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Original Research

Impact of Biogas Slurry on Physiological and Antioxidant Mechanisms of Wheat Under Drought Stress

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Abstract

Drought, a significant abiotic stressor, exerts harmful effects on crop productivity on a global scale. Researchers have investigated various strategies aimed at mitigating the detrimental impacts of drought on crop productivity. Biogas slurry (BGS) improves soil fertility and water retention, leading to increased crop output. The study aims to assess how biogas slurry improves wheat crop development and yield during drought stress. A pot experiment was carried out at the agronomic research area of IUB Bahawalpur to investigate the effect of biogas slurry on wheat crop growth under restricted water availability. The present study employed four distinct treatments of biogas slurry, namely T_0 (control), T_1 (450 kg ha^{-1}), T_2 (550 kg ha^{-1}), and T_3 (650 kg ha^{-1}), in conjunction with the imposition of drought conditions. The results of the study suggest that the presence of drought conditions during the grain-filling phase exerts a negative influence on the final yield outcome. Furthermore, it was observed that biogas slurry at a dosage of 650 kg ha^{-1} has a pronounced impact on both the growth and yield of the cultivated crop. Soil amendment of biogas slurry at a 650 kg ha^{-1} rate yielded notable improvements in multiple plant growth parameters. The experimental results indicate a remarkable (6.18%) elevation in plant height, a substantial (13.26%) augmentation in the count of fertile tillers, a significant (6.90%)

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extension in spike length, a noteworthy (7.95%) rise in the number of grains, and a remarkable (16.97%) increase in grain yield pot^{-1}. Furthermore, physiological parameters such as stomatal conductance (40.76%), chlorophyll content (20.25%), water use efficiency (35.41%), transpiration rate (38.71%), photosynthesis rate (23.68%), SOD (42%), POD (26%), CAT (33%), and APX (16%) were also improved with biogas slurry application. In conclusion, biogas slurry at a rate of 650 kg ha^{-1} could be an effective strategy for boosting wheat cereal production by minimizing the harmful effects of drought stress.

Keywords: biogas slurry, drought, wheat, cereals, yield

Introduction

Agricultural growth faces numerous challenges, such as the reduction of available farmland, climate change impacts, water scarcity, fluctuations in temperature, changes in rainfall patterns, rising input costs, and the migration of populations from rural to urban areas. Consequently, there is an urgent need to enhance agricultural productivity by adopting new strategies in crop production [1, 2]. By 2050, global food production must quadruple to meet the demands of the world's fast-expanding population [3]. According to Araus et al. [4], rather than increasing agricultural land to enhance wheat production, the emphasis should be placed on attaining optimum yields through other methods. Limited water resources pose a significant problem as they severely affect agricultural production, with recurring droughts affecting more than half of the wheat-growing regions [5, 6].

Drought is a widely recognized and severe abiotic stress, contributing substantially to the decline in global crop productivity [7]. Among crops, wheat stands out for its heightened vulnerability to drought-induced stress, particularly during the advanced growth stages known as terminal drought. This condition is characterized by diminished atmospheric and soil moisture levels and elevated air temperatures, culminating in a disparity between evapotranspiration and water absorption from the soil [8].

In bolstering plant resilience, adopting innovative techniques presents a formidable challenge in cultivating plant tolerance to oxidative stress [9]. Diverse factors converge to influence a plant's reaction to drought significantly. These include the intensity and duration of the stress, the developmental stage, physiological processes, and the genetic constitution of the plant [10, 11]. Additionally, environmental factors [12, 13], the activation of photosynthetic mechanisms [14, 15], gene expression patterns, and respiratory activity [16] collectively contribute to the intricate mosaic of a plant's response to drought stress.

Biogas slurry (BGS) is a residual substance generated due to the anaerobic digestion process within biogas plants. According to Nasir et al. [17], it is generated through the anaerobic fermentation of organic matter after the methane production process is completed. Around 25 to 30% of the overall dry mass of animal and human waste transforms into a flammable gas, with the remaining substance referred to as biogas slurry or processed slurry. According to Islam [18], biogas slurry is an advantageous organic fertilizer due to its substantial nutrient content and abundance of organic matter.

Applying biogas slurry as a fertilizer offers notable benefits for biogas facilities. The biogas plant retains or discharges methane and inorganic materials, which cannot be converted into methane. According to Gupta [19], biogas slurry contains a substantial amount of nutrients, including nitrogen (N), phosphorus (P), and potassium (K), as well as trace elements such as calcium (Ca), zinc (Zn), nickel (Ni), iron (Fe), sodium (Na), boron (B), cobalt (Co), chromium (Cr), and cadmium (Cd). The provided diagram, labeled Fig. 1, is presented for reference. Furthermore, it was noted that biogas slurry consists of organic nitrogen, mineral elements, and bioactive substances such as hormones, humic acids, and vitamins, as highlighted by Liu et al. [20].

Nonetheless, the precise and optimal application rate of biogas slurry under drought conditions requires further clarification. The objective of the present study was to investigate the impact of different dosage levels of biogas slurry on the performance of wheat (*Triticum aestivum* L.) when confronted with conditions of drought-induced stress.

Experiment

Experimental Design

The experiment was executed utilizing a complete randomized design (CRD) comprising three replicates. Various doses of biogas slurry, specifically 450, 550, and 650 kg ha^{-1}, were administered to the experimental pots. The imposition of the drought stress treatment will involve the deliberate withholding of irrigation after specific growth stages. A comprehensive soil physiochemical analysis was conducted before the commencement of sowing activities. The analysis findings indicated that the soil composition comprised 23% sand, 19% silt, and 65% clay.

Additionally, the analysis revealed the presence of 0.89% organic matter, with nitrogen (N) content measuring at 0.35 mg kg^{-1}, phosphorus at 3.8 mg kg^{-1}, potassium at 122 mg kg^{-1}, and calcium at 105 mg kg^{-1}. Furthermore, the pH of the soil was determined to be 7.6. The application of fertilizer was carried out uniformly across all pots, with a dosage of nitrogen (N), phosphorus (P), and potassium (K) of 145, 115, and 63 kg ha^{-1}, respectively. Before the sowing process, the seeds underwent a sterilization procedure involving treatment with a 70% ethanol solution for 2 minutes. The experimental setup involved using pots measuring 30×30 cm, meticulously filled with a standardized quantity of 10 kg of soil. Subsequently, a total of nine seeds were carefully sown within each pot. The sowing activity was carried out on the 17th of November, 2020. The experimental protocol involved the maintenance of three plants per pot. The growth, yield, and physiological parameters were assessed after the harvest from each pot, following established protocols. The measurement of leaf chlorophyll content is facilitated by using a device known as a chlorophyll meter. The stomatal conductance was measured using the LI-600 Porometer/Fluorometer, a commonly employed instrument in plant physiology research for assessing the gas exchange properties of leaves. Determining water use efficiency (WUE) was conducted by applying the formula described in the study conducted by Raza et al. [21]. Water use efficiency (WUE) is the quotient obtained by dividing the grain yield by the total quantity of water applied. Stomatal conductance (SC) was assessed using an automated porometer MK-3 Delta-T Devices, Burwell Cambridge, England.

Application of Biogas Slurry and Drought Stress

Before planting, the biogas slurry was applied to the respective treatment pots. The drought stress would be induced by withholding irrigation at three growth stages (tillering, flowering, and grain filling). Since the drought started, all pots have received the same water; however, during the tillering (D.T.S.), flowering (D.F.S.), and grain filling (DGFS) stages, thirty percent water holding capacity (W.H.C.) was maintained in drought pots. All the pots were watered equally until the start of drought stress. In comparison, 80 percent of the pot's W.H.C. was kept as a control. Tap water is used for irrigation purposes. The soil moisture content is monitored regularly to maintain appropriate stress levels.

Estimation of Antioxidant Activity

Using the method described by Kar and Mishra [22], we measured CAT, POD, and SOD activities. As per Cakmak [23], the activity of APX (ascorbate peroxidase) was measured.

Statistical Analysis

The pot experiment was statistically analyzed using Statistix 8.1 computer software at the 5% probability level.

Results

Growth and Yield Related Parameters

The experimental conditions involving drought stress impacted the observed plant height significantly. Specifically, the plant height decreased by 49, 41.5, and 26% during the tillering, flowering, and grain-filling stages compared to the control treatment. Furthermore, it is noteworthy that the length of spikes exhibited a significant decrease, with reductions of 42, 36, and 32% observed at the corresponding stages. Using biogas slurry (T_1, T_2, and T_3) demonstrated notable amelioration impacts caused by drought, resulting in a significant augmentation in plant height and the length of their spikes. The utilization of biogas slurry in this study led to notable enhancements in plant height, with increases of 9 and 13.7% observed during the tillering and flowering stages, respectively. Additionally, spike length experienced significant improvements, with increments of 11.7 and 15.6% recorded at the tillering and flowering stages (as shown in Table 1.).

During critical growth stages, tillering, flowering, and grain filling, the imposition of drought stress was

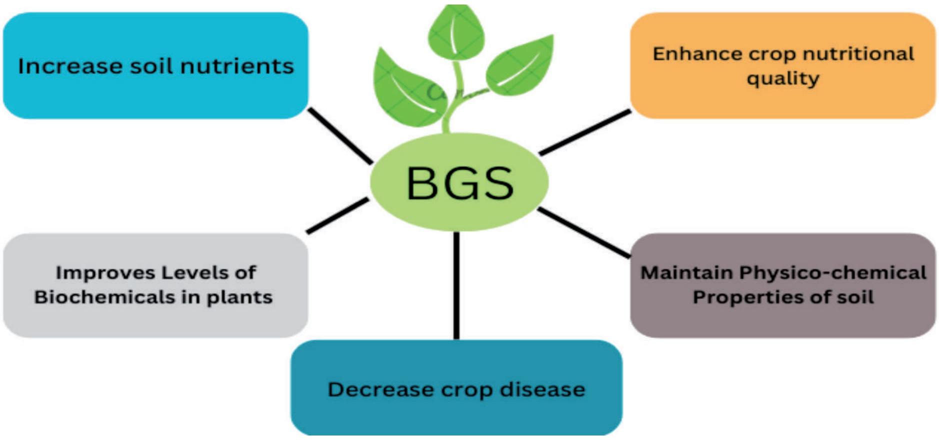

Fig. 1. Biogas Slurry effect on plants.

Table 1. Effect of biogas slurry on different growth and yield-related parameters of wheat under drought stress.

Treatments	BGs Doses	PH	SL	NGPS	GW	GY	BY
Do	T_0	62.70 b	13.14 a	36.00	30.21	12.45	17.00
	T_1	60.12 c	12.11 g	33.55	27.00	10.13	15.81
	T_2	61.08 d	12.41 d	34.10	28.25	11.00	16.10
	T_3	66.21 a	12.82 b	35.15	29.42	11.85	16.81
DTS	T_0	57.12 h	12.71 c	34.61	29.60	12.10	16.60
	T_1	58.17 l	12.00 h	31.12	26.40	9.85	14.40
	T_2	60.31 m	12.11 g	32.20	28.15	10.82	15.39
	T_3	61.72 o	12.20 f	23.33	29.00	11.40	16.05
DFS	T_0	58.21 f	12.22 e	34.55	29.10	11.90	15.90
	T_1	55.81 k	11.45 k	31.10	25.10	9.31	13.31
	T_2	53.91 n	11.53 j	31.20	27.09	10.25	13.31
	T_3	61.26 o	11.81 i	33.00	28.51	11.00	15.41
DGF	T_0	60.43 b	12.82 b	32.70	26.31	10.41	14.31
	T_1	62.55 e	9.91 n	29.24	23.45	7.90	12.08
	T_2	63.32 g	10.19 m	30.00	25.15	9.20	13.29
	T_3	65.12 i	10.72 l	31.21	27.00	10.51	14.55
LSD ($p \leq 0.05$)		2.05	0.50	2.15	1.47	0.42	1.06

Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

observed to have harmful effects on various parameters of wheat. These parameters include the number of spikelets per spike, which exhibited reductions of 28.4, 32.2, and 41.9% during drought at the tillering, flowering, and grain-filling stages, respectively. Similarly, fertile tillers experienced reductions of 22.6, 24.7, and 31.4% during the growth stages. Furthermore, the number of grains per spike was reduced by 17.9, 18.6, and 28.8% during DTS, DFS, and DGFS, respectively. Additionally, the 1000 grain weight, grain yield, and biological yield were all adversely affected by drought stress, with reductions of 28.3, 32.5, and 41.6% observed in grain yield and reductions of 11.2, 18.4, and 23.7% followed in biological yield during DTS, DFS, and DGFS, respectively. The application of biogas slurry demonstrated positive effects in drought and control conditions, effectively mitigating the adverse impacts of drought and enhancing the values of the above parameters. Significantly, biogas slurry (T_3) utilization exhibited a discernible increase in grain yield (11.4%) when contrasted with the application of T_1 and T_2. Implementing biogas slurry has been observed to be an effective strategy for mitigating the adverse effects of drought on multiple yield-related characteristics of wheat.

Water Use Efficiency (WUE)

Fig. 2 illustrates that using biochar at various growth stages enhanced wheat's water efficiency, mitigating the effects of water scarcity. The WUE exhibited a 39, 42, and 33% reduction under drought stress during the tillering, flowering, and grain-filling stages compared to the control treatment. Biogas slurry application (T_1, T_2, and T_3) significantly reduced the drought impact by 23, 22, and 33%, respectively, compared to T_0.

Stomatal Conductance

During the tillering, flowering, and grain-filling stages, stomatal conductance decreased by 11.1, 19.6, and 25.3%, respectively, during drought stress compared to the control treatment. Furthermore, the utilization of biogas slurry (T_1, T_2, and T_3) demonstrated a notable alleviation of the adverse effects caused by the drought. This was evident through a substantial reduction in stomatal conductance, with reductions of 26.3 and 39.5% observed in T_1 and T_2, respectively, compared to the control treatment. These findings are visually represented in Fig. 3.

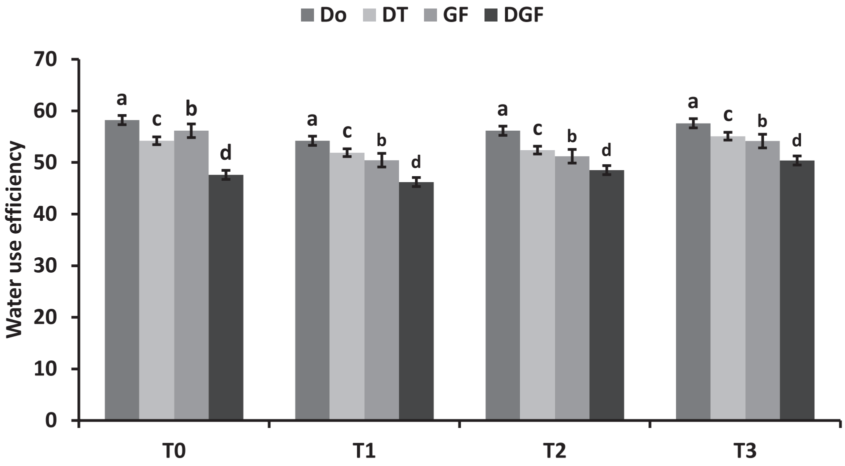

Fig. 2. Effect of biogas slurry on water use efficiency during periods of drought stress at different growth stages of wheat. Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

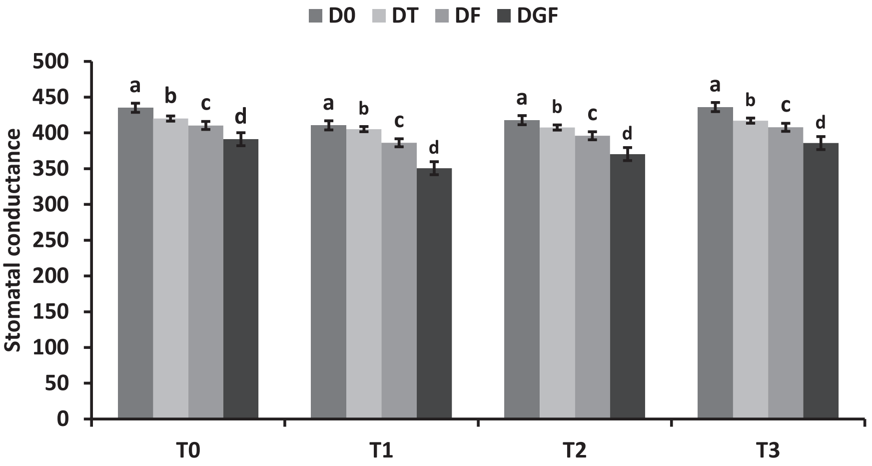

Fig. 3. Effect of biogas slurry on Stomatal conductance during periods of drought stress at different growth stages of wheat. Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

Chlorophyll Content

The utilization of biogas slurry had a significant impact on the chlorophyll content of drought-stressed wheat leaves, as depicted in Fig. 4. As compared to the control treatment, biogas slurry application increases the chlorophyll content by 9, 15, and 21%, respectively.

Photosynthesis and Transpiration Rate

Fig. 5 shows the data regarding the rate of photosynthesis. All treatments were found to be significantly affected. Biogas slurry (T_1, T_2, and T_3) enhanced the photosynthesis and transpiration rate by 22, 28, 32, 27.6, 31.2, and 36% as compared to control. Under drought, significant reductions were noticed in transpiration and photosynthesis rates. Specifically, transpiration rates dropped by 74.5, 69.5, and 62.8% at the tillering, flowering, and grain-filling stages, as compared to the control application. Similarly, photosynthesis rates drop to 43.5, 32.4, and 31.1% at the respective growth stages.

Enzymatic Activity

The investigation into the effects of utilizing biogas slurry in drought conditions revealed a noteworthy influence on the antioxidant enzymatic activities of wheat, as illustrated in Fig. 6. The observed phenomenon of increased activities of catalase (CAT) and superoxide dismutase (SOD) enzymes, accompanied by a decrease in peroxidase (POD) enzyme activity, can be attributed

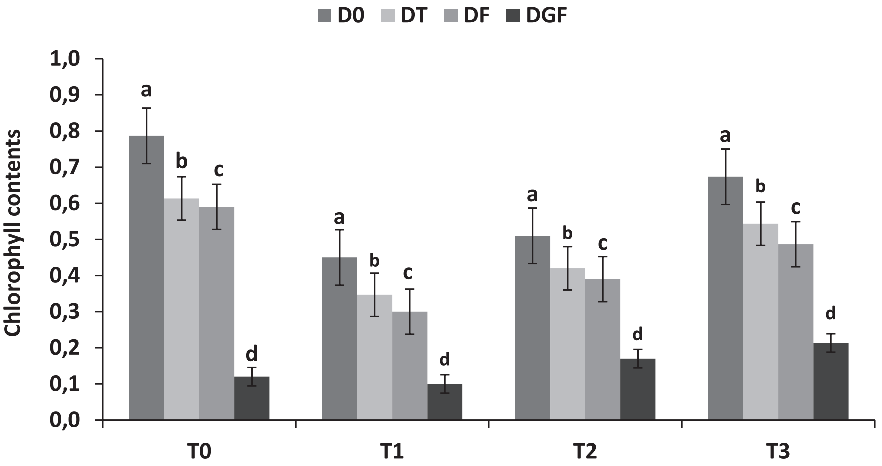

Fig. 4. Effect of biogas slurry on Chlorophyll content during periods of drought stress at different growth stages of wheat. Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

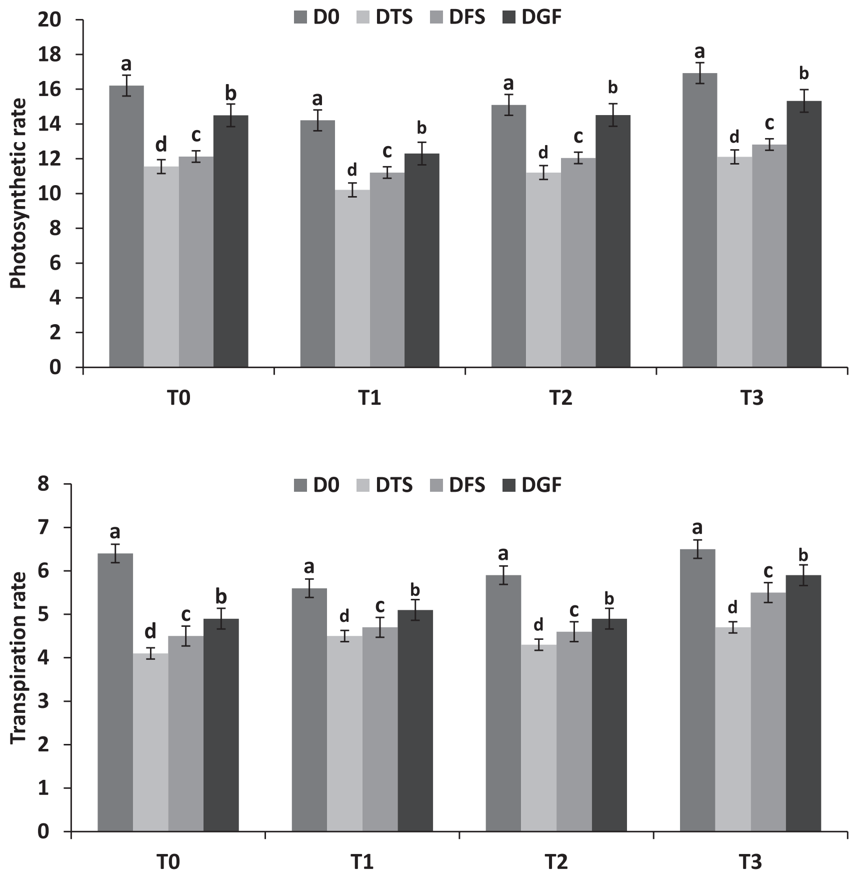

Fig. 5. Effect of biogas slurry on photosynthetic and transpiration rates during periods of drought at different growth stages of wheat. Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

to the prevailing drought conditions. CAT and SOD activities were reduced by 18.1, 12.4, 13.5, 22.3, 17.6, and 30.3%, respectively, whereas POD enzyme levels decreased by 4.8, 3.9, and 2.4%, respectively. Moreover, the BGs application at 650 kg ha^{-1} (T_3) was more effective than other treatments in drought and under normal conditions.

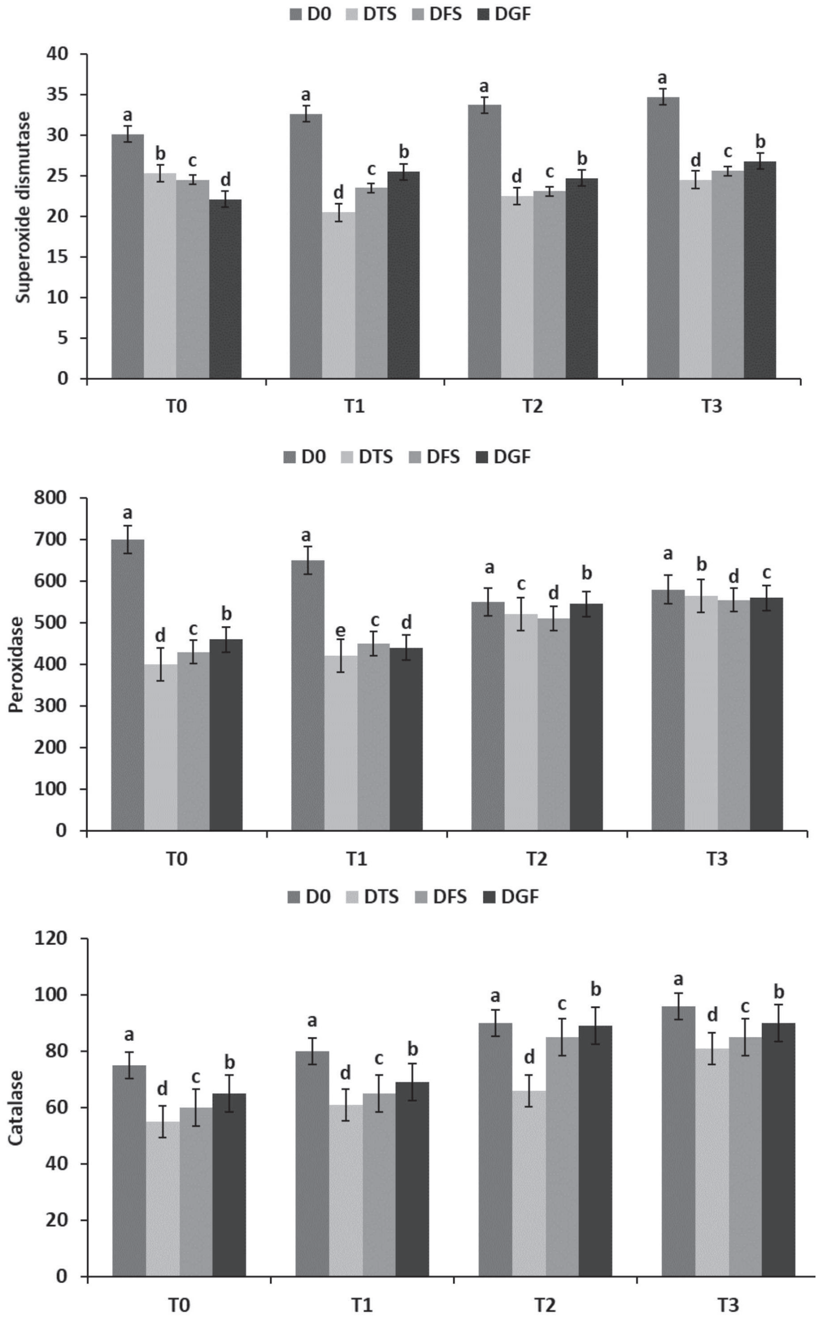

Fig. 6. Antioxidant enzyme activities superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) affected by biogas slurry application during periods of drought at different stages of growth of wheat. Where Do = control, DTS = drought at tillering, DFS = drought at flowering, and DGF = drought at grain filling stage. T_0, T_1, T_2, and T_3 indicate control, 450, 550, and 650 kg ha^{-1}. The mean values in a column sharing the same letter/s are statistically non-significant with each other at a probability level of 5%.

Discussion

The application of biogas slurry has exhibited a favorable impact on the growth of wheat plants. The research conducted by Saleem et al. [24] underscores the notable influence of water stress on wheat development and yield parameters. The diminution of water content within plant cells, resulting in reduced turgidity, has been identified as a factor disrupting critical protoplasmic activities. Consequently, this disruption leads to diminished rates of cell division and a corresponding decrease in plant height. Raza et al. [25] corroborate these findings by highlighting the adverse impact of drought stress on plant height across distinct developmental stages.

The equilibrium of hormonal levels emerges as a pivotal determinant governing plant height, as emphasized by Zhao et al. [26]. Water scarcity can significantly perturb this hormonal balance. Following applying biogas slurry into the soil at a dosage of 650 kg ha^{-1}, a noteworthy surge of 13.26% in plant height was evident. Shahid et al. [27] conducted a study involving the utilization of biogas slurry in combination with chemical fertilizers in a balanced 50% proportion. This amalgamation yielded a marked enhancement in plant height.

Moreover, the research by Raza et al. [25] highlights the intricate relationship between spike length and yield potential. A longer spike length indicates a higher likelihood of increased spikelet production and subsequent yield. However, Ihsan et al. [28] counterbalance this notion by demonstrating that water limitations can curtail spikelet numbers due to suppressed metabolic processes arising from inadequate water availability for diverse plant functions.

Our experiment found that maximum spike length (4.5%) was obtained when BGS was applied at 650 kg ha^{-1} (Table 1.) compared to the control one. Similarly, when drought occurs at the grain-filling stage, spike length is decreased by up to 14%. A harmonious outcome was reported by Rizwan et al. [29]. The actual yield of a crop is intricately tied to its grain weight, with an increase in grain weight expected to amplify crop yield correspondingly. Within this context, the control treatment yielded a peak grain weight of 1000 units, mirroring a similar achievement in treatment T4, where applying Biogas Slurry (BGS) at a rate of 650 kg ha^{-1} yielded comparable results. Incorporating insights from a comprehensive investigation, it is discernible that the manifestation of drought conditions during the critical grain-filling stage results in a substantial reduction of approximately 22% in grain weight. A distinct study by Shahid et al. [27] underscores the potential of improving the 1000-grain weight by integrating biogas slurry with chemical fertilizers, balanced at a 50% ratio each. The decline in grain yield is a direct consequence of inadequate water supply. Raza et al. [25] suggest that restricted water availability affects plant growth and nutrient uptake. Such implications are echoed in findings demonstrating the influence of drought stress on the equilibrium between nutrient source and sink within plants. This imbalance disrupts crop growth rates and critical metabolic processes, reducing grain yield [30]. Biogas slurry positively contributes to soil systems by elevating nutrient content and carbon availability and bolstering plant metabolic functions. Gurung et al. [31] corroborate crop quantity and quality enrichment resulting from BGS application. Notably, biological yield stands to be influenced by variations in grain weight and plant height. In light of Raza et al.'s [25] findings, the impact of drought stress is evident in the observable decrease in biological yield. Intriguingly, our study suggests that applying BGS at a rate of 650 kg under drought-stress conditions positively affects the biological yield of wheat.

The results of the present study are consistent with the findings reported by Rizwan et al. [29]. The WUE can be improved by enhancing plant growth and increasing photosynthetic material, as demonstrated by Zhang et al. [32]. WUE is decreasing due to the limited availability of water [25]. WUE was increased to 4% when BGS was applied at 650 kg ha^{-1} compared to control and 7% for drought treatment. WUE at the grain-filling stage is most affected by drought and decreases up to 13% compared to other growth stages (Fig. 2). The present study has provided evidence indicating that the limited availability of water has a detrimental impact on the process of photosynthesis and the ability of stomata to conduct water vapor.

Consequently, this leads to a decrease in moisture content under conditions of drought-induced stress. Nevertheless, incorporating biogas slurry into the soil resulted in enhancements in cation exchange capacity and physicochemical characteristics, thereby resulting in an augmentation of the photosynthetic rate and availability of soil water. The results of this study align with the findings of prior research conducted by Khan et al. [33] and Hafez et al. [34]. In drought conditions, there was a noticeable reduction in chlorophyll content, gas exchange, WUE, stomatal conductance (SC), and transpiration rate compared to adequately watered plants. Partial stomatal closure has been attributed to similar observations in wheat crops, as reported by Zaheer et al. [35] and Raza et al. [36]. Ali et al. [37] reported that biogas slurry effectively alleviated the adverse consequences of drought by facilitating water retention within the soil pores and releasing it gradually during dry periods. The effect of biogas slurry on soil pH is noteworthy, as it has implications for nutrient uptake and availability within the rhizosphere.

The scientific literature extensively documents the prevalence of drought-induced stress, known to elicit a physiological response in plants termed oxidative stress. This phenomenon is characterized by generating ROS, molecules with high reactivity that can damage various cellular components. ROS have been recognized for their capability to induce cellular harm and disrupt normal physiological processes, as outlined by Ahanger et al. [38] and Rashid et al. [39]. Nonetheless, a burgeoning body of research is illuminating the potential of specific agricultural approaches, such as the integration of biogas slurry, as plausible strategies to ameliorate the harmful consequences of drought on plant organisms.

During unfavorable circumstances, plants grapple with the challenge of neutralizing ROS, thereby incurring oxidative stress Rizwan et al. [40]. This oxidative stress can adversely affect plants by catalyzing lipid peroxidation and impairing nucleic acids, disrupting their fundamental biological processes [38]. The research conducted by Rizwan et al. [40] underscores various plant species' susceptibility to oxidative stress due to water scarcity.

Under such adverse circumstances, plants exhibit a restricted ability to counteract the effects of ROS and their harmful consequences. The investigation reveals that the application of biogas slurry led to an elevation in the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in wheat plants subjected to drought conditions (Fig. 6). This observation suggests that supplementing wheat plants with biogas slurry effectively reinforces their antioxidant defense mechanism, enhancing their capacity to withstand the oxidative stress triggered by drought.

Drought-induced oxidative stress negatively impacts wheat but can be mitigated through enzymes like SSD, APX, POD, and CAT, increasing tolerance against drought-induced adverse effects.[41-43]. Drought stress in wheat raised EL, H_2O_2 concentration, and POD activity while decreasing SOD and CAT activity. SOD converts O_2 directly to H_2O_2, while CAT converts H_2O_2 and O_2 directly. POD may also contribute to H_2O_2 catalysis. Plants can detoxify ROS and require a balance between generation and breakdown for optimal growth [39, 44].

Conclusions

The application of biogas slurry as an organic amendment has been demonstrated to be a beneficial strategy for enhancing the growth and yield of wheat crops, particularly under drought-stress conditions. This enhancement in plant physiological parameters contributes to the simultaneous amelioration of wheat quality and yield. Thus, within the context of mitigating the detrimental impacts of water deficit stress, incorporating biogas slurry (BGS) emerges as a viable strategy to bolster the growth and productivity of wheat crops.

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Conflict of Interest

The authors declare no conflict of interest.

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