

# Effects of Ethanol and Zinc Sulfate Foliar Application on Yield and Drought Tolerance of Peanut (*Arachis hypogaea* L.)

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## Research Article

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# Abstract

Water scarcity is a major constraint in peanut production, necessitating cost-effective strategies to mitigate yield losses. This two-year study (2018–2019) is the first to investigate the synergistic effects of foliar-applied ethanol (0, 3, 6% v/v) and zinc sulfate (0, 1500, 3000 ppm) under three irrigation regimes [well-watered after 50 mm evaporation (IR50), mild drought stress after 100 mm evaporation (IR100), and severe drought stress after 150 mm evaporation (IR150)] using a split-factorial experiment based on randomized complete block design. The combined application of 3% ethanol and 3000 ppm zinc sulfate was the most effective treatment, reducing severe drought damage by 40–50%. This synergy effectively maintained pod, kernel, and oil yields under severe stress at 2587.71, 1944.41, and 975.51 kg ha<sup>-1</sup>, respectively, while also maximizing yields under well-watered conditions. The physiological basis for this resilience was a fortified antioxidant system, evidenced by a 29% increase in catalase activity and a 15–30% reduction in malondialdehyde content, indicating lower oxidative stress. Crucially, the marginal yield gain from 3000 ppm zinc over 1500 ppm was negligible (~1%). Therefore, we recommend the foliar co-application of 3% ethanol with 1500 ppm zinc sulfate as a highly efficient and economically viable strategy to bolster drought tolerance, reduce irrigation dependency, and secure peanut oil production in water-limited environments.

## 1. Introduction

Peanut (*Arachis hypogaea* L.) is a globally significant industrial crop, prized as a primary source of edible oil and plant-based protein. Its productivity, however, is highly vulnerable to water deficit, particularly in semi-arid regions where drought can cause yield reductions of 30–60% by impairing photosynthesis, seed filling, and metabolic processes (Ratnakumar and Vadez, 2011; Devi et al., 2019). With increasing water scarcity, developing low-input, sustainable strategies to enhance crop resilience is paramount for securing the supply of industrial commodities like peanut oil.

Foliar application of stress-mitigating compounds presents a promising approach. Ethanol (C<sub>2</sub>H<sub>5</sub>OH), an endogenous metabolite produced under hypoxia, has been shown to enhance drought resilience in C<sub>3</sub> plants by suppressing photorespiration, improving carbon-use efficiency, scavenging reactive oxygen species (ROS), and inducing stomatal closure to conserve water (Gout et al., 2000; Devireddy et al., 2019; Takahashi et al., 2020). Concurrently, zinc (Zn) is an essential micronutrient that acts as a cofactor for over 300 enzymes. Zinc deficiency, widespread in croplands (Cakmak, 2009), severely limits yield by disrupting chlorophyll synthesis, auxin metabolism, and critically, the activity of key antioxidant enzymes like superoxide dismutase (SOD) (Cakmak, 2000; Hajiboland and Beiramzadeh, 2010).

While the individual benefits of ethanol and zinc are documented, their interactive potential for industrial crop production remains entirely unexplored. We hypothesize that the foliar co-application of ethanol and zinc sulfate will act synergistically to enhance drought tolerance in peanut beyond the additive effects of either treatment alone. This hypothesized synergy is predicated on complementary mechanisms: ethanol improving internal carbon availability and water-use efficiency, thereby facilitating zinc uptake and utilization, while zinc fortifies the antioxidant enzyme system, potentially enhancing the activity of ethanol-metabolizing enzymes like alcohol dehydrogenase (ADH) and providing a more robust defense against drought-induced oxidative stress.

This study is, to our knowledge, the first to comprehensively evaluate the combined foliar application of ethanol and zinc sulfate on peanut under varying irrigation regimes. We aim to determine their interactive effects on yield, oil production, and key physiological markers of drought tolerance, including oxidative damage and antioxidant enzyme activity. The findings are expected to provide a novel, practical, and sustainable strategy for enhancing the productivity and stability of peanut as an industrial oil crop in water-limited agroecosystems.

## 2. Materials and methods

### 2.1. Experimental site, design, and soil properties

A two-year field experiment was conducted in 2018 and 2019 on a private farm in Astara, Iran, located at 48° 52' E longitude and 38° 28' N latitude, with an elevation of -21 m. The site experiences a humid subtropical climate (Köppen classification: Cfa), characterized by high annual precipitation (~1800 mm), elevated relative humidity (>80%), mild winters, and warm, humid summers. The mean annual temperature is ~16°C, with growing season temperatures averaging 15–28°C.

The experiment employed a split-factorial arrangement within a Randomized Complete Block Design (RCBD) with three replications. Main plots were assigned to three irrigation regimes based on cumulative evaporation from a Class A pan: IR50 (50 mm, well-watered), IR100 (100 mm, mild stress), and IR150 (150 mm, severe stress). Subplots consisted of factorial combinations of three ethanol concentrations (0, 3, 6% v/v; denoted E0, E3, E6) and three zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) concentrations (0, 1500, 3000 ppm; denoted Zn0, Zn1500, Zn3000).

Prior to planting, composite soil samples (0–30 cm depth) were collected, air-dried, and analyzed for key physicochemical properties (Table 1). Soil pH and electrical conductivity (EC) were measured in a saturated paste extract. Total nitrogen was determined by the Kjeldahl method, available phosphorus by the Olsen bicarbonate method, and exchangeable potassium by ammonium acetate extraction followed by flame photometry. Organic carbon was quantified by wet oxidation.

Table 1  
Initial physicochemical properties of the experimental soil (0–30 cm depth)

| Soil depth<br>(cm) | Soil texture | Clay<br>(%) | Silt<br>(%) | Sand<br>(%) | pH  | Organic<br>Carbon (%) | Total<br>Nitrogen (%) | Available<br>phosphorus<br>(mg kg <sup>-1</sup> ) | Available<br>potassium<br>(mg kg <sup>-1</sup> ) | Zinc<br>(mg kg <sup>-1</sup> ) |
|--------------------|--------------|-------------|-------------|-------------|-----|-----------------------|-----------------------|---------------------------------------------------|--------------------------------------------------|--------------------------------|
| 0–30               | Sandy clay   | 31.7        | 27.1        | 41.2        | 5.7 | 1.65                  | 0.171                 | 9.3                                               | 287                                              | 0.82                           |

Based on the initial soil analysis, which indicated low available phosphorus, triple superphosphate (150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) and urea (60 kg ha<sup>-1</sup> N) were applied pre-sowing to meet crop nutrient requirements. Individual plots measured 15 m<sup>2</sup>, consisting of six 5-meter-long rows with 0.5-meter row spacing. A 3-meter bare buffer strip separated main plots to prevent lateral movement of water between different irrigation regimes.

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Soil physicochemical properties were analyzed prior to experimental establishment. Composite soil samples (0 to 30 cm depth) were collected in a zigzag pattern at field capacity moisture, homogenized, and air dried. Samples were sieved (2 mm) before analysis. Phosphorus content was determined via Olsen bicarbonate extraction (Olsen & Sommers, 1982), total nitrogen by Kjeldahl digestion (Bremner, 1996), and exchangeable potassium through 1 M ammonium acetate extraction followed by flame photometry using a Jenway PFP7 instrument (Simard, 1993). Organic carbon was quantified by wet oxidation (Nelson & Sommers, 1982), electrical conductivity (EC) was measured in saturated paste extract using an EC meter (Bohn et al., 1985), and pH was determined in saturated paste using a pH meter (Page et al., 1982). Initial soil physicochemical properties are summarized in Table 1. Based on the initial soil analysis, which indicated low available phosphorus, triple superphosphate (150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) and urea (60 kg ha<sup>-1</sup> N) were applied pre-sowing to meet crop nutrient requirements. Individual plots measured 15 m<sup>2</sup>, consisting of six 5-meter-long rows with 0.5-meter row spacing. A 3-meter bare buffer strip separated main plots to prevent lateral movement of water between different irrigation regimes.

## 2.2. Plant material

The experiment utilized seeds of the peanut (*Arachis hypogaea* L.) cv. 'Goli' [released as 'NC 2' in the USA (Mozingo et al., 1991)]. This cultivar has a runner (indeterminate) growth habit and is considered medium-maturing (110–120 days) (Gholi et al., 2016; Noormohammadi & Safarzadeh Vishkaei, 2009). It is known for its high yield potential (up to 4500 kg ha<sup>-1</sup> under optimal irrigation), high oil content (48–52%), large kernel size, and a high shelling percentage, which makes it suitable for mechanized harvesting (Mozingo et al., 1991; Noormohammadi & Safarzadeh Vishkaei, 2009). Seeds were obtained from the Seed and Plant Improvement Institute of Iran.

## 2.3. Crop establishment and management

Groundnut seeds were hand-sown on 20 May in both 2018 and 2019 at a density of 20 seeds per meter of row. Plants were later thinned to establish a stand density of 10 plants per square meter (10 plants m<sup>-2</sup>). Individual plots measured 15 m<sup>2</sup>, consisting of six 5-meter-long rows with 0.5-meter row spacing. A 3-meter bare buffer strip separated main plots to prevent lateral movement of water. Weed control was maintained through two hand-weedings during flowering and pod-formation stages. Foliar sprays contained ethanol (0–6% v/v) and zinc sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O; 0–3000 ppm Zn), applied with 0.1% Tween 20 as a surfactant. Applications occurred at BBCH<sup>1</sup>-stage 50 Inflorescence emergence (flower buds visible), BBCH<sup>11</sup> 65 Full flowering (> 50% open flowers), and BBCH 71: Pod development (gynophores penetrating soil) (Meier et al., 2001) using a backpack sprayer calibrated to deliver 200 L ha<sup>-1</sup>. Commercial-grade ethanol (96% purity, Pars Alcohol Co.) was diluted to target concentrations based on doses established from existing literature.

Based on regional climatic conditions, the total irrigation water volume for the full irrigation treatment was established at 4800 m<sup>3</sup> ha<sup>-1</sup>. The irrigation regimes were defined based on cumulative Class A pan evaporation. The well-watered control treatment (IR50) was irrigated after 50 mm of evaporation, receiving 12 irrigation events totaling 4800 m<sup>3</sup> ha<sup>-1</sup>. The moderate drought stress treatment (IR100) was irrigated after 100 mm of evaporation, which resulted in a reduced total volume of 2400 m<sup>3</sup> ha<sup>-1</sup>. The severe drought stress treatment (IR150) was irrigated after 150 mm of evaporation, receiving 4 irrigation events with a total volume of 1600 m<sup>3</sup> ha<sup>-1</sup>.

All crop protection measures and agronomic practices were uniformly applied across all plots, with no diseases or insects observed during the growing season. Groundnut pods were harvested on 21 September in both years. The following traits were evaluated: pod yield, plant height, pods per plant, seeds per pod, 100-seed weight, biological yield, seed yield, oil content, and oil yield.

## 2.4. Assessment of pod and seed yield and associated traits

Both years' harvests occurred on September 21. Following the removal of border areas (the first and last rows, plus 0.5 meters from each end of the plots), trait measurements were taken from the middle rows. Plant height, pods per plant, and seeds per pod were recorded using ten randomly selected plants per plot.

Biological yield (BY), pod yield (PY), and seed yield (SY) were determined through destructive harvesting of the four center rows, representing an 8 m<sup>2</sup> sampling area. For biological yield, the sampled plants from each plot were oven-dried at 75°C for 72 hours until a constant weight was achieved. The dried biomass was then weighed, and BY was calculated per unit area.

Peanut pods were harvested at physiological maturity, indicated by leaf yellowing and wilting. At this stage, sampled pods excavated from the soil displayed key maturity markers: when split open, the papery inner pericarp surrounding the kernels had darkened from white/cream to dark brown or black. Simultaneously, fully mature pods readily detached from the underground pegs. The total pod weight from the middle rows was measured, converted to a per-hectare basis, and recorded as PY (pod yield, kg ha<sup>-1</sup>). Seeds were subsequently hand-shelled, weighed, and recorded as SY (seed yield, kg ha<sup>-1</sup>) per plot.

## 2.5. Determination of oil content

Oil content was determined using a Foss NIRS DS21000 near-infrared spectrometer calibrated with 50 groundnut samples of known oil content (NIR) (Wan et al., 2017).

## 2.6. Determination of oil yield

Oil yield was calculated as: Seed yield (kg ha<sup>-1</sup>) × [Oil content (%) / 100] (Weiss, 2000, p. 102).

## 2.7. Lipid peroxidation assay (Malondialdehyde content)

Malondialdehyde (MDA) content, as a marker of lipid peroxidation, was quantified using the thiobarbituric acid reactive substances (TBARS) assay according to Heath and Packer (1968) with minor modifications. Briefly, approximately 0.2 g of fresh leaf tissue was homogenized in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12,000 × g for 15 min. Then, 0.5 mL of the supernatant was reacted with 1.5 mL of 0.5% (w/v) thiobarbituric acid (TBA) solution prepared in 20% TCA. The mixture was incubated in a water bath at 95°C for 30 min, and the reaction was stopped by placing the tubes on ice. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The MDA concentration was calculated using its extinction coefficient (155 mM<sup>-1</sup> cm<sup>-1</sup>) and expressed as nmol g<sup>-1</sup> fresh weight.

## 2.8. Antioxidant enzyme activity assay (Catalase activity)

Catalase activity was measured spectrophotometrically by monitoring the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 240 nm according to the method of Aebi (1984). For the enzyme extraction, 0.1 g of fresh leaf tissue was ground to a fine powder in liquid nitrogen and homogenized in 2 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 × g for 20 min at 4°C, and the resulting supernatant was used as the crude enzyme extract. The reaction mixture for the assay contained 50 µL of the enzyme extract in 1.5 mL of 50 mM phosphate buffer (pH 7.0) and 500 µL of 15 mM H<sub>2</sub>O<sub>2</sub>. The decrease in absorbance at 240 nm was recorded for 60 seconds. Enzyme activity was defined as the amount of enzyme required to decompose 1 µmol of H<sub>2</sub>O<sub>2</sub> per minute and was expressed as µmol mg<sup>-1</sup> protein min<sup>-1</sup>. Total soluble protein content in the extracts was determined by the Bradford (1976) method using bovine serum albumin as a standard.

## 2.9. Statistical analyses

All data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). Bartlett's test confirmed homogeneity of error variances across all traits between years ( $p > 0.05$ ). Following this verification, a combined two- year ANOVA was performed using PROC GLM. Mean comparisons used Duncan's Multiple Range Test (DMRT) at  $P \leq 0.05$ . Statistically significant relationships between trait means and yield were assessed via Pearson correlation analysis (PROC CORR). To draw the graphs Excel Software was used.

## 3. Results and Discussion

### 3.1. Results of Homogeneity of Variances (Bartlett's Test)

Homogeneity of error variances between the two years was confirmed for all measured traits using Bartlett's test ( $p > 0.05$ ). Consequently, the data from both years were combined and subjected to a combined analysis of variance (Table 2).

Table 2  
Results of Bartlett's test for homogeneity of error  
variances across two growing seasons (2018 and  
2019)

| Traits               | $\chi^2$ | <i>Pr</i> > ChiSq |
|----------------------|----------|-------------------|
| Plant Height         | 2.36 ns  | 0.15              |
| Pod number per plant | 2.84 ns  | 0.12              |
| Seed number per pod  | 2.93 ns  | 0.10              |
| 100-Seed weight      | 1.48 ns  | 0.36              |
| MDA                  | 1.07 ns  | 0.42              |
| Catalase             | 1.26 ns  | 0.39              |
| Pod Yield            | 3.18 ns  | 0.08              |
| Seed Yield           | 3.02 ns  | 0.09              |
| Oil content          | 1.86 ns  | 0.31              |
| Oil yield            | 2.88 ns  | 0.11              |
| ns: non- significant |          |                   |

The combined analysis of variance (ANOVA) revealed significant ( $p < 0.01$ ) main effects of irrigation regimes (IR), zinc (Zn), and ethanol (E), along with their two-way and three-way interactions, for all traits (Table 3).

Table 3  
Combined variance analysis of (Mean Squares) measured agronomic characteristics in groundnut as affected by irrigation regimes, ethanol and

| Source of variation | DF | Plant Height | Pod number per plant | Seed number per pod | 100-Seed weight | Biological yield | Seed Yield    | oil content | Oil Yield    | Pod Yield   | Catalase |
|---------------------|----|--------------|----------------------|---------------------|-----------------|------------------|---------------|-------------|--------------|-------------|----------|
| Y                   | 1  | 14.02 ns     | 152.124 ns           | 3.18 ns             | 93.52 ns        | 19411.67 ns      | 123046.3 ns   | 1.302 ns    | 30425.38 ns  | 950104.1 ns | 13.8 r   |
| Rep (Y)             | 4  | 255.494      | 278.767              | 12.557              | 65.649          | 2462789.44       | 924178.24     | 93.591      | 11993.25     | 461979.38   | 19.58    |
| I                   | 2  | 983.17**     | 62.602**             | 27.191*             | 304.816**       | 15972661.33**    | 16776095.68** | 385.8**     | 5912572.71** | 219078.24** | 721.6    |
| Y × I               | 2  | 8.09 ns      | 0.35 ns              | 1.3 ns              | 2.074 ns        | 7665.18 ns       | 1651.23 ns    | 2.32 ns     | 1044.67 ns   | 820.44 ns   | 6.38 r   |
| Error A             | 8  | 2.17         | 0.2798               | 0.915               | 1.5803          | 7789.63          | 1477.62       | 1.9324      | 4011.15      | 1361.74     | 5.38     |
| E                   | 2  | 59.69*       | 21.045**             | 415.449**           | 49.259*         | 6493831.74**     | 449529.91**   | 8.89*       | 174979.33**  | 862951.98*  | 208.6    |
| Y × E               | 2  | 2.65 ns      | 0.25 ns              | 3.17 ns             | 2.32 ns         | 8344.92 ns       | 181.13 ns     | 1.35 ns     | 937.64 ns    | 3159.74 ns  | 2.67 r   |
| Z                   | 2  | 410.24**     | 425.863**            | 302.448**           | 19.029**        | 252546.36**      | 592025.46**   | 44.12**     | 22911.85**   | 101493.35*  | 57.08    |
| Y × Z               | 2  | 2.55 ns      | 4.66 ns              | 2.26 ns             | 0.37 ns         | 629.69 ns        | 197.69 ns     | 0.86 ns     | 135.18 ns    | 4618.46 ns  | 2.08 r   |
| I × E               | 4  | 32.87*       | 94.465**             | 22.031**            | 18.801**        | 153445.22**      | 15797.07**    | 19.104*     | 61904.40**   | 57698.33**  | 70.81    |
| Y × I × E           | 4  | 3.01 ns      | 5.41 ns              | 1.41 ns             | 2.658 ns        | 2249.26 ns       | 113.18 ns     | 2.37 ns     | 67.59 ns     | 1801.28 ns  | 3.81 r   |
| I × Z               | 4  | 29.13*       | 79.10**              | 70.13**             | 105.462**       | 178410.5**       | 109232.25**   | 96.548*     | 57581.49**   | 136246.75** | 41.66    |
| Y × I × Z           | 4  | 3.55 ns      | 4.23 ns              | 3.58 ns             | 2.104 ns        | 802.55 ns        | 482.25 ns     | 10.5 ns     | 118.77 ns    | 2519.17 ns  | 3.05 r   |
| E × Z               | 4  | 9.46*        | 48.617**             | 32.86*              | 91.183**        | 22765.72**       | 3738.38**     | 50.418**    | 552858.92**  | 31786.64*   | 12.03    |
| Y × E × Z           | 4  | 1.534 ns     | 5.16 ns              | 3.39 ns             | 3.60 ns         | 117.14 ns        | 133.1 ns      | 2.41 ns     | 127.43 ns    | 2256.37 ns  | 1.04 r   |
| I × E × Z           | 8  | 13.94*       | 18.507**             | 26.327**            | 41.684**        | 27183.62**       | 7235.17**     | 32.131*     | 24206.19**   | 48276.26*   | 32.98    |
| Y × I × E × Z       | 8  | 3.691 ns     | 2.014 ns             | 1.14 ns             | 2.70 ns         | 123.33 ns        | 256.56 ns     | 6.47 ns     | 104.07 ns    | 3306.55 ns  | 5.17 r   |
| Error B             | 97 | 2.0952       | 3.586                | 2.258               | 1.9572          | 5497.7           | 824.65        | 7.0123      | 422.15       | 4037.88     | 6.39     |
| C.V. (%)            | -  | 11.27        | 9.32                 | 14.35               | 6.76            | 13.85            | 16.86         | 7.63        | 15.69        | 17.55       | 11.57    |

ns, \*, \*\*: Non-significant or significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

Abbreviations: Y: Year, Rep: Replication, I: Irrigation, Error A: Main plot error, E: Ethanol, Z: Zinc, Error B: Subplot error, C.V: Coefficient of Variation, MDA: Malondialdehyde

## 3.2. Malondialdehyde (MDA) Content

Malondialdehyde (MDA) content increased dramatically with drought intensity, with the highest accumulation (59.68 nmol g<sup>-1</sup> FW) occurring under severe drought (IR150) combined with high ethanol (E6) and zinc (Zn3000) (Table 4). This represented a 7.4-fold increase over the well-watered control, confirming severe oxidative damage.

This rise in MDA aligns with established literature on drought-induced oxidative stress, where water deficit disrupts cellular homeostasis and triggers lipid peroxidation (Hasanuzzaman et al., 2019). The dose-dependent effect of ethanol was evident; while moderate concentrations may stabilize membranes, the high concentration (E6) exacerbated damage, supported by findings that elevated ethanol can be toxic (Rodríguez-Gamir et al., 2019).

Zinc application effectively mitigated MDA accumulation, reducing it by 15–30% across stress regimes. This protective role is consistent with its known function in activating antioxidant enzymes and stabilizing membranes (Khan et al., 2020). A key novel finding was the synergistic interaction between ethanol and zinc. The combination of moderate ethanol (E3) and high zinc (Zn3000) produced the lowest MDA levels, indicating a combined effect greater than the sum of individual applications.

Notably, the highest MDA was found in the IR150/E6/Zn3000 treatment, suggesting that excessive ethanol can overwhelm zinc's protective capacity under severe stress. This underscores the need for optimized application rates.

A strong negative correlation between MDA and catalase activity ( $r = -0.68$ ) confirmed the crucial role of the antioxidant system in limiting oxidative damage. Furthermore, the positive correlations between catalase activity and key yield components highlight how reduced oxidative stress directly supports productivity and oil quality under drought (Hasanuzzaman et al., 2019; Prasad et al., 2019).

## 3.3. Catalase (CAT) Activity

Catalase (CAT) activity reached 66.19 nmol mg<sup>-1</sup> protein min<sup>-1</sup> under severe drought (IR150) with high ethanol (E6) and zinc (Zn3000), exhibiting a 29% increase over the well-watered control (Table 4). This elevated activity demonstrates a robust antioxidant response to stress, consistent with findings in other legumes (Silva et al., 2020).

Ethanol showed a dualistic effect: moderate concentration (E3) stimulated CAT activity in well-watered plants, whereas high concentration (E6) suppressed it under drought, suggesting cultivar-specific sensitivity compared to model plants like *Arabidopsis*. Zinc application consistently boosted CAT activity by 8–15%, demonstrating strongest synergy with E3. This Zn-organic collaboration mirrors synergism reported in other crops (Liu et al., 2023).

The strong negative correlation between CAT and MDA ( $r = -0.68$ ) confirms its role in reducing oxidative damage. Positive correlations with yield components (SY, PY, OY, BY, PNP) and oil content (OC) demonstrate that enhanced CAT activity preserves productivity and lipid biosynthesis under drought, aligning with known mechanisms of antioxidant protection (Wang et al., 2021). The combined E3 + Zn3000 treatment most effectively induced this synergistic antioxidant response.

### 3.4. Synergistic Interaction of Ethanol and Zinc in Mitigating Oxidative Stress

This study reveals a novel synergistic interaction between moderate ethanol (E3) and high zinc (Zn3000), representing the most effective strategy for minimizing oxidative stress under drought conditions. This combination produced the lowest MDA levels and highest CAT activity, demonstrating superior stress mitigation compared to individual applications.

The synergy arises from complementary mechanisms: ethanol enhances internal CO<sub>2</sub> concentration and reduces photorespiration, while zinc acts as an essential cofactor for antioxidant enzymes like SOD (Cakmak, 2000). We propose a multi-faceted interaction where ethanol may facilitate zinc uptake through enhanced membrane permeability (Gutierrez & Boerema, 2023), while zinc promotes efficient ethanol metabolism via alcohol dehydrogenase activation (Vallee & Auld, 1990). This reciprocal relationship converts ethanol into beneficial energy sources under drought (Ismond et al., 2003).

Furthermore, both compounds collectively enhance antioxidant defense through different pathways. Ethanol induces stomatal closure to reduce ROS generation (Devireddy et al., 2019), while zinc provides direct enzymatic ROS scavenging through Cu/Zn-SOD. This dual approach of limiting ROS production while enhancing scavenging capacity creates a powerful protective system that preserves cellular integrity under drought conditions.

### 3.5. Pod yield (PY)

Severe drought stress (IR150) reduced pod yield by 40–50%, consistent with previous reports (Puangbut et al., 2015; Aydinsakir et al., 2016). The novel finding is the synergistic effect between foliar-applied ethanol (3%) and zinc sulfate (3000 ppm). The E3 + Zn3000 combination maintained yield at 2587.71 kg ha<sup>-1</sup> under severe stress, reducing potential losses by 59% compared to controls.

This synergy surpassed individual treatments. Zinc improved yield components by maintaining membrane integrity and photosynthate translocation (Prasad et al., 2019), while ethanol enhanced carbon use efficiency and induced stomatal closure to conserve water (Devireddy et al., 2019; Pereira et al., 2018), thereby improving plant water status and photosynthate allocation under stress. Their combination provided superior oxidative stress reduction and yield component preservation.

Strong correlations confirmed pods per plant ( $r = 0.97$ ) as the primary yield determinant (Puangbut et al., 2015). The negative correlation between MDA and PY ( $r = -0.58$ ) and positive correlation between CAT and PY ( $r = 0.61$ ) demonstrate the role of antioxidant pathways in yield protection (Wang et al., 2021; Cakmak, 2000). The negative correlation between SNP and 100-SW ( $r = -0.45$ ) confirms trade-offs between yield components (Dwivedi et al., 1993), supporting prioritization of high-pod-count genotypes in breeding programs.

### 3.6. Plant height (PH)

Maximum plant height (85.7 cm) was achieved under optimal irrigation (IR50) with E3 and Zn3000, while severe stress (IR150) reduced height by 10% (60.0 cm) in control treatments (Table 4). This reduction aligns with established drought responses in legumes where water deficit limits cell expansion and division (Boote et al., 1982). The combined E3 + Zn3000 treatment significantly alleviated stress effects, resulting in plants 18% taller than untreated controls under severe drought.

Zinc application mitigated growth reduction by promoting auxin biosynthesis and maintaining turgor pressure (Rizwan et al., 2019), while ethanol improved carbon partitioning and photosynthetic efficiency (Pereira et al., 2018). Their combination demonstrated synergistic growth enhancement, supporting more sustained vegetative development under drought than either treatment alone, consistent with findings on combined stress mitigators in legumes (Lanjewar et al., 2021). The strong correlations between PH and both biological yield ( $r = 0.90$ ) and seed yield ( $r = 0.86$ ) underscore the agronomic importance of maintaining plant architecture under stress conditions.

### 3.7. Pod number per plant (PNP)

Pod number per plant (PNP) significantly decreased under water deficit, with maximum values (47.8 pods) occurring under optimal irrigation (IR50) with E3 and Zn3000 (Table 4). This treatment maintained superior PNP under stress (42.0 pods under IR100; 38.2 pods under IR150). The observed reduction aligns with previous reports of 25–50% PNP decrease under drought due to flower and peg abortion (Puangbut et al., 2015; Aydinsakir et al., 2016).

Zinc application mitigated PNP reduction by improving reproductive success through enhanced pollen viability and fertilization (Prasad et al., 2019), potentially via auxin metabolism and oxidative stress protection (Cakmak, 2000). Ethanol (E3) maintained PNP by improving water use efficiency and carbon metabolism (Nonomura & Benson, 1992; Pereira et al., 2018). The E3 + Zn3000 combination demonstrated superior synergistic protection of pod set by

acting on complementary pathways: ethanol enhancing carbon fixation and osmotic adjustment, while zinc improving enzyme function and antioxidant defense.

Strong correlations with plant height ( $r = 0.77$ ) and biological yield ( $r = 0.90$ ) confirm that maintained vegetative architecture supports reproductive capacity under drought (Pandey et al., 2012).

### **3.8. Seed number per pod (SNP)**

Drought stress significantly reduced seed number per pod (SNP) across all treatments. Maximum SNP (1.65 seeds) occurred under optimal irrigation ( $IR_{50}$ ) with foliar E3 and 3000 ppm Zn (Table 4). This treatment maintained the highest SNP values under both mild stress ( $IR_{100}$ : 1.23 seeds) and severe stress ( $IR_{150}$ : 1.12 seeds; Table 4). Similarly, a study by Aydinsakir et al. (2016) found that irrigation regimes significantly affecting SNP, with severe stress leading to the greatest reduction. The efficacy of the combined E3 + Zn3000 treatment in mitigating SNP loss is supported by its role in bolstering key reproductive processes. Zinc nutrition is crucial for pollen germination, pollen tube growth, and seed development, which are all highly sensitive to drought-induced inhibition (Prasad et al., 2020). Concurrently, foliar ethanol application is known to enhance osmotic adjustment and photosynthate supply to developing reproductive sinks, thereby reducing seed abortion under moisture stress (Nonomura & Benson, 1992). The synergistic effect observed suggests that the combination effectively addresses both the physiological and biochemical constraints that limit SNP under drought conditions (Puangbut et al., 2018).

### **3.9. 100-seed weight (100-SW)**

Drought stress significantly reduced 100-seed weight by 15–30%, consistent with previous reports of impaired photo-assimilate supply during seed filling (Hamidou et al., 2012; Puangbut et al., 2015). The combined E3 + Zn3000 treatment effectively mitigated these reductions, maintaining 61.5 g under mild stress and 57.8 g under severe stress compared to the maximum 69.8 g under optimal conditions.

Zinc application improved seed weight by maintaining membrane integrity and facilitating carbohydrate mobilization to developing seeds (Prasad et al., 2019), supported by zinc's role as a cofactor for carbohydrate metabolism enzymes (Cakmak, 2000). Ethanol enhanced carbon availability for sink organs, similar to methanol effects reported in C3 plants (Nonomura & Benson, 1992). The synergistic combination of both treatments provided superior protection against drought-induced seed weight reduction.

### **3.10. Biological yield (BY)**

Drought stress severely reduced groundnut biological yield (BY) by 40 to 50 percent. Foliar ethanol (3%) and zinc (3000 ppm) maximized BY across all conditions: 8100 kg ha<sup>-1</sup> under full irrigation, 6354 kg ha<sup>-1</sup> under mild stress, and 5204 kg ha<sup>-1</sup> under severe stress (Table 4). Strong BY correlations with plant height ( $r = 0.94$ ; Table 5), pod number per plant ( $r = 0.90$ ; Table 5), seed yield ( $r = 0.92$ ; Table 5), and oil yield ( $r = 0.88$ ; Table 5) confirm its integrative role in yield architecture. The profound reduction in biological yield observed under drought stress in this study is consistent with previous reports in groundnut and other leguminous crops. Hamidou et al. (2012) similarly reported that drought stress could reduce the total biomass production in peanut by up to 60%, depending on the intensity and duration of the stress period. This drastic reduction is primarily attributed to drought-induced inhibition of canopy development, leaf expansion, and photosynthetic efficiency, which collectively limit the cumulative biomass accumulation over the growing season (Anjum et al., 2011).

**Table 4**  
Mean comparison of measured agronomic characteristics in groundnut influenced by the interaction effect of irrigation regimes × ethanol × Zn

| Irrigation regimes | ethanol | Zinc               | PH<br>(cm)            | PY<br>(kg ha <sup>-1</sup> ) | PNP                 | SNP                  | 100-SW<br>(g)      | BY<br>(kg ha <sup>-1</sup> ) | SY<br>(kg ha <sup>-1</sup> ) | OC<br>(%)           | OY<br>(kg ha <sup>-1</sup> ) | CAT<br>(nmol mg <sup>-1</sup> pro min <sup>-1</sup> ) | MDA<br>(µg g <sup>-1</sup> FW) |
|--------------------|---------|--------------------|-----------------------|------------------------------|---------------------|----------------------|--------------------|------------------------------|------------------------------|---------------------|------------------------------|-------------------------------------------------------|--------------------------------|
| IR <sub>50</sub>   | E0      | Zn <sub>0</sub>    | 76.09 <sup>cd</sup>   | 3133.33 <sup>c</sup>         | 39.40 <sup>j</sup>  | 1.31 <sup>f</sup>    | 61.09 <sup>e</sup> | 6984.36 <sup>d</sup>         | 2288.46 <sup>d</sup>         | 48.64 <sup>op</sup> | 1113.11 <sup>e</sup>         | 51.07 <sup>klm</sup>                                  | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 79.13 <sup>c</sup>    | 3547.54 <sup>b</sup>         | 42.73 <sup>d</sup>  | 1.36 <sup>e</sup>    | 64.13 <sup>d</sup> | 7359.48 <sup>c</sup>         | 2574.33 <sup>c</sup>         | 50.60 <sup>fg</sup> | 1302.61 <sup>c</sup>         | 52.95 <sup>hij</sup>                                  | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 81.96 <sup>b</sup>    | 3667.47 <sup>b</sup>         | 44.64 <sup>b</sup>  | 1.43 <sup>d</sup>    | 66.96 <sup>c</sup> | 7697.26 <sup>b</sup>         | 2618.18 <sup>c</sup>         | 51.72 <sup>bc</sup> | 1354.12 <sup>b</sup>         | 54.80 <sup>ghi</sup>                                  | 1.10 <sup>g</sup>              |
|                    | E3      | ZN <sub>0</sub>    | 79.44 <sup>c</sup>    | 3472.12 <sup>b</sup>         | 41.35 <sup>f</sup>  | 1.51 <sup>c</sup>    | 64.94 <sup>d</sup> | 7221.95 <sup>c</sup>         | 2858.77 <sup>b</sup>         | 49.78 <sup>ik</sup> | 1373.32 <sup>b</sup>         | 47.94 <sup>no</sup>                                   | 8.10 <sup>g</sup>              |
|                    |         | ZN <sub>1500</sub> | 82.85 <sup>b</sup>    | 4366.53 <sup>a</sup>         | 44.26 <sup>c</sup>  | 1.59 <sup>b</sup>    | 67.65 <sup>b</sup> | 7791.66 <sup>b</sup>         | 3175.35 <sup>a</sup>         | 52.99 <sup>a</sup>  | 1682.62 <sup>a</sup>         | 48.98 <sup>mno</sup>                                  | 7.10 <sup>g</sup>              |
|                    |         | ZN <sub>3000</sub> | 85.65 <sup>a</sup>    | 4412.66 <sup>a</sup>         | 47.81 <sup>a</sup>  | 1.65 <sup>a</sup>    | 69.83 <sup>a</sup> | 8103.14 <sup>a</sup>         | 3235.78 <sup>a</sup>         | 53.05 <sup>a</sup>  | 1716.58 <sup>a</sup>         | 50.12 <sup>lmn</sup>                                  | 7.10 <sup>g</sup>              |
|                    | E6      | Zn <sub>0</sub>    | 75.03 <sup>efg</sup>  | 3005.49 <sup>d</sup>         | 36.94 <sup>l</sup>  | 1.19 <sup>i</sup>    | 57.03 <sup>j</sup> | 6350.17 <sup>f</sup>         | 2097.61 <sup>e</sup>         | 48.69 <sup>op</sup> | 1026.90 <sup>fg</sup>        | 52.87 <sup>ijkl</sup>                                 | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 77.38 <sup>cd</sup>   | 3179.55 <sup>c</sup>         | 38.33 <sup>j</sup>  | 1.225 <sup>h</sup>   | 59.28 <sup>h</sup> | 6785.40 <sup>e</sup>         | 2295.33 <sup>d</sup>         | 49.38 <sup>lm</sup> | 1133.43 <sup>de</sup>        | 53.95 <sup>hij</sup>                                  | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 79.43 <sup>c</sup>    | 3281.75 <sup>c</sup>         | 40.14 <sup>h</sup>  | 1.27 <sup>g</sup>    | 59.73 <sup>g</sup> | 7074.28 <sup>d</sup>         | 2341.66 <sup>d</sup>         | 50.30 <sup>hi</sup> | 1177.85 <sup>d</sup>         | 55.01 <sup>ghi</sup>                                  | 1.10 <sup>g</sup>              |
| IR <sub>100</sub>  | E0      | Zn <sub>0</sub>    | 71.93 <sup>hi</sup>   | 2633.44 <sup>j</sup>         | 37.52 <sup>k</sup>  | 1.10 <sup>klm</sup>  | 55.93 <sup>l</sup> | 5608.79 <sup>i</sup>         | 1658.46 <sup>i</sup>         | 49.15 <sup>mn</sup> | 815.13 <sup>jk</sup>         | 58.20 <sup>defg</sup>                                 | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 74.23 <sup>efgh</sup> | 2883.28 <sup>d</sup>         | 39.88 <sup>h</sup>  | 1.14 <sup>j</sup>    | 57.54 <sup>j</sup> | 5981.32 <sup>h</sup>         | 1884.67 <sup>f</sup>         | 50.89 <sup>ef</sup> | 959.11 <sup>gh</sup>         | 62.11 <sup>cd</sup>                                   | 1.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 75.15 <sup>def</sup>  | 3105.53 <sup>c</sup>         | 40.74 <sup>g</sup>  | 1.187 <sup>i</sup>   | 58.95 <sup>h</sup> | 6173.25 <sup>g</sup>         | 2047.27 <sup>e</sup>         | 52.06 <sup>b</sup>  | 1065.81 <sup>ef</sup>        | 64.16 <sup>ab</sup>                                   | 1.10 <sup>g</sup>              |
|                    | E3      | Zn <sub>0</sub>    | 74.03 <sup>fgh</sup>  | 2883.33 <sup>d</sup>         | 38.81 <sup>i</sup>  | 1.14 <sup>j</sup>    | 58.03 <sup>i</sup> | 5896.41 <sup>h</sup>         | 1796.88 <sup>g</sup>         | 50.43 <sup>gh</sup> | 906.17 <sup>hi</sup>         | 54.04 <sup>ijk</sup>                                  | 3.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 76.44 <sup>de</sup>   | 3174.58 <sup>c</sup>         | 40.28 <sup>g</sup>  | 1.19 <sup>i</sup>    | 60.44 <sup>f</sup> | 6108.75 <sup>g</sup>         | 2007.36 <sup>e</sup>         | 52.82 <sup>a</sup>  | 1060.29 <sup>ef</sup>        | 54.91 <sup>hij</sup>                                  | 3.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 77.14 <sup>cd</sup>   | 3302.66 <sup>c</sup>         | 41.99 <sup>e</sup>  | 1.23 <sup>h</sup>    | 61.47 <sup>e</sup> | 6354.09 <sup>f</sup>         | 2276.94 <sup>d</sup>         | 53.12 <sup>a</sup>  | 1209.51 <sup>cd</sup>        | 56.17 <sup>fgh</sup>                                  | 2.10 <sup>g</sup>              |
|                    | E6      | Zn <sub>0</sub>    | 68.08 <sup>j</sup>    | 2384.21 <sup>f</sup>         | 35.17 <sup>m</sup>  | 1.08 <sup>lmn</sup>  | 53.08 <sup>n</sup> | 5425.19 <sup>j</sup>         | 1458.67 <sup>j</sup>         | 48.15 <sup>q</sup>  | 702.35 <sup>kl</sup>         | 61.15 <sup>def</sup>                                  | 3.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 70.97 <sup>hi</sup>   | 2468.56 <sup>e</sup>         | 37.24 <sup>k</sup>  | 1.11 <sup>ijkl</sup> | 44.94 <sup>r</sup> | 5641.46 <sup>i</sup>         | 1605.33 <sup>i</sup>         | 50.97 <sup>de</sup> | 818.24 <sup>jk</sup>         | 63.09 <sup>gh</sup>                                   | 3.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 71.93 <sup>fgh</sup>  | 2698.11 <sup>d</sup>         | 39.06 <sup>h</sup>  | 1.12 <sup>jk</sup>   | 56.93 <sup>k</sup> | 5791.13 <sup>i</sup>         | 1789.81 <sup>g</sup>         | 51.49 <sup>cd</sup> | 921.57 <sup>hi</sup>         | 65.22 <sup>a</sup>                                    | 3.10 <sup>g</sup>              |
| IR <sub>150</sub>  | E0      | Zn <sub>0</sub>    | 63.68 <sup>cd</sup>   | 2083.24 <sup>g</sup>         | 32.73 <sup>r</sup>  | 0.99 <sup>op</sup>   | 51.09 <sup>o</sup> | 4325.67 <sup>n</sup>         | 1101.26 <sup>m</sup>         | 44.14 <sup>u</sup>  | 486.10 <sup>o</sup>          | 61.95 <sup>cde</sup>                                  | 4.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 66.81 <sup>lm</sup>   | 2233.68 <sup>f</sup>         | 34.91 <sup>n</sup>  | 1.05 <sup>n</sup>    | 52.13 <sup>o</sup> | 4683.85 <sup>m</sup>         | 1455.28 <sup>j</sup>         | 47.68 <sup>r</sup>  | 693.88 <sup>kl</sup>         | 62.93 <sup>bcd</sup>                                  | 4.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 68.91 <sup>jk</sup>   | 2413.38 <sup>e</sup>         | 37.90 <sup>jk</sup> | 1.08 <sup>lmn</sup>  | 52.96 <sup>n</sup> | 4802.49 <sup>m</sup>         | 1705.60 <sup>h</sup>         | 49.75 <sup>kl</sup> | 848.54 <sup>ij</sup>         | 63.87 <sup>abc</sup>                                  | 3.10 <sup>g</sup>              |
|                    | E3      | Zn <sub>0</sub>    | 68.04 <sup>kl</sup>   | 2207.27 <sup>g</sup>         | 33.81 <sup>p</sup>  | 1.02 <sup>o</sup>    | 54.94 <sup>m</sup> | 4783.33 <sup>m</sup>         | 1401.47 <sup>j</sup>         | 47.61 <sup>r</sup>  | 667.24 <sup>l</sup>          | 58.86 <sup>defg</sup>                                 | 4.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 70.75 <sup>ij</sup>   | 2396.18 <sup>e</sup>         | 36.18 <sup>l</sup>  | 1.06 <sup>mn</sup>   | 55.85 <sup>l</sup> | 4942.85 <sup>l</sup>         | 1752.15 <sup>g</sup>         | 48.70 <sup>no</sup> | 853.30 <sup>ij</sup>         | 60.41 <sup>efg</sup>                                  | 4.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 71.08 <sup>ghi</sup>  | 2587.71 <sup>e</sup>         | 38.24 <sup>j</sup>  | 1.12 <sup>jk</sup>   | 57.83 <sup>i</sup> | 5204.08 <sup>k</sup>         | 1944.41 <sup>f</sup>         | 50.17 <sup>ij</sup> | 975.51 <sup>gh</sup>         | 61.85 <sup>cde</sup>                                  | 4.10 <sup>g</sup>              |
|                    | E6      | Zn <sub>0</sub>    | 59.97 <sup>n</sup>    | 1905.55 <sup>h</sup>         | 30.89 <sup>s</sup>  | 0.94 <sup>q</sup>    | 49.03 <sup>q</sup> | 4101.27 <sup>o</sup>         | 951.71 <sup>n</sup>          | 46.69 <sup>t</sup>  | 444.35 <sup>p</sup>          | 63.01 <sup>bc</sup>                                   | 5.10 <sup>g</sup>              |
|                    |         | Zn <sub>1500</sub> | 61.82 <sup>mn</sup>   | 2094.63 <sup>g</sup>         | 33.14 <sup>q</sup>  | 0.96 <sup>pq</sup>   | 50.38 <sup>p</sup> | 4287.49 <sup>n</sup>         | 1121.55 <sup>l</sup>         | 47.04 <sup>s</sup>  | 527.58 <sup>n</sup>          | 64.67 <sup>ab</sup>                                   | 5.10 <sup>g</sup>              |
|                    |         | Zn <sub>3000</sub> | 62.57 <sup>m</sup>    | 2297.86 <sup>f</sup>         | 34.10 <sup>o</sup>  | 0.98 <sup>p</sup>    | 50.93 <sup>p</sup> | 4434.63 <sup>n</sup>         | 1263.27 <sup>k</sup>         | 48.55 <sup>p</sup>  | 613.32 <sup>m</sup>          | 66.19 <sup>a</sup>                                    | 5.10 <sup>g</sup>              |

Means followed by the same letters in each column are not significantly different at 5% probability level on the basis of D'MRT test.

PH: Plant height, PY: Pod yield, PNP: Pod number per plant, SNP: Seed number per pod, 100-SW:100-Seed weight, BY: Biological yield, SY: Seed yield, OC: Oil content and OY: Oil yield, CAT: Catalase, MDA: Malondialdehyde

**Table 5.** Correlation coefficient between grain yield and measured agronomic traits in groundnut influenced by the interaction effect of irrigation regimes × ethanol × Zn

| Traits                                                                                                                                                                                              | SY      | OY      | PH                 | BY                 | PNP                | SNP                | 100-SW  | OC                 | PY      | MDA     | Catalase |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|---------|--------------------|--------------------|--------------------|--------------------|---------|--------------------|---------|---------|----------|
|                                                                                                                                                                                                     | 1       | 2       | 3                  | 4                  | 5                  | 6                  | 7       | 8                  | 9       | 10      | 11       |
| 1                                                                                                                                                                                                   | 1       |         |                    |                    |                    |                    |         |                    |         |         |          |
| 2                                                                                                                                                                                                   | 0.91**  | 1       |                    |                    |                    |                    |         |                    |         |         |          |
| 3                                                                                                                                                                                                   | 0.86**  | 0.64**  | 1                  |                    |                    |                    |         |                    |         |         |          |
| 4                                                                                                                                                                                                   | 0.92**  | 0.88**  | 0.94**             | 1                  |                    |                    |         |                    |         |         |          |
| 5                                                                                                                                                                                                   | 0.95**  | 0.62**  | 0.77**             | 0.90**             | 1                  |                    |         |                    |         |         |          |
| 6                                                                                                                                                                                                   | 0.75**  | 0.57**  | 0.66**             | 0.79**             | 0.18 <sup>ns</sup> | 1                  |         |                    |         |         |          |
| 7                                                                                                                                                                                                   | 0.89**  | 0.81**  | 0.36*              | 0.79**             | -0.53**            | -0.45*             | 1       |                    |         |         |          |
| 8                                                                                                                                                                                                   | 0.67**  | 0.72**  | 0.28 <sup>ns</sup> | 0.26 <sup>ns</sup> | 0.24 <sup>ns</sup> | 0.26 <sup>ns</sup> | 0.53**  | 1                  |         |         |          |
| 9                                                                                                                                                                                                   | 0.93**  | 0.69**  | 0.88**             | 0.94**             | 0.97**             | 0.28 <sup>ns</sup> | 0.76**  | 0.21 <sup>ns</sup> | 1       |         |          |
| 10                                                                                                                                                                                                  | -0.56** | -0.51** | -0.41*             | -0.52**            | -0.49**            | -0.37*             | -0.53** | -0.32              | -0.58** | 1       |          |
| 11                                                                                                                                                                                                  | 0.68**  | 0.66**  | 0.63**             | 0.67**             | 0.69**             | 0.34*              | 0.51**  | 0.54**             | 0.61**  | -0.68** | 1        |
| <sup>ns</sup> , * and **: non-significant, significant at 5% and 1% probability levels, respectively.                                                                                               |         |         |                    |                    |                    |                    |         |                    |         |         |          |
| Abbreviations: SY: Seed yield, OY: Oil yield, PH: Plant height, BY: Biological yield, PNP: Pod number per plant, SNP: Seed number per pod, 100-SW: 100-Seed weight, OC: Oil content, PY: Pod yield. |         |         |                    |                    |                    |                    |         |                    |         |         |          |

The efficacy of the combined ethanol and zinc foliar application in mitigating BY loss aligns with, yet significantly expands upon, the known effects of these compounds studied in isolation. Previous research has demonstrated that zinc application can enhance drought tolerance by improving leaf turgor maintenance, chlorophyll synthesis, and photosynthetic rates, thereby supporting greater biomass production under stress (Cakmak, 2000; Prasad et al., 2019). The role of ethanol in enhancing drought tolerance and biomass accumulation is linked to its function in improving water-use efficiency, inducing antioxidant defense systems, and potentially modulating membrane properties for better nutrient uptake under stress (Pereira et al., 2021; Gutierrez & Boerema, 2023). While early studies on similar short-chain alcohols like methanol suggested mechanisms such as photorespiration inhibition (Nonomura & Benson, 1992; Gout et al., 2000), recent research provides more direct evidence for ethanol's role in activating specific physiological and biochemical pathways that confer drought resilience.

### 3.11. Seed yield (SY)

Seed yield declined significantly under progressive water deficit, with peak yields of 3235.78 kg ha<sup>-1</sup> (E3 + Zn3000) under full irrigation (Table 4). The E3 + Zn3000 treatment maximized SY across stress regimes, maintaining 1944.41 kg ha<sup>-1</sup> under severe stress. SY showed strong correlations with pod number per plant ( $r = 0.95$ ), biological yield ( $r = 0.92$ ), and 100-seed weight ( $r = 0.89$ ).

The yield decline under drought aligns with established mechanisms of disrupted photosynthate partitioning (Boote et al., 2019). Zinc application mitigated losses by enhancing antioxidant activity and pollen viability, reducing pod abortion (Prasad et al., 2019). Ethanol improved water use efficiency and photosynthetic assimilation (Pereira et al., 2018), sustaining both source and sink functions under stress.

The demonstrated synergy between E3 and Zn3000 exceeds previously reported effects of single applications (Lanjewar et al., 2021). This superior performance results from complementary mechanisms: ethanol preserving carbon fixation and turgor maintenance, while zinc fortifying enzymatic and antioxidant functions, creating a holistic protective strategy against drought-induced yield losses.

### 3.12. Oil content (OC)

The combined E3 + Zn3000 treatment maximized oil content, achieving 53.05% under full irrigation and 53.12% under mild stress (Table 4). Severe drought reduced OC by 15–20% in controls, consistent with known disruption of lipid biosynthesis pathways under water deficit (Dwivedi et al., 1996). The optimal treatment maintained OC at 50.17% under severe stress, 3–6% higher than alternatives.

Zinc application sustained OC by activating lipid biosynthesis enzymes (dehydrogenase, alcohol dehydrogenase) and stabilizing membranes (Prasad et al., 2019; Cakmak, 2000). The novel synergy with ethanol suggests complementary mechanisms: ethanol providing carbon substrates for oil synthesis while zinc optimizes the enzymatic conversion environment. This dual approach addresses both source (carbon supply) and sink (metabolic conversion) limitations imposed by drought, offering superior quality preservation alongside yield stability.

### 3.13. Oil yield (OY)

Oil yield declined significantly under progressive drought, with maximum values of 1716.58 kg ha<sup>-1</sup> (E3 + Zn3000) under full irrigation (Table 4). The E3 + Zn3000 treatment consistently maximized OY across stress regimes, maintaining 975.51 kg ha<sup>-1</sup> under severe stress. This decline aligns with known disruption of carbohydrate allocation to kernels under drought (Boote et al., 2019).

Zinc improved OY by enhancing activity of key enzymes like ACCase in lipid biosynthesis (Prasad et al., 2019), influencing both seed yield and oil content. Ethanol provided carbon precursors (e.g., acetyl-CoA) for fatty acid synthesis (Pereira et al., 2018), particularly crucial under photosynthetic limitation. The novel synergy between these compounds resulted in superior OY compared to individual applications, simultaneously improving seed number (zinc effect) and seed filling (ethanol effect), as evidenced by strong correlations with 100-seed weight ( $r = 0.81$ ).

This strategy effectively mitigates economic losses by preserving both yield and quality under drought. However, limitations include testing on a single cultivar (NC2), necessitating validation across genotypes. Future research should incorporate photosynthetic parameters and molecular approaches to fully elucidate the synergy mechanisms.

## 4. Conclusion

This study establishes the foliar co-application of 3% ethanol and zinc sulfate (1500–3000 ppm) as a novel, synergistic, and sustainable strategy for peanut production. This combination significantly enhances drought tolerance by fortifying the antioxidant defense system, thereby mitigating oxidative damage and preserving photosynthetic capacity and reproductive success. This translates directly into higher and more stable yields of pods, kernels, and most importantly, oil, under water deficit conditions.

We specifically recommend the use of 3% ethanol combined with 1500 ppm zinc sulfate due to its cost-effectiveness and high efficiency. This strategy offers a practical tool for farmers to reduce irrigation water use without compromising yield or profitability, contributing to the sustainable intensification of peanut cultivation for the oil industry. Future research should focus on elucidating the molecular mechanisms of this synergy and validating these results across a wider range of cultivars and environments.

## Declarations

### Funding Declaration

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## Author Contribution

S.S. and N.S. analyzed data and wrote the main manuscript text. Z.R. ran the experiment. S.M. prepared figures and tables. K. F. and K. A. selected data. All authors reviewed the manuscript.

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## Footnotes

1. <sup>1</sup>BBCH stands for Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie – the three German organizations that originally developed this universal scale for plant growth staging.

## Figures

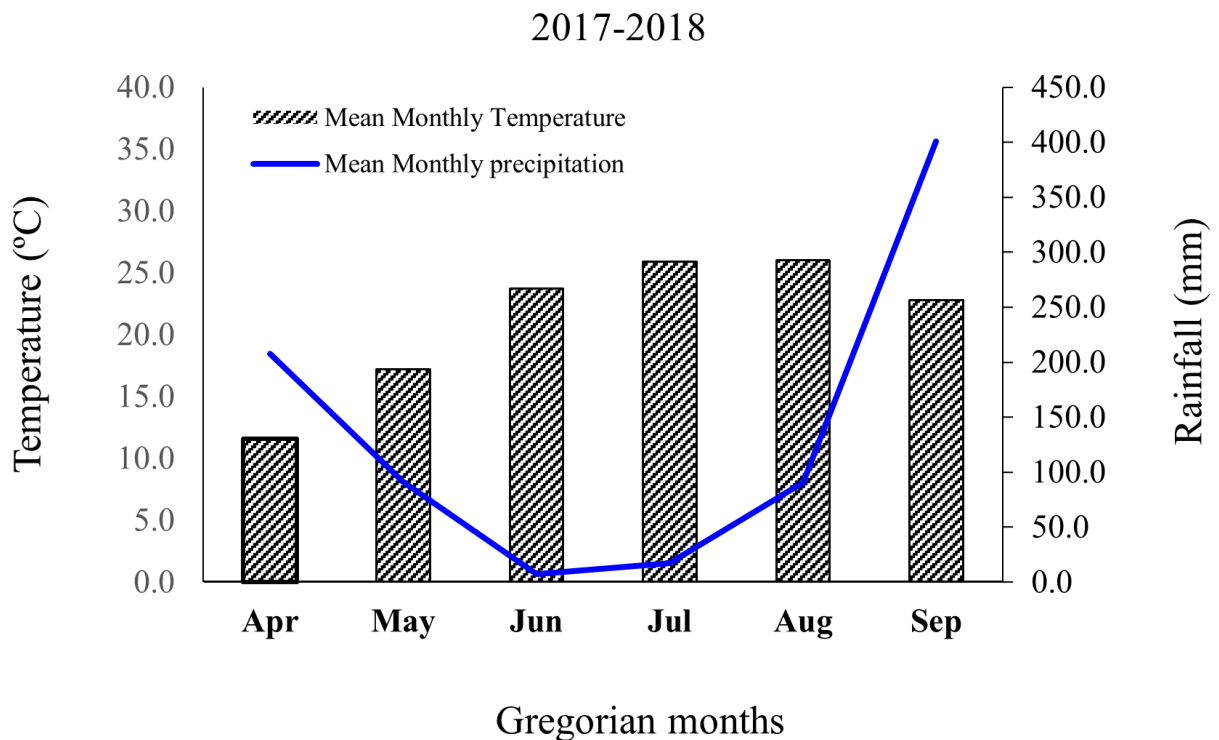


Figure 1

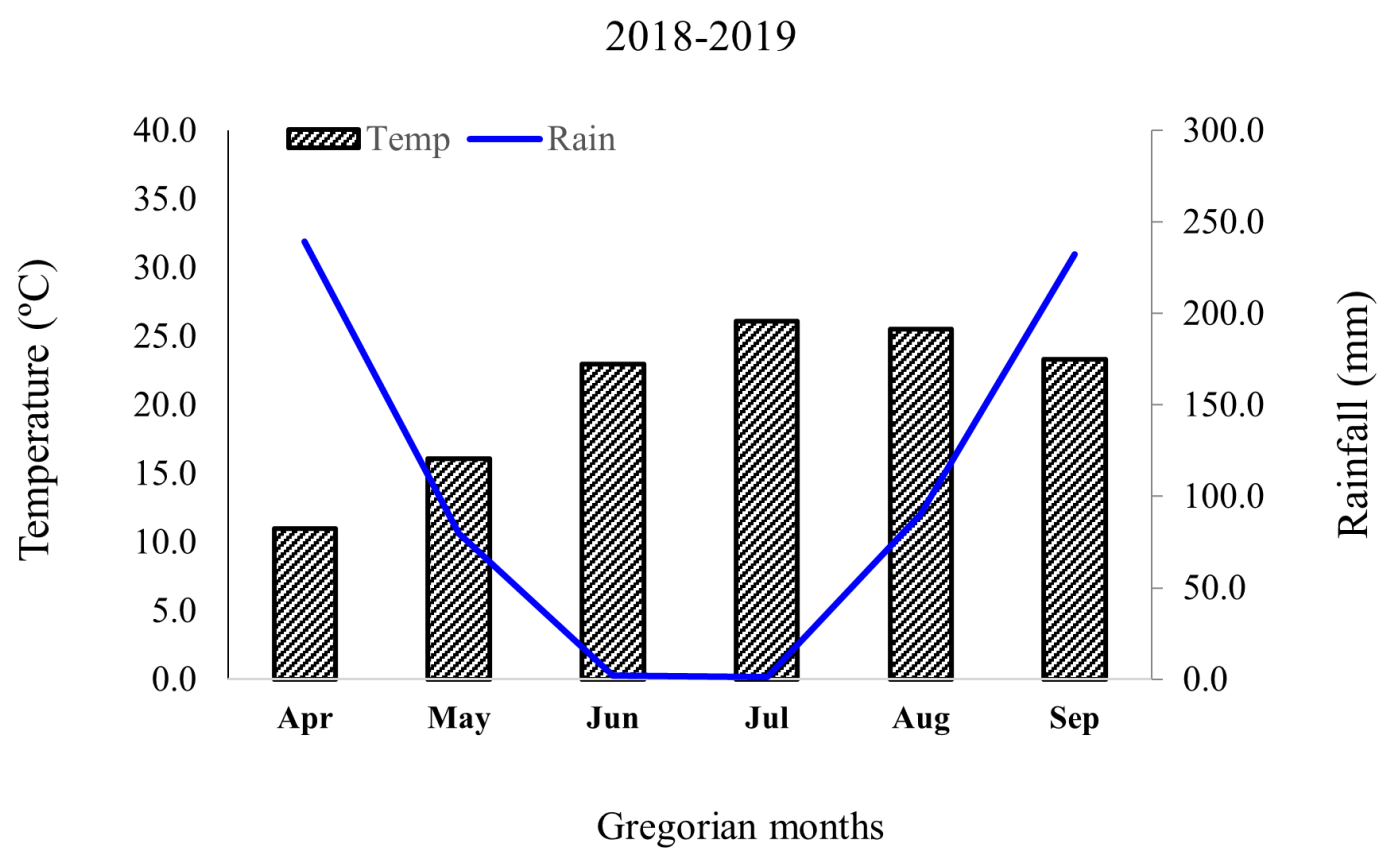


Figure 2

Mean monthly precipitations and temperature in the years 2018–2019.