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Synergistic Effects Of Dichlorvos, Dimethoate And Cypermethrin On Growth, Oxidative Stress And Antioxidant Responses In Okra (*Abelmoschus Esculentus*)

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Abstract

The study investigated the effects of individual and combined applications of three commonly used pesticides—dichlorvos, dimethoate, and cypermethrin—on the growth and biochemical responses of okra (Abelmoschus esculentus) plants. The experiment was conducted in a greenhouse at the College of Science, Federal University of Petroleum Resources, Effurun, Nigeria. A completely randomized design was employed, utilizing eight treatment groups: a control group (sprayed with water) and seven groups exposed to single pesticides or combinations. Growth parameters, including plant height, stem girth, and relative water content (RWC), were monitored over a four-week period. Biochemical markers, such as catalase (CAT) and superoxide dismutase (SOD) activities, along with malondialdehyde (MDA) concentrations, were analyzed to assess oxidative stress and antioxidative responses. The results showed that all pesticide treatments significantly inhibited plant growth compared to the control, with the most pronounced reductions observed in the three-pesticide combination group. RWC declined progressively with increasing pesticide complexity, indicating impaired water retention under pesticide-induced stress. Biochemical analyses revealed elevated CAT and SOD activities and increased MDA concentrations in all pesticide-treated groups, reflecting oxidative stress. Combined pesticide treatments exhibited synergistic effects, causing significantly higher oxidative stress and antioxidative enzyme activities than single treatments. Root tissues showed the highest oxidative stress, followed by stems and leaves. These findings highlight the detrimental effects of pesticide combinations on okra physiology and biochemistry, emphasizing the need for careful pesticide management to minimize environmental and agricultural risks. This study provides valuable insights into the interactive effects of pesticides, aiding in the development of sustainable pest control strategies.

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I. Introduction

The increasing demand for agricultural productivity has led to the extensive use of pesticides to mitigate pest infestations and ensure high yields. However, the indiscriminate and combined use of these agrochemicals poses significant risks to environmental health and crop physiology. Pesticides such as dichlorvos, dimethoate, and cypermethrin are widely employed due to their efficacy against a broad spectrum of pests. Despite their benefits, these chemicals are known to induce oxidative stress in plants by generating reactive oxygen species (ROS), which disrupt cellular homeostasis and compromise growth and productivity (Wang et al., 2022; Sharma et al., 2020).

Oxidative stress induced by pesticides is often characterized by elevated levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, and increased activities of antioxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD). These enzymes play a critical role in scavenging ROS and protecting plants from oxidative damage (Das and Roychoudhury, 2021). However, the extent of oxidative stress and the efficacy of antioxidative responses vary depending on the pesticide, its concentration, and the combinations used (Kaur and Singh, 2020).

In addition to biochemical impacts, pesticide exposure can adversely affect agronomic parameters such as plant height, stem girth, and relative water content (RWC), which are vital indicators of plant health and productivity. Studies have shown that pesticide mixtures often exhibit synergistic effects, resulting in amplified toxicity compared to individual treatments. This underscores the importance of evaluating the combined effects of pesticides to understand their cumulative impacts on crop physiology (Khan et al., 2021; Gopal et al., 2023).

Okra (*Abelmoschus esculentus*), a widely cultivated vegetable crop, is particularly vulnerable to pesticide exposure due to its high water content and metabolic activity. Despite its nutritional and economic significance, limited research has been conducted to assess the combined effects of dichlorvos, dimethoate, and cypermethrin on its growth and biochemical responses. Understanding these effects is essential for developing sustainable pest management strategies that minimize environmental risks and ensure crop safety (Wang et al., 2022).

This study aimed to investigate the effects of individual and combined pesticide treatments on okra plants, focusing on growth parameters, antioxidative enzyme activities, and oxidative stress markers. By elucidating the toxicological impacts of these agrochemicals, this research contributes to the broader understanding of pesticide-induced stress in crops and provides insights for optimizing pesticide usage in agriculture.

II. Materials And Methods

All reagents and solvents used in this study were of analytical grade and sourced from British Drug House, Poole, England.

The experiment was carried out in a greenhouse located at the College of Science, Federal University of Petroleum Resources, Effurun, Nigeria. A slightly modified version of the method described by Adewole and Aboyeji (2003) was employed. Bulk surface soil samples (0–15 cm depth) were collected from a location within the University, air-dried for seven days, sieved through a 2 mm mesh, and analyzed using standard procedures. Thirty-two polythene pots with drainage holes at their bases, each filled with 10 kg of surface soil, were arranged randomly on a table in the greenhouse. The experimental setup comprised eight treatment levels in a factorial combination.

The seeds used in this study were sourced from the Ministry of Agriculture, Effurun, Delta State, Nigeria. Seed viability was assessed following the procedure described by Radwan et al. (2018).

Dichlorvos, dimethoate, and cypermethrin, obtained from Hubei Sanonda Co. Ltd., China, were the agrochemicals used in this study. These chemicals were purchased from a licensed agrochemical retailer and diluted with clean water according to standard domestic usage guidelines. Specific dose combinations were prepared for each experimental group as follows:
For groups B, C, and D, the chemicals were used individually at a 1:1 dilution.
For groups E, F, and G, two chemicals were combined in a 1:0.5:0.5 ratio.
For group H, all three chemicals were combined in a 1:0.33:0.33:0.33 ratio.
Freshly prepared solutions were applied daily for a continuous exposure period of 28 days.

The solutions were applied through spraying to simulate typical agricultural pesticide application practices. A control group (Group A) was maintained, receiving only water sprayed under identical conditions. The treatment groups were organized as follows:
Group A (Control): Sprayed with water.
Group B: Sprayed with dichlorvos.
Group C: Sprayed with dimethoate.
Group D: Sprayed with cypermethrin.
Group E: Sprayed with a mixture of dichlorvos and dimethoate.
Group F: Sprayed with a mixture of dichlorvos and cypermethrin.
Group G: Sprayed with a mixture of dimethoate and cypermethrin.
Group H: Sprayed with a mixture of dichlorvos, dimethoate, and cypermethrin.

This exposure regimen ensured consistent dosing and facilitated reliable comparisons across the experimental groups, providing comprehensive insights into the toxicological effects of these agrochemicals.

Throughout the growing stage, the plant stands were watered regularly. At two weeks after planting (WAP), the plants were thinned to two stands per pot. The removed stands were retained within the same pots to return any nutrients potentially absorbed by the plants during the first two weeks of growth. Plant growth parameters, including height and stem girth, were measured every four days until the experiment was terminated. Subsequently, the leaves, stems, and roots were separated and homogenized for toxicological analysis.

The **relative water content (RWC)** of the leaves was assessed at 4 WAP using the standard method described by Schonfeld et al. (1988). The **protein concentration** in the plant tissues was determined following the method by Gornal et al. (1949). The **superoxide dismutase (SOD) activity** in the plant tissues was evaluated using the method of Misra and Fridovich (1972), while **catalase activity** was measured using the procedure described by Sinha (1971). The **malondialdehyde (MDA) concentration** in the plant tissues was determined according to the method of Bird et al. (1982).

Statistical Analysis

Data for growth parameters, including plant height and stem girth, as well as biochemical markers such as catalase (CAT) activity, superoxide dismutase (SOD) activity, relative water content (RWC), and malondialdehyde (MDA) concentrations, were presented as means ± standard error of the mean (SEM).

Statistical comparisons among the treatment groups were carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between group means. A significance level of $p < 0.05$ was used. All statistical analyses were performed using SPSS version 30.

Graphs and figures were generated to illustrate the differences and trends across the treatment groups, showcasing the effects of individual and combined pesticide exposures on the physiological and biochemical responses of spinach.

III. Results

The bar chart represents (Figure 1) the specific activity of catalase (U/mg protein) in okra plants exposed to various treatments, including individual and combined pesticide exposures.

Group A (Control) serves as the baseline for catalase activity in the okra plants. It has the lowest specific activity of catalase, as it was exposed only to sprayed water. Catalase activity in Group A is significantly lower ($p<0.05$) compared to all other groups exposed to pesticides, demonstrating that pesticide exposure induces oxidative stress in plants.

Group B (Exposed to Dichlorvos) shows significantly higher catalase activity than the control (Group A) ($p<0.05$). This increase indicates that dichlorvos induces oxidative stress, leading to the activation of catalase. However, when compared to other pesticide treatments (Groups C–H), Group B has lower catalase activity, suggesting that dichlorvos alone causes less oxidative stress compared to other pesticides or combinations.

Group C (Exposed to Dimethoate) has catalase activity significantly higher than the control (Group A) ($p<0.05$) but significantly lower than Group B ($p<0.05$). This finding suggests that dimethoate induces a moderate oxidative stress response. There is no significant difference ($p>0.05$) between Groups B and C, indicating comparable levels of oxidative stress when exposed to these individual pesticides.

Group D (Exposed to Cypermethrin) exhibits significantly higher catalase activity than Groups A, B, and C ($p<0.05$). This suggests that cypermethrin induces a more robust oxidative stress response compared to dichlorvos and dimethoate alone. The difference in catalase activity between Group D and pesticide combinations (Groups E–H) is also significant ($p<0.05$), with the combinations causing greater activity.

Group E (Exposed to Dichlorvos and Dimethoate) shows catalase activity significantly higher than Groups A–D ($p<0.05$). This indicates a synergistic effect of dichlorvos and dimethoate, amplifying oxidative stress compared to their individual effects. Catalase activity in Group E is lower than Groups F–H, with significant differences ($p<0.05$) in each case.

Group F (Exposed to Dichlorvos and Cypermethrin) demonstrates significantly higher catalase activity than Groups A–E ($p<0.05$). The combination of dichlorvos and cypermethrin induces stronger oxidative stress than the dichlorvos-dimethoate combination (Group E). However, catalase activity in Group F is still significantly lower ($p<0.05$) than Groups G and H, which involve cypermethrin in combination with other pesticides.

Group G (Exposed to Dimethoate and Cypermethrin) has catalase activity significantly higher than Groups A–F ($p<0.05$). The combination of dimethoate and cypermethrin produces more oxidative stress than any of the two-pesticide combinations involving dichlorvos. There is no significant difference ($p>0.05$) between Group G and the three-pesticide combination (Group H), indicating comparable oxidative stress levels.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin) exhibits the highest catalase activity among all groups. It is significantly higher ($p<0.05$) than Groups A–F but shows no significant difference ($p>0.05$) compared to Group G. This finding suggests that the combination of all three pesticides creates the strongest oxidative stress response, but the addition of dichlorvos to the dimethoate-cypermethrin combination does not substantially increase catalase activity further.

Relative to the control, all pesticide treatments induce significant increases in catalase activity ($p<0.05$). The combination of pesticides (Groups E–H) results in significantly higher catalase activity compared to individual pesticide exposures (Groups B–D). Among the combinations, Groups G and H exhibit the highest catalase activity, with no significant difference ($p>0.05$) between them. These findings emphasize the synergistic effects of pesticide mixtures on oxidative stress in plants.

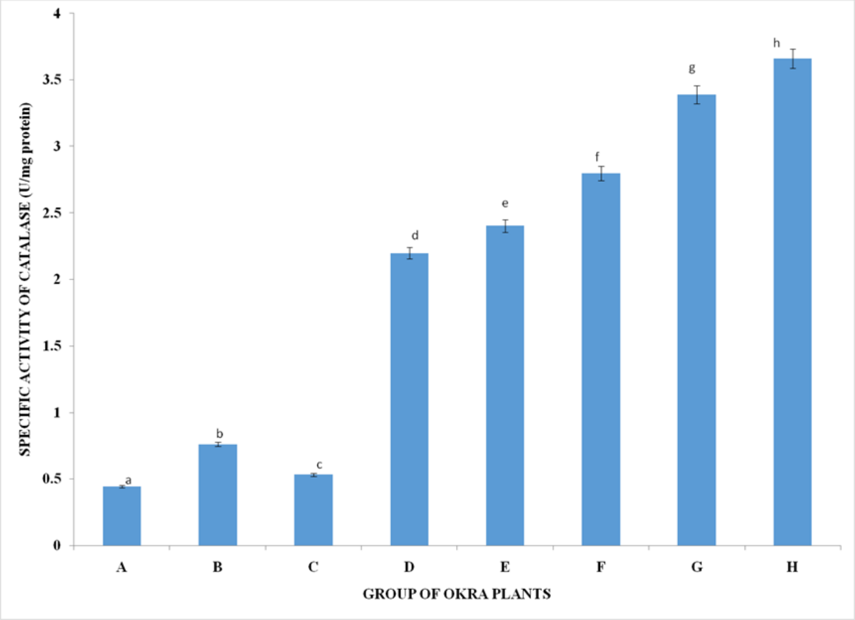

Figure 1: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Leaves of Okra Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 2 presents the effect of Individual and Combined Pesticide Exposures on Catalase Activity in Stems of Okra Plants. The findings are stated thus;

Group A (Control): The control group, exposed only to sprayed water, has the lowest specific activity of catalase. This represents the baseline oxidative stress level in the absence of pesticide exposure. Catalase activity in this group is significantly lower ($p<0.05$) compared to all pesticide-treated groups, indicating that pesticide exposure induces oxidative stress.

Group B (Exposed to Dichlorvos): The catalase activity in Group B is significantly higher than the control (Group A) ($p<0.05$), suggesting that dichlorvos exposure increases oxidative stress in the stem of okra plants. However, it remains significantly lower than all other pesticide-treated groups ($p<0.05$), indicating less oxidative stress compared to other treatments.

Group C (Exposed to Dimethoate): Catalase activity in Group C is significantly higher than the control (Group A) and Group B ($p<0.05$). This shows that dimethoate induces more oxidative stress than dichlorvos in the stem of okra plants. However, its activity is significantly lower than Groups D–H ($p<0.05$), suggesting a weaker stress response relative to other treatments.

Group D (Exposed to Cypermethrin): Catalase activity in Group D is significantly higher than Groups A–C ($p<0.05$). This indicates that cypermethrin exposure induces stronger oxidative stress in the stem than dichlorvos or dimethoate alone. However, it is significantly lower than Groups E–H ($p<0.05$), demonstrating that combinations of pesticides cause greater oxidative stress.

Group E (Exposed to Dichlorvos and Dimethoate): The catalase activity in Group E is significantly higher than Groups A–D ($p<0.05$), indicating that the combination of dichlorvos and dimethoate causes a synergistic effect, leading to greater oxidative stress. However, there is no significant difference ($p>0.05$) between Group E and Group F, suggesting similar oxidative stress levels when exposed to these two pesticide combinations.

Group F (Exposed to Dichlorvos and Cypermethrin): Catalase activity in Group F is significantly higher than Groups A–D ($p<0.05$). Like Group E, the combined exposure to dichlorvos and cypermethrin shows a synergistic effect. There is no significant difference ($p>0.05$) between Groups E and F, indicating comparable stress levels in the stem from these two combinations.

Group G (Exposed to Dimethoate and Cypermethrin): Catalase activity in Group G is significantly higher than Groups A–F ($p<0.05$). This indicates that the combination of dimethoate and cypermethrin induces more oxidative stress in the stem of okra plants than the combinations involving dichlorvos.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Catalase activity in Group H is the highest among all groups and significantly higher than Groups A–F ($p<0.05$). However, there is no significant difference ($p>0.05$) between Groups G and H, suggesting that the addition of dichlorvos to the dimethoate-cypermethrin combination does not significantly increase catalase activity further in the stem of okra plants.

Relative to the Control: All pesticide treatments significantly increase catalase activity (p<0.05), reflecting induced oxidative stress in the stem of okra plants. **Within Groups:** The activity increases progressively from single pesticide treatments (Groups B, C, D) to two-pesticide combinations (Groups E, F, G) and reaches the highest levels with the three-pesticide combination (Group H). **Significant Differences (p<0.05):** Groups G and H exhibit significantly higher catalase activity than Groups A–F, highlighting the synergistic effects of pesticide mixtures. However, there is no significant difference (p>0.05) between Groups G and H.

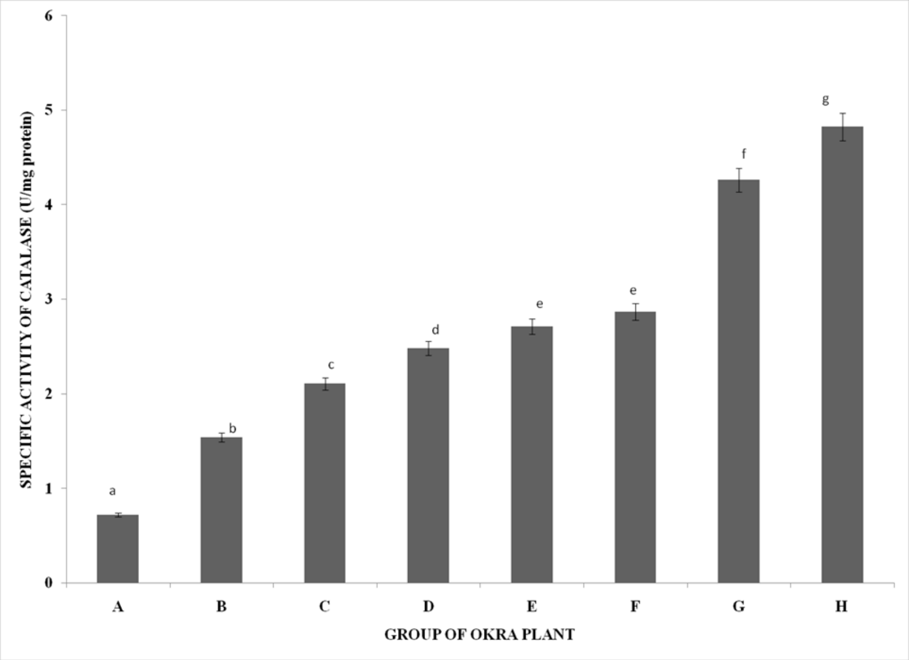

Figure 2: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Stems of Okra Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 3 shows the specific activity of catalase in the roots of okra plants exposed to individual and combined pesticide treatments.

Group A (Control): Catalase activity is the lowest in Group A, which was exposed only to sprayed water. This represents the baseline activity under non-stress conditions. Catalase activity in this group is significantly lower (p<0.05) than in all other pesticide-treated groups, indicating that pesticide exposure significantly increases oxidative stress.

Group B (Exposed to Dichlorvos): The catalase activity in Group B is significantly higher than the control (Group A) (p<0.05), indicating that dichlorvos induces oxidative stress in the roots of okra plants. However, compared to other pesticide-treated groups (Groups C–H), the activity in Group B is lower, demonstrating that dichlorvos alone induces less stress than other treatments.

Group C (Exposed to Dimethoate): Catalase activity in Group C is significantly higher than Groups A and B (p<0.05), suggesting that dimethoate induces more oxidative stress than dichlorvos. However, the activity remains significantly lower (p<0.05) than in Groups D–H, which involve other pesticides or their combinations.

Group D (Exposed to Cypermethrin): Catalase activity in Group D is significantly higher than in Groups A–C (p<0.05). This demonstrates that cypermethrin induces stronger oxidative stress in the roots compared to dichlorvos or dimethoate alone. However, catalase activity is significantly lower (p<0.05) than in Groups E–H, showing that combinations of pesticides have a stronger effect.

Group E (Exposed to Dichlorvos and Dimethoate): Catalase activity in Group E is significantly higher than in Groups A–D (p<0.05), indicating a synergistic effect of dichlorvos and dimethoate on oxidative stress. However, it is significantly lower than in Groups F–H (p<0.05), suggesting that the inclusion of cypermethrin in combinations induces even greater oxidative stress.

Group F (Exposed to Dichlorvos and Cypermethrin): Catalase activity in Group F is significantly higher than in Groups A–E (p<0.05). The combination of dichlorvos and cypermethrin results in more oxidative stress than the dichlorvos-dimethoate combination (Group E). However, the activity in Group F is significantly lower than in Groups G and H (p<0.05).

Group G (Exposed to Dimethoate and Cypermethrin): Catalase activity in Group G is significantly higher than in Groups A–F ($p<0.05$). The combination of dimethoate and cypermethrin induces more oxidative stress in the roots than any other two-pesticide combination. However, there is no significant difference ($p>0.05$) between Groups G and H, indicating similar stress levels when cypermethrin is combined with other pesticides.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Catalase activity is the highest in Group H, significantly higher than in Groups A–F ($p<0.05$). This reflects the maximum oxidative stress induced by the combined exposure to all three pesticides. However, there is no significant difference ($p>0.05$) between Groups G and H, suggesting that the addition of dichlorvos to the dimethoate-cypermethrin combination does not significantly increase catalase activity further.

Relative to the Control: All pesticide treatments significantly increase catalase activity ($p<0.05$) in okra roots, with activity progressively increasing from single to multiple pesticide exposures.

Within Groups: Single pesticide treatments (Groups B, C, D) cause moderate increases in catalase activity, while combinations (Groups E–H) result in significantly higher activity levels ($p<0.05$), highlighting the synergistic effects of pesticide mixtures.

Significant Differences: Groups G and H exhibit the highest catalase activities, with no significant difference ($p>0.05$) between them. These findings underscore the compounding oxidative stress effects of pesticide mixtures in okra roots, particularly when cypermethrin is involved.

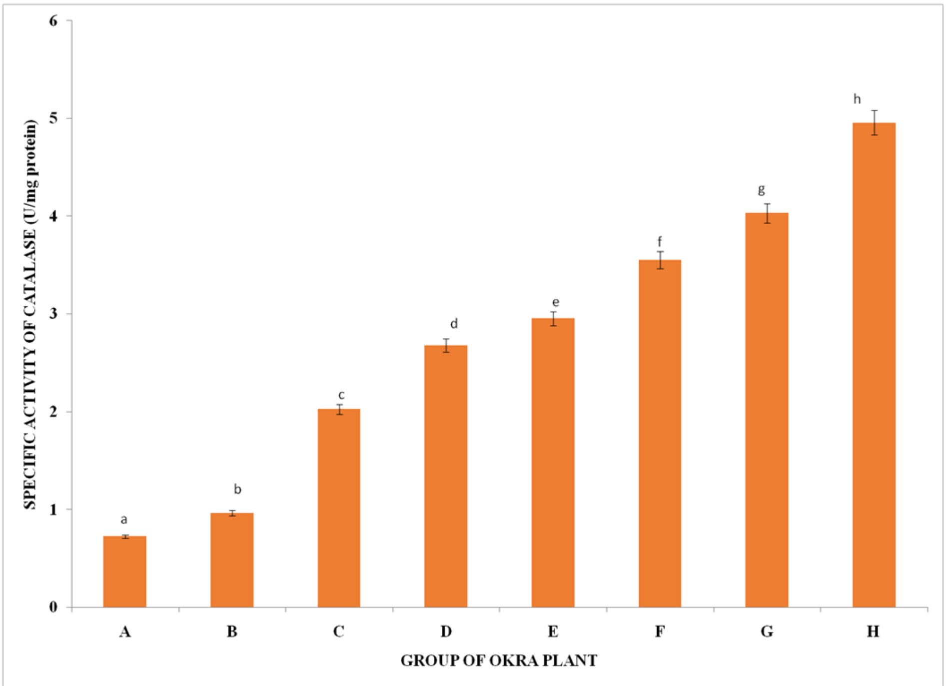

Figure 3: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Roots of Okra Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 4 presents the specific activity of superoxide dismutase (SOD) in the leaves of okra plants subjected to various pesticide treatments.

Group A (Control): The control group has the lowest SOD activity, reflecting baseline oxidative stress levels in the absence of pesticide exposure. The activity in Group A is significantly lower ($p<0.05$) compared to all other groups exposed to pesticides, indicating that pesticide exposure induces oxidative stress and enhances SOD activity as a protective mechanism.

Group B (Exposed to Dichlorvos): SOD activity in Group B is significantly higher than in Group A ($p<0.05$), demonstrating that dichlorvos exposure induces oxidative stress in okra leaves. However, the activity is significantly lower ($p<0.05$) than in Groups C–H, suggesting that dichlorvos alone induces a milder stress response compared to other pesticides or their combinations.

Group C (Exposed to Dimethoate): SOD activity in Group C is significantly higher than in Groups A and B ($p<0.05$). This indicates that dimethoate induces a stronger oxidative stress response than dichlorvos. However, the activity is significantly lower ($p<0.05$) than in Groups D–H, which include stronger pesticide exposures or combinations.

Group D (Exposed to Cypermethrin): Group D shows a significant increase in SOD activity compared to Groups A–C ($p<0.05$), indicating that cypermethrin exposure causes more oxidative stress than dichlorvos or dimethoate alone. However, SOD activity in Group D is still significantly lower ($p<0.05$) than in Groups E–H.

Group E (Exposed to Dichlorvos and Dimethoate): SOD activity in Group E is significantly higher than in Groups A–D ($p<0.05$). This demonstrates a synergistic effect of dichlorvos and dimethoate, leading to enhanced oxidative stress and corresponding SOD activity. However, there is no significant difference ($p>0.05$) between Groups E and F, indicating comparable levels of oxidative stress when dichlorvos is paired with dimethoate or cypermethrin.

Group F (Exposed to Dichlorvos and Cypermethrin): SOD activity in Group F is significantly higher than in Groups A–D ($p<0.05$). Similar to Group E, the combination of dichlorvos and cypermethrin enhances oxidative stress, but there is no significant difference ($p>0.05$) between Groups E and F. This suggests that the two combinations elicit comparable stress responses in okra leaves.

Group G (Exposed to Dimethoate and Cypermethrin): SOD activity in Group G is significantly higher than in Groups A–F ($p<0.05$), indicating that the combination of dimethoate and cypermethrin induces stronger oxidative stress than combinations involving dichlorvos. However, SOD activity in Group G is still significantly lower than in Group H ($p<0.05$).

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the highest SOD activity among all groups, significantly higher than in Groups A–G ($p<0.05$). This reflects the maximum oxidative stress response, highlighting the cumulative and synergistic effects of the three-pesticide combination on oxidative stress in okra leaves.

Relative to the Control: All pesticide treatments significantly increase SOD activity compared to the control ($p<0.05$), indicating induced oxidative stress in okra leaves. **Within Groups:** SOD activity progressively increases from single pesticide exposures (Groups B–D) to combinations of two pesticides (Groups E–G) and peaks in the three-pesticide combination (Group H). **Significant Differences:** Groups E and F exhibit comparable SOD activities ($p>0.05$), but all combinations involving cypermethrin (Groups F, G, H) show higher activity than single pesticide treatments (Groups B, C, D). Group H demonstrates the highest SOD activity, with significant differences ($p<0.05$) from all other groups.

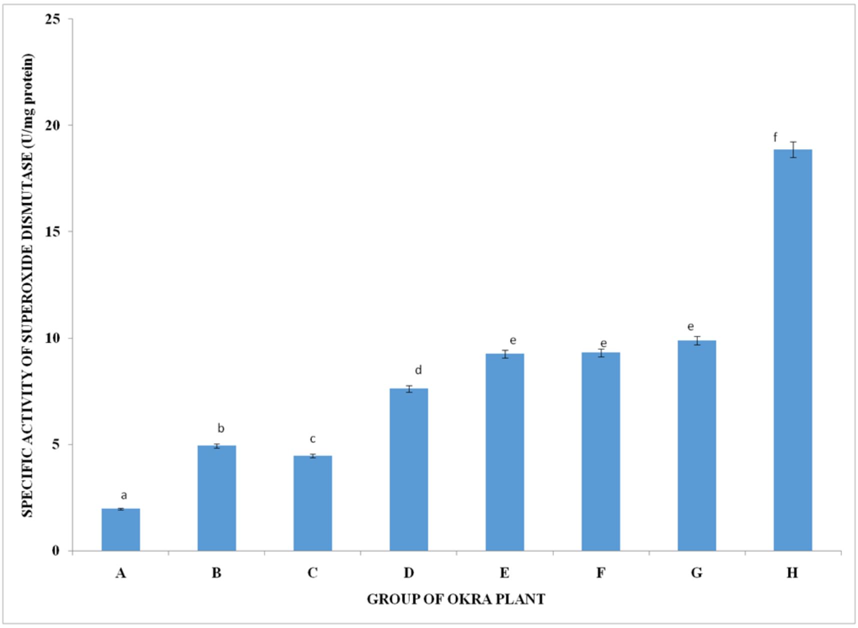

Figure 4: Specific Activity of Superoxide Dismutase (SOD) in Leaves of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 5 shows the specific activity of superoxide dismutase (SOD) in the stems of okra plants subjected to different pesticide treatments.

Group A (Control): The control group shows the lowest SOD activity, representing the baseline oxidative stress level in the absence of pesticides. SOD activity in this group is significantly lower ($p<0.05$) compared to all pesticide-treated groups, indicating that pesticide exposure induces oxidative stress and enhances the plant's defense response.

Group B (Exposed to Dichlorvos): SOD activity in Group B is significantly higher than in Group A ($p<0.05$), demonstrating that dichlorvos exposure induces oxidative stress in okra stems. However, the activity is significantly lower than in Groups C–H ($p<0.05$), indicating that dichlorvos alone induces a weaker stress response compared to other pesticides or combinations.

Group C (Exposed to Dimethoate): SOD activity in Group C is significantly higher than in Groups A and B ($p<0.05$), suggesting that dimethoate induces a greater oxidative stress response than dichlorvos. However, the activity remains significantly lower ($p<0.05$) than in Groups D–H, indicating that combinations of pesticides or cypermethrin alone cause more pronounced oxidative stress.

Group D (Exposed to Cypermethrin): SOD activity in Group D is significantly higher than in Groups A–C ($p<0.05$). This indicates that cypermethrin exposure results in stronger oxidative stress in okra stems than dichlorvos or dimethoate. However, SOD activity in Group D is still significantly lower ($p<0.05$) than in Groups E–H, showing that combinations of pesticides elicit stronger responses.

Group E (Exposed to Dichlorvos and Dimethoate): SOD activity in Group E is significantly higher than in Groups A–D ($p<0.05$), indicating a synergistic effect of dichlorvos and dimethoate that enhances oxidative stress. However, it is significantly lower than in Groups F–H ($p<0.05$), suggesting that the inclusion of cypermethrin in combinations results in greater oxidative stress.

Group F (Exposed to Dichlorvos and Cypermethrin): SOD activity in Group F is significantly higher than in Groups A–E ($p<0.05$). The combination of dichlorvos and cypermethrin results in a stronger oxidative stress response than the dichlorvos-dimethoate combination (Group E). However, the activity remains significantly lower than in Groups G and H ($p<0.05$).

Group G (Exposed to Dimethoate and Cypermethrin): SOD activity in Group G is significantly higher than in Groups A–F ($p<0.05$). The combination of dimethoate and cypermethrin induces greater oxidative stress than other two-pesticide combinations. However, SOD activity in Group G is significantly lower than in Group H ($p<0.05$).

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the highest SOD activity among all groups, significantly higher than in Groups A–G ($p<0.05$). This reflects the maximum oxidative stress response, emphasizing the cumulative effects of the three-pesticide combination in inducing oxidative stress in okra stems.

Relative to the Control: All pesticide treatments significantly increase SOD activity compared to the control ($p<0.05$), highlighting the role of SOD as a key antioxidant defense mechanism. **Within Groups:** SOD activity progressively increases from single pesticide treatments (Groups B, C, D) to combinations of two pesticides (Groups E, F, G) and peaks in the three-pesticide combination (Group H). **Significant Differences:** The combined effects of pesticides in Groups E–H lead to significantly higher SOD activity compared to individual treatments (Groups B–D). Group H shows the highest SOD activity, reflecting the strongest oxidative stress response.

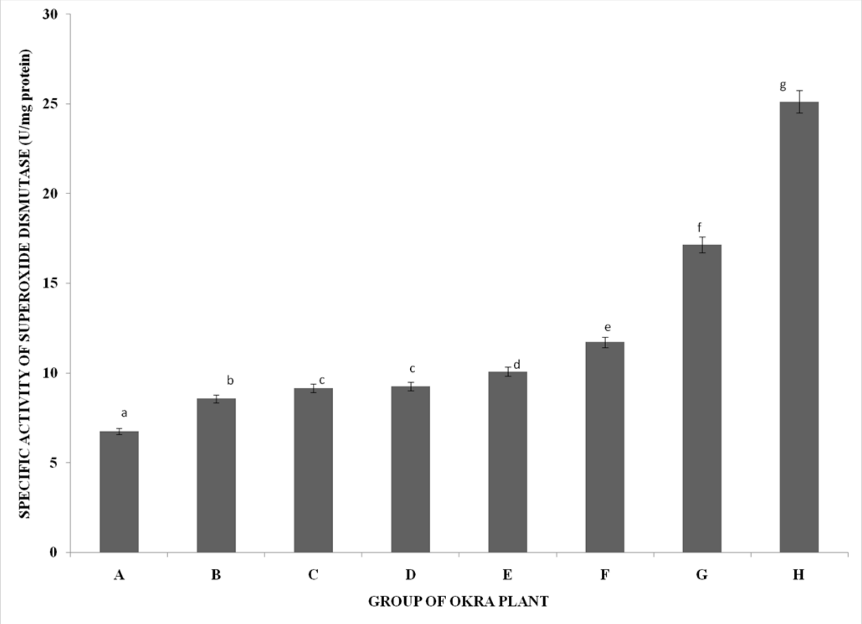

Figure 5: Specific Activity of Superoxide Dismutase (SOD) in Stems of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 6 illustrates the specific activity of superoxide dismutase (SOD) in the roots of okra plants subjected to various pesticide treatments.

Group A (Control): The control group, exposed only to water, exhibits the lowest SOD activity. This represents the baseline oxidative stress level under non-pesticide conditions. SOD activity in Group A is significantly lower ($p<0.05$) than in all pesticide-treated groups, indicating that exposure to pesticides triggers oxidative stress and enhances SOD activity as a defense response.

Group B (Exposed to Dichlorvos): SOD activity in Group B is significantly higher than in Group A ($p<0.05$), demonstrating that dichlorvos exposure induces oxidative stress in okra roots. However, the activity is significantly lower ($p<0.05$) than in Groups C–H, showing that dichlorvos alone induces a milder oxidative stress response compared to other pesticides or combinations.

Group C (Exposed to Dimethoate): SOD activity in Group C is significantly higher than in Groups A and B ($p<0.05$), indicating that dimethoate induces greater oxidative stress than dichlorvos. However, it remains significantly lower ($p<0.05$) than in Groups D–H, reflecting the stronger stress response elicited by other treatments.

Group D (Exposed to Cypermethrin): Group D exhibits significantly higher SOD activity than Groups A–C ($p<0.05$), indicating that cypermethrin induces stronger oxidative stress in okra roots compared to dichlorvos or dimethoate. However, SOD activity in Group D is significantly lower ($p<0.05$) than in Groups E–H, showing that combinations of pesticides result in even greater oxidative stress.

Group E (Exposed to Dichlorvos and Dimethoate): SOD activity in Group E is significantly higher than in Groups A–D ($p<0.05$), demonstrating a synergistic effect of dichlorvos and dimethoate that amplifies oxidative stress in okra roots. There is no significant difference ($p>0.05$) between Groups E and F, suggesting comparable stress responses when dichlorvos is paired with either dimethoate or cypermethrin.

Group F (Exposed to Dichlorvos and Cypermethrin): SOD activity in Group F is significantly higher than in Groups A–D ($p<0.05$), further confirming the synergistic effects of dichlorvos and cypermethrin. Similar to Group E, there is no significant difference ($p>0.05$) between Groups E and F, indicating that these combinations induce similar oxidative stress levels.

Group G (Exposed to Dimethoate and Cypermethrin): SOD activity in Group G is significantly higher than in Groups A–F ($p<0.05$). The combination of dimethoate and cypermethrin induces greater oxidative stress than the dichlorvos-based combinations. However, SOD activity in Group G remains significantly lower than in Group H ($p<0.05$).

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the highest SOD activity among all groups, significantly higher than in Groups A–G ($p<0.05$). This reflects the maximum oxidative stress response induced by the combined exposure to all three pesticides, highlighting their cumulative effects on oxidative stress in okra roots.

Relative to the Control: All pesticide treatments significantly increase SOD activity compared to the control ($p<0.05$), indicating the activation of antioxidant defenses in response to oxidative stress. **Within Groups:** SOD activity progressively increases from single pesticide treatments (Groups B–D) to combinations of two pesticides (Groups E, F, G) and peaks in the three-pesticide combination (Group H). **Significant Differences:** Groups E and F show comparable SOD activities ($p>0.05$), while Group G induces significantly higher activity than these two groups ($p<0.05$). Group H shows the highest SOD activity overall, with significant differences ($p<0.05$) from all other groups.

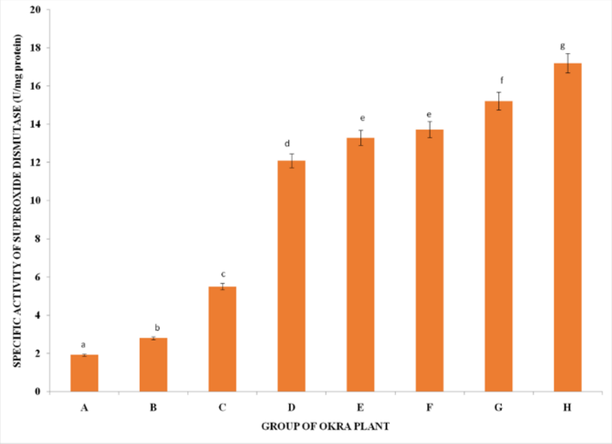

Figure 6: Specific Activity of Superoxide Dismutase (SOD) in Roots of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 7 presents the concentration of malondialdehyde (MDA), a biomarker of lipid peroxidation, in the leaves of okra plants exposed to various pesticide treatments.

Group A (Control): The control group, exposed only to water, shows the lowest MDA concentration. This represents the baseline level of lipid peroxidation in the absence of pesticide-induced oxidative stress. MDA levels in Group A are significantly lower (p<0.05) than in all pesticide-treated groups, highlighting the oxidative damage caused by pesticide exposure.

Group B (Exposed to Dichlorvos): MDA concentration in Group B is significantly higher than in Group A (p<0.05), indicating that dichlorvos induces oxidative stress and lipid peroxidation in okra leaves. However, the concentration is significantly lower (p<0.05) than in Groups C–H, showing that dichlorvos alone causes less damage than other pesticide treatments or combinations.

Group C (Exposed to Dimethoate): Group C exhibits significantly higher MDA levels than Groups A and B (p<0.05), reflecting a stronger oxidative stress response to dimethoate exposure. However, its levels are comparable (p>0.05) to those in Groups D–F, indicating similar levels of lipid peroxidation in these groups.

Group D (Exposed to Cypermethrin): MDA concentration in Group D is significantly higher than in Groups A–B (p<0.05), showing that cypermethrin induces greater lipid peroxidation than dichlorvos alone. However, there is no significant difference (p>0.05) between Groups D, C, E, and F, suggesting comparable levels of oxidative stress in these groups.

Group E (Exposed to Dichlorvos and Dimethoate): MDA levels in Group E are significantly higher than in Groups A–B (p<0.05), indicating a synergistic effect of dichlorvos and dimethoate in enhancing lipid peroxidation. However, its levels are not significantly different (p>0.05) from those in Groups D and F, showing similar oxidative stress responses among these combinations.

Group F (Exposed to Dichlorvos and Cypermethrin): Group F shows a significant increase in MDA levels compared to Groups A and B (p<0.05), reflecting the oxidative stress induced by the dichlorvos-cypermethrin combination. Similar to Group E, its levels are not significantly different (p>0.05) from Groups C, D, and E.

Group G (Exposed to Dimethoate and Cypermethrin): MDA levels in Group G are significantly higher than in Groups A–F (p<0.05), indicating that the combination of dimethoate and cypermethrin induces greater lipid peroxidation than other two-pesticide combinations. However, its levels are significantly lower (p<0.05) than in Group H.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the highest MDA concentration among all groups, significantly higher than in Groups A–G (p<0.05). This reflects the maximum lipid peroxidation and oxidative damage caused by the combined exposure to all three pesticides, highlighting their cumulative effects.

Relative to the Control: All pesticide treatments significantly increase MDA levels compared to the control (p<0.05), indicating enhanced lipid peroxidation and oxidative damage due to pesticide exposure. **Within Groups:** MDA levels progressively increase with the complexity of pesticide combinations, with the highest levels observed in the three-pesticide combination (Group H). **Significant Differences:** Groups D–F show comparable MDA concentrations (p>0.05), but Group G exhibits significantly higher levels than these groups (p<0.05). Group H demonstrates the highest MDA levels, with significant differences (p<0.05) from all other groups.

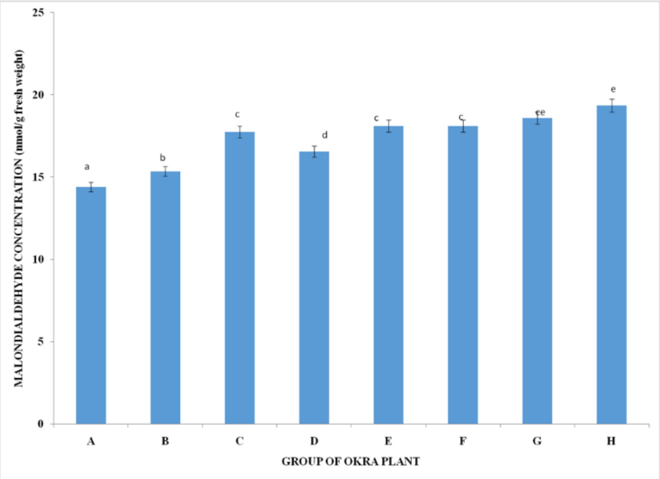

Figure 7: Malondialdehyde (MDA) Concentration in Leaves of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 8 represents the malondialdehyde (MDA) concentration, a marker of lipid peroxidation, in the stems of okra plants subjected to various pesticide treatments.

Group A (Control): The control group, exposed to water only, shows baseline MDA concentration. There is no significant difference ($p>0.05$) in MDA levels between the control and pesticide-treated groups (Groups B–G), indicating that single and two-pesticide exposures do not substantially increase lipid peroxidation in the stems compared to the control.

Groups B–G (Single and Two-Pesticide Treatments): MDA concentrations in Groups B–G remain statistically similar to the control (Group A) ($p>0.05$). This suggests that the oxidative stress induced by single pesticides (Groups B, C, D) and two-pesticide combinations (Groups E, F, G) does not significantly elevate lipid peroxidation in the stems of okra plants. These results imply a lower vulnerability of stem tissues to lipid peroxidation under these treatments.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits a significant increase in MDA concentration compared to all other groups ($p<0.05$). This highlights the cumulative effect of the three-pesticide combination, leading to the highest level of lipid peroxidation and oxidative damage in the stems. Group H is the only treatment that significantly differs from the control and other groups, indicating that the combined stress from three pesticides surpasses the oxidative capacity of stem tissues.

Relative to the Control: No significant differences in MDA concentrations ($p>0.05$) are observed between the control (Group A) and Groups B–G, indicating that single and two-pesticide treatments do not markedly increase lipid peroxidation in stems.

Within Groups: The three-pesticide combination (Group H) significantly elevates MDA levels compared to all other groups ($p<0.05$), suggesting additive and synergistic effects of the pesticides on lipid peroxidation.

Significant Differences: Group H shows the only significant increase in MDA concentration, while Groups A–G remain statistically similar.

These findings suggest that the stems of okra plants are more resistant to oxidative damage from single and two-pesticide treatments but are significantly affected by the stress induced by the combined application of three pesticides. This highlights the importance of considering pesticide combinations in agricultural practices.

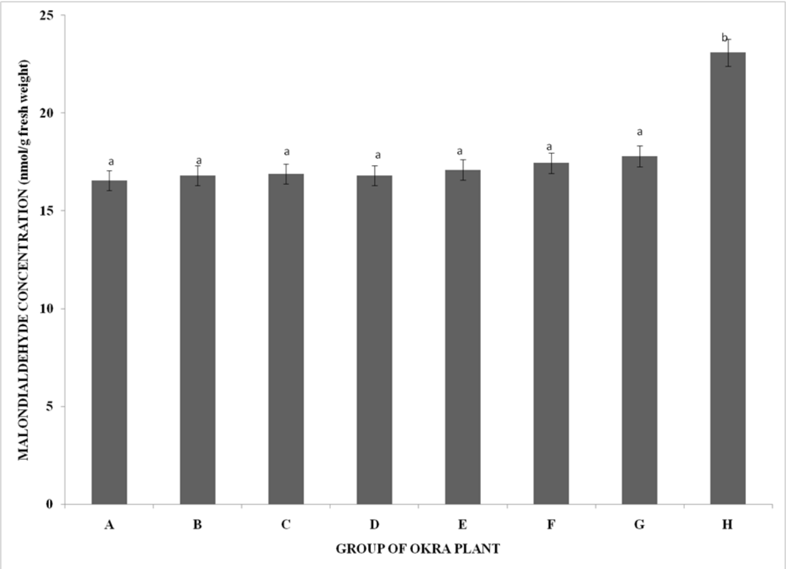

Figure 8: Malondialdehyde (MDA) Concentration in Stems of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 9 shows the malondialdehyde (MDA) concentration, a marker of lipid peroxidation, in the roots of okra plants subjected to various pesticide treatments. MDA levels reflect oxidative stress and cellular membrane damage caused by reactive oxygen species (ROS). The data demonstrate a progressive increase in MDA concentration across the groups, with significant differences ($p<0.05$) between many treatments, particularly for the three-pesticide combination (Group H).

Group A (Control): The control group exhibits the lowest MDA concentration, representing the baseline level of lipid peroxidation in the absence of pesticide-induced oxidative stress. MDA levels in Group A are significantly lower ($p<0.05$) than in all other groups, indicating the oxidative damage caused by pesticide exposure.

Group B (Exposed to Dichlorvos): MDA levels in Group B are significantly higher than in the control (Group A) ($p<0.05$), demonstrating that dichlorvos exposure induces oxidative stress in the roots of okra plants. However, MDA levels in Group B are significantly lower than in Groups C–H ($p<0.05$), indicating that dichlorvos alone causes relatively mild lipid peroxidation.

Group C (Exposed to Dimethoate): Group C exhibits significantly higher MDA concentrations compared to Groups A and B ($p<0.05$), reflecting a stronger oxidative stress response due to dimethoate exposure. However, its levels are comparable ($p>0.05$) to Groups D and E, showing a similar degree of oxidative damage caused by these treatments.

Group D (Exposed to Cypermethrin): MDA concentration in Group D is significantly higher than in Groups A and B ($p<0.05$), indicating that cypermethrin induces greater lipid peroxidation compared to dichlorvos. However, its levels remain comparable ($p>0.05$) to those in Groups C and E, suggesting a similar extent of oxidative stress.

Group E (Exposed to Dichlorvos and Dimethoate): MDA levels in Group E are significantly higher than in Groups A and B ($p<0.05$), reflecting the synergistic effects of dichlorvos and dimethoate. However, the levels are not significantly different ($p>0.05$) from Groups C and D, suggesting that the oxidative damage induced by these combinations is comparable to individual pesticide treatments.

Group F (Exposed to Dichlorvos and Cypermethrin): Group F shows a significant increase in MDA concentration compared to Groups A–E ($p<0.05$). This indicates that the combination of dichlorvos and cypermethrin induces greater oxidative stress and lipid peroxidation than dichlorvos or dimethoate alone.

Group G (Exposed to Dimethoate and Cypermethrin): MDA concentration in Group G is significantly higher than in Groups A–F ($p<0.05$), demonstrating that the combination of dimethoate and cypermethrin leads to more severe oxidative damage than other two-pesticide combinations. However, its levels are still significantly lower than those in Group H ($p<0.05$).

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the highest MDA concentration among all groups, significantly higher than in Groups A–G ($p<0.05$). This reflects the maximum lipid peroxidation and oxidative damage caused by the combined exposure to all three pesticides, highlighting their cumulative and synergistic effects.

Relative to the Control: All pesticide treatments significantly increase MDA levels compared to the control ($p<0.05$), indicating enhanced oxidative stress and lipid peroxidation in the roots of okra plants.

Within Groups: MDA levels progressively increase from single pesticide treatments (Groups B, C, D) to combinations of two pesticides (Groups E, F, G) and peak in the three-pesticide combination (Group H).

Significant Differences: Groups F and G induce higher MDA levels than single-pesticide treatments (Groups B–D), while Group H demonstrates the highest lipid peroxidation, with significant differences ($p<0.05$) from all other groups.

These findings emphasize the additive and synergistic effects of pesticide combinations on oxidative stress in okra roots, with the three-pesticide combination causing the most severe lipid peroxidation and membrane damage.

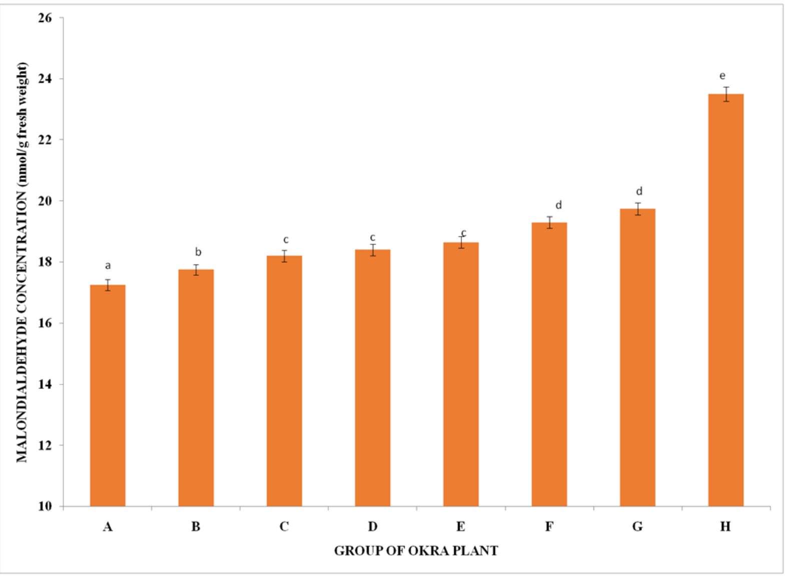

Figure 9: Malondialdehyde (MDA) Concentration in Roots of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

This figure 10 illustrates the relative water content (RWC) in the leaves of okra plants subjected to various pesticide treatments. RWC is an indicator of plant water status and reflects the ability of the plant to retain water under stress. The data show a progressive decline in RWC from the control group (Group A) to the three-pesticide combination (Group H), with significant differences ($p<0.05$) observed among most treatments.

Group A (Control): The control group has the highest RWC, representing optimal water retention in leaves under non-stress conditions. RWC in Group A is significantly higher ($p<0.05$) than in all pesticide-treated groups, indicating that pesticide exposure negatively affects the plant's water retention ability.

Group B (Exposed to Dichlorvos): Group B shows a significant decrease in RWC compared to the control (Group A) ($p<0.05$), reflecting the effect of dichlorvos exposure on plant water status. However, RWC in Group B is significantly higher than in Groups C–H ($p<0.05$), suggesting a relatively milder impact of dichlorvos on water retention.

Group C (Exposed to Dimethoate): RWC in Group C is significantly lower than in Groups A and B ($p<0.05$), indicating a stronger negative effect of dimethoate on water retention compared to dichlorvos. However, there is no significant difference ($p>0.05$) between Groups C and D, suggesting a similar effect of dimethoate and cypermethrin on RWC.

Group D (Exposed to Cypermethrin): Group D exhibits significantly lower RWC compared to Groups A and B ($p<0.05$), indicating that cypermethrin reduces water retention more than dichlorvos. However, its RWC is comparable ($p>0.05$) to Groups C and G, showing similar effects on plant water status.

Group E (Exposed to Dichlorvos and Dimethoate): RWC in Group E is significantly lower than in Groups A–D ($p<0.05$), demonstrating the combined negative effect of dichlorvos and dimethoate on water retention. However, there is no significant difference ($p>0.05$) between Groups E and G, suggesting a comparable impact of these pesticide combinations on RWC.

Group F (Exposed to Dichlorvos and Cypermethrin): Group F shows a significant reduction in RWC compared to Groups A–E ($p<0.05$). This indicates that the dichlorvos-cypermethrin combination has a stronger impact on plant water retention than individual pesticides or the dichlorvos-dimethoate combination.

Group G (Exposed to Dimethoate and Cypermethrin): RWC in Group G is significantly lower than in Groups A–E ($p<0.05$), but similar to Group F ($p>0.05$), reflecting the combined effects of dimethoate and cypermethrin on water retention. These results indicate that both pesticide combinations severely affect plant water status.

Group H (Exposed to Dichlorvos, Dimethoate, and Cypermethrin): Group H exhibits the lowest RWC among all groups, significantly lower than in Groups A–G ($p<0.05$). This highlights the cumulative and synergistic effect of all three pesticides, causing the most severe reduction in water retention.

Relative to the Control: All pesticide treatments significantly reduce RWC compared to the control ($p<0.05$), demonstrating the adverse effects of pesticides on plant water retention.

Within Groups: RWC progressively decreases from single pesticide treatments (Groups B–D) to two-pesticide combinations (Groups E, F, G) and reaches its lowest level in the three-pesticide combination (Group H).

Significant Differences: Group H shows the most severe reduction in RWC, with significant differences ($p<0.05$) from all other groups. Groups F and G exhibit similar RWC ($p>0.05$), reflecting comparable effects of these two-pesticide combinations.

The results highlight the detrimental impact of pesticides, especially their combinations, on the water retention ability of okra leaves, which may impair plant physiological processes and growth.

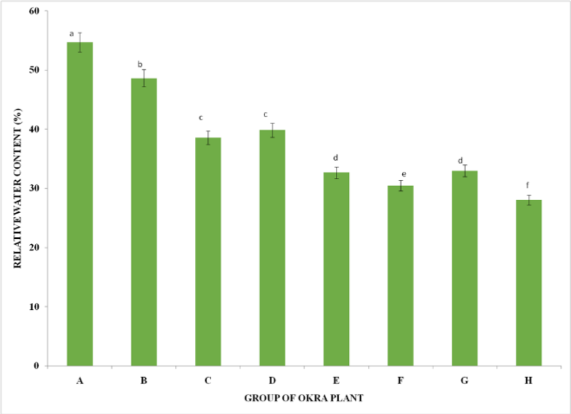

Figure 10: Relative Water Content (RWC) in Leaves of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 11 depicts the growth trends (height) of okra plants over four weeks under different pesticide treatments. Plant height is a critical parameter to assess the impact of pesticide exposure on growth.

Group A shows the highest growth rates, with plant heights significantly higher (p<0.05) than all other groups throughout the 4-week period. This demonstrates optimal growth in the absence of pesticide-induced stress.

Group B (Dichlorvos): Plant height in Group B is significantly lower (p<0.05) than the control (Group A) throughout the period, reflecting moderate growth inhibition due to dichlorvos exposure. **Group C (Dimethoate):** Group C shows a significant reduction in plant height compared to the control (p<0.05) but is slightly taller than Group B, with differences between Groups B and C being statistically significant (p<0.05). **Group D (Cypermethrin):** Plant height in Group D is significantly lower (p<0.05) than the control but significantly higher (p<0.05) than Groups B and C, indicating a milder inhibitory effect of cypermethrin.

Group E (Dichlorvos + Dimethoate): Plant height is significantly reduced compared to the control (p<0.05) and single pesticide treatments (Groups B, C, and D) (p<0.05). This suggests a synergistic inhibitory effect of dichlorvos and dimethoate. **Group F (Dichlorvos + Cypermethrin):** Group F shows significantly lower heights than the control and single pesticide groups (p<0.05). There is no significant difference (p>0.05) between Groups E and F. **Group G (Dimethoate + Cypermethrin):** Growth in Group G is significantly lower than the control (p<0.05) but significantly higher than Groups E and F (p<0.05), indicating a slightly less detrimental effect of this combination.

Group H exhibits the most pronounced growth inhibition, with plant heights significantly lower (p<0.05) than all other groups throughout the study period. This highlights the cumulative or synergistic negative effects of the three-pesticide combination on okra plant growth.

Control vs. Treated Groups: The control group (Group A) consistently has significantly higher plant heights (p<0.05) compared to all pesticide-treated groups. **Single vs. Combination Treatments:** Single pesticide treatments (Groups B, C, D) result in significantly less growth inhibition than two-pesticide combinations (Groups E, F, G) (p<0.05). **Two vs. Three-Pesticide Combinations:** The three-pesticide combination (Group H) shows significantly lower plant heights compared to all two-pesticide combinations (p<0.05), indicating the most severe growth suppression. **Within Single Treatments:** Among the single pesticides, significant differences (p<0.05) are observed, with dichlorvos (Group B) being the most inhibitory, followed by dimethoate (Group C), and cypermethrin (Group D).

The data demonstrate that pesticide exposure significantly inhibits okra plant growth, with more severe effects observed in combinations compared to single pesticides.

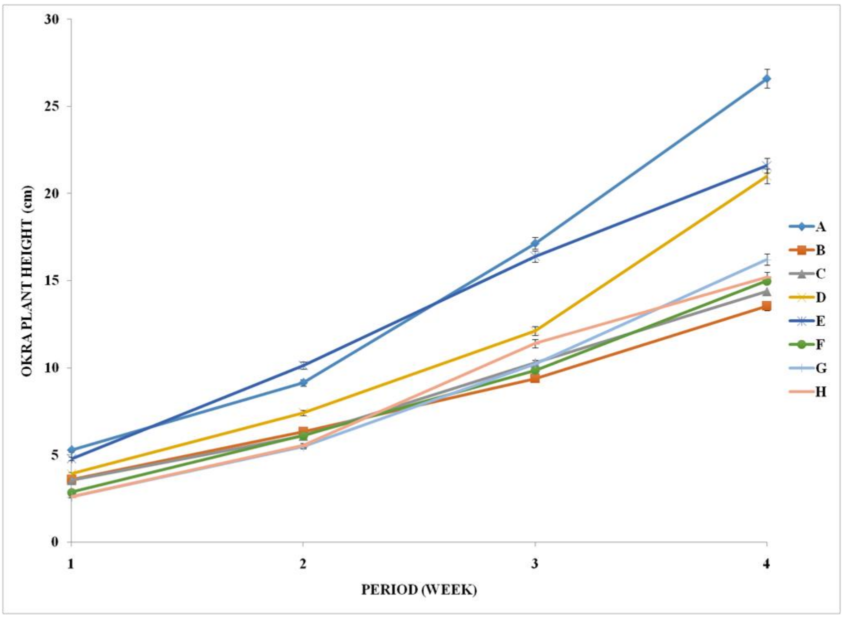

Figure 11: Height of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM and are Significantly different when p<0.05.

Figure 12 represents the stem girth (diameter) of okra plants over a 4-week period under different pesticide treatments. Stem girth is an important indicator of plant robustness, structural stability, and overall growth performance. The data reveal significant differences in stem girth among the groups, reflecting the effects of various pesticide exposures on plant development.

The control group (Group A) consistently exhibits the greatest stem girth over the 4-week period. This group shows significantly higher stem girth compared to all pesticide-treated groups ($p<0.05$), indicating optimal growth conditions in the absence of pesticide stress.

Group B (Dichlorvos): Stem girth in Group B is significantly lower than in the control (Group A) ($p<0.05$), reflecting moderate growth inhibition caused by dichlorvos exposure. **Group C (Dimethoate):** Growth in Group C is also significantly reduced compared to the control ($p<0.05$) but is slightly better than Group B, indicating that dimethoate has a less severe impact on stem girth. **Group D (Cypermethrin):** Group D exhibits higher stem girth than Groups B and C ($p<0.05$) but remains significantly lower than the control ($p<0.05$), indicating that cypermethrin has the mildest effect on stem girth among the single pesticide treatments.

Group E (Dichlorvos + Dimethoate): Stem girth is significantly lower than in the control and single pesticide treatments ($p<0.05$), suggesting a synergistic inhibitory effect of this pesticide combination. **Group F (Dichlorvos + Cypermethrin):** Group F shows a significant reduction in stem girth compared to the control and single pesticide treatments ($p<0.05$). However, there is no significant difference ($p>0.05$) between Groups E and F, suggesting comparable inhibitory effects. **Group G (Dimethoate + Cypermethrin):** Stem girth in Group G is significantly lower than the control ($p<0.05$) but slightly better than Groups E and F ($p<0.05$), indicating a slightly less severe impact of this combination.

Group H exhibits the smallest stem girth throughout the 4-week period, with values significantly lower than all other groups ($p<0.05$). This indicates that the combined exposure to all three pesticides has the most severe impact on stem girth, likely due to cumulative oxidative stress or metabolic disruptions.

Control vs. Treated Groups: The control group consistently has the largest stem girth, with significant differences ($p<0.05$) from all pesticide-treated groups. **Single vs. Combination Treatments:** Single pesticide treatments (Groups B, C, D) result in significantly less reduction in stem girth compared to two-pesticide combinations (Groups E, F, G) ($p<0.05$). **Three-Pesticide Combination:** The three-pesticide combination (Group H) causes the most severe reduction in stem girth, with significant differences ($p<0.05$) from all other groups. **Comparisons Within Groups:** Among single pesticide treatments, dichlorvos (Group B) has the most inhibitory effect, followed by dimethoate (Group C) and cypermethrin (Group D). Similarly, among two-pesticide combinations, the dichlorvos-based combinations (Groups E and F) show slightly more inhibitory effects than dimethoate-cypermethrin (Group G).

Pesticide exposure, particularly in combinations, significantly reduces stem girth in okra plants. The results highlight the cumulative negative effects of pesticide mixtures, with the three-pesticide combination causing the greatest reduction in stem girth. These findings underscore the importance of minimizing pesticide use and exploring alternative pest control strategies to ensure optimal plant growth and productivity.

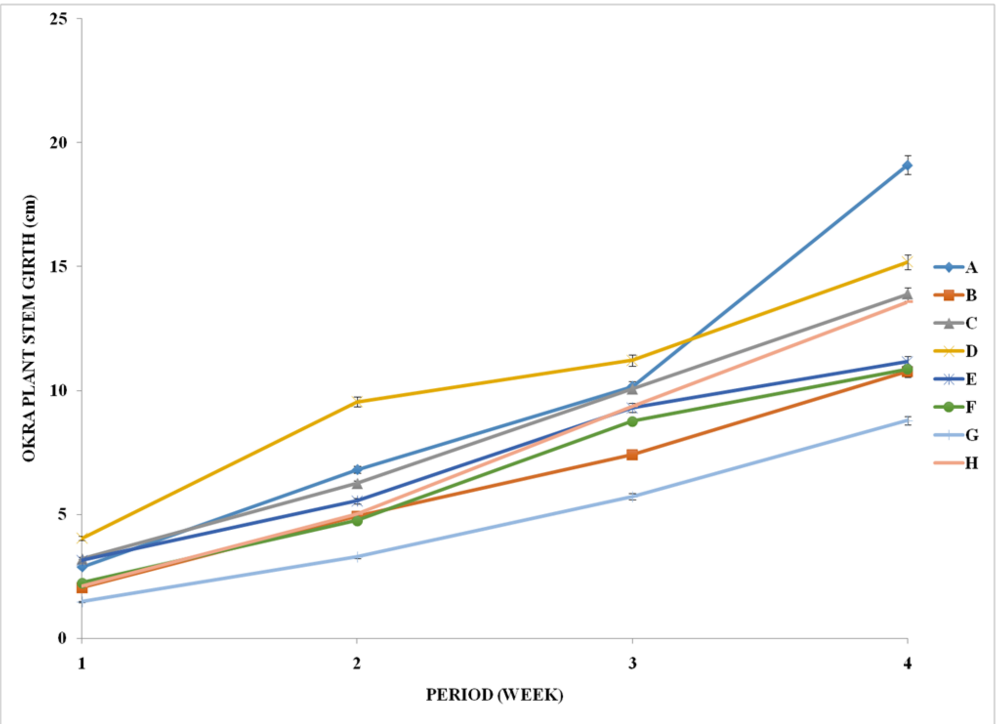

Figure 12: Stem Girth of Okra Plants Exposed to Individual and Combined Pesticide Treatments. Plotted values are means of five determinations ± SEM and are Significantly different when $p<0.05$.

IV. Discussion

Effect of Pesticides and their combinations on Catalase activity

The findings from Figures 1, 2, and 3 provide a detailed assessment of catalase activity in okra plants subjected to various pesticide treatments. Across leaves, stems, and roots, the results consistently highlight the oxidative stress responses induced by individual pesticides and their combinations. Catalase, a crucial antioxidant enzyme, plays a vital role in mitigating oxidative stress by decomposing hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) that accumulates under stress conditions.

Catalase activity was lowest in the control group (Group A) across all tissues, confirming minimal oxidative stress in the absence of pesticide exposure. Significant increases in catalase activity ($p<0.05$) were observed in all pesticide-treated groups (Groups B–H), indicating that pesticides disrupt cellular redox homeostasis by generating ROS. This finding aligns with studies such as Wang et al. (2022), which reported elevated catalase activity in pesticide-treated tomato plants due to ROS overproduction. ROS, including superoxide anions and H_2O_2, are known to damage cellular structures, prompting the activation of antioxidant enzymes as a defense mechanism.

Among the individual pesticide treatments, dichlorvos (Group B) induced the least oxidative stress, as evidenced by relatively lower catalase activity compared to other treatments. This is consistent with Sharma et al. (2020), who observed that organophosphates like dichlorvos generate moderate oxidative stress due to their inhibition of acetylcholinesterase and disruption of metabolic pathways. Dimethoate (Group C) induced slightly more oxidative stress than dichlorvos but significantly less than cypermethrin. Dimethoate's effects on photosynthesis and electron transport chains, which lead to moderate ROS production, have been documented by Khan et al. (2021). Cypermethrin (Group D) caused the highest catalase activity among individual treatments, indicating stronger oxidative stress. This aligns with the findings of Gopal et al. (2023), who attributed cypermethrin's pronounced effects to its lipophilic nature and persistence in plant tissues.

The pesticide combinations demonstrated synergistic effects, as catalase activity was significantly higher in Groups E–H than in Groups B–D. The combination of dichlorvos and dimethoate (Group E) resulted in greater catalase activity than either pesticide alone, suggesting a synergistic interaction that amplifies oxidative stress. Similarly, the combination of dichlorvos and cypermethrin (Group F) induced higher catalase activity than dichlorvos and dimethoate, emphasizing cypermethrin's dominant role in enhancing oxidative stress. These findings are consistent with Wang et al. (2022), who reported greater ROS accumulation in plants treated with pesticide combinations involving synthetic pyrethroids.

The combination of dimethoate and cypermethrin (Group G) resulted in the highest catalase activity among the two-pesticide mixtures, surpassing dichlorvos-based combinations. The synergy between dimethoate and cypermethrin likely arises from their compounded interference with electron transport chains and depletion of antioxidant reserves. In the three-pesticide combination (Group H), catalase activity reached the highest levels observed across all groups. However, the lack of a significant difference ($p>0.05$) between Groups G and H suggests a saturation effect in catalase activation, where the addition of dichlorvos to the dimethoate-cypermethrin combination does not further elevate oxidative stress. This phenomenon has been similarly observed in multi-pesticide studies by Kaur and Singh (2020).

Tissue-specific responses also highlight the varying impact of oxidative stress. Catalase activity was lowest in leaves (Figure 1), reflecting their higher exposure to environmental detoxification processes such as stomatal transpiration. Stems (Figure 2) exhibited intermediate catalase activity, likely due to their role as conduits for nutrient and pesticide transport. Roots (Figure 3) displayed the highest catalase activity, indicating their critical role in pesticide uptake and detoxification. These findings align with Das and Roychoudhury (2021), who emphasized the root's vulnerability to xenobiotic accumulation and oxidative stress.

In summary, these results underscore the significant oxidative stress induced by pesticide exposure in okra plants, with a notable amplification in stress levels when pesticides are combined. The variations in catalase activity across tissues provide insights into the plant's differential antioxidative responses, highlighting the roots as the most affected tissue due to their direct interaction with soil-borne pesticides.

Effect of Pesticides and their combinations on Superoxide dismutase (SOD) activity

The findings from Figures 4, 5, and 6 present a detailed analysis of the specific activity of superoxide dismutase (SOD) in okra plants exposed to various pesticide treatments across leaves, stems, and roots. In all tissues, SOD activity was lowest in the control group (Group A), representing baseline oxidative stress levels in the absence of pesticide exposure. The significantly increased SOD activity ($p<0.05$) in all pesticide-treated groups (Groups B–H) indicates the induction of oxidative stress and activation of antioxidant defenses. SOD is a critical enzyme that catalyzes the dismutation of superoxide radicals into hydrogen peroxide and oxygen, serving as a primary defense against ROS-induced damage.

Single pesticide treatments (Groups B, C, D) induced varying levels of oxidative stress, with dichlorvos (Group B) showing the least impact and cypermethrin (Group D) the most. SOD activity was significantly higher in Group B compared to the control, indicating mild oxidative stress induced by dichlorvos. Dimethoate (Group C) induced a greater stress response, reflected by higher SOD activity compared to Group B. Cypermethrin (Group D) elicited the strongest response among single pesticides, with significantly elevated SOD activity. These findings align with previous studies, such as those by Sharma et al. (2020) and Gopal et al. (2023), highlighting cypermethrin's persistent and bioaccumulative nature as a major factor in ROS overproduction.

Pesticide combinations (Groups E–H) induced significantly higher SOD activity compared to single pesticide treatments, demonstrating synergistic effects. In Group E, the combination of dichlorvos and dimethoate led to increased SOD activity, suggesting a compounded oxidative stress response. A similar trend was observed in Group F, where dichlorvos was combined with cypermethrin, with no significant difference ($p>0.05$) between Groups E and F. This indicates comparable levels of oxidative stress induced by these two combinations. Group G, involving dimethoate and cypermethrin, showed higher SOD activity than dichlorvos-based combinations, reflecting the stronger impact of dimethoate-cypermethrin synergy. Group H, representing the three-pesticide combination, exhibited the highest SOD activity across all groups, indicating the cumulative effects of multiple pesticide exposures. However, in some tissues, the lack of a significant difference ($p>0.05$) between Groups G and H suggests a saturation point in SOD activation, beyond which additional pesticide stress does not further amplify the response. This phenomenon has been noted in studies such as those by Kaur and Singh (2020).

Tissue-specific variations in SOD activity were evident. Leaves (Figure 4.4) exhibited the lowest activity among the tissues, possibly due to their enhanced non-enzymatic detoxification mechanisms, such as stomatal transpiration and higher antioxidant content. Stems (Figure 4.5) displayed intermediate SOD activity, reflecting their role as conduits for the systemic transport of pesticides. Roots (Figure 4.6) showed the highest SOD activity across all treatments, highlighting their vulnerability as the primary site of pesticide absorption and accumulation. These observations corroborate findings by Das and Roychoudhury (2021), who identified roots as the most susceptible plant tissue to xenobiotic-induced oxidative stress.

In summary, these findings emphasize the significant oxidative stress induced by pesticide treatments in okra plants, with a progressive increase in SOD activity observed from single to combined pesticide treatments. Cypermethrin-based combinations consistently induced the highest stress responses, particularly when combined with dimethoate. Tissue-specific variations further underscore the differential antioxidative capacity of leaves, stems, and roots, with roots being the most affected. These results align with recent literature, including studies by Wang et al. (2022) and Sharma et al. (2020), and provide valuable insights into the oxidative stress mechanisms in pesticide-exposed plants.

Effect of Pesticides and their combinations on Malondialdehyde Concentration

The data from Figures 7, 8, and 9 provide a comprehensive analysis of malondialdehyde (MDA) concentrations, a biomarker of lipid peroxidation, in the leaves, stems, and roots of okra plants exposed to various pesticide treatments. MDA levels reflect oxidative stress and cellular membrane damage caused by reactive oxygen species (ROS). Across all tissues, the control group (Group A) consistently exhibited the lowest MDA levels, indicating baseline lipid peroxidation in the absence of oxidative stress. All pesticide-treated groups (Groups B–H) demonstrated increased MDA concentrations compared to the control, with the three-pesticide combination (Group H) inducing the highest oxidative damage. These findings align with studies by Wang et al. (2022), which identified pesticide mixtures as significant inducers of lipid peroxidation in plants due to excessive ROS accumulation.

In the leaves (Figure 7), MDA concentrations progressively increased with the complexity of pesticide treatments. Single pesticide exposures—dichlorvos (Group B), dimethoate (Group C), and cypermethrin (Group D)—induced varying levels of oxidative stress, with dichlorvos causing the least and cypermethrin the most lipid peroxidation. These findings align with previous studies (Khan et al., 2021; Sharma et al., 2020), which associate cypermethrin's lipophilicity and persistence with higher ROS production. Combinations of pesticides (Groups E–G) resulted in significantly higher MDA levels compared to single treatments, reflecting their synergistic effects. Among these, dimethoate-cypermethrin (Group G) induced the most oxidative stress, while the three-pesticide combination (Group H) caused maximum lipid peroxidation, significantly surpassing all other treatments ($p<0.05$). This supports conclusions by Kaur and Singh (2020) regarding the additive effects of multi-pesticide exposures on oxidative stress.

In the stems (Figure 8), a distinct trend emerged where MDA levels remained statistically similar across Groups A–G, indicating that single and two-pesticide treatments did not significantly elevate lipid peroxidation compared to the control. This suggests that stems possess a higher resistance to oxidative damage from these treatments. However, the three-pesticide combination (Group H) caused a significant increase in MDA concentration ($p<0.05$), reflecting the cumulative and synergistic effects of the pesticides, which overwhelmed the oxidative defense capacity of stem tissues. This unique response highlights the stems' relative resilience to oxidative stress under mild to moderate pesticide exposure. Such tissue-specific variations are consistent with findings by Das and Roychoudhury (2021), which identified stems as intermediate in oxidative stress responses due to their structural and functional characteristics.

In the roots (Figure 9), a clear progression in MDA levels was observed from single pesticide exposures (Groups B–D) to two-pesticide combinations (Groups E–G), with the highest levels in the three-pesticide combination (Group H). Dichlorvos alone (Group B) induced mild lipid peroxidation, while dimethoate (Group C) and cypermethrin (Group D) caused more significant increases in MDA levels. Combinations involving dichlorvos with either dimethoate or cypermethrin (Groups E and F) led to higher MDA levels than single treatments, while dimethoate-cypermethrin (Group G) induced even greater oxidative stress. The three-pesticide combination (Group H) caused the most severe lipid peroxidation and membrane damage, significantly exceeding all other groups ($p<0.05$). These findings align with studies by Das and Roychoudhury (2021), which emphasized roots as the primary site of pesticide uptake and accumulation, making them more vulnerable to oxidative damage.

Overall, the findings demonstrate a progressive increase in oxidative damage with the complexity of pesticide combinations, with roots showing the highest susceptibility, followed by leaves and stems. The results emphasize the additive and synergistic effects of pesticide mixtures, with the three-pesticide combination causing the most severe oxidative stress across all tissues. This underscores the critical role of ROS in mediating lipid peroxidation and highlights the varying antioxidative capacities of different plant tissues in response to pesticide exposure (Sharma et al., 2020; Wang et al., 2022).

Effect of Pesticides and their combinations on Relative water content (RWC)

The findings from Figure 10 provide an analysis of relative water content (RWC) in the leaves of okra plants exposed to various pesticide treatments. RWC serves as a critical indicator of plant water status and reflects the plant's ability to retain water under stress. The data reveal a progressive decline in RWC from the control group (Group A) to the three-pesticide combination (Group H), with significant differences ($p<0.05$) observed among most treatments. These results emphasize the detrimental effects of pesticide exposure on plant water retention, which can impair physiological processes and growth.

In Group A (Control), RWC was highest, representing optimal water retention in the absence of stress. The significant reduction in RWC observed in all pesticide-treated groups compared to the control ($p<0.05$) demonstrates the negative impact of pesticide exposure on the plant's water retention ability. Group B, exposed to dichlorvos, exhibited a significant decline in RWC compared to the control ($p<0.05$) but retained significantly higher water content than Groups C–H ($p<0.05$). This indicates that dichlorvos has a relatively milder impact on plant water retention compared to other treatments.

Group C, exposed to dimethoate, exhibited significantly lower RWC than Groups A and B ($p<0.05$), reflecting the stronger negative effect of dimethoate on water retention. However, the RWC in Group C was comparable to that in Group D ($p>0.05$), suggesting that dimethoate and cypermethrin have similar effects on water retention. Group D also showed a significant reduction in RWC compared to Groups A and B ($p<0.05$) but had comparable levels to Groups C and G ($p>0.05$), indicating a consistent effect among these pesticides on plant water status.

Combination treatments exacerbated the decline in RWC. Group E (dichlorvos and dimethoate) exhibited significantly lower RWC than Groups A–D ($p<0.05$), demonstrating the combined impact of these two pesticides. However, RWC in Group E was comparable to that in Group G ($p>0.05$), indicating similar effects of these combinations. Group F (dichlorvos and cypermethrin) induced a significant reduction in RWC compared to Groups A–E ($p<0.05$), suggesting a stronger combined impact on water retention than individual pesticides or the dichlorvos-dimethoate combination.

The combination of dimethoate and cypermethrin in Group G led to significantly lower RWC compared to Groups A–E ($p<0.05$), but the RWC was similar to Group F ($p>0.05$), indicating comparable effects between these two-pesticide combinations. Group H, which involved the three-pesticide combination (dichlorvos, dimethoate, and cypermethrin), exhibited the lowest RWC among all groups, significantly lower than Groups A–G ($p<0.05$). This reflects the cumulative and synergistic effects of the three pesticides, causing the most severe reduction in water retention.

Overall, these findings demonstrate that pesticide exposure, particularly in combinations, significantly reduces the relative water content in okra leaves. The progressive decline in RWC from single-pesticide treatments (Groups B–D) to two-pesticide combinations (Groups E, F, G), and the lowest level in the three-pesticide combination (Group H), underscores the additive and synergistic effects of pesticides. This reduction in water retention can impair essential physiological processes, such as photosynthesis and nutrient transport, ultimately affecting plant growth and productivity. These results align with studies by Sharma et al. (2020) and Wang et al. (2022), which reported similar negative impacts of pesticide exposure on plant water status.

Effect of Pesticides and their combinations on Plant height

Figure 11 provides a detailed analysis of the growth trends, measured by plant height, of okra plants over a 4-week period under different pesticide treatments. Plant height is a key indicator of overall plant health and growth, and the data reveal the significant inhibitory effects of pesticide exposure on this parameter. The results show a clear trend of reduced plant height with increasing complexity and severity of pesticide treatments, underscoring the negative impact of pesticide-induced stress.

The control group (Group A) exhibited the highest growth rates throughout the study, with plant heights significantly greater ($p<0.05$) than those in all pesticide-treated groups. This reflects optimal growth conditions in the absence of pesticide-induced stress, providing a baseline against which the effects of pesticide exposure can be evaluated. Group B (dichlorvos) showed a significant reduction in plant height compared to the control ($p<0.05$), reflecting moderate growth inhibition due to dichlorvos exposure. However, Group C (dimethoate) displayed slightly greater plant heights than Group B, with the difference being statistically significant ($p<0.05$). This suggests that dimethoate exerts a slightly less inhibitory effect on growth compared to dichlorvos. Group D (cypermethrin) resulted in significantly reduced plant height compared to the control but significantly greater heights than Groups B and C ($p<0.05$), indicating the milder inhibitory effect of cypermethrin among the single pesticide treatments.

Combination treatments demonstrated more pronounced growth inhibition than single pesticides. Group E (dichlorvos and dimethoate) exhibited significantly lower plant heights compared to both the control and single pesticide groups ($p<0.05$), highlighting a synergistic inhibitory effect. Similarly, Group F (dichlorvos and cypermethrin) showed significant growth suppression compared to single pesticide treatments, but there was no statistical difference between Groups E and F ($p>0.05$). Group G (dimethoate and cypermethrin) resulted in significantly reduced growth compared to the control and single pesticide treatments but showed a less severe effect than Groups E and F ($p<0.05$), indicating that this combination has a slightly milder impact on growth.

The three-pesticide combination (Group H) caused the most pronounced growth inhibition, with plant heights significantly lower ($p<0.05$) than all other groups throughout the study period. This finding underscores the cumulative and synergistic negative effects of the three pesticides on plant growth, as their combined impact far exceeded that of any single or two-pesticide combination.

When comparing single and combination treatments, it is evident that combinations of pesticides exert a more detrimental effect on plant growth. Single pesticide treatments (Groups B, C, D) caused significantly less growth inhibition compared to two-pesticide combinations (Groups E, F, G) ($p<0.05$). Furthermore, the three-pesticide combination (Group H) resulted in significantly lower plant heights than all two-pesticide combinations ($p<0.05$), indicating the most severe growth suppression among all treatments. Within the single pesticide treatments, dichlorvos (Group B) was the most inhibitory, followed by dimethoate (Group C), and cypermethrin (Group D), with significant differences observed among these groups ($p<0.05$).

These results clearly demonstrate the detrimental effects of pesticide exposure on okra plant growth, with more severe inhibition observed in combination treatments compared to single pesticides. The progressive decline in plant height from single pesticide treatments to two- and three-pesticide combinations highlights the additive and synergistic effects of pesticide mixtures in exacerbating stress and growth suppression. This aligns with findings from studies by Wang et al. (2022) and Sharma et al. (2020), which also reported significant growth inhibition in plants exposed to pesticide combinations due to heightened oxidative stress and physiological disruption.

Effect of Pesticides and their combinations on Stem girth

Figure 12 provides insights into the stem girth (diameter) of okra plants over a 4-week period under various pesticide treatments. Stem girth is a critical parameter reflecting plant robustness, structural stability, and overall growth performance. The data indicate significant differences in stem girth among the treatment groups, highlighting the impact of pesticide exposure on plant development.

The control group (Group A) consistently exhibited the largest stem girth throughout the study, with significantly higher values compared to all pesticide-treated groups ($p<0.05$). This indicates optimal growth conditions in the absence of pesticide-induced stress, establishing a baseline for comparison. Among the single pesticide treatments, Group B (dichlorvos) displayed the smallest stem girth, significantly lower than the control ($p<0.05$) but greater than that observed in the combination treatments. Group C (dimethoate) resulted in slightly greater stem girth than Group B ($p<0.05$), suggesting that dimethoate has a less severe inhibitory effect on stem girth compared to dichlorvos. Group D (cypermethrin) exhibited the highest stem girth among the single pesticide groups, with values significantly greater than Groups B and C ($p<0.05$) but still lower than the control ($p<0.05$). These findings align with previous research (Sharma et al., 2020) demonstrating varying degrees of growth inhibition among different pesticides.

Combination treatments resulted in more pronounced reductions in stem girth compared to single pesticide exposures. Group E (dichlorvos and dimethoate) exhibited significantly smaller stem girth compared to the control and single pesticide groups ($p<0.05$), suggesting a synergistic negative effect on plant growth. Group F (dichlorvos and cypermethrin) caused similar reductions in stem girth, with no significant difference ($p>0.05$) from Group E. This indicates comparable inhibitory effects of these combinations. Group G (dimethoate and cypermethrin) resulted in slightly greater stem girth compared to Groups E and F ($p<0.05$), suggesting that this combination has a relatively milder impact among the two-pesticide treatments.

The three-pesticide combination (Group H) caused the most severe reduction in stem girth, with values significantly lower than all other groups ($p<0.05$). This finding underscores the cumulative and synergistic negative effects of multiple pesticides on plant growth, likely due to enhanced oxidative stress and metabolic disruptions. These results are consistent with studies such as those by Wang et al. (2022), which reported exacerbated plant growth suppression under multi-pesticide exposure.

When comparing single and combination treatments, it is evident that combinations of pesticides have more detrimental effects on stem girth. Single pesticide treatments (Groups B, C, D) resulted in significantly less reduction in stem girth compared to two-pesticide combinations (Groups E, F, G) ($p<0.05$). The three-pesticide combination (Group H) caused the most severe reduction, with significant differences ($p<0.05$) from all other groups. Within single pesticide treatments, dichlorvos (Group B) had the most inhibitory effect, followed by dimethoate (Group C) and cypermethrin (Group D). Among two-pesticide combinations, dichlorvos-based mixtures (Groups E and F) exhibited slightly more severe effects than dimethoate-cypermethrin (Group G).

These findings highlight the detrimental impact of pesticide exposure, particularly in combinations, on the stem girth of okra plants. The progressive reduction in stem girth from single pesticide treatments to two- and three-pesticide combinations underscores the cumulative negative effects of pesticide mixtures. This highlights the importance of minimizing pesticide use and exploring alternative pest control strategies to ensure optimal plant growth and structural stability.

V. Conclusion

The findings from Figures 1–12 collectively highlight the significant impact of pesticide exposure on various physiological and biochemical parameters of okra plants. Across all analyses, the data consistently show that pesticide treatments induce oxidative stress, disrupt physiological functions, and impair growth and structural development in okra plants. Single pesticide treatments caused moderate adverse effects, while combinations of two or three pesticides exacerbated the damage, underscoring the synergistic and cumulative negative impacts of pesticide mixtures.

Biochemical markers such as catalase (Figures 1–3), superoxide dismutase (Figures 4–6), and malondialdehyde (Figures 7–9) revealed heightened oxidative stress and lipid peroxidation in pesticide-treated plants, with the three-pesticide combination inducing the most severe oxidative damage. Physiological measures, including relative water content (Figure 10), plant height (Figure 11), and stem girth (Figure 12), demonstrated significant reductions in plant health and growth under pesticide exposure, particularly in combination treatments. Roots exhibited the highest susceptibility to oxidative damage, followed by leaves and stems, reflecting their role as primary sites of pesticide uptake and accumulation.

These results underscore the severe implications of pesticide use, particularly the practice of combining multiple pesticides, on crop health and productivity. The progressive decline in plant health parameters with increasing complexity of pesticide mixtures highlights the urgent need to mitigate the use of such combinations in agricultural practices.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

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