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From Fertilizer to Disease Control: Study of the Relationship between Fertilizer Applications and Bacterial Fruit Blotch Disease

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Abstract

In this research, the effects of different doses of nitrogen (Ammonium sulfate) and phosphorus (Triple superphosphate) fertilizers given in combination in melon cultivation on the growth of bacterial fruit blotch caused by *Acidovorax citrulli* were examined. The experiment was conducted with a randomized plot design that consisted of three replications. The study included recommended optimal doses, 25%, 50%, and 100% more than the optimal dose, as well as a control group that did not receive any fertilizer application. The findings of the study revealed that the application of the optimal fertilizer dose caused the least disease severity (27.78%), while 55.56% disease severity was determined in the pathogen (control) application only, while the disease severity was highest when the fertilizer dose was increased by 100% more than the optimal dose. The results indicate a direct association between the amount of fertilizer applied and the intensity of disease severity, where in a greater severity of disease is noted with higher doses of fertilizer.

Keywords: *Acidovorax citrulli*, Disease severity, Fertilizer dosage, Nitrogen fertilizer, Phosphorus fertilizer

INTRODUCTION

Bacterial fruit blotch disease caused by *Acidovorax citrulli* is a serious and destructive disease that affects various cucurbit crops, particularly melons and watermelons, during seedling and fruiting stages (Schaad et al., 2003). This seed-borne pathogen is responsible for significant economic losses in many parts of the world (Latin and Hopkins, 1995; Demir, 1996; Burdman and Walcott, 2012). Environmentally friendly methods that increase yield and improve product quality are crucial for plant disease control (Batish et al., 2007; Camprubí et al., 2007). Therefore, it is necessary to determine practices that prevent or minimize the occurrence of bacterial fruit spot disease in cucurbit crops during the growing period. Plant nutrition is an integral component of an integrated plant protection program in sustainable agriculture (Dordas, 2008). Proper nutrient application via fertilization is critical for improved agricultural production. While plant resistance to disease is largely genetically controlled, nutrient deficiencies or excesses can also affect resistance. Plant nutrients can function as attractants or repellents for pathogens and can trigger resistance or tolerance mechanisms in the host plant (Agrios, 2005). Furthermore, they influence the growth rate of the host plant, protecting seedlings from infection during the stage when they are most vulnerable to pathogens (Krauss, 1999).

Optimal fertilization is known to reduce plant susceptibility to disease, as fertilization has a significant impact on plant resistance to disease. However, nutrients are also essential for the growth and development of microorganisms, which utilize these nutrients in substantial amounts (Agrios, 2005). Pathogens can cause a reduction in the availability of essential nutrients for the plant they infect, leading to increased plant susceptibility to disease due to nutrient deficiency (Dordas, 2008). The three most important nutrients that influence plant response to microbial pathogens are nitrogen (N), phosphorus (P), and potassium (K), which are directly related to plant resistance (Huber and Haneklaus, 2007; Rahman and Punja, 2007). Nitrogen is a crucial element in the production of essential compounds and

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is used by plants in the production of compounds involved in defense against pathogens, such as phytoalexins and other antimicrobial molecules (Huber and Thompson, 2007). Phosphorus is the second most widely used nutrient in many agricultural crops and is part of many organic molecules of the cell, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP), and phospholipids. It also plays a significant role in many metabolic processes in plants and pathogens (Bolat and Kara, 2017). Potassium (K) is an essential nutrient that plays an active role in most of the biochemical and physiological processes that affect plant growth. It increases the resistance of plants to various biotic and abiotic stress factors (Marschner, 2011; Wang et al., 2013).

Studies have reported that the impact of elements on the development of disease agents can vary, and some elements can have a positive or negative effect depending on the specific forms and concentrations of nutrients (Saygılı et al., 2006; Marschner, 2011). Therefore, it is important to understand the specific effects of nutrients on a particular pathosystem, and it is not possible to directly apply the knowledge from one pathosystem to another. Additionally, determining the optimal nutrient amounts for disease control is crucial. However, there is limited research on the role of nutrients in *A. citrulli* and melon pathosystems (Zimerman-Lax et al., 2016). Thus, the present study aimed to investigate the impact of different doses of nitrogen and phosphorus solutions on the severity of bacterial fruit spot disease in melon seedlings.

MATERIALS and METHODS

Pathogen strain and plant material used in the study

In this study, *Acidovorax citrulli* KVN 21 strain, isolated by İnik (2019) from melon plants showing bacterial fruit blotch symptoms in Iğdır province and determined to have the highest virulence within the scope of his master's thesis study, was selected as the pathogen (İnik, 2019). The commercial Sürmeli F1 melon variety was chosen as plant material.

Soil preparation for pots

Before the study, soil samples were dried in an oven at 105°C until reaching a constant weight. The moisture content of the soil was kept constant by weighing the pots at three-day intervals and adding water when needed to maintain a field capacity of at least 70%. Routine analysis of the pot soils revealed that 2 kg da^{-1} N, 1.6 kg da^{-1} P_2O_5, and 9 kg da^{-1} K_2O were present, and these values were used to determine the appropriate N and P nutrient levels for the melon plants. Soil physical and chemical properties were determined by commonly used methods for calcareous soils (Kacar, 2009). It has been reported that melon plant needs 10 kg da^{-1} N and 9 kg da^{-1} P_2O_5 and 10 kg da^{-1} K_2O nutrients during the growing period (Güçdemir, 2006). As a result of the analysis, potassium fertilizer was not required since the existing soil was sufficient to meet the potassium needs of the plant. In the light of this information, the nitrogen, ammonium sulphate and phosphorus required by the melon were met by using Triple super phosphate fertilizers. The experiment was set up according to the random plot design, arranged to contain 1 kg of soil in each pot, and was carried out in 3 replications. Fertilizers were applied to the soil in combination with planting. The study included 5 different applications: optimum fertilizer dose, 25%, 50%, 100% more than the appropriate fertilizer dose and pathogen (control). The optimum dose for nitrogen fertilizer was determined as 2 g and for phosphorus fertilizer as 2.2 g, and other doses were adjusted accordingly. No fertilizer was given in the pathogen application.

Preparation of pathogen inoculum and determination of disease severity in plants

To prepare the pathogen inoculum, the strains were streaked onto Nutrient Agar (NA) medium and incubated at 27°C for 48 hours. After the melon seeds were disinfected with 2% sodium hypochlorite, two seeds were planted in each pot containing sterile soil and the experiment was set up with 3 replications. Then, fertilizer solutions prepared at different doses were added to the pots. The bacterial colonies were then transferred to Nutrient Broth (NB) medium and incubated overnight at 150 rpm min^{-1} on a shaker at 27°C. The resulting bacterial solution was diluted with sterile distilled water (sdH_2O) to a concentration of 10^8 CFU ml^{-1} using a turbidimeter. Plants were kept under laboratory conditions and inoculated by spraying the pathogen solution with a density of 10^8 CFU ml-1 on the above-ground parts of the plants when they reached the 4-5 leaf stage. The pots were then covered with polyethylene bags for 48 hours. Disease severity values were determined two weeks after pathogen inoculation using the 0-6 scale (0=no symptoms; 1=1-5% symptomatic leaf; 2=6-12% symptomatic leaf; 3=13-37% symptomatic leaf; 4=38-62% symptomatic leaf; 5=% 63-87 symptomatic leaves and 6=88-100% symptomatic leaves or dead plants) developed by Carvalho et al. (2013). The % disease severity in seedlings was calculated according to the equation 1 by Thowsend and Heuberger (1943), and % effect values were determined using the Abbott (1925) formula and compared to the control (Equation 2).

Severity Disease %= Σ[E(scale value×number of plants evaluated on the scale)/ (highest scale value×total number of plants)]x100 (1)

Effect %=[(X-Y)/X]x100

X; Disease Severity in Control, Y; Disease Severity in Application (2)

Statistical analysis

The disease severity data obtained as a result of the experiment were analyzed by one-way ANOVA using R studio program and the difference between the applications was determined by Duncan multiple comparison test ($p \leq 0.01$) Rstudio version 4.3.0 software (R Core Team, 2023).

RESULTS and DISCUSSION

In this study, the effect of different fertilizer doses on the development of bacterial fruit spot disease caused by *Acidovorax citrulli* in melon was evaluated. The data obtained as a result of the experiment are given in Table 1. The severity of the disease was found to be significantly different among the applied fertilizer doses, with the lowest disease severity occurring at the optimum fertilizer dose and the highest at 100% more than the optimum dose. The study showed that disease development increased with increasing fertilizer dose, and the optimum dose was necessary to prevent the development of *A. citrulli*. The % effect of different fertilizer doses on disease development is presented in Figure 1. The control treatment without fertilizer showed 55.56% disease severity, which was considered normal, as plants require the necessary nutrients for growth and development.

Table 1. *The influence of nutrient management on Acidovorax citrulli growth and disease development in melon*

Applications	Scale Values	Disease Severity (%)
OFD**	1.67±0.33b*	27.78
25% more than OFD	3.17±0.45ab	52.78
50% more than OFD	4.33±0.17a	72.22
100% more than OFD	4.50±0.29a	75.00
Control	3.33±0.73ab	55.56

*: There is no statistical difference between the values shown with the same letter and the values are the average of three replicates (p≤0.01)., OFD**: Optimum fertilizer dose

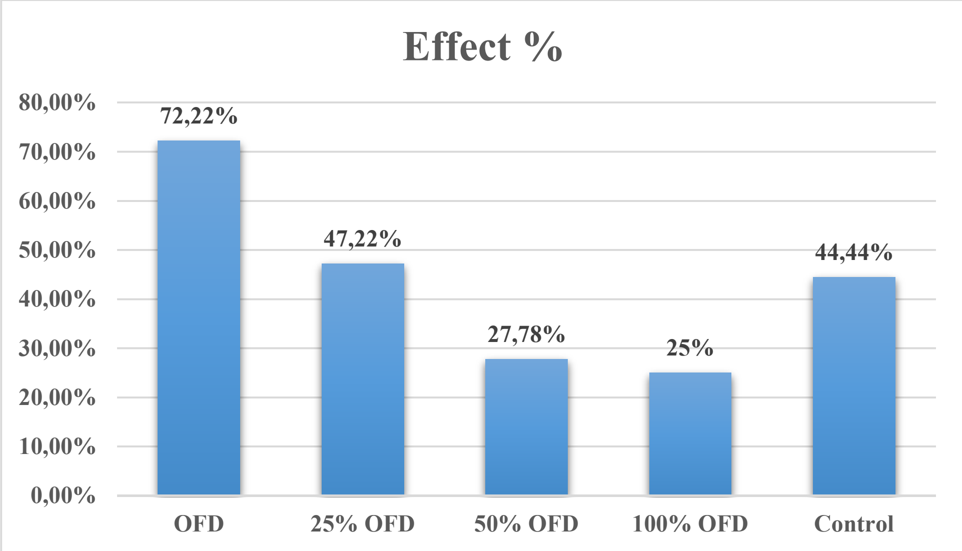

Figure 1 *Effect of different doses of fertilizer applications on bacterial fruit blocth disease in melon.*

In the current study, the optimum fertilizer dose recommended in melon cultivation and the effect of different levels of this dose on the development of *A citrulli* were investigated and it was determined that the optimum dose of fertilizer was the most effective application in suppressing the disease. In the study, the disease severity was determined at a rate of 55.56% in the control application without fertilizer. Since the growth and development problems that occur in plants in the absence of the nutrients needed by the plants are known, this result was accepted as usual. It was determined that as the applied fertilizer dose increased, the disease severity increased and the highest disease severity (75%) occurred in the application where 100% more than the optimum dose was given.

It has been reported in various studies that plant nutrients play a critical role in stress (biotic and abiotic) resistance of plants (Cakmak, 2005; Amtmann et al., 2008; Römheld and Kirkby, 2010) as well as contributing to plant growth by increasing soil fertility (Esringü et al., 2016). Zimerman-Lax et al. (2016) investigated the effect of different nitrogen fertilizer applications against *A. citrulli* and reported that the treatments affected the expression of resistance genes such as HPL (hydroperoxide lyase), PDF1.4 (defensin) and PRX34 (peroxidase) and nitrate fertilization as a nitrogen source significantly reduced disease development. In various studies, it has been reported that the appropriate doses of nutrients required for plants reduce the incidence of disease in plants and this is due to the role of nutrients in the tolerance or resistance mechanisms of the host plant (Celar, 2003; Öborn et al., 2003; Sharma et al., 2005; Dordas, 2008; Nicolas et al., 2019). Therefore, it is necessary to provide sufficient amounts of nutrients necessary for the stimulation and synthesis of antimicrobial compounds present in the plant. Thus, phytoalexins, phenols, flavonoids and other defense compounds with inhibitory effects accumulate around the site of infection (Huber and Haneklaus, 2007).

CONCLUSION

In this study, we aimed to investigate the effectiveness of different fertilizer doses in controlling bacterial fruit spot disease caused by *Acidovorax citrulli* in cucurbitaceae plants, which is known to cause significant yield losses. The results of the study showed that the application of fertilizer at the recommended optimum dose was the most effective in preventing the pathogen. The findings highlight the crucial role of plant nutrients in modulating the interplay between host plant resistance and pathogen virulence and emphasize the importance of maintaining an adequate level of nutrient supply to sustain high levels of disease resistance. Notably, our results also indicate that excessive fertilizer use can lead to increased disease severity, as higher nutrient concentrations can enhance the susceptibility of plants to pathogen attacks. Overall, our findings suggest that careful nutrient management is essential for maintaining plant health and preventing disease outbreaks in Cucurbitaceae crops.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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