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Physiological and Biochemical Disruption of Maize *(Zea mays* L.) under Zinc Sulphide-induced Stress

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ABSTRACT

Zinc sulphide (ZnS) application can influence plant growth, with effects varying by concentration and plant variety. This study evaluated the impact of ZnS on two maize (*Zea mays* L.) cultivars, AS-5277 and 30-K08, focusing on biomass production, photosynthetic pigments, and biochemical traits. Five ZnS concentrations (0.1–0.3 g/100 ml, denoted T1–T5) were applied, compared to a control. Growth parameters (plant height, root length, fresh and dry weight, moisture content, leaf width) and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) were measured, alongside flavonoid, sugar, and phenol content. Lower ZnS concentrations (T1: 0.1 g/100 ml, T2: 0.15 g/100 ml, T3: 0.2 g/100 ml) significantly enhanced plant height, root length, fresh and dry weight, and leaf width in both varieties, with AS-5277 showing greater improvements than 30-K08. Chlorophyll a, chlorophyll b, and carotenoid contents increased at lower concentrations, peaking at T3, but declined at higher concentrations (T4: 0.25 g/100 ml, T5: 0.3 g/100 ml). Similarly, flavonoid, sugar, and phenol contents increased at lower concentrations, with AS-5277 exhibiting more pronounced enhancements. Higher ZnS levels inhibited growth, reduced pigment levels, and lowered biochemical contents, indicating toxicity. These findings underscore ZnS's dual role as a beneficial micronutrient at low doses and a toxicant at high doses, with varietal differences in response. Optimizing ZnS application rates is crucial to enhance maize growth and biochemical activity while minimizing toxicity risks.

INTRODUCTION

Maize or Corn (*Zea mays* L.) is an icon of agriculture around the world, and an essential crop that feeds millions of people around the world. Maize is a member of the Poaceae family, originated in south America around 55-70 million years back and has been cultivated during the past 6000 and 10000 years (Bolot et al., 2009). Being the third most widespread growing product across the world, it covers 118 million hectares, providing approximately 600 million metric tons a year (Yakadri et al., 2015). Being adaptable and tolerant to different environmental conditions ranging across coastal to irrigated lands, nutritious, rich in carbohydrates, essential amino acids, vitamins, and minerals, it finds its irreplaceable quality on the human and animal food tables (Rose, 2011; Amegbor et al., 2022). In addition to its caloric value, maize is used in industrial contexts, including corn oil, corn syrup, and ethanol, which highlights the economic importance of the substance (Chen et al., 2019). Maize supplies calories in balanced diets and feeds around half a billion people in developing countries (Frelat et al., 2016). There is a problem in its production, however, such as environmental stresses and soil contamination, especially heavy metals.

Some of these elements are the heavy metals, stable elements of high mass and density found as traces in soils but in toxic levels with anthropogenically related factors like mining, industrial wastes, and agricultural applications (Koutsospyros et al., 2006). Some heavy metals, such as zinc (Zn), copper (Cu), and iron (Fe), are crucial micronutrients in plant growth, whereas others, such as cadmium (Cd), lead (Pb), and mercury (Hg), can have serious and detrimental effects on the physiology of the plant and the health of the human when their levels are not within the safe limits (Mushtaq et al., 2022).

Zinc, especially, plays a crucial role in maize because it affects the processes of metabolism including photosynthesis, protein synthesis, enzyme activity (Broadley et al., 2007). Nevertheless, insufficient supply of Zn as well as its excessive doses are harmful to the growth of the plant, and it slows down yield and jeopardizes the nutrition (Noulas et al., 2018). The issue of heavy metal contamination in the soils especially Zn pollution is potential because maize is a priority cereal crop grown in one million hectares in Pakistan (Karim et al., 2010).

This paper deals with the influence of zinc on the maize production, discussing the uptake, transport, and effects of zinc on growth and nutritional quality concerning the agricultural situation in Pakistan. By considering the existence of the paradox between the vital of an element and its potential toxicity, this paper is going to contribute information about the optimization of the process of maize cultivation in terms of its production and food security in the regions exceeding the level of heavy metal contamination.

MATERIALS AND METHODS

The experiment was carried out at the Abdul Wali Khan University of Mardan, Pakistan to examine the two maize varieties (*Zea Mays L.* var. AS-5277 and 30-K08) on the impact of zinc sulphide (ZnS) on the vegetative growth and photosynthetic efficiency. The seeds of both species were obtained in the local market and before sowing, the healthy and of the same size seeds were taken. Before planting, the seeds were sterilized on a surface using 80% ethanol sterilized within 8 minutes as this can get the microbial contaminants killed and rinsed the seeds three times with distilled water. They were prepared in five concentrations of ZnS (in different flasks) 1000 ppm (0.1 g/100 mL), 1500 ppm (0.15 g/100 mL), 2000 ppm (0.2 g/100 mL), 2500 ppm (0.25 g/100 mL); 3000 ppm (0.3 g/100 mL) using three replicates of each treatment (ppm calculated as Seeds had been post-sterilized and soaked overnight, and planted in separate cups with seven sets of seeds per cup, and three reps of each concentration to measure germination and early growth reaction under controlled conditions.

Experimental Procedure

Soil was measured accurately using measuring balance and 280 grams of soil was taken in 36 separate disposable cups each. Seven seeds of both varieties (30-K08 and AS-5277) were sown in separate cups and thoroughly watered and wetted. In the first 3 days maximum germination of both maize varieties (var.30-K08 and AS-5277) was observed. Zinc sulphide treatments were applied after 9 days of germination. Before application of zinc sulphide treatments growth parameters such as shoot length, root length, fresh weight, dry weight and moisture content were measured. The plants were kept under zinc sulphide stress for 10 days and then all the growth parameters were measured again to evaluate the effect of zinc sulphide.

Measurement of Chlorophyll and Carotenoids

For measuring chlorophyll and carotenoid content of maize 0.5 grams of leaf material was crushed using mortar and pestle in 10 ml acetone (80%). The solution was taken in falcon tubes and centrifuged for 30 minutes at 4000 rpm. The supernatant was transferred to clean falcon tubes and the pellet was discarded. The leaf extract (supernatant) was used to measure chlorophyll content. Chlorophyll and carotenoids content was measured using a 721-vis spectrophotometer. Leaf extract was taken in cuvette and absorption was measured at 664 nm, 645 nm, and 510 nm and 480nm wavelengths. The chlorophyll (a and b) and carotenoids were calculated using the underlying formulas:

Assessment of Growth Parameters in Maize (*Zea mays* L.) Under Zinc Sulphide Treatment

To evaluate the effects of zinc sulphide on maize (*Zea mays L.*) varieties AS-5277 and 30-K08, several growth and physiological parameters were assessed. Measurements were taken before and after a 10-day treatment period with zinc sulphide, following an initial 9-day germination phase. Plant height was measured from the soil surface to the tip of the longest leaf using a calibrated ruler (cm), with the height of all seven plants per replicate cup recorded and averaged. Root length was determined by carefully uprooting plants, washing roots to remove soil, and measuring the primary root from base to tip (cm), with mean values calculated per replicate. Fresh weight was obtained by harvesting whole plants (shoots and roots), blotting excess moisture, and weighing immediately on an analytical balance (g), followed by calculation of mean fresh weight per replicate. For dry weight, the same plants were oven-dried at 70°C for 48 hours until constant weight was achieved, then weighed and averaged. Leaf width was measured at the widest point of the third fully expanded leaf from the top using a digital caliper (mm), and mean leaf width per replicate was recorded. These parameters collectively provided a comprehensive assessment of zinc sulphide impact on maize growth and development.

Determination of Metabolites in Maize Seedlings

Biochemical analyses of maize leaf samples were conducted to quantify total carbohydrate, phenolic, and flavonoid contents using established protocols. For carbohydrate estimation, a 0.5-gram fresh leaf sample was macerated in 10 mL distilled water, centrifuged at 3000 rpm for 5 minutes, and the supernatant was mixed with 80% phenol and concentrated H_2SO_4, with optical density measured at 485 nm (Bates and Hatcher, 1992). Phenolic content was determined by grinding 5 grams of leaf material in 80% ethanol, filtering through Whatman No. 1 paper, diluting with sterile water, heating, and adding Na_2CO_3 and Folin-Ciocalteu reagent, with optical density recorded at 650 nm (Kumar et al., 2014). Flavonoid content was assessed by crushing a 0.5-gram leaf sample in 80% ethanol, incubating for 24 hours, centrifuging at 10,000 rpm for 15 minutes, and adding $NaNO_2$, $AlCl_3$, NaOH, and sterile water to the supernatant, with optical density measured at 415 nm (Peñarrieta et al., 2007).

Statistical Analysis

The obtained data were analyzed using SPSS and the graph were prepare using Microsoft excel.

RESULTS

Plant Height

Zinc sulphide treatments significantly influenced plant height in maize variety AS-5277 (Figure 1 a). Lower concentrations (T1: 0.1 g/100 ml, T2: 0.15 g/100 ml, T3: 0.2 g/100 ml) increased plant height to 36.633 cm 37.067 cm, and 40.597 cm, respectively, compared to the control (35.167 cm). The highest plant height was recorded in T3, while higher concentrations (T4: 0.25 g/100 ml, T5: 0.3 g/100 ml) reduced height to 32.747 cm and 29.47 cm, respectively. For variety 30-K08, root length (reported as plant height in the data) increased at lower concentrations (T1: 29.633 cm, T2: 31.433 cm, T3: 34.073 cm) compared to the control (29.057 cm), but decreased at higher concentrations (T4: 29.5 cm, T5: 27.373 cm). AS-5277 exhibited a greater plant height increase than 30-K08 at lower concentrations.

Root Length

Root length in AS-5277 was enhanced at lower zinc sulphide concentrations (T1: 21.683 cm, T2: 24.9 cm, T3: 26.487 cm) compared to the control (20.56 cm), with the highest value in T3 (Figure 1b). Higher concentrations (T4: 18.79 cm, T5: 18.487 cm) inhibited root growth. For 30-K08, root length increased at T1 (21.71 cm), T2 (22.727 cm), and T3 (24.12 cm) compared to the control (19.03 cm), but decreased at T4 (19.43 cm) and T5 (17.5 cm). AS-5277 showed a more pronounced root length increase at lower concentrations than 30-K08.

Fresh Weight

Fresh weight in AS-5277 increased at lower concentrations (T1: 3.0533 g, T2: 3.1467 g, T3: 3.62 g) compared to the control (2.9067 g), with the highest value in T3 (Figure 1c). Higher concentrations (T4: 2.54 g, T5: 2.3133 g) significantly reduced fresh weight. For 30-K08, fresh weight increased at T1 (2.4067 g), T2 (2.5033 g), and T3 (2.6 g) compared to the control (2.33 g), but decreased at T4 (2.35 g) and T5 (2.25 g).

Dry Weight

Dry weight in AS-5277 was enhanced at lower concentrations (T1: 0.4337 g, T2: 0.4947 g, T3: 0.68 g) compared to the control (0.4193 g), with the highest value in T3 (Figure 1d). Higher concentrations (T4: 0.3733 g, T5: 0.342 g) reduced dry weight. For 30-K08, dry weight increased at T1 (0.3613 g), T2 (0.366 g), and T3 (0.3837 g) compared to the control (0.3487 g), but decreased at T4 (0.3517 g) and T5 (0.327 g).

Moisture Content

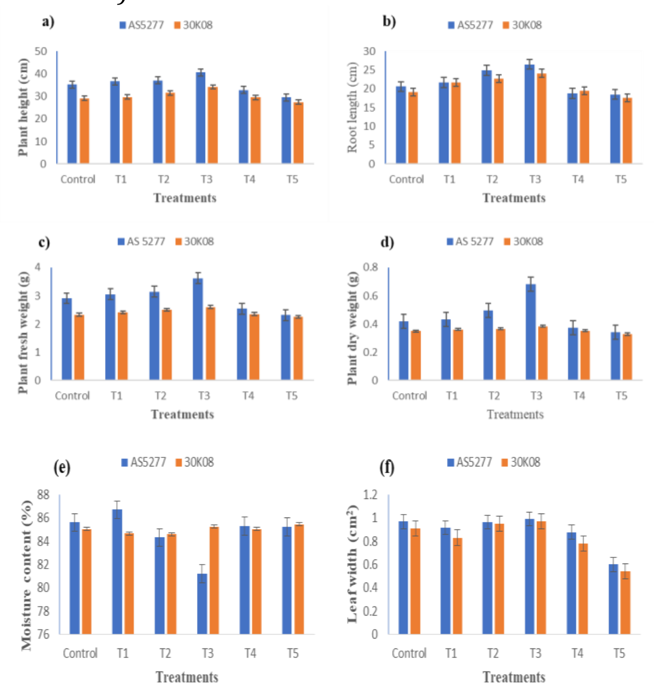
Moisture content in AS-5277 was highest at T1 (86.69%) but decreased at T2 (84.317%) and T3 (81.21%) compared to the control (85.603%) (Figure 1e). Higher concentrations (T4: 85.297%, T5: 85.203%) increased moisture content. For 30-K08, moisture content increased at T3 (85.24%), T4 (85.033%), and T5 (85.46%) compared to the control (85.03%), but decreased at T1 (84.317%) and T2 (84.587%). Lower concentrations reduced moisture content in both varieties.

Leaf Width

Leaf width in AS-5277 increased at lower concentrations (T1: 0.916 cm, T2: 0.9638 cm, T3: 0.9899 cm) compared to the control (0.9683 cm), with the highest value in T3 (Figure 1f). Higher concentrations (T4: 0.8769 cm, T5: 0.5991 cm) reduced leaf width. For 30-K08, leaf width increased at T1 (0.83 cm), T2 (0.95 cm), and T3 (0.97 cm) compared to the control (0.91 cm), but decreased at T4 (0.7799 cm) and T5 (0.5392 cm). AS-5277 showed a greater leaf width increase at lower concentrations than 30-K08.

Figure 1

The effect of varying concentrations of ZnS on growth parameters of two maize varieties (AS 5277 and 30K08.) The figure shows the effect of five foliar ZnS nanoparticle concentrations (1000, 1500, 2000, 2500, 3000 ppm, denoted by T1 to T5) compare with control for (a) plant height, (b) root length, (c) fresh weight (d) dry weight (e) moister content and (f) leaf width of two maize varieties (AS 5277 and 30K08).



Chlorophyll a

The effect of zinc sulphide treatments on chlorophyll a content in maize varieties AS-5277 and 30K08 was evaluated (Figure 2a). For variety AS-5277, lower concentrations (T1, T2, T3) increased chlorophyll-A content (18.45, 15.927, and 19.13, respectively) compared to the control (18.423), with the highest value in T3. However, higher concentrations (T4, T5) reduced chlorophyll-A content (16.95 and 14.433, respectively). Similarly, for variety 30K08, lower concentrations (T1, T2, T3) enhanced chlorophyll-A content (15.053, 15.13, and 15.227, respectively) compared to the control (15.123), with the peak at T3 (15.226). Higher concentrations (T4, T5) decreased chlorophyll-A content (14.777 and 13.273, respectively). Overall, zinc sulphide treatments had a more positive impact on chlorophyll-A content in variety AS-5277 compared to 30K08.

Chlorophyll b

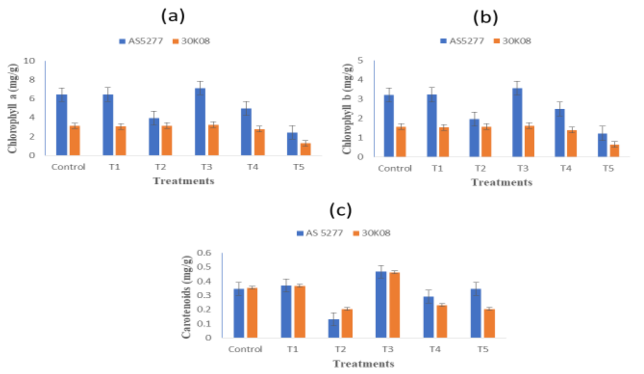
The effect of zinc sulphide on chlorophyll-B content is shown in (Figure 2b). For variety AS-5277, lower concentrations (T1, T2, T3) increased chlorophyll-B content (8.2667, 7.3667, and 9.2833, respectively) compared to the control (8.3667), with the highest value in T3. Higher concentrations (T4, T5) reduced chlorophyll-B content (7.543 and 7.222, respectively). For variety 30K08, lower concentrations (T1, T2, T3) also increased chlorophyll-B content (7.3, 7.5, and 7.7667, respectively) compared to the control (7.4667), peaking at T3 (7.7667). Higher concentrations (T4, T5) significantly decreased chlorophyll-B content (6.9333 and 5.9, respectively). Zinc sulphide treatments showed a more pronounced positive effect on chlorophyll-B content in variety AS-5277 compared to 30K08.

Carotenoid

The impact of zinc sulphide treatments on carotenoid content is presented in (Figure 2c). For variety AS-5277, lower concentrations (T1, T2, T3) increased carotenoid content (0.37, 0.13, and 0.4667, respectively) compared to the control (0.3467), with the highest value in T3. Higher concentrations (T4, T5) decreased carotenoid content (0.29 and 0.3467, respectively). For variety 30K08, lower concentrations (T1, T2, T3) similarly increased carotenoid content (0.3667, 0.2033, and 0.463, respectively) compared to the control (0.353), peaking at T3 (0.463). Higher concentrations (T4, T5) significantly reduced carotenoid content (0.13 and 0.0333, respectively). Zinc sulphide treatments had a more positive effect on carotenoid content in variety AS-5277 compared to 30K08.

Figure 2

Effects of ZnS on physiological and biochemical traits of maize varieties AS 5277 and 30K08. The figures show the effects of ZnS at the concentrations of 1000, 1500, 2000, 2500, and 3000 ppm (T1 to T5) as compared to the control on (a) moisture content, (b) leaf width, (c) chlorophyll a and (d) chlorophyll b contents in the maize varieties AS 5277 and 30K08.



Determination of Flavonoid, Sugar and Phenol

The zinc sulfide treatments had major effect on contents of flavonoid, sugar, and phenol in particular in the maize varieties AS-5277 and 30-K08 as shown in Figures 3a, 3b and 3c. In AS-5277, concentrations on T1: 0.1 g/100 ml, T2: 0.15 g/100 ml, T3: 0.2 g/100 ml increased flavonoid to about 60 mg/g, 50 mg/g, and 40 mg/g (control 15 mg/g), sugar to around 90 mg/100ml, 70 mg/100ml, and 65 mg/100ml (control 60 mg The flavonoid (~45 mg/g, ~20 mg/g), sugar (~50 mg/100ml, ~75 mg/100ml), and phenol (~270 mg/100ml, ~255 mg/100ml) content were lower at higher concentrations (T4 0.25 g/100 ml and T5 0.3 g/100 ml). In 30-K08, the concentration improvements increased the flavonoid to around 65 mg/g, 50 mg/g, and 35 mg/g (control: ~20 mg/g), sugar to around 100 mg/100ml, 85 mg/100ml, and 75 mg/100ml (control: ~80 mg/100ml), and phenol to around 300 mg/100ml, 200 mg/100ml and 275 A more significant change at lower concentrations was a general pattern in S-5277 compared to 30- K08 in over all the measured contents.

Figure 3

Effects of ZnS on biochemical traits of maize varieties AS 5277 and 30K08. The figures show the effects of ZnS at the concentrations of 1000, 1500, 2000, 2500, and 3000 ppm (T1 to T5) as compared to the control on (a) carotenoids, (b) flavonoids, (c) sugar and (d) phenols contents in the maize varieties AS 5277 and 30K08.

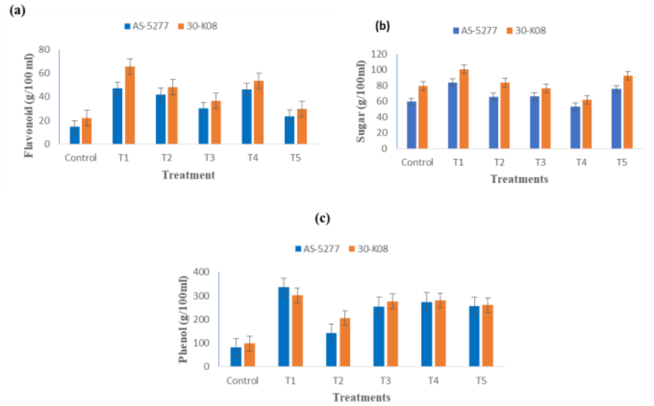
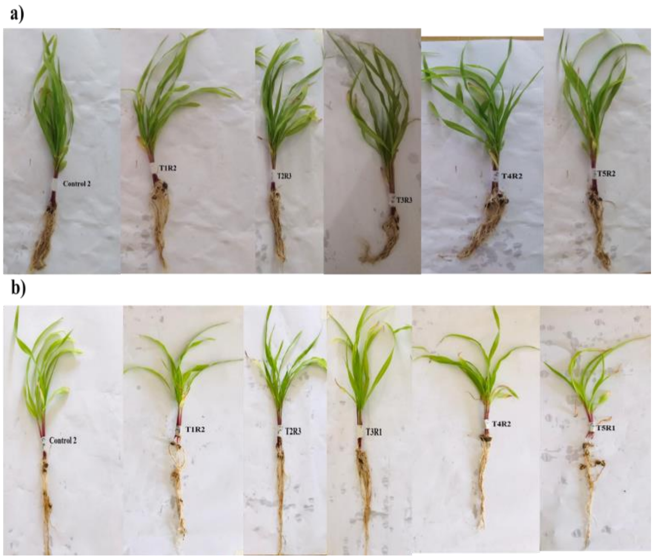


Figure 4

The figure presents morphological responses of maize (a) var. AS-5277 and (b) var. 30-K08 across various treatments (ZnS at the concentrations of 1000, 1500, 2000, 2500, and 3000 ppm (T1 to T5) and control).



DISCUSSION

Heavy metals, characterized by high atomic weight and density, are naturally present in soils in trace amounts and can induce toxicity in living organisms (Koutsospyros et al., 2006). The transition metals of the periodic table, encompassing approximately 60 elements, are often classified as heavy metals due to their potential to influence biological systems. This study investigated the effects of zinc sulfide (ZnS) on the vegetative growth and photosynthetic efficiency of two varieties of *Zea mays* L., focusing on parameters such as plant height, root length, fresh and dry weight, moisture content, leaf width, and photosynthetic pigments (chlorophyll b chlorophyll b and carotenoids).

The results indicated that low concentrations of zinc sulfide positively influenced several growth parameters. As shown in Figure 4.1, plant height increased with lower ZnS concentrations, consistent with findings by Harris et al. (2007), who reported enhanced plant height under similar conditions. This suggests that trace levels of ZnS may stimulate cellular elongation or nutrient uptake, promoting vertical growth. Similarly, root length (Figure 4.2) was maximized at lower ZnS concentrations, aligning with Seregin et al. (2011), who observed increased root elongation under low ZnS exposure. However, some studies, including Seregin et al. (2011), have reported contradictory outcomes, potentially due to variations in soil properties, ZnS bioavailability, or plant genotypes.

The measurements of fresh and beaten weight (Figures 4.3 and 4.4) also showed an increase at the low concentrations of ZnS. The effects in this improvement could be due to the increased biomass accumulation brought about by an optimum supply of zinc that is critical in enzymatic reactions and metabolism. Nonetheless, these results are opposite to those made by Rajaei et al. (2009) and Adiloglu (2006), who documented the decreases in fresh and dry weight exposed to higher loads of ZnS. Such inconsistencies might relate to the variances in the ZnS concentration levels which at a higher concentration, is toxic thereby affecting growth and biomass formation.

With low ZnS concentrations, the moisture content was on the rise, and this observation is aligned with Vazin (2012), who had indicated the same. This could mean better water uptake or water flow in plants subjected to trace amounts of ZnS, which would be related to a better root system. Leaf width similarly peaked at lower ZnS concentration, but in a study undertaken by Aref (2011), other results were obtained contrary to this argument, indicating that there might be variability in terms of the effects of leaf morphology, across the Zea mays varieties or even experimental conditions.

It also showed increased content on photosynthetic pigments like chlorophyll a, chlorophyll b and carotenoids where ZnS concentrations were low. These findings align with those of Paula et al. (2015) who reported high carotenoid levels at comparable concentration levels, which could probably be due to stabilization of photosynthetic complexes or riddance against oxidative damage caused by zinc. Conversely, Arough et al. (2016) found that the concentration of chlorophyll a and b decreased in high ZnS concentration, and this effect demonstrates a concentration-dependent effect which indicates that high concentrations of ZnS can either change the structure of chloroplast or biosynthesis of pigments.

Interpretation of the stimulatory effect of a low concentration of ZnS on the growth and photosynthetic activity of Zea mays demonstrates the two-sided role of zinc serving in both as essential micronutrient and as a poison. The contradictory findings of the literature reflect the importance of site-specific influence parameters such as zinc sulfide concentration, soil quality, and variety of plants on formations of physiological responses. Further research will focus on molecular mechanism of such responses and long-term effects of exposure to ZnS in an effort to establish the intensity of agricultural activity in zones which are water contaminated with heavy metals.

The results demonstrate that treatments with ZnS at different concentrations (from 1000 to 3000 ppm, T1 to T5) affect the biochemical components differently in both genotypes, AS-5277 and 30K08. Carotenoids have shown a declining trend in both genotypes from the control values to all the treatments, with AS-5277 consistently higher than 30K08. Flavonoid content gets significantly increased due to ZnS application, peaking at T1, with major influence by 30K08. This means it is probably indicative of induced antioxidant response. Rising sugar contents are also noted with the increase of ZnS, most clearly in 30K08. This possibly reflects the changed carbohydrate metabolism under stresses. Phenol content shows a slight initial increase at T1 with not much variation thereafter between the two genotypes. Thus, both respond to it in a similar way. Altogether, ZnS creates metabolic changes like stress, causing an increase in flavonoids and sugars as well as a reduction in carotenes, with 30K08 more showing a strong biochemical reaction, indicating more sensitivity or adaptive shifts toward it compared to AS-5277. This type of pattern matches the famous modulation of secondary metabolites of plants through zinc, where antioxidant compounds are stimulated to nullify extent stress effects.

The results showed that ZnS treatment with an increasing concentration. On the basis of carotenoids, both types decreased from control to all treatments, whereas AS-5277 was consistently higher than 30K08. There was a considerable increase in flavonoid content as a result of ZnS application, which was at its highest level at T1, with 30K08 showing the greatest effect, and is suggestive of an induced antioxidant response. Sugar contents also increased more due to the effect of ZnS, especially in 30K08, which may reflect altered carbohydrate metabolism under stress. At T1, phenol content showed an initial increase and has displayed only minor variation thereafter in the two genotypes (Ashraf et al., 2010). Thus, both respond to it in a similar way. In short, the effect of ZnS is metabolic stress changes that increase flavonoids and sugars while decreasing carotenes, with high response to biochemical generally seen in 30K08, indicating possible higher sensitivity or adaptive shift over AS-5277 (Fatemeh et al., 2020). This arrangement matched the famous zinc-modulated secondary compounds of plants where antioxidant compounds are stimulated to nullify extent stressed effects (Cheng, 2009).

CONCLUSION

The experiment has shown that low levels of zinc sulfide (ZnS) positively influenced the growth and physiological capabilities of 2 Zea mays L. varieties (AS-5277 and 30-K08). Particularly, reduced ZnS treatments stimulated fresh weight, dry weight, shoot length, root length, leaf width, chlorophyll, and carotenoid content. Contrarily, an increase in concentrations of ZnS was found to have inhibitory effects on these parameters, with no substantial impact on moisture content. These results emphasize the beneficial/harmful dialecticism role of ZnS as a trace element that, at higher concentrations, phytotoxicity might occur due to an imbalance of oxidative stress. The dose-related functions of ZnS indicate that it would be essential to optimize its dosage within the agricultural systems to achieve maximum yields of maize.

Recommendations

Further studies are needed to explore the molecular processes underlying zinc sulfide (ZnS) tolerance to high doses in Zea mays, in regards to the alteration of metabolism and oxidative stress. It is important to develop mitigation approaches that minimize phytotoxicity in polluted soils e.g. through amendment or through maize that is resistant to ZnS. To determine the prolonged impacts on the amount of grain and grain quality, long term field tests are required to determine the chronic effects of ZnS application, whilst the determination of the application-level thresholds of ZnS that can be applied to diverse varieties and soil types would aid sustainable agriculture.

*Authors' Contribution

Muhammad Usama Khan and **Wasim Khan** conducted the experiment. The manuscript was written by **Saima Maqbool Safia Gul,** and **Syed Maqsood Ali**. **Muhammad Dawood, Muhammad Waseem,** and **Saqib Amin** conducted analysis and data visualization. **Awais Ahmad** critically reviewed the manuscript and suggested corrections.

Ethics Approval

This study received the approval of the Ethical Committee of the Department of Agronomy, Abdul Wali Khan University Mardan. All procedures were performed in line with the standards protocols set out by the institutional and national research committee.

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Data Availability Statement

The supporting data for the findings of the current study is available from the corresponding author.

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