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Full Length Research Article

Physiological Modifications of Growth, Biochemical Compositions and Anti-Oxidant Activities in Water-Stressed Beniseed (*Sesamum indicum*) as Affected by Glycine Betaine

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ABSTRACT
Water imbalance limits growth and antioxidant production in plants. This study assessed varying concentrations (1, 2, 3 and 4 g/L) of glycine betanine (GB) on growth, nutritional contents, and antioxidant production in beniseed under drought conditions. Plant height (49.40 cm), number of leaves (27), leaf area (231.93 cm^2), specific leaf area (57.98 m^2kg^{-1}), and leaf area index (0.62 m^2m^{-2}) were higher in beniseed treated with 4g/L GB compared with lower concentrations of the osmolyte. However, the well-watered treatment improved significantly the relative growth rate (0.06 $mgg^{-1}day^{-1}$) and net assimilation rate (0.02 $gm^{-2}day^{-1}$). Chlorophyll-a (3.38 mg/g), chlorophyll-b (3.81mg/g) and total chlorophyll (7.20 mg/g) showed a significant increase in the plant under 3g/LGB. Moisture (91.07%), fat (2.74%), ash (3.55%), crude fiber (3.51%), and crude protein (4.47%) were significantly higher ($p<0.05$) in the treatments grown under 4g/LGB. The observation was consistent in sodium, potassium, calcium and magnesium as well as vitamin A, vitamin B5 and vitamin C. Superoxide dismutase (SOD) (1.58 u/gt), APX (1.18 u/g), CAT (1.58 mg^{-1}), GR (1.59 u/g), GST (14.07 mg^{-1}) and SP (19.06 u/ml) were higher in the roots of beniseed grown under drought. Conclusively, beniseed grown under,3 and 4g/L GB improved growth attributes and nutritional contents while high antioxidant production was observed in the roots of beniseed droughted compared with other parts.

Keywords: Osmolyte, Osmoregulation, Amelioration, Antioxidative enzymes, Water deficit, Drought-induced stress

INTRODUCTION
Sesame (*Sesamum indicum* L.) is an erect annual herb known as beniseed which belongs topedaliaceae family. It is one of the oldest and most traditional oilseed crops valued for quality seed oil. Studies have shown that beniseed cultivation was native to South Asia and spread west to Mesopotamia (Fuller, 2003; Scott *et al*., 2021; Purseglove, 2016). It is commonly used for food, ointments, medicine, sweets, and as bakery products or milled to get high-grade edible oil. Despite the economic relevance of the plant, the erratic nature of meteorological conditions has drastically reduced the production of the crop (Global Agrisystem, 2010).

Studies have also proven that plants including beniseed are being faced with water limitation. The condition often results in water stress (Umair *et al*., 2020; Chaudhry *et al.,* 2022) and results in a decline in the production potentials of plants due to their low water use efficiency (Kapoor *et al.,* 2020). Gene pools of many plants including beniseed gradually being eroded and extricated rapidly due to irregularity being observed in rainfall patterns and its associated effects on the reproductive cycle of the crops(Monja-Mio *et al.,* 2019). Also, lack of water or dehydration has been reported to contribute to desiccation, disruption of stable water configuration, changes in cell pH, increased concentration of cell sap, decrease in water potential, and modulation of biochemical activities of plants(Kumar *et al.,* 2019; Wahab *et al*., 2022).

In an attempt to withstand the resultant effects of water stress on plants including beniseed, many of them develop several natural mechanisms to cope with the conditions. Some of the mechanisms are the production of hydrophilic substances such as polyhydric alcohol, osmolytes, and antioxidative enzymes, desiccation postponement and desiccation tolerance, drought avoidance and drought tolerance, drought escape and hydropasive and hydroctive stomata closure among others (Zadehbagheri *et al.,* 2014; Hussain *et al*., 2021). Sugars such as glycine batanine and sucrose are usually increased in drought conditions in such plants since their presence regulates the water potential of the cells (Chen *et al.,* 2020). According to Kurepin *et al.* (2017), GB treatment modulated growth, survival, regulated metabolic processes, improved net CO_2 assimilation rate, enzymes, and lipids of photosynthetic machinery of plants at various levels of water deficit. Despite the natural coping mechanisms displayed by plants against water stress, the condition is still being reported to account for 70% of crop failure (Zhang *et al.,* 2019) therefore, there is a need to argument the water stress coping mechanisms of plants in order to protect plants from injury and maintain their food values even under abiotic stresses. In addition, only a few studies have been conducted on coping mechanisms of beniseed to water deficit therefore, this study investigated the effect of GB on growth, biochemical compositions, and anti-oxidant activities in the roots and leaf of water-stressed beniseed.

MATERIALS AND METHODS
Study Site of Field Experiment
This experiment was conducted at Botanical Garden, Lagos State University, Ojo Nigeria (Latitude 6.46663, N $6^0$27'59.8662'', Longitude 3.20004, E $3^0$12'0.14652'') (F682+G27, Ojo 102101, Lagos, Nigeria)

Seeds Collection
Seeds of beniseed were purchased from Farmers' market in Asero, Abeokuta Ogun State.

The seeds were identified at the Forest Herbarium Ibadan (FHI-38198) of Forestry Research
Institute of Nigeria, Ibadan.

Soil Collection and Nursery Preparation
Top soil was randomly collected 500m apart at the Botanical Garden of the Department of Botany, of Lagos State University, Ojo-Lagos following the methods of Vijay *et al.* (2019), Ojewumi *et al.* (2022) and Ojewumi *et al.* (2023). The soil was poured into eighteen perforated buckets. Seedlings of the plant were raised in the greenhouse of the Department for three weeks. Uniform seedlings (13-14cm) were selected and transplanted into each planting bucket and thereafter watered for another week which facilitated the hardening of the seedlings on the planting bucket.

Experimental Design and Preparation of Treatments
The planting buckets (one seedling per bucket) were arranged in a completely randomized design of four replicates. Thereafter, 1 2, 3, and 4 g GB were measured separately and diluted in 1 liter of water. Hundred (100mL) of each treatment was applied once daily on the seedling exogenously according to Ojewumi *et al*. (2022). Drought-imposed vegetables (Droughted) were treated with 100 mL of distilled water once in a week using foliar application just to ensure their survival. The treatments were extended for eight weeks after which data were collated from the vegetable under each treatment before the flowering stage of the vegetable.

Data Collection
Plant height, number of leaves, leaf area, leaf concentrations, shoot, and root dry weight were measured according to methods of Ojewumi *et al.* (2023)

Assessment of Growth Components in Beniseeds:
The total leaf area was measured using a leaf area metre. Specific leaf area (SLA) and leaf area index (LAI), relative growth rate, net assimilation rate, and leaf area ratio of the plants were determined as shown below (Alireza *et al.,* 2012).

$$SLA = \frac{Leaf\,area}{\text{Corresponding weight of leaf}}$$

$$LAI = \frac{Leaf\,area}{Area\,of\,litter\,fall}$$

$$NAR = \frac{W_2 - W_1}{A_2 - A_1} \cdot \frac{Log_e A_2 - Log_e A_1}{t_2 - t_1}$$

$$LAR = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{Log_e A_2 - Log_e A_1}{W_2 - W_1}$$

$$RGR = \frac{Log_e W_2 - Log_e W_1}{t_2 - t_1}$$

Where A_1= Area of leaf at t_1, A_2= Area of leaf at t_2, W_1= first measured weight (g), W_2= second measured weight (g), T_1= initial time (weeks) and T_2= final or second time (weeks)

Determination of Photosynthetic Pigments in Beniseed Leaves
Photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoid were determined using a spectrophotometry method described in Metzner *et al.* (1965) and Dawood *et al.* (2014).

Determination of Nutritional Contents in Beniseed Leaves
Ash content: Five (5.0 g) of the samples were added to a known weight crucible, weighed and dried (932F) for 4 hours and reweighed. Ash was determined as shown below (AOAC, 2000 cited in Ojewumi *et al.* (2021).

$$\text{Ash (\%)} = \frac{weight\,of\,ash}{weight\,of\,sample} \times 100$$

Crude Fat: Two grams of the beniseed sample were kept using a paper thimble in a weighed fat extractor. Ninety (90 mL) of C_6H_{14} was further added, refluxed and weighed and crude fat was determined.

$$\text{Crude fat (\%)} = \frac{weight\,of\,flask - weight\,of\,empty\,flask}{weight\,of\,original\,sample} \times 100$$

Moisture: moisture in the sample was calculated using the formula below:

$$\text{Moisture (\%)} = \frac{weight\,of\,sample\,before\,drying - weight\,of\,sample\,after\,drying}{weight\,of\,sample\,before\,drying} \times 100$$

Crude fiber: Two grams each of the samples were boiled in 20ML of 1.25% H_2SO_4 for 30min, filtered, washed thoroughly in hot water, and boiled using 200ML of 1.25% NaOH for 30 min. Spotless beaker was dried (100±5°C), cooled, and the weights of the contents were estimated. Spotless beakers with its content were dried at 9320F-11120F for 3 hours and weighed. Crude fiber was determined as shown below;
Crude fibre (%) =

$$\frac{Weight\,of\,beaker\,and\,crude\,fibre - weight\,of\,spoutless\,beaker\,and\,crude\,fibre}{weight\,of\,original\,sample} \times 100$$

Crude protein: The total nitrogen was determined using Micro-Kjeldahl (Ojewumi and Oyebanji (2020). Protein content (%) was determined as shown below
Protein (%) =

$$\frac{\text{Titer value} X 1.4 X\ 6.25 X 01 NHCL X volused}{\text{Weight of sample x Allquot digested sample used for distillation}} \times 100$$

Determination of Mineral Elements and Vitamins in the Beniseed Leaves
Elemental contents such as calcium, potassium, magnesium, zinc, phosphorus, and sodium in the samples were determined using an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2280) as reported by (Harborne, 1973). Vitamin A was measured using a Spectrophotometer (MetrohmSpectronic 21D

Model) at a wavelenght 328 nm according to the procedure of AOAC (2000). Vitamin B3 was determined as described by Iqbal *et al*. (2012). Vitamin B5; and Vitamin B6 were determined following the standard procedure of AOAC (2000). Ascorbic and vitamin E and vitamin K pantothenic acid absorbances were read at 760 nm and 570 nm, respectively; whereas the absorbances of tocopherol, pyridoxine, and phylloquinone were read at 470 nm, 415 nm, and 480 nm using Spectrophotometer (MetrohmSpectronic 21D Model).

Determination of Antioxidant Activities in Roots and Leaves of Beniseed

Catalase Activity (CAT)

CAT was assayed by measuring the initial rate of disappearance of H_2O_2 (Hafez *et al.,* 2012). Catalase reaction solution contained 50 $mmolL^{-1}$sodium-potassium phosphate buffer (pH 7.0), 10 $mmolL^{-1}H_2O_2$, and 20 µL enzyme extract in a final assay volume of 1 mL. The decrease in H_2O_2 was measured following the changes in the absorbance of the reaction solution at 240 nm. Extinction coefficient ε = 0.036 $Lmmol^{-1}cm^{-1}$ was used to calculate the concentration of CAT. One unit of CAT is defined as the enzymatic activity that catalyses the degradation of 1 µmol H_2O_2 per minute.

Glutathione Reductase (GR)

Glutathione reductase activity was determined using a reaction solution. The reaction solution contained 50 $mmolL^{-1}$sodiumphosphatebuffer (pH 7.5), 5 $mmolL^{-1}$ EDTA, 1 $mmolL^{-1}$ NADPH, 1 $mmolL^{-1}$ oxidized glutathione and 300 µl enzyme extract in a final assay volume of 1 mL. NADPH oxidation was determined at 340 nm (Foyer *et al.,* 1991). Activity of the enzyme was calculated using an extinction coefficient ε = 6.22 $Lmmol^{-1}cm^{-1}$ for NADPH. One unit of GR is defined as the enzyme activity that oxidizes 1 µmol NADPH per min.

Superoxide Dismutase (SOD)

Superoxide dismutase activity in the root and leaves of the vegetable was determined by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) to blue formazan by flavins under illumination. Superoxide dismutase reaction solution contained 50 $mmolL^{-1}$ sodium-potassium phosphate buffer (pH8.0), 300 $\mu molL^{-1}$ methionine,1.5 $mmol{\cdot}L^{-1}$ NBT, 120 µ molL−M1 riboflavin,100 $mmolL^{-1}$ Na_2EDTA,300 µ $molL^{-1}$ potassium cyanide and 100 µL enzyme extract in a final assay volume of 1 mL. The riboflavin was added last. The reaction was started by illuminating the test tubes under 4 fluorescent lamps for 10 min. The absorbance was measured using spectrophotometry at 560 nm. One unit of SOD activity was regarded as the amount of enzyme that inhibited 50% of NBT photo-reduction versus a blank cell containing no enzymatic extract

Superoxide Peroxidase (SP)

The peroxidase activity was determined using 4-methylcatechol as substrate. The increase in the absorption caused by oxidation of 4-methylcatechol by H_2O_2 was measured at 420 nm spectrophotometrically. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol, 5 mM H_2O_2 and 500 µL of crude extract in a total volume of 3.0 mL at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions.

Glutathione S-Transferase

Glutathione S-transferase (GST) activity was assayed with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced GSH in 1 mL phosphate buffer (pH 6.5). The reaction was monitored and an increase in OD_{340} was measured. Also, activities on other substrates were assayed according to Hafez *et al.* (2020).

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed using a statistical analysis system. The means were determined and separated using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

RESULTS

Effects of inclusion of varying concentrations of glycine batanine (GB) on morphological characters of water-stressed beniseed is presented in Figures1-2. Plant height and number of leaf length of the beniseed were influenced by inclusion of varying concentrations of GB from 2 through 8 weeks after treatment (WAT). Highest plant height (49.40cm) and number of leaf (27.0) were observed in beniseed treated with 100 mL of 4g/l GB compared with other treatments.

Physiological indices such as leaf area, specific leaf area, and leaf area index on the leaves of beniseed were significantly modulated by varying concentrations of GB. Highest LA (231.93cm^2), Leaf area index (0.62 m^2m^{-2}), and specific leaf area (57.98 m^2kg^{-1}) were observed in the leaves of beniseed treated with 100 ml of 4g/l GB followed by well-watered while least of the parameters were recorded in droughted. A similar observation was noticed in leaf area ratio (0.10 $gm^{-2}day^{-1}$), net assimilation rate (0.02 $gm^{-2}day^{-1}$) and relative growth rate (0.06$gm^{-2}day^{-1}$) of well-watered (Table 1). Although all the treatments produced effects on the physiological attributes of the plant, however, 100 ml of 4g/L GB was more impactful on the improvement of the physiological attributes of the plant.

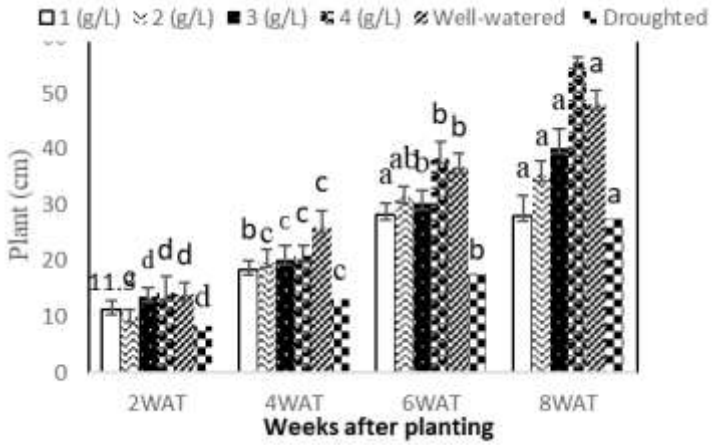

Figure 1: Effects of varying concentrations of glycine batanine on heights of beniseed
Differences in lowercase letters on bars indicate significant differences among treatments at p<0.05

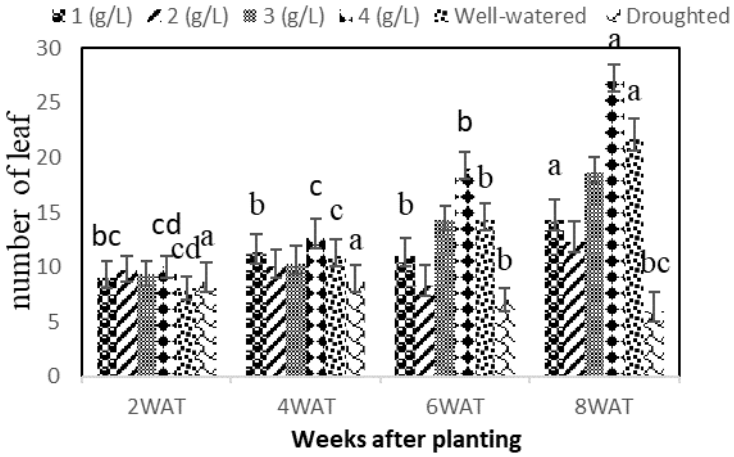

Figure 2: Effects of varying concentrations of glycine batanine on number of leaf of beniseed.
Differences in lowercase letters on bars in each week indicate significant differences among treatments at p<0.05

The pigment and its types were significantly higher in the leaves of the; plant treated with 100ml of 3g/l GB. Highest chlorophyll a (3.38 µgcm2), chlorophyll b (3.81 µgcm2) and total chlorophyll (7.20) were recorded in the leaves of the plant treated with 100ml of 3g/l GB, followed by 100 ml of 4g/l GB while least value of them was observed in the leaves of beniseed droughted (Table 2).

Moisture (91.07%), dry matter (91.07 %) and fat (2.74%) were significantly higher (p<0.05) in the leaves of beniseed treated with 100ml of 3 g/L GB. Similar significant increase was obtained in ash (3.55 %), crude fiber (3.51%), and crude protein (4.47%) in the leaves of the plant treated with 4g/LGB (Table 3). In addition, sodium (15.15 mg/100g), potassium (398mg/100g), calcium (124.89 mg/100g), magnesium (64.89mg/100g), phosphorous (98.88 mg/100g) and zinc (2.55 mg/100g) were higher(P<0.05) in the leaves of the plant treated with 100ml of 4g/l GB followed by well-watered while lowest of the parameters were noticed in the droughted (Table 4).

In the same vein, Vitamin A (49.68 mg/100g), Vitamin B5 (0.15 mg/100g), vitamin B6 (0.69 mg/100g) vitamin C (16.03 mg/100g) vitamin E (1.75 mg/100g) and vitamin K (62.92 mg/100g) were significantly higher in the leaves of the plants treated with 100 ml of 4g/l GB compared with other treatments (Table 5). The treatment produced significant effects on quantities and variations of the antioxidative enzymes in the leaves and roots of the vegetable. SOD (1.58 u/g), APX (1.18 u/g), CAT (1.58 mg^{-1}), GR (1.591 u/g), GST (14.07 mg^{-1}) and SP (19.06 u/ml) were recorded in the roots of beniseed droughted while higher proline (2.47 mm/l) was recorded in the roots of beniseed treated with 4 g/l GB (Table 6).

Table 1: Effects of varying concentrations of glycine batanine on the physiological parameters of beniseed

GLYCINE (g/L)	LA	LAI (m^2m^{-2})	SLA (m^2kg^{-1})	NAR ($gm^{-2}day^{-1}$)	LAR ($gm^{-2}day^{-1}$)	RGR ($mgg^{-1}day^{-1}$)
1	216.96±5.79^{c}	0.61±0.10^{ab}	54.24±13.94^{b}	0.01±0.01^{a}	0.08±0.04^{a}	0.04±0.003^{a}
2	157.00±1.61^{d}	0.34±0.07^{d}	34.45±24.38^{cd}	0.01±0.01^{a}	0.06±0.01^{a}	0.06±0.014^{a}
3	231.93±4.57^{a}	0.56±0.14^{b}	56.58±25.04^{b}	0.01±0.01^{a}	0.02±0.01^{a}	0.05±0.007^{a}
4	226.33±1.15^{b}	0.62±0.26^{a}	57.98±10.14^{a}	0.01±0.01^{a}	0.05±0.008^{a}	0.04±0.008^{a}
Well-Watered	137.83±9.53^{c}	0.14±0.04^{c}	39.25±4.65^{c}	0.02±0.01^{a}	0.10±0.03^{a}	0.06±0.006^{a}
Droughted	130.86±3.38^{f}	0.36±0.25^{d}	32.71±7.59^{d}	0.01±0.01^{a}	0.06±0.01a	0.078±0.02^{a}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Duncans Multiple Range Test (DMRT). WAT= weeks of treatment, LA= Leaf area, SLA=Specific leaf area, LAI=Leaf area index, RGR= Relative growth rate, NAR=Net assimilation rate, LAR=Leaf area ratio

Table 2: Effects of varying concentrations of glycine betanine on levels of chlorophyll contents in the leaves of water-stressed beniseed

	CHLOROPHYLL (μgcm^2)		
GLYCINE (g/L)	**CHLOROPHYLL-a**	**CHLOROPHYLL-b**	**TOTAL CHLOROPHYLL**
1	2.98±0.00^{c}	2.59±0.01^{c}	5.58±0.01^{cd}
2	3.00±0.01^{bc}	2.60±0.01c	5.61±0.01^{c}
3	3.38±0.03^{a}	3.81±0.03^{a}	7.20±0.06^{a}
4	3.08±0.03^{b}	2.68±0.01^{b}	5.77±0.04^{b}
Well-Watered	2.95±.01^{c}	2.52±0.01^{d}	5.48±0.01^{d}
Droughted	1.55±0.03^{d}	1.16±0.01^{e}	2.69±0.02^{e}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Duncans Multiple Range Test (DMRT).

Based on the findings of this study, 100 ml of 3-4g/L GB improved growth attributes and physiological parameters, chlorophyll and nutrition contents of beniseed therefore the use of the osmolytes is recommended for cultivation of the plants, especially during water deficit conditions.

DISCUSSION

Drought stress is one of the restrictive factors hindering sustainable production of agricultural products. It has a devastating influence on morphological and physiological activities as well as the production efficiency of plants (Guidi *et al*., 2013). Significant increase observed in height and number of leaf of beniseed treated with 100 ml of 4g/L GB indicates that the application of the osmolyte can alleviate the negative effects of water stress on the morphology of beniseed (Oloyede *et al.,* 2021). Also, the substantial increase observed in biomass of beniseed modulated by exogenous application of GB may be attributed to the roles of the osmolytes in osmotic adjustment, enhancement of turgor pressure and water potential for water translocation and nutrient uptake and channelization of metabolites to improve the morphological characters of the plant (Yan *et al.,* 2011).

Higher leaf area recorded in the plant treated with 100 ml of 4g/l GB may suggest that the treatment enhanced the leaf surface area of the plant for light energy absorption and photosynthetic efficiency (Serapicos *et al.,*2022). This is consistent with the findings of Khodary and Moussa (2013) which posited that GB improved leaf area in sesame plants under water stress conditions. The significant effect the treatment exerted on the leaf area index may be proof of the contributions of the treatments toward the active photosynthetic area of the plant (Ahmed *et al.,* 2019). High specific leaf area recorded in the plant under 100 ml of the treatments may indicate roles of the osmolyte in nutrients and dry mass indices formation in the leaves, a unit of biomass production, and as an estimation of nutrient balance for growth of the plant (Farooq *et al.,* 2019). The leaf area ratio being significantly induced by the application of 100ml 4g/L GB could signify the modulation influence of the treatment on the photosynthetic capacity of the leaves of the plant. This observation informs that the treatment-modulated leaf area ratio of the plant could be a predictor of competitiveness, rate of resource acquisition, and level of mineralization of photosynthetic products (Gupta *et al.,* 2021).

High chlorophyll a, chlorophyll b, and total chlorophyll recorded in the leaves of the plant treated with 100ml 3g/l GB may indicate that the treatment can be used to predict the health status of thylakoid and nutrition level, for the production of pigments and as a measure of photosynthetic capacity the plant. This submission is in agreement with the submissions of Dawood *et al*. (2014) and Tari *et al.,* (2016) who opined that among different biochemical attributes, leaf chlorophyll is the most important attribute that reflects the health status of plants. However, the reduction observed in the pigments in beniseed droughted may denote a level of disorganization in thylakoid membranes, with more degradation than synthesis of chlorophyll via the formation of proteolytic enzymes such as chlorophyllase (Dawood *et al*., 2014, Jaleel *et al.,* 2008; Mafakheri *et al.,* 2009). High chlorophyll contents determined in the plants treated with 100 ml of 3 and 4g/L GB may be precursors for the production of secondary metabolites such as proximate, minerals and vitamins as products of photosynthesis (Bratosin *et al.,* 2021). Reverse was the case in droughted plants where lower chlorophyll recorded might have accounted for reduced photosynthates as the plant experienced a hydroactive situation (Bratosin *et al.,* 2021). It has been reported that in plants, a rise in lack of water due to heat slows down chlorophyll production, photosynthesis, and nutrient channelization but when some coonhounds such as osmolyte are added the process is restarted

Moreover, the treatment also significantly influenced the antioxidative enzymes in the leaves and roots of beniseed. High SOD, APX, CAT, GR, GST, and SP recorded in the roots of the droughted plant could indicate that although plants lose water through their leaves by stomata opening; however, during water stress, antioxidative enzymes are produced more in roots compared with leaves as means of regulating water exchange with the environment via root hair or lenticels. It may also signify the activation of defense mechanisms of roots of droughts against oxidative damage caused by water deficit. The GB may act as an antitranspirant by allowing plants to absorb more water for a long period to facilitate metabolic activities. These attributes may make the application of GB an economically feasible approach to counteract the adverse effects of environmental stresses on the production of *S. indicum* in unfavorable environmental conditions. This observation suggests that GB at 100 ml of 4g/L plays a role in osmotic adjustment against dehydration (Yan *et al.,* 2011).

CONCLUSION

Based on the results of this study, 100 mL of 3-4g/L GB improved growth and physiological attributes as well as nutritional contents in glycine betanine-treated-beniseed while beniseed droughted followed by 4g/LGB enhanced production of antioxidants against water stress. The use of the osmolyte is recommended for the cultivation of the plants most especially during water deficit conditions.

Table 3: Effects of varying concentrations of glycine betanine on proximate contents in the leaves of water stressed beniseed

GLYCINE (g/L)	PROXIMATE (%)						
	MOISTURE	DRY MATTER	FAT	ASH CONTENT	CRUDE FIBER	CRUDE PROTEIN	CARBON HYDRATE
1	82.21±0.46^{c}	8.00±0.51^{c}	2.58±0.04^{abc}	3.37±0.07^{b}	3.29±0.07^{b}	4.14±0.01^{bc}	4.88±0.14^{a}
2	81.61±0.03^{cd}	8.57±0.02^{bc}	2.48±0.03^{bc}	3.19±0.02^{c}	3.41±0.02^{ab}	4.31±0.13^{ab}	5.03±0.02^{a}
3	91.07±0.39^{a}	9.70±0.33^{a}	2.74±0.04^{a}	3.530±.04^{ab}	3.07±0.02^{c}	3.91±0.03^{c}	5.33±0.58^{a}
4	80.82±0.02^{d}	9.31±0.02^{ab}	2.69±0.01^{ab}	3.55±0.03^{a}	3.51±0.01^{a}	4.47±0.02^{a}	5.34±0.05^{a}
Well-Watered	82.55±0.57^{bc}	8.58±0.02^{bc}	2.46±0.13^{c}	3.40±0.01^{cb}	3.37±0.01^{d}	4.24±0.01^{ab}	5.19±0.01^{a}
Droughted	83.54±0.62^{b}	6.70±0.05^{d}	0.77±0.02^{d}	1.57±0.01^{d}	1.49±0.01^{ab}	2.43±0.02^{d}	3.20±0.02^{b}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Duncan's Multiple Range Test (DMRT).

Table 4: Effects of varying concentrations of glycine betanine on levels of mineral contents in the leaves of water stressed beniseed

GLYCINE (mg/L)	MINERAL (mg/100g)					
	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	PHOSPHOROUS	ZINC
1	12.41±0.06^{d}	309.18±0.03^{e}	106.01±0.03^{e}	50.95±0.03^{d}	78.87±0.03^{c}	2.42±.03^{b}
2	13.18±0.03^{c}	324.32±0.01^{d}	114.29±0.00^{d}	52.85±0.01^{c}	81.27±0.03^{d}	2.51±.03^{ab}
3	13.20±0.02^{c}	353.65±0.02^{c}	115.46±0.01^{c}	53.05±0.01^{c}	82.75±0.07^{c}	2.54±.03^{a}
4	15.15±0.01^{a}	398.88±0.06^{a}	124.86±0.05^{a}	64.89±0.29^{a}	98.88±0.02^{a}	2.55±.01^{a}
Well-Watered	13.62±0.02^{b}	368.41±0.03^{b}	116.19±0.10^{b}	54.32±0.05^{b}	84.23±0.33^{b}	2.52±.01^{ab}
Droughted	12.03±0.04^{e}	298.92±0.03^{f}	101.51±0.04^{f}	50.30±0.04^{e}	79.54±0.03^{e}	2.43±.03^{b}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Duncan's Multiple Range Test (DMRT).

Table 5: Effect of varying concentrations of glycine betanine on levels of vitamin contents in the leaves of water stressed beniseed

GLYCINE (g/l)	VITAMINS (mg/100g)						
	Vitamin A	Vitamin B3	Vitamin B5	Vitamin B6	Vitamin C	Vitamin E	Vitamin K
1	175.25±0.03^{d}	2.55±0.30^{ab}	0.10±0.00^{bc}	0.66±0.03^{b}	14.00±0.01^{d}	1.58±0.11^{b}	60.05±0.06^{c}
2	78.68±0.39^{c}	2.62±0.04^{a}	0.10±0.01^{bc}	0.65±0.00^{b}	15.17±0.03^{c}	1.59±0.00^{b}	61.54±0.02^{b}
3	84.49±0.03^{b}	2.64±0.03^{a}	0.12±0.00^{b}	0.64±0.01^{c}	15.93±0.03^{b}	1.69±0.09^{ab}	61.71±0.01^{b}
4	49.68±0.31^{a}	2.66±0.03^{a}	0.15±0.99^{a}	0.69±0.00^{a}	16.03±0.05^{a}	1.75±0.05^{a}	62.92±0.02^{a}
Well-Watered	57.25±0.04^{e}	2.49±0.04^{b}	0.11±0.01^{ab}	0.65±0.03^{c}	13.24±0.03^{e}	1.55±0.05^{c}	58.48±0.04^{d}
Droughted	51.87±0.25^{f}	2.27±0.02^{c}	0.09±0.00^{c}	0.60±0.03^{d}	11.95±0.04^{f}	1.47±0.07^{d}	48.03±0.32e

Means ± standard errors in columns with different superscripts are significantly different at; p<0.05 using Duncan's Multiple Range Test (DMRT)

Table 6: Effects of varying concentrations of glycine betanine on levels of antioxidative enzymes in the leaves of water stressed beniseed

	ANTIOXIDATIVE ENZYMES					
Glycine (g/l)	**SOD (u/g)**	**APX(u/g)**	**CAT (mg^{-1})**	**GR(u/g)**	**GST (mg^{-1})**	**SP(u/ml)**
1 Leaf	0.93 ± 0.02^{g}	1.29 ± 0.01^{f}	1.09 ± 0.01^{g}	0.96 ± 0.02^{gh}	6.47 ± 0.03^{j}	14.28 ± 0.05^{i}
2 Leaf	1.13 ± 0.02^{e}	1.36 ± 0.00^{e}	1.17 ± 0.02^{f}	1.19 ± 0.00^{f}	7.70 ± 0.03^{i}	17.11 ± 0.02^{g}
3 Leaf	1.18 ± 0.00^{de}	1.43 ± 0.02^{d}	1.19 ± 0.01^{ef}	1.26 ± 0.02^{e}	8.07 ± 0.03^{h}	18.24 ± 0.03^{f}
4 Leaf	1.23 ± 0.02^{cd}	1.45 ± 0.00^{d}	1.26 ± 0.01^{d}	1.29 ± 0.00^{e}	8.52 ± 0.02^{f}	20.04 ± 0.04^{c}
Well-Watered Leaf	0.77 ± 0.01^{h}	0.98 ± 0.00^{g}	1.10 ± 0.03^{g}	0.93 ± 0.03^{h}	6.01 ± 0.14^{k}	8.08 ± 0.00^{k}
Droughted Leaf	1.37 ± 0.032^{b}	$1.66\pm.02^{b}$	1.42 ± 0.03^{b}	1.46 ± 0.02^{b}	9.94 ± 0.10^{c}	18.24 ± 0.08^{f}
1 Root	1.06 ± 0.008^{f}	1.37 ± 0.03^{d}	1.28 ± 0.00^{d}	1.07 ± 0.01^{h}	8.33 ± 0.00^{g}	16.92 ± 0.01^{h}
2 Root	1.21 ± 0.033^{cd}	1.44 ± 0.00^{d}	1.36 ± 0.02^{c}	1.26 ± 0.01^{e}	9.04 ± 0.03^{e}	18.45 ± 0.02^{f}
3 Root	1.25 ± 0.015^{c}	1.52 ± 0.020^{c}	1.38 ± 0.00^{bc}	1.31 ± 0.00^{d}	9.51 ± 0.02^{d}	20.44 ± 0.02^{b}
4 Root	1.37 ± 0.01^{b}	1.62 ± 0.02^{b}	1.39 ± 0.02^{bc}	1.36 ± 0.01^{c}	10.21 ± 0.01^{b}	21.91 ± 0.01^{f}
Well-Watered Root	0.81 ± 0.00^{h}	1.01 ± 0.00^{g}	1.24 ± 0.01^{d}	1.00 ± 0.00^{h}	7.95 ± 0.06^{h}	10.11 ± 0.02^{g}
Droughted Root	1.58 ± 0.010^{a}	1.81 ± 0.02^{a}	1.58 ± 0.03^{a}	1.59 ± 0.01^{a}	14.07 ± 0.03^{a}	19.06 ± 1.00^{a}

Means ± standard errors in columns with different superscripts are significantly different at $p<0.05$ using Duncan's Multiple Range Test (DMRT). CAT=catalase, SOD= Superoxide Dismutase, APX= Ascorbate Peroxide, GPX= Glutathione Peroxidase

REFERENCES

Global Agrisystem (2010) Evaluation Study of Agriclinics and agribusiness centre Scheme, Global Agrisystem Private Limited, New Delhi.

Ahmed, N., Zhang, Y., Li, K., Zhou, Y., Zhang, M. and Li, Z. (2019). Exogenous application of glycine betaine improved water use efficiency in winter wheat (*Triticumaestivum*L.) via modulating photosynthetic efficiency and antioxidative capacity under conventional and limited irrigation conditions. *Crop Journal,* **7**(5): 635-650.

Alireza, P. M. K., Ata, A. S., Ghodrat, A. F. and Abdolmehdi, B. (2012). *Pimpinella anisum* under different irrigation regimes and amounts of super absorbent analysis system institute, Cary, Nc; p 556

Bates, L. S., Waldan, R. P. and Teare, L. D. (1973). Rapid determination of free proline under water stress studies. *Plant Soil,* **39**:205–207.

Bratosin, B. C., Darjan, S. and Vodnar, D. C. (2021). Single cell protein: A potential substitute in human and animal nutrition. *Sustainability*, **13**(16):9284.

Chaudhry, S. and Sidhu, G. P. S. (2022). Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Reports* **41(**1): 1-31.

Chen, X., Zhao, P., Ouyang, L., Zhu, L., Ni, G. and Schäfer, K. V. (2020). Whole-plant water hydraulic integrity to predict drought-induced Eucalyptus urophylla mortality under drought stress. *Forest Ecology and Management,* **468**: 118179.

Dawood, M. G., Taie, H. A. A., Nassar, R. M. A., Abdelhamid, M. T. and Schmidhalter, U. (2014). The changes induced in the physiological, biochemical and anatomical characteristics of Viciafaba by the exogenous application of proline under seawater stress. *South African Journal of Botany,* **93**:54-63.

Farooq, M., Wahid, A., Kobayashi, N. S. M. A., Fujita, D. B. S. M. A. and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. In *Sustainable Agriculture*, Springer Netherlands 153-188. doi: 10.1007/978-90-481-2666-8_12.

Foyer, C., Maud, L., Camille, G., Karl, J. K, (1991). Effects of Elevated Cytosolic Glutathione Reductase Activity on the Cellular Glutathione Pool and Photosynthesis in Leaves under Normal and Stress Conditions, *Plant Physiology*, **97**(3); 863–872,

Fuller, D. Q. (2003). African crops in prehistoric South Asia: a critical review. *Food, fuel and fields: Progress in African Archaeobotany*, **15**: 239-271.

Guidi, L., DEgl'innocenti, E., Genovesi, V. and Giordano, C. (2013). Effect of water stress on some morpho-physiological traits of tomato. *Journal of. Agronomy,* **12**(1):1-8.

Gupta, S., Schillaci, M., Walker, R., Smith, P. M., Watt, M. and Roessner, U. (2021). Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. *Plant and Soil*, **461**: 219-244.

Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., Rashwan, E., El-Maghraby, L., Awad, A. and Abdelaal, K. (2020). Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agronomy*, **10**(5): 630.

Hussain, S., Hussain, S., Ali, B., Ren, X., Chen, X., Li, Q. and Ahmad, N. (2021). Recent progress in understanding salinity tolerance in plants: Story of Na+/K+ balance and beyond. *Plant Physiology and Biochemistry*, **160**:239-256.

Iqbal, H., Riaz, U., Rooh U., Muhammad, K., Naseem, U., Abdu, B., Farhat, A.K, Muneebur, R K, Mohammad,Z, Jehangir, K, Naeem K. (2012). Phytochemical analysis of selected medicinal plants, *African Journal of Biotechnology*; **10**(38): 7487-7492.

Jaleel, C. A., Sankar, B., Sridharan, R. and Panneerselvam, R. (2008). Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in Catharanthus roseus. *Turkish Journal of Biology*, ***32***(2): 79-83.

Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M. and Sharma, A. (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Applied Sciences*, **10**(16): 5692.

Khodary, S. and Moussa, H. R. (2013). Effect of glycine betaine on growth, photosynthesis and some metabolic pathways of salt-stressed maize (*Zea mays* L.). *Journal of Stress Physiology and Biochemistry,* **9**(4): 229-251.

Kumar, R., Berwal, M. and Saroj, P. L. (2019).Morphological, physiological, biochemical andmolecular facet of drought stress in horticultural crops, *International Journal of Bio-resource and Stress Management,* **10**(5):545-560.

Kurepin, L. V., Ivanov, A. G., Zaman, M., Pharis, R. P., Hurry, V. and Hüner, N. P. (2017). Interaction of glycine betaine and plant hormones: Protection of the photosynthetic apparatus during abiotic stress. *Photosynthesis: Structures, Mechanisms, and Applications*, **9**: 185-202.

Mafakheri, A., Siosemardeh, A. F., Bahramnejad, B., Struik, P. C. and Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian Journal of Crop Sscience*, **4**(8): 580-585.

Metzner, H., Rau, H. and Senger, H. (1965). Determination of photosynthetic pigment. *MangelMutanten Von Chlorella Plant*, **65** 186-191.

Monja-Mio, K. M., Herrera-Alamillo, M. A., Sánchez-Teyer, L. F., and Robert, M. L. (2019). Breeding strategies to improve production of Agave (*Agave spp.*). *Advances in Plant Breeding Strategies: Industrial and Food Crops,* **6:**319-362.

Ojewumi, A. W., Junaid, O. E. and Feyibunmi, G. O. (2022). Morpho-physiological assessment of water stress ameliorative potential of some osmoprotectants on growth performance and chlorophyll contents of cayenne pepper (Capsicum annum). *FUOYE Journal of Pure and Applied Sciences, (FJPAS)*, **7**(3): 13-20.

Ojewumi, A. W., Ayoola, S. A., Ope, A. A. and Iyanda, T. M. (2023). Proline as an osmolyte modulates changes in morphological and physiological attributes of underwater stress. *Annals of Science and Technology*, **8** (1): 51-58

Ojewumi, A. W. and Kadiri, M. (2021). Physiological responses of photosynthetic and respiratory rates of some leafy vegetables to Spent engine oil contamination. *Jewel Journal of Scientific Research (JJSR)*, **6**(2): 188-189.

Ojewumi, A. W. and Oyebanji, E. O. (2020). Ethnopharmacological survey and physiological evaluation of nutritional and phytochemical contents of indigenous plants used for treatment of toothache and mouth odour in Ijebu Ode Local Government Area, Ogun state, Nigeria, *Nigerian Journal of Pure and Applied Sciences,* **33**(1): 3559-3576.

Ojewumi, A. W., Keshinro, O. M., Mabinuori. L. F. and Makinde S. C. O., (2023). Oxidative defense mechanisms of proline on growth, nutritional compositions and antioxidant activities in water-stressed *Solanum aethiopicum L. Journal of Plant Stress Physiology,* **2023**, 9:1-9.

Oloyede, F. M., Egbewole, S. O. and Ogundele, O. M. (2021). Effect of glycine treatments on the morphological, physiological and nutritional characteristics of *Sesamum indicum* under water stress conditions. *Journal of Applied Sciences and Environmental Management*, **25**(5): 931-939.

Purseglove, J.W. (1972) Tropical Crops, Monocotyledons. Longman Group Limited, London, 52-54.

Scott, A., Power, R. C., Altmann-Wendling, V., Artzy, M., Martin, M. A., Eisenmann, S. and Warinner, C. (2021). Exotic foods reveal contact between South Asia and the Near East during the second millennium BCE. *Proceedings of the National Academy of Sciences*, **118**(2), e2014956117.

Serapicos, M., Afonso, S., Gonçalves, B. and Silva, A. P. (2022). Exogenous Application of Glycine Betaine on Sweet Cherry Tree (Prunusavium L.): Effects on Tree Physiology and Leaf Properties. *Plants*, **11**(24): 3470.

Tari, I., Csiszár, J., Szalai, G., Horváth, F., Pecsvaradi A., Kiss, G. and Szepesi, A. (2016). Acclimation of photosynthetic processes to high irradiance in rice: changes in photosynthesis, antioxidant activity and plant architecture. *Plant. Physiology and Biochemistry,* **109**:244-251

Umair Hassan, M., Aamer, M., UmerChattha, M., Haiying, T., Shahzad, B., Barbanti, L. and Guoqin, H. (2020). The critical role of zinc in plants facing the drought stress. *Agriculture*, **10(**9): 396.

Vijay, A; Dhotare, V D; Guldekar, SMB; Sagar, NI. (2019) Evaluation of Soil Nutrient Index and their Relation with Soil Chemical Properties of Washim Road Farm of Dr.PDKVAkkola, Maharashtra, India, *International Journal of Current Microbiology and Applied Sciences*, **8**(9): 1773-1779.

Wahab, A., Abdi, G., Saleem, M. H., Ali, B., Ullah, S., Shah, W. and Marc, R. A. (2022). Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. *Plants*, **11(**13): 1620.

Yan, Z., Guo, S., Shu, S., Sun, J. and Tezuka, T. (2011). Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. *African Journal of Biotechnology*, **10**(80): 18381-18390.

Zadehbagheri, M., Azarpanah, A. and Javanmardi, S. (2014). Proline metabolite transport an efficient approach in corn yield improvement as response to drought conditions. *Nature*, **566**: 76-485.

Zhang, Q., Yu, H., Sun, P., Singh, V. P. and Shi, P. (2019). Multisource data based agricultural drought monitoring and agricultural loss in China. *Global and planetary change*, **172:** 298-306.