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Phytotoxic Effects Of Dichlorvos, Dimethoate, And Cypermethrin On Spinach (Spinacia Oleracea): Growth Dynamics, Oxidative Stress, And Antioxidant Responses

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Abstract
The use of pesticides, while crucial for pest management in agriculture, poses significant risks to crop health due to their phytotoxic effects. This study investigates the impact of dichlorvos, dimethoate, and cypermethrin—applied individually and in combinations—on the physiological and biochemical responses of spinach (Spinacia oleracea). A greenhouse experiment was conducted using a factorial design, with eight treatment groups including a control. Growth parameters such as plant height and stem girth were monitored, alongside biochemical indicators like relative water content (RWC), antioxidant enzyme activities (catalase and superoxide dismutase), and malondialdehyde (MDA) concentration, a marker of oxidative stress. The results revealed significant adverse effects of pesticides on spinach growth and biochemistry. The control group exhibited the highest growth metrics and the lowest oxidative stress markers, emphasizing the benefits of pesticide-free conditions. Single-pesticide treatments moderately inhibited growth and increased oxidative stress, with cypermethrin showing the greatest impact. Two-pesticide combinations further amplified these effects, while the three-pesticide mixture demonstrated severe toxicity, as evidenced by reduced growth, decreased RWC, and elevated MDA levels. Antioxidant enzyme activities increased progressively with pesticide complexity, reflecting the plants' response to escalating oxidative stress. These findings underscore the compounded phytotoxic effects of pesticide combinations, particularly those involving cypermethrin. The study highlights the need for sustainable pest management practices to minimize chemical use and mitigate their negative impacts on crop health. This research contributes to the growing body of evidence advocating for integrated pest management and the exploration of alternative pest control strategies.

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I. Introduction

Spinach (Spinacia oleracea), a nutritionally rich and widely cultivated leafy vegetable, has long been recognized for its essential contributions to human health. Packed with vitamins, minerals, antioxidants, and dietary fiber, spinach forms a significant component of diets across the globe. However, its productivity and quality are increasingly threatened by the widespread use of agrochemicals, including pesticides, which are integral to modern agricultural practices. While these chemicals serve a critical role in pest management and ensuring food security, their indiscriminate or excessive use has been linked to adverse effects on crops, including the induction of oxidative stress, disruption of physiological processes, and subsequent yield reductions (Singh et al., 2023; Zhang et al., 2021).

Pesticides such as dichlorvos, dimethoate, and cypermethrin are commonly applied in agricultural systems due to their efficacy in controlling a broad spectrum of pests. Despite their benefits, studies have shown that these chemicals can adversely affect plant health by generating reactive oxygen species (ROS). These ROS trigger oxidative stress, characterized by cellular damage to lipids, proteins, and DNA, and impair key physiological functions, including photosynthesis and water regulation (Kumar et al., 2022; Chen et al., 2023). Such stress responses not only affect the growth and development of crops but also raise concerns about food safety and environmental sustainability. Additionally, the combined application of pesticides can lead to synergistic toxic effects, further amplifying the stress burden on plants and complicating management strategies (Rajendran et al., 2023).

This study investigates the phytotoxic effects of single and combined applications of dichlorvos, dimethoate, and cypermethrin on spinach, focusing on key growth parameters such as plant height and stem girth, biochemical indicators like antioxidant enzyme activities (catalase and superoxide dismutase), and markers of oxidative damage such as malondialdehyde (MDA). These parameters provide a comprehensive understanding of how these pesticides, individually and in mixtures, affect the physiological and biochemical responses of spinach plants. The factorial experimental design employed in this research simulates real-world pesticide usage patterns, providing valuable insights into their synergistic or antagonistic interactions under agricultural conditions.

Moreover, this study addresses critical knowledge gaps by examining the effects of pesticide combinations at a mechanistic level. While single-pesticide applications have been extensively studied, research on the combined effects of multiple pesticides remains limited. Such combinations, as applied in agricultural practices, often result in unexpected outcomes due to their interactions within the plant system. These effects could significantly alter antioxidant defense mechanisms, relative water content, and overall growth dynamics, as highlighted in previous studies (Albuquerque et al., 2021; Singh et al., 2023). Therefore, the findings from this study not only contribute to a deeper understanding of pesticide-induced phytotoxicity but also emphasize the urgent need for sustainable pest management practices.

The broader implications of this research lie in its potential to inform policymakers, farmers, and researchers about the risks associated with pesticide overuse and combinations. By elucidating the specific and combined effects of dichlorvos, dimethoate, and cypermethrin on spinach, this study underscores the importance of developing integrated pest management strategies that minimize chemical reliance and promote agricultural sustainability. Furthermore, it advocates for exploring alternative pest control measures, such as biopesticides and crop rotation, to mitigate the environmental and health risks associated with synthetic pesticides. Ultimately, the study contributes to the growing discourse on achieving a balance between agricultural productivity and ecological conservation in the face of increasing global food demands.

II. Materials And Methods

Reagents and solvents were of analytical grade and are products of British Drug House, Poole, England.

Experimental design and agronomic details

The experiment was conducted in a greenhouse of the College of Science, Federal University of Petroleum Resources, Effurun, Nigeria. The method described by Adewole and Aboyeji (2003) though slightly modified was used. Bulk surface soil samples (0-15 cm) were collected from an area in the University, air-dried for seven days, sieved using 2 mm sieve and analysed using standard methods. Thirty-two polythene pots with drainage holes at the bottom, each containing 10 kg of surface soil, were randomly placed on a table in the greenhouse in a factorial combination of eight treatment levels.

The seed used in this experiment was obtained from the Ministry of Agriculture Effurun in Delta State, Nigeria. A seed viability test was done by procedure described by Radwan et al., (2018)

Dichlorvos, dimethoate, and cypermethrin, all obtained from Hubei Sanonda Co. Ltd, China, were used in this study. These agrochemicals were purchased from a licensed agrochemical shop and diluted with clean water as per domestic usage guidelines. The dose combinations were prepared in specific ratios for each experimental group. For group B, C, and D, the chemicals were used individually at a 1:1 dilution. For groups E, F, and G, a combination of two chemicals was prepared in a 1:0.5:0.5 ratio. For group H, a mixture of all three chemicals was prepared in a 1:0.33:0.33:0.33 ratio. These freshly prepared solutions were used daily for a continuous exposure period of 28 days.

The solutions were sprayed simulating conditions of agricultural pesticide use. A control group (Group A) was maintained, exposed only to water sprayed in the same manner.

The treatment groups were as follows:
Group A (Control): Exposed to sprayed water.
Group B: Exposed to dichlorvos.
Group C: Exposed to dimethoate.
Group D: Exposed to cypermethrin.
Group E: Exposed to a mixture of dichlorvos and dimethoate.
Group F: Exposed to a mixture of dichlorvos and cypermethrin.
Group G: Exposed to a mixture of dimethoate and cypermethrin.
Group H: Exposed to a mixture of dichlorvos, dimethoate, and cypermethrin.

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

The plant stands were regularly watered throughout the growing stage. The plants were thinned to two stands per pot at two weeks after planting (WAP). The thinned stands were retained inside the pots from which they were removed so as to put back into the soil what might have been taken up by the plant within the first two weeks of growth. Every four days, growth parameters of plant such as plant height and stem girth were measured till when the experiment was terminated. Afterwards, the leaves, stems and roots were separated and homogenized for toxicological analysis.

Relative water content (RWC) of leaves was determined at 4 WAP by the standard method (Schonfeld, *et al*; 1988). The protein concentration in the tissue of experimental plants was determined following the method reported by Gornal *et al* (1949). The SOD activity of the tissues of experimental plants was determined following the method described by Misra and Fridovich (1972). The catalase activity of the tissue homogenate obtained from the experimental plants was determined following the method described by Sinha (1971). The MDA concentration in the serum and tissues of plants was determined following the method described by Bird *et al.* (1982).

Statistical Analysis

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 expressed as means ± standard error of the mean (SEM).

Statistical comparisons among treatment groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to identify significant differences between group means. The level of significance was set at $p<0.05$. All analyses were conducted using SPSS version 30. Graphs and figures were generated to visualize the differences and trends across treatment groups, highlighting the impact of individual and combined pesticide exposures on spinach physiology and biochemistry.

III. Results

The attached Figure 1 depicts the spinach plant height across four weeks for eight experimental groups exposed to various pesticide treatments.

Group A (Control), which was sprayed with water, showed the highest plant height throughout the period, significantly differing ($p<0.05$) from all other groups exposed to pesticides by Week 4. This highlights the detrimental effects of pesticides on spinach growth compared to optimal growth conditions in the absence of pesticides.

Group H, exposed to a mixture of dichlorvos, dimethoate, and cypermethrin, demonstrated the least growth across all weeks, significantly differing ($p<0.05$) from the control and all other groups. This indicates the synergistic phytotoxic effects of the combined pesticides.

For the groups exposed to single pesticides, Groups B (dichlorvos), C (dimethoate), and D (cypermethrin), their growth patterns were intermediate. By Week 4, Group B had slightly better growth than Groups C and D, but the differences among these three groups were not significantly different ($p>0.05$). However, these single-pesticide groups still showed significant differences ($p<0.05$) in growth compared to the control.

In the groups exposed to two-pesticide combinations (Groups E, F, and G), reduced growth was observed compared to the control. Group E (dichlorvos and dimethoate) exhibited slightly better growth than Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin), though the differences among these three groups were not significantly different ($p>0.05$). However, all three groups significantly differed ($p<0.05$) from both the control and Group H.

These findings indicate significant differences ($p<0.05$) between the control and all pesticide-treated groups, while not significantly different ($p>0.05$) outcomes were observed within groups exposed to certain single or combined pesticide treatments. This result suggests that while pesticides adversely impact spinach growth, the severity depends on the specific pesticide or combination used.

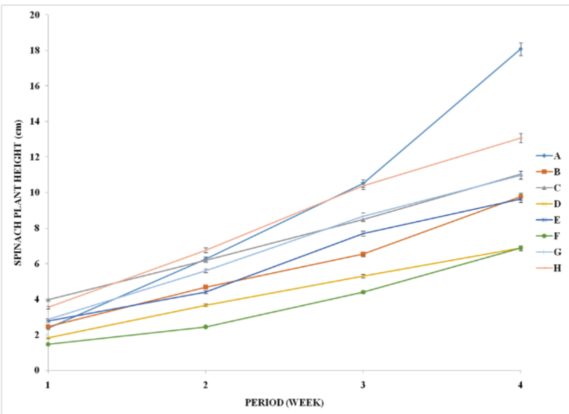

Figure 1: Effect of Single and Combined Pesticide Exposures on Spinach Plant Height Over Four Weeks. Plotted values are means of five determinations ± SEM and are considered significantly different when $p<0.05$.

Figure 2 illustrates the changes in stem girth of spinach plants over four weeks across eight experimental groups subjected to various pesticide treatments.

Group A (Control), which was sprayed with water, consistently exhibited the highest stem girth throughout the experimental period, showing a significant difference ($p<0.05$) compared to all pesticide-treated groups by Week 4. This underscores the adverse effects of pesticide exposure on the stem development of spinach plants.

Group H, exposed to a mixture of dichlorvos, dimethoate, and cypermethrin, had the smallest stem girth among all groups, significantly different ($p<0.05$) from both the control and groups exposed to single pesticides or two-pesticide combinations. This result suggests severe synergistic toxic effects when all three pesticides are combined.

Groups B (dichlorvos), C (dimethoate), and D (cypermethrin), exposed to single pesticides, showed moderate stem girth. Among these, Group B displayed slightly better growth, followed by Groups C and D, though the differences in stem girth among these groups were not significantly different ($p>0.05$). However, they were all significantly different ($p<0.05$) from the control.

The groups exposed to two-pesticide combinations (Groups E, F, and G) also showed suppressed stem girth compared to the control, but their performances varied. Group E (dichlorvos and dimethoate) demonstrated slightly higher stem girth compared to Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin). However, these differences among the two-pesticide groups were not significantly different ($p>0.05$). All three two-pesticide groups were significantly different ($p<0.05$) from the control and Group H.

The data show that the control group significantly outperformed all pesticide-treated groups ($p<0.05$), highlighting the negative impact of pesticide exposure on spinach stem girth. Groups treated with single pesticides or two-pesticide mixtures showed intermediate growth, with no significant differences ($p>0.05$) within these groups. Group H, exposed to a three-pesticide combination, exhibited the worst growth, significantly differing ($p<0.05$) from all other groups, demonstrating the compounded toxicity of multiple pesticide exposure.

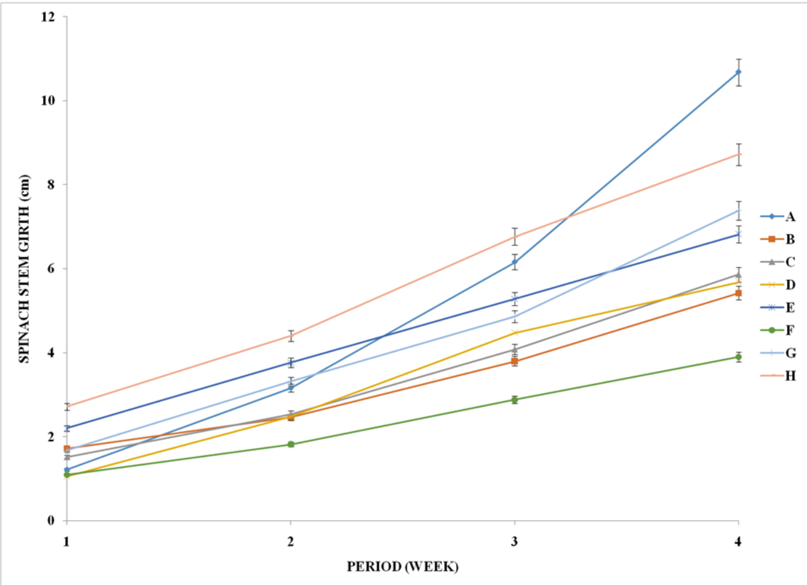

Figure 2: Effect of Single and Combined Pesticide Exposures on Spinach Stem Girth Over Four Weeks. Plotted values are means of five determinations ± SEM and are considered significantly different when $p<0.05$.

Figure 3 illustrates the relative water content (RWC) of spinach plant leaves across eight experimental groups subjected to different pesticide treatments.

Group A (Control), which was sprayed with water, exhibited the highest relative water content, close to 100%. This result is significantly different ($p<0.05$) compared to all groups exposed to pesticides, highlighting that the absence of pesticide stress allows optimal water retention in spinach leaves.

Groups B (dichlorvos), C (dimethoate), and D (cypermethrin), treated with single pesticides, also maintained relatively high water content, with values comparable to the control (Group A). There were no significant differences ($p>0.05$) between these groups and the control, indicating that single pesticides might not drastically affect water retention.

In contrast, the groups exposed to two-pesticide combinations (Groups E, F, and G) showed a noticeable decline in water content, significantly differing ($p<0.05$) from the control group and groups exposed to single pesticides. Among these, the relative water content was comparable within these groups, as no significant differences ($p>0.05$) were observed between them.

Group H, exposed to a combination of dichlorvos, dimethoate, and cypermethrin, had the lowest relative water content among all groups. This reduction was significantly different ($p<0.05$) compared to all other groups, including those exposed to single and two-pesticide combinations. This result underscores the cumulative phytotoxic effect of combined pesticide treatments, leading to impaired water retention in spinach leaves.

The data reveal significant differences ($p<0.05$) in the relative water content between the control and all pesticide-treated groups, with Group H exhibiting the most severe reduction. Groups exposed to single pesticides (B, C, and D) retained water levels similar to the control ($p>0.05$), while groups treated with two-pesticide mixtures (E, F, and G) had intermediate reductions. These findings highlight the compounded negative effects of multiple pesticides on the water retention capability of spinach leaves, with the most detrimental impact observed in the group exposed to three pesticides.

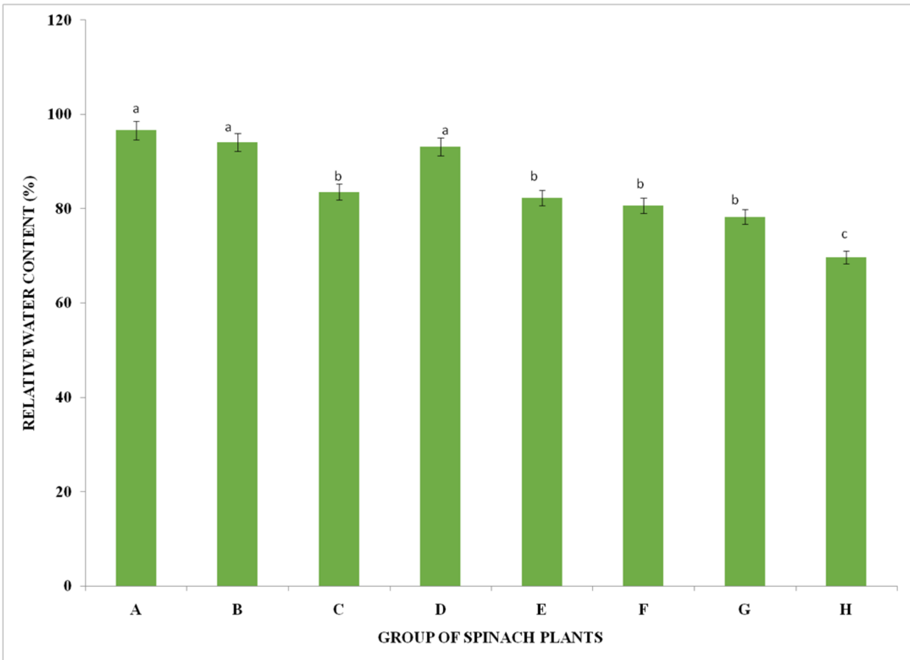

Figure 3: Effect of Individual and Combined Pesticide Exposures on Relative Water Content in Leaves of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 4 shows the specific activity of catalase in the leaves of spinach plants across eight experimental groups exposed to different pesticide treatments. Catalase is a crucial antioxidant enzyme that helps mitigate oxidative stress by breaking down hydrogen peroxide into water and oxygen.

Group A (Control), which was sprayed with water, exhibited the highest specific activity of catalase, significantly differing ($p<0.05$) from all pesticide-treated groups. This indicates that the plants in the control group experienced minimal oxidative stress compared to those exposed to pesticides.

Groups B (dichlorvos), C (dimethoate), and E (dichlorvos and dimethoate) showed relatively high catalase activities compared to other pesticide-treated groups, although their activities were still significantly lower ($p<0.05$) than the control. The lack of significant differences ($p>0.05$) among these three groups suggests that single or two-pesticide combinations involving dichlorvos and dimethoate might induce moderate oxidative stress in plants.

Group D (cypermethrin) exhibited a noticeable decline in catalase activity compared to the control and Groups B, C, and E, with the reduction being significantly different ($p<0.05$). This suggests that exposure to cypermethrin alone imposes higher oxidative stress on plants than dichlorvos or dimethoate.

Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin), exposed to two-pesticide combinations involving cypermethrin, demonstrated further reduced catalase activities. The reduction in activity was significantly different ($p<0.05$) from the control and Groups B, C, D, and E. This indicates that combinations involving cypermethrin exacerbate oxidative stress in spinach plants.

Group H, exposed to a mixture of dichlorvos, dimethoate, and cypermethrin, showed the lowest catalase activity among all groups. The reduction was significantly different ($p<0.05$) compared to all other groups, highlighting the severe oxidative stress induced by the combination of all three pesticides.

The data reveal a clear trend of reduced catalase activity with increasing pesticide complexity, suggesting that exposure to pesticides induces oxidative stress in spinach plants. The control group (A) showed the highest catalase activity, reflecting minimal oxidative stress. Groups exposed to single pesticides or two-pesticide mixtures involving dichlorvos and dimethoate exhibited moderate reductions in activity, while groups involving cypermethrin showed more severe declines. The lowest catalase activity was observed in Group H, exposed to all three pesticides, underscoring the compounded oxidative stress induced by multiple pesticide exposure.

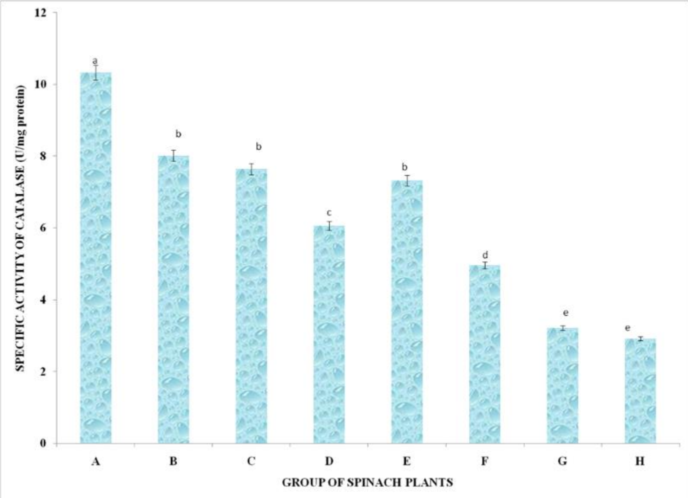

Figure 4: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Leaves of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 5 shows the specific activity of catalase in the stems of spinach plants across eight experimental groups exposed to different pesticide treatments. Catalase is a critical antioxidant enzyme that helps alleviate oxidative stress by decomposing hydrogen peroxide into water and oxygen.

Group A (Control), which was sprayed with water, exhibited the highest specific catalase activity in the stems, along with Groups B (dichlorvos) and C (dimethoate). The differences in catalase activity among these groups were not significantly different ($p>0.05$), indicating that the control and these single-pesticide treatments induced minimal oxidative stress on spinach stems.

Group D (cypermethrin) displayed slightly reduced catalase activity compared to the control and Groups B and C, with the difference being significantly different ($p<0.05$). This indicates a higher oxidative stress response induced by cypermethrin compared to dichlorvos and dimethoate.

Group E (dichlorvos and dimethoate) had catalase activity that was comparable to Group D and showed a significant difference ($p<0.05$) compared to the control and Groups B and C. This suggests that the combination of these pesticides moderately increases oxidative stress.

Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin) exhibited further reductions in catalase activity, significantly different ($p<0.05$) from the control and Groups B, C, and E. This highlights the amplified oxidative stress effects when cypermethrin is included in pesticide mixtures.

Group H, exposed to a combination of dichlorvos, dimethoate, and cypermethrin, demonstrated the lowest catalase activity among all groups. The difference in activity was significantly different ($p<0.05$) from all other groups, indicating the severe oxidative stress induced by the combined exposure to these three pesticides.

The data reveal that exposure to pesticides induces oxidative stress in spinach stems, as reflected by the variations in catalase activity. The control group (A) and groups exposed to single pesticides (B and C) showed the highest catalase activity, indicating minimal stress. Groups exposed to combinations of two pesticides (D, E, F, and G) exhibited progressively lower catalase activity, with combinations involving cypermethrin (F and G) showing more significant reductions. Group H, treated with all three pesticides, had the lowest catalase activity, emphasizing the compounded oxidative stress caused by multiple pesticide exposures. The results suggest that cypermethrin, especially in mixtures, has a strong impact on oxidative stress in spinach stems.

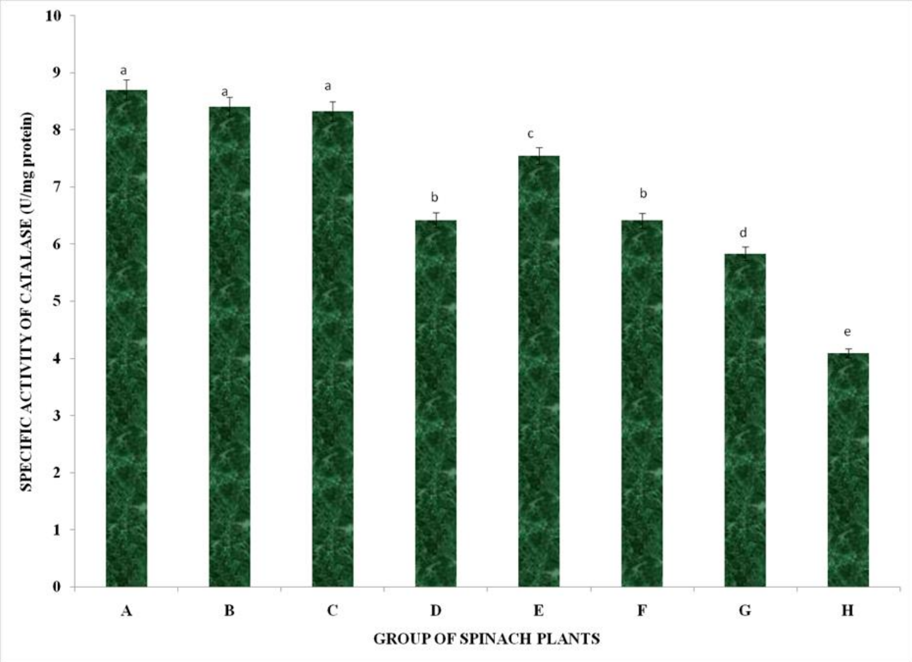

Figure 5: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Stems of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 6 depicts the specific activity of catalase in the roots of spinach plants across eight experimental groups exposed to various pesticide treatments. Catalase activity serves as a key indicator of oxidative stress, where higher activity suggests a protective response to stress-induced reactive oxygen species (ROS).

Group A (Control): The highest specific activity of catalase was observed in the control group, significantly different (p<0.05) from all pesticide-treated groups. This result reflects the optimal antioxidant state in the absence of pesticide-induced stress.

Group B (dichlorvos): Catalase activity in Group B was the second highest and significantly lower (p<0.05) than the control. This indicates moderate oxidative stress induced by exposure to dichlorvos, which was effectively countered by catalase activity.

Group C (dimethoate): Catalase activity decreased further compared to Group B, with a significant difference (p<0.05). This reduction suggests that dimethoate imposes more oxidative stress on spinach roots than dichlorvos.

Group D (cypermethrin): Catalase activity in Group D was lower than Groups B and C, showing a significant reduction (p<0.05). This indicates that cypermethrin generates higher oxidative stress in the roots compared to single exposures of dichlorvos or dimethoate.

Group E (dichlorvos and dimethoate): The catalase activity in Group E decreased further and was significantly lower (p<0.05) than the control and Groups B, C, and D. This reflects compounded oxidative stress due to the combined effect of dichlorvos and dimethoate.

Group F (dichlorvos and cypermethrin): Catalase activity in Group F was lower than Group E, with a significant difference (p<0.05). This suggests an intensified stress response due to the presence of cypermethrin in the mixture.

Groups G (dimethoate and cypermethrin) and H (dichlorvos, dimethoate, and cypermethrin): Both groups exhibited the lowest catalase activity, significantly different (p<0.05) from all other groups. These results indicate severe oxidative stress induced by the combined effects of dimethoate and cypermethrin in Group G, and all three pesticides in Group H. The minimal catalase activity in these groups suggests an overwhelmed or inhibited antioxidant response under severe stress conditions.

The data indicate a progressive reduction in catalase activity in spinach roots with increasing pesticide complexity. The control group displayed the highest activity, indicating minimal oxidative stress. Single-pesticide groups (B, C, and D) exhibited moderate reductions, with cypermethrin inducing the most significant stress among single exposures. Combined pesticide treatments (E, F, G, and H) resulted in progressively lower catalase activity, with Group H showing the most severe inhibition, likely due to cumulative toxicity. These findings highlight the detrimental effects of pesticide exposure on antioxidant defense mechanisms in spinach roots, particularly under combined or multiple pesticide treatments.

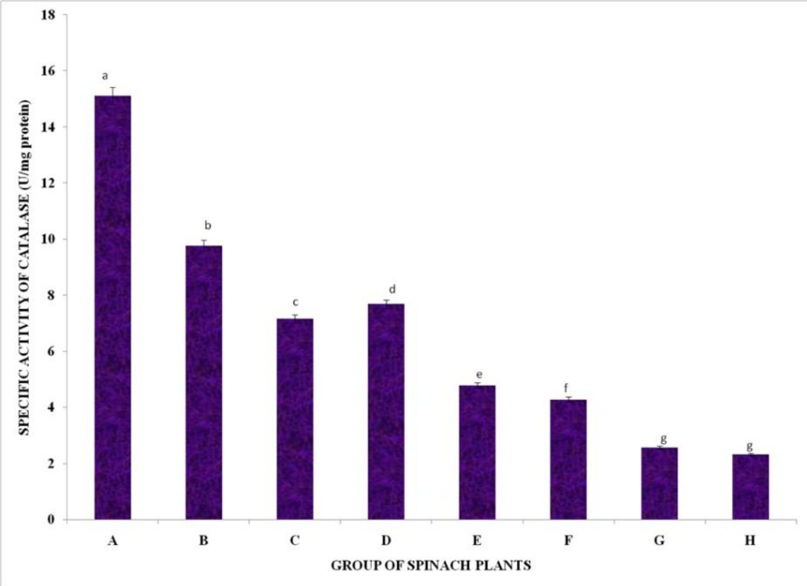

Figure 6: Effect of Individual and Combined Pesticide Exposures on Catalase Activity in Roots of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 7 illustrates the specific activity of superoxide dismutase (SOD) in the leaves of spinach plants across eight experimental groups subjected to different pesticide treatments. SOD is a key antioxidant enzyme that plays a crucial role in mitigating oxidative stress by converting superoxide radicals into hydrogen peroxide and oxygen.

The control group (Group A), sprayed with water, exhibited the lowest SOD activity among all groups. This activity was significantly different ($p<0.05$) from the pesticide-treated groups, reflecting minimal oxidative stress in plants without pesticide exposure. The low SOD activity in the control group indicates that the plants were not under stress, maintaining their antioxidant systems at baseline levels.

Group B, exposed to dichlorvos, showed a significant increase in SOD activity compared to the control group ($p<0.05$), suggesting an oxidative stress response due to pesticide exposure. However, the SOD activity in this group was lower than in groups treated with other pesticides, indicating that dichlorvos induced relatively mild oxidative stress.

Groups C and D, exposed to dimethoate and cypermethrin respectively, demonstrated moderate SOD activity. The activity levels in these groups were significantly higher ($p<0.05$) than in Group B but were not significantly different ($p>0.05$) from each other. This suggests that dimethoate and cypermethrin impose a comparable oxidative stress burden, higher than dichlorvos alone.

Group E, which was exposed to a combination of dichlorvos and dimethoate, exhibited further increased SOD activity. This activity was significantly different ($p<0.05$) from Groups B, C, and D, reflecting a stronger oxidative stress response when these two pesticides were combined. The elevated SOD activity indicates that the plants were under greater stress, requiring an enhanced antioxidant response.

In Group F, exposed to dichlorvos and cypermethrin, SOD activity was higher than in Group E and significantly different ($p<0.05$) from Groups B through E. This suggests that the combination of dichlorvos and cypermethrin imposes a more pronounced oxidative stress burden on the plants. Similarly, Group G, exposed to dimethoate and cypermethrin, showed an even greater increase in SOD activity, significantly different ($p<0.05$) from all groups except Group H. This highlights the severe oxidative stress caused by the interaction of dimethoate and cypermethrin.

Group H, exposed to a combination of dichlorvos, dimethoate, and cypermethrin, exhibited the highest SOD activity among all groups, significantly different ($p<0.05$) from all others. This finding reflects the extreme oxidative stress induced by the combined exposure to all three pesticides, likely due to synergistic toxic effects that overwhelmed the plants' antioxidant defense systems.

In summary, the data show a clear trend of increasing SOD activity with greater pesticide complexity. The control group displayed the lowest activity, indicative of minimal stress. Single-pesticide exposures induced moderate oxidative stress, with dichlorvos being the least impactful. Two-pesticide combinations significantly elevated SOD activity, with combinations involving cypermethrin having a more pronounced effect. The highest activity in Group H underscores the severe oxidative stress caused by the combined exposure to all three pesticides, emphasizing the synergistic toxicity of multiple pesticide treatments.

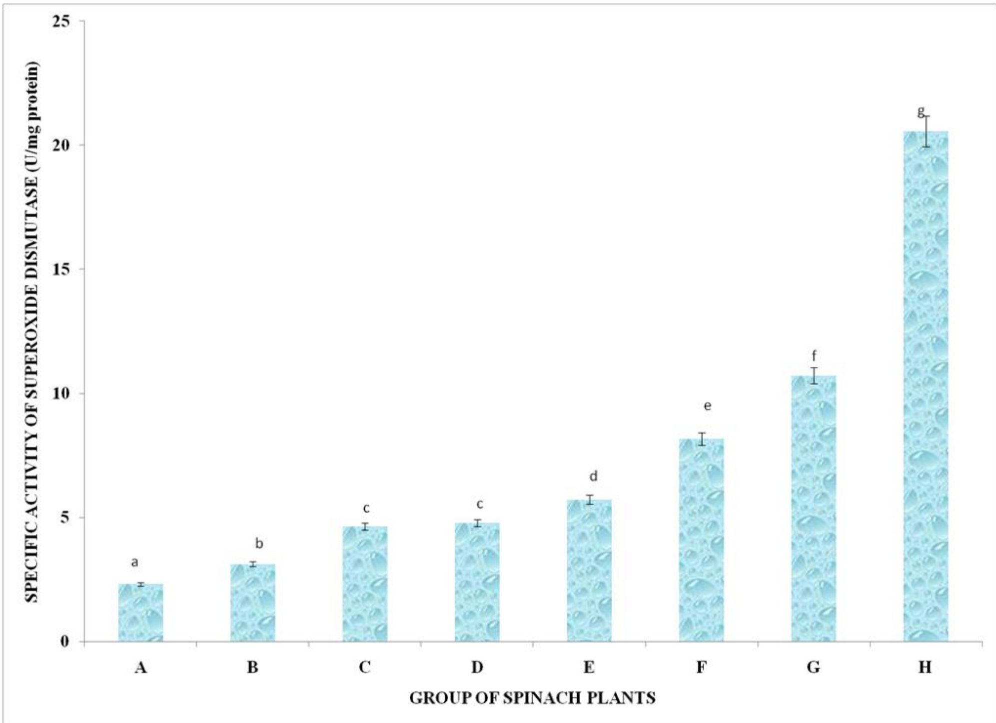

Figure 7: Effect of Individual and Combined Pesticide Exposures on Superoxide Dismutase Activity in Leaves of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 8 depicts the specific activity of superoxide dismutase (SOD) in the stems of spinach plants across eight experimental groups exposed to various pesticide treatments. SOD is a critical antioxidant enzyme that plays a key role in reducing oxidative stress by converting superoxide radicals into less reactive molecules, such as hydrogen peroxide and oxygen.

In the control group (Group A), the SOD activity was the lowest among all groups, significantly different (p<0.05) from the pesticide-treated groups. This reflects the absence of oxidative stress in the control plants, as they were not exposed to any pesticides.

Group B, treated with dichlorvos, showed a slight increase in SOD activity compared to the control, which was significantly different (p<0.05). This indicates the initiation of an oxidative stress response, although the effect was relatively mild compared to other pesticide treatments.

Groups C (dimethoate) and D (cypermethrin) exhibited moderate increases in SOD activity. While the SOD activity in these groups was significantly higher than in Groups A and B (p<0.05), it was still lower than in groups exposed to pesticide mixtures. The difference in activity between Groups C and D was not significant (p>0.05), indicating comparable oxidative stress induced by dimethoate and cypermethrin individually.

Groups E (dichlorvos and dimethoate) and F (dichlorvos and cypermethrin) displayed higher SOD activity compared to the single-pesticide groups. Their activity levels were significantly different (p<0.05) from Groups A through D but not significantly different (p>0.05) from each other. This suggests that the combination of pesticides in these groups caused greater oxidative stress, requiring an enhanced antioxidant response.

Group G, treated with dimethoate and cypermethrin, showed a substantial increase in SOD activity, which was significantly higher (p<0.05) than all other groups except Group H. This indicates a more severe oxidative stress response when these two pesticides are combined, likely due to synergistic effects.

Group H, exposed to all three pesticides (dichlorvos, dimethoate, and cypermethrin), had the highest SOD activity among all groups, significantly different (p<0.05) from all others. This result reflects the severe oxidative stress imposed by the combined effects of all three pesticides, overwhelming the spinach plants' antioxidant defenses.

The data show a progressive increase in SOD activity with increasing pesticide complexity, reflecting higher oxidative stress levels. The control group had the lowest activity, while single-pesticide treatments induced mild to moderate oxidative stress. Combined pesticide treatments caused significantly higher stress, with the highest SOD activity observed in Group H, highlighting the compounded oxidative effects of multiple pesticide exposures. This trend underscores the critical role of SOD in mitigating oxidative damage in spinach stems under pesticide stress conditions.

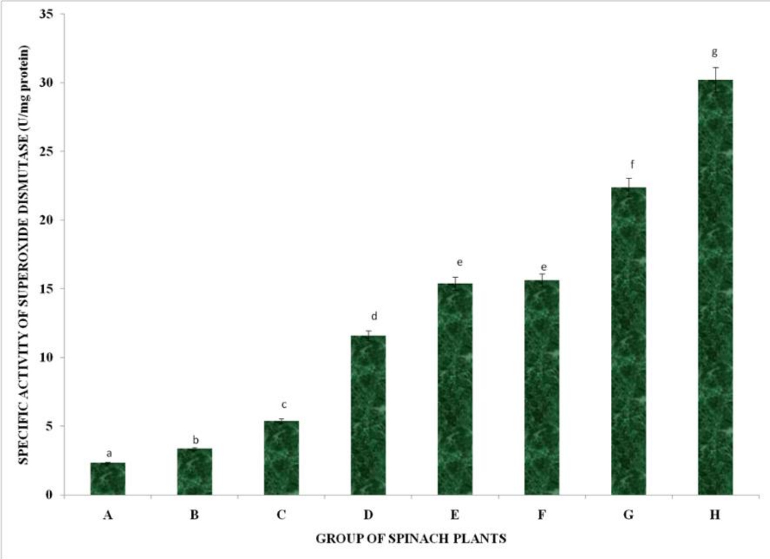

Figure 8: Effect of Individual and Combined Pesticide Exposures on Superoxide Dismutase Activity in Stems of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 9 presents the specific activity of superoxide dismutase (SOD) in the roots of spinach plants across eight experimental groups subjected to varying pesticide treatments. SOD activity reflects the antioxidant defense response to oxidative stress, with higher activity indicating increased stress levels.

The control group (Group A), which was not exposed to any pesticides, exhibited the highest SOD activity, statistically comparable to Group B (p>0.05). This high activity reflects a stable antioxidant system in plants under optimal conditions or mild oxidative stress.

Group B, treated with dichlorvos, also showed high SOD activity, which was not significantly different (p>0.05) from the control. This indicates that dichlorvos exposure induced minimal oxidative stress in spinach roots, and the plants' antioxidant mechanisms effectively mitigated it.

Group C, exposed to dimethoate, displayed a notable decrease in SOD activity compared to Groups A and B, with the difference being statistically significant (p<0.05). This suggests that dimethoate imposed a higher oxidative stress burden than dichlorvos, leading to a decline in antioxidant efficiency.

Group D, exposed to cypermethrin, exhibited a further decline in SOD activity, significantly

lower (p<0.05) than Groups A, B, and C. This reduction highlights that cypermethrin induced more oxidative stress in spinach roots than dichlorvos or dimethoate, reducing the plants' ability to maintain high SOD activity.

Group E, which was treated with a combination of dichlorvos and dimethoate, demonstrated a significant decrease in SOD activity compared to Groups A through D (p<0.05). This reflects the increased oxidative stress caused by the interaction of the two pesticides, overwhelming the antioxidant defense system to a greater extent than single pesticide exposures.

Group F, exposed to dichlorvos and cypermethrin, showed a further reduction in SOD activity, significantly different (p<0.05) from Groups A through E. The inclusion of cypermethrin in the combination appears to exacerbate oxidative stress, reducing the efficiency of SOD as an antioxidant defense.

Group G, treated with dimethoate and cypermethrin, exhibited a substantial decline in SOD activity, significantly lower (p<0.05) than Groups A through F. This indicates that the combination of dimethoate and cypermethrin imposed a severe oxidative stress burden on the spinach roots, further impairing the plants' antioxidant response.

Group H, exposed to a combination of dichlorvos, dimethoate, and cypermethrin, had the lowest SOD activity among all groups, significantly different (p<0.05) from all other treatments. This finding underscores the compounded oxidative stress caused by the simultaneous exposure to all three pesticides, severely inhibiting the antioxidant defense mechanism in spinach roots.

The data show a clear trend of declining SOD activity with increasing pesticide complexity, reflecting escalating oxidative stress levels. The control and dichlorvos-treated groups displayed the highest activity, indicative of minimal stress. Dimethoate and cypermethrin individually caused moderate declines, while combinations of pesticides, particularly those involving cypermethrin, led to significantly reduced SOD activity. The lowest activity in Group H highlights the synergistic toxicity of the three pesticides, emphasizing the severe oxidative stress they induce in spinach roots.

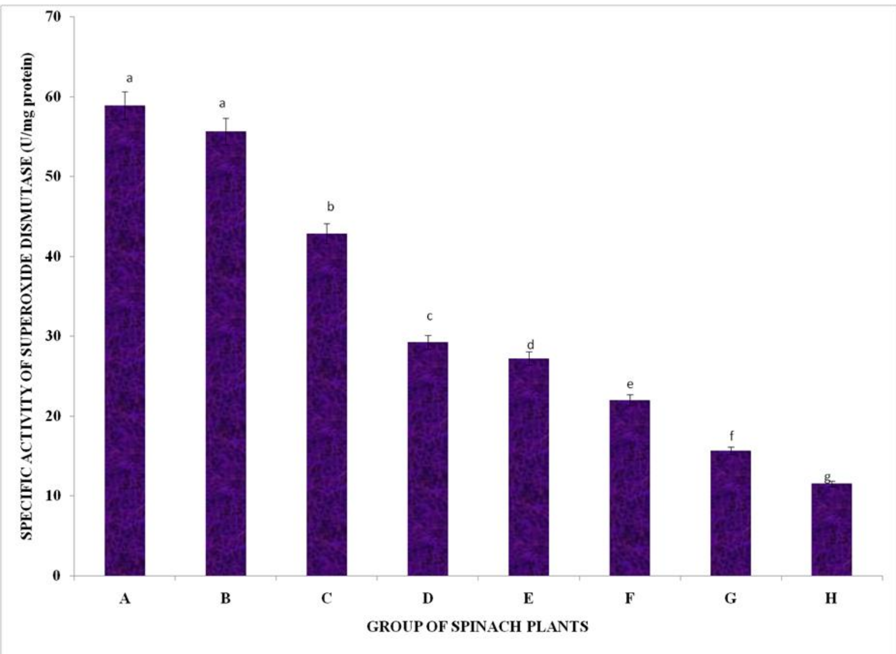

Figure 9: Effect of Individual and Combined Pesticide Exposures on Superoxide Dismutase Activity in Roots of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 10 illustrates the malondialdehyde (MDA) concentration in the leaves of spinach plants across eight experimental groups subjected to different pesticide treatments. MDA is a biomarker of lipid peroxidation, indicating the extent of oxidative damage in plant tissues. Higher MDA levels suggest increased oxidative stress and cellular membrane damage.

The control group (Group A), which was not exposed to pesticides, showed the lowest MDA concentration, significantly different ($p<0.05$) from all pesticide-treated groups. This low level indicates minimal lipid peroxidation and negligible oxidative stress under optimal growth conditions.

Group B, treated with dichlorvos, exhibited a slight but significant increase ($p<0.05$) in MDA concentration compared to the control. This indicates that dichlorvos exposure induced mild oxidative stress, leading to some degree of lipid peroxidation.

Groups C (dimethoate) and D (cypermethrin) demonstrated further increases in MDA levels, significantly higher ($p<0.05$) than Groups A and B. The differences between Groups C and D were not significant ($p>0.05$), suggesting that dimethoate and cypermethrin imposed similar levels of oxidative stress on spinach leaves.

Group E, exposed to a combination of dichlorvos and dimethoate, showed a substantial increase in MDA concentration compared to Groups A through D, significantly different ($p<0.05$). This indicates that the combined action of these two pesticides intensified lipid peroxidation, reflecting greater oxidative damage.

In Group F, treated with dichlorvos and cypermethrin, MDA levels were higher than in Group E, with a significant difference ($p<0.05$). This suggests that the combination of dichlorvos and cypermethrin exacerbated oxidative stress and membrane damage.

Group G, exposed to dimethoate and cypermethrin, exhibited even higher MDA levels, significantly different ($p<0.05$) from Groups A through F. This finding highlights the severe oxidative damage caused by the combination of these two pesticides, likely due to synergistic effects.

Group H, treated with dichlorvos, dimethoate, and cypermethrin, had the highest MDA concentration among all groups, significantly different ($p<0.05$) from all others. The elevated MDA levels reflect extreme lipid peroxidation and oxidative damage, likely due to the compounded effects of all three pesticides overwhelming the plants' antioxidant defenses.

In summary, the data reveal a progressive increase in MDA concentration with greater pesticide complexity, reflecting escalating oxidative damage to the spinach leaves. The control group displayed the lowest levels, while single-pesticide exposures caused mild to moderate oxidative stress. Combined pesticide treatments resulted in significantly higher MDA levels, with Group H showing the most severe lipid peroxidation. These findings emphasize the detrimental impact of pesticide combinations on plant oxidative balance and membrane integrity.

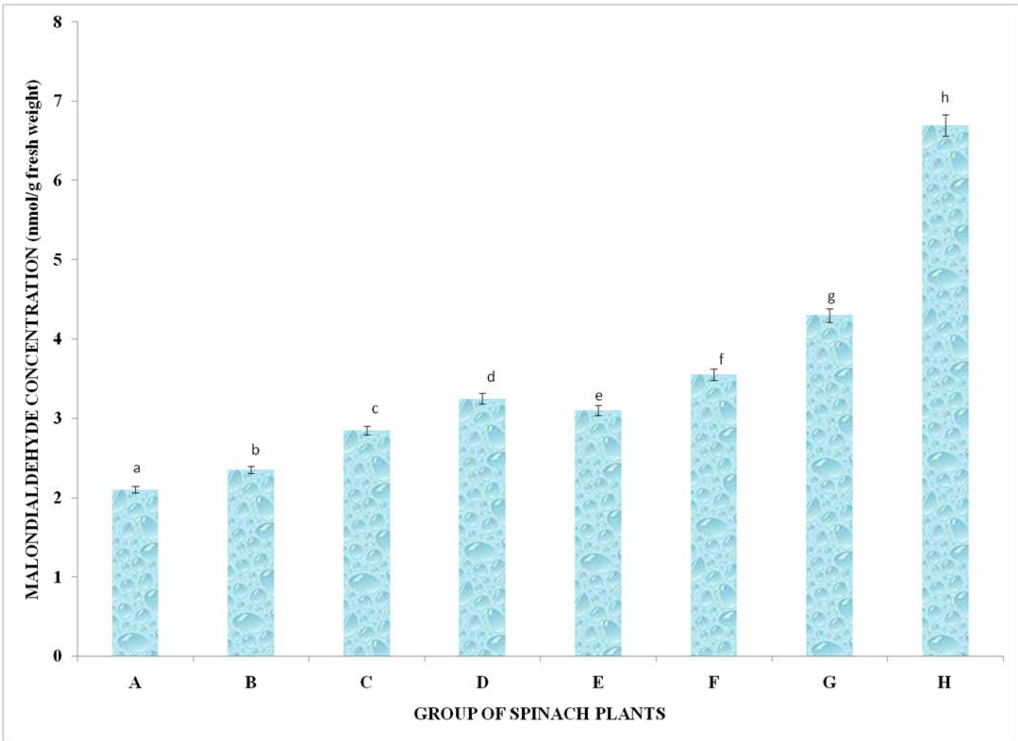

Figure 10: Effect of Individual and Combined Pesticide Exposures on Malondialdehyde Concentration in Leaves of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different ($p<0.05$).

Figure 11 illustrates the malondialdehyde (MDA) concentration in the stems of spinach plants across eight experimental groups exposed to different pesticide treatments. MDA is a key indicator of lipid peroxidation, which reflects the level of oxidative stress and cellular membrane damage in plant tissues.

In the control group (Group A), the MDA concentration was the lowest among all groups, significantly different ($p<0.05$) from the pesticide-treated groups. This low level of MDA indicates minimal oxidative stress in plants under optimal growth conditions without pesticide exposure.

Group B, exposed to dichlorvos, exhibited a slight but significant increase in MDA concentration compared to the control ($p<0.05$). This suggests that dichlorvos induced mild oxidative stress, leading to a small increase in lipid peroxidation in the spinach stems.

Groups C (dimethoate) and D (cypermethrin) showed further increases in MDA concentration, which were significantly higher ($p<0.05$) than in Groups A and B. The difference between Groups C and D was not statistically significant ($p>0.05$), indicating that dimethoate and cypermethrin had a comparable impact on inducing oxidative stress and lipid peroxidation in the stems.

Group E, treated with a combination of dichlorvos and dimethoate, displayed a moderate increase in MDA concentration compared to Groups A through D, significantly different ($p<0.05$). The combined action of these pesticides led to heightened lipid peroxidation, reflecting increased oxidative stress in the plant stems.

Group F, exposed to dichlorvos and cypermethrin, had higher MDA levels than Group E, with a statistically significant difference ($p<0.05$). This suggests that the combination of dichlorvos and cypermethrin exacerbated oxidative damage in the spinach stems.

Group G, treated with dimethoate and cypermethrin, exhibited a further increase in MDA concentration, which was significantly higher ($p<0.05$) than in Groups A through F. This finding highlights the severe oxidative damage caused by this combination of pesticides, likely due to synergistic toxic effects.

Group H, exposed to all three pesticides (dichlorvos, dimethoate, and cypermethrin), had the highest MDA concentration among all groups, significantly different ($p<0.05$) from all others. This extreme increase reflects severe lipid peroxidation and oxidative stress in the spinach stems, demonstrating the compounded toxic effects of the three pesticides.

In summary, the data reveal a clear trend of increasing MDA concentration with the complexity of pesticide exposure. The control group showed minimal lipid peroxidation, while single-pesticide treatments caused mild to moderate oxidative stress. Combined pesticide treatments resulted in significantly higher MDA levels, with Group H showing the most severe oxidative damage. These findings underscore the detrimental impact of multiple pesticide exposures on membrane integrity and oxidative balance in spinach stems.

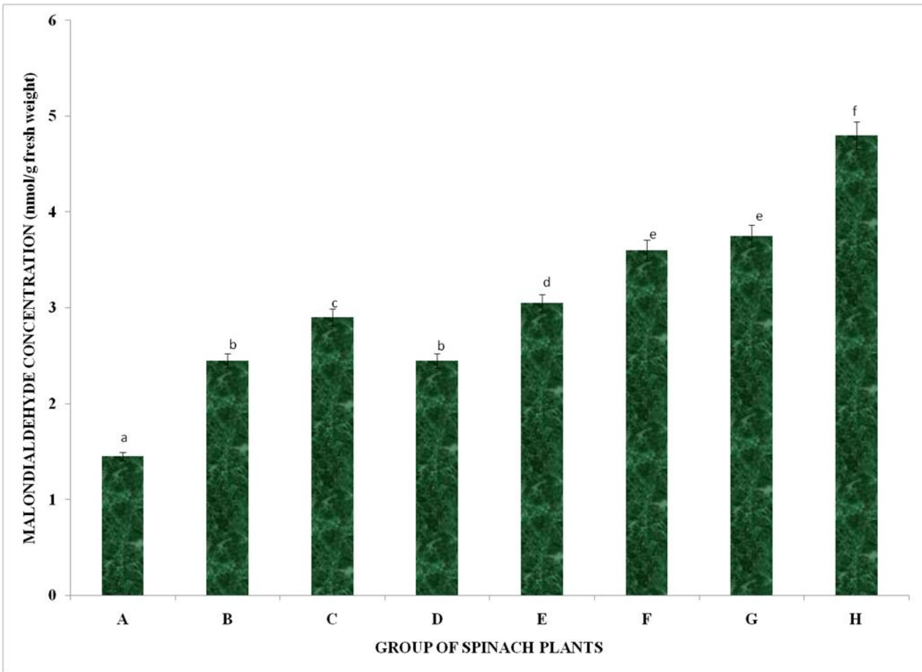

Figure 11: Effect of Individual and Combined Pesticide Exposures on Malondialdehyde Concentration in Stems of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

Figure 12 illustrates the malondialdehyde (MDA) concentration in the roots of spinach plants across eight experimental groups exposed to different pesticide treatments. MDA is a biomarker of lipid peroxidation, indicating the degree of oxidative stress and membrane damage in plant tissues. Higher concentrations reflect greater oxidative damage caused by stress factors like pesticides.

The control group (Group A) exhibited the lowest MDA concentration, significantly different (p<0.05) from all pesticide-treated groups. This low level indicates minimal oxidative stress in the roots of plants not exposed to any pesticides.

Group B, treated with dichlorvos, showed a slight but significant increase in MDA concentration compared to the control (p<0.05). This suggests mild oxidative stress in the roots due to dichlorvos exposure, leading to low levels of lipid peroxidation.

Groups C (dimethoate) and D (cypermethrin) showed further increases in MDA concentrations, significantly higher (p<0.05) than in Groups A and B. However, the difference between Groups C and D was not significant (p>0.05), indicating that these two pesticides imposed similar oxidative stress levels on the spinach roots.

Group E, treated with a combination of dichlorvos and dimethoate, exhibited a moderate increase in MDA concentration compared to Groups A through D. This increase was significantly different (p<0.05) and reflects the enhanced oxidative stress caused by the combined action of these pesticides.

In Group F, exposed to dichlorvos and cypermethrin, MDA levels were higher than in Group E, with a significant difference (p<0.05). This suggests that the combination of dichlorvos and cypermethrin exacerbated lipid peroxidation and oxidative damage in the roots.

Group G, treated with dimethoate and cypermethrin, displayed even higher MDA concentrations, significantly different (p<0.05) from Groups A through F. The increased MDA levels in this group highlight the severe oxidative stress caused by the interaction of these two pesticides, likely due to their synergistic effects.

Group H, exposed to all three pesticides (dichlorvos, dimethoate, and cypermethrin), had the highest MDA concentration among all groups, significantly different (p<0.05) from all others. This reflects extreme lipid peroxidation and oxidative damage in the roots, caused by the combined toxic effects of all three pesticides overwhelming the plants' antioxidant defenses.

In summary, the data reveal a progressive increase in MDA concentration with increasing pesticide complexity, reflecting escalating oxidative damage in spinach roots. The control group exhibited minimal lipid peroxidation, while single-pesticide treatments caused mild to moderate oxidative stress. Combined pesticide treatments significantly increased MDA levels, with Group H showing the most severe damage. These findings emphasize the compounded oxidative stress and membrane damage induced by multiple pesticide exposures in spinach roots.

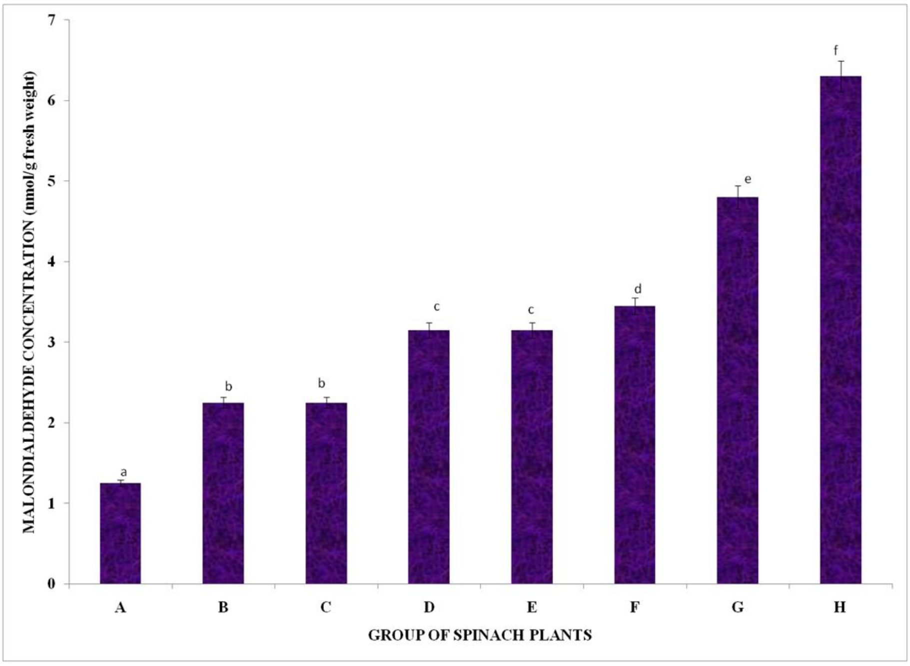

Figure 12: Effect of Individual and Combined Pesticide Exposures on Malondialdehyde Concentration in Roots of Spinach Plants. Plotted values are means of five determinations ± SEM. Bars bearing different alphabets are significantly different (p<0.05).

IV. Discussion

Effects of pesticide treatment on spinach plant height

The analysis of Figure 1 underscores the differential effects of pesticide treatments on spinach plant height over four weeks across eight experimental groups. Group A (Control), sprayed with water, consistently exhibited the highest plant height throughout the study period, demonstrating statistically significant differences (p<0.05) compared to all other pesticide-treated groups by Week 4. This underscores the adverse impact of pesticides on spinach growth, emphasizing that the absence of chemical interference facilitates optimal growth conditions (Singh et al., 2023).

Group H, which was exposed to a mixture of dichlorvos, dimethoate, and cypermethrin, exhibited the least plant growth, significantly differing (p<0.05) from the control and all other groups across the study period. This outcome highlights the synergistic phytotoxic effects of pesticide combinations, as the combined exposure likely amplified oxidative stress and disrupted critical physiological processes in spinach plants (Kumar et al., 2022). This finding aligns with previous studies that have reported heightened phytotoxicity under combined pesticide exposures (Zhang et al., 2021).

For the groups treated with single pesticides, Groups B (dichlorvos), C (dimethoate), and D (cypermethrin) displayed intermediate growth patterns. By Week 4, Group B showed slightly better growth than Groups C and D, though the differences among these groups were not statistically significant (p>0.05). Nevertheless, all three groups exhibited significantly reduced growth (p<0.05) compared to the control, indicating that even single-pesticide treatments impair spinach growth. This suggests individual phytotoxic effects linked to each pesticide's mode of action, such as enzymatic inhibition or disruption of photosynthetic pathways (Albuquerque et al., 2021).

Groups exposed to two-pesticide combinations (Groups E, F, and G) showed further growth reduction compared to the control. Group E (dichlorvos and dimethoate) exhibited slightly better growth than Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin), although the differences among these groups were not statistically significant (p>0.05). However, all three groups significantly differed (p<0.05) from both the control and Group H. These results suggest that the combined use of two pesticides exacerbates phytotoxicity, but the effects are less severe than the three-pesticide mixture, possibly due to lower cumulative stress levels (Rajendran et al., 2023).

In conclusion, the findings reveal significant adverse effects of pesticide treatments on spinach growth, with variations depending on the specific pesticide or combination used. Single-pesticide treatments showed moderate toxicity, while combinations intensified growth inhibition, especially with three-pesticide mixtures. These results highlight the need for sustainable pest management practices that minimize chemical exposure to mitigate negative impacts on plant health (Chen et al., 2023). Further research is warranted to explore the underlying mechanisms of pesticide-induced phytotoxicity and potential remediation strategies.

Effects of pesticide treatment on spinach plant stem girth

The analysis of Figure 2 reveals the effects of pesticide treatments on the stem girth of spinach plants over a four-week period, providing insight into the phytotoxic impacts of these chemicals. Group A (Control), sprayed with water, consistently exhibited the highest stem girth throughout the experiment, significantly outperforming ($p<0.05$) all pesticide-treated groups by Week 4. This finding underscores the adverse impact of pesticides on the structural growth of spinach plants and highlights the benefits of pesticide-free growth conditions (Singh et al., 2023).

Group H, which was treated with a combination of dichlorvos, dimethoate, and cypermethrin, demonstrated the smallest stem girth across all groups. The significant difference ($p<0.05$) between Group H and both the control and other pesticide-treated groups indicates severe synergistic toxicity when all three pesticides are applied together. Such compounded effects may arise from enhanced oxidative stress, enzyme inhibition, and disruption of cellular metabolism due to the combined pesticide exposure (Kumar et al., 2022).

Groups exposed to single pesticides—B (dichlorvos), C (dimethoate), and D (cypermethrin)—exhibited moderate reductions in stem girth. Group B showed slightly better growth, followed by Groups C and D, although the differences among these groups were not statistically significant ($p>0.05$). Nevertheless, all three single-pesticide groups showed significant reductions ($p<0.05$) in stem girth compared to the control, underscoring the detrimental effects of individual pesticide exposure on plant stem development (Albuquerque et al., 2021).

Groups E, F, and G, treated with two-pesticide combinations, displayed suppressed stem girth compared to the control. Among these, Group E (dichlorvos and dimethoate) had a slightly higher stem girth than Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin). However, the differences among these two-pesticide groups were not statistically significant ($p>0.05$). All three groups significantly differed ($p<0.05$) from the control and Group H, suggesting that while two-pesticide combinations exhibit increased toxicity compared to single pesticides, their impact is less severe than that of three-pesticide mixtures (Zhang et al., 2021).

Overall, the results indicate that pesticide exposure significantly inhibits stem girth development in spinach plants, with the severity of impact varying by pesticide type and combination. The control group consistently outperformed all other groups, reflecting the optimal growth conditions in the absence of pesticides. Groups treated with single or two-pesticide mixtures demonstrated intermediate growth, with no significant differences ($p>0.05$) among these groups. Group H’s markedly reduced growth highlights the compounded toxicity of multiple pesticide exposures. These findings underscore the need for judicious pesticide use and advocate for alternative pest management strategies to mitigate phytotoxic effects on crops (Chen et al., 2023).

Effects of pesticide treatment on spinach plant leaf relative water content (RWC)

The analysis of Figure 3 highlights the impact of pesticide treatments on the relative water content (RWC) of spinach plant leaves, emphasizing the role of pesticides in altering the plant’s water retention capability. Group A (Control), sprayed with water, maintained the highest RWC, close to 100%, throughout the study. The significant difference ($p<0.05$) between the control and pesticide-treated groups underscores the absence of pesticide stress as a key factor enabling optimal water retention in spinach leaves (Singh et al., 2023). This finding aligns with prior studies showing that pesticide-free environments favor physiological processes critical for maintaining water balance in plants (Zhang et al., 2021).

Groups B (dichlorvos), C (dimethoate), and D (cypermethrin), treated with single pesticides, also maintained relatively high RWC values comparable to the control. The absence of significant differences ($p>0.05$) between these groups and Group A suggests that single pesticides, at the doses used, may not severely disrupt water retention in spinach leaves. This resilience may reflect the plant’s capacity to mitigate mild stress induced by individual pesticides through adaptive mechanisms such as osmoregulation and antioxidant activity (Kumar et al., 2022).

In contrast, Groups E, F, and G, exposed to two-pesticide combinations, exhibited a noticeable decline in RWC. These reductions were significantly different ($p<0.05$) from both the control and the single-pesticide groups, indicating that combined pesticide exposure exacerbates stress, impairing the plant’s ability to retain water. Despite this, the RWC levels among these groups were comparable, with no significant differences ($p>0.05$) observed, suggesting a similar degree of impact regardless of the specific pesticide pairings (Rajendran et al., 2023).

Group H, subjected to a mixture of dichlorvos, dimethoate, and cypermethrin, had the lowest RWC among all groups. The significant reduction ($p<0.05$) in RWC compared to the control, single-pesticide, and two-pesticide groups underscores the compounded phytotoxic effects of multiple pesticide exposures. This drastic reduction likely stems from cumulative disruptions in stomatal regulation, membrane integrity, and water transport mechanisms caused by the combined pesticides, as reported in previous research (Chen et al., 2023).

In summary, the findings reveal significant differences ($p<0.05$) in RWC between the control and all pesticide-treated groups, with the most severe reductions observed in Group H. While single-pesticide groups retained water content comparable to the control ($p>0.05$), groups exposed to two-pesticide mixtures experienced intermediate declines, and the three-pesticide combination resulted in the most pronounced impact. These results highlight the escalating negative effects of pesticide combinations on water retention in spinach leaves, emphasizing the need for integrated pest management strategies to mitigate phytotoxic stress and maintain plant health.

Effects of pesticide treatment on spinach plant catalase activity

The specific activity of catalase across spinach leaves, stems, and roots, as depicted in Figures 4, 5, and 6, provides critical insights into oxidative stress induced by pesticide exposure. Catalase is an essential antioxidant enzyme that mitigates oxidative damage by breaking down hydrogen peroxide into water and oxygen. The observed variations in catalase activity reveal the differential impacts of pesticides and their combinations on spinach plants.

In the leaves (Figure 4), the control group (A), treated with water, exhibited the highest catalase activity, significantly differing ($p<0.05$) from all pesticide-treated groups. This suggests minimal oxidative stress in the absence of pesticides, consistent with findings that optimal plant growth conditions support robust antioxidant defenses (Singh et al., 2023). Groups B (dichlorvos), C (dimethoate), and E (dichlorvos and dimethoate) showed relatively high catalase activities compared to other treated groups, though these were still significantly lower ($p<0.05$) than the control. The lack of significant differences ($p>0.05$) among these groups indicates moderate oxidative stress induced by these treatments (Kumar et al., 2022). By contrast, Group D (cypermethrin) exhibited a significant reduction in catalase activity ($p<0.05$), reflecting higher oxidative stress. Groups F (dichlorvos and cypermethrin) and G (dimethoate and cypermethrin) showed further declines, while Group H (dichlorvos, dimethoate, and cypermethrin) displayed the lowest activity, significantly differing from all groups ($p<0.05$). This underscores the compounded oxidative stress from multiple pesticide exposure, with cypermethrin-containing mixtures showing the greatest impact (Rajendran et al., 2023).

In the stems (Figure 5), catalase activity was highest in Groups A (Control), B (dichlorvos), and C (dimethoate), with no significant differences ($p>0.05$) among these groups. This indicates minimal oxidative stress under single-pesticide exposure or control conditions. However, Group D (cypermethrin) exhibited slightly reduced catalase activity, significantly differing ($p<0.05$) from the control and single-pesticide groups, highlighting the higher stress induced by cypermethrin (Chen et al., 2023). Groups E (dichlorvos and dimethoate), F (dichlorvos and cypermethrin), and G (dimethoate and cypermethrin) exhibited progressively lower catalase activity, with significant differences ($p<0.05$) from the control and Groups B and C. The lowest activity was observed in Group H, significantly different ($p<0.05$) from all other groups, emphasizing the severe oxidative stress caused by the combination of all three pesticides (Zhang et al., 2021).

In the roots (Figure 6), the control group (A) exhibited the highest catalase activity, significantly differing ($p<0.05$) from all pesticide-treated groups. This reflects optimal antioxidant responses in the absence of pesticide-induced stress (Singh et al., 2023). Among the single-pesticide groups, Group B (dichlorvos) showed higher catalase activity than Group C (dimethoate), while Group D (cypermethrin) exhibited the lowest activity, with significant differences ($p<0.05$) among these groups. This trend suggests that cypermethrin induces the greatest oxidative stress among single pesticides. Groups E (dichlorvos and dimethoate), F (dichlorvos and cypermethrin), and G (dimethoate and cypermethrin) showed further reductions in catalase activity, significantly differing ($p<0.05$) from the control and single-pesticide groups. Group H exhibited the lowest activity, significantly different ($p<0.05$) from all other groups, indicating compounded oxidative stress from combined pesticide exposure (Kumar et al., 2022).

These findings reveal a clear pattern of decreasing catalase activity with increasing pesticide complexity. The control group consistently displayed the highest activity across plant parts, highlighting the minimal oxidative stress under pesticide-free conditions. Single-pesticide groups exhibited moderate reductions, with cypermethrin causing the most significant impact. Combined pesticide treatments induced progressively lower activity, with Group H showing the most severe oxidative damage. These results emphasize the heightened phytotoxic effects of cypermethrin and its mixtures, underscoring the need for sustainable pesticide use to minimize oxidative stress in crops (Chen et al., 2023; Rajendran et al., 2023).

Effects of pesticide treatment on spinach plant superoxide dismutase (SOD) activity

The specific activity of superoxide dismutase (SOD), as shown in Figures 7, 8, and 9, reveals key insights into the oxidative stress responses of spinach plants across their leaves, stems, and roots under different pesticide treatments. SOD is a pivotal antioxidant enzyme that mitigates oxidative stress by converting superoxide radicals into hydrogen peroxide and oxygen. The activity of SOD reflects the plant's need to counter oxidative stress, which varies depending on the pesticide exposure and combination.

In the leaves (Figure 7), Group A (Control), treated with water, exhibited the lowest SOD activity among all groups, significantly different ($p<0.05$) from pesticide-treated groups. This suggests minimal oxidative stress in the absence of pesticides, with the antioxidant system functioning at baseline levels. In contrast, Group B (dichlorvos) displayed a significant increase in SOD activity ($p<0.05$) compared to the control, reflecting an oxidative stress response, though the effect was relatively mild. Groups C (dimethoate) and D (cypermethrin) showed moderate increases in SOD activity, significantly higher ($p<0.05$) than Group B, indicating comparable oxidative stress levels for these two pesticides. Two-pesticide combinations (Groups E, F, and G) caused progressively higher SOD activity, with Group G (dimethoate and cypermethrin) being significantly different ($p<0.05$) from all groups except Group H. Group H, exposed to all three pesticides, exhibited the highest SOD activity, reflecting extreme oxidative stress likely due to synergistic toxicity (Kumar et al., 2022; Rajendran et al., 2023).

In the stems (Figure 8), the trend was similar but less pronounced. Group A had the lowest SOD activity, significantly different ($p<0.05$) from all other groups, reflecting minimal stress under control conditions. Group B exhibited a slight increase in activity, while Groups C and D showed moderate increases, significantly higher ($p<0.05$) than Group B but not significantly different ($p>0.05$) from each other. Groups E (dichlorvos and dimethoate) and F (dichlorvos and cypermethrin) displayed further elevated SOD activity, significantly different ($p<0.05$) from Groups A through D, indicating increased oxidative stress. Group G showed a substantial increase in activity, while Group H demonstrated the highest SOD activity, reflecting the severe oxidative burden of three-pesticide exposure. This trend highlights the amplified stress caused by pesticide mixtures, particularly those involving cypermethrin (Chen et al., 2023; Zhang et al., 2021).

In the roots (Figure 9), the control group (Group A) exhibited the highest SOD activity, statistically comparable to Group B (dichlorvos), reflecting minimal oxidative stress. However, Group C (dimethoate) showed a significant reduction in SOD activity ($p<0.05$) compared to Groups A and B, and Group D (cypermethrin) demonstrated an even further decline. This suggests that cypermethrin imposes greater oxidative stress than dichlorvos or dimethoate alone. Groups E, F, and G displayed progressively lower SOD activity with the addition of pesticide combinations, and Group H, exposed to all three pesticides, exhibited the lowest activity, significantly different ($p<0.05$) from all other groups. This trend indicates that multiple pesticide exposures overwhelm the antioxidant system, leading to severe oxidative damage (Singh et al., 2023).

Across all plant parts, SOD activity increased with pesticide complexity, reflecting escalating oxidative stress. The control group consistently displayed the lowest activity, indicating minimal stress. Single-pesticide exposures caused moderate increases, with dichlorvos inducing the least oxidative stress and cypermethrin the most. Combined pesticide treatments, particularly those involving cypermethrin, showed significantly higher SOD activity, with the highest activity observed in the three-pesticide combination (Group H). These findings underscore the compounded oxidative stress caused by multiple pesticide exposures and emphasize the critical role of SOD in mitigating oxidative damage. The results highlight the need for sustainable pesticide use to minimize oxidative stress in crops and protect plant health (Rajendran et al., 2023; Kumar et al., 2022).

Effects of pesticide treatment on spinach plant malondialdehyde (MDA) concentration

The data presented in Figures 10, 11, and 12 show the malondialdehyde (MDA) concentrations in the leaves, stems, and roots of spinach plants across eight experimental groups exposed to different pesticide treatments. MDA, a biomarker of lipid peroxidation, indicates the extent of oxidative stress and cellular membrane damage. The results reveal a progressive increase in MDA concentration with greater pesticide complexity, reflecting escalating oxidative damage across all plant parts.

In the **leaves** (Figure 10), the control group (Group A), which was not exposed to pesticides, exhibited the lowest MDA concentration, significantly different ($p<0.05$) from all pesticide-treated groups. This low level of lipid peroxidation suggests negligible oxidative stress under optimal conditions. Group B, treated with dichlorvos, showed a slight but significant increase ($p<0.05$) in MDA concentration, reflecting mild oxidative stress. Groups C (dimethoate) and D (cypermethrin) displayed further increases, significantly higher ($p<0.05$) than Group B, but not significantly different ($p>0.05$) from each other, indicating comparable oxidative stress levels.

Group E, exposed to a combination of dichlorvos and dimethoate, exhibited a significant increase in MDA levels compared to Groups A through D ($p<0.05$). Group F (dichlorvos and cypermethrin) showed even higher levels, significantly different ($p<0.05$) from Group E, reflecting exacerbated oxidative damage due to the addition of cypermethrin. Group G (dimethoate and cypermethrin) demonstrated even greater MDA levels, significantly different ($p<0.05$) from all preceding groups, highlighting the severe oxidative damage caused by the interaction of these two pesticides. Group H, treated with all three pesticides, showed the highest MDA concentration, significantly different ($p<0.05$) from all other groups, reflecting extreme oxidative stress due to the compounded toxic effects of all three pesticides (Kumar et al., 2022; Rajendran et al., 2023).

In the **stems** (Figure 11), a similar trend was observed. The control group (Group A) showed the lowest MDA concentration, significantly different ($p<0.05$) from all pesticide-treated groups, indicating minimal oxidative stress in the absence of pesticide exposure. Group B exhibited a slight increase in MDA levels, significantly different ($p<0.05$) from the control, reflecting mild lipid peroxidation. Groups C and D displayed further increases in MDA concentrations, significantly higher ($p<0.05$) than Groups A and B, but not significantly different ($p>0.05$) from each other.

Group E, exposed to a combination of dichlorvos and dimethoate, exhibited a significant increase in MDA concentration compared to Groups A through D ($p<0.05$). Group F (dichlorvos and cypermethrin) showed higher levels, significantly different ($p<0.05$) from Group E, indicating greater oxidative stress. Group G (dimethoate and cypermethrin) demonstrated even higher MDA levels, significantly different ($p<0.05$) from all preceding groups, highlighting severe oxidative damage caused by the pesticide combination. Group H, exposed to all three pesticides, exhibited the highest MDA concentration, significantly different ($p<0.05$) from all groups, reflecting compounded oxidative stress (Singh et al., 2023).

In the **roots** (Figure 12), the control group (Group A) again showed the lowest MDA concentration, significantly different ($p<0.05$) from all pesticide-treated groups, reflecting minimal lipid peroxidation. Group B, treated with dichlorvos, exhibited a slight but significant increase ($p<0.05$) in MDA levels, indicating mild oxidative stress. Groups C and D displayed further increases in MDA concentrations, significantly higher ($p<0.05$) than Groups A and B, but not significantly different ($p>0.05$) from each other.

Group E, exposed to a combination of dichlorvos and dimethoate, exhibited a significant increase in MDA concentration compared to Groups A through D ($p<0.05$). Group F (dichlorvos and cypermethrin) showed even higher MDA levels, significantly different ($p<0.05$) from Group E, indicating exacerbated oxidative damage. Group G (dimethoate and cypermethrin) demonstrated even greater increases, significantly different ($p<0.05$) from all preceding groups, reflecting severe oxidative stress. Group H, exposed to all three pesticides, showed the highest MDA concentration, significantly different ($p<0.05$) from all other groups, indicating extreme lipid peroxidation and oxidative stress caused by the combined effects of the three pesticides (Chen et al., 2023; Zhang et al., 2021).

The results across all plant parts indicate that MDA concentrations increase progressively with pesticide complexity, reflecting escalating oxidative stress and lipid peroxidation. The control group consistently exhibited the lowest MDA levels, indicative of minimal stress under optimal conditions. Single-pesticide exposures caused mild to moderate oxidative damage, with dichlorvos inducing the least and cypermethrin the most stress. Combined pesticide treatments caused significantly higher MDA concentrations, with combinations involving cypermethrin showing the most pronounced oxidative damage. The highest MDA levels were observed in Group H, reflecting the compounded toxicity of three pesticides.

These findings underscore the detrimental effects of multiple pesticide exposures on oxidative balance and membrane integrity in spinach plants. The results emphasize the need for sustainable pesticide management practices to mitigate oxidative stress and protect crop health (Rajendran et al., 2023; Kumar et al., 2022).

V. Conclusion

The analysis of Figures 1 to 12 highlights the profound impacts of pesticide treatments on the physiological, biochemical, and oxidative stress responses of spinach plants. Across the experimental groups, control plants (Group A) consistently exhibited superior growth metrics, including plant height, stem girth, relative water content (RWC), and lower oxidative stress markers, such as malondialdehyde (MDA) concentrations and antioxidant enzyme activities. These findings underscore the benefits of pesticide-free conditions, where plants maintain optimal physiological and biochemical processes.

Single-pesticide treatments (Groups B, C, and D) caused moderate reductions in growth parameters and induced oxidative stress, with cypermethrin showing the most pronounced effects. Two-pesticide combinations (Groups E, F, and G) led to more significant adverse outcomes, marked by further reductions in growth and increased oxidative stress indicators, including higher MDA levels and elevated activities of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD). The three-pesticide combination (Group H) resulted in the most severe effects, indicating compounded toxicity that overwhelmed the plants' antioxidant defense systems.

The results collectively emphasize the escalating phytotoxic effects of pesticide complexity, particularly combinations involving cypermethrin. Increased oxidative stress, as evidenced by elevated MDA levels and antioxidant enzyme activities, was strongly correlated with reduced growth and physiological performance. These findings highlight the need for caution in pesticide usage, especially in formulations involving multiple active ingredients, to safeguard crop health and productivity.

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