

Original article

Investigation of the Effects of Some Herbicides Used in Rice Agriculture on Photosynthetic Pigments and Proteins in Osmancık-97 Cultivar

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Abstract

Rice (*Oryza sativa* L.) is an important cereal species that meets the basic nutritional needs of a large portion of the world's population through its processed product (rice). One of the problems encountered during its production is weeds. Weed control in production areas is widely carried out through herbicide use in our country and worldwide. In this study, it was aimed to determine the effects of herbicide types used in rice agriculture and different dose applications of these herbicides on photosynthetic pigments (total chlorophyll, chlorophyll a, chlorophyll b, carotenoid), total protein, and malondialdehyde (MDA) amounts in the widely cultivated Osmancık-97 rice variety. Quinclorac, cyhalofop butyl, and bentazon+MCPA were used as herbicides, and D dose (recommended dose) and 2D dose (double dose) applications were performed. Additionally, a mixture (mix) application combining equal amounts of these three herbicides was also carried out. Plant samples from both control and treatment groups were collected at seedling, stem elongation, and panicle formation stages, and analyses were performed. The findings of this study indicate that unconscious use of excessive herbicide doses and the use of multiple herbicides in mixture during production negatively affect the development of rice plants due to decreases in photosynthetic pigments. Therefore, to reduce the negative effects of herbicides used in weed control in production areas, polyculture farming instead of the widely preferred monoculture farming in rice production as a practice to prevent weed proliferation, elimination of weeds before seed formation, weed cleaning in irrigation channels, use of certified seeds, and in chemical control, use of herbicides at recommended doses following product instructions should be ensured.

Keywords: *Oryza sativa* L., Herbicide, Photosynthetic pigment, Malondialdehyde

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INTRODUCTION

The fact that agricultural areas worldwide have reached their final limits, the decline in rural population, the rapidly increasing world population, and the malnutrition problems and scarcity occurring especially in less developed and developing countries have made it necessary to increase the possibilities of obtaining high yields per unit area. At this point, the use of quality seeds in agricultural areas, increasing irrigation facilities, timely and proper application of chemical fertilizers, and the use of pesticides in necessary amounts have become important factors in increasing yield and product quality (Bayramoğlu, 2010).

Pesticides are used in agricultural production to control or eliminate diseases, pests, and weeds, thereby increasing agricultural production quantities and product quality. Due to their positive characteristics such as ease of use compared to other agricultural control methods, providing effective results in a short time, and being economical when used consciously and in a controlled manner, they have become more preferred by producers today. As in the world, pesticide use in our country is also increasing every day (Katip, 2019).

With the use of pesticides in agricultural production, both the global food production quantity has increased and higher quality products have been obtained. As a result of conscious and controlled use, mostly positive results have been achieved, and effective control of harmful organisms has been enabled (Tiryaki et al., 2010). It has been stated that if pesticide use in production is abandoned, quality and yield of the product would decrease by up to 60%, and production value would significantly decrease (Zhang, W. 2018). Currently, pesticides are used to protect products from harmful organisms and also during storage and transportation. In addition to all these benefits, the toxic effects of pesticides and their residues on the environment and living organisms continue to be a source of concern. Studies have shown that a significant portion of pesticides applied in agricultural areas cannot reach the target organisms and cause contamination in air, water, and soil (Tudi et al. 2021).

Rice (*Oryza sativa* L.), classified under the tribe Oryzeae belonging to the subfamily Pooideae of the family Poaceae, is an important agricultural plant that meets the basic nutritional needs of more than approximately half of the world's population through its product (rice) obtained after processing in the manufacturing process (Sezer et al., 2012). Although it contains a small amount of protein, it is the second most used cereal type in human nutrition after wheat due to its richness in amino acids necessary for nutrition (Yılmaz & Avkiran 2019). Rice production occupies an important place in our country's agriculture as well.

The main objective of agriculture is to obtain as much high-quality product as possible per unit area without disrupting the ecological balance. One of the most important factors limiting this objective is weeds. Weeds, considered as plants we do not want to grow in agricultural production areas, develop in the same period as the cultivated plant and compete for light, nutrients, water, and space. Studies on

this subject have shown that, if necessary, precautions are not taken, weeds can cause yield losses of 15-50% in most cultivated plants (Sürek et al., 2016).

In rice cultivation, weed control carried out at the right time and with the right methods is a matter that needs to be emphasized for achieving better yields. One of the fundamental principles for effective control is the identification of weed species present in that production area. Then, depending on the intensity of weeds, control methods should be determined for those causing yield decreases and those hosting diseases and pests, leading to secondary damages (Özaslan, C. 2015).

Cultural control of weeds is carried out through the use of certified seeds, cleaning of tools and equipment used to prevent contamination of weed seeds, controlling the weeds present within the field and its surroundings before seed formation, timely field preparation, crop rotation, and prevention of weed seed contamination through irrigation water. In mechanical control methods, weeds are removed by hoeing or hand pulling during the growing period of the cultivated plant.

Chemical control has become the most preferred method by rice producers due to its positive characteristics such as ease of use, providing effective results in a short time, and being economical when used consciously and in a controlled manner. The use of herbicides selected according to the type and intensity of weeds is very important for obtaining effective results (Demirkan, 2013; Yazlık et al., 2020). For example, selective herbicides such as quinclorac and cyhalofop butyl, which are widely used against grass species frequently encountered in rice cultivation areas and have the same grass structure as cultivated rice species, are frequently preferred.

Herbicides are classified differently according to their application time, efficacy, selectivity, mode of action, and chemical family (Herrera-Herrera et al., 2016). In the classification based on modes of action, there are many herbicides including photosynthesis inhibitors, auxin growth regulators, lipid synthesis inhibitors, protein synthesis inhibitors, specific enzyme inhibitors, carotenoid biosynthesis inhibitors, and those effective on cell membranes. These herbicides are applied at different growth stages of the plant in agricultural areas according to their properties.

Before using herbicides in mixture form, checking the modes of action and miscibility of herbicides is important for obtaining desired results. When herbicides are mixed, they show three different types of effects: synergistic, antagonistic, and neutral (Blouin et al., 2010; Rustom et al., 2018). Neutral effect is when each herbicide in the mixture shows the same performance when applied in combination as when applied separately (Fish et al., 2016; Rustom et al., 2018). Synergistic effect indicates that the mixture provides a greater effect than the expected effect from the herbicides' combined activities when applied separately. Antagonistic effect is the opposite of synergistic effect and indicates that the mixture provides less effect than the sum of individual herbicide applications (Akobundu et al., 1975; Pellerin et al., 2003).

Plants are in constant interaction with the environment in which they live. Throughout their life cycles, as a result of changes in environmental conditions, they encounter many stress factors, both biotic and abiotic (Table 1). Plants, which have no option to escape from these factors due to their sessile nature, are directly exposed to this factor. This direct effect negatively affects plant growth and development, and if the stress factor continues for a long time, it may result in the death of the plant (Büyük et al. 2012).

Table 1. Biotic and abiotic stress factors in plants (Özen & Onay, 2007)

Abiotic Factors		Biotic Factors
Physical Factors	Chemical Factors	
• Drought (Water Deficiency)	• Air Pollution	• Weeds
• Temperature	• Plant Nutrients	• Animal Pests (Insects, Red Spiders, Nematodes)
• Radiation	• Pesticides	• Diseases (Fungal, Bacterial, and Viral)
• Flooding	• Toxins	• Microorganisms
• Mechanical Effects (Wind, snow, and ice cover)	• Salts	
	• Soil Solution pH	

Herbicides, which are one of the abiotic stress factors, are an important stress factor especially for plants cultivated in agricultural areas. Morphological, physiological, and molecular responses of plants to herbicides containing different active ingredients have been studied by various researchers. Each herbicide has a target site called its mode of action. These targets can be enzymes, proteins, or sites in the metabolic pathways of plants (Caverzan et al. 2019). According to their modes of action, herbicides are classified into groups, and the responses of plants to herbicides may vary depending on the species and variety of the plant, the applied dose of the herbicide, the application time, and environmental factors may vary.

MATERIALS and METHODS

In this study, *Oryza sativa* L. cv. Osmancık-97, whose seeds were obtained from the Republic of Türkiye Ministry of Agriculture and Forestry, Trakya Agricultural Research Institute Directorate, was used as plant material. Osmancık-97 is a variety registered by the Trakya Agricultural Research Institute Directorate and is the most cultivated and preferred variety in most of the rice production areas in Türkiye due to its high yield potential and quality characteristics.

Surface sterilization of Osmancık-97 rice variety seeds was performed by keeping them in 5% sodium hypochlorite solution for 20 minutes. The seeds, which were then washed three times with distilled water, were kept in water for two days for pre-germination and then sown in pots filled with soil in the greenhouse.

The seeds left to germinate in pots in the greenhouse were watered regularly every day. Hoagland solution was used for irrigation to support the growth and development of germinated rice seedlings. This solution provides the seedlings with necessary nutrients.

Herbicide applications were performed when rice plants reached approximately 10 cm in height. Herbicides containing quinclorac, cyhalofop butyl, and bentazon+MCPA active ingredients and the mix (mixture) obtained by mixing these three herbicides at certain proportions were applied to a total of eight different groups as D dose (recommended dose) and 2D dose (double dose) (Table 2).

Table 2. Applied herbicide amounts

Herbicide Types	Application Amount
Control	(0)
Quinclorac 1. Dose	3 ml
Quinclorac 2. Dose	6 ml
Cyhalofop Butyl 1. Dose	3 ml
Cyhalofop Butyl 2. Dose	6 ml
Bentazon+MCPA 1. Dose	4 ml
Bentazon+MCPA 2. Dose	8 ml
Mix 1. Dose (Quinclorac+Cyhalofop Butly+Bentazon+MPCA)	3 ml+3 ml+4 ml
Mix 2. Dose (Quinclorac+Cyhalofop Butly + Bentazon+MPCA)	6 ml+6 ml+8 ml

After herbicide application, considering the developmental stages of rice plants, plant samples from both control and treatment groups were collected at seedling, stem elongation, and panicle formation stages. Commercial kits and spectrophotometric methods were used in the analyses.

Photosynthetic pigment analysis was performed according to the Arnon (1949) method (Arnon, 1949). Fresh leaf samples taken at seedling, stem elongation, and panicle formation stages were cut into small pieces before starting the analysis. From each control and treatment group, 200 mg of leaf samples were weighed and placed in porcelain mortars. Liquid nitrogen was added to the porcelain mortars to pulverise the samples. Approximately 4 ml of 80% acetone was added to the homogenised samples and kept in the dark for 2 hours. The supernatants of centrifuged samples were taken and measured spectrophotometrically at 470, 645, 652, and 663 nm wavelengths. The results were calculated by applying the obtained absorbance values to the formulas given below.

$$\text{Total chlorophyll} = A_{652} \times 27.8 \times 20 / \text{mg sample weight}$$

$$\text{Chlorophyll a (Chl a)} = (11.75 \times A_{663} - 2.35 \times A_{645}) \times 20 / \text{mg sample weight}$$

$$\text{Chlorophyll b (Chl b)} = (18.61 \times A_{645} - 3.96 \times A_{663}) \times 20 / \text{mg sample weight}$$

$$\text{Carotenoid} = ((1000 \times A_{470} - 2.27 \times \text{Chl a} - 81.4 \times \text{Chl b}) / 227) \times 20 / \text{mg sample weight}$$



Figure 1. General appearance of rice plants at the seedling stage



Figure 2. General appearance of rice plants at the panicle formation stage

RESULTS

The detailed numerical results are summarized in the table Table 3 below.

Photosynthetic pigments (mg/g), total protein (mg/mL), and MDA (nmol/g) in Osmancık-97 rice at seedling, stem elongation, and panicle formation stages under herbicide treatments (mean $\pm$ SE).

Table 3. Analysis results

Stage	Parameter	Control	Quinclorac (D)	Quinclorac (2D)	Cyhalofop-butyl (D)	Cyhalofop-butyl (2D)	Bentazon + MCPA (D)	Bentazon + MCPA (2D)	Mixture (D)	Mixture (2D)
Seedling stage	Total chlorophyll	3,97±0,01	3.22±0.04*,#	1.85±0.01*	2.15±0.02*,#	3.92±0.04	2.43±0.01*,#	2.17±0.02*	2.12±0.01*,#	3,13±0,02*
	Chlorophyll a	2,54±0,06	2.22±0.04*,#	1.42±0.00*	1.62±0.00*,#	2.49±0.11	1.81±0.00*,#	1.64±0.01*	1.59±0.00*,#	2.16±0.08*
	Chlorophyll b	0,73±0,03	0.52±0.03*,#	0.19±0.01*	0.26±0.01*,#	0.74±0.03	0.31±0.01*	0.26±0.01*	0.26±0.01*,#	0.52±0.02*
	Carotenoids	0,82±0,01	0.72±0.01*,#	0.46±0.00*	0.51±0.00*,#	0.82±0.02	0.58±0.00*,#	0.53±0.00*	0.52±0.00*,#	0.72±0.02*
	Total protein	9,28±0,27	10.10±0.22*,#	10.88±0.12*	11.02±0.16*,#	9,5±0.04	10.61±0.13*	10.44±0.09*	12.58±0.25*,#	9,62±0.07
	MDA	0,31±0,02	0.38±0.05	0.31±0.02	0.24±0.01*,#	0.32±0.01	0.23±0.02*,#	0.32±0.03	0.25±0.02	0.29±0.02
Stem elongation stage	Total chlorophyll	7,17±0,49	6.33±0.01	4.92±0.04*,#	3.94±0.16*	5.75±0.24*,#	4.45±0.42*	4.83±0.08*	7.22±0.13	5.97±0.20*,#
	Chlorophyll a	2,63±0,19	3.16±0.00*	3.00±0.04*,#	2.58±0.13	2.79±0.15	2.77±0.20	2.87±0.14	3.07±0.01	2.92±0.16
	Chlorophyll b	2,76±0,06	1.72±0.01*	1.11±0.01*,#	0.82±0.03*	1.62±0.20*,#	0.98±0.12*	1.13±0.08*	2.26±0.06*	1.68±0.06*,#
	Carotenoids	1,00±0,04	1.08±0.00	1.02±0.01	0.94±0.03	1.01±0.02	0.98±0.04	1.01±0.02	1.07±0.00	1.03±0.03
	Total protein	14,49±0,32	16.05±0.20*	12.67±0.21*,#	18.68±0.12*	12.66±0.08*,#	12.46±0.08*	16.15±0.24*,#	15.62±0.34*,#	13.59±0.23
	MDA	0,51±0,01	0.45±0.04	0.32±0.01	0.34±0.03*	0.26±0.02*	0.29±0.01*	0.44±0.02*,#	0.42±0.01*	0.44±0.01*
Panicle formation stage	Total chlorophyll	7,26±0,49	4.74±0.13*	5.35±0.05*	3.35±0.02*	3.97±0.03*	6.11±0.04*	3.77±0.03*,#	5.48±0.01*	4.11±0.05*,#
	Chlorophyll a	2,62±0,20	2.95±0.14	2.83±0.20	2.27±0.06	2.62±0.09	3.01±0.17	2.47±0.07	3.11±0.05*,#	2.64±0.07
	Chlorophyll b	2,89±0,03	1.04±0.04*	1.39±0.07*,#	0.66±0.03*	0.80±0.03*,#	1.67±0.10*	0.78±0.02*,#	1.32±0.02*,#	0.88±0.04*
	Carotenoids	0,99±0,05	1.02±0.03	1.02±0.03	0.86±0.01*,#	0.97±0.02	1.06±0.03*	0.90±0.02*,#	1.06±0.01*	0.96±0.02*,#
	Total protein	14,23±0,04	13.27±0.10*	15.21±0.09*,#	10.51±0.16*,#	14.44±0.05	17.89±0.05*	12.53±0.03*,#	15.58±0.04*,#	12.58±0.13*
	MDA	0,35±0,01	0.41±0.02	0.85±0.04*,#	0.38±0.01	0.43±0.02*,#	0.44±0.02*	0.58±0.02*,#	0.40±0.02*,#	0.48±0.01*

Note: Values are presented as mean ± standard error. Statistical symbols (*, #) are retained as provided in the original table output (Duncan's test, $P \leq 0.05$).

As a result of the statistical analysis performed with total chlorophyll amounts at the seedling stage, a significant decrease was observed compared to the control in all herbicide applications except the 2D dose cyhalofop butyl application ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, bentazon+MCPA showed the greatest reducing effect in the D dose application groups, while the mix application had the greatest reducing effect in the 2D dose application groups (Figure 3).

When examining the seedling stage chlorophyll a analysis results, as a result of statistical analyses performed with chlorophyll a amounts, a significant decrease was observed compared to the control in

all herbicide applications except the 2D dose cyhalofop butyl application ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, quinclorac showed the greatest reducing effect in the D dose application groups, while the mix application showed the greatest reducing effect in the 2D dose application groups.

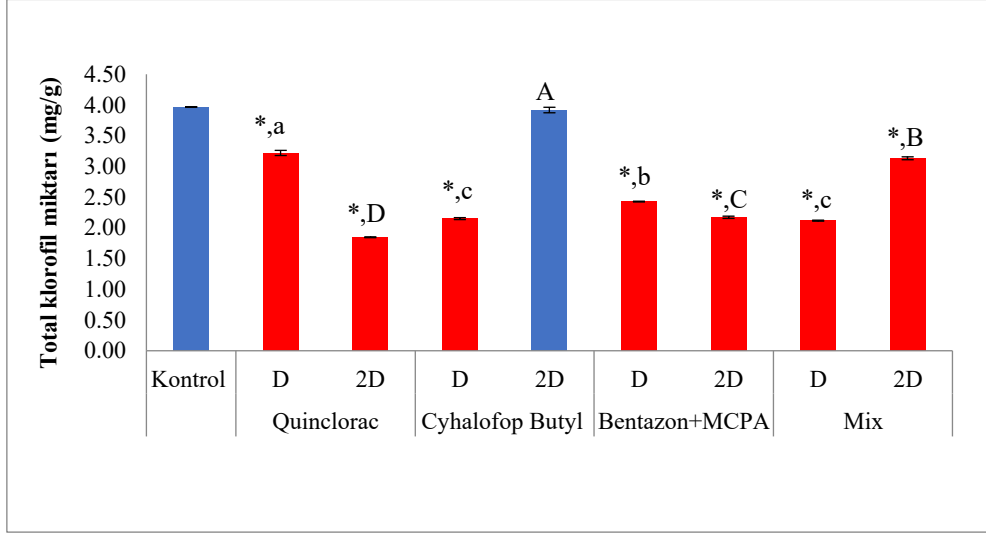


Figure 3. Osmancık-97 seedling stage total chlorophyll analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

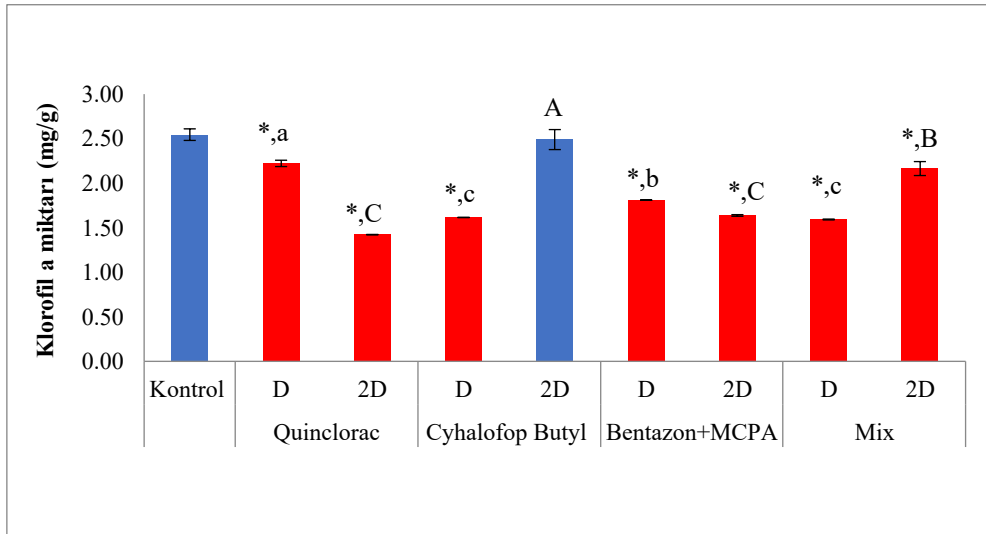


Figure 4. Osmancık-97 seedling stage chlorophyll a analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

As a result of the statistical analyses performed with chlorophyll b amounts at the seedling stage, a significant decrease was observed compared to the control in all herbicide applications except the 2D dose cyhalofop butyl application ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, bentazon+MCPA showed the greatest reducing

effect in the D dose application groups, while the mix application had the greatest reducing effect in the 2D dose application groups.

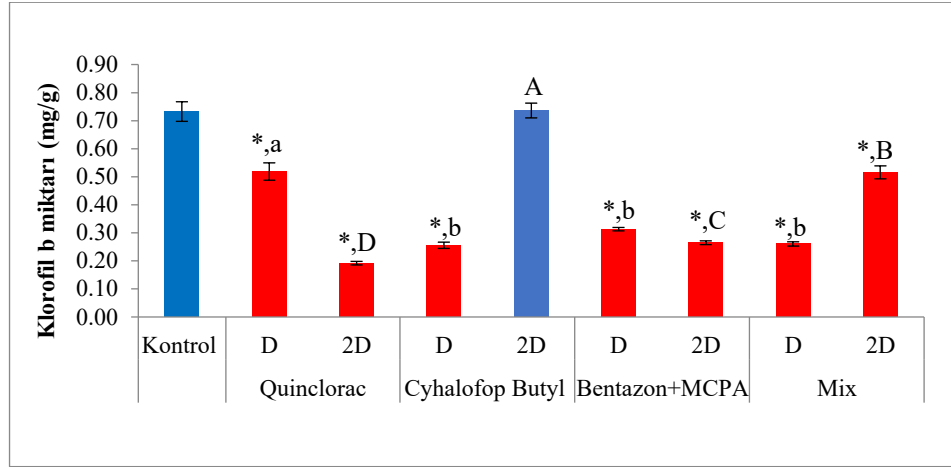


Figure 5. Osmancık-97 seedling stage chlorophyll b analysis results.

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the seedling stage carotenoid analysis results, as a result of the statistical analysis performed with carotenoid amounts, a significant decrease was observed compared to the control in all herbicide applications except the 2D dose cyhalofop butyl application ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, bentazon+MCPA and mix showed the greatest reducing effect in D dose applications, while the mix application showed the greatest reducing effect in 2D dose applications.

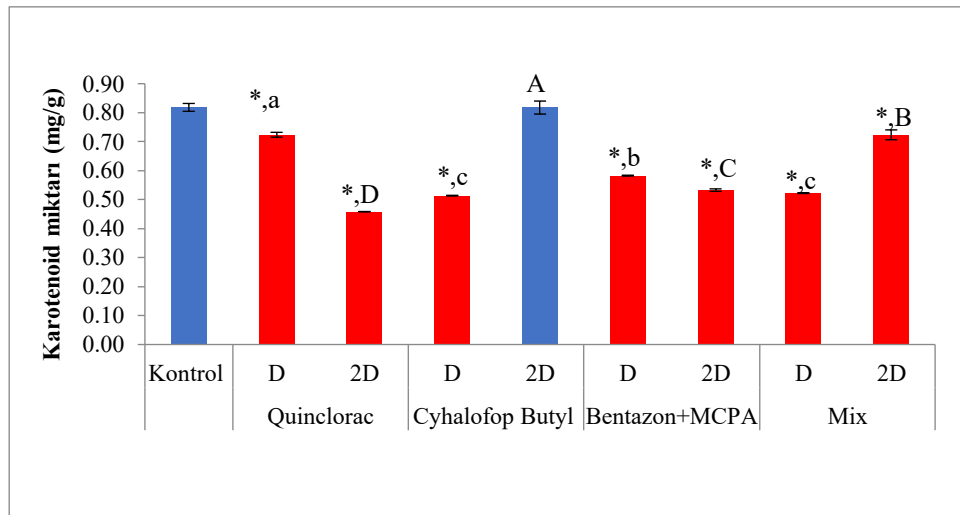


Figure 6. Osmancık-97 seedling stage carotenoid analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

When examining the stem elongation stage total chlorophyll results of the Osmancık-97 rice variety, a significant decrease was observed in all herbicide applications compared to the control group except for D dose quinclorac, D dose mix, and 2D dose mix applications ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, bentazon+MCPA showed the greatest reducing effect in D dose applications, while cyhalofop butyl showed the greatest reducing effect in 2D dose applications.

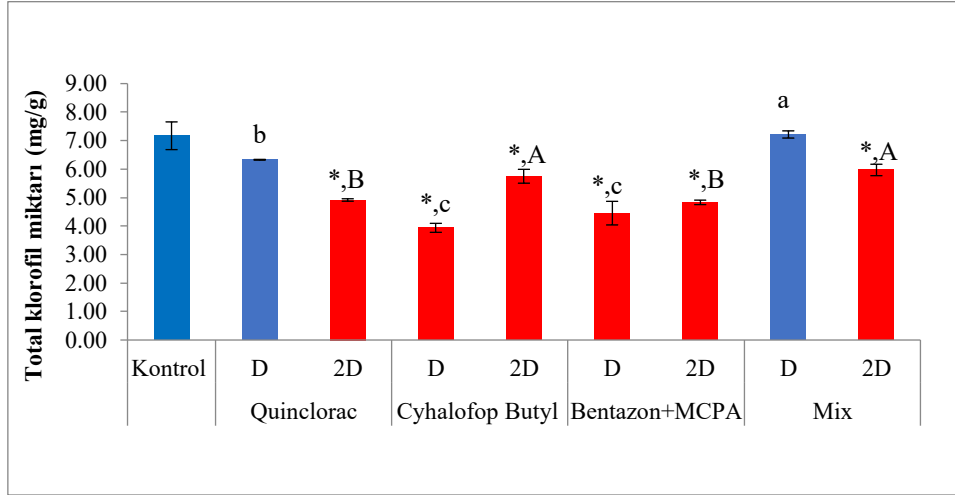


Figure 7. Osmancık-97 stem elongation stage total chlorophyll analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the stem elongation stage chlorophyll a results, as a result of statistical analyses, a significant increase was observed in D and 2D dose quinclorac applications compared to the control ($P \leq 0.05$). In other herbicide applications, a decrease was observed compared to the control.

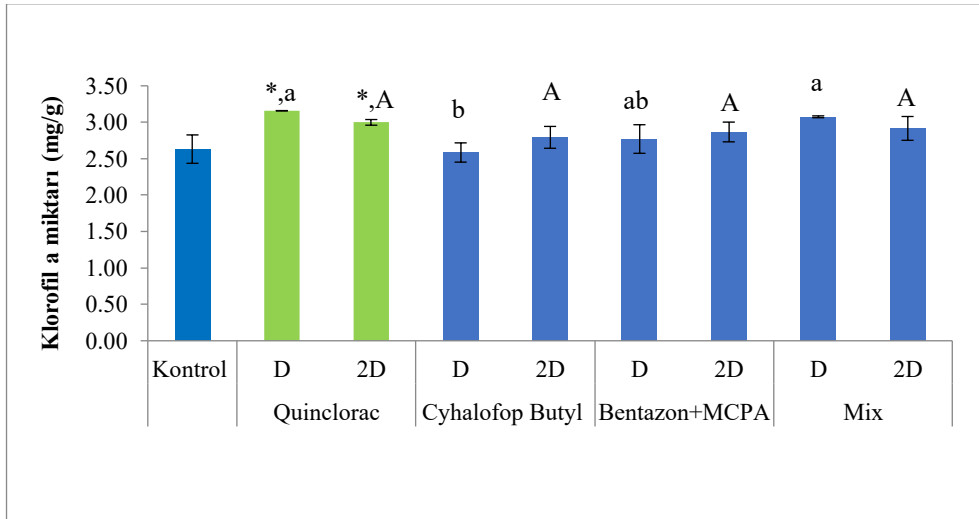


Figure 8. Osmancık-97 stem elongation stage chlorophyll a analysis results

*Means shown in green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the stem elongation stage chlorophyll b analysis results, as a result of statistical analysis, a statistically significant decrease was observed in all herbicide-treated groups compared to the control ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, bentazon+MCPA showed the greatest reducing effect in D dose applications, while cyhalofop butyl showed the greatest reducing effect in 2D dose applications.

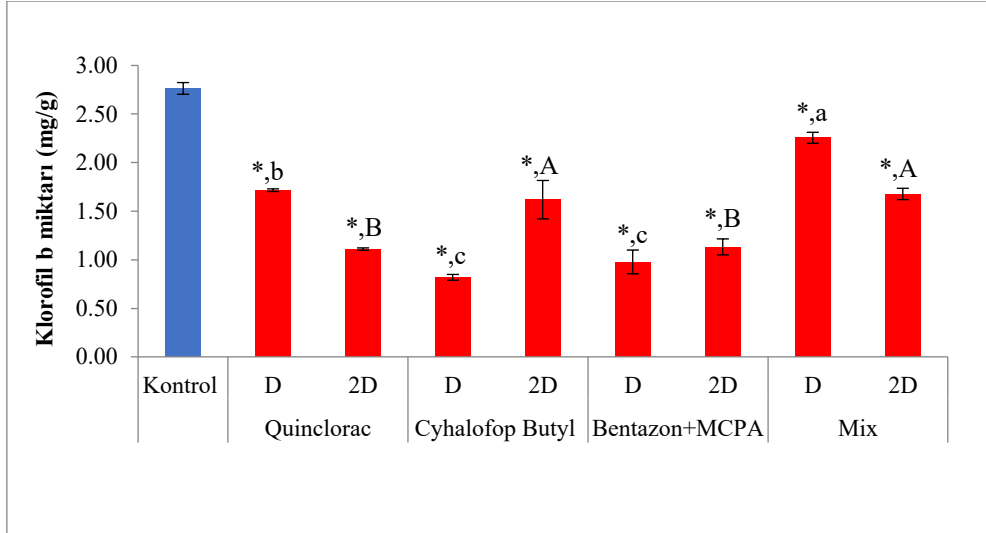


Figure 9. Osmancık-97 stem elongation stage chlorophyll b analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

When examining the stem elongation stage carotenoid analysis results, as a result of statistical analysis, no significant change was observed in groups treated with D and 2D dose herbicides compared to the control ($P \leq 0.05$).

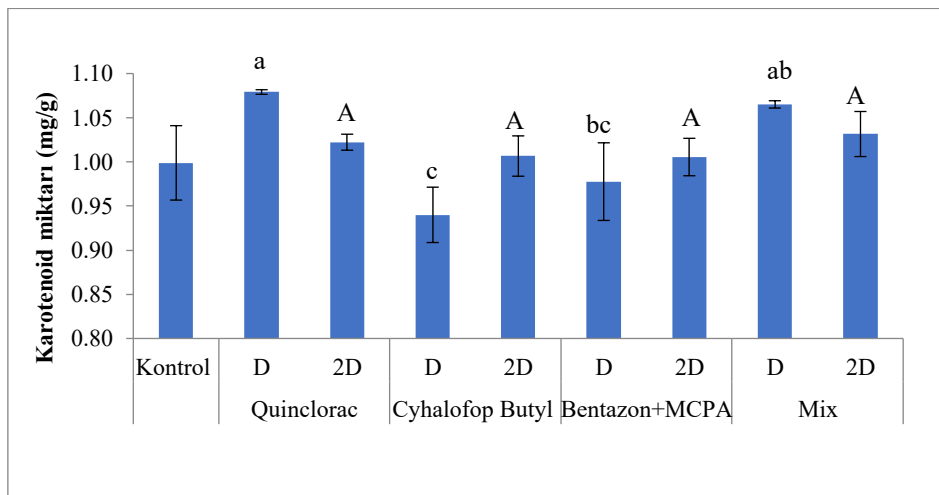


Figure 10. Osmancık-97 stem elongation stage carotenoid analysis results

Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

As a result of the statistical analysis of the panicle formation stage total chlorophyll analysis results of the Osmancık-97 rice variety, a significant decrease was observed in all herbicide applications compared to the control ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, mix showed the greatest reducing effect in D dose applications, while cyhalofop butyl showed the greatest reducing effect in 2D dose applications.

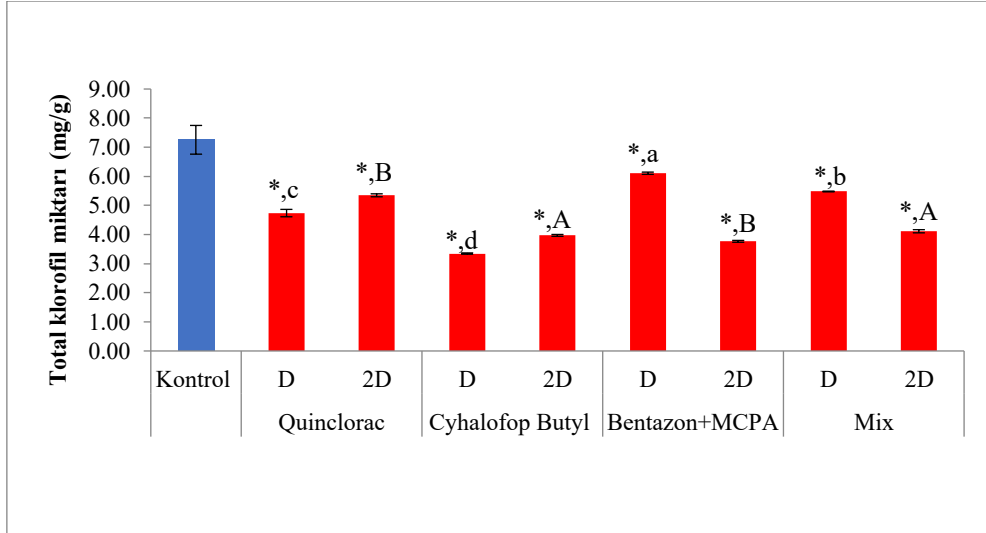


Figure 11. Osmancık-97 panicle formation stage total chlorophyll analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the statistical analysis results of the panicle formation stage control group chlorophyll a amount, a significant increase was observed in D dose mix application compared to the control ($P \leq 0.05$), while a decrease was observed in other herbicide applications.

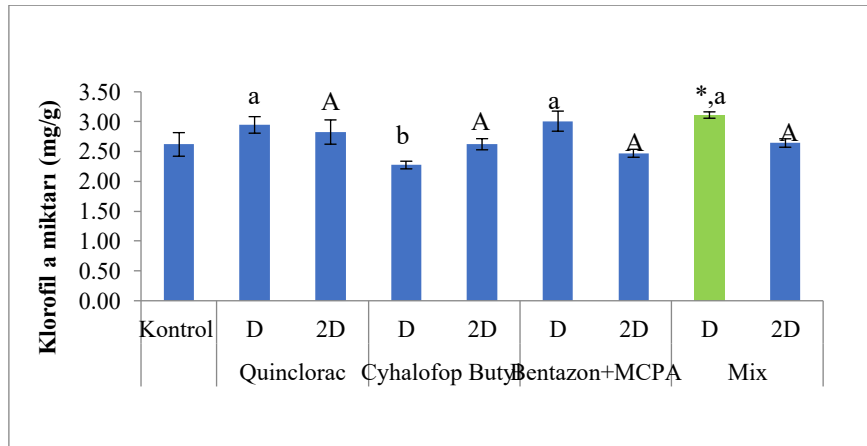


Figure 12. Osmancık-97 panicle formation stage chlorophyll a analysis results

*Mean shown in green colour is statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

As a result of the statistical analysis of the panicle formation stage control group chlorophyll b amount, a significant decrease was observed in all herbicide applications compared to the control ($P \leq 0.05$). As a result of the comparison between herbicide applications containing different active ingredients, mix showed the greatest reducing effect in D dose applications, while cyhalofop butyl showed the greatest reducing effect in 2D dose applications.

According to the statistical analysis performed with the control group carotenoid amount at the panicle formation stage, no significant change was observed in groups treated with D and 2D dose quinclorac compared to the control. In D dose cyhalofop butyl application, an increase was observed, while in 2D dose application, a decrease was observed. In bentazon+MCPA and mix applications, a decrease was generally observed compared to the control ($P \leq 0.05$).

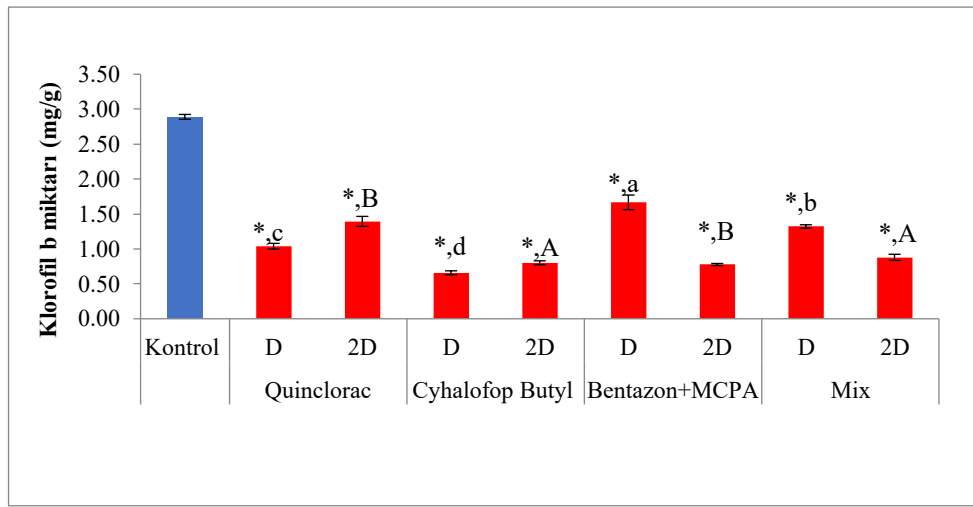


Figure 13. Osmancık-97 panicle formation stage chlorophyll b analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

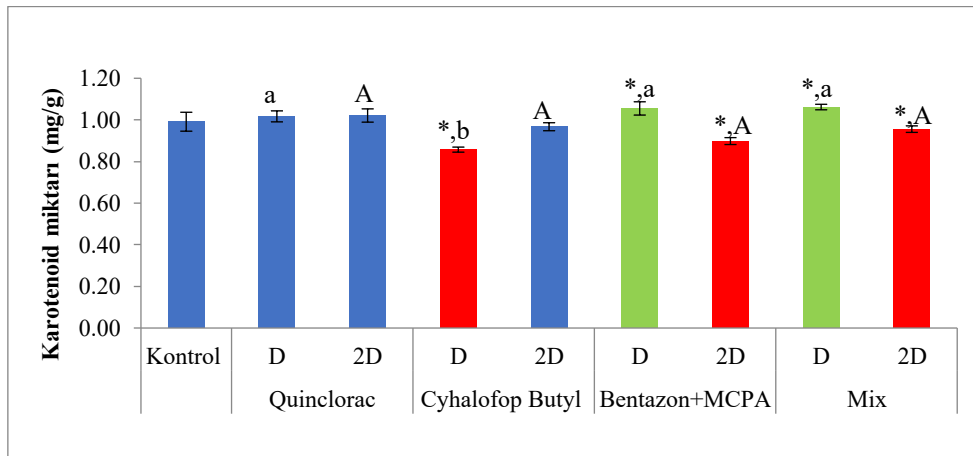


Figure 14. Osmancık-97 panicle formation stage carotenoid analysis results

*Means shown in red and green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

The MDA analysis results of the Osmancık-97 rice variety for seedling, stem elongation, and panicle formation stages are provided in Appendix 2. According to the statistical analysis based on the MDA analysis results at the seedling stage, no significant change was observed in groups treated with quinclorac and mix compared to the control. In D dose cyhalofop butyl and bentazon+MCPA applications, a significant increase was observed compared to the control ($P \leq 0.05$).

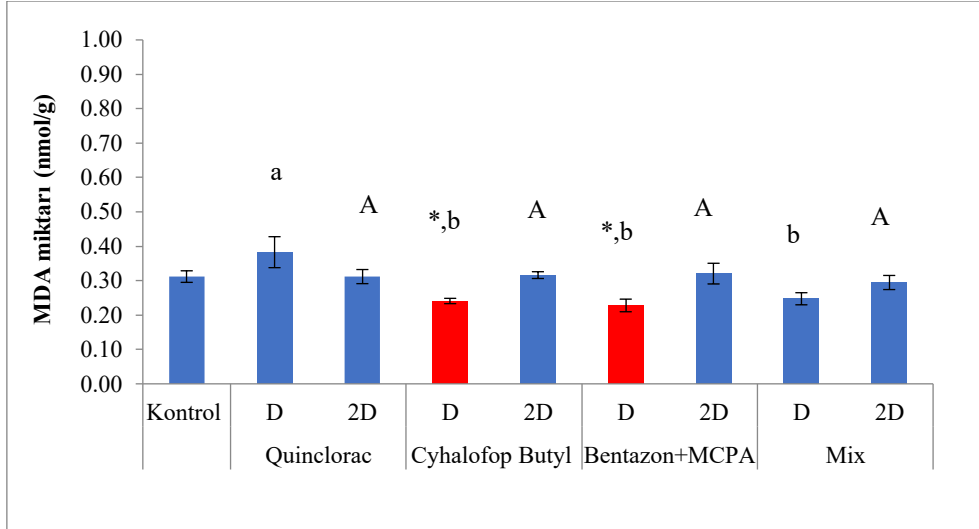


Figure 15. Osmancık-97 variety seedling stage MDA analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

When examining the stem elongation stage MDA analysis results, the MDA amount of the control group was determined as 0.51 ± 0.01 nmol/g. According to the statistical analysis, in D and 2D dose cyhalofop butyl, bentazon+MCPA, and mix applications, a significant increase was observed compared to the control ($P \leq 0.05$).

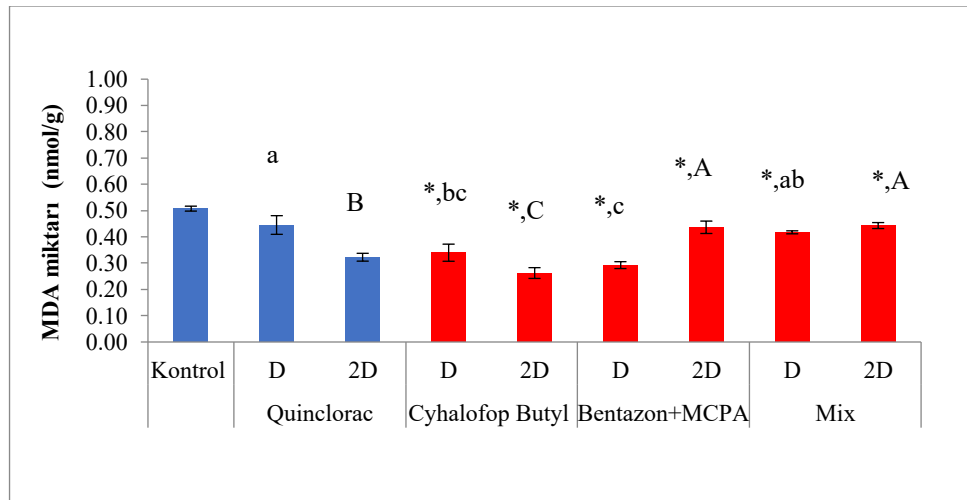


Figure 16. Osmancık-97 variety stem elongation stage MDA analysis results

*Means shown in red colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

Panicle Formation Stage MDA Analysis Results

At the panicle formation stage, the control group MDA amount was determined as 0.35 ± 0.01 nmol/g. According to the statistical analysis, in groups treated with D and 2D dose bentazon+MCPA and mix applications, significant changes were observed compared to the control ($P \leq 0.05$).

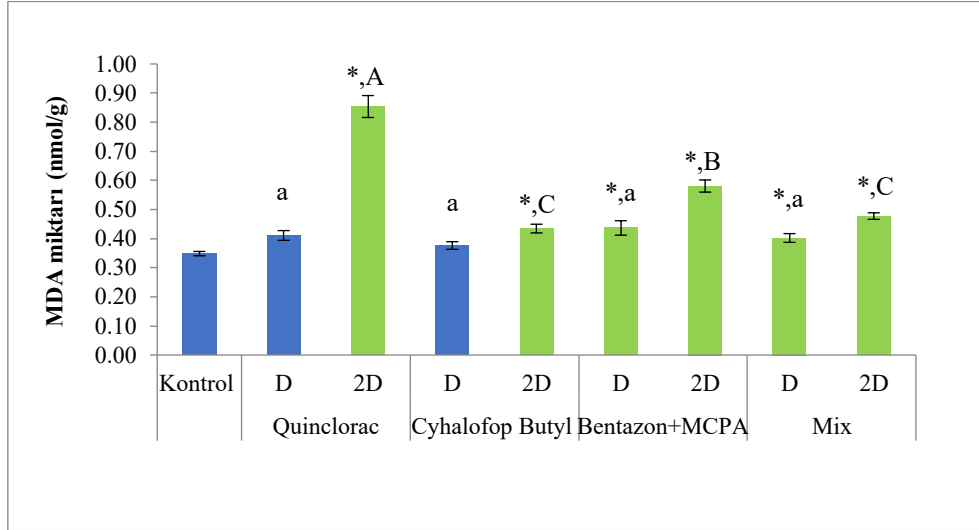


Figure 17. Osmancık-97 variety panicle formation stage MDA analysis results

*Means shown in green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

Total Protein Analysis Results

The total protein analysis results of the Osmancık-97 rice variety for seedling, stem elongation, and panicle formation stages are given in Appendix 2.

When examining the results at the seedling stage, the total protein amount of the control group was determined as 9.28 ± 0.27 mg/ml. As a result of the statistical analysis, in D and 2D dose quinclorac, cyhalofop butyl, and bentazon+MCPA applications, a significant increase was observed compared to the control ($P \leq 0.05$).

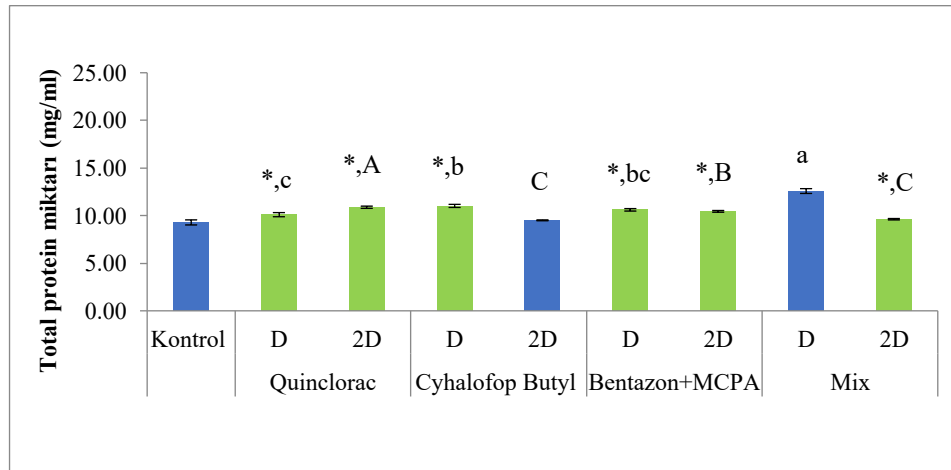


Figure 18. Osmancık-97 variety seedling stage total protein analysis results

*Means shown in green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the stem elongation stage analysis results, as a result of the statistical analysis of total protein amount, a significant increase was detected in the D dose quinclorac application group compared to the control. In the 2D dose application, a decrease was observed. In cyhalofop butyl, bentazon+MCPA, and mix applications, decreases were generally observed ($P \leq 0.05$).

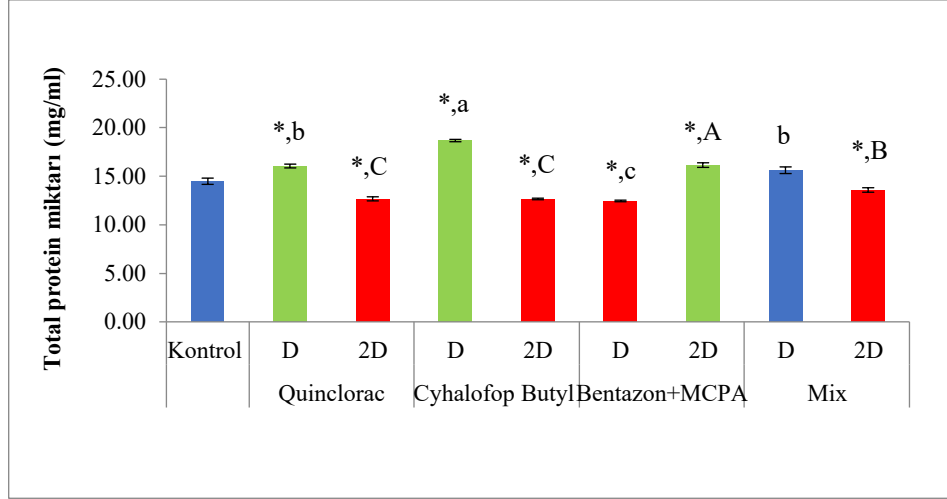


Figure 19. Osmancık-97 variety stem elongation stage total protein analysis results

*Means shown in red and green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

According to the statistical analysis performed with the results at the panicle formation stage, a significant decrease was detected in the D dose quinclorac application group compared to the control, while an increase was detected in the 2D application group ($P \leq 0.05$). Variable results were obtained in other herbicide applications.

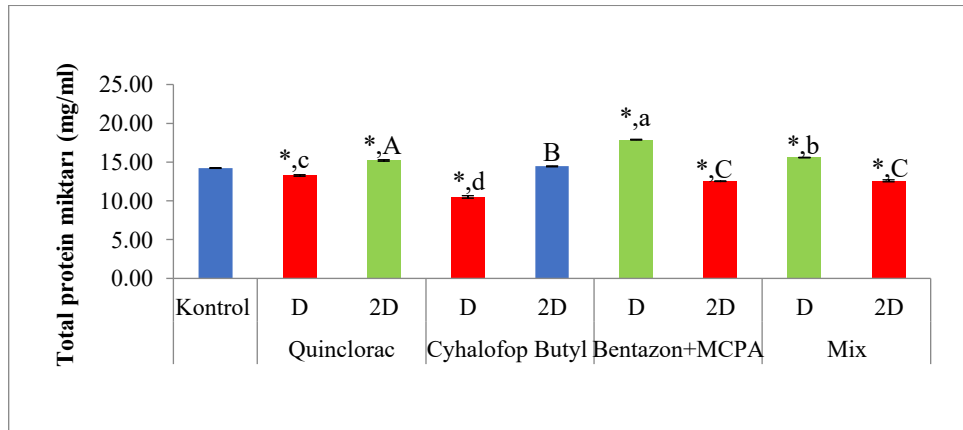


Figure 20. Osmancık-97 variety panicle formation stage total protein analysis results

*Means shown in red and green colour are statistically different compared to the control. Different lowercase letters indicate differences among D dose herbicide application groups, while different uppercase letters indicate differences among 2D dose application groups (Duncan's test, $P \leq 0.05$).

DISCUSSION

As with many cultivated crops, weeds are among the leading factors causing yield and quality losses in rice production. To control weeds and consequently achieve high yields and economic returns, an effective control method must be chosen. Today, herbicides, which are part of chemical control methods, have become frequently preferred in weed control due to their ease of application, rapid and effective results, and economic advantages. Many herbicides have been developed for use in rice production areas and exhibit selectivity among weed species. These herbicides are widely used both alone and in combination with multiple herbicides to expand weed control (Marchezan et al., 2017; Peng et al., 2021; Wang et al., 2022; Yazlık et al., 2020).

In obtaining effective results from herbicide use, the use of appropriate tools and equipment during herbicide applications, calibration of the tools to be used before application, following the instructions of herbicides, and not using herbicides in excessive doses are of great importance (Torun, H., & Uygur, S. 2012).

The change in photosynthetic pigment amounts in leaves is an important indicator of plant resistance and environmental quality (Carter & Spiering, 2002). Stress factors affect photosynthetic pigment amounts. In our study, total chlorophyll amounts of the Osmancık-97 variety generally showed a decrease in all herbicide-treated groups at the seedling stage. At the same stage, a significant increase compared to the control was observed only in the 2D dose cyhalofop butyl application group. At the stem elongation stage, a significant decrease was observed in all herbicide-treated groups except the D dose quinclorac and mix, and 2D dose mix application groups. At the panicle formation stage, a significant decrease compared to the control was determined in all herbicide-treated groups.

According to the seedling stage chlorophyll a result, a decrease was observed in all herbicide-treated groups in the Osmancık-97 variety. At the stem elongation stage, chlorophyll a amount of the Osmancık-97 variety showed an increase with D and 2D dose quinclorac applications. At the panicle formation stage, an increase was observed in the D dose mix application group.

Seedling stage chlorophyll b amounts of the Osmancık-97 variety generally showed a decrease in all herbicide applications. According to the stem elongation stage results, a decrease was observed in all herbicide-treated groups in the Osmancık-97 variety. Similar results were also obtained at the panicle formation stage. In one of the studies examining the effects of herbicides on photosynthetic pigment amounts, Langaro et al. (2016) investigated the effects of imazapic+imazapyr, quinclorac, bentazon, cyhalofop-butyl, penoxsulam, bispyribac-sodium herbicides on rice plants and reported variable responses.

Carotenoids are among the non-enzymatic components of the antioxidant system and protect chlorophylls by preventing both singlet oxygen and triplet chlorophyll formation in plants under stress conditions (Doğanlar, 2012).

MDA is one of the end products of lipid peroxidation and is considered a general indicator of cellular damage in plants under stress. In our study, the MDA results obtained at the seedling stage showed variable responses depending on herbicide types. (Wang et al. 2016) reported that applying increasing concentrations of quinclorac to different rice varieties resulted in a significant increase in MDA levels in the roots and leaves of both rice varieties under quinclorac stress.

One of the mechanisms affected by herbicides in plants is protein synthesis. It is known that soluble protein content is an important indicator of the physiological status of plants (Piotrowska et al., 2010).

Conclusion

The findings of this study determined the effects of herbicides containing quinclorac, cyhalofop butyl, and bentazone+MCPA, as well as their mixed application, on the Osmancık 97 rice variety. Changes in photosynthetic pigment, total protein, and MDA levels were identified as usable parameters for monitoring herbicide-induced physiological responses in rice. The findings from these parameters indicate that the indiscriminate overuse of herbicides during production and the mixed application of multiple herbicides can negatively impact rice plant development. To prevent this situation and help reduce weed growth in rice production areas, we recommend that rice producers prefer polyculture farming practices.

Additional Declaration

Author Contributions

All authors have read and approved the final manuscript and agree to be accountable for the integrity of the work.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Approval

This study was conducted in accordance with the principles of Pen Academic Publishing Research Ethics Policy throughout all stages of the process.

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