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Plant defense elicitor, 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) and its mode of action against fungal pathogen *Alternaria solani* in tomato (*Solanum lycopersicum* L.)

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Abstract: Biotic stress factors are one of the major constraints plants face, and they significantly affect production and yield. There are multiple ways to cope with stress factors, including genetic enhancement. When they cannot provide sufficient protection, pesticides are commonly applied. Plant defense elicitors are a new approach for boosting plants' natural immune responses and tolerance levels. The newly identified promising plant defense elicitor; 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) was previously studied against the oomycete *Hyaloperonospora arabidopsidis*, the bacterial pathogens *Pseudomonas syringae* and *Clavibacter michiganensis* ssp *michiganensis* and found to induce disease resistance against these phytopathogens. However, it was not tested against fungal pathogens. Here for the first time, DPMP was evaluated against one of the most destructive fungal pathogens, *Alternaria solani*. Disease severity and plant development were evaluated. The results revealed that DPMP neither inhibited nor enhanced the disease severity of *A. solani*. Gene expression of several salicylic acid, jasmonic acid, and ethylene pathway-related genes (*Pti4, TPK1b, Pto kinase, PRB1-2, SABP2*, and *PR3*) were also analyzed. According to the results, while DPMP induces *PRB1-2, TPK1b*, and *Pto kinase* gene expressions, the protection against *A. solani* does not occur via these genes. *PR3* is one of the most important genes for defense responses against necrotrophic pathogens, and DPMP downregulated gene expression of *PR*3. These results demonstrated that DPMP mostly takes a role through the SA-related defense pathway and was effective against biotrophic and hemibiotrophic pathogens. However, it is not suitable for protection against the necrotrophic pathogen *A. solani*. Further research may pinpoint the activity of DPMP on the defense pathway and provide a better understanding of the mode of action for DPMP and other plant elicitors for specific plant protection solutions.

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1. Introduction

Plants provide a rich source of nutrients for heterotrophic microorganisms and are, therefore, subject to attack by many pathogens. These attacks cause significant yield and quality losses in agriculture (Onaga and Wydra, 2016). Chemical substances called 'pesticides' are used against various pests to protect plants against pathogens and reverse the yield loss caused by biotic stress. Pesticides

can be classified as acaricides, insecticides, fungicides, herbicides, etc., according to the organism they target (Nicolopoulou-Stamati et al., 2016). Pesticides act directly against the target organism and aim to kill or stop the pest from spreading. Today, pesticides are used intensively and unconsciously in many agricultural fields. As a result of the intensive use of pesticides, the pesticide itself or its transformation products can remain in the food, soil, water, and air, which endangers all living organisms, especially humans (Pretty, 2008; Nicolopoulou-Stamati et al., 2016). The negative effects of pesticides on humans and the environment have been revealed extensively (Nicolopoulou-Stamati et al., 2016). The health problems and environmental pollution caused by the use of pesticides have forced many countries, especially the United Nations and non-governmental organizations, to take some precautions (Skevas et al., 2013). The high level of unwanted side effects of pesticides has led researchers to use plant activators as an alternative method for pest control (Walters et al., 2013; Villaverde et al., 2014).

Plant activators are stimulants that are given to the plant from the outside and strengthen the plant's natural immune system by stimulating and making it more resistant or tolerant against plant pests. While stimulants trigger the plant defense system, making the plant stronger against the pathogen, they do not have direct toxicity against the pathogen or other organisms (Bektas and Eulgem, 2015). For this reason, plant activators are used as an alternative to pesticides to eliminate the side effects of pesticides on the environment and other organisms. The "good agricultural practices" targets of the Ministry of Agriculture and Forestry of Turkey also support the use and formulation of products within this framework (Ministry of Agriculture and Forestry 2018-2022 Strategic Plan, 2018).

Various studies have been carried out since the 1970s regarding plant activators that stimulate the plant immune system, and some chemicals have been shown to stimulate it. Polyacrylamide acid compounds were tested against Tobacco Mosaic Virus (TMV) as plant activators and it was found to increase *PR1* gene expression (Gianinazzi and Kassanis, 1974). Since this first study, other synthetic chemicals have been shown to trigger the plant immune system over the years (Langcake and Wickins, 1975; Watanabe et al., 1977). Probenazole (PBZ), discovered in 1977, increased the defense response against *Magnaporthe oryzae* by activating enzymes related to plant immunity (Watanabe et al., 1977). In a study conducted by Ciba-Geigy (Syngenta) company in 1987, the contributions of 2,6-dichloro-isonicotinic acid (INA) and the later discovered acibenzolar-S-methyl (ASM/BTH) to plant protection were demonstrated (Metraux et al., 1990 and 1991; Ward et al., 1991; Uknes et al., 1992). BTH Bion® has been used as a pesticide for many years in many countries with this trademark. While plant activators can be synthetic, various studies have shown that pathogen-derived or plant-derived products have effects on the plant immune system (Kishimoto et al., 2006; Serrano et al., 2010).

Tomato (*Solanum lycopersicum* L.), a nutritious and delicious vegetable, is widely consumed in fresh or canned form and constitutes an important part of vegetable production. Pathogens emerging in tomato production areas can cause significant yield losses. As a result of these diseases and pests, growth retardation in the plant, deterioration of product quality, and death may occur (Foolad and Panthee, 2012). *Alternaria solani*, which is a necrotrophic fungal pathogen, causes early blight in tomatoes. It is an important disease agent that causes root rot and root collar blight, as well as early leaf blight (Rao et al., 2007; Ray et al., 2015). Early blight (EB) causes more than 10% yield loss in the world and Turkey (Boyno et al., 2020; Çevik et al., 2021). *A. solani* infects almost all Solanaceae members, including tomatoes and potatoes mostly through dead plant tissues. It has also been reported to infect vegetables, ornamental plants, and fruit species (apple, orange) (Foolad et al., 2008). EB is seen on the leaves, stems, and fruits of the tomatoes and causes severe damage throughout the season. The disease first appears in the field as small, irregular, brownish-black spots on old leaves. The spots take a round or elongated shape over time and look like intertwined rings, with the central part open (Adhikari et al., 2017). Diseased spots can spread to the whole fruit, and infected fruits are shed over time. Due to the ability of disease agents to survive under adverse conditions, there are difficulties in their control (Jindo et al., 2021). While cultural practices are important for the control of *A. solani*, prominent control is provided by chemical applications (Adhikari, et al., 2017).

Plant activators are powerful alternatives to the use of pesticides in agriculture. The introduction of plant activators in agriculture may contribute to the reduction of environmental pollution caused by the use of pesticides and their toxic effects on human health and will also be compatible with environmentally friendly agricultural policies implemented in the world and our country (Skevas et al., 2013; Nicolopoulou-Stamati et al., 2016; Ministry of Agriculture and Forestry 2018-2022 Strategic Plan, 2018). Even though there have been some scientific studies on plant activators, very few synthetic

stimulants have been identified, and new research in this area is essential. Developments in today's technology and molecular biology may enable more plant activators to be found and defined and their mechanisms of action to be revealed in this field. Recently 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl}e phenol (DPMP) is described as a novel synthetic elicitor. Recent studies showed its activity against some pathogens, including the biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) on *Arabidopsis thaliana* (Bektas et al., 2016). Also, its activity against two distinct bacterial pathogens*; Pseudomonas syringae* pv *tomato (Pst)* and *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) were revealed (Bektas et al., 2016; Bektas, 2021) with significant potential as a plant protection agent. While this research showed that DPMP is a robust synthetic elicitor against some tested biotrophic and hemibiotrophic pathogens, its activity against necrotrophic pathogens was not revealed. Therefore the goal of this study was the elicit the activity of DPMP against early blight caused by *A. solani* on the molecular level. Understanding the effect of DPMP on necrotrophic disease response can give us clues about DPMP's mode of action on the plant defense induction pathway.

2. Material and Methods

2.1. Plant material and growth conditions

The study was conducted under controlled conditions in the Department of Agricultural biotechnology, Siirt University, Siirt, Turkey. "Moneymaker" Tomato (*Solanum lycopersicum* L.) cultivar was used as plant material. Seeds were surface sterilized with 5 % sodium hypochlorite (NaOCl), and 70% ethanol, followed by rinsing under sterile water excessively. Sterilized seeds were germinated in Petri dishes at 25-27 °C and 16 h/8 h light/dark regimes. Seedlings were transplanted into pots containing peat and perlite mixture with a ratio of 2:1. Relative humidity and mean temperatures of the growth environment ranged between 60-70% and 25-27 °C, respectively. Chemical applications were initiated when plants reached three to the four-leaf stage in the 5th week.

2.2. Fungal material, disease assessments, and growth measurements

One of the most commonly found disease agents, *A. solani* was used for disease assessment. The *A. solani EAb 1* isolate (Boyno et al., 2020) was obtained from Dr. Semra Demir, Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yıl University, Turkey. *A. solani* isolates were grown on potato dextrose agar (PDA), and a sterilized water solution with 5 × 105 conidia mL^{-1} for the foliar spray was prepared. 24 hours after the second (last) application of the DPMP or control treatment, Each plant (5 weeks old) was sprayed with the stated concentration using a manual hand sprayer.

The disease severity (DS) of the infected plants was evaluated on the 18th, 23rd, 28^{th,} and 33rd days after the experiment started. A commonly applied 0-5 scale was used for disease severity scoring (Pandey et al., 2003). The percentage of the necrotic lesions on the leaf surface is used for the scoring. With this scale, 0 equals no symptoms, 1 equal 1-11%, 2 equals 11-25%, 3 equals 26-50%, 4 equals 51-75%, and 5 equals 76-100% symptoms on the leaves. The disease progress curve (AUDPC) value was calculated according to Pandey et al. (2003). Plant growth parameters, plant height (PH), shoot fresh weights (SFW), and shoot dry weights (SDW) were collected to obtain plants development under disease and chemical applied conditions. PH was measured manually with a ruler, SFW was determined with a precision scale (Weightlab instruments), while SDW was obtained after drying samples at 70 ˚C for 48 h in an oven (Nüve, TR).

2.3. Plant defense elicitor treatments

DPMP was used as a plant elicitor against *A. solani* inoculation. DPMP was generously provided by Dr. Thomas Eulgem, University of California, Riverside, USA. Since DPMP needs to be dissolved in a specific solvent, DMSO (100%) is used to dissolve DPMP. Stock DPMP solution was diluted to 10 µM with 0.2% DMSO in it. DMSO at 0.2% without DPMP is also applied to control plants to observe any possible effects of the solvent. Final concentrations of DPMP (10 µM) and DMSO (0.2%) were applied to plants one week and one day before pathogen application. Twenty-four hours after the second application of the DPMP or DMSO, the pathogen application was assessed. Each experiment was replicated three times with three plants per replication.

2.4. Gene expression analysis

Samples for gene expression analysis were collected 24 hours after the second chemical application, before pathogen application. A total of nine leaves per plant were collected and grounded in liquid nitrogen. Total RNA was isolated using PureLink RNA Mini Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA concentration and purity were determined using a Multiskan GO spectrophotometer (Thermo Scientific). RNase-Free DNase I (Thermo Scientific) was used to remove any DNA contamination. RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used for cDNA synthesis. Selected genes listed in Table 1 were used for the gene expression analysis. The experiments were repeated with three different biological and three different technical replicates. Real-time reverse transcription-quantitative PCR (RT-qPCR) was used for quantification of gene expression patterns using the PicoReal Real-Time PCR system (Thermo Scientific). Average Ct values were normalized to *Actin* for each gene of interest, and relative transcript levels were calculated according to Livak and Schmittgen (2001).

Table 1. Plant defense-related genes, forward and reverse primers, and references

Gene	Forward	Reverse	Reference
Pti4	CAACAGTTACCACCGACGAAC	GACCAATAGTTGATGGACACC TG	(Rasool et al., 2021)
PRB1-2	CGGTGAACACTGGAAATGTG	GGAGCATCGCCATTAATCAT	(Nehela et al., 2021)
SABP2	AACGGACACCAGCAGAGAAT	TGGCCTTTGACAAATCTTCC	(Nehela et al., 2021)
Pto kinase	AGATTGAACCATGGCAGACC	GATACTCTCACGCCGTAGCC	(Khan et al.,2012)
PR3	CAATTCGTTTCCAGGTTTTG	ACTTTCCGCTGCAGTATTTG	(Khan et al., 2012)
TPK1b	ATGGGGATATGTTTGAGTGCTA GAA	GAACGTGTTCTCGTCGATCCA CCCT	(Ray et al., 2015)
Actin	TGTCCCTATTTACGAGGGTTATG C	CAGTTAAATCACGACCAGCAA GAT	(Zhou et al., 2015)

2.5. Statistical analysis

Data collected in the study were analyzed according to Analysis of Variance (ANOVA). Means were compared following Tukey's Honest Significant Difference (HSD) test. Significance (*$p<0.05$) in each figure and table was indicated with different letters. Statistics software V10 (Analytical Software, Tallahassee, FL) was used for the statistical analyses.

3. Results

3.1. Evaluation of the Effects of DPMP on *A. solani* Disease Severity and Progress

Tomato plants at 5 weeks were subjected to foliar spraying of DPMP two times (1 and 7 days before inoculation (dbi)), to elaborate their responses under *A. solani* infected conditions. The results of four consecutive disease scoring clearly indicated that DPMP did not reduce the disease severity of *A. solani* in tomato (Figure 1a). A similar outcome was obtained in AUDPC values, for which DPMP and DMSO had similar AUDPC values (Figure 1b).

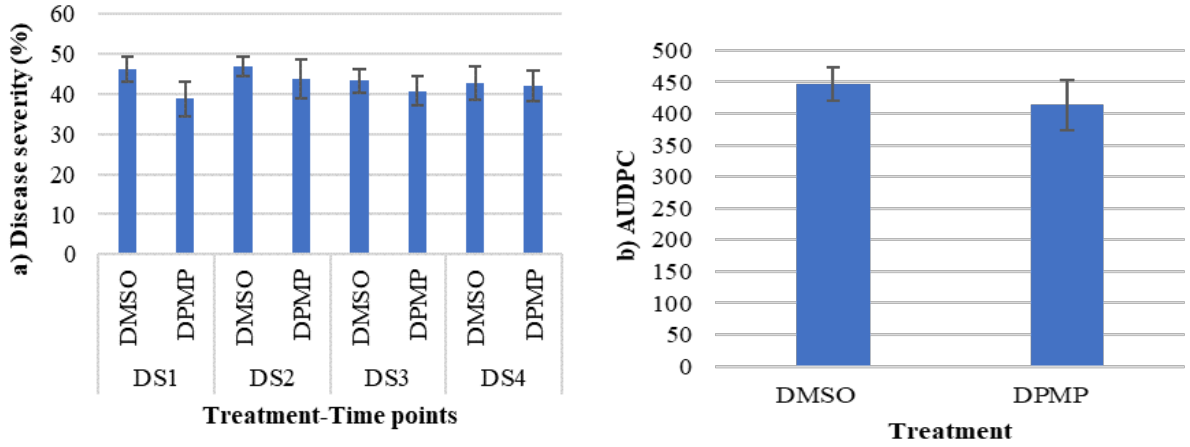

Figure 1. The effects of DPMP on *Alternaria solani* disease severity and progress. DPMP or DMSO was applied before pathogen inoculation. The AUDPC value was obtained with disease severity scores (%) at 4-time points. Three independent trials were analyzed using ANOVA and the means were separated according to Tukey's HSD multiple comparison test. Significant differences ($p < 0.05$) within each group are shown with different letters, otherwise, the data was Non-Significant (NS).

To make a precise comparison of DMSO, DPMP, and negative control (NK), four different morphological traits were evaluated. Plant height (PH), number of leaves, and plant fresh and dry weights were compared. Plant height was the tallest ($p<0.05$) in NK, while DPMP and DMSO had similar PH values and DMSO applied plants had slightly taller statures (Figure 2a). The number of leaves was also the highest in NK, followed by DMSO and DPMP, but the difference was not significant. Plant fresh (PFW) and dry (PDW) weights were measured to obtain the biomass potential of each application. Accordingly, NK had the highest PFW and PDW values that were predicted (Figures 2c and d). Since DPMP did not provide any protection against *A. solani*, it did not cause any change in the negative effect of pathogens on plant development as well.

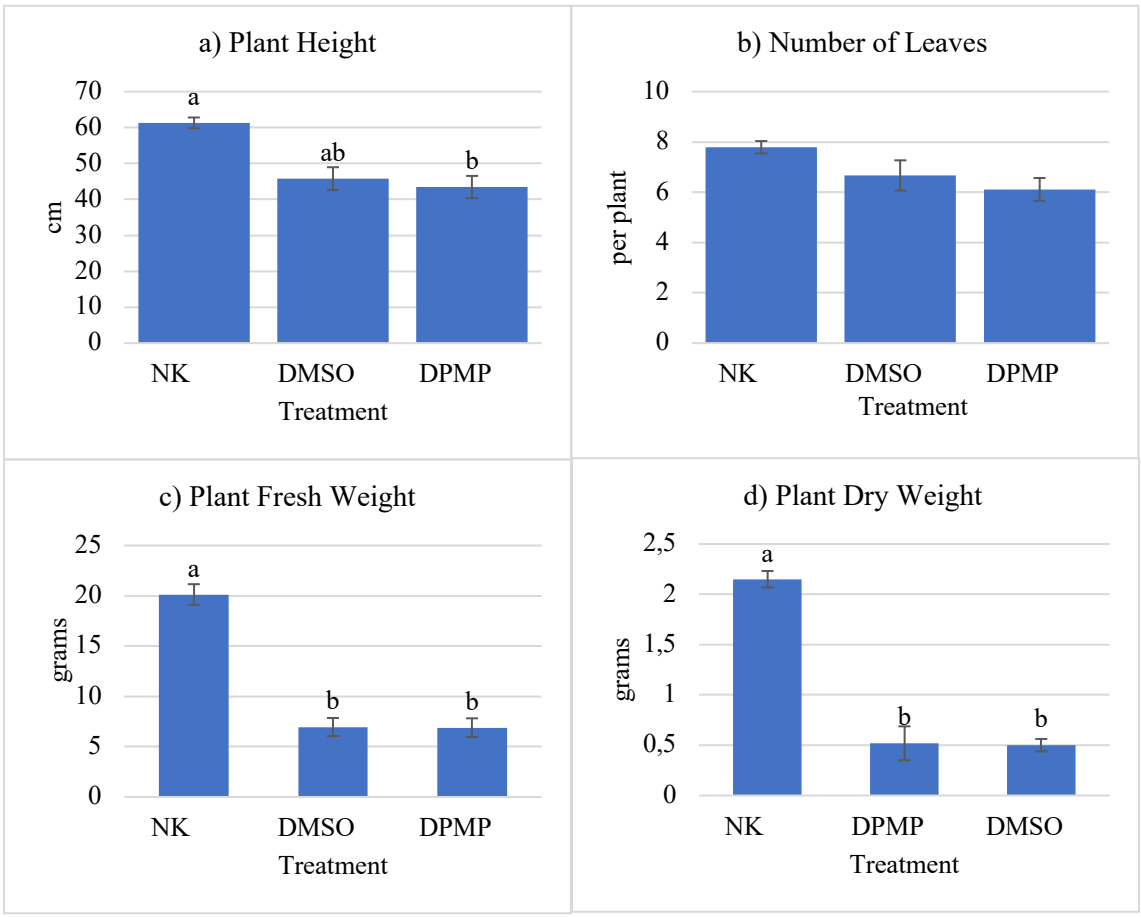

Figure 2. The effects of DPMP on a) Plant height, b) Number of leaves, c) Plant fresh weight, and d) Plant dry weight on *Alternaria solani* infected or uninfected (negative control (NK)) tomato plants. Significant differences within each group are shown with different letters according to Tukey's HSD test ($p < 0.05$).

3.2. Gene expression profiles of tomato plants sprayed with DPMP and DMSO (Control) under *A. solani* inoculated conditions

In the first part of this study, the effectiveness of DPMP against *A. solani* was evaluated with morphological observations and disease severity scoring in comparison with DMSO (inoculated control), and NK (non-inoculated, negative control). Since DPMP did not reduce disease severity under any of the four data points, we aimed to see the molecular mechanism of the disease response induction by monitoring selected marker defense response genes. Six different plant disease response-related genes, *Pti4, TPK1b, Pto kinase, PRB1-2, SABP2,* and *PR3*, were compared with RT-qPCR between DMSO and DPMP sprayed plants. Gene expression profiles of DPMP and DMSO sprayed plants were compared and normalized with *Actin.* According to the results, DPMP application down-regulated the expression of *Pti4* and *PR3,* on the other hand, overexpressed the activity of *PRB1-2, TPK1b,* and *Pto kinase.* The gene expression level of *SABP2* remained the same though (Figure 3). These results showed that DPMP had a remarkable effect on the gene activity of *PRB1-2, TPK1b,* and *Pto kinase* that was induced 3-9 fold compared to control, however, the induction of these genes did not affect the plant protection against *A. solani*.

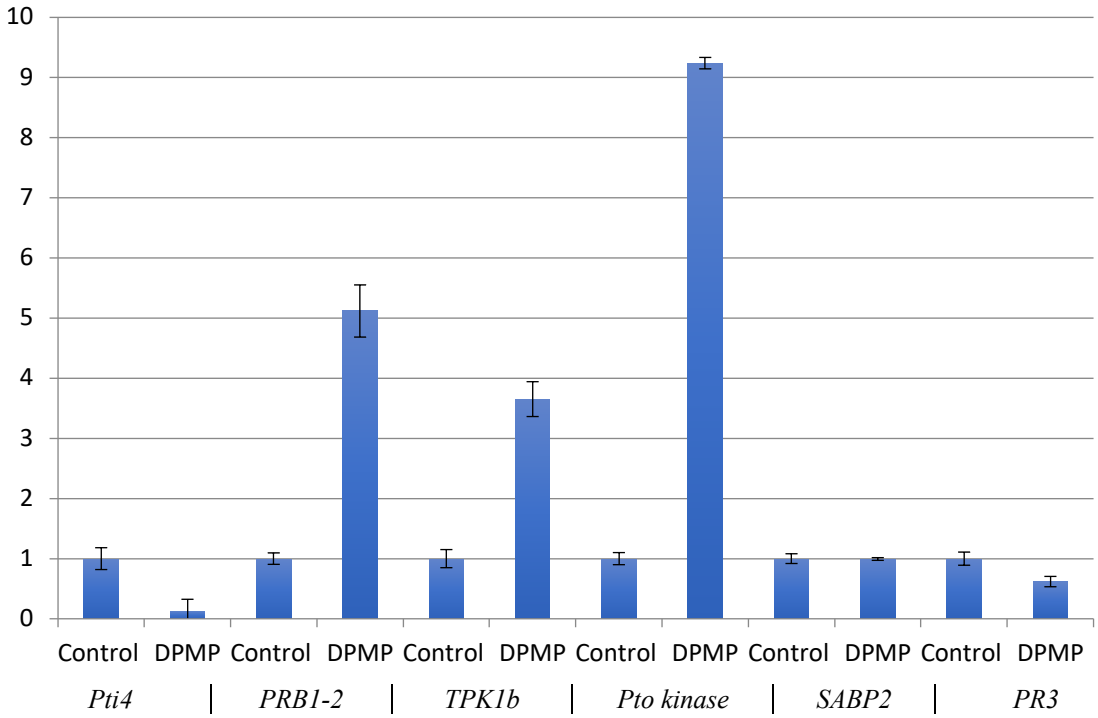

Figure 3. Transcriptional regulation of defense-related genes with the application of DPMP on tomato. Analysis of *Pti4*, *PRB1-2, TPK1b*, *Pto kinase*, *SABP2,* and *PR3* genes after DPMP or control (DMSO) applications that were normalized to *Actin*. Values presented mean ± SE of 3 biological replicates per treatment.

4. Discussion

Biotic stress factors are one of the major constraints in plant production and sustainability. One of the many ways to cope with stress factors is to improve disease tolerance or resistance genetically (Foolad et al., 2008; Singh et al., 2017). If plants do not have a natural allelic structure for disease resistance, exogenous applications, such as pesticides, are commonly used for plant protection (Nicolopoulou-Stamati et al., 2016). Even though pesticides are the most common way to fight disease agents, alternative ways are emerging with the new developments in agriculture and biotechnology. Plant defense elicitors are relatively new substances that can be obtained synthetically or organically (Bektas and Eulgem, 2015; Cohen et al., 2016). DPMP is a new plant defense elicitor with significant promise against *Pst* and *Cmm,* and *Hpa* on tomatoes and *Arabidopsis* (Bektas et al., 2016; Bektas, 2021). However, It has not been tested against many different pathogens, and the mode of action and effectiveness range is not fully known yet. *A. solani* (Early blight disease of Solanaceae family: EB), a necrotrophic fungal pathogen, causes a significant economic impact on tomato production worldwide (Adhikari et al., 2017). Even though there are pesticides commonly used to fight EB, they are not the best choice due to environmental and health-related side effects. These constraints force researchers and farmers to find new-novel approaches that are more environmentally friendly and cost-effective (Gerage et al., 2017; Nicolopoulou-Stamati et al., 2016). A novel approach is the enhancement of a plant defense system with an exogenous application, and DPMP is one of the candidates for this new approach (Bektas and Eulgem, 2015). Here, to elucidate possible roles of DPMP against *A. solani*, 5 weeks old tomato plants (cv. Moneymaker) were sprayed with DPMP, and the disease severity of *A. solani* was analyzed. Also, the molecular basis of the plant defense induction was elicited with relative expression levels of six different defense-related genes.

DPMP with a concentration of 10 µM was applied to plants before *A. solani* inoculation. It has been shown that DPMP did not reduce the disease severity of the pathogen (Figure 1). Compared to the control group, DPMP-applied plants also showed a similar defense response against pathogens and did not show any significant induction on any disease severity evaluation time points (Figure 1a). Correlated with that, the AUDPC values were not significantly different compared to the control (Figure 1b). On the other hand, previous research showed that DPMP induced plants' defense mechanism

against *Pst* (Bektas et al., 2016) and *Cmm* (Bektas, 2021*).* These indicated bacterial pathogens are hemibiotroph, but *A. solani* is a necrotrophic pathogen (Foolad et al., 2008). Previous research also provides information about the protective effect of DPMP against biotrophic oomycetes (Bektas et al., 2016). Based on these results, DPMP may induce defense responses related to biotrophic and hemibiotrophic pathogens, but plant defense response against necrotrophic pathogens might be through different pathways (Glazebrook, 2005; Lai and Mengiste, 2013). Previous findings demonstrated that the salicylic acid (SA) pathway is one of the central elements of plant defense induction against biotrophic and hemibiotrophic pathogens, and the jasmonic acid pathway is most crucial for necrotrophic pathogens (Bektas and Eulgem, 2015; Brouwer et al., 2020; Glazebrook, 2005; Vernooij et al., 1995). However, recent studies have provided controversial information to argue that SA-, ET-, and JA-related defense responses involve extensive transcriptional reprogramming against *A. solani* (Brouwer et al., 2020; Nehela et al., 2021; Spletzer and Enyedi, 1999).

To understand why the DPMP did not have any effect on *A. solani*, we tried to elicit some marker defense-related genes from different defense pathways, including salicylic acid, jasmonic acid, and ethylene-related defense responses. Six genes were evaluated with RT-qPCR to see which pathways are active under the current scenario. Of these, *Pto kinase* is a gene that encodes serine/threonine kinase. Previous research revealed that its overexpression activity inhibits *Pst* infection through effector-triggered immunity (ETI) (Oh and Martin, 2011). In this study, the relative gene expression level of *Pto kinase* was up-regulated more than 9 folds in response to DPMP spraying; however, it did not provide any protection against *A. solani*. This finding is consistent with previous research that suggested that *Pto kinase* is important in ETI and coordinated with SA-related pathways (Glazebrook, 2005; Oh and Martin, 2011). As a result, *Pto kinase* is not required for plant protection against necrotrophic pathogen *A. solani. Tomato protein kinase 1* (*TPK1b)* was another gene that was over-expressed through the application of DPMP. *TPK1b* encodes receptor-like cytoplasmic kinase and is known to be induced by infection, wounding, and oxidative stress (Smith et al., 2014). DPMP application induced *TPK1b* gene expression in about 3 to 4 folds (Figure 3). This finding demonstrated that *TPK1b* is not a key element for plant protection against early blight.

JA and ethylene-induced defense mechanisms are more active when the plant is infected with necrotrophic pathogens (Glazebrook, 2005; Rasool et al., 2021). *Pti4* encodes a transcription factor in the ethylene-responsive element-binding factor (ERF) family of proteins (Gu et al., 2000). So, we analyzed the gene expression of *Pti4* after DPMP application. Relative gene expression of *Pti4* was slightly downregulated with the DPMP spraying compared to DMSO (control), suggesting that *Pti4* may not be the key element for *A. solani* defense responses. Accordingly, previous reports suggest that *Pti4* takes a role in the activation of GCC-box *PR* genes against hemibiotroph pathogen *Pst* in tomatoes (Gu et al., 2000 and 2002; Wang et al., 2021) and does not involve in the disease response against this pathogen.

It was previously reported that benzoic acid and its hydroxylated derivatives increase *SABP2* and *pathogenesis-related protein* (*PRB1*-2) gene expressions and reduce the severity of *A. solani* (Nehela et al., 2021). Thus, we evaluated the relative expression levels of these genes. *SABP2* plays a role in the transformation of methyl salicylic acid (MeSA) into salicylic acid (SA) and induces systemic acquired resistance (SAR) (Tripathi et al., 2010). Here, DPMP did not affect the gene expression level of *SABP2.* On the contrary, DPMP applications up-regulated *PRB1-2* expression (Figure 3), but *PRB1-2* over-expression did not protect plants against the *A. solani*. The controversies of *PRB1*-2 activity against *A. solani* might be due to downstream activity of *PRB1*-2 gene activation.

To make a diverse comparison, we also evaluated *PR3,* which is one of the most important players in the plant defense system for necrotrophic pathogens. *PR3* is one of the pathogenesis-related genes (PR genes) (Edreva, 2005; Sinha et al., 2014). PR genes may differ in structure, mechanisms of action, and pathogen specificity (Anisimova et al., 2021). Some are hydrolytic enzymes (like chitinases (*PR3*)), others are antimicrobial proteins (like defensins), phytoalexins, anti-fungal proteins, etc. (Anisimova et al., 2021; Edreva, 2005). Biotrophic pathogens activate the SA pathway and related PR genes (*PR1, PR2* & *PR5*), while necrotrophic pathogens stimulate the JA pathway and activate specific PR genes (*PR3, PR4* & *PR12*) (Ali et al., 2018). Here, *PR3* (Chitinase/Chi3) encoding chitinase involved in the ethylene/jasmonic acid-mediated signaling pathway was evaluated with DPMP treatment. Accordingly, *PR3* gene expression was neither up nor down-regulated with DPMP application (Figure 3) and therefore did not contribute any protection against early blight. Previous research showed that DPMP induces *PR1* gene expression and provides significant protection against *Cmm*. All of these results showed that DPMP induces plant defense responses against biotrophic and hemibiotrophic pathogens through SA-related defense responses but not necrotrophic pathogens.

5. Conclusions

Early blight disease (*A. solani*), causes significant yield losses in tomato production. Plant protection against the disease is provided by Fungicides, but their side effect on the environment and living organisms forced researchers to find alternative ways. In this study, the promising plant defense elicitor DPMP as an alternative to pesticides was evaluated at disease severity and molecular levels. In our research, for the first time, we provided a report that DPMP-regulated defense activation did not provide effective protection against necrotrophic pathogen *A. solani*. On the contrary, it provides significant protection against some biotrophic and hemibiotrophic pathogens. This finding provides us some foresight about what is the mode of action of DPMP as a plant defense elicitor. These findings contribute valuable information for a researcher to come up with molecular mechanisms of defense activation against distinct pathogens.

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