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MITIGATING DROUGHT STRESS IN MAIZE TO EXPLORING THE GROWTH-PROMOTING EFFECTS OF *CONOCARPUS ERECTUS* L. SMOKE

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ABSTRACT

This study investigated the use of smoke solutions and ash from *Conocarpus erectus* leaves to mitigate drought stress in maize (*Zea mays* var. Azam). Drought significantly reduced plant growth, relative water content, and chlorophyll, while increasing carotenoids, phenolics, and antioxidants. Three concentrations of smoke solution (full strength SM1, half strength SM2, quarter strength SM3) were applied as seed primers, and ash was applied to soil at 4 g/kg. The combined application of smoke solution and ash increased plant survival under drought by improving the root system, leaf pigments, and shoot length. It also prevented significant changes in antioxidant and phenolic content. The full-strength smoke solution (SM1) combined with ash was the most effective treatment. The study concludes that using a full-strength *Conocarpus erectus* smoke solution as a seed primer and its ash as a soil amendment can effectively minimize drought impacts on maize growth and serve as a beneficial bioregulator.

Key words: Smoke, ash, *Conocarpus erectus*, bioregulator, drought, maize

INTRODUCTION

Smoke is a colloidal solution of suspended dust particles in air. The smoke is a mixture of solid dust particles, and the air which is a mixture of gases. Plant-derived smoke has a famous substance for increasing plant growth and development (Kepczynski and Kepczynska, 2023). Plant species from different ecosystems can be affected by the smoke solution derived from plants, which is a substance for inspiring plant development and growth (Dayamba, 2010). The initial proof that dissolved smoke and smoke extracts in water significantly improved seed germination was first discovered by Lange and Boucher's (1990). The post-germination growth of maize was positively impacted by smoke produced from plants (Kamran *et al.,* 2014). Research regarding the post- germination of crops that have been exposed to the smoke solution derived from plants revealed that the treatment had an impact on the phases of plant growth and development in addition to the seed germination stage (Guzzo *et al.,* 2021). The well-known substance known as "smoke derived from plants" has a favorable impact on different types of plant species (Van Staden *et al.,* 2004). It has been determined that heat, temperature, chemicals, and smoke are the indicators of seed germination connected to fire or post-fire conditions (Anas *et al.* ,2025; Khattak *et al.,* 2025). The smoke solution derived from plants proved to be more valuable in terms of its timely availability due to its long-lasting efficiency, which eliminated any doubt regarding its storage period. Smoke derived from plants has been found to be a possible factor for breaking seed dormancy, rapid up seed propagation, and improving the growth of seedlings, length and mass, germination of pollen, root cuttings, and growth of pollen tubes among other growth-related plant phenomena (Papenfus *et al.,* 2015). It decreased the negative impacts of salt, drought, heavy metals, and fluctuations in temperature on plant development (Akhtar *et al.,* 2017).

The active ingredients in the plants derived smoke solution include butanolides, which include cyanohydrin and karrikins (Lange and Boucher's 1990). The discovery of karrikin provided clarification on the functioning processes of smoke solution derived from plants during the seed propagation stage (Bose *et al.,* 2020). The compounds found in smoke are water soluble, persistent at high temperatures, and highly effective even at low quantities (Flematti *et al.,* 2004). Furthermore, a research identifying in environments that are prone to fire or have just experienced one, heat, temperature, chemicals, and smoke are all important elements in seed germination. Smoke has an active effect on plant growth hormones, speeding up the cell cycle and eventually improving seed germination Jain and Staden (2006). Plant-derived smoke contains certain significant chemicals, such as butanolides, also known as karrikins, and cyanohydrins, which are important in thawing seed dormancy (Pennacchio *et al.,* 2007). The active component, butanolide was isolated to burn Cellulose (Van Staden *et al.,* 2004). These substances are water soluble, firm at high temperatures, and highly effective even at low strength. Recent research has shown the existence of

cyanohydrins in smoke, which also significantly relieve seed germination and seedling growth (Nelson *et al.*, 2012). The effects of fires and the smoke they produce include harmful outcomes such as the elimination of beneficial microbes and soil insects, the loss of essential minerals, and the introduction of air pollution (Kaliramana *et al.*, 2019). Because burning vegetation releases more carbon dioxide (CO_2) into the atmosphere, it has a major negative impact on air quality (Todorovic, 2015). Smoke functions through chemical interacting with endosperm, embryo, and seed coat inhibitors. Through the activation of signal molecules specific to smoke, called promotive hormones, these interactions promote seed germination (Mackellar, 2008).

Conocarpus erectus, also known as the Buttonwood or Button Mangrove, is a perennial plant belonging to the family *Combretaceae*. It normally takes on a shrub-like form when its height varies between 1.5 and 4 meters and can convert into a tree when it reaches a height of 20 meters or more. It grows on shorelines in humid and subtropical areas of the Americas and West Africa and recently introduced in Pakistan. A variety of compounds have been extracted from the leaves of *Conocarpus erectus*, including Gallic acid, 3,3-Dimethoxyellagic acid, ellagic acid,12 other phenols, and a novel trimethoxy-ellagic glycoside known as 3,3,4-tri-0-methyiellagic acid, or 4-0- B glucupyranuronide. These compounds appear as an amorphous yellow powder. In the present climate change scenario, the prolonged drought stress is badly affecting agriculture in different parts of Pakistan. To minimize the adverse effects of drought stress on agricultural production a number of agro-chemicals have been used. However, the frequent uses of agrochemicals such as synthetic growth regulators, pesticides and chemical fertilizers have been reported for their toxic effects on the environment and human health. Therefore, the use of environmentally friendly, economic and highly effective alternatives of synthetic agrochemicals is highly encouraged. *Zea mays* L. is one of Pakistan's major crops. After rice and wheat, maize is Pakistan's third-most significance crop of maize. Across the world, it is cultivated for industrial use as well as food and feed. Impact of drought on maize crop adversely affects their growth and yield. Since maize typically grows large and broad-leaved, a firm drought at the seedling or developing stage will cause the leaves to curl and hinder the plant's development. Maize output is also affected by water scarcity both before and after blooming. Consequently, throughout this time, enough water is needed. Lack of moisture causes drought stress in maize, has an impact on the development of seeds, yield, photosynthetic ability, early vegetative growth, seedling emergence and fertilization (Musaddaq *et al.*, 2021). Maize is farmed at an average grain yield of 2,864 kg/hectare on 1.016 million hectares, producing 3.037 million tons annually. Area under cultivation Approximately, 65% of Pakistan's maize is irrigated, with the remaining grown under strictly regulated conditions (Tariq and Iqbal, 2010). This research study was designed to determine the effects of smoke solutions of *C. erectus* leaves on growth and biochemical constituents of maize plants under drought stress.

MATERIALS AND METHODS

Preparation of smoke solutions

About 2 kg healthy and mature leaves of *C. erectus* were handpicked and air dried for seven days (moisture content 10 % dry weight), were crushed into fine powder with the use of an electric grinder equipped with a 5 mm sieve plate. Aqueous (water) smoke solutions were prepared by burning *C. erectus* leaves powder in a furnace according to the method of Lange and Boucher (1990) with slight modifications. A total of 150 g of dry plant material (7% moisture content) was burnt and the smoke was trapped in 500 mL distilled water in a sealed glass bottle unstill the whole plant material was burnt to ash. The resultant smoke solution was called full strength smoke solution (SM1). The SM1 was later diluted with distilled water to obtain half strength (SM2) and quarter strength (SM3) smoke solutions. Afterwards, these smoke solutions were filtered and kept cold at 4°C. The resulting ash was carefully collected and converted to fine powder in a pestle and mortar. The pH of the resultant smoke solution SM1 was 4.2 which was adjusted to pH 7 by drop wise addition of 1N NaOH solution before the formation of SM2 and SM3.

Sterilization of Seeds

The healthy *Zea mays* seeds variety Azam were carefully washed with 0.5% mercuric chloride solution, the seeds were cleaned from traces of mercuric chloride by two times washing with distilled water and allowed to dry on a blotting paper.

Pot Experiment

The plant growth experiment was started by soaking the seeds in smoke-based solutions (full, half and quester strength) for a duration of 12 hours in a beaker fitted with an aeration facility. Afterwards, they were planted in pots with a sand and clay mixture (1:1), with the soil to pot ratio being constant. Moreover, the ash obtained after burning the *C. erectus* leaves was mixed with soil at 4 g/kg soil. In each pot five seeds were planted, the pots were arranged in a

complete randomized design (CRD).Each treatment and control had three replicates. After achieving growth for 15 days, the plants were treated with drought stress maintaining soil moisture content 7.5% (dry weight basis) for 7 days. The pots kept as control group were regularly receiving normal water supply. Various treatments included smoke solution at full strength, smoke solution at half strength, and smoke solution at quarter strength, control (normally irrigated) and ash with and without drought stress were applied.

Soil moisture content

The moisture content of the soil was calculated by measuring soil fresh and dry weight.

For dry weight measurements, the 10 g soil sample was oven dried at 102 ^{o}C for 72 hours.

$$\text{Soil moisture content \%} = \frac{\text{Fresh weight of soil} - \text{dry weight of soil}}{\text{Dry weight of soil}} \times 100$$

Assessment of Shoot and Root Biomass Under Different Treatments

Shoot and root length was measured by using a measuring scale, and a digital balance was used to measure fresh root and shoot weights. For root width measurements a digital Vernier caliper was used.

Relative water content assessment

Leaf relative water content was measured by determining leaf fresh, turgid and dry weight (Anas *et al*., 2024; Anas *et al.*, 2024).

$$Leaf\ relative\ water\ content\ (\%) \quad \frac{Leaf\ fresh\ weight - leaf dry\ weight}{Leaf\ turgid\ weight - leaf\ dry\ weight} \times 100$$

2.7 Evaluation of Chlorophyll *a* and *b* Content Under Different Treatments

For the estimation of leaf photosynthetic pigments, leaf green tissues (0.1g) were crushed in 10ml of acetone (80%) and afterwards subjected to centrifugation at 4000*g* for 20 minutes. The supernatant was collected and transferred to test tubes to determine the concentration of photosynthetic pigments by using a spectrophotometer (SP at the wavelengths of 645 nm, 663 nm (Arnon, 1949).

Determination of Total Phenolic Content

To determine total phenolic content, 0.1g of the plant material was broken down, mixed with 5ml of methanol, and left in falcon tubes for the night. Using different test tubes, 200 µL of the Folin-Ciocalteu component, 2.5 ml of a 7.5% Na_2CO_3 solution, 0.5 mL of deionized water (DH_2O), and 200 of both root and leaf and extracts were made. After 90 minutes of darkness, a spectrophotometer was used to detect absorbance at 765 nanometers (Adom and Liu, 2002). The total phenolic content was calculated using the following formula:

$$Leaf/Root\ Phenolics\ Content = (Absorbance\ of\ the\ sample - 0.0123/\ 0.0095)$$

Determination of antioxidant assay

We evaluated the antioxidant value of both root and leaf tissue using the Blois method. The tissues of the leaves and roots were completely ground in 5ml methanol and stored in falcon tubes for the night. Afterward, we combined 2ml solutions of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) along with the supernatant from a one-milliliter sample extract. After 30 minutes of darkness incubation, the spectrophotometer measured the optical density (O.D) at 517nm (Brand-Williams *et al*., 1995).

$$DPPH\ (\%) Inhibition\ of\ Free\ Radicals = \frac{Control\ reading - Sample\ reading}{Control\ reading} \times 100$$

Statistical analysis

For data analysis, one-way ANOVA and least significant difference (LSD) tests were used. Statistical analyses were performed using Statistix-10 software, version 2010.

RESULTS

Study of Phenotypic traits

The applied drought stress, *C. erectus* leaf smoke solutions and ash significantly affected the growth traits of maize. The application of full-strength (SM1) and half-strength (SM2) smoke solutions significantly enhanced the germination percentage of *Zea mays* seeds compared to the smoke-free control. In control the seed germination show 80 % rate and the seeds treated with SM1 and SM2 exhibited a 20% higher germination rate, indicating that smoke-derived compounds may have positively influenced seed viability and early growth. The enhanced germination response suggests that bioactive molecules in the smoke solutions, such as karrikins and other growth- promoting agents, likely played a role in triggering germination processes (Fig. 1)

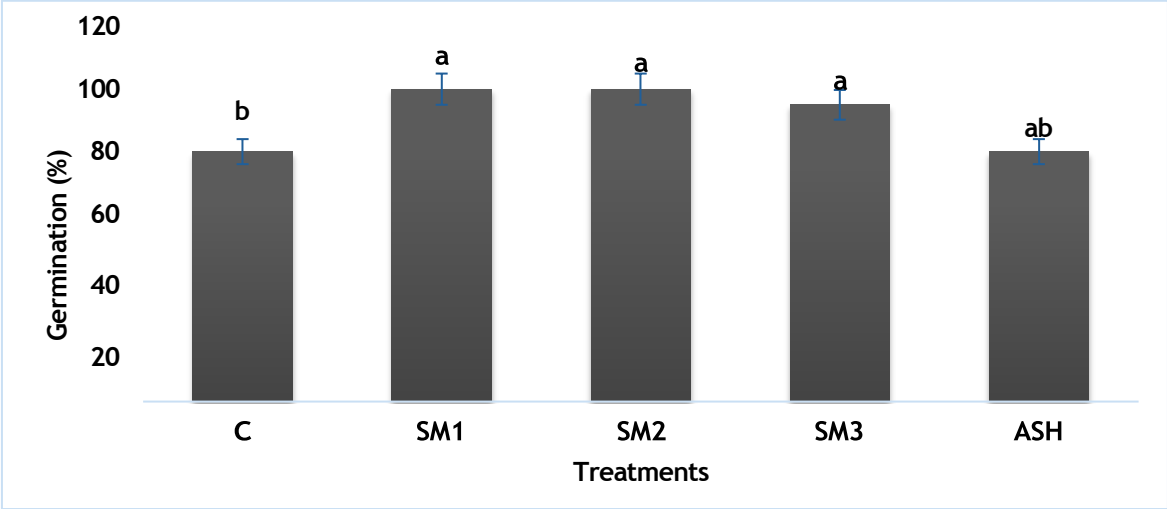

Fig. 1. Germination Response of Zea mays Under Different Treatments.

Effect of Smoke Solutions on Growth and Biomass Accumulation Under Drought Stress

The shoot length, root length, and leaf area varied across different treatments compared to the control (C). The highest shoot length was observed in SM1 and SM2, showing an increase of approximately 26% over the control, followed by SM2 (22%) and SM3 (18%). Ash application alone contributed to a 15% increase in shoot length. Under drought conditions, the combined treatments of SM1+D, SM2+D, and SM3+D enhanced shoot length by 12%, 10%, and 8%, respectively, while D+ASH resulted in a 6% increase. Similarly, root length was highest in SM1, exhibiting a 30% increase over the control, with SM2 and SM3 showing improvements of 25% and 20%, respectively. Ash application alone led to a 17% increase. Under drought stress, SM1+D, SM2+D, and SM3+D improved root length by 14%, 12%, and 10%, respectively, whereas D+ASH contributed to an 8% increase. For leaf area, SM1 treatment resulted in a 28% increase compared to the control, followed by SM2 (24%) and SM3 (20%). Ash treatment alone improved leaf area by 16%. Under drought conditions, SM1+D, SM2+D, and SM3+D enhanced leaf area by 14%, 12%, and 10%, respectively, while D+ASH resulted in an 8% increase. These results suggest that smoke solutions and ash significantly contribute to improved plant growth under both normal and drought-stressed conditions. Additionally, treatments SM1+D, SM2+D, and SM3+D recorded superior shoot weights compared to the drought-only treatment, underscoring the role of smoke solutions in stress resilience. In terms of root biomass, SM1 and SM3 significantly increased both fresh and dry weight over the smoke-free control, while the ash treatment had no impact. Overall, the smoke solutions, particularly SM1 and SM2, demonstrated considerable potential in mitigating drought-induced reductions in growth and biomass, reinforcing their role as biostimulants for enhancing maize resilience under water stress (Fig 2).

Effect of Smoke Solutions on Leaf Relative Water Content Under Drought Stress

Drought stress resulted in a significant reduction in leaf relative water content, with a 35% decrease compared to the control. However, the application of smoke solutions effectively mitigated this decline. SM1 treatment showed the highest improvement, increasing leaf relative water content by 93% compared to drought-stressed plants, followed by SM2 (88%) and SM3 (84%). Ash application alone enhanced water retention by 82%, demonstrating its positive impact on plant hydration. The combined treatments of SM1 + D, SM2 + D, and SM3 + D improved leaf relative water content by 78%, 76%, and 74%, respectively. The drought-stressed plants treated with ash (D + ASH)

exhibited a 72% increase in water content, indicating a moderate but significant improvement. These findings emphasize the potential of *Conocarpus erectus* leaf smoke and ash in enhancing drought tolerance by improving leaf water status in maize (Fig 3).

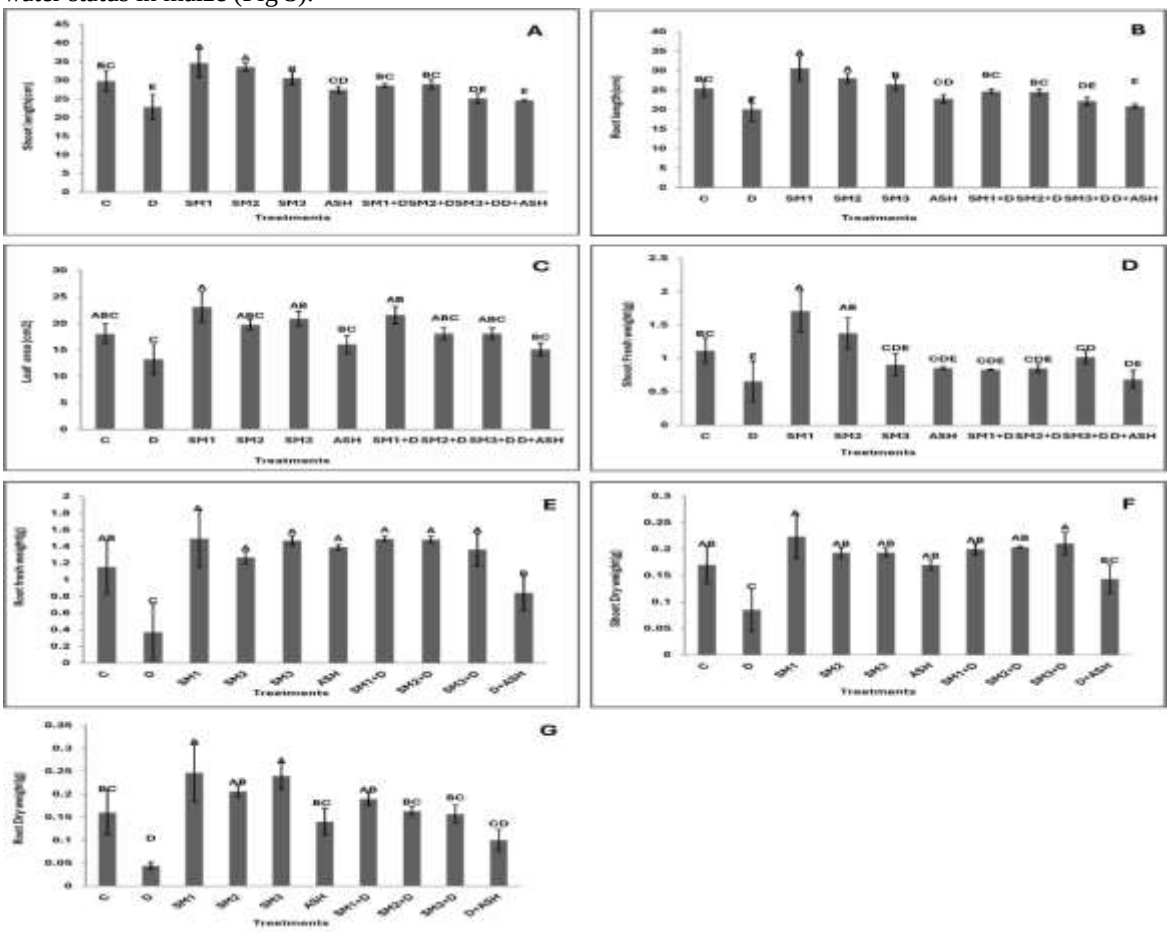

Fig. 2. Examined morphological changes in maize treated with *C. erectus* derived smoke (SM). The seeds were soaked in different strength of smoke for 12 hours, and the changes were measured using one-way ANOVA.
SM1=Full strength ,SM2 =Half strength, SM3=quartor strength SM1+D =Full strength+ Drought ,SM2+D=Half strength+ Drought, SM3+D= Quartor strength+ Drought and D+ Ash=Drought+ Ash**.**

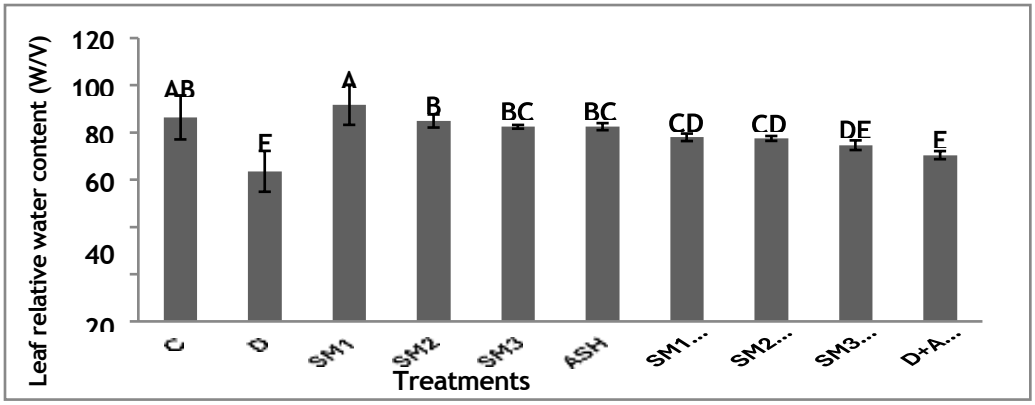

Fig. 3. Effects of Plant-Derived Smoke and Drought Stress on Leaf Relative Water Content in Maize Leaf relative water content (% W/V) in maize plants subjected to different treatments, including control (C), drought stress (D), smoke solutions of varying strengths (SM1, SM2, SM3), ash application, and combinations of smoke solutions or ash with drought stress. Different letters indicate statistically significant differences among treatments as determined by one-way ANOVA ($p < 0.05$). Error bars represent standard deviation.

Impact of Smoke Solutions on Chlorophyll Content Under Drought Stress

Drought stress significantly reduced the production of chlorophyll a and b, leading to lower values compared to the irrigated control (Figure 4). However, the application of SM1 and SM2 resulted in the highest chlorophyll a content relative to the control, while *C. erectus* leaf ash had no significant impact. Notably, among the three smoke treatments, only SM1 and SM2 effectively mitigated the adverse effects of drought stress on chlorophyll a level. In contrast, chlorophyll b content was particularly responsive to SM1 and SM3 under drought conditions, suggesting their potential role in sustaining photosynthetic efficiency during water deficit stress.

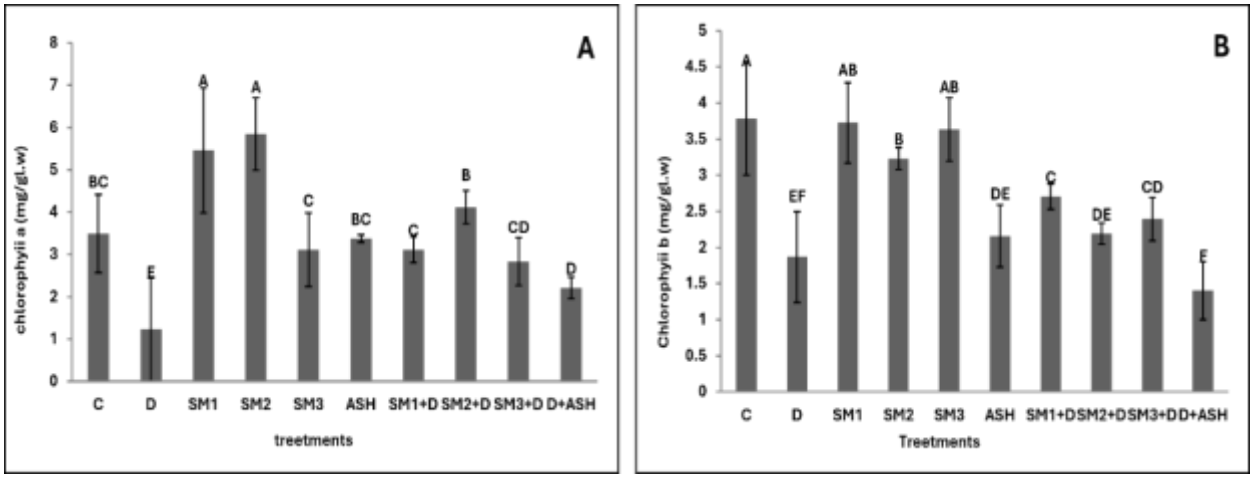

Fig. 4. Effects of Plant-Derived Smoke and Drought Stress on Chlorophyll a and b Content in Maize (A) Chlorophyll (a) and (B) Chlorophyll (b) content (mg/g fresh weight) in maize leaves subjected to different treatments, including control (C), drought stress (D), smoke solutions of varying strengths (SM1, SM2, SM3), ash application, and combinations of smoke solutions or ash with drought stress. Different letters indicate statistically significant differences among treatments as determined by one-way ANOVA ($p < 0.05$). Error bars represent standard deviation.

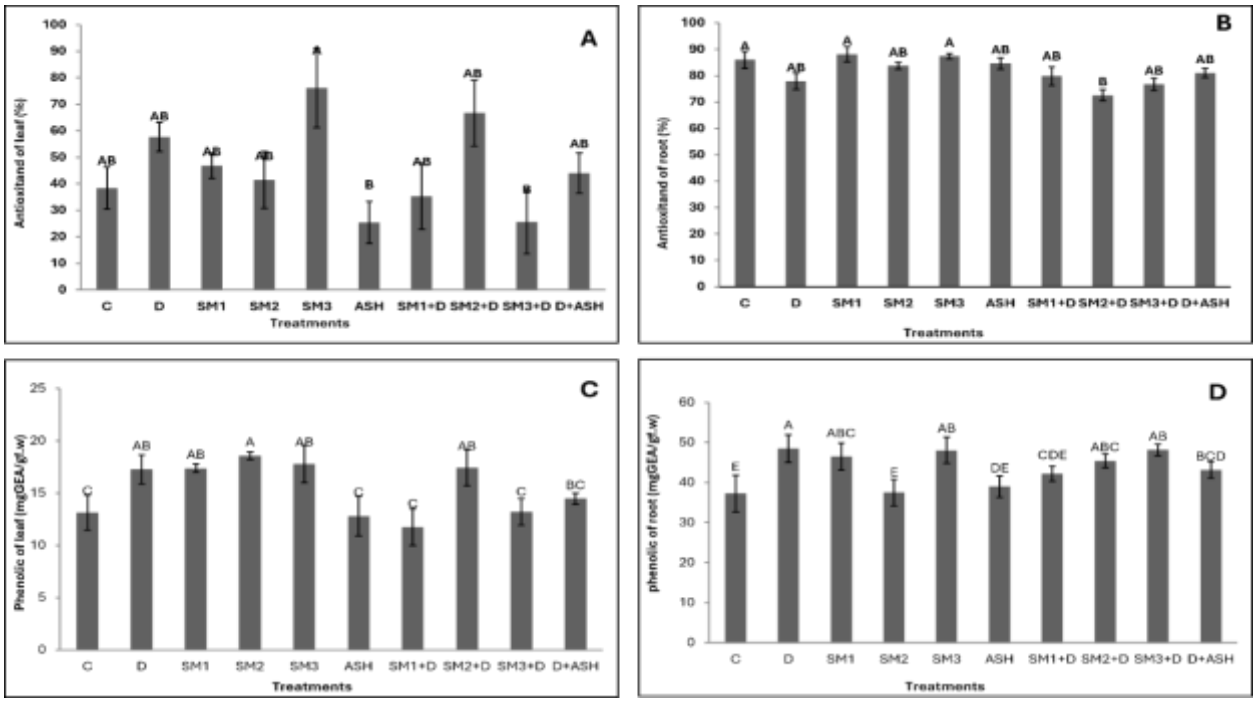

Fig. 5. Maize seeds were soaked for 12 hours in plant-derived smoke solutions of varying concentrations (full strength, half strength, and quarter strength) before being sown in sand. (A) Antioxidant activity and (B) phenolic content were measured in both leaves and roots under different treatments. Different letters indicate statistically significant differences as determined by one-way ANOVA ($p < 0.05$). Error bars represent standard deviation.

Influence of Smoke Solutions on Antioxidant and Phenolic Content in Maize

Smoke solutions (SM1, SM2, and SM3) significantly enhanced antioxidant activity in maize shoots and roots under non-stress conditions, with SM1 showing the highest increase of approximately 35–40% compared to the control. However, under drought stress, the antioxidant potential declined by 20–25% when lower concentrations of smoke solutions (SM2 + D) were applied. Root phenolic content increased by 30% under drought stress alone, while the application of smoke solutions further elevated this response, with SM1 leading to a 50–55% increase, followed by SM2 and SM3 with 40–45% increases. Ash treatment alone resulted in a moderate 25% rise in root phenolic content, though it was less effective than smoke solutions. The combination of SM1 with drought stress (SM1 + D) showed the highest phenolic content, marking a 60% increase over the control, while medium and lower concentrations of smoke solutions with drought stress exhibited a 45–50% enhancement. Similarly, drought stress combined with ash led to a 35% increase in phenolic content, underscoring the role of these treatments in improving maize stress resilience (Fig 5).

DISCUSSION

The experiment result revealed that smoke solution of *Conocarpus erectus* (SM1 and SM2) stimulated seed germination percentage in maize as compared to the smoke free control. The smoke solution riches of ethylene and Butenolide which stimulated the germination % in Papaya plant (Chumpookam *et al.,* 2012). The SM1 and SM2 increased seed germination percentage by 20% as compared to smoke free control. Treatment with drought stress reduced the morphological growth parameters of maize plants, including shoot length, root length, root width, and leaf area. Dry soil initiates a decrease in the intake of water by root, which lowers the turgor pressure and water potential of the leaves and decreases leaf area (Rolline *et al.,* 2013). Reduced production of both fresh and dried biomass is a typical adverse impact of water stress on crop plants (Farooq *et al.,* 2009). When maize plants were treated for drought, there was a documented decrease in plant length and leaf area (Latif *et al.,* 2016). The total growth-related properties of maize exhibited beneficial effects from C. erectus smoke. Under drought stress, the plants treated with smoke performed better. Less of a decrease in maize shoot and root weight, and leaf area was observed under drought stress. In maize, Sparg *et al., (*2006) discovered that smoke created from plants increased the fresh weight, shoot length, and germination percentage of roots and shoots. On the other hand, too much smoke originating from plants prevented lettuce from sprouting and growing branches (Light *et al.,* 2002).

Drought stress affects plant water status, which is determined by recording the relative water content of plants (Makbul *et al.,* 2011). Because of the drought treatment, LRWC was significantly reduced during the current study. It has been discovered that drought has lowered LRWC in maize (Bibi *et al.,* 2016). Under drought stress, the smoke treatments reduced the negative effects of leaf relative water content and increased LRWC. This suggested that the bioactive chemicals in the smoke of C. erects leaves controlled the amount of water that plants absorbed during drought stress. Stressed plants have lower RWCs than unstressed ones. The limit RWC is from 85 to 90% in stressed plants, whereas it can be as low as 30% in plants that are not impacted by drought (Reddy *et al.,* 2003).

Drought stress changed the ratios of carotenoids to chlorophyll "a" and "b" (Farooq *et al.,* 2009). In this research, drought stress reduced the chlorophyll content of leaf in maize. However, the plants which are treated by smoke had higher chlorophyll levels than both the plants which are treated by drought and normally irrigated plants. Photosynthetic rate and chlorophyll concentration have a positive correlation with grain yield and biomass production (Pandey and Singh, 2010). Water stress has a direct impact on the chemical response of PSII (Nori *et al.,* 2011). According to research, chlorophyll levels decrease during drought stress (Kuroda *et al.,* 1990). Furthermore, cultivars that were resistant and susceptible to the drought and heat stress plants decreased chlorophyll content (Shamsi, 2010). In all smoking treatments, the amount of chlorophyll a and chlorophyll b in terms of synthesis and accumulation increased, enhancing the light reaction's efficacy in plants. Plant-derived smoke solutions increased the process of ribulose-1,5- bisphosphate carboxylase/oxygenase production in chickpea and maize (Baxter and Staden 1994). Smoke solution improved chlorophyll a and b and carotenoids levels in rice, which are required for photosynthesis (Jamil *et al.,* 2014).

Drought stress increases plant antioxidant content (Camaille *et al.,* 2021). Our research found that antioxidant levels in the leaves and roots in maize do not rise during drought stress. According to the study by (Guzzo *et al.,* 2020), leaf activity peaked and then rapidly fell when the antioxidant levels increased during the drought. Plants with organs roots during a drought, tissue is adversely affected because it comes into direct contact with drying soil. One of the most typical responses to water stress is electron leakage through the thylakoid membrane, which damages chlorophyll, reduces antioxidants, and reduces photosynthesis (Qureshi *et al.,* 2007). The plants treated with smoke solutions had normal levels of antioxidants, demonstrating that the drought strictness was reduced by the *C. erectus* smoke application. According to research on the impact of smoke of *Bacillus safensis* on rice seedlings produced

under salt stress, smoke did not considerably influence antioxidant activity (Khan *et al.,* 2017).

The varied irrigation intervals had a significant impact on the total phenolic content of shoots and roots (Okello *et al.,* 2017). They also propose that irrigating the plants at 85 c.bars produced the best results since the phenolic content of the roots and leaves increased as the degree of water stress improved. Under common irrigation situations, plants treated with smoke solutions had a greater phenolic content. However, in drought-stress conditions, plants treated with smoke solutions had lower phenol content. Plants introduce the synthesis of phenolic compounds in response to water shortage (Mandal et al., 2009). As a result, phenolic compounds play important biological and ecological roles, because they provide protection against several types of stress (Ayaz *et al.,* 2000). Strong antioxidants known as phenolic compounds help plants resist harsh situations (Mittler, 2002). However, the responses of *Solanum villosum* and *Solanum scabrum* to the water stress conditions differed. Higher phenolic content was detected in *Solanum scabrum* roots and *Solanum villosum* shoots (Okello *et al.,* 2017). Additionally, studies on how smoke solution stimulation impact phytochemicals in several plant species, such as flavonoids, phenolics, and indigo (Kulkarni *et al.,* 2013). In addition to its phenolic content, plant smoke solution improved the absorption of growth nutrients, promoting protein production (Jamil *et al.,* 2014). This study found that C. erectus smoke solutions released drought stress impact, as evidenced by the phenolic content of maize leaves and roots.

Conclusions

Drought stress negatively impacted the growth characteristics of maize. However, the application of smoke solutions (SM1 and SM2) along with ash derived from *Conocarpus erectus* leaves significantly improved all measured growth parameters. These findings suggest that *C. erectus* leaf-derived smoke solutions can serve as effective bioregulators, enhancing drought resistance in maize, particularly in arid and drought-prone regions. Furthermore, the bioactive compounds responsible for these growth-promoting effects should be isolated, purified, and identified using advanced chromatographic and analytical techniques for potential agricultural applications.

Conflicts of Interest

The authors declare no conflict of interest.

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