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Investigation of The Protective Properties of Bacteria (*Agrococcus citrus*) and Fungus (*Fusarium oxysporium*) Pigments ın Lettuce Plant Exposed to UV Stress

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ABSTRACT

As a result of climate change and the ozone layer spoilage, harmful rays of the sun such as UV reach the world more and harm agricultural production. To be protected from the harmful effects of UV, not only human beings, but all living organisms have developed different characteristics. In recent years, pigments with radiation absorbing and antioxidant properties have been used against UV damages. In this study, the effect of carotenoid pigments obtained from bacteria and fungi on the lettuce plant (Lettuce Yedikule 5701) was investigated due to its high antioxidant and UV protection properties. Pigment solutions partially purified from microorganisms were sprayed onto the plants. While an increase was detected in the amounts of hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO) in the lettuce plant with the effect of UV, a decrease was observed in these parameters when applied with pigment solutions. With the same application, microbial pigments protected the plant against the harmful effects of UV by increasing the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and the amount of chlorophyll. As a result of this study; It has been determined that microbial pigments, which can be obtained easily and with low costs, have protection properties against the harmful effects of UV and provide the plants with properties to resist the stress.

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Introduction

The harmful rays of the sun which with climate change and the deterioration of the ozone layer, are reached the world. Ultraviolet rays come first among these rays. UV causes skin cancer and aging in humans, and causes both molecular and biochemical changes in higher plants, thus increasing reactive oxygen species (Czégény et al., 2016; Coffey et al., 2017; Sevindik et al., 2017; Pramono et al., 2020). ROS are also produced under normal conditions, but their levels increase with UV exposure and even cause death (Jiang et al., 2012; Krupodorova et al., 2022). UV radiation changes germination, flowering, photosynthesis, leaf and stem thickness in plants. It also causes changes in the chloroplast structure. It increases the number of peroxisomes in the cell (Sarghein et al., 2011; Kına et al., 2021). The chance of survival of a plant exposed to UV stress depends on the amount of ROS and the change in the amount of antioxidant enzymes (Kına et al., 2021; Akgül et al., 2022). UV-induced photomorphological changes and synthesis of phenolic compounds affect plant yield due to photosynthesis. In recent years, pigments with natural antioxidant properties have been used against UV damage. Pigments are preferred in many industries such as food and medicine because of these properties (Boric et al., 2011; Mohana et al., 2013; Poorniammal et al, 2018; Venil et al. 2020; Mohammed et al., 2022). Natural pigments are obtained from insects, plants and microorganisms. Pigments obtained from microorganisms are preferred because of their stabilization, high efficiency and availability in all conditions (Morales-Oyervides et al., 2017; Pehlivan et al., 2021). Bacterial and fungal pigments such as melanin and carotenoid protect organisms by preventing the harmful effects of UV rays (Pailliè-Jiménez et al., 2020).

Considering the UV protective properties of the pigments of these organisms, in this study, the protection of the pigments of two different organisms obtained such

as bacteria and fungi against the lettuce plant UV exposure which is consumed all over the world and has commercial value was investigated.

Material and Method

Microorganisms

Bacterium *Agrococcus citrus* (OG4) and fungus *Fusarium oxysporium* (OG11) used in the study were obtained from the microbiology culture collections of Atatürk University, Faculty of Science, Department of Biology, Microbiology Laboratory.

Extraction of Pigments

Bacterial pigment; 3 g peptone was placed in Nutrient Broth and it was adjusted to have 100 mL broth in 250 mL flasks. The media were autoclaved at 121 ˚C and 1.5 atm. Bacteria were inoculated in an amount of OD_{600} = 1 and incubated for 3 days at 30 ˚C at 150 rpm. After incubation, the culture was centrifuged at 6000 rpm for 15 minutes. This process was repeated twice by washing with distilled water so that there would be removed nutrient residue. Then, ethanol was added to the precipitated bacteria and the pigment was allowed to pass into the solvent at 28 ˚C for 1 hour, and the bacteria were removed by centrifugation at 6000 rpm for 15 minutes (Shatila et al. 2013).

Fungal pigment**;** after the selected fungus was grown on Potato Dextrose Agar at 28°C for 7 days, its spores were transferred to a culture broth containing 100 ml of Potato Dextrose Broth and 3 g of peptone in a 250 mL flask. It was incubated at 28°C for 7 days. The micelles formed were removed from the environment and treated with ethanol in a separate place, allowing the pigment to pass into the solvent. The pigment in the supernatant was treated with ethyl acetate: chloroform (1:1) and used to study the orange pigment (Narendrababu and Shishupala 2017).

UV-Visible Spectrophotometry of Microbial Pigment Extracts

The absorption spectrum of the extracted pigments was measured in a visible wavelength region of 200 - 700 nm and their graphs were obtained.

Growth of Plants and Pigment Applications

Lettuce seeds (Yedikule 5701) were supplied from the commercially purchased. Seeds were sterilized with 5 % sodium hypochlorite for 15 min and rinsed thoroughly with distilled water, then germinated on moist filter paper in the dark at 25 ˚C for 3 days. Germinated seedlings were grown in a hydroponic environment at 25 ˚C in an environment-controlled chamber at a light intensity of 120 µmoL photons m^{-2} s^{-1} and a 14/10 h light/dark photoperiod.

Eighteen-day-old healthy seedlings were used in this experiment. Seedlings; 1 g/L of microbial pigment was dissolved in 2 mL of ethanol and it was completed with 23 mL of distilled water and sprayed on the leaves. For the control, 2 mL of ethanol and distilled water were used. After 24 hours, they were exposed to a UV-B lamp (Philips TL100W/12) with a brightness of 3.3 Wm^{-2} to UV-B irradiation for 3 minutes. After one day the plants were harvested for various analyses.

Determination of Lipid Peroxidation and Hydrogen Peroxide

Lipid peroxidation was determined by Heath and Packer (1968) method, and the amount of H_2O_2 was determined according to Patterson et al. (1984).

Determination of Antioxidant Enzyme Activity

To determine the activities of antioxidant enzymes, fresh leaves (0.5 g) were ground with a mortar and pestle under chilled conditions in the presence of phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 12.000 g for 10 min at 4 ˚C, and the resulting supernatant was used for the enzyme assay. SOD activity was assayed using the method of Agarwal and Pandey (2004) that spectrophotometrically measures inhibition of the photochemical reduction of nitro-blue tetrazolium at 560 nm. POX activity was measured according to the method of Zhang and Kirkham (1994). The enzyme extract (20 mL) was added to the reaction mixture containing 20 mL guaiacol solution and 10 mL H_2O_2 solution in 3 mL of phosphate buffer solution (pH 7.0). The addition of enzyme extract started the reaction, and the increase in absorbance was recorded at 470 nm for 5 min. Enzyme activity was quantified by the amount of tetra-guaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}). CAT activity was performed according to Qiu et al. (2011); the reaction mixture in a total volume of 2 mL contained 0.1 mL enzyme extract, 100 mM phosphate buffer (pH 7), 0.1 µM EDTA, and 0.1 % H_2O.

Determination of Photosynthetic Pigments

Fresh leaf tissues (0.1 g) were homogenized in chilled 80 % (v/v) acetone. The homogenate was centrifuged at 8800 g for 10 min at 4 ˚C in dark. The absorbance of the acetone extract was measured at 663, 645, and 470 nm using a spectrometer (Shimadzu UV mini-1240). The contents of chlorophyll a, chlorophyll b, and total carotenoids were calculated according to Arnon (1949).

Statistical Analysis

All experiments were performed 6 times and the average of values was presented. The data were analyzed by analysis of variance, and means were compared by using Duncan's Multiple Range Test at $p < 0.05$ significance level.

Results and Discussion

In this study; the abs values of the bacterial (*A. citrus*) and fungal (*F. oxysporium*) pigments we used in order to prevent the harmful effects of UV on the lettuce plant were measured at 200-700 nm wavelengths. It was determined that the highest abs value of the bacterial pigment was A_{473} and that of the fungal pigment was A_{457}. When these results were compared with the literature, it was determined that the pigments were carotenoid group pigments (Figure 1).

It was determined by the measured H_2O_2 and LPO amount that UV, which is an abiotic stress factor, caused stress in the lettuce plant. Bacterial and fungal carotenoids we use to reduce UV stress; It was observed that the amount of H_2O_2 decreased by approximately 23 % and 31 %, respectively.

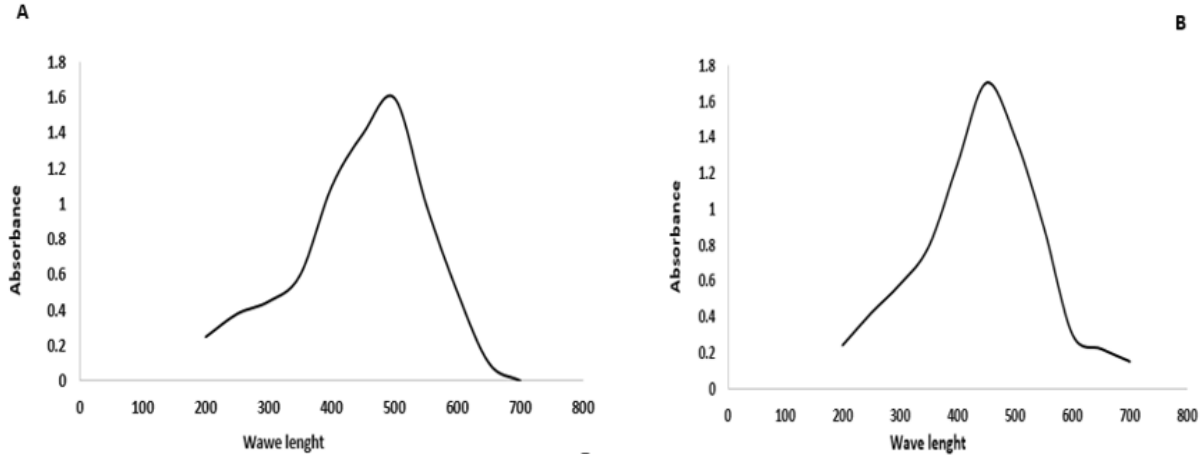

Figure 1. The abs values of bacterial and fungal pigments. A; bacterial pigment, B: fungal pigment

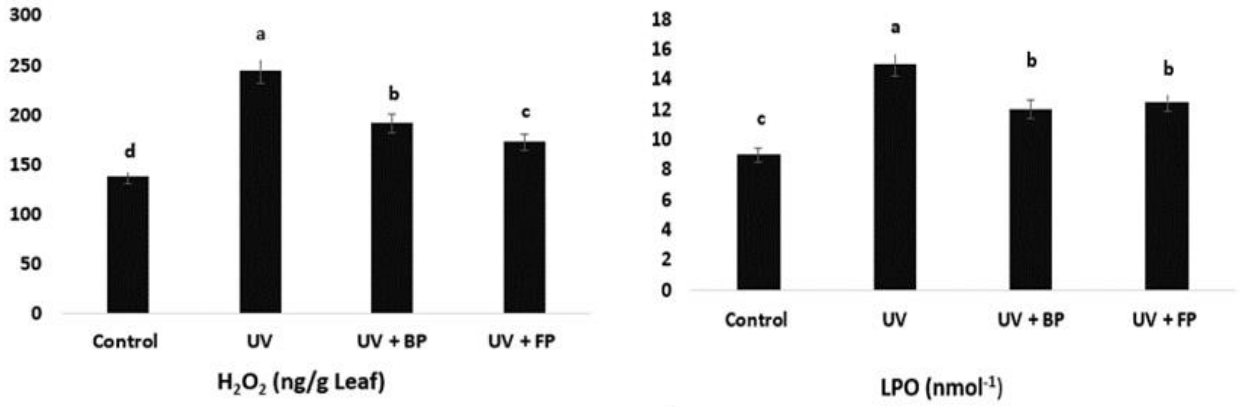

Figure 2. H_2O_2 and LPO amounts of pigments applied in lettuce plant exposed to UV stress Letters mean the significant difference from its control and UV application value at P<0.05 level according to Duncan Test.

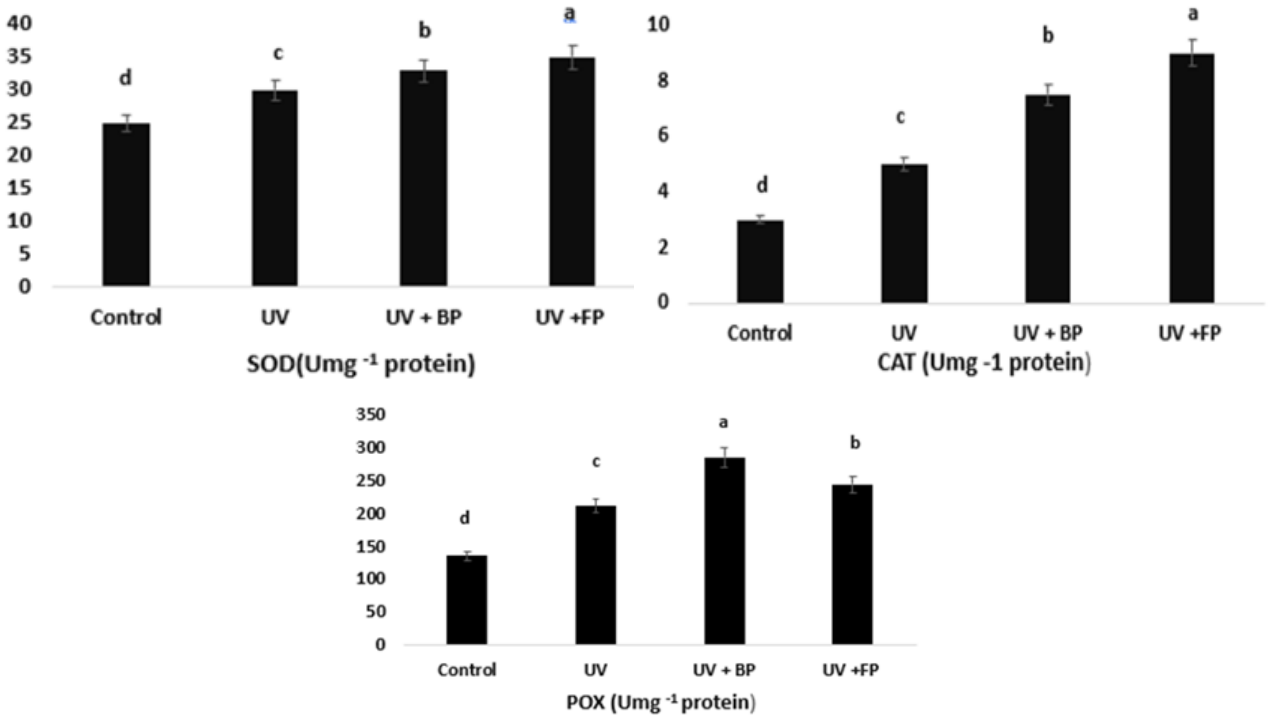

Figure 3. Antioxidant enzyme amounts

Letters mean the significant difference from its control and UV application value at P<0.05 level according to Duncan Test.

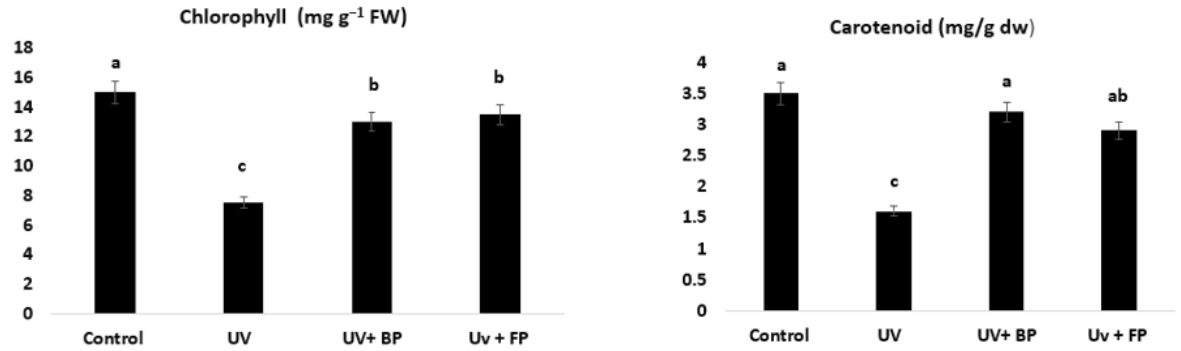

Figure 4. Changes in chlorophyll and carotenoid in lettuce seedlings applied bacterial and fungal carotenoid pigments.

Letters mean the significant difference from its control and UV application value at P<0.05 level according to Duncan Test.

At the same time, the amount of LPO that increased under UV stress decreased significantly with microbial carotenoid applications (Figure 2).

It has been observed that antioxidant enzymes (SOD, CAT and POX), which are used to cope with stress factors and increase the chance of survival of the plant, increase under UV stress compared to control conditions. Accordingly, it was determined that bacterial and fungal carotenoid applications increased the amount of these enzymes even more. As a result, it was determined that microbial carotenoids showed protective properties against UV stress (Figure 3).

In our study, it was determined that the amount of chlorophyll and carotenoid decreased with UV application. It was observed that bacterial and fungal pigment applications increased the amount of chlorophyll and carotenoids (Figure 4).

Environmental stress factors cause yield loss in agriculture worldwide. In particular, abiotic stress factors such as UV, drought, and salinity directly affect crop production (Nakabayashi et al., 2014). UV rays that come to the world more due to changing climatic conditions and the thinning of the ozone layer affect all organisms as well as plants. It causes the production of large amounts of reactive oxygen species (ROS), especially in mitochondria and chloroplasts (Calzada et al., 2019; Haskirli et al., 2021). Increased ROS level in the cellular system in response to various biotic and abiotic stresses leads to lipid peroxidation, inactivation of enzymes and DNA damage (Akula and Ravishankar 2011). Plants have different defense systems to overcome stress conditions. Antioxidant defense system comes first among these defense systems and helps plants survive in adverse conditions (Khare et al., 2020; Uysal et al., 2021; Unal et al., 2022). One of these protective mechanisms is pigments produced against the harmful effects of UV. In particular, carotenoids have photoprotective potential and prevent the production of free radicals formed by excessive light energy. Carotenoids have antioxidant effects, high biological activity and are produced by many microorganisms (Cristina et al. 2019).

It is known that carotenoids are used in food, cosmetics and health industries due to their UV protection feature. The few or no toxicities, easily degradable and non-polluting reasons have made it attractive to use carotenoids in these industries (Mahana et al., 2021). Carotenoids produced by bacteria and fungi are used in many industries for commercial purposes. Produced carotenoids protect microorganisms against the harmful effects of UV with their bioactive properties (Narendarababu and Shislupala 2017; Jimenez et al., 2020). Because; In this study, it was investigated whether bacterial and fungal carotenoids have a protective feature against UV stress on the lettuce plant consumed all over the world.

Bacterial carotenoids act as antioxidants by scavenging free radicals formed by the effect of UV-A and UV-B, and by enabling bacteria to form an adaptation to UV stress, they support the survival of bacteria. Fungal carotenoids also protect various fungi against the destructive effect of UV (Avalos and Limon, 2015). As is known, carotenoids are orange-colored pigments. Bacterial and fungal carotenoids absorption wavelengths between A_{400} and A_{500} best in spectrophotometric measurements (Shatila et al. 2013; Narendrababu, and Shishupala 2017). In our study, we used orange pigments obtained from *A. citrus* as bacterial pigment and *F. oxysporium* as a fungal pigment. The highest abbreviation value of bacterial pigment in spectrophotometric measurements; was determined that the absorbance value of the fungal pigment was A_{473}, and the absorbance value of the fungal pigment was A_{457} (Figure 1). These results are in line with the literature data.

In this study, it was observed that UV, which is an abiotic stress factor, increased the amount of H_2O_2 and decreased the amount of by 23 % and 31 %, respectively, in bacterial and fungal pigment application (Figure 2). The amount of LPO increased in the lettuce plant exposed to UV stress, and a decrease in the amount of LPO was observed in bacterial and fungal pigment applications. According to Tiryaki et al. (2019), cold stress, which is an abiotic stress factor, increased the amount of LPO and H_2O_2, and a significant decrease was observed in bacterial applications. Our study shows parallelism with this study. Considering these results, it can be accepted as an indicator of the protective feature of the applied pigments against UV in the plant. At the same time, this effect was also seen in the amounts of enzymes (SOD, CAT, POX), which are antioxidant defense system elements (Figure 3). In previous studies, it was determined that antioxidant enzymes increased, decreased or remained the same in different plants under UV stress (Ozgur et al., 2021). This study is similar to previous studies, and the applied microbial pigments were effective in coping with the UV stress of the plant.

It is known that UV destroys the chloroplast structure in plants. When the chloroplast structure deteriorates, the amount of photosynthesis decreases, thus negatively

affecting plant growth and development. In addition, it causes short root stems, increase in thicker leaves, deterioration of leaf structure, decrease in biomass and decrease in total protein amount in plants (Robson et al., 2015). In this study, it was determined that the amount of chlorophyll and carotenoid decreased with UV application (Figure 4). In bacterial and fungal pigment applications, it increased the amount of chlorophyll and carotenoids and encouraged plant growth and development.

When considered as a whole, it can be thought that the increase in the amount of enzymes, which are seen as stress-fighting factors, and the increase in the amount of chlorophyll and carotenoid, which are parameters that have a significant effect on plant productivity, have a positive effect on pigment applications. With pigments obtained easily and cheaply, applications related to plant productivity can be made and studies can be expanded by increasing trials. Our work in preparing the preparations that will benefit agricultural production by diversifying microbial products is pioneering and unique.

Conclusion

With climate change, the harmful rays of the sun reach the earth. Ultra violet rays come first among these rays. UV causes many diseases in humans, especially cancer. In plants, UV radiation changes germination, flowering, photosynthesis, leaf and stem thickness. It also causes changes in the chloroplast structure. It is known to accelerate cell fragmentation by increasing the number of peroxisomes in the cell. To eliminate these harmful effects of UV, pigments with high antioxidant and UV protection are used. Natural pigments; It is obtained from plants, animals and microorganisms and is used in various fields. In particular, pigments obtained from microorganisms have been used more recently due to their easy and cheap availability and high stability. Based on this information, microbial pigments from the same pigment group and obtained from two different organism groups were applied to the lettuce plant in order to save the lettuce plant, which has commercial value and is consumed almost all over the world, from the harmful effects of UV rays, and the protective properties of these pigments were determined. As a result of the study, it was seen that these pigments protect plants under UV stress and develop a defense mechanism against this stress. Based on these results, it has been found that inexpensive microbial pigments can be used against UV stress.

Conflict of Interest

The author declares that there is no conflict of interests.

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