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Frequency of Application of Salicylic Acid and Its Impact on Growth Aspects and Biochemical Quality In Lettuce Plants (*Lactuca sativa*L.)

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Abstract

Background: Lettuce is grown and consumed practically all over the world, constituting an important source of minerals, especially calcium and vitamin A. It is one of the hardwood greens preferred for salads, because it has a pleasant and refreshing flavor. However, it presents short post-harvest life, being subject to the attack of phytopathogens, which can shorten its shelf life. **Objective:** The goal of this work was to evaluate the effect of 1 mL of salicylic acid (SA) solution at 1 mM concentration on lettuce plants (*Lactuca sativa* L.) under the crop`s physical, physiological, biochemical and post-harvest. To achieve that, two experiments (I and II) designed completely randomly were carried out in a greenhouse, each with four replicates and the same six treatments. In the experiment I, the variables physical responses were: number of leaves per plant, percentage of leaves with some injury per plant, shoot green mass (g), root green mass (g), root volume (mL).For the variables physiological and biochemical responses, three leaves of different positions (lower, intermediate and superior) were collected and evaluated: total chlorophyll, total protein, total sugars, peroxidase, phenylalanine ammonia-lyase (PAL) and total phenols. The second experiment occurred after 10 days of collection and storage in a BOD incubator at 10°C; the number of leaves with some injury or rot per plant was quantified and, after removal of these leaves, the final mass (m, g) was obtained, where mass loss (m_0-m_f, g) was calculated.**Results:**It was verified that the SA did not interfere in the physical, physiological and biochemical characteristics, therefore, it was not responsible for a metabolic loss during the growth and development of the culture, as well, it did not reduce the post-harvest shelf life.**Conclusion:**It is concluded that from application frequencies every 24 h with 1 mL of SA solution at 1 mM concentration, sprayed on leaves of lettuce (*Lactuca sativa* L.), there is no metabolic loss during growth and development of the crop, as well, did not reduce post-harvest shelf life.The results point to the possibility that the volatile effect of salicylic acid had an effect on adjacent plants.

Key words: Metabolic loss, Elicitors, Salicylic acid, Lactuca sativa.

INTRODUCTION

The development of more sustainable agricultural systems that can produce quality food in adequate quantities without polluting the environment is one of the biggest challenges facing us today (Edaroyati et al 2017). The production of lettuce (Lactuca sativa L.) is of great importance in practically all the world, which is very consumed and with tradition of being cultivated by small farmers by different way (Makhadmeh et al, 2017) such soilless and soil and in regions close to the great urban centers. With this, discovering new forms of cultivation and production is of economic and social importance, as it may in the future impact on the lives of producing families. Among the new forms of production are those aimed at compounds that may induce resistance to plants, such as salicylic acid (SA) (Monteiroet al., 2013). SA is a phenolic compound that occurs naturally in plants, playing an important role in regulating plant growth, development, maturation and defense responses (Almeida, 2012; Sánchez-Rangel *et al*.,2015; Cueto-Ginzoa *et al*., 2016). In addition to the defense responses, SA acts in the response to abiotic stress, including drought, low temperature, salinity, among others. Studies have suggested that SA has great agronomic potential to improve the stress tolerance of crops of agricultural importance (Jayakannan *et al*., 2015; Zaidi *et al*., 2015; Hajer *et al*., 2016). However, their usefulness is dependent on the concentration applied, the mode of application and the condition of the plants (Miura and Tada, 2014).

SA is a key signaling molecule that is necessary for the induction of defense genes and rapid and localized cell death at the site of pathogenic infection (hypersensitivity response) during incompatible host-pathogen interactions (Sánchez-Rangel *et al*., 2015). Changes in the activities of some enzymes are important for the definition of the state of induced resistance in the presence of pathogens. Among these enzymes, the most important are: Phenylalanine Ammonia-Liase (PAL), peroxidases, chitinases, lipoxygenases and β-1,3-glucanases (Almeida, 2012).

For instance, the effect of SA on tomato plants (*Solanumlycopersicum*) infected with Tomato Potato (PV) X Virus (strain SPCP1) was examined by Cueto-Ginzoa (2016). According to the author, through the stabilization of photosystem II, SA partially compensated the reduction in the photosynthetic rate during the

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infection by increasing the conductance of the mesophyll, increased the proteins related to thermotolerance and stress, in addition to decreasing the proteins related to the aperture the strongest effects of SA occur at the beginning of the cycle of pathogenesis.

According to Zaidi *et al*. (2015), the role of SA is very important in the mechanisms of reduction of plant water deficiency. Sayyari *et al*. (2004) evaluated the effect of salicylic acid (SA) on lettuce (*L. sativa* L.) under water stress, in which there was a reinforcement in the growth rate and change in the physiological parameters that diminished the adverse effects of the water stress of the plants.

The accumulation of total phenolic compounds bound to the soluble cell wall and total soluble proteins in maize (*Zea mays*) plants exposed to water stress and foliar spray of salicylic acid (SA) at 10^{-4} mol-L^{-1} and 10^{-5} mol-L^{-1} was investigated by Latif *et al*. (2016). The author suggests that the accumulation of both cell wall soluble soluble phenolic compounds by SA foliar spraying may be a mechanism related to drought tolerance by the induced stress of SA in economically viable corn.

Already in conditions of saline stress, Hajer *et al*. (2016) suggests that salicylic acid can also be considered as a potential growth regulator to improve plant resistance. For Jayakannan *et al*. (2015) SA is related to signaling to control the entry of sodium into the tissue of the Arabidopsis root and this prevents the loss of potassium; being important for the tolerance to the salts and oxidative stress.

The objective of this work was to evaluate the frequency of application of salicylic acid and the impact on physical, physiological and biochemical characteristics in lettuce plants (*Lactuca sativa* L.), as well as on physical characteristics in the post-harvest period.

MATERIAL AND METHODS

The experiment was carried out in an automated greenhouse and the analyzes carried out at the Phytosanitary and Biochemical Laboratories of the Federal Technological University of Paraná (UTFPR), DoisVizinhos campus. Two experiments (I and II) were carried out, both in completely randomized design, with 4 replicates and 6 treatments, totalizing 24 experimental units (EUs). In the experiment I, physical, physiological and biochemical variables were evaluated at the time of closure. In the second experiment, physical variables were evaluated in the post-harvest period (shelf life).

Lettuce seedlings (*L. sativa* L.) with approximately 5 cm height were obtained, which were transplanted in 8 L polyethylene pots. The substrate used consisted of the homogenized mixture of 50% of red latosol and 50% of poultry manure duly treated.

After five days of transplanting, it was verified that there was no loss/death of any of the plants and that they were already vigorous for implantation of the experiment. Previous test showed that 1 mL of solution with distilled water and salicylic acid (SA) at 1 mM concentration would not cause plant phytotoxicity.

The treatments, applied with hand spray on the leaves of the plants, were: **T1** - control witness 1mL of water every 24 h; **T2** - 1mL solution with distilled water and SA at 1 mM concentration every 24 h (1 day); **T3** - 1 mL of SA solution every 48 h (2 days); **T4** - 1 mL of SA solution every 72 h (3 days); **T5** - 1 mL of SA solution every 168 h (7 days); and **T6** - 1 mL of SA solution every 336 h (14 days).

The treatments were applied in the 48 EUs for 37 days (approximate time of the culture cycle duration), and in the initial day, with the exception of the witness, 1 mL of solution was sprayed in all the experimental units with distilled water and acid salicylic acid (SA) at 1 mM concentration. All plants, in addition to the witness, that on a given day did not receive the SA solution, were then sprayed with 1mL of water in order to offer the same conditions of foliar humidity, thus avoiding systematic error.

At the end of the experiment (37th day), in the greenhouse, with the help of a PALker digital chlorophyllometer, model CFL 1030, the chlorophyll content of mature leaves was measured. Five measurements were carried out per plant, the extreme measurements being discarded (minor and major), and the average was calculated based on three readings for each EU.

After the collection of these data the harvesting was performed, cutting the plants in the region of insertion of the lower leaves. The plants harvested were conditioned in plastic bags and immediately taken to the laboratory for the other analyzes. After harvesting the aerial part, the roots were collected and washed.

In the laboratory, for each of the 24 EUs of the first experiment, the following data were collected: mass (g) of fresh matter of aerial part and root (g); the number of leaves; the number of leaves with some injury or senescent (%) and root volume (mL). The volume of the root was obtained by the method of displacement of liquid proposed by Archimedes. Three leaves of different positions (lower, intermediate and superior) of the plant were collected, which were immediately frozen at -4 °C and packed in foil for the following biochemical analyzes: total protein determination (mg/g tissue); total sugars (mg/g tissue); peroxidase (EU/min); quantification of the enzyme Phenylalanine Ammonia-Liase (PAL, UAbs/min/mg protein); and total phenols (EU/min).

The determination of the total proteins of the samples was performed according to the methodology described by Bradford (1976). The samples were macerated in a pestle with 6 mL of 0.2 M phosphate buffer (pH 7.5). Thereafter, the material was centrifuged at 11,700 rpm for 10 min at 4 ° C, and the supernatant was collected. The total protein reading was performed in a spectrophotometer at 590 nm, using bovine serum albumin as standard.

For the determination of total sugars, the sulfuric phenol method was used (Dubois, *et al*., 1956). 20 µl of the supernatant from each sample obtained above was transferred to properly identified test tubes, plus 480 µl of phosphate buffer, 500 µl of 5% phenol and 2.5 ml of sulfuric acid. After the mixture cooled to room temperature, the spectrophotometer was read at 490 nm. The standard curve of total sugars from glucose (100 µg/mL)was also obtained.

The peroxidases present in the plant tissue were determined by the method of Matsumo and Uritami (1972). 3 ml of the supernatant from each sample was transferred into properly identified test tubes, where the following preparation was present: 5 mL citrate buffer pH 5.0 plus 0.5 mL 3% hydrogen peroxide plus 0.5 mL guaiacol, 5%. The mixture was vortexed and placed for 15 min in a water bath at 30 °C and then for 10 min on ice. Add 0.5 mL of sodium bisulfite and vortex again. The reading was carried out in a spectrophotometer at 450 nm.

The activity of the PAL was determined by colorimetric quantification of the trans-cinnamic acid released from the phenylalanine substrate, according to Kuhn (2007), using 0.25 g of the sample plus 3.0 mL of the TRIS – HCl pH 8,0 buffer. This extract was packed in Eppendorf® tubes and centrifuged for 10 min at 4 °C and 6,000 rpm. Then, 200 µL aliquot of the supernatant was transferred into test tubes, adding another 3.0 mL of the extraction buffer. The solution was vortexed, whereby the enzyme extract was obtained. From this extract, 1.5 mL was transferred to another test tube, plus 1.0 mL of the extraction buffer and 0.5 mL of phenylalanine. Again, the vortex solution was stirred for homogenization. The tubes were then placed in a water bath for 45 min at 40 °C. Finally, the tubes were removed from the water bath and placed in an ice bath for 5 min to interrupt the reaction. The spectrophotometer was read at 290 nm.

Total phenols were obtained using the method adapted from Bieleski and Tuner (1966) and Jennings (1981). The procedures were divided into two phases. In phase 1, 1 g of plant material was weighed, which was macerated with 4 mL of MCW solution (methanol, chloroform, water 6/2.5/1.5). The macerate was placed in tubes and centrifuged at 6,000 rpm at 20 °C for 20 min.The supernatant was collected and transferred to properly identified test tubes containing 1mL of chloroform and 1.5 mL of distilled water. Another centrifugation was performed at 6,000 rpm, 20 °C for 15 min. In step 2, 0.5 mL of the upper part of the supernatant was removed and transferred to tubes containing 0.5 mL of water and 0.5 mL of the Folin-Ciocalteau reagent (1:10 dilution). The preparation was vortexed. After 15 min, 5 mL of alkaline reagent A (prepared from 2% sodium carbonate in 0.1 N sodium hydroxide solution) was added and vortexed again. After 50 min the spectrophotometer was read at 760 nm.

In experiment II, the roots and leaves with some injury were removed when harvest was done. Then, the initial mass (m_0, g) of the 24 UEs were weighed. Then the lettuce plants were placed in transparent plastic bags for hortifruti (35 cm x 50 cm) and stored in a BOD incubator (Biochemical Oxigen Demand) at 10 °C for 10 days, simulating the shelf conditions. At the end of this period, the number of leaves with some injury or rot per plant was quantified and, after removal of these leaves, the final mass was obtained (m_f, g), where mass loss (m_0- m_f, g).

For data analysis, the computational tool R (Team, 2018) version 3.4.3 was used. To verify the assumptions of the mathematical model of the experimental design, the Shapiro-Wilk test (for residue normality) and Bartlett's test (for homogeneity of variances) were used at a 5% significance level. When the assumptions of the model were not met, the Box-Cox transformation was applied (Box and Cox, 1964). Then, the data were submitted to analysis of variance (ANOVA) and when significant, the Tukey averages comparison test was performed, at the 5% level of significance.

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RESULTS AND DISCUSSION

Tables 1, 2 and 3 present the results of the experiment I against the physical, physiological and biochemical variables as a function of the frequency of application of 1 mL of SA solution at 1 mM concentration, respectively. The results of experiment II, with post-harvest data, are presented in Table 4.

Table 1: Physical variables in function of the frequency of application of 1 mL of SA solution at concentration of 1 mM in lettuce (*L. sativa* L.): number of leaves/plant, percentage of leaves with some injury or senescent/plant, fresh mass of aerial part (g), fresh root mass (g), root volume (mL) and root length (cm).DoisVizinhos - PR, 2016.

Physical Response VariableAverages						
Treatments	Number of leaves/plant	Leaves with some injury or senescent/plant(%)	Fresh mass of aerial part (g)	Fresh root mass(g)[I]	Root volume(mL)[II]	Root length (cm)[III]
T1	19,25[ns]	20,83[ns]	183[ns]	5,80[ns]	8,25[ns]	14,00[ns]
T2	16,00	19,10	125	3,51	6,50	10,75
T3	18,50	23,25	175	5,64	8,75	11,75
T4	18,50	16,03	200	7,13	9,00	15,25
T5	16,00	16,85	149	4,44	7,00	10,25
T6	18,75	19,83	174	5,95	10,00	12,50
CV(%)	10,86	27,05	24,64	30,88	33,62	15,68

ns: Not significant by the F test at the 5% level of significance. [III]Transformedby Box-Cox with□=-0,8.

Table 2: Physiologicalvariables in function of the frequency of application of 1 mL of SA solution at concentration of 1 mM in lettuce (*L. sativa* L.):chlorophyll A,chlorophyll B andtotal chlorophyll. DoisVizinhos-PR, 2016.

PhysiologicalResponse Variable Averages (Chlorophyll content)			
Treatments	Chlorophyll A	Chlorophyll B	Total chlorophyll
T1	23,78[ns]	2,98[ns]	26,75[ns]
T2	23,56	2,58	26,13
T3	24,19	2,94	27,13
T4	24,37	2,58	26,95
T5	23,34	2,65	25,99
T6	28,40	3,79	32,19
CV(%)	12,26	23,98	13,17

ns: Not significant by the F test at the 5% level of significance.

Table 3: Biochemichal variables in function of the frequency of application of 1 mL of SA solution at concentration of 1 mM in lettuce (*L. sativa* L.):total proteins, total sugars, peroxidase, PAL and phenols. DoisVizinhos-PR, 2016.

Averages of Biochemical Responses Variables					
Treatments	Total proteins[I] (mg/g.tissue)	Total sugars (mg/g.tissue)	Peroxidase[II] (EU/min)	PAL[III](UAbs/min/ mg.protein)	Phenols[IV](mg/g.tissue)
T1	1,23 ab	211,65 a	30,08 a	0,062[ns]	0,4187[ns]
T2	1,29 ab	169,33 ab	29,32 ab	0,054	0,3841
T3	1,16 b	113,84 bc	29,11 b	0,051	0,3441
T4	1,13 b	88,40 c	29,13 ab	0,063	0,6508
T5	1,28 ab	73,09 c	29,53 ab	0,065	0,4072
T6	1,52 a	73,54 c	28,75 b	0,065	0,4738
CV(%)	12,25	21,28	25,80	49,78	18,09

Treatments with averages not linked by the same letter in the column differ by the Tukey averages comparison test at the 5% level of significance. Transformedby Box-Cox with: [I]□=-0.5; [II]□=15.5; [III]□=2.7; e [IV]□=0.4, respectively.

Table 4: Post-harvest physical variablesin function of the frequency of application of 1 mL of SA solution at concentration of 1 mM in lettuce (*L. sativa* L.):initial mass, number of leaves with sore or rot per plant, final mass and mass loss. DoisVizinhos-PR, 2016.

Averages of Post-Harvest Physical Response Variables				
Treatments	Initial mass(g)	Number of leaves with sore or rot/plant	Final mass(g)	Mass loss(g)
T1	165,25[ns]	4,25[ns]	103,75[ns]	61,50[ns]
T2	188,00	3,75	120,00	68,00
T3	145,50	3,25	100,00	61,50
T4	158,00	3,00	105,75	52,25
T5	149,25	3,00	101,25	48,00
T6	181,75	4,50	117,50	64,25
CV(%)	24,50	28,53	23,38	38,33

ns: Not significant by the F test at the 5% level of significance.

When the normality assumptions of the residues and/or homogeneity of the variances were not met, the use of the *boxcox()* function of the MASS package (Team, 2018) allowed investigating a transformation for the response variables of interest of this work. In general, the Box-Cox transformation has the practical advantage of estimating the maximum likelihood value for f (□);thus, we obtain □ such that the response variable of interest under this transformation can mainly meet the residue normality assumption (Box and Cox,1964), a fact that occurred for all variables. The assumption of homogeneity of the variances was not only observed for: fresh root mass (g), root volume (mL) and PAL activity (UAbs/min/mg.protein); however, the F test of the analysis of variance is more sensitive to the normality of the data.

It was verified that the sprinkling of SA did not contribute to the reduction of leaves per lettuce plant, loss of mass (g) of fresh plant material, volume (mL) or length (cm) of root, as well as, did not influence the plant defense considering the percentage of leaves with some injury or senescent (Table 1).

The frequency of application of SA also did not influence the levels of chlorophyll A, B and total with higher averages for chlorophyll content in T6 (Table 2). Tatagiba *et al*. (2014) verified that plants in saline or water stress may have the content of diminished photosynthetic pigments. For chlorophyll A the T5 treatment was lower and for chlorophyll B, the treatments T2 and T4. More detailed studies should be carried out to elucidate the behavior of chlorophyll production in lettuce plants, because the data inferred that a small frequency of SA could encourage the production of total chlorophyll.

On the other hand, protein analysis (Table 3) showed that total protein concentration was higher in T6, which is not significantly different from T1, T2 and T5. The lowest concentrations of proteins were in T3 and T4 treatments, which are not significantly different from T1, T2 and T5. The elevation of protein content is related to the synthesis of PR-proteins (proteins related to pathogenesis) which are responsible for plant defense (Kuhn, 2007).

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Zaidi *et al*. (2015) also investigated the effect of applying four levels of SA (0, 0.375, 0.75 and 1.5 mM) under water deficiency in tolerant and sensitive sunflower hybrids. It verified that there was a decrease in the protein content under water stress, but, when applying SA, there was an increase in the protein contents. The author concluded that the SA contributed to increase the tolerant capacity of the plant to water stress. In another study, SA foliar spraying in 10^{-5} L^{-1} improved the adverse effects of water stress on the reduction of leaf water relative content, fresh weight, dry weight, fresh root weight, dry root weight , root length and root area (Latif *et al*., 2016).

Already Renault *et al*. (2015) studied pathogenesis-related proteins (PRPs) using leaves of vines obtained from woody cuttings grown in greenhouses. According to the author, the elicitation with SA induced the production of a new protein presenting 30 kDa.

As for total sugars, there was a higher concentration in T1, which does not differ statistically from T2, followed by T3, which does not differ from T4, T5 and T6. These changes in total sugars (reducers and sucrose) may be related to increased metabolic activity of the seedlings, since the metabolic cycles are integrated. A process of induction of compounds of secondary metabolism can affect the primary metabolism of carbon such as glycolysis, pentose phosphate or citric acid cycle (Mazaro, 2007).

In relation to the quantification of peroxidase, there was greater expressiveness in T1, which did not differ statistically from T2, T4 and T5. The lowest value was for T3 followed by T6, which differed only from T1. Peroxides are glycoproteins with the capacity to catalyze many reactions such as lignin formation, incorporation of glycoproteins into the cell wall, peroxidative destruction of indolylacetic acid in addition to other growth regulators.The increase in peroxidase activity is a sign of alteration of plant metabolism, in the process of plant defense, as in the formation of lignin by the polymerization of phenols (Labanca, 2002). According to Kuhn (2007), the alteration of peroxidase activity is related to disease reduction mechanisms. Campos *et al*. (2004), observed a positive correlation between peroxidase and polyphenoloxidase activities, and phenolic compound contents with anthracnose resistance in beans.The activity of peroxidase increases when it is related to wounds present in the plants, which can generate an increase in lignin biosynthesis, which will act as a barrier to infection by pathogens and in this way causes an increase in the concentration of oxidation products of the phenolic compounds.

Bertoncelli *et al*. (2015), in cucumber studies, demonstrated that SA influenced plant growth and maturation, but not enzymatic characteristics. Already Mazaro *et al*. (2015), attributed that the application of SA can give maintenance and quality in certain plants, delaying maturation and reducing rot, an unproven fact with lettuce.

In a study by Deuner *et al*. (2015), it was suggested that resistance inducers could signal the formation of many cellular metabolites in plants, among them: proteins, total and reducing sugars. Kumar *et al*. (2015), also observed that a low frequency of SA application encouraged the production of total proteins and reducing sugars. In particular, T6 presented higher protein content, ie, it is the treatment with a lower frequency of application among the others.

Biochemical analyzes of the enzymatic activity of PAL and phenols showed that SA did not significantly influence (Table 3). Mazaro *et al*. (2008), it was observed that the action of elicitors in different concentrations contributed to a higher content of total phenols in acerola when compared to the control.New analyzes should be performed to elucidate the behavior of the lettuce in relation to the frequency of application of the SA, since for total sugars and peroxidase it was observed a tendency to reduce the levels obtained, and in the other variables the non-statistical significance.

The hypothesis raised for this phenomenon is the volatization of salicylates, since SA, when exogenously applied in vegetables, induces the production of its methyl ester, the metilsalicylate, which is the precursor of the synthesis of the salicylic acid itself, being a potent induced resistance (Resende*et al*., 2003).Studies suggest that methyl salicylate acts as a volatile signal, being transmitted to distant parts of the plant and even to adjacent plants (Shulaev*et al*., 1997).

In view of this evidence, as the experimental units were very close, it is not to be ruled out that volatilization of salicylates produced from SA could have occurred. If this hypothesis is considered, it is possible that all treatments were affected, which would justify the non-significance between most of the analyzed variables and could be applied in the lettuce culture, applying SA only in some plants, facilitating if the management.

Post-harvest physical variables also indicate that there was no loss as a function of the frequency of application of SA (Table 4). Mengiste (2014), Shuping and Eloff (2017), argue that understanding the mechanisms of post-harvest plant immunity is limited and that studies are needed to reduce losses.

Conclusion:

The evolutionary success of vegetables has certainly been involved with the emergence, development and enhancement of defense mechanisms. Throughout its evolutionary history, the performance of natural selection, inherited mutations and evolutionary changes have made plants increasingly adapted, resistant and able to coexist and interact with different types of organisms, but undoubtedly the harmful interactions (herbivory, pathogens, parasites, etc.) were the most challenging during the process of coevolution (TAIZ *et al*., 2017), because in nature resistance seems to be the rule and not the exception.

Each plant when under stress, abiotic or biotic, responds differently. Responses range from preformed or postformed forms of defense. And, to induce resistance in plants requires the activation of mechanisms that are initially latent. For example, in the presence of an aggressive agent or even a resistance-activating molecule there is the triggering of a cascade of signals that can activate physical and chemical defense barriers. Induction of metabolites such as proteins and carbohydrates may be related to pathogenesis by contact with chemical or biological agents.

According to MiuraandTada (2014), in general, low concentrations of SA tend to decrease sensitivity to abiotic stress, and elevated concentrations may induce elevated levels of oxidative stress, leading to decreased tolerance to abiotic stress.The results point to the possibility that the volatile effect of salicylic acid influenced adjacent plants. Thus, future studies are needed to verify if there was communication between plants through volatiles produced and shared between the experimental units.

The public has expressed growing interest in the processes of obtaining the raw materials of the products they consume, as well as appeals of environmental preservation policies. Considering that L. sativa is an indicator plant in relation to allelopathic effects in plant production systems, this study also contributes with future research regarding the use of natural products such as SA.

Added to this, inducer treatment may be a useful tool to improve the quality of health promotion in lettuce plants without loss of sensory quality (Zlotek *et al*., 2014). In this context, SA is a substance that induces these processes; having the benefit of occurring natuaralmente in the plants (Sánchez-Rangel *et al*., 2015).

In this work, it is concluded that from application frequencies every 24 h with 1 mL of SA solution at 1 mM concentration, sprayed on leaves of lettuce (*Lactuca sativa* L.), there is no metabolic loss during growth and development of the culture. Also, salicylic acid applications did not reduce post-harvest shelf life. The induction of resistance in plants is an alternative and effective method for activation of defense mechanisms, and can be used in several cultures, especially in those where there are no naturally resistant organisms.

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