

EFFECT OF SALT (NaCl) AGAINST WILT DISEASE OF COTTON CAUSED BY *VERTICILLIUM DAHLIAE* KLEB. UNDER *IN VITRO* CONDITIONS

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ABSTRACT

Verticillium wilt disease causes economic yield losses in cotton growing areas in the world. In addition, an increase in salinity is observed in the soils of our country due to improper agricultural practices. This present study was carried out to find out the efficiency of NaCl with *Verticillium dahliae* Kleb. for two cotton cultivars following Completely Randomized Design (CRD). In this study, cotton cultivars (*Gossypium hirsutum* L.) of Nazilli 84 and Gloria were cultured in MS medium (40 ml per all magenta) containing 3 mycelial disc of *Verticillium dahliae* (0.5 mm) and different NaCl concentrations (0, 25, 50, 100, 150 mM) under *in vitro* conditions. The lowest disease severity value was found in the cv. Nazilli 84 in 150 mM NaCl + *Verticillium dahliae* application (48%), and in 100 mM NaCl + *Verticillium dahliae* application (50%) in cv. Gloria. In control plants, the disease severity was found to be 83% for cv. Nazilli 84 and 62% for cv. Gloria. Different results regarding plant height and leaf count were obtained with NaCl + *Verticillium dahliae* in cvs. Nazilli 84 and Gloria. Only with salt application, while the highest plant height was determined to be 3.92 cm for cv. Nazilli 84 at 100 mM NaCl application, at the same salt concentration, cv. Gloria was adversely affected by this application and plant height was measured at 1.83 cm determined.

In conclusion, NaCl causes inhibition of *V. dahliae* in both the cultivars of cotton at particular concentration. The analysis of variance for all the parameters (Plant height and number of leaves) were found to be significant at $P \leq 0.05$. These results show that salinity-tolerant cotton plants can increase their tolerance to *V. dahliae* infection. *V. dahliae* disease can be suppressed by applying the NaCl concentration in the soil at appropriate doses depending on the cotton cultivars.

Key words: *Verticillium dahliae* Kleb., NaCl, disease severity, plant height, number of leaves, cotton

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Published first online June 28, 2023

Published final September 30, 2023

INTRODUCTION

Cotton, a natural fiber product, is produced by millions of farmers around the world and is an important source of raw materials, providing income in the textile industry, cotton seed oil, feed production, import and export (Huang *et al.*, 2021; Tokel *et al.*, 2022). While China ranks first in cotton production, India, USA, Brazil, Pakistan, Turkey, Uzbekistan, Australia, Turkmenistan and Mexico follow. In Turkey, cotton is grown on a total area of 477 thousand hectares in four main regions (Aegean, Southeastern Anatolia, Çukurova and Antalya) and 2.2 million tons of seed cotton yield are produced (TSI, 2021).

Verticillium dahliae Kleb. is a soil-borne pathogen that plays an important role in cotton disease. The pathogen causes root rot in seedlings in the early period and vascular wilt in late period (Zhang *et al.*, 2012; Sakci *et al.*, 2017). The life of the pathogen is

divided into dormant, parasitic and saprophytic periods (Baytar *et al.*, 2017). The pathogen survives for many years in the soil through its microsclerotia and causes new infections (Zhang *et al.*, 2016). Verticillium wilt also negatively affects the fiber and technological properties of cotton (Liu, 2017). It is reported that the average yield loss of cotton crop caused by Verticillium wilt is approximately 10–35% (Song *et al.*, 2020). Today, an effective chemical control against this disease has not been developed yet (Turktaş and Koral, 2017).

Salt stress is an increasing threat to agriculture worldwide. Salinity is increasing day by day, especially in the agricultural areas of the Mediterranean Region (Bellaifore *et al.*, 2021). Incorrect agricultural practices in Turkey's agricultural lands, salinity in irrigation, excessive water consumption and excessive fertilizer use are among the reasons for the increase in soil salinity (Oz and Karasu, 2007). Salinity is the accumulation of soluble salts at the cellular level and affects the development of

plants through morphological and physiological means (Shabala, 2013). Salt content inhibits the activities of enzymes involved in germination and reduces vegetative growth (Sharif *et al.*, 2019; Oguzhan *et al.*, 2021). Salinity affects the net assimilation rate by reducing stomatal conductance and water uptake, which generally results in increased intercellular CO₂ concentration (Nayem *et al.*, 2020). Plant salt resistance depends on plant species, soil, water and environmental variables (Barros *et al.*, 2021).

The effects of salt and disease on plants have been studied by researchers individually, and the effect as a combination is not fully understood. Some researchers have reported that salinity affects pathogens, hosts, or microbial activity in soil (Morissey *et al.*, 2014; Ali *et al.*, 2017). Depending on the plant cultivar, salt stress has been found to increase the susceptibility of plants to root pathogens (Chojak *et al.*, 2012; Bai *et al.*, 2018). Conversely, some researchers reported that antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), which are involved in plant defense activities, are closely related to the abiotic and biotic stress in many plants (Shoab *et al.*, 2018; Wang *et al.*, 2021). The role of NaCl in suppressing soil-borne diseases has also been studied in crown and root rot (*Fusarium oxysporum asparagi*) of asparagus. It was reported that the crown and root rot of asparagus were decreased significantly (51%) in fungal root lesions exposed to NaCl compared to the control plant treated with *Fusarium oxysporum asparagi* only (Elmer, 2004).

In this study, it was aimed to determine the effect of *Verticillium dahliae* and different salt concentrations (NaCl) on the development and changes of cotton cultivars (Nazilli 84 and Gloria). The reason for establishing the experiment under *in vitro* conditions is to minimize soil/climatic factors and to get results in a shorter time.

MATERIALS AND METHODS

The study was carried out in the tissue culture laboratory and climate chambers of the Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, Turkey.

Plant materials: The mid-early and moderately susceptible Nazilli 84 (Karcilioglu *et al.*, 1992) and early and tolerant Gloria cotton cultivars (Erdogan and Kurt, 2013) of the species *G. hirsutum* L. were used as planting material in the study.

Pathogen: Isolated from cotton and a high virulence pathotypes, Vd11 isolate (Non-defoliating pathotype, Nazilli Cotton Research Institute) was used for an artificial inoculum (Erdogan, 2009).

Cultivation of the pathogen: *V. dahliae* isolate was cultured in petri dishes (90 mm diameter) containing Potato Dextrose Agar (PDA-Difco) medium and incubated at 24°C for 14 days.

Sterilization of seeds: For clonal propagation of cotton plantlets *in vitro*, cotton seeds were first soaked in 70% ethanol for 3 minutes and then kept in 15% sodium hypochlorite (NaOCl) solution containing 1-2 drops of tween 20 for 15 minutes, then rinsed with sterile distilled water three times washed for five minutes each time. The sterilized seeds were dried thoroughly on sterilized blotting papers.

Clonal propagation of cotton plants: In the first stage of tissue culture studies, ½ MS (Murashige and Skoog, 1962) + 0.1 g/l myo inositol + 20 g/l sucrose + 7 g/l agar containing medium was used for seeds. After the seeds germinated, a medium containing MS (Sigma-Aldrich Company) + 0.1/l g myo inositol + 1 mg/l kinetin + 20 g/l sucrose + 2 g phytoagar was used to propagate the plants. The pH of both media was adjusted to 5.7-5.8 and they were distributed as 40 ml in magenta containers with 250 ml inner volume. Cotton plantlets were cultured at 25±1°C at all periods, in climate chambers with 16 hours light/8 hours dark and 3000 lux light intensity.

Effect of different levels of NaCl on Verticillium wilt disease of cotton cultivars under *in vitro* conditions:

After the cotton seeds were germinated *in vitro* conditions, they were subcultured four times in MS medium and the cotton plantlets were multiplied. Cotton plantlets (approximately 20 mm) were cultured for two weeks in MS medium containing different salt (NaCl) concentrations (0, 25, 50, 100, 150 mM). After two weeks, three disc pieces (0.5 mm diameter) of *V. dahliae* isolate cultured for 14 days in PDA medium were placed in MS medium containing cotton plantlets cultured at different salt concentrations. Cotton plantlets cultured only in MS medium were used as a negative control, whereas cotton plantlets cultured in MS medium containing only *V. dahliae* were used as a positive control. The development of disease symptoms was observed for two weeks. The plantlets were evaluated according to the 0-3 scale (Table 1) (Bugbee and Presley, 1967).

Table 1: Wilt scale for *V. dahliae* on cotton plants.

Scale value	Disease symptom
0	Healthy plant
1	50% foliar symptoms on leaves
2	51-75 % foliar symptoms on leaves
3	76-100 % foliar symptoms on leaves and plant died

Disease index was calculated according to the obtained scale values.

Disease Index formula = (0 (a) + (1) (b) + (2) (c) + (3) (d))/n=a, b, c, d= number of plants included in each scale value n: total number of plants. Disease severity was calculated with the Townsend-Heuberger formula (Bora and Karaca, 1970). The plant height (cm) of the surviving plants was measured and the number of leaves was determined.

Statistical Analysis: The experiment was designed in a completely randomized design (CRD) with five replicates in each treatment and each replica had five plants. The percentage of diseased plants was submitted to angular transformation before statistical analysis. The data were analysed by ANOVA (one-way analysis of variance) using the SPSS 22 package program and the differences between the applications were determined by the Tukey - Multiple comparison test ($P \leq 0.05$). All experiments were repeated at least twice.

RESULTS

In this study, the effect of NaCl + *V. dahliae* application on disease severity and plant growth in cv. Nazilli 84 was found statistical differences. In the case of cv. Nazilli 84, the lowest disease severity value was 48% with 150 mM NaCl + *V. dahliae* application, while the highest disease severity value was 83% with the control *V. dahliae* application was determined. For the salt concentration application, the highest disease severity value was determined with 100 mM NaCl+ *V. dahliae* application (65%) (Figure 1). While a statistical difference was observed in Nazilli 84 cotton cultivar plant height, the highest plant was found in 150 mM NaCl + *V. dahliae* application (2.76 cm). The maximum number of leaves was determined in 25 mM NaCl + *V. dahliae* application (3.55), and the lowest number of leaves was determined only in the pathogen test (2.83) (Table 2).

Table 2: The effect of NaCl + *V. dahliae* application on disease severity and plant growth in cv. Nazilli 84.

Application	Disease Index	Disease severity (%)	Plant height (cm)	Number of leaves
25 mM NaCl + <i>V. dahliae</i>	2.11 b	54.00	2.73 ab	3.55 a
50 mM NaCl + <i>V. dahliae</i>	2.39 bc	59.00	2.17 b	3.11 ab
100 mM NaCl + <i>V. dahliae</i>	2.60 bc	65.00	2.28 ab	2.61 bc
150 mM NaCl + <i>V. dahliae</i>	1.94 b	48.00	2.76 a	2.94 b
Control (+)	3.33 c	83.00	2.36 ab	2.83 b
Control (-)	0.00 a	0.00	2.68 ab	3.22 ab

Note: In the same column, different letters indicate significant difference ($P \leq 0.05$)

The highest disease scale value in cv. Gloria was determined in the control *V. dahliae* (2.73) and 50 mM NaCl + *V. dahliae* application (2.67). The disease severity was found to be 62% in plants treated with only *V. dahliae* and the disease severity was found to be 56%, 58%, 39%, and 50%, respectively in the salt applications (25, 50, 100, 150 mM salt + *V. dahliae* Kleb.) The lowest disease scale value was determined in 100 mm NaCl+ *V.*

dahliae application (1.59) and plants were affected by the disease at a low rate (Figure 1). The maximum plant height was determined in untreated plants (3.14 cm) and 150 mM NaCl + *V. dahliae* application in cv. Gloria (2.78 cm). In plants treated with NaCl + pathogen, the number of leaves increased only in 150 mM NaCl + *V. dahliae* application (3.16). Other applications were statistically included in the same group. (Table 3).



Figure 1: The effect of NaCl + *V. dahliae* application on Nazilli 84 and Gloria cotton cultivars.

Table 3: The effect of NaCl + *V. dahliae* application on disease severity and plant growth in cv. Gloria.

Application	Disease index	Disease severity (%)	Plant height (cm)	Number of leaves
25 mM NaCl + <i>V. dahliae</i>	2.25 bc	56.00	2.71 ab	2.67b
50 mM NaCl + <i>V. dahliae</i>	2.67 c	58.00	2.16 b	2.67 b
100 mM NaCl + <i>V. dahliae</i>	1.59 b	39.00	2.49 ab	2.75 b
150 mM NaCl + <i>V. dahliae</i>	2.00 bc	50.00	2.78 ab	3.16 a
Control (+)	2.73 c	62.00	2.52 ab	2.67 b
Control (-)	0.00 a	0.00	3.14 a	2.67 b

Note: In the same column, different letters indicate significant difference ($P \leq 0.05$)

When the effect of salt applications on cotton cultivars was examined, the highest plant height was determined as 3.92 cm in 100 mM NaCl application in cv. Nazilli 84, while cv. Gloria was adversely affected, and the plant height was determined as 1.83 cm in the same dose of salt application. The longest plant height in cv. Gloria was determined as 3.15 and 3.13 cm at 150 mM NaCl and 25 mM NaCl applications, respectively, and they were in the same group statistically. When the

effect of NaCl applications on the number of leaves was examined, an increase in the number of leaves was observed in the application of 100 mM NaCl in cv. Nazilli 84, and the average number of leaves was determined as 5.00. In cv. Gloria, on the other hand, the highest leaf number was determined at 150 mM NaCl application and the number of leaves was recorded as 3.88 (Table 4).

Table 4: The effect of NaCl application on plant growth in cotton cultivars

Application	Plant height (cm)		Number of leaves	
	Nazilli 84	Gloria	Nazilli 84	Gloria
25 mM NaCl	3.20 ab	3.13 a	3.62 ab	2.50 ab
50 mM NaCl	2.35 b	2.33 ab	2.37 b	2.50 ab
100 mM NaCl	3.92 a	1.83 b	5.00 a	2.00 b
150 mM NaCl	2.41 b	3.15 a	2.62 b	3.88 a
Control (-)	2.97 ab	3.38 a	2.62 b	3.50 ab

Note: In the same column, different letters indicate significant difference ($P \leq 0.05$)

According to the results obtained from this study, application of low doses of NaCl promotes plant growth, while application of NaCl at high rates has a toxic effect on plants and causes the plant death. Salt resistance in plants was found to depend on plant species. In the study, it is thought that low-dose salt application increases the pH in the environment and causes the plant to be stressed, and *V. dahliae* disease seen in the cotton plant is suppressed.

DISCUSSION

Salt stress disrupts the physiological and morphological structure of plants. *V. dahliae* is an important fungal disease in cotton and causes significant crop losses. In this study, NaCl application was found to suppress the severity of *V. dahliae* disease and the development of the disease. Salt stress has been observed to negatively affect the development of the pathogen *V. dahliae* in cotton. Similar results have been reported by other researchers.

Boumaaza *et al.* (2015), applied different concentrations of NaCl against *B. cinerea* and examined their effect on fungal mycelial growth. According to the results obtained, it was reported that 50 and 100 ppm NaCl concentrations enhanced the growth of *B. cinerea* mycelium, while 150 ppm NaCl concentration prevented the growth of *B. cinerea* mycelium. While the concentration of NaCl applied in low doses affects mycelial growth, application of NaCl above a certain dose inhibits mycelial growth of fungi. Turkkan and Erper (2014), the effect of 12 different salt solutions *in vitro* conditions investigated against *Fusarium oxysporum* f.sp. *cepa* on onion. According to the results obtained, they stated that salt solutions inhibited mycelial growth of *F. oxysporum* f.sp. *cepa*. In addition, the root and shoot development of the plant is adversely affected at high salt concentration, but an increasing salt concentration causes an increase in phenolic substances in plants and confers disease resistance on plants. Elevated phenolic compounds in plants due to salt stress inhibit fungal mycelium germination and increase lignin synthesis (Liu *et al.*, 2017). In a study of Rauf *et al.* (2014), they found that root diseases settle more on the

roots and that these pathogens are suppressed as the amount of salt increases when low concentrations of NaCl are applied to various root diseases. The overproduction of reactive oxygen species (ROS) in plant cells under stress contributes to the damage of cell components such as DNA, proteins and membrane lipids, while plants use both enzymatic and non-enzymatic defense systems to eliminate ROS, the enzymatic antioxidant defense system developed by plant cells. It contains and increases various enzymes such as SOD, APX, GPX, GR, CAT, etc. In addition, salt stress contributes to a significant increase in root catalase and guaiacol peroxidase activities (Panda and Khan, 2009; Lu *et al.*, 2016; Dikilitas *et al.*, 2017; Hanacchi *et al.*, 2022). It was reported that NaCl application to the asparagus plant reduced *F. oxysporum* f. sp. *asparagus* and *F. proliferatum* root diseases. It is stated by some researchers that fungal diseases in plants are suppressed due to increased soil pH as a result of NaCl application in soil (Elmer *et al.*, 2004; Boumaaza *et al.*, 2015).

Apart from the results we have obtained, it has been reported by some researchers that NaCl application has a synergistic effect with plant disease agents. Nayem *et al.* (2020), found in their study that the application of NaCl in citrus plants enhanced the severity and development of the citrus disease *Xanthomonas campestris* pv. *citri*. NaCl application is an important factor in the increasing root diseases and makes the plant more susceptible to pathogen attack. While the application of low doses of NaCl ensures the survival of the plant cell by promoting its growth, the high rate of NaCl increases the plant's susceptibility to attack by pathogens (You *et al.*, 2011; Goudarzi *et al.*, 2011).

Salinity had a positive effect on the development of pathogens of Oidium and Phytophthora diseases in tomato plants (Kissoudis *et al.*, 2014). In addition, Haller *et al.* (2020), found in their study that the susceptibility of the Arabidopsis plant to pathogens from *Pseudomonas syringae*, *Alternaria brassicicola* and *B. cinerea* increased as a result of high salt application. The reason for this is that it is known to increase disease development by destroying the plant defense system due to deterioration of osmotic balance by salt stress (Chojak *et al.*, 2012; Nayem *et al.*, 2020).

The lowest disease scale value was determined in 100 mM NaCl+ *V. dahliae* application in the tolerant Gloria cotton cultivar than the susceptible Nazilli 84 cotton cultivar. In addition, an increase in the number of leaves was observed in cv. Gloria in these combined applications. The effect of only NaCl applications on the number of leaves was examined, an increase in the number of leaves and plant height (cm) was observed in the application of 100 mM NaCl in cv. Nazilli 84 more than cv. Gloria. In a previous study conducted by Izci (2009), cv. Nazilli 84 was reported as a NaCl tolerant cultivar. Under nonsaline conditions, cv. Nazilli 84 was

susceptible to *V. dahliae*. It seems that the salt tolerance mechanism active in cv. Nazilli 84 did not protect the plants very much from the stresses that enhanced the disease.

The *V. dahliae* tolerant Gloria cotton cultivar maintained more tolerance than cv. Nazilli 84 to *V. dahliae* regardless of salinity level. In contrast, disease severity increased with increasing salinity levels in cultivars susceptible Nazilli 84 cotton cultivar to *V. dahliae*. This result indicates that the tolerance of Gloria cotton cultivar to *V. dahliae* was stable in the presence of salinity stress that predisposed the cv. Nazilli 84. Our results are in agreement with those reported by Levin *et al.* (2007). It was to evaluate the influence of salinity on *V. dahliae* expression in olive stem cuttings of different olive cultivars (Picual (highly susceptible to *V. dahliae*, resistant to salt), Barnea (moderately susceptible to *V. dahliae*, highly sensitive to salt), Manzanillo (highly susceptible to *V. dahliae*, mediumhigh sensitivity to salt), Arbequina (intermediate resistant to *V. dahliae* and to salt) and Frantoio (resistant to *V. dahliae*, resistant to salt). In cv. Frantoio, significantly high senescence symptoms were observed only in cuttings exposed to the combination of *V. dahliae* and the high saline concentration. It has been established that that different olive cultivars may respond differently to *V. dahliae* or salinity but develop in many cases a clear synergistic response when subjected to a combination of both.

In this study, it was found that the application of NaCl in low doses had a positive effect on plant growth and suppressive of *V. dahliae* in cotton plant, while the application of NaCl in high doses had a toxic effect on the plant. Similar results have been reported by other researchers. Sattar *et al.* (2010), found in their study that while plants showed normal growth at 50-100 mM NaCl application, cotton plant growth decreased at concentrations above 100 mM and plants died at 600 mM NaCl concentration. Ergin *et al.* (2021), in their study, while seed germination is observed at low doses of NaCl application at a normal level, cotton seeds do not germinate with increasing salt concentration and seed germination is completely inhibited at a salt concentration above 200 mM. Salt stress causes nutrient ion balance, decrease in stomata conductivity, low photosynthetic activity, etc., deterioration, morphological changes (decrease in leaf count, plant size, root length and fruit production) and changes in secondary metabolites (signaling molecules, hormones and oxidative compounds) in plants, and this varies from plant to plant (Nayem *et al.*, 2020; Hanacchi *et al.*, 2022).

Conclusion: It is concluded that the salt application partially suppressed *V. dahliae* in the cotton plant. Moreover, the salt applied in high doses to the cotton plant has a toxic effect on the plants. This study is relevant to the management of *V. dahliae* in a saline

environment in that it provides an understanding of the direct and indirect effects of salinity on *V. dahliae*, cotton and their interaction. In light of this study, an effective management of *V. dahliae* with high level of salinity should partly include the selection of cultivars with tolerance to resistance to *V. dahliae*.

Conflict of interests: The authors declare that they have no competing interests.

Authors' contribution: SEA supervised the work, carried out research work, statistical analysis, also finalized the manuscript, OE: carried out research work and contributed in paper, ZNT: carried out research work,

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