesis process and dry matter yield production is the improving of chlorophyll content in plant tissues and activation the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation (Ghai *et al*., 2002 and Moharekar *et al*., 2003).

Exogenous application of SA was used to be effective in plant physiological and metabolic pathways which may develop the resistance to water scarcity.

Further, addition of SA at various concentrations through roots, seed soaking and foliar spraying in a concentration-dependent teqniuce increased the negative influence of water stress on tissue water status, stomatal conductance, membrane properties and plants physiological activities and chlorophyll content, (Horváth *et al*. 2007; Hayat *et al*. 2010). The supply of SA in heat production in plants is well documented, and this is through its action on respiration which increase rate elevates the surface temperature (Van der Straeten *et al*., 1995)

With regard to muskmelon plant, the application of SA either through seed soaking or foliar application, enhanced protection against drought (Marisha *et al*., 2017). In addition, minimum concentrations within the range of

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0.1–0.5 mM were more effective in minimizing the negative effect of drought than maximum concentration (1 mM). However, the method of application did not found to be significant (Korkmaz *et al.* 2007). In cucumber, the application of SA by seed soaking or foliar spray ameliorated injury caused by water deficit. Application of SA was more effective within seeds soaking and the best results were obtained using 0.5 mM SA solution (Baninasab 2010). However, application of SA (0.7 mM) in rice plants was more effective in ensuring better resistance to water stress when applied by foliar spray than seed treatment (Farooq *et al.* 2009). Higher concentrations of SA were effective in protecting Satureja hortensis plants with 1.0 - 3.0 mM and amaranth, tomato plants at 3 mM resistant to water deficit (Umebese *et al.* 2009; Yazdanpanah *et al.* 2011).

The very first physiological response, ever attributed to SA in plants, was its impact on flower induction, supplemented with kinetin and indole acetic acid (Eberhard *et al*., 1989)

Concerning the effect of SA on nitrate metabolism, it could be concluded that SA increase the activity of nitrate reductase enzyme which will decrease the accumulation of free nitrate in plant tissues, and increase the protein content (Hayat *et al*., 2005).

On the other hand, it is stated that SA has a vital role on stress tolerance in plant including toxic metals tolerance (Yang *et al*., 2003), drought tolerance (Hamada and Al-Hakimi 2001), heat tolerance (Dat *et al*., 1998), cold tolerance (Kang and Saltveit 2002), salinity resistant (Szepesi *et al*., 2005), ozone stress (Rao and Davis 1999) and ultraviolet radiation (Ervin *et al*., 2004). This study was carried out to determine the rate of salicylic acid degradation in the soil and study the effect of salicylic acid spraying on its translocation in lettuce (*Lactuca sativa L.*) tissues, as well as its effect on fresh yield and chemical analysis.

MATERIALS AND METHODS

Salicylic acid degradation experiment

To determine the rate of salicylic acid degradation in the experimented peat soil, five concentrations of ^{14}C radioactive salicylic acid were prepared (i.e. 0.001, 0.01, 0.1, 1.0 and 10 µM). These concentrations were prepared from of a stock solution of 20 mM salicylic acid**.** A 250 µL from each solution were added to a 5 g of field-moist soil in a 60 mL polypropylene tubes. Soil was mixed gently with the radioactive solution by shacking. Polypropylene vials (5 mL volume) of 1M NaOH (1mL) were added to the tubes to act as traps for $^{14}CO_2$, which generated from salicylic acid degradation. Vials of NaOH traps were removed after 0.5, 1.5, 3, 6, 24, 48 and 168 h. Then, a 4 mL of scintillation fluid (Wallac optiphase 3; Wallac EG&G, Milton Keynes, UK) was added to NaOH vials and mixed well on a vortexer. Amounts of ^{14}C trapped in NaOH vials were determined by liquid scintillation counter (Wallac 1404 scintillation counter, Wallac EG&G, Milton Keynes, UK).

A pot experiment was carried out in Bilqas city, Dakhalia Governorate in the summer season of 2017 to study the effect of salicylic acid spraying and translocation in lettuce (*Lactuca sativa L.*) and its effect on yield quantity, and chemical analysis.

Lettuce was chosen to be the experimented plant, because it is a fresh edible plant, so it has the priority to examine comparable with other crops.

The used soil in the experiment was peat soil, its saturation percentage was determined using the method described by Richards (1954), and it was 215%.

Total soluble salts were determined by measuring the electrical conductivity in the extract of saturated sample as explained by Jackson (1967), and it were 0.35 dS/m.

Organic matter content was 90.25%, and it was determined by Walkley and Black method which described by Hesse (1971).

The used experimental design was split plot design, main plots were salicylic acid concentrations which were 0, 0.0001, 0.001, 0.01, 0.1, 1.0 and 10 mM, and the sub plots were solution pH at values 4 and 7.

Lettuce seedlings were transplanted in 29th June 2017, irrigated to reach the field capacity, two times per week.

Plants were sprayed with salicylic acid after one week from cultivation once a week, and stopped two weeks before harvesting.

Chlorophyll content was determined one week before harvesting with the portable chlorophyll meter (SPAD-502, Minolta).

Lettuce plants were harvested in29th August 2017, fresh weight was weighted immediately and calculated per gram.

After one week from foliar application, representative discs from lettuce leaves were subjected to radiographic imaging analysis to ensure salicylic acid translocation into veins.

Free nitrate concentration in lettuce leaves were determined colourimetrically in the fresh tissue according to the method described by Singh (1988).

Samples were taken from each pot, dried at 70°, and grounded manually to avoid contamination.

To determine nutrients concentration, 0.2 g of each sample was digested using 5 cm^3 from the mixture of sulfuric (H_2SO_4) and perchloric ($HCLO_4$) acids (1:1) as described by Peterburgski (1968).

Phosphorus was determined colorimetrically at wave length 680 nm using spectrophotometer (Spekol) as described by Jackson (1967), and Potassium was determined by using Gallen Kamp flame photometer as mentioned by Jackson (1967).

Data of the present study were statistically analyzed and the differences between the means of the treatments were considered significantly when they were more than least significant differences (LSD) at the levels of 5% according to Duncan (1955), using CoStat (Version 6.303, CoHort, USA, 1998-2004).

RESULTS AND DISCUSSION

Salicylic acid degradation in soil

Data presented in Fig. 2 revealed that salicylic acid showed a fast degradation during the first 48 h. Then, the rate of degradation became slow until the last reading (168 h). After one week (168 h) the final degradation rate of salicylic acid ranged from 60.6% with the concentration of

0.0001 mM to 93.9% with the concentration of 10 mM. Therefore, it is revealed that the rate of salicylic acid degradation increased as the concentration raised from 0.0001 to 10 mM.

Dissolved LMW organic acids also form an important source of labile C for soil microorganisms (Jones and Darrah, 1994). Typically, low molecular weight organic acids are rapidly degraded in soil with half lives ranging from 0.5 to 12 h (Jones and Darrah, 1994; Jones *et al*., 2001). The rate of mineralization, however, is likely to be dependent upon factors such as microbial biomass and community structure as well as microbial transporter expression (Jones, 1998).

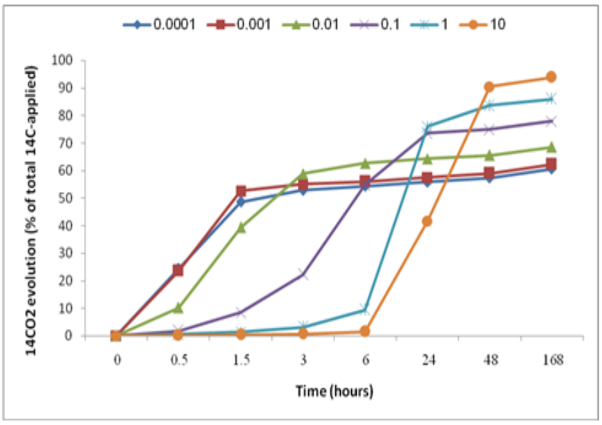

Fig. 2. Salicylic acid degradation at different time periods (hours).

This interpretation is supported by the fumigated soil with chloroform. It is well known that chloroform fumigation resulted in a lethal effect on soil microorganisms. The survival of soil microorganisms is associated with increasing time after fumigation (White *et al*., 1994). As illustrated in Fig. 3, the rate of SA degradation was approximately zero until 24 h. Thereafter, the rate of SA degradation increased slightly to reach about 28.7% after one week (168 h).

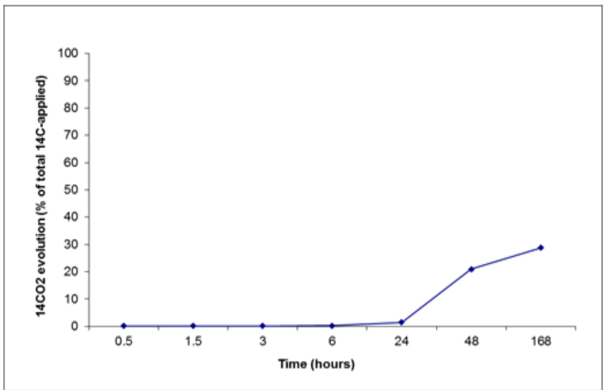

Fig. 3. Effect of chloroform fumigation on SA mineralization.

Concerning the rest of SA, which did not degraded by soil microorganisms, it could be adsorbed on soil particles. Adsorption has been found to significantly reduce the decomposition rates of organic acids in model systems (Boudot, 1992; Jones and Edwards, 1998). In this respect, it is cleared that the rate of adsorption is decreased as the concentration of SA increased.

Effect of salicylic acid treatments.

Chlorophyll content

The illustrated data in Table 1 indicated that there is a highly significant effect of salicylic acid concentrations on chlorophyll content (SPAD), and this is because of the positive effect of salicylic acid on improving chlorophyll content in plant tissues and activation the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation (Ghai *et al*., 2002; Moharekar *et al*., 2003 and Francxoise *et al*., 2009).

Results indicated that salicylic acid concentration of 0.01 mM was the best concentration treatment on enhancing chlorophyll formation under pH 7 value, but concentrations content, and these results were matched with the same results which obtained by (Fariduddin *et al*.,2003).

Concerning to the effect of pH value of the foliar solution, it can be stated that decreasing pH value from 7 to 4 had a high significant effect on decreasing chlorophyll content (SPAD), and this may be attributed to the corrosion effect of low pH value on plant tissues, meanwhile, the interaction between salicylic acid concentration and pH value was not significant.

Fresh weigh yield

The effect of salicylic acid treatments on chlorophyll content reflected on lettuce fresh weight yield (g), and it is cleared that low concentrations (0.01, 0.001 and 0.0001 mM) of salicylic acid enhanced lettuce fresh yield, while high concentrations (10, 1.0 and 0.1 mM) were attributed with a reduction in lettuce fresh yield, moreover, the concentrations of 1.0 and 10 mM caused a necrotic symptoms on lettuce leaves. The highest mean value of fresh weight was 90.83 g plant^{-1} obtained from the integrated with spraying of SA 0.01 mM concentration and pH 7 value. Meanwhile, the lowest mean value of the aforementioned trait was 48.33 g plant^{-1} occurred with the combined spraying of 10 mM concentration and pH 4 value. These results were in great harmony with Hayat *et al*., (2005).

It is stated that salicylic acid may function as plant growth regulator (Arberg, 1981; Horváth *et al*. 2007; Hayat *et al*. 2010), consequently salicylic acid will accelerate the leaf area and dry mass production. Moreover, salicylic acid spraying will accelerate photosynthesis process and dry matter yield production (Khan *et al*., 2003).

On the other hand, pH value resulted in a high significant effect on fresh weight and this is may be attributed to the loss of organic substrates which reflected on decreasing the yield (Murata *et al*., 2003), but the interaction between treatments was not significant.

Phosphorus concentration percentage

Tabulated data in Table (1) show that increasing salicylic acid concentrations gradually decreased phosphorus concentrations in lettuce tissues, and this is due to depolarisation of cell membranes (Glass 1974 b), these results also obtained by (Glass 1973).

Concerning the effect of pH solution spray in phosphorus concentration in lettuce tissues, it could be concluded that, decreasing pH value from 7 to 4 was attributed with a high significant reduction in phosphorus concentration percentage, and this is may be attributed to the adverse effect of low pH value on lettuce which reflected on its ability on phosphorus uptake. Meanwhile, the interaction between salicylic acid concentrations and pH value was not significant.

The maximum mean value of P concentration was 0.416% obtained from untreated plants with SA under pH 7 value. Meanwhile, the minimum mean value of the same element was 0.243% occurred with the combined spraying of 10 mM and pH 4 value.

Potassium concentration percentage

Potassium concentration in plant tissues took the same trend of phosphorus uptake suggesting that the protonated form of SA is more active than its charged form (Glass 1973), similar results were also stated by (Glass 1974 a).

Decreasing pH value was attributed with a high significant reduction on potassium concentration in lettuce tissues, and this is also because low pH value had a negative effect on lettuce reflected on decreasing potassium uptake, also the interaction between salicylic acid concentrations and pH value was not significant. The maximum mean value of K % in lettuce plant was 4.17% obtained from untreated plants with SA under pH 7 value. Meanwhile, the minimum mean value of the same element was 2.33% occurred with the combined spraying of 10 mM and pH 4 value.

Table 1. Effect of salicylic acid concentrations on lettuce yield and analysis

Treatments		Chlorophyll	Fresh weight	Phosphorus Conc.	Potassium Conc.	Nitrate Conc.
SA Conc. (mM)	**pH value**	**(°Spad)**	**(g)**	**(%)**	**(%)**	**(mg kg^{-1} DW)**
0	4	25.07^{f}	66.33^{e}	0.386^{b}	3.90^{b}	20.12^{cde}
	7	27.80^{cde}	74.33^{c}	0.416^{a}	4.17^{a}	22.83^{bcd}
0.0001	4	26.20^{ef}	69.00^{de}	0.383^{bc}	3.70^{c}	17.57^{ef}
	7	28.07^{cd}	76.16^{c}	0.396^{b}	4.07^{a}	21.13^{bcde}
0.001	4	27.37^{de}	76.10^{c}	0.353^{e}	3.40^{e}	17.19^{ef}
	7	30.80^{ab}	81.83^{b}	0.370^{cd}	3.77^{c}	19.67^{cdef}
0.01	4	29.30^{bc}	82.83^{b}	0.326^{f}	3.17^{f}	16.08^{f}
	7	32.27^{a}	90.83^{a}	0.356^{de}	3.57^{d}	18.38^{ef}
0.1	4	24.87^{f}	52.57^{f}	0.307^{g}	2.97^{g}	18.13^{ef}
	7	27.47^{de}	71.00^{d}	0.327^{f}	3.23^{f}	19.15^{def}
1	4	22.1^{g}	56.67^{g}	0.280^{h}	2.63^{h}	22.47^{bcd}
	7	24.84^{f}	67.00^{e}	0.310^{g}	2.90^{g}	23.48^{abc}
10	4	19.50^{h}	48.33^{h}	0.243^{i}	2.33^{i}	24.37^{ab}
	7	21.97^{g}	57.33^{g}	0.273^{h}	2.60^{h}	26.93^{a}
SA concentration	F-Test	**	**	**	**	**
	LSD at 0.05	0.78	1.85	0.013	0.099	1.70
pH value	F-Test	**	**	**	**	**
	LSD at 0.05	0.59	1.20	0.005	0.047	1.3

***In the same column, means followed by a same letter are not significantly different at 5% level by Duncan (1955).**

Free nitrate accumulation

Data illustrated in Table (1) revealed that spraying with salicylic acid at low concentrations had a high significant effect on decreasing nitrate accumulation in plant tissues, while high concentrations caused a negative effect, this is because of the activation of nitrate reductase enzyme in plant tissues at low concentrations of salicylic acid, meanwhile, high concentrations were inhibitory (Jain and Srivastava 1981).

On the other hand, pH at value 4 had a high significant effect on decreasing nitrate accumulation in lettuce tissues comparable with pH 7, and this is revealed to the activation of antioxidative enzymes in plant tissues (Quan *et al*., 2002), nevertheless, the interaction between salicylic acid concentrations and pH value was not significant.

The maximum mean value of nitrate content was 26.93 mg kg^{-1} DW obtained from the sprayed plants with 10 mM of SA under pH 7. Meanwhile, the minimum mean value of the same character was 16.08 mg kg^{-1} DW occurred with the combined spraying of 0.01 mM and pH 4 values.

Data presented in Fig. () showed the translocation of SA into leaves (veins in particular) through the noticeable dark colures. This finding confirmed the translocation of SA inside plant tissues and stimulating several physiological indices in plant (e.g. chlorophyll synthesis and thus assimilation of the generated products). Furthermore, SA might have a beneficial effect on alleviating nitrate accumulation in plant tissues through activating nitrate reductase enzyme as shown in Fig. 4 These results are coincided with ((Hayat *et al*., 2005). These images revealed that the dispersal of SA was obviously noticeable through major vein of leaf tissues as presented in Fig 4 A. However, the distribution of SA appeared pale in Fig. 4 suggesting the marginal translocation of SA during the initial spraying phase. These results are coincided with Francxoise *et al*., (2009) and Kang *et al*., (2012).

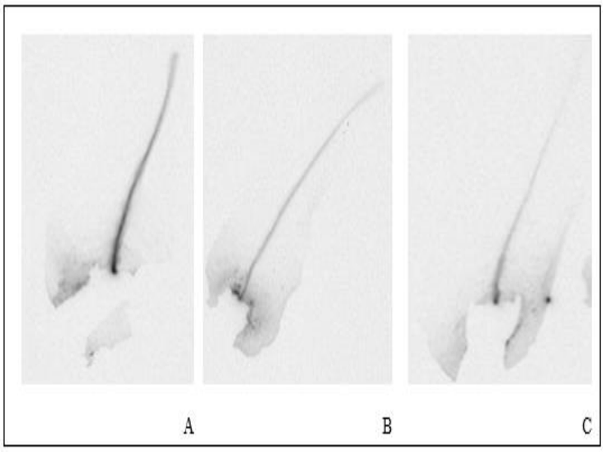

Fig. 4. Auto radiographic images of the Lettuce leaves.

CONCLUSION

The obtained results concluded that the rate of salicylic acid degradation increased as the concentration raised from 0.0001 to 10 mM and the rate of SA degradation increased slightly to reach about 28.7% after one week (168 h). Further, spraying with salicylic acid at concentration of 0.01 mM was the best concentration on enhancing chlorophyll content, fresh weight yield, phosphorus and potassium concentrations, in addition, alleviation of free nitrate accumulation in lettuce tissues. It was noticed that pH value 7 was better than pH value 4 on enhancing chlorophyll content, fresh weight yield, phosphorus and potassium concentrations, while pH value 4 was better than pH 7 on alleviation of free nitrate accumulation in plant tissues

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حمض السلسليك كتقنية فعالة صديقة للبيئة

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إجريت تجربة معملية لدراسة معدل تحلل حمض الساليسيليك (SA) في تربة البيت بواسطة خمسة تركيزات من حمض الساليسيليك المشع C14 (أي 0.001 ، 0.01 ، 0.1 ، 1.0 و 10 ميكرومتر) في فترات زمنية مختلفة بالمعمل. أظهرت النتائج أن معدل تدهور حمض الساليسيليك زاد مع زيادة التركيز من 0.0001 إلى 10 ملم. بعد ذلك ، ارتفع معدل تدهور SA بشكل طفيف ليصل إلى حوالي 28.7 ٪ بعد أسبوع واحد (168 ساعة). كما تم إجراء تجربة أصص بمدينة بلقاس ، بالدقهلية خلال الموسم الصيفي 2017 لدراسة تأثير رش حمض الساليسيليك و انتقاله في أنسجة الخس (Lactuca sativa L.) ، وكذلك تأثيره على المحصول الطازج والتحليل الكيميائي . تم رش حمض الساليسيليك بتركيزات من 0 ، 0.0001 ، 0.001 ، 0.01 ، 0.1 ، 1.0 و 10 ملليمول عند قيم الأس الهيدروجيني 4 و 7 بعد أسبوع واحد من الزراعة مرة واحدة في الأسبوع ، وتم التوقف قبل أسبوعين من الحصاد. أظهرت النتائج أن الرش بحامض الساليسيليك بتركيز 0،01 ملليمول كان أفضل تركيز على تحسين محتوى الكلوروفيل ، وزيادة الوزن الطازج ، وتركيزات الفوسفور والبوتاسيوم ، بالإضافة إلى تخفيف تراكم النترات الحر في أنسجة الخس. فيما يتعلق بتأثير قيمة الرقم الهيدروجيني ، لوحظ أن قيمة الرقم الهيدروجيني 7 كانت أفضل من قيمة الرقم الهيدروجيني 4 في تعزيز محتوى الكلوروفيل ، وإنتاجية الوزن الطازج ، وتركيزات الفوسفور والبوتاسيوم ، بينما كانت قيمة الرقم الهيدروجيني 4 أفضل من الرقم الهيدروجيني 7 في تخفيف تراكم النترات الحرة في الأنسجة النباتية