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Research Article

Induced Resistance of Shallot (*Allium cepa* L. var *aggregatum*) against Twisted Disease Using Ultraviolet-B Light

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ABSTRACT

Ultraviolet-B (UV-B) light induces plant resistance against disease infection. Its ability to induce plant resistance has been widely used as an inducer of plant resistance. This study aimed to determine the ability of UV-B light to suppress twisted shallot disease, the potency of plant resistance induction through the accumulation of peroxidase enzymes, salicylic acid, and chlorophyll content, and to observe shallot growth after treatment. Bulb irradiation was treated daily for 4 hours using UV-B light 65 μW/cm^2 for 3, 5, and 7 days and was compared to inoculated plants treated using fungicide (Benomyl). The results showed that UV-B irradiation for seven days reduced the incidence of shallot twisted disease. However, the incubation period treatments showed similar results. UV-B irradiation of 4 hours/day for seven days increased salicylic acid content and maintained the same chlorophyll content as the negative control. However, the peroxidase enzyme content was similar. Treatment of UV-B, 4 hours/day for seven days of irradiation did not inhibit the growth and production of shallot plants. Therefore, bulbs irradiation treatment of UV-B 65 μW/cm^2 in 4 hours/day for seven days could be recommended to reduce shallot twisted disease.

Keywords: induce resistance; shallot; twisted disease; ultraviolet-b light

INTRODUCTION

Shallot twisted disease caused by *Fusarium* spp. is one of the main diseases of shallot plants that causes significant yield loss (Lestiyani *et al*., 2015). In general, farmers will usually spray pesticides to suppress the plant diseases. However, the intensive use of pesticides could cause some problems, for example chemical residue in the environment which influence the imbalance population of ecosystem inhabitants (Sudewa *et al*., 2008). Shallot twisted disease in several shallot production centers in Indonesia can cause yield losses of up to 50%. According to Wiyatiningsih *et al.* (2009), shallot twisted disease showed characteristic symptoms in which their pseudostems and leaves grow taller, twisted, chlorosis and finally all leaves dried. Therefore, efforts to control by using safe, inexpensive and environmentally friendly disease-control technology are needed.

UV radiation can be divided into three types, namely UV-A light with a wavelength of 320−400 nm, UV-B light with a wavelength of 290−320 nm, and UV-C light with a wavelength of 200−290 nm. Previous report showed that UV-B induced resistance of rose and cucumber against fungal disease (Suthaparan *et al*., 2012). Ultraviolet B irradiation generally reduces growth parameters such as plant height, fresh weight, and dry weight, but does not significantly affect leaf area and leaf moisture content (Alexieva *et al*., 2001). Ultraviolet B irradiation does not cause damage to plants, but slightly inhibits growth (Demkura & Ballare, 2012).

Ultraviolet B lamps can be produced by artificial techniques using fluorescent ultraviolet lamps. Elevated UV-B radiation (UV-B) has pleiotropic effects on plant development, morphology, and physiology. The morphological consequences of UV-B-supplemented white-light treatment include reduced growth, thickening of leaves and of cuticular

wax layers (Jansen *et al.*, 1998). However, UV-B light irradiation can induce rose plant resistance from powdery mildew with irradiation of 65−140 mW/m^2 2h-nights/day (Kobayashi *et al.*, 2013).

The high activity of peroxidase indicates the enormous amount of resistance towards pathogens as reported previously by research on yardlong bean (Pujihartati *et al.,* 2006). Along with that, salicylic acid which naturally can be found inside the plant, has been proved to play an important role as a plant defense (Vlot *et al.* 2009). The example of some parameters which show plant resistance against various pathogens is chlorophyl content (Andari & Nurcahyani, 2018). Therefore, this study was conducted to determine the ability of UV-B light in suppressing shallot twisted disease, the potency of plant resistance induction through accumulation of peroxidase enzymes, salicylic acid, chlorophyl content and to observe the effect of UV-B light on shallot growth.

MATERIALS AND METHODS

Research Layout

This research was conducted using a completely randomized design (CRD). This experiment used 9 treatments which were repeated 3 times and 5 bulbs per polybag, at the greenhouse of the Faculty of Agriculture, Universitas Gadjah Mada, from September to November 2021. Analysis of salicylic acid content was conducted at the Agricultural Environmental Agrochemical Residue Laboratory, Agricultural Environmental Research Institute (*Balai Penelitian Lingkungan Pertanian* [BALINGTAN]), Bogor. Peroxidase enzyme analysis was carried out at the Laboratory of Plant Pathology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. The shallot variety used in this study was the Tajuk variety. *Fusarium acutatum* KP2 used in this study was collection of Laboratory of Plant Pathology, UGM. The treatment were as follows: UV 3 (bulb was exposed to UV-B lamp for 4 hours/day for 3 consecutive days); UV 5 (bulb was exposed to UV-B lamp for 4 hours/day for 5 consecutive days); UV 7 (bulb was exposed to UV-B lamp for 4 hours/day for 7 consecutive days); UV 3 + INOC (4 hours/day for 3 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil); UV 5 + INOC (4 hours/day for 5 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil); UV 7 + INOC (4 hours/day for 7 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil); FUNGICIDE + INOC (bulb was treated with fungicide [Benlate®, ai: Benomyl] and then inoculation with *F. acutatum* KP2); Control + (which has been inoculated with *F. acutatum* KP2); Control – (without any inoculation).

Ultraviolet-B Light Application

This study used a 24 watt Panasonic UV-B lamp and a box made of cardboard for irradiating shallot bulbs with a size of 1 × 1 m (l × w) and a distance of 0.3 meters above the treated bulbs with a dose of 65 µW/cm^2 in measure using the SP-82UV (Figure 1).

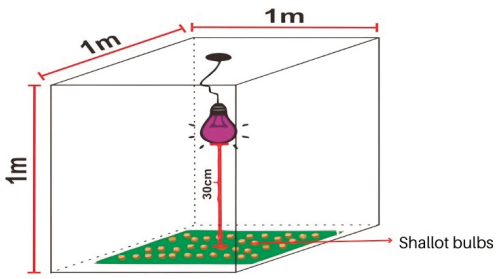

Figure 1. UV-B lamp installation and irradiation box for shallot bulbs (1×1×1 m) and a distance of 0.3 meters

Pot Preparation, Pathogen Inoculation and Shallot Planting

The planting medium was sterile soil mixed with organic fertilizer with a ratio of 1: 4 using polybags with a size of 40 cm × 40 cm. *Fusarium acutatum* KP2 inoculum was prepared by growing a culture of *Fusarium acutatum* KP2 on PDA media, then inoculum was made by spore suspension with a density of 10^5 microconidia/ml. Inoculation was carried out by pouring the spore suspension into the planting medium 3 days before planting shallot seedlings, in the afternoon, as many as 20 ml/polybag.

Determination of Twisted Disease Incidence

Twisted disease incidence was observed at 7 day after planting (DAP) with 7 day intervals for 8 weeks. Observations were made by counting the number of diseased plants per polybag and then determined

using the following formula (Korlina & Baswarsiati, 1995):

$$\text{Disease incidence} = \frac{\text{total number of infected plant with twisted symptom}}{\text{total number of plant perpolybags}} \times 100$$

Incubation Period Observation

The incubation period is the time between the time of inoculation of pathogenic fungi until the onset of symptoms of twisted disease. After inoculation of pathogenic fungi, observations were made every day to find out when the symptoms of twisted disease appeared for the first time on shallots (Purwantisari *et al.*, 2016).

Analysis of Peroxidase Enzyme Activity

The activity of the peroxidase enzyme was analyzed on 14 days after planting (DAP) by the method of Saravanan *et al.* (2004). In the test tube, a mixture of 1.5 mL of 0.05 M pyrogallol, 0.5 mL of enzyme extract from shallot leaves, and 0.5 mL of 1% H_2O_2 was made. The mixture was precipitated at room temperature and put into a 0.5 mL cuvette. The absorbance was measured with a UV spectrophotometer at a wavelength (λ) of 420 nm and read from zero, with each sample being replicated 3 times. Enzyme activity was calculated in U/mg/min. One unit is the change in the activity of the Optimal Density (OD) of 420 nm on the spectrophotometer per minute.

Before and after incubation, absorbance values were obtained, The enzyme activity was determined by the following formula:

$$\text{Unit ml-1 enzyme} = \frac{(\text{rA420nm/20SecSample} - \text{rA420nm/20SecBlank})(3)(\text{df})}{(12)(0.2)}$$

3 = total volume (mL)
df = dilution factor
12 = coefficient of 1 mg/mL, purpurogalin at 420 nm.
0.2 = volume (mL) used

One unit describes the change of 1 mg of pyrogallol to 1 mg of purpurogallin in seconds at 20ºC, pH 6.

Analysis of Salicylic Acid Content

Analysis of signaling compound content was carried out on 21 DAP plant samples. Extraction and analysis of metabolite used modified methods from Tenhaken and Rubel (1997). First, the plant sample (1.0 g) was ground in a mortar-pastel while adding 3 ml of a mixture of methanol and acetone (1:1, v/v) then transferred to an eppendorf tube. The Eppendorf tube containing the suspension was centrifuged at 5,000 rpm for 10 minutes. The supernatant in this step was combined with the supernatant obtained previously. The supernatant was centrifuged at a speed of 5,000 rpm for 10 minutes. The supernatant was then separated from the precipitate. The precipitate was extracted again by adding 1 mL of a mixture of methanol and acetone (1:1 v:v), then centrifuged at 5000 rpm for 10 minutes. The supernatant was combined with the supernatant obtained previously. The centrifuged supernatant was dried in a vacuum with a freeze dryer, the dry residue was then suspended by adding 30% methanol. The suspension was then centrifuged at 5,000 rpm for 10 minutes. The precipitate formed was removed with the supernatant used for analysis.

The content of metabolites in the sample plants was analyzed qualitatively and quantitatively using High-Performance Liquid Chromatography (HPLC). The mobile phase used was methanol:50 mM sodium acetate buffer pH 4.5 (30:70) (methanol:50 mM sodium acetate buffer (30:70) = 500 ml homogenized for 10 minutes by stirring with a magnetic stirrer) with a flow rate of 0.6 ml/minute. Sample and solution were filtered using a 0.45 μm RC cellulose acetate filter membrane for the mobile phase. The chromatopaque used was C-R7A plus. The wavelength used in the analysis of the content of these metabolites is 280 nm with the type of column used is VP-ODS ultra, UV detector at 280 nm.

Quantitative analysis was carried out to determine the content of metabolites in the sample, by converting the sample area to a standard area whose concentration is known on the calibration curve. Calibration curves were obtained from concentrations of standard compounds.

Observation of Chlorophyll Levels

Observation of the chlorophyll content was carried out once at 35 DAP. As much as 1 g of young shallot leaves were sampled, crushed, and 20 mL of 80% acetone was added until all the color

was removed from the tissue. The extract was centrifuged for 10 minutes at 1,500 rpm. The surface of the cuvette was cleaned, then inserted into the spectrophotometer. Measurements using a spectrophotometer with absorbance values of chlorophyll solutions at wavelengths of 663 nm and 645 nm (Agustina *et al.,* 2019). Concentrations was calculated using the following formula: Leaf chlorophyll content = 17.3A645 +7.18A663 mg/l (Harborne, 1987).

Growth Observation of Shallot

Growth was observed on all plants by counting and measuring the number of leaves and plant height every week with an interval of 7 days up to 56 days. Plant fresh weight, tuber wet weight, tuber dry weight, leaf wet weight and leaf dry weight were calculated after harvest.

RESULTS AND DISCUSSION

Effect of Application Using Ultraviolet-B Light in Disease Suppression

Results showed that UV-7+INOC significantly reduced disease incidence compared to K+ and was not significantly different from K- while UV-3+ INOC only suppressed it slightly reduced disease incidence (Table 1). UV-B irradiation may induced plant resistant mechanisms against pathogens attacks and it was in accordance with the results of previous research UV-B irradiation increases plant resistance (Demkura & Ballaré, 2012). Suthaparan *et al.* (2012) showed that UV-B radiation suppressed powdery mildew development with a direct effect on powdery mildew in tea plants. Shallot twisted disease also occured in negative control (K-) and untreated plants assuming that pathogens was already attached on bulbs. The shallot bulbs used in this study were from certified bulbs, but certification still focused on agronomic parameters and not whether pathogens are detected on them. Consequently, the twisted disease possibly occurred although the bulbs were certified.

The incubation period between treated and inoculated plants was not significantly different indicating that the twisted symptoms appeared around 23−29 DAP. Only treatment using UV-7 demonstrated significant differences.

Table 1. Effect of UV-B light irradiation on disease incidence and incubation period in shallots 8 weeks after planting (WAP)

Treatments	Disease Incidence (%)	Incubation Period
UV-3	26.67 de	28.7 ab
UV-5	26.67 de	26.3 abc
UV-7	20.00 e	29 a
UV-3+INOC	60 b	24 bc
UV-5+INOC	46.67 bc	23.7 c
UV-7+INOC	26.67 de	25 abc
FUNGICIDA+INOC	40 cd	24.3 abc
K+	66 a	23.7 c
K-	26.67 de	26.7 abc

Notes: UV-3 (bulb was exposed to Ultraviolet B [UV-B] lamp for 4 hours/day for 3 consecutive days), UV-5 (bulb was exposed to UV-B lamp for 4 hours/day for 5 consecutive days), UV-7 (bulb was exposed to UV-B lamp for 4 hours/day for 7 consecutive days), UV-3 + INOC (4 hours/day for 3 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV-5 + INOC (4 hours/day for 5 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV-7 + INOC (4 hours/day for 7 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), FUNGISIDA + INOC (bulb was treated with fungicide [Benlate®, ai: Benomyl] and then inoculation with *F. acutatum* KP2), Control + (inoculated with *F. acutatum* KP2), Control – (without inoculation). The numbers followed by the same letter indicate there are no differences according to DMRT 5%.

Peroxidase, Salicylic Acid, and Chlorophyll Content

Results showed that some UV-B treatments had an impact on shallot plants (Table 2). Peroxidase (POX) enzymes was not significantly different between treatments and at 14 DAP. Since activity of POX enzyme was only observed once, it was assumed that the influence of POX was unobservable on 14 DAP. Peroxidase enzymes act as impulses in monolignol polymerization which are useful for plant defense. Lignin infiltration in the interior of the cell defense system can increase the mechanical acuity of plant cells against pathogen infiltration (Huang, 2001; Strange, 2003). Therefore, further research to find the increasing of POX enzyme activity needs to be conducted by time course experiment.

Content of Salicylic acid in UV-7+INOC was significantly higher from K+, UV-7+INOC resulted

Table 2. Effect of UV-B irradiation on shallot plant biochemistry

Treatment	POX 14 DAP unit/mL	SA 21 DAP mg/Kg	Chlorophyll 35 DAP mg/L
UV-3	4.30 a	0.85 a	19.20 abc
UV-5	3.95 a	0.86 cd	18.84 cd
UV-7	4.74 a	0.92 c	16.76 e
UV-3+INOC	3.36 a	0.86 cd	19.90 abc
UV-5+INOC	4.18 a	1.17 b	19.22 bcd
UV-7+INOC	3.90 a	1.35 a	20.56 ab
FUNGICIDA+INOC	4.02 a	1.14 b	17.83 de
K+	5.99 a	0.62 f	18.67 cd
K-	5.33 a	0.69 e	21.25 a

Notes: UV-3 (bulb was exposed to ultraviolet B [UV-B] lamp for 4 hours/day for 3 consecutive days), UV-5 (bulb was exposed to UV-B lamp for 4 hours/day for 5 consecutive days), UV 7 (bulb was exposed to UV-B lamp for 4 hours/day for 7 consecutive days), UV 3 + INOC (4 hours/day for 3 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV 5 + INOC (4 hours/day for 5 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV 7 + INOC (4 hours/day for 7 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), FUNGISIDA + INOC (bulb was treated with fungicide [Benlate®, ai: Benomyl] and then inoculation with *F. acutatum* KP2), Control + (inoculated with *F. acutatum* KP2), Control – (without inoculation). The numbers followed by the same letter indicate there are no differences according to DMRT 5%. POX refers to peroxidase enzyme activity. SA refers to Salicylic acid content. chlorophyll to chlorophyll content.
POX 14 DAP : Observations were made 14 days after planting (DAP).
SA 21 DAP : Observations were made 21 days after planting (DAP).
Chlorophyll 35 DAP : Observations were made 35 days after planting (DAP).

in a value of 1.35 while K+ was only 0.62. This was assumed that UV-B irradiation at UV-7+ INOC could activate the salicylic acid signal to accumulate higher amount of salicylic acid which contribute to the protection of plants against *F. acutatum* KP2 infection. According to (Gaffney *et al.*, 1993) Salicylic acid is a signal transduction that ends with Systemic Acquired Resistance (SAR). Salicylic acid is one of the compounds that indicate a well-proven defense response of plants. In this study, sufficient salicylic acid content could protect plants from pathogen attacks.

The UV-7 treatment was significantly different from the K+ treatment, while the UV-7+INOC treatment was not significantly different from the K- (Table 2). Result showed that daily UV-B treatment for 4 hours done repeatedly for 7 days protected plants against *F. acutatum* KP2 inoculation and contributed to better shallot growth. Therefore disease incidence of UV-7+INOC was low and not significantly different from K- and other non-treated plants. Observation of leaf chlorophyll content was carried out at 35 DAP because maximum vegetative phase during that age, where nitrogen absorption is also maximal and begins to decrease when entering the generative phase due to lower nitrogen requirements during generative phase (Yuningsih, 2002). Low chlorophyll levels in shallot plants is thought to be due to infection from pathogens that cause diseased plants, causing chlorosis (yellowing of leaves) so that the leaves curl and curl. Loss of chlorophyll content in plants causes plants to become stunted and even do not produce marketable shallots. Observation of chlorophyll content needs to be carried out to determine the effect of twisted disease on chlorophyll due to chlorosis, which causes a decrease in yield in plants.

Shallot Growth Observation

Growth parameters of UV-7+INOC were not significantly different from K- in each observation (Table 3). This indicates that UV-7 treatment can induce plant resistance against pathogen attacks and was in accordance with research from Kobayashi *et al.* (2013) that tested UV-B irradiation on roses.

UV-5; UV-7 and UV-7+INOC treatments showed no significant difference between fresh and dry weight both on the leaves and the bulbs (Table 3). However, UV-3 and UV-5+ INOC demonstrated significantly lower yield compared to the negative control, but were not significantly different from the positive control (inoculated plants). This im-

Table 3. Observation of results shallot growth 8 weeks after planting (WAP)

Treatment	Fresh Weight of The Shallot Leaves (g)	Dry Weight of The Shallot Leaves (g)	Fresh Weight of The Shallot Bulbs (g)	Dry Weight of Shallot Bulbs (g)
UV-3	3.00 c	1.00 c	14.77 c	11.77 cd
UV-5	3.94 abc	2.47 ab	16.20 bc	12.2 bcd
UV-7	5.22 a	2.79 a	19.07 a	14.81 a
UV-3+INOC	2.73 c	0.73 c	12.69 d	9.69 e
UV-5+INOC	3.60 bc	1.60 bc	14.54 c	10.54 de
UV-7+INOC	4.67 ab	2.66 ab	17.36 ab	13.36 abc
FUNGICIDA+INOC	3.31 bc	1.3 c	14.67 c	11.67 cd
K+	2.51 c	0.51c	9.23 e	6.23 f
K-	5.12 a	3.12 a	18.18 a	13.98 ab

Notes: UV-3 (bulb was exposed to Ultraviolet B [UV-B] lamp for 4 hours/day for 3 consecutive days), UV-5 (bulb was exposed to UV-B lamp for 4 hours/day for 5 consecutive days), UV-7 (bulb was exposed to UV-B lamp for 4 hours/day for 7 consecutive days), UV-3 + INOC (4 hours/day for 3 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV-5 + INOC (4 hours/day for 5 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), UV-7 + INOC (4 hours/day for 7 consecutive days, bulbs were exposed to UV-B lamp then were planted in the inoculated soil), FUNGISIDA + INOC (bulb was treated with fungicide [Benlate®, ai: Benomyl] and then inoculation with *F. acutatum* KP2), Control + (inoculated with *F. acutatum* KP2), Control – (without inoculation). The numbers followed by the same letter indicate there is no difference according to DMRT 5%.

plied that shorter UV-B irradiation time (3 days) has the potential to suppress the growth of shallot plants, but on the other hand, after increasing the irradiation time to 7 days, UV-B treatment resulted in plants that were resistant against pathogen infection. Although it is not yet clear what causes this mechanism to occur, it appears that the length of UV-B irradiation period needs to be considered for further research.

CONCLUSION

UV-B lamp irradiation 65 μW/cm^2 for 4 hours/day and 7 days significantly suppressed the incidence of twisted disease. UV-B lamp irradiation 4 hours/day for 7 days significantly increase salicylic acid accumulation and maintain the chlorophyll content similar to non-inoculated plants that may explain their disease resistance against twisted disease. Moreover, treatment of UV-B lamp irradiation 4 hours/day for 7 days on bulbs showed that plants could grew normally as of untreated plants that implies that UV-B lamp irradiation 4 hours/day for 7 days on bulbs could be recommended to reduce the disease on shallots.

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