Evaluation of oxidative stress indices in two rice (*Oryza sativa* L.) varieties subjected to post-emergence application of bensulfuron-methyl and bispyribac-sodium herbicides at different times

Abstract

This study focused on evaluating indices of oxidative stress in Faro 51 and Faro 59 rice varieties subjected to bensulfuron-methyl and bispyribac-sodium herbicide treatments at different application times. Using a split-plot experimental design replicated three times, a field study was carried out during the 2024 rainy season at the Research Farm of Adamawa State University, Mubi. The study treatments consisted of: two rice varieties, three periods of herbicide application, bensulfuron-methyl and bispyribac-sodium herbicides each at 200 and 400 g/ha and 200 and 400 ml/ha concentrations respectively and hoe weeded and weedy check were used as controls. The leaf samples of the rice plants from each treatment plot were obtained at the first and third Weeks after Treatment (WAT). The results showed that weedy check treatment resulted to lowest production of superoxide dismutase and catalase at the first and second sampling than the hoe weeded and herbicide treatments. The malondialdehydes (MD) rate due to hoe weeded (6.08 µmol/g) and 200 g/ha bispyribac-sodium (4.50 µmol/g) treatments were the lowest at first and second sampling respectively when compared with that of other treatments. Also, the 2, 3 and 4 Weeks after Sowing (WAS) application times had significant difference in their effect against the rate of antioxidants production in the rice plants. The 2 WAS caused the highest rate of MD, ascorbic acid (AA) and hydrogen peroxide (HP) than 3 and 4 WAS application times. The highest rate of superoxide dismutase (SD) and catalase at the first and second sampling were on the other hand recorded at 4 WAS application time. Again, the herbicide effect based on the rice variety showed that, Faro 51 & Faro 59 rice varieties had significant difference in their rate of antioxidants production. Faro 51 had the highest rate of MD, AA and HP than Faro 59. However, Faro 59 had the highest of SD and catalase than Faro 51. The study concluded that, Faro 59 rice variety has more resistance to oxidative stress than Faro 51 rice variety when subjected to bensulfuron-methyl and bispyribac-sodium herbicides. The application of the two herbicides at 2 WAS induce more oxidative stress on the two rice varieties than at 3 and 4 WAS.

Keywords: Level of antioxidants, Oxidative stress in rice plant, herbicides effect on oxidative stress of plants, Plants resistant to herbicides, Impact of plant variety on oxidative stress

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1.1 Introduction

Oxidative stress is a complex chemical and physiological processes that occurs in plants when there is an imbalance between the production of reactive oxygen species (ROS) and the plant's ability to detoxify or repair any resulting damage (Giasson *et al*., 2002 and Demidchik, 2015). This often happens when there is too much ROS as well as reactive molecules and free radicals to the extent that the antioxidant defense system of the plant body is overpowered (Giasson *et al*., 2002). Usually, plants are predisposed to high production of ROS when exposed to abiotic stress like drought, salinity, UV radiation, heavy metals toxicity, high temperature, herbicides, hypoxia, pollutants, fungicides, and topography (Teotia and Singh, 2014; Quamer *et al*., 2021). It might also be as a result of biotic stresses that include microbial attacks, competition with weeds for available soil resources, and insect attacks (Sahu *et al*., 2022). The oxidative stress which is usually characterized by the increase in ROS and alteration of the antioxidant system, is determined by the level of hydrogen peroxide, superoxide, and hydroxyl radicals (Marchenzan *et al*., 2017). This oxidative stress has a damaging effect on plants as it causes cell death due to DNA damage, lipid peroxidation, and protein oxidation. This subsequently affects the growth and quality of plants and could lead to the death of plants (Chaki *et al*., 2020; Quamer *et al*., 2021). Therefore, to neutralize or counter the damaging effects of ROS, plants developed a defense mechanism that could be enzymatic or not, to curtail or get rid of ROS and thus protect themselves from oxidative damage (Marchenzan *et al*., 2017). The major enzymatic antioxidants that plants use for protection against oxidative stress include superoxide dismutase, ascorbate peroxidase, and catalase. The non-enzymatic system, however, are phenolic compounds, carotenoids, and reduced glutathione which are usually produced by plants in response to stress (Michalak, 2006).

The use of herbicides on rice and other crop fields has no doubt contributed a lot to easing the stresses involved in the control of weeds. This as a result has culminated in plants easily accessing soil resources like water, nutrients and space due to less competition with weeds consequently enhancing their growth and yield characteristics. Prominent among the herbicides used on rice fields are herbicides like bensulfuron-methyl, which is a selective systemic herbicide used as pre-emergence or mostly as post-emergence for the control of sedges, grasses, and broad leaf weeds on rice and wheat fields. The herbicide, which falls under the category of sulfonylurea family of chemicals, usually acts by inhibiting the activity of acetolactate synthase, an enzyme involved in the biosynthesis of essential amino acids that include isoleucine and valine in targeted plants. The action of the herbicide could eventually lead to the interruption of normal growth and development of the targeted plants and consequently their death (NCBI, 2024). Bispyribac-sodium is also another common herbicide used on rice fields. It is an organic sodium salt of bispyribac that is also a selective systemic herbicide used for the post-emergence control of sedges, grasses, and broadleaf weeds on rice fields. The herbicide, which belongs to pyrimidinyloxybenzoic acid class e 78';of chemicals, acts by halting the activity of the enzyme acetolactate synthase which as a result inhibits the biosynthesis of branched-chain amino acids. This as a result disrupts the normal growth and development of the targeted plants thereby leading to their death (NCBI, 2024). Notwithstanding the significant improvement in the yield of rice plants due to the application of herbicides on rice fields (Onwuchekwa-Henry *et al*., 2023), oxidative stress that could eventually affect the growth and yield performance of rice plants has been linked to herbicides applied either as pre- or post-emergence (Langaro *et al*., 2016). This was substantiated by Kaur (2019) when he found that 71 % of the group of herbicides classified based on their mode of action caused excess production of ROS after the inhibition of their target site. The nature and gravity of the oxidative stress, however, vary according to the herbicide type, the variety of rice, the prevailing environmental conditions, and the age of herbicide application (Langaro *et al*., 2016; Islam *et al*., 2016). Some herbicides are made for application at the early growth stage of plants; however, others are made for application at the mature stage of plant development. This means that the application of herbicides at the growth stage other than the one the herbicide is made for would have a detrimental effect on the physiological processes of the plant. Kurtz and Street (2003) recorded the least visible foliar injury of rice plants when glyphosate treatments at 70, 140, and 280 g a.i/ha were applied at the boot stage than the glyphosate treatments that were applied at three-four leaf stage, midtiller and panicle initiation stages of the rice plant. Faro 51 and Faro 59 are lowland and upland rice varieties respectively preferred for cultivation in the study area due to their high yield and adaptability in the region. Therefore, the use of bensulfuron-methyl and bispyribac-sodium as post-emergence herbicides in the control of weeds on rice fields has gain acceptance among the farmers in Mubi region of Adamawa State due to their reliability and effectiveness in the control of weeds, it became imperative to evaluate the level of oxidative stress that these herbicides might cause in those rice varieties at varied concentrations and time of application, as there was paucity of information on that regard.

1.2 Materials and Methods

1.2.1 Study area

The study was carried out on the research farm of the Department of Crop Science, Adamawa State University, Mubi during the wet season of 2024. The study area falls within Latitude $10^{\circ}16'06''$ N and Longitude $13^{\circ}16'01''$ E; and has an elevation of 882 m above sea level. The area has an average annual temperature of 32 $^{\circ}$C and relative humidity ranging from 28-45 % during the wet season; and lies within the Sudan Savannah vegetation zone of Nigeria (Adebayo, 2004; ADSU Metrological station, 2023).

1.2.2 Experimental treatments

The treatments consisted of two post-emergence herbicides (Bensulfuron-methyl 5.0 % and Bispyribac-sodium) at two concentrations each (i.e Bensulfuron-methyl at 200 and 400 g/ha and Bispyribac-sodium at 200 and 400 ml/ha), hoe weeding and weedy check, two varieties of rice (Faro 51 and Faro 59) and three periods of herbicides application (i.e 2, 3, 4 weeks after sowing).

1.2.3 Experimental design

The experiment was laid out in a split-plot design with three replications. The experimental field, which was 35 x 20.5 m^2 in size, was divided into 36 plots each of which was 1 x 1.5 m^2 in size, and was replicated three times, thus giving a total of 108 subplots. The gap between the main plots and subplots was 1 and 0.5 m respectively. The rice varieties and time of the herbicide application were assigned to the main plots, and the herbicide concentrations were accommodated in the subplots.

1.2.4 Source of seeds for planting

The seeds of the Faro 51 and Faro 59 rice varieties were obtained from Premier Seed Company, Zaria, Kaduna State, Nigeria.

1.2.5 Land preparation

The experimental field was ploughed and harrowed using a tractor.

1.2.6 Seed planting

About 5-6 seeds of the Faro 51 and Faro 59 rice varieties were planted in a hole of about 2 cm depth at an intra-row and inter-row spacing of 25 and 20 cm respectively, and was after germination thinned to 2 seedlings per stand at 2 weeks after sowing.

1.2.7 Application of herbicides

The herbicides (Bensulfuron-methyl and Bispyribac-sodium) were applied each at two concentrations (Bensulfuron-methyl at 200 and 400 g/ha and Bispyribac-sodium at 200 and 400 ml/ha) using a back-mounted Knapsack sprayer at 2, 3 and 4 WAS.

1.2.8 Sampling of plants

The sampling of rice plants for the determination of the rate of antioxidant production was carried out twice, that was at the first and third weeks after Treatment (WAT). At the 1st and 3rd WAT, four rice plants, one from each hill in each treatment plot were randomly selected and placed in a well-labeled polythene bag; and thereafter taken to the laboratory for determination of antioxidant content.

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1.2.9 Data collection:

1.2.9.1 Determination of catalase activity

The method described by Sadasivam and Manickam (1997) was used for the determination of catalase activity. About 1 g of the fresh leaf sample was cut into small pieces and homogenized in 10 ml of 0.067 M phosphate buffer (pH 7.0) with a pre-cooled mortar and pestle. The homogenate was centrifuged at 18000 g for 15 minutes. The sediment was stirred with cold phosphate buffer and allowed to stand in the cold with occasional shaking and then repeat the extraction once again. The supernatants were combined and used for the assay of catalase activity. The catalase activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a 3 ml reaction mixture containing 0.16 ml of 10 % W/V H_2O_2 diluted to 100 ml with 0.067 M phosphate buffer and 0.1 ml of enzyme extract.

1.2.9.2 Determination of malondialdehyde

The malondialdehyde was determined using Thiobarbituric Acid Reactive Substances (TBARS) method as described by Heath and Packer (1968). About 0.5 g of the fresh plant leaf was grounded in 5.0 ml of 0.1 % thiobarbituric acid (TCA) containing 0.5 % butylated hydroxytoluene containing 1.0 % PVP. The homogenate was centrifuged at 12,000 g for 30 minutes. Four millilitres of the supernatant were mixed with 4.0 ml of the substrate (0.5 % thiobarbituric acid and 20 % TCA). The mixture was boiled for 30 minutes, chilled on ice, and centrifuged at 12,000 g for 10 minutes. The absorbance of the supernatant at 532 nm was measured and the non-specific absorbance at 600 nm was subtracted. The malondialdehyde content was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$.

1.2.9.3 Determination of superoxide dismutase

The method described by Beyer and Fridovich (1987) with modification was used for the determination of the superoxide dismutase. The fresh leaf sample of the plant was weighed and transferred into a pre-chilled tube. Phosphate buffer (pH 7.8) was added to the tube at a ratio of approximately 1 mL buffer per 100 mg of the tissue. The plant tissue was then homogenized using a homogenizer or by grinding the tissue with a pestle and mortar. The homogenate was then centrifuged at a low temperature (4 $^{\circ}$C) to remove cellular debris and collect the supernatant. In a quartz cuvette, a reaction mixture was prepared by combining 0.9 mL of phosphate buffer (pH 7.8), 0.1 mL of riboflavin solution, 0.1 mL of methionine solution, 0.1 mL of EDTA solution (0.1 M) and 0.1 mL of nitroblue tetrazolium (NBT) solution. The contents were gently mixed after which the cuvette was placed in a spectrophotometer. The 0.1 mL of the supernatant obtained from the sample preparation step was added to the cuvette and mixed gently. The absorbance was then measured at the wavelength of 560 nm to establish a baseline. The cuvette was illuminated with a light source (e.g. fluorescent lamp) for 15 minutes to induce superoxide production. The final absorbance reading was recorded at the same wavelength. A control reaction was performed without the sample (i.e. using buffer instead) to account for non-enzymatic reactions. The superoxide dismutase activity was calculated by calculating the percentage inhibition of NBT reduction in the presence of the sample by using the formula:

% inhibition = $\frac{Abscontrol - Abssample}{Abscontrol}$ x 100

And then compare the percentage inhibition to a calibration curve generated using known superoxide dismutase (SOD) activity standards. The SOD was expressed as units per gram of fresh weight.

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1.2.9.4 Determination of hydrogen peroxide

The method described by Velikova *et al.* (2000) was used for the determination of the hydrogen peroxide. About 500 mg of the fresh plant leaf and root tissue was homogenized separately in an ice bath with 5 ml of 0.1 % (w/v) trichloroacetic acid. The homogenate was then centrifuged at 10,000 rpm for 15 minutes, and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium iodide. The absorbance of the supernatant was measured at 390 nm.

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1.2.9.5 Determination of ascorbic acid

The method of Sadasivam and Manickam (1997) was used for the determination of the ascorbic acid. The plant tissue was homogenized in 4 % oxalic acid and centrifuged at 10,000 g for 10 minutes. The assay mixture consisted of 0.1 ml of brominated sample extract that was made up to 3.0 ml with distilled water, 1.0 ml of 2 % 2,4-dinitrophenylhydrazine reagent, and 1-2 drops of thiourea. This was incubated at 37 °C for 3 hours. The orange-red osazone crystals formed were dissolved by adding 7.0 ml of 80 % sulphuric acid and the absorbance was read at 540 nm.

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1.2.10 Data Analysis

The data generated from this study were subjected to two-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 26, and means with significant difference were separated using Duncan's Multiple Range Test (DMRT).

1.3 Results

It was revealed that the bensulfuron-methyl and bispyribac-sodium herbicides had a significant effect (at $p \leq 0.05$) on the rate of antioxidant production in leaves of the Faro 51 and Faro 59 rice varieties at both the first and second sampling. The weedy check treatment resulted in lowest production of antioxidants like superoxide dismutase and catalase at both the first and second sampling than the hoe weeded and herbicides treatments. Similarly, the hoe weeded and 200 g/ha bispyribac-sodium treatments caused the lowest production of malondialdehyde at the first and second sampling respectively than other treatments. Also, the bensulfuron-methyl treatment resulted in the lowest production of ascorbic acid at both the first and second sampling when compared with that of other treatments (Table 1). The results also showed that, the 2, 3 and 4 weeks after sowing (WAS) application times had significant difference in their effect against the production of antioxidants in leaves of the rice plants at both the first and second sampling. The 2

WAS application time resulted in the highest production of most of the antioxidants including: malondialdehyde, ascorbic acid, and hydrogen peroxide than at 3 and 4 WAS application times. The 4 WAS application time, however, resulted in the lowest production of the aforementioned antioxidants than the other periods of application. The significantly highest and lowest production of superoxide dismutase and catalase antioxidants was due to 4 and 2 WAS application times respectively (Table 1). It was also indicated that the Faro 51 and Faro 59 rice varieties subjected to the different herbicide treatments differ significantly in their antioxidants production with Faro 51 having the highest rate of malondialdehyde, ascorbic acid and hydrogen peroxide than Faro 59 rice variety. The Faro 59 rice variety, however, had the highest rate of superoxide dismutase and catalase than Faro 51 rice variety (Table 1).

The interaction effect of time of application and herbicides was observed to have a significant effect on the rate of antioxidant production in leaves of the rice plants with the 2 WAS application time resulting in the highest production of antioxidants that include malondialdehyde, ascorbic acid, and hydrogen peroxide than other application times due to weedy check treatment especially at the second sampling. The highest production of superoxide dismutase at both the first and second sampling was due to 400 ml/ha bispyribac-sodium treatment applied at 4 WAS. The lowest production of these antioxidants, however, was observed at the 4 WAS application. Also, the highest production of catalase at the first and second sampling was as a result of 400 g/ha bensulfuron-methyl and 200 ml/ha bispyribac-sodium treatment respectively applied at 4 WAS. However, the weedy check treatment at 2 WAS application time resulted in the lowest production of the two antioxidants (Table 2).

Also, interaction effect of variety and herbicides on antioxidant production was significant at both the first and second sampling with Faro 51 rice variety having the highest production rate for antioxidants like malondialdehyde, ascorbic acid, and hydrogen peroxide than Faro 59 rice variety. The Faro 59 rice variety, however, had the highest rate of superoxide dismutase and catalase than Faro 51 especially due to 400 ml/ha bispyribac-sodium treatment especially at first and second sampling (Table 3).

Similarly, the interaction effect of variety and time of herbicide application on antioxidant production was significant at the first and second sampling. Faro 51 rice variety had the highest production of malondialdehyde, ascorbic acid, and hydrogen peroxide, especially at 2 WAS application than Faro 59 rice variety. The Faro 59 rice variety, however, had the highest production of superoxide dismutase and catalase at 4 WAS application than Faro 51 rice variety (Table 4).

Again, the interaction effect of variety, time of application, and herbicides on antioxidant production of rice plants was significant at both the first and second sampling. Faro 51 rice variety subjected to especially 200 ml/ha bensulfuron-methyl treatment at 2 WAS had the highest rate of malondialdehyde than Faro 59 rice variety in the first and second samples. However, Faro 59 rice variety subjected to bispyribac-sodium treatments had the highest rate of superoxide dismutase and catalase than Faro 51 rice variety at 4 WAS application (Table 5).

1.4 Discussion

Plants in an effort to combat stress that might arise as a result of herbicides application and weed infestations produce antioxidants (Darmanti *et al*., 2016 & Harre *et al*., 2018) which could be enzymatic or non-enzymatic to curtail or regulate the accumulation of reactive oxygen species (ROS), a highly reactive substances or molecules responsible for the occurrence of oxidative stress (Marchenzan *et al*., 2017). The rate and level of antioxidant production in plants in response to ROS production depended upon the nature or level of stress that a plant is subjected to. Lower production of certain antioxidants in plants is an indication of oxidative stress in plants, however, lower production of some others could be a sign of lower stress in plants (Kumar *et al*., 2024; Radwan *et al*., 2019; Agostinetto *et al*., 2016). In this study, lower production of antioxidants like superoxide dismutase and catalase was observed as a result of weedy check treatment. This was an indication of reduced stress response in the rice plants imposed by the weeds which as a result reduced their capability to allocate energy in synthesizing antioxidants thus leading to stress in the rice plants. The lower level of ascorbic acid in the rice plants as a result of bensulfuron-methyl treatment when compared with that of other treatments showed that this herbicide predisposed the rice plant to stress than the bispyribac-sodium, hoe weeded and weedy check treatments especially at the first week after treatment thereby making the rice plants vulnerable to oxidative stress. This finding was in agreement with the discovery of Nohatto *et al*. (2016) who also reported reduction in the activity of some antioxidants like catalase as a result of application of some herbicides like bentazon and penoxsulam on rice fields. They attributed the reduction in activity of those antioxidants to oxidative stress. The lower rate of malondialdehyde recorded due to hoe weeding and 200 g/ha bispyribac-sodium treatments was a prove of lower stress in the rice plants. This is because a higher rate of malondialdehyde in plants is usually attributed to high rate of lipid peroxidation in plants due to a high accumulation of ROS that could cause damage to cells' membranes (Morales and Munne-Bosch, 2019). Therefore, the lower stress in the rice plants caused by the hoe weeded and 200 g/ha bispyribac-sodium treatments culminated in lower lipid peroxidation thus leading to a lower rate of malondialdehyde in rice plants. Nohatto *et al*. (2016) similarly reported lower levels of malondialdehyde in rice plants due to the application of cyhalofop-butyl and attributed to lower stress in the rice plants.

The age of the plant could greatly influence the plant response to herbicide effects or stressors (Khaliq and Matloob, 2012). This can be attributed to variations in the physiological processes at the different stages of plant growth and development (Hasanuzzaman *et al*., 2020). This explains why in this study, the significantly highest and lowest number of antioxidants like malondialdehyde, ascorbic acid, and hydrogen peroxide were observed at the 2 and 4 WAS respectively in the rice plants at both the first and third weeks after the application of both the bensulfuron-methyl and bispyribac-sodium herbicides. The study of Kurtz and Street (2003) agreed with the findings of this study when they recorded a least visible foliar injury of rice plants treated with glyphosate at the boot stage than at three leaf stage, midtiller, and panicle initiation stages of the rice plant as a result of oxidative stress. Unlike at the 2 WAS where there was an increased in some antioxidants, at the 4 WAS, there was a significant increase in the production of antioxidants like catalase and superoxide dismutase. The catalase aids in catalyzing the decomposition of hydrogen peroxide into water and oxygen (Palma *et al*., 2020). Superoxide dismutase on the other hand catalyzes the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Therefore, the highest rate of these antioxidants at 4 WAS than at 2 and 3 WAS was an indication of a high rate of hydrogen peroxide and superoxide radicals at that age of the rice plant which as a result necessitated their production as was similarly discovered by Nohatto *et al*. (2016) in rice plant subjected to bentazon, penoxsulam, and cyhalofop-butyl herbicides.

The variety of plant plays a key role in determining the intensity and level of oxidative stress in plants. This is because some plant varieties due to variations in certain genes might have special stress adaptation mechanisms like improved metabolic adjustments, good water retention, and root architecture than others thus enhancing their capabilities to withstand oxidative stress through the production of antioxidants (Begovic *et al*., 2023). This explains why in this study, the rice varieties

Faro 51 and Faro 59 responded differently to bensulfuron-methyl and bispyribac-sodium herbicides effect as evident by the level of antioxidant production in the two rice varieties. The Faro 51 (CISADANE) rice variety is a lowland rice variety as it thrives primarily in lowland areas; and Faro 59 (NERICA 8) rice variety on the other hand is termed an upland rice because of its ability to thrive and being cultivated in upland areas (NSPI, 2024). This means that the Faro 59 could withstand drought than the Faro 51 rice variety. The higher production of hydrogen peroxide in the Faro 51 rice variety than in Faro 59 due to the application of the two herbicides especially bensulfuron-methyl was an indication of oxidative stress. This probably led to lipid peroxidation that eventually triggered high production of malondialdehyde and ascorbic acid so as to detoxify the effect of ROS. The higher rate of superoxide dismutase and catalase antioxidant enzymes in Faro 59 than in Faro 51 rice, however, was an indication that the Faro 59, unlike the Faro 51 rice variety, had higher capabilities to produce a high amount of catalase that aided in significantly converting a sufficient amount of hydrogen peroxide produced from the actions of superoxide dismutase into oxygen and water. This discovery was in agreement with the findings of Hartatik *et al*. (2024) who found that, different rice varieties responded differently to oxidative stress. They discovered that, due to varietal differences, the rice plants subjected to similar stress conditions had significant difference in rate of antioxidants production with some having higher production of certain antioxidants than others. Similar observation was also reported by Wang *et al*. (2019) in two rice varieties.

Plants based on variety portray different levels of antioxidant activity in reaction to herbicide effect (Begovic *et al*., 2023). Also, the age of plant at which herbicides are applied significantly affects and influence the response of plants to oxidative stress through its capability to produce antioxidants (Hasanuzzaman *et al*., 2020). Similarly, herbicides, depending upon the type and concentrations greatly influence antioxidant production in plants by inducing oxidative stress due to generation of ROS (Caverzan *et al*., 2019). This explain why in this study, a significant effect in the interaction of herbicide and age of plant at which the herbicide was applied; herbicide and variety of rice plant, variety of rice plant and age of herbicide application; and herbicide, age of herbicide application and variety on the antioxidant production of rice plants were observed. This shows that the herbicide, time of application, and variety of the rice plant all together play a role in impacting the rice plant's ability to respond to oxidative stress via antioxidant production as was similarly observed by some researchers (Eceiza *et al*., 2023).

1.5 Conclusion

Faro 59 rice variety has more resistance to oxidative stress than Faro 51 rice variety when subjected to bensulfuron-methyl and bispyribac-sodium herbicides. The level of oxidative stress due to application of bensulfuron-methyl and bispyribac-sodium herbicides depended upon the age of plant. Their application at 2 WAS induce more oxidative stress on Faro 51 and Faro 59 rice varieties than at 3 and 4 WAS. Variety, concentrations of herbicides and time of application together play a role in inducing oxidative stress on Faro 51 and Faro 59 rice varieties.

1.6 Authors' Contributions

All the authors contributed in conducting this research and in writing the research paper. Authors T. D. Tizhe, C. S. Yusuf and R. Umar designed and collected the data of the research work, N. N. Zakawa analyzed the data collected from the study, S. M. Mallum write the first draft of the manuscript.

1.7 Acknowledgements

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Table 1: Effect of Post-emergence application of bensulfuron-methyl and bispyribac-sodium herbicides on antioxidant synthesis of rice plants at different sampling weeks

		Antioxidant									
		MDA (µmol/g)		Ascorbic acid (µmol/g)		SD (IU/g)		HP (µmol/g)		Catalase (IU/g)	
		Sampling Week (WAT)									
Treatment	**Rate**	1	2	1	2	1	2	1	2	1	2
Herbicides – H											
Weedy check	-	6.35^{a}	4.87^{ab}	5.80^{c}	5.19^{a}	1721.00^{d}	3584.98^{f}	5.83^{b}	3.40^{b}	5.00^{c}	5.04^{c}
Hoe weeded	-	6.08^{e}	4.85^{b}	5.93^{a}	5.21^{a}	1844.01^{c}	3640.68^{d}	5.90^{a}	3.34^{c}	5.17^{b}	5.09^{b}
Bensulfuron-methyl	200 g/ha	6.28^{b}	4.90^{a}	5.67^{d}	5.06^{c}	1916.56^{b}	3637.41^{e}	5.88^{a}	3.22^{d}	5.33^{a}	5.05^{c}
Bensulfuron-methyl	400 g/ha	6.23^{c}	4.69^{c}	5.70^{d}	4.87^{e}	1895.56^{bc}	3725.63^{b}	5.69^{c}	3.19^{d}	5.20^{b}	5.09^{b}
Bispyribac-sodium	200 ml/ha	6.19^{d}	4.50^{e}	5.78^{c}	4.93^{d}	1938.54^{b}	3717.20^{c}	5.44^{d}	3.46^{a}	5.21^{b}	5.15^{a}
Bispyribac-sodium	400 ml/ha	6.16^{d}	4.64^{d}	5.89^{b}	5.15^{b}	2071.77^{a}	3741.86^{a}	5.34^{e}	3.45^{a}	5.17^{b}	5.18^{a}
SE±	-	0.01	0.01	0.01	0.01	22.68	0.52	0.02	0.01	0.01	0.01
A. Time (WAS) - T											
2		8.09^{a}	6.18^{a}	7.22^{a}	7.19^{a}	1268.35^{c}	2615.31^{c}	6.06^{a}	5.06^{a}	3.87^{c}	3.94^{c}
3		6.18^{b}	4.99^{b}	6.04^{b}	5.51^{b}	1636.60^{b}	3932.94^{b}	5.52^{b}	2.72^{b}	5.51^{b}	5.16^{b}
4		4.37^{c}	3.06^{c}	4.12^{c}	2.50^{c}	2788.77^{a}	4475.63^{a}	5.46^{c}	2.25^{c}	6.17^{a}	6.20^{a}
SE±		0.01	0.01	0.01	0.01	16.04	0.37	0.01	0.01	0.01	0.01
Variety - V											
Faro 51		6.25^{a}	5.25^{a}	6.17^{a}	5.83^{a}	1750.96^{b}	3496.33^{b}	5.77^{a}	3.57^{a}	4.65^{b}	4.74^{b}
Faro 59		6.18^{b}	4.24^{b}	5.43^{b}	4.30^{b}	2044.85^{a}	3852.92^{a}	5.59^{b}	3.11^{b}	5.71^{a}	5.46^{a}
SE±		0.01	0.01	0.01	0.01	13.10	0.30	0.01	0.01	0.01	0.01
Interaction											
H x T		*	*	*	*	*	*	*	*	*	*
H x V		*	*	*	*	*	*	*	*	*	*
T x V		*	*	*	*	*	*	*	*	*	*
H x T x V		*	*	*	*	*	*	*	*	*	*

Means along the column with similar superscript alphabet are not significantly different at p>0.05.

Key: A. Time = Application Time; **WAT =** Weeks after Treatment**; MDA =** Malondialdehydes; **SD** = Superoxide dismutase; **HP** = Hydrogen peroxide; *** =** Significant; **WAS =** Weeks after Sowing

Table 2: Interaction effect of time of application and herbicides on antioxidants synthesis of rice plants

		Antioxidant									
		MDA (µmol/g)		**Ascorbic acid (µmol/g)**		**SD (IU/g)**		**HP (µmol/g)**		**Catalase (IU/g)**	
		Sampling Week (WAT)									
Time (WAS)	**Herbicide**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**
2	WC	8.29^{b}	6.59^{a}	7.69^{a}	7.71^{a}	1242.25^{h}	2512.60^{r}	6.25^{a}	4.74^{e}	3.72^{h}	3.89^{ij}
	HW	7.95^{c}	6.38^{b}	7.30^{b}	7.34^{b}	1287.17^{gh}	2533.51^{q}	6.30^{a}	5.55^{a}	3.79^{h}	3.90^{ij}
	BM200ml/ha	8.41^{a}	6.44^{b}	7.12^{c}	7.00^{d}	1192.41^{h}	2572.00^{p}	6.06^{b}	5.39^{b}	4.00^{g}	3.85^{j}
	BM400ml/ha	7.85^{d}	5.97^{c}	7.14^{c}	6.98^{d}	1182.44^{h}	2666.97^{o}	6.06^{b}	4.77^{e}	3.72^{h}	3.95^{i}
	BS 200g/ha	8.01^{c}	5.84^{d}	7.12^{c}	7.07^{c}	1273.65^{gh}	2711.66^{m}	5.76^{d}	5.01^{c}	3.97^{g}	4.02^{h}
	BS 400g/ha	8.02^{c}	5.83^{d}	6.98^{d}	7.07^{c}	1432.15^{f}	2695.12^{n}	5.91^{c}	4.92^{d}	4.01^{g}	4.06^{h}
3	WC	5.95^{g}	4.95^{g}	5.63^{h}	5.54^{f}	1370.91^{fg}	3808.41^{l}	5.63^{e}	3.08^{g}	5.21^{f}	5.35^{e}
	HW	5.96^{g}	4.94^{g}	6.00^{g}	5.54^{f}	1650.21^{e}	3908.57^{k}	5.87^{c}	2.27^{lm}	5.58^{de}	5.29^{e}
	BM 200g/ha	6.13^{f}	5.21^{e}	6.16^{f}	5.48^{fg}	1724.57^{de}	3915.42^{j}	5.45^{gh}	2.31^{l}	5.58^{de}	5.10^{f}
	BM 400 g/ha	6.41^{e}	5.09^{f}	6.00^{g}	5.45^{g}	1664.16^{de}	4016.71^{g}	5.40^{h}	2.57^{i}	5.55^{de}	5.09^{fg}
	BS 200 ml/ha	6.21^{f}	4.63^{h}	6.17^{f}	5.44^{g}	1636.69^{e}	3959.70^{i}	5.53^{fg}	2.92^{h}	5.64^{d}	5.06^{g}
	BS 400 ml/ha	6.45^{e}	5.14^{ef}	6.27^{e}	5.61^{e}	1773.08^{d}	3988.83^{h}	5.28^{i}	3.15^{f}	5.49^{e}	5.08^{g}
4	WC	4.82^{h}	3.05^{j}	4.09^{k}	2.32^{j}	2549.84^{c}	4433.91^{e}	5.62^{e}	2.38^{k}	6.07^{bc}	5.88^{d}
	HW	4.35^{ij}	3.25^{i}	4.49^{i}	2.75^{hi}	2594.64^{c}	4479.96^{d}	5.52^{g}	2.21^{m}	6.16^{b}	6.08^{c}
	BM 200g/ha	4.30^{j}	3.05^{j}	3.74^{m}	2.70^{i}	2832.69^{b}	4424.80^{f}	6.14^{b}	1.95^{n}	6.42^{a}	6.20^{b}
	BM 400 g/ha	4.42^{i}	3.02^{j}	3.94^{l}	2.19^{k}	2840.09^{b}	4493.21^{b}	5.61^{ef}	2.22^{m}	6.34^{a}	6.24^{b}
	BS 200 ml/ha	4.34^{ij}	3.02^{j}	4.06^{k}	2.27^{j}	2905.29^{ab}	4480.25^{c}	5.03^{j}	2.45^{j}	6.01^{c}	6.38^{a}
	BS 400 ml/ha	4.02^{k}	2.94^{k}	4.42^{j}	2.77^{h}	3010.08^{a}	4541.63^{a}	4.84^{k}	2.27^{lm}	6.01^{c}	6.39^{a}
	SE±	0.03	0.03	0.02	0.02	39.28	0.91	0.03	0.02	0.03	0.02

Means along the column with similar superscript alphabet are not significantly different at $p>0.05$.

Key: WAT = Weeks after Treatment**; MDA =** Malondialdehydes; **SD =** Superoxide dismutase; **HP =** Hydrogen peroxide; **WAS =** Weeks after Sowing

Table 3: Interaction effect of variety and herbicides on antioxidants synthesis of rice plants

		Antioxidant									
		MDA (µmol/g)		**Ascorbic acid (µmol/g)**		**SD (IU/g)**		**HP (µmol/g)**		**Catalase (IU/g)**	
		Sampling Week (WAT)									
Variety	**Herbicide**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**
Faro 51	WC	6.40^{b}	5.47^{b}	6.08^{d}	5.77^{d}	1564.86^{f}	3356.59^{l}	5.70^{e}	3.74^{a}	4.26^{h}	4.67^{e}
	HW	6.16^{ef}	5.25^{c}	6.17^{c}	6.09^{a}	1670.84^{e}	3436.09^{j}	6.01^{a}	3.40^{d}	4.58^{g}	4.73^{e}
	BM200ml/ha	6.19^{def}	5.57^{a}	6.01^{e}	5.99^{b}	1720.73^{e}	3392.07^{k}	5.91^{bc}	3.34^{de}	4.90^{d}	4.70^{e}
	BM400ml/ha	6.50^{a}	5.10^{d}	6.08^{d}	5.51^{e}	1720.98^{e}	3546.18^{i}	5.69^{e}	3.49^{c}	4.82^{e}	4.71^{e}
	BS 200g/ha	6.15^{f}	4.99^{e}	6.27^{b}	5.75^{d}	1834.58^{d}	3604.46^{h}	5.74^{de}	3.80^{a}	4.68^{f}	4.83^{d}
	BS 400g/ha	6.07^{g}	5.10^{d}	6.39^{a}	5.88^{c}	1993.77^{c}	3642.58^{g}	5.54^{f}	3.67^{b}	4.65^{f}	4.81^{d}
Faro 59	WC	6.30^{c}	4.26^{g}	5.53^{g}	4.60^{f}	1877.14^{d}	3813.36^{f}	5.96^{ab}	3.06^{g}	5.74^{ab}	5.40^{c}
	HW	6.00^{h}	4.45^{f}	5.69^{f}	4.33^{h}	2017.17^{bc}	3845.27^{c}	5.78^{d}	3.29^{ef}	5.77^{a}	5.45^{bc}
	BM 200g/ha	6.37^{b}	4.23^{gh}	5.33^{hi}	4.13^{j}	2112.39^{ab}	3882.74^{b}	5.86^{c}	3.09^{g}	5.77^{a}	5.40^{c}
	BM 400 g/ha	5.95^{h}	4.29^{g}	5.31^{i}	4.23^{i}	2070.15^{ab}	3905.08^{a}	5.69^{e}	2.88^{h}	5.59^{c}	5.47^{b}
	BS 200 ml/ha	6.22^{de}	4.00^{i}	5.30^{i}	4.11^{j}	2042.50^{bc}	3829.94^{e}	5.14^{g}	3.11^{g}	5.74^{ab}	5.47^{b}
	BS 400 ml/ha	6.25^{cd}	4.18^{h}	5.39^{h}	4.41^{g}	2149.77^{a}	3841.15^{d}	5.14^{g}	3.23^{f}	5.68^{b}	5.54^{a}
	SE±	0.02	0.02	0.02	0.02	32.08	0.74	0.02	0.02	0.02	0.02

Means along the column with similar superscript alphabet are not significantly different at $p>0.05$.

Key: WAT = Weeks after Treatment**; MDA =** Malondialdehydes; **SD =** Superoxide dismutase; **HP =** Hydrogen peroxide

Table 4: Interaction effect of variety and time of application on antioxidants synthesis of rice plants

		Antioxidant									
		MDA (μmol/g)		Ascorbic acid (μmol/g)		SD (IU/g)		HP (μmol/g)		Catalase (IU/g)	
		Sampling Week (WAT)									
Variety	**Time (WAS)**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Faro 51	2	8.58^{a}	6.76^{a}	7.41^{a}	7.53^{a}	1227.70^{f}	2468.07^{f}	6.09^{a}	4.99^{b}	3.13^{f}	3.64^{f}
	3	5.46^{d}	5.29^{c}	6.54^{c}	6.52^{c}	1456.66^{d}	3628.84^{d}	5.25^{d}	2.93^{c}	5.30^{d}	4.85^{d}
	4	4.70^{e}	3.69^{e}	4.55^{e}	3.45^{e}	2568.52^{b}	4392.07^{b}	5.96^{b}	2.79^{d}	5.51^{c}	5.74^{b}
Faro 59	2	7.60^{b}	5.59^{b}	7.04^{b}	6.86^{b}	1308.99^{e}	2762.55^{e}	6.03^{a}	5.13^{a}	4.60^{e}	4.24^{e}
	3	6.91^{c}	4.70^{d}	5.54^{d}	4.50^{d}	1816.54^{c}	4237.04^{c}	5.80^{c}	2.50^{e}	5.72^{b}	5.47^{c}
	4	4.05^{f}	2.43^{f}	3.70^{f}	1.54^{f}	3009.02^{a}	4559.18^{a}	4.96^{e}	1.70^{f}	6.82^{a}	6.65^{a}
	SE±	0.01	0.01	0.01	0.01	22.68	0.52	0.02	0.01	0.01	0.01

Means along the column with similar superscript alphabet are not significantly different at $p>0.05$.

Key: WAT = Weeks after Treatment**; MDA =** Malondialdehydes; **SD =** Superoxide dismutase; **HP =** Hydrogen peroxide; **WAS =** Weeks after Sowing

Table 5: Interaction effect of variety, time of application and herbicides on antioxidants synthesis of rice plants

			Antioxidant									
			MDA (µmol/g)		Ascorbic acid (µmol/g)		SD (IU/g)		HP (µmol/g)		Catalase (IU/g)	
			Sampling Week (WAT)									
Variety	**Time (WAS)**	**Herbicide**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Faro 51	2	WC	8.66^{b}	7.17^{b}	7.63^{b}	7.85^{a}	1172.24^{i-k}	2241.94^{z}	6.23^{b}	5.17^{d}	2.72^{o}	3.54^{s}
		HW	8.87^{a}	7.02^{c}	7.25^{cd}	7.55^{b}	1142.21^{k}	2255.27^{z}	6.16^{b}	5.67^{a}	2.85^{n}	3.56^{s}
		BM200ml/ha	8.96^{a}	7.52^{a}	7.22^{d}	7.39^{c}	1142.66^{k}	2266.33^{z}	6.16^{b}	5.56^{b}	3.28^{m}	3.53^{s}
		BM400ml/ha	8.67^{b}	6.52^{d}	7.36^{c}	7.24^{d}	1152.77^{jk}	2545.13^{yz}	5.86^{c-e}	4.32^{g}	3.27^{m}	3.66^{r}
		BS 200g/ha	8.17^{c}	6.16^{e}	7.68^{ab}	7.57^{b}	1213.16^{i-k}	2765.35^{x}	6.17^{b}	4.76^{e}	3.32^{m}	3.78^{q}
		BS 400g/ha	8.18^{c}	6.21^{e}	7.33^{cd}	7.57^{b}	1543.16^{fg}	2734.43^{y}	5.96^{c}	4.47^{f}	3.36^{m}	3.77^{q}
	3	WC	5.27^{j}	5.37^{i}	6.25^{ij}	6.46^{jk}	1087.82^{k}	3445.67^{s}	5.27^{ij}	3.17^{j}	4.74^{k}	4.95^{k}
		HW	5.26^{j}	5.02^{k}	6.23^{j}	6.96^{f}	1435.17^{gh}	3655.92^{pq}	5.96^{c}	2.02^{q}	5.44^{fg}	4.94^{k}
		BM200ml/ha	5.27^{j}	5.67^{g}	6.36^{i}	6.71^{gh}	1465.19^{gh}	3587.43^{r}	5.02^{j}	2.01^{q}	5.43^{gh}	4.83^{l}
		BM400ml/ha	5.86^{h}	5.16^{j}	6.77^{f}	6.05^{l}	1454.00^{gh}	3670.44^{q}	5.06^{j}	3.17^{j}	5.37^{g-i}	4.72^{m}
		BS 200g/ha	5.44^{i}	5.02^{k}	6.67^{f-h}	6.55^{ij}	1624.93^{f}	3681.27^{p}	5.34^{hi}	3.67^{h}	5.56^{f}	4.85^{l}
		BS 400g/ha	8.18^{c}	5.52^{h}	6.97^{e}	6.39^{k}	1672.87^{f}	3732.33^{o}	4.84^{k}	3.56^{i}	5.26^{ij}	4.83^{l}
	4	WC	5.27^{j}	3.87^{o}	4.37^{q}	3.01^{u}	2434.52^{d}	4382.17^{i}	5.62^{fg}	2.87^{l}	5.32^{hi}	5.54^{h}
		HW	4.36^{m}	3.72^{pq}	5.02^{o}	3.78^{qr}	2435.15^{d}	4397.10^{h}	5.92^{cd}	2.51^{o}	5.45^{fg}	5.68^{f}
		BM200ml/ha	4.35^{m}	3.54^{r}	4.46^{q}	3.87^{q}	2554.35^{cd}	4322.47^{k}	6.54^{a}	2.46^{o}	5.98^{c}	5.75^{f}
		BM400ml/ha	4.99^{k}	3.62^{q}	4.11^{r}	3.24^{s}	2556.17^{cd}	4422.96^{g}	6.16^{b}	2.97^{k}	5.82^{d}	5.76^{f}
		BS 200g/ha	4.85^{l}	3.81^{op}	4.46^{q}	3.13^{t}	2665.65^{b}	4366.78^{j}	5.73^{fg}	2.98^{k}	5.15^{j}	5.88^{e}
		BS 400g/ha	4.37	3.56^{r}	4.87^{p}	3.70^{r}	2765.28^{b}	4460.97^{f}	5.83^{d-f}	2.97^{k}	5.35^{g-i}	5.85^{e}

Means along the column with similar superscript alphabet are not significantly different at $p>0.05$.

Key: WAT = Weeks after Treatment**; MDA =** Malondialdehydes; **SD =** Superoxide dismutase; **HP =** Hydrogen peroxide; **WAS =** Weeks after Sowing

Table 5 continued: Interaction effect of variety, time of application and herbicides on antioxidants synthesis of rice plants

			Antioxidant									
			MDA (μmol/g)		Ascorbic acid (μmol/g)		SD (IU/g)		HP (μmol/g)		Catalase (IU/g)	
			Sampling Week (WAT)									
Variety	**Time (WAS)**	**Herbicide**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Faro 59	2	WC	7.92^{d}	6.02^{f}	7.76^{a}	7.56^{b}	1312.26^{h-j}	2783.27^{w}	6.27^{b}	4.32^{g}	4.72^{k}	4.24^{op}
		HW	7.02^{f}	5.74^{g}	7.34^{c}	7.14^{e}	1432.13^{gh}	2811.76^{u}	6.43^{a}	5.43^{c}	4.72^{k}	4.23^{op}
		BM200ml/ha	7.87^{d}	5.37^{i}	7.02^{e}	6.62^{hi}	1242.17^{i-k}	2877.67^{t}	5.96^{c}	5.21^{d}	4.72^{k}	4.16^{p}
		BM400ml/ha	7.04^{f}	5.42^{hi}	6.92^{e}	6.72^{g}	1212.12^{i-k}	2788.82^{v}	6.27^{b}	5.22^{d}	4.17^{l}	4.23^{op}
		BS 200g/ha	7.86^{d}	5.52^{h}	6.57^{h}	6.57^{i}	1334.13^{hi}	2657.97^{z}	5.36^{hi}	5.26^{d}	4.62^{k}	4.26^{no}
		BS 400g/ha	7.87^{d}	5.46^{hi}	6.63^{gh}	6.57^{i}	1321.15^{hi}	2655.82^{z}	5.87^{c-e}	5.37^{c}	4.67^{k}	4.35^{n}
	3	WC	6.63^{g}	4.54^{m}	5.01^{o}	4.62^{n}	1654.00^{f}	4171.16^{m}	5.98^{c}	2.99^{k}	5.68^{e}	5.74^{f}
		HW	6.66^{g}	4.86^{l}	5.78^{l}	4.13^{p}	1865.26^{e}	4161.23^{n}	5.78^{ef}	2.53^{no}	5.73^{de}	5.64^{g}
		BM200ml/ha	6.99^{f}	4.75^{l}	5.96^{k}	4.25^{o}	1983.96^{e}	4243.41^{l}	5.87^{c-e}	2.61^{n}	5.73^{de}	5.37^{i}
		BM400ml/ha	6.97^{f}	5.02^{k}	5.24^{n}	4.85^{m}	1874.32^{e}	4362.97^{j}	5.74^{fg}	1.97^{qr}	5.74^{de}	5.46^{h}
		BS 200g/ha	6.97^{f}	4.25^{n}	5.67^{lm}	4.34^{o}	1648.45^{f}	4238.13^{l}	5.71^{fg}	2.17^{p}	5.72^{de}	5.26^{j}
		BS 400g/ha	7.22^{e}	4.76^{l}	5.57^{m}	4.84^{m}	1873.28^{e}	4245.34^{l}	5.72^{fg}	2.74^{m}	5.72^{de}	5.34^{ij}
	4	WC	4.36^{m}	2.23^{v}	3.81^{t}	1.62^{x}	2665.17^{bc}	4485.65^{e}	5.62^{g}	1.89^{r}	6.83^{a}	6.23^{d}
		HW	4.33^{m}	2.77^{s}	3.97^{s}	1.72^{w}	2754.12^{b}	4562.82^{c}	5.13^{j}	1.91^{r}	6.86^{a}	6.49^{c}
		BM200ml/ha	4.26^{m}	2.57^{t}	3.02^{v}	1.52^{y}	3111.04^{a}	4527.14^{d}	5.75^{ef}	1.44^{t}	6.85^{a}	6.66^{b}
		BM400ml/ha	3.85^{n}	2.43^{u}	3.77^{t}	1.14^{z}	3124.00^{a}	4563.47^{c}	5.06^{j}	1.47^{t}	6.86^{a}	6.73^{b}
		BS 200g/ha	3.84^{n}	2.24^{v}	3.67^{u}	1.42^{yz}	3144.93^{a}	4593.72^{b}	4.34^{l}	1.92^{r}	6.87^{a}	6.88^{a}
		BS 400g/ha	3.68^{o}	2.32^{uv}	3.97^{s}	1.83^{v}	3254.87^{a}	4622.28^{a}	3.84^{m}	1.57^{s}	6.67^{b}	6.93^{a}
		SE±	0.04	0.04	0.04	0.03	55.56	1.28	0.04	0.03	0.04	0.03

Means along the column with similar superscript alphabet are not significantly different at $p>0.05$.

Key: WAT = Weeks after Treatment**; MDA =** Malondialdehydes; **SD =** Superoxide dismutase; **HP =** Hydrogen peroxide; **WAS =** Weeks after Sowing

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