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Evaluation of Exogenous Salicylic Acid Application on White Mould Disease (*Sclerotinia sclerotiorum*) and Photosynthetic Pigments in Lettuce (*Lactuca sativa* L.)

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Abstract: The aim of this study was to investigate the effectiveness of salicylic acid (SA) treatment against white mould disease (*Sclerotinia sclerotiorum*) (SS) disease in lettuce (*Lactuca sativa* L.) plant. In this study, two lettuce cultivars (Melina and Fuzila) were used and four treatments were applied to the plants (control, SS, SA, and +SA+SS treatments). Weight (g), the number of marketable leaf per plant, the number of non-marketable leaf per plant, leaf ratio (%), relative water content (%), chlorophyll parameters (chlorophyll a, b and total chlorophyll (mg $100g^{-1}$), colour parameters (L, a and b) and disease severity (%) were determined. According to the results, SA treatment increased the weight (563.65 g $plant^{-1}$ for cv. Melina and 574.67 g $plant^{-1}$ for cv. Fuzila) and number of marketable leaves (26.83 leaf per plant for cv. Melina and 31.33 leaf per plant for cv. Fuzila) whereas it decreased the number of non-marketable leaves (8.66 leaf per plant for cv. Melina and 6.67 leaf per plant for cv. Fuzila) in both cultivars. Besides, it was found that SA treatment reduced disease severity by 19% and 14% for cv. Melina and cv. Fuzila, respectively. Total chlorophyll contents ranged from 0.421 mg $100g^{-1}$ to 0.484 mg $100g^{-1}$ for Melina and 0.467 mg $100g^{-1}$ to 0.593 mg $100g^{-1}$ for Fuzila cvs. These results demonstrated that there was an increasing tendency due to SA applications for the total chlorophyll content in all cultivars. Consequently, it has been determined that SA treatment is effective and can be used against white mold disease in lettuce plants.

Keywords: Disease severity, *Lactuca sativa* L., marketable yield, salicylic acid, *Sclerotiorum sclerotinia*, quality

1. Introduction

Lettuce (*Lactuca sativa* L.) is one of the most economically important leafy vegetables cultivated worldwide. The total world production of lettuce was 29.1 million tonnes in 2019 and, Turkey had approximately 1.7% part of this production with 520.1 thousand tonnes (Anonymous, 2021). In the present study, lettuce was used as a model plant, since it is one of the most important dietary leafy vegetables which is primarily consumed worldwide fresh or fresh-cut (Putnam et al., 2000). Also, lettuce is an important source of dietary antioxidants especially considering its high reactive oxygene species (ROS) scavenging activity. It contains several nutritive and health-promoting compounds such as phenolic components; Vitamin A, C, and E; calcium; lutein; and dietary fiber (Kim et al., 2016).

As with all agricultural products, some diseases and pests limit lettuce cultivation. While some of these diseases cause economic damage, some do not. One of the most important limiting factors of growing lettuces is white mold disease which is a soil-borne fungal disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Sclerotinia sclerotiorum* (SS) is a pathogen known to be effective on 64 subgroups, 225 stocks, and 361 strains including imported vegetables (Smolińska and Kowalska, 2018). The disease causes to occur soft and dark lesions in the root region of plants. However, fungus sometimes starts on leaves and then is carried to the stems (Agrios, 1997; Tozlu, 2008). Fungus comes up during the advanced vegetation period and affects mainly the seedlings and it may cause the death of the plants (Dixon, 1984; Bruehl, 1987). *Sclerotinia sclerotiorum* fungal species can live in the soil for 3 years (Xia et al., 2020). Considering this information SS is one of the most important diseases of lettuce both in field and greenhouse production. It can infect every growing period. White mold disease might cause great losses in countries where lettuce cultivation is very important as Turkey (Melzer and Boland, 1994; Uğurcan, 1997; Subbarao, 1998; Mert Türk and Mermer, 2004; Chitrampalam et al., 2008; Lahoz et al., 2009). A lack of resistant varieties for this disease is a significant problem for lettuce production. The producers have been trying mechanical and cultural methods for struggling against this fungal disease. Using chemicals that are an easier and more effective way of disposing of this disease has been mostly preferred by lettuce producers. However, this method can cause negative effects in terms of economy, human health, and the environment. Since lettuce is a freshly consumed vegetable, chemicals directly affect human health. Therefore, natural materials that are not damaging to nature and human health have been preferred in recent years instead of using chemicals to increase resistance to diseases in plants.

Salicylic acid (SA) is a major phenylpropanoid compound that has been described as a plant growth regulator and signal transducer or messenger. It plays an important physiological and biological role in plant metabolism (Zheng et al., 2019, Sabagh et al., 2021). For instance, seed germination, fruit yield, glycolysis, flowering, and heat production in thermogenic plants (Basit et al., 2018; Özyazıcı, 2021; Özyazıcı and Açıkbaş, 2021), ion uptake, and transport (Maruri-Lopez et al., 2019), photosynthesis rate, stomatal conductivity, and transpiration (Khan et al., 2003, Özyazıcı, 2021; Özyazıcı and Açıkbaş, 2021) could also be affected by SA application. In previous studies, the amount of SA increased in plants with different disease inoculations (Hayat et al., 2010, Koo et al., 2020). SA is involved in the resistance response of plants such as by stimulating adventitious root formation, resistance against biotic and abiotic stresses (Mohammed et al., 2020). Hence, this study aimed to observe the effects of SA treatments on SS resistance, the quality, and marketable yield parameters in lettuce (*L. sativa* L.) plants.

2. Materials and Methods

2.1. Plant growth conditions

The experiment was carried out under greenhouse conditions in soil. Two types of curly lettuce cvs. Melina and Fuzila (AG Tohum, Antalya, TURKEY) were used in this study (Anonymous, 2020). Lettuce cvs. were exposed to photoperiod 16 h of light /8 h of dark and temperature of 22±2 /18±2 °C. The seeds were sown to the Klassman (Potgrond H, EU) peat in a tray. Lettuce seedlings were transplanted into 1.5-liter pots consisting of 50% soil, 25% peat, and 25% perlite when the true leaves of lettuce were formed.

2.2. Growth condition of pathogen

One pathogenic isolate of SS obtained from lettuce was cultured in Petri plates containing Potato Dextrose Agar (PDA) medium at 25 °C for 10 days. Leaves were inoculated with 10 mL of a conidial suspension (106 conidia m L^{-1}) per plant using a small-calibrated hand sprayer (1.5 L capacity). One-month-old plants were inoculated with SS. After inoculation, the plants were held in closed polyethylene bags for one day and the disease caused by the pathogen progressed over a month.

2.3. Plant treatments and pathogen inoculation

Sclerotinia sclerotiorum (SS) (Lib.) de Bary *(sclerote)* was inoculated to all lettuce leaves excluding control groups and plants were used only in SA applications (Yanar and Miller, 2003; Anonymous, 2016). Sclerotes were infected to four leaves of each plant.

In this experiment, four groups were constituted as follows:

1. -SA-SS (Control): The plants were treated with water.
2. +SA-SS (SA): The plants were treated with salicylic acid
3. -SA+SS (SS): The plants were inoculated with *S. sclerotiorum*
4. +SA+SS: The plants were treated both with SA and inoculated with *S. sclerotiorum*

The leaves were applied by spraying 8 µM SA (Sigma-Aldrich) solution on the 15th day after planting (in the active development period) (Özgönen et al., 2001). In this study, each treatment was replicated four times.

2.4. Yield parameters

In order to evaluate the effects of SA and SS on lettuce yield variables, 15 lettuce plants were collected from each group after 60 days of the seedling. Harvested plants were weighed and mean weight was calculated (Scales; Radwag PS 3500/C/1, Radom, Poland) after non-marketable leaves having physiological disorders were separated. For the analyses of yield parameters, leaves were counted to determine the non-marketable and marketable yield of all plants in each treatment.

2.4.1. Leaf ratio

For the determination of leaf ratio; the ratio of the number of non-marketable leaves to the number of marketable leaves was calculated.

2.4.2. Leaf relative water content

Relative water content (RWC) at midday was determined with Equation 1.

$$\mathrm{RWC} = 100 \text{ x } \frac{FW - DW}{TW - DW} \quad (1)$$

FW, TW, and DW are the fresh, turgor, and dry weight respectively. In the laboratory, lettuce leaves were weighted (FW), then leaves were saturated with water in pure water at 4 °C in the dark for 18 hours and weighted (TW). Lastly, leaves were dried in an oven at 80 °C for 24 hours and weighed (Goicoechea et al., 2004).

2.4.3. Photosynthetic pigments

Total chlorophyll, chlorophyll a, and b were determined from four fresh, fully mature, symptomless leaves by extraction with 90% acetone determining levels with a spectrophotometer (Shimadzu UV-120-01) at 645, 652, and 663 nm determined as mg $100g^{-1}$ fresh weight (Lichtenthaler and Wellburn, 1983).

2.4.4. Leaf colour measurement

Four pieces of lettuce leaves were used for each treatment and the leaf colour of the leaves was measured by using a Minolta Colorimeter CR-300 (Konica Minolta, Inc., Tokyo, Japan). Leaves were measured at 4 locations of each piece of lettuce and a total of 4 readings for each treatment were carried out. According to CIELAB color scale, L * brightness (L *= 0, black; L *= 100, white), a * red and greenness (+60, red; -60, green) and b * value is yellowness and blue (+60, yellow; -60, blue) (Akbulut and Çoklar, 2008).

2.4.5. Determination of disease severity

The following scale was used to determine disease severity. The color scale is numbered between 0-4 and evaluated according to these numbers, 0= There is no disease in the leaf. 1= 1/3 of the leaf has disease. 2= 1/2 of the leaf has disease. 3= 2/3 of the leaf has disease. 4= All leaves have disease. Harvested plants and diseased plants were determined as visual according to the scale.

The formula of Towsend -Hauberger was used to determine disease severities on the plants and then the formula of Abbatt was utilized obtain to the activity of SA (Townsend and Heuberger, 1943). The effect of SA was determined by the Abbott formula (Erdiller, 1992).

2.5. Statistical analysis

The experiment was conducted as four parallel sets, in a completely randomized design with 6 replications. The results were statistically evaluated by one-way analysis of variance (ANOVA) using the JMP software package version 7.0. Based on the F test results, the differences between the groups were determined by the least significant difference (LSD) multiple comparison test.

3. Results and Discussion

Bioproductivity or marketable yields, total biomass (weight), leaf amount of lettuce were influenced by root performance and nutrient content in the soil (Larqué-Saavedra and Martin-Mex, 2007). In this study, it was determined that SA treatments increased the number of marketable leaves in both varieties and had positive effects on the number of marketable leaves which is an important criterion for leafy vegetables. The maximum count of marketable leaves per plant was found at 26.83 and 31.33 for cv. Melina and Fuzila, respectively in the +SA-SS treatments. Besides, non-marketable leaf counts were found to be considerably low in +SA-SS treatments while –SA+SS applications provided an increase in the number of non-marketable leaves in both cultivars.

It is known that an increase in leaf count also provides an increase in yield. Therefore, the maximum values of weight per plant were obtained to be 563.65 g and 574.67 g, respectively for cv. Melina and cv. Fuzila at +SA-SS treatment. However, no statistical difference was found between +SA-SS and -SA-SS treatment in cv. Fuzila. In other words, SA applications increased the total weight by about 8.5% and 5%, respectively in cv. Melina and cv. Fuzila compared to -SA-SS (control) plants (Table 1).

The results of this research proved that SA applications had positive effects on both yield and quality parameters while SS applications had negative effects on both cultivars (Table 1, 2 and 3). Similarly, Larqué-Saavedra and Martin-Mex (2007), reported that SA applications improved yield parameters of *Brassica napus*.

The ratio of non-marketable leaves to marketable leaves also shows the effectiveness of SA applications (Table 1). However, it was found that there was no significant difference between the treatments for the leaf ratio in both cultivars. Leaf ratio values ranged from 0.32% (+SA-SS) to 0.53% (-SA+SS) in cv. Melina and varied from 0.21

Table 1. Quality and yield parameters of cvs. Melina and Fuzila*

Cultivars	Treatments	Weight (g plant^{-1})	Marketable leaf (leaf plant^{-1})	Non-marketable leaf (leaf plant^{-1})	Leaf ratio (%)	RWC (%)
Melina	-SA-SS	519.55 c	21.83 b	8.50 b	0.39	76.34 b
	+SA-SS	563.65 a	26.83 a	8.66 b	0.32	75.58 b
	+SA+SS	548.14 b	21.50 b	9.16 b	0.42	81.71 a
	-SA+SS	529.80 c	21.00 b	11.33 a	0.53	75.41 b
$LSD_{0.05}$		13.75	3.83	2.21		4.18
Fuzila	-SA-SS	548.33 ab	25.83 b	11.00 b	0.42	67.13 b
	+SA-SS	574.67 a	31.33 a	6.67 c	0.21	69.23 ab
	+SA+SS	543.16 b	24.83 bc	12.50 ab	0.50	72.32 a
	-SA+SS	527.83 b	21.00 c	14.00 a	0.66	70.54 a
$LSD_{0.05}$		28.52	4.15	2.06		3.09

*: The difference between the means indicated by the same letter in the same column is not significant

Table 2. Chlorophyll parameters of cv. Melina and cv. Fuzila*

Cultivars	Treatments	Chlorophyll a (mg 100g^{-1})	Chlorophyll b (mg 100g^{-1})	Total chlorophyll (mg 100g^{-1})
Melina	-SA-SS	0.313	0.116 ab	0.429
	+SA-SS	0.361	0.123 a	0.484
	+SA+SS	0.323	0.098 c	0.421
	-SA+SS	0.348	0.100 bc	0.448
$LSD_{0.05}$		0.048	0.017	0.059
Fuzila	-SA-SS	0.330	0.138	0.468
	+SA-SS	0.441	0.152	0.593
	+SA+SS	0.333	0.133	0.467
	-SA+SS	0.382	0.134	0.516
$LSD_{0.05}$		0.127	0.034	0.150

*: The difference between the means indicated by the same letter in the same column is not significant

Table 3. Colour parameters of cvs. Melina and Fuzila*

Cultivars	Treatments	L	a	b
Melina	-SA-SS	71.47 a	-29.59 a	9.79
	+SA-SS	70.87 a	-29.18 a	9.83
	+SA+SS	69.56 b	-31.20 b	9.53
	-SA+SS	71.71 a	-29.80 ab	11.17
$LSD_{0,05}$		1.27	1.45	1.83
Fuzila	-SA-SS	71.11	-30.61	7.40
	+SA-SS	72.14	-30.35	8.69
	+SA+SS	72.55	-29.00	9.47
	-SA+SS	72.32	-30.12	9.47
$LSD_{0,05}$		2.56	2.65	2.26

*: The difference between the means indicated by the same letter in the same column is not significant

(+SA-SS) to 0.66% (-SA+SS) in cv. Fuzila. These results demonstrated that -SA+SS applications had the best values for both varieties. Similar results were also obtained by Gorni and Pacheco (2016) that the number of leaves and leaf area values of yarrow plants treated with 0.25 and 1.00 mM SA applications did not differ from control plants (Table 1).

Relative water content (RWC) ranged from 75.41% (-SA+SS) to 81.71% (+SA+SS) in cv. Melina and differed from 67.13% (-SA-SS) to 72.32% (+SA+SS) in cv. Fuzila. Therefore, our results showed that SS applications increased RWC values in both cultivars (Table 1). On the other hand, Nouriyani (2021) determined that RWC increased by 26% in the foliar application of salicylic acid from maize under water deficiency stress.

When all yield parameters were considered, our results were in agreement with the results reported by Türkyılmaz et al. (2005) that SA applications on beans had a positive effect on yield and quality. Similarly, Kaydan et al. (2006) also observed positive effects of SA treatments on wheat.

There was no statistically significant difference between the applications for chlorophyll a, chlorophyll b, and total chlorophyll content of both lettuce cultivars in this study (Table 2). Total chlorophyll content in Melina cv. varied between

0.484 and 0.421 mg 100g^{-1}; in Fuzila cv. were 0.467 and 0.593 mg 100g^{-1} for all treatments. These results demonstrated that there was an increasing tendency due to SA applications for the total chlorophyll content in all cultivars.

When the chlorophyll a content was evaluated, the numerically best values were obtained from +SA-SS treatments for cvs. Melina (0.361 mg 100g^{-1}) and Fuzila (0.441 mg 100g^{-1}). Besides, in both lettuce cultivars in this study, the results showed that SS applications decreased chlorophyll b contents although there was no statistically significant difference and chlorophyll b values were increased by +SA-SS applications for cvs. Melina (0.123 mg 100g^{-1}) and Fuzila (0.152 mg 100g^{-1}). Many studies have reported that SA application increases leaf chlorophyll content (Ghai et al., 2002; Moharekar et al., 2003; Yildirim et al., 2008).

Leaf colour is one of the most important quality parameters of leafy vegetables and is observed with L, a, and b values. There was no statistical difference in the L values in cv. Fuzila which were varied from 71.11 (-SA-SS) to 72.55 (SA+SS). Similarly, there was no statistically significant difference between the treatments in the b values for both cultivars, and b values differed from 9.53 (+SA+SS) and 11.17 (-SA+SS) in cv. Melina and 7.40 (-SA-SS) and 9.47 (+SA+SS and -SA+SS) in cv. Fuzila (Table 3).

When SA applications on disease severity on lettuce plants were examined, it was observed that +SA applications decreased disease severity in cv. Melina by 19% and by 14% in cv. Fuzila (Figure 1). It was also obtained that percent activity values for white mold disease were determined in cv. Melina as 22.35% and in cv. Fuzila as 20.29%. Therefore, these results proved that SA applications created a beneficial effect on decreasing the severity effects of this disease. Plants limited the infected area to prevent the spread of the disease agents. The hypersensitive defence response is found in all higher plants and is characterized by a rapid cell death at the point of pathogen ingress (Balint-Kurti, 2019). According to Alkahtani et al. (2011) 0.2% SA application significantly reduced the disease against powdery mildew on cucumber. AL-Saleh (2011) applied 0.109 µl SA against bacterial spot disease in tomato plants and observed a significant reduction in the disease. Wang et al. (2011) reported that 5 mM SA application against gray mold in tomato plant reduced the disease and increased fruit quality. Mert Türk and Mermer Doğu (2009) reported that 5, 10, and 100 mM of SA affected the white mold disease rate and severity which was caused by SS by enabling mycelial growth on cabbage. Similarly, Nováková et al. (2014) supported that SA-treated canola was protected against SS.

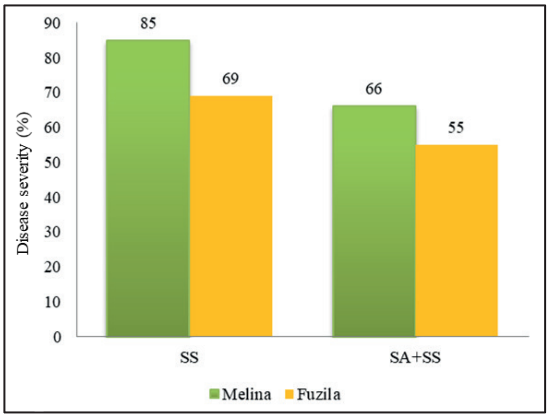

Figure 1. Disease severity in cvs. Melina and Fuzila

4. Conclusions

In this research, the results determined that SA treatments had the potential to decrease white mold disease, and SA applications positively affected the yield by increasing the number of marketable leaves. Besides, when SA applications were used by SS treatments on lettuce plants, it also positively affected the increase in the number of non-marketable leaves. In our study, we aimed to protect lettuce plants against white mold disease caused by SS by the means of SA treatments. However, in future research, salicylic acid dozes could be standardized and also applied to different vegetables. This research also offers an insight into the studies related to other vegetable diseases which will be done in the future.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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