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Exogenous application of humic acid or chitosan mitigates drought stress on *Paspalum vaginatum* turfgrass

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Abstract: The current study was carried out to examine the impact of humic acid or chitosan applications on morphology and physiology attributes of *Paspalum vaginatum* Swartz. cv. Salam grown under drought stress. Drought stress was enforced by various watering intervals (2, 5, 8 and 11). The plants subjected to various watering intervals were sprayed biweekly with either humic acid (HA) or chitosan (CHT) each at concentrations of 300 and 600 ppm, whereas the tap water was used as control. The findings indicated that drought stress decreased all growth traits (such as, sward height, lawn density and dry weights of clippings and underground parts), total chlorophylls, total carbohydrates, N, P and K%, while proline, phenols content and Enzyme activity (CAT, APX and SOD) were raised. The plants foliar sprayed with HA or CHT at higher doses led to boost in the tested growth traits, total chlorophylls, total carbohydrates, N, P and K%, proline, phenols and enzyme activity with superiority of HA. Based on the outcome of the present research it can be inferred that, foliar application of HA at 600 ppm can ameliorates the harmful impacts of drought stress on physiology and growth traits of *Paspalum vaginatum*.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

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1. Introduction

Seashore paspalum (*Paspalum vaginatum* Swartz.) is one of the most extensively utilized grasses for lawn establishment in Egypt. It widely utilized in new towns, as well as in golf courses, sports fields, coastal resorts, tourist villages, and home lawns. It is suited to warm subtropical and tropical regions. It produces a dark green turf that is thick and finely textured. It can be applied to golf course greens as well as sport turfs and utility lawns (Barsoom *et al.*, 2024).

The primary environmental factor affecting the quality, growth, and production of turfgrass is water scarcity, which is a biotic stressor restricting agricultural productivity in the majority of countries, particularly in dry and semi-arid regions. Turfgrass undergo a number of physiological and biochemical changes as a result of water stress, including a decrease in plant morphology (Cui *et al*., 2020; Katuwal *et al*., 2021; Errickson *et al*., 2023; Taleb *et al*., 2023; Porcelli *et al*., 2024; Hejl *et al*., 2024), decreases in the absorption of nutrients (Shen *et al*., 2024), decreases in total chlorophyll content (Sheikh Mohammadi *et al*., 2017), rising levels of phenols and prolin (Fariaszewska *et al*., 2020), as well as rising levels of in CAT, SOD or APX (Bandurska and Jozwiak, 2010; Salehi *et al*., 2014; Katuwal *et al*., 2020).

Bio-stimulators have been used recently as a result of research into biological ways to prevent the use of chemical products and mitigate the negative effects of water scarcity in agriculture. Among the different types of bio-stimulators are Humic acid and chitosan. Humic acid (HA) is a natural polymer organic compound which can be utilized to improve plant growth, nutrient availability in the soil. Under normal conditions previous authors reported HA had a favorable impact on enhancing growth and nutrient uptake of turfgrass species (Shahin, *et al*., 2015; Taher *et al*., 2023). Under drought stresse conditions, HA has the ability to alleviate the deleterious impacts of drought by enhancing chlorophyll, and carotenoids, total sugars, indoles, phenols, nutrient uptake as well as antioxidant activities (El-Sayed *et al*., 2016; Aalipour *et al*., 2019).

Chitosan (CHT) is another bio- stimulators chitin derivative. It is a naturally occurring polymer that is environmentally benign and biodegraded by biological agents in agriculture (Shafiei-Masouleh, 2019). Under normal conditions, previous studies augmented CHT had a suitable impact like increase growth parameters, chlorophylls content, photosynthesis, and nutrient uptake (Byczyńska, 2018; Abd-El-Hady, 2020). Under stressed conditions, the detrimental impacts of drought can be effectively alleviated by CHT by increasing proline content, antioxidant activity, chlorophyll and carbohydrates (Pirbalouti *et al*., 2017; Zhao *et al*., 2019; Almeida *et al*., 2020; Abou dahab *et al*., 2023). The beneficial effects of CHT is increase stomatal closure through ABA synthesis, photosynthetic rate, and the synthesis of carbohydrates, amino acids, organic acids, and other metabolites that are necessary for energy metabolism under stress, osmotic adjustment, and stress signaling. They also stimulate antioxidant enzymes via the signaling pathways for hydrogen peroxide and nitric oxide (Hidangmayum, *et al*., 2019).

Although bio-stimulators have been shown to have positive effects on ornamental plants and to increase growth parameters, there is insufficient information on how they can mitigate the negative effects of drought on turfgrass. Therefore, the purpose of this study is to assess how foliar application of HA or CHT affects the quality of *Paspalum vaginatum* grown under drought stress.

2. Materials and Methods

The present experiment (8.5 months) was undertaken in the experimental nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two growing season of 2023 and 2024. The aim of this work was to evaluate the response of seashore paspalum grown under drought stress to foliar application of HA or CHT.

Plant material

On 1st of March, 2023 and 2024 growing seasons, sods of seashore paspalum were obtained from a commercial turf nursery and planted in the experimental area which divided into sixty beds (1 m x 1 m), with distance 30-cm between them. Compost was incorporated into the soil to a depth of 12 to 15 cm at a rate of 2 $m^3/100\ m^2$ to thoroughly prepare the beds. The physical and chemical characteristics of the experimental soil are presented in Table 1. The conditions of temperature, relative humidity and total rainfall from the beginning to the end of the experiment are reported in Table 2.

Experimental procedures

Starting from 15th of March in both seasons, respectively the beds were irrigated once every 2, 5, 8 and 11 days with 6 L of fresh water/bed for imposing drought stress. The beds were irrigated 127.5, 51, 32, and 23.2 times throughout the course of the study. This implies that when the experiment is finished (after 8.5 months), beds watered every 2, 5, 8 and 11 days interval were given 765, 306, 191.25and 139.09 water liters, in the order.

Stating from 31st March till to 15th October (in both seasons), the beds were sprayed every 2 weeks with either HA or CHT each at concentrations of 300 and 600 ppm, while only tap water was used to spray the control plants. Both HA and CHT were purchased from Tecknogreen company, Egypt. Using a plastic

Table 1 - Soil physical and chemical properties utilized for growing *Paspalum vaginatum* (mean of two seasons)

Soil properties	Data
Physical characteristics	
Soil texture	
Clay	55.2
Coarse sand	4.99
Fine sand	17.08
Silt	22.76
Chemical characteristics	
Soluble anions (meq/l)	
Cl-	3.13±0.03
SO4-	2.52±0.02
Ca++	7.39±0.04
Mg++	2.76±0.03
K+	0.39±0.01
Na+	5.15±0.05
N (ppm)	93.35±3.00
P (ppm)	21.13±0.13
O. m (%)	1.61±0.20
EC (dS/m)	1.54±0.12
pH	7.15±0.04

atomizer, the turfgrass was sprayed until the runoff threshold (100 ml/beds) was reached after adding 1 ml/L of bio-new film as a wetting agent. All the turfgrass beds were monthly fertilized with kristalon-quick fertilizer (NPK 20:20:20 + micronutrients) at a dose of 280 kg/ha. Additionally, manual picking of weeds, disease and pest control has also been carried out.

Layout of experimental

The layout of the experiment was a split-plot design with 20 treatments [4 watering frequency x 5 plant bio-stimulators (including the control)] with 3 blocks (replicates), each replicate consisting of 20 beds (1 bed/treatment). Irrigation frequency were assigned to the main plots in a randomized complete blocks design, while plant bio-stimulators treatments were randomly assigned to the sub-plots within irrigation frequency.

The data recorded

Vegetative growth traits. On the 15th of April till the end of the study (in two seasons, respectively), the turfgrass was mowed biweekly to a height of 3 cm using scissors. Sward height (cm) was recorded immediately before each mowing (every 2 weeks) using the method described by Dernoeden (1984), in which a ruler was set upright on the soil surface and a cardboard disk with a hole in its center was dropped freely over the ruler onto the top of the vegetative turf canopy. Sward height was calculated as the distance between soil surface and the disk, three sward height measurements were recorded in each plot, and the average sward height was calculated. Fresh weights of the clippings (g/m^2) after mowing (every 2 weeks) were collected manually and dried then the average of dry weights of the clippings (g/m^2) were calculated. At the end of each growing season, turf density (number of tillers/100 cm^2) was recorded using a 10 x 10 cm wooden frame which randomly tossed three times per plot, the number of tillers inside the frame was counted manually each time and the average lawn density was then calculated. Root length and dry weight of underground parts (g/m^2) were recorded according to the method of Hussein *et al*. (2012) in which the underground parts (roots + rhizomes) were taken from two sod and soil cores, each with an area of 400 cm^2 (20x20 cm) and a depth of 20 cm. The

Table 2 - Summary of forward selection for predicting embryo fresh weight in *Acer monspessulanum*

Period	Temperature Max. (°C)	Temperature Min. (°C)	Average of RH (%)	Total rain fall (mm)
March	23.5±1.50	11.6±0.06	53±2.50	1.9±0.20
April	28.3±1.30	14.6±2.30	47±1.00	0.9±0.20
May	32.0±1.00	17.7±0.07	46±1.00	0.5±0.10
June	33.9±1.30	20.1±2.10	49±2.00	0.1±0.00
July	34.7±0.07	22.0±1.50	58±±1.00	0±0.00
August	34.2±0.20	22.1±0.10	61±2.00	0±0.00
September	32.6±1.94	20.5±0.60	60±2.00	0±0.00
October	29.2±0.20	17.4±0.40	60±0.00	0.5±0.10

underground parts were washed and weighed, and then recorded weights were used to calculate the average dry weight of underground parts per square meter (g/m^2). Dry weight of the clippings and underground parts were assessed by allowing them to dry at 70°C until their weight was consistent.

Chemical Analysis. At the end of the growing seasons total chlorophylls (a + b) (mg/g fresh weight) in fresh clipping were determined according to the method of Lichtenthaler and Buschmann (2005). In accordance with Dubois *et al*. (1956), the total amount of carbohydrates in the clipping (as a percentage of dry matter) was determined. Nitrogen, phosphorus and potassium content in clipping were determined according to Estefan *et al*. (2013). Nitrogen (%) was determined by using the micro-Kjeldahl method. Phosphorus (%) was determined calorimetrically by using the chlorostannous molybdophosphoric blue colour method in sulphuric acid. Potassium (%) was determined by using the flame photometer apparatus (CORNING M 410, Germany). A method developed by Bates *et al*. (1973) was used to determine the proline content (µ moles/g fresh matter of clipping). According to Selim *et al*. (1978), the total phenol content of three grams of fresh clipping was measured after it was crushed and extracted with 80% ethanol at 0°C for seventy-two hours, with the ethanol being replaced every twenty-four hours. Antioxidant enzyme extraction were carried out using clipping at 40°C in a buffer solution (3: 1 buffer: fresh weight v/v) in a pastel. It was mortared with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 *g* for 30 min and then the supernatants were stored in separate aliquots at 8°C. Antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) were assayed as described by Haida and Hakiman, (2019). Activities of the enzymes were reported in units/min/mg protein.

Statistical analysis

Statistical analysis of variance (ANOVA) was performed on the mean of all collected data using a split plot design. Combined analysis was done on both growing seasons together. Snedecor and Cochran (1989) Duncan's multiple range tests at the 5% level was used to compare the data means.

3. Results and Discussion

Growth traits

Data presented on Table 3 showed that the tested growth traits (sward height, lawn density, dry weights of clippings and underground parts) were significantly affected by irrigation frequency, bio-stimulators treatment and their interaction. Data in Table 4 indicted that within each level of HA or CHT, in most instances, extending the intervals between irrigations daily from 2 to 5, 8 or 11 days resulted in gradual significant decrease in all evaluated growth traits compared to the brief interval (2 days). The detrimental effects of drought surrounding the underground parts, reduced soil moisture availability from water stress, and decreased root absorption of water and nutrients can all contribute to the

Table 3 - Mean square for the impact of irrigation frequency and bio-stimulators treatments and their interaction on vegetative growth traits of *Paspalum vaginatum*

Parameters	Source of variation						
	Treatment			Error		CV	
	Irrigation frequency (A)	Bio-stimulators (B)	(A × B)	(A)	(B)	(A)	(B)
Sward height (cm)	28.04 **	4.74 **	23.31 *	1.91	2.83	13.2	16.09
Lawn density (number of tillers/100 cm^2)	6.59 *	0.75 *	4.30 *	-	-	13.26	13.53
Root length (cm)	10.05 **	9.10 **	0.29 *	0.90	0.66	16.78	14.41
Dry weight of clippings (g/m^2/2 weeks)	159.62 **	41.27 *	101.93 *	4.75	22.56	7.29	15.89
Dry weight of underground parts (g/m^2/2 weeks)	1594.67 **	80.00 *	648.78 *	54.18	88.94	8.73	11.18

*, **, *** significant at P ≤ 0.05, P≤0.01, P≤0.001, respectively.

Table 4 - Sward height, Lawn density and dry weight of clippings and underground parts of *Paspalum vaginatum* as affected by the interactions between irrigation frequency and bio-stimulators treatments (mean of two seasons)

Irrigation frequency	Bio-stimulators	Sward height (cm)	Lawn density (number of tillers/100 cm^2)	Root length (cm)	Dry weight of clippings (g/m^2/2 weeks)	Dry weight of underground parts (g/m^2/2 weeks)
2 days	Control	8.28±0.46 e-g	204.23±8.75 a-d	5.04±1.01 e-g	25.85±0.98 f-h	81.33±3.78 d-f
	HA (1)	14.80±0.29 ab	207.57±10.82 a-d	7.03±0.1 a-c	36.08±4.29 a-d	96.90±8.64 bc
	HA (2)	16.05±0.66 a	220.90±14 a	8.11±0.53 a	40.82±1.1 a	114.30±2.69 a
	CHT (1)	10.52±1.72 de	212.72±19.37 a-c	7.03±0.49 a-c	36.90±2.64 a-c	92.70±5.3 b-e
	CHT (2)	13.55±1.12 a-c	208.92±12.2 a-d	6.50±0.25 b-d	34.62±0.66 a-e	100.5±4.71 ab
5 days	Control	8.10±0.74 e-g	161.03±11.74 e-g	4.44±0.7 fg	24.24±1.44 g-i	65.40±2.49 gh
	HA (1)	11.78±1.36 cd	204.92±24.87 a-d	5.47±0.95 d-f	29.99±3.03 c-h	91.57±1.36 b-e
	HA (2)	12.25±0.82 b-d	217.78±4.01 ab	7.16±0.47 ab	39.18±2.83 ab	100.07±7.89 ab
	CHT (1)	8.50±1.44 e-g	187.83±15.48 a-f	5.43±0.36 d-f	31.73±0.78 c-f	82.40±6.75 c-f
	CHT (2)	11.82±0.82 cd	202.23±11.48 a-e	5.44±0.33 d-f	32.01±3.36 b-f	89.60±2.13 b-e
8 days	Control	7.10±0.29 fg	136.70±2.2 gh	3.95±0.79 g	23.13±2.2 hi	59.70±4.24 hi
	HA (1)	11.73±1.4 cd	199.72±16.75 a-e	5.72±1.2 c-f	31.49±4.9 c-g	85.73±10.61 b-f
	HA (2)	11.78±1.26 cd	203.60±9.99 a-d	6.16±0.93 b-e	31.46±4.03 c-g	94.27±1.82 b-d
	CHT (1)	8.15±0.63 e-g	178.75±19.01 b-f	5.01±0.22 e-g	28.68±1.07 e-h	79.07±2.44 e-g
	CHT (2)	10.68±0.35 de	196.75±19.56 a-e	5.36±0.19 d-f	29.52±1.15 d-h	86.13±2.74 b-f
11 days	Control	6.85±0.47 g	112.00±3.45 h	3.68±0.87 g	18.49±1.17 i	47.83±3.13 i
	HA (1)	9.70±0.52 d-f	171.48±23.3 c-g	4.85±0.35 e-g	25.28±2.25 f-i	83.27±7.76 c-f
	HA (2)	11.38±0.97 cd	182.30±10.45 a-f	6.00±0.18 b-e	25.98±3.29 f-h	83.93±4.13 c-f
	CHT (1)	7.53±0.97 fg	153.10±8.88 f-h	4.93±0.22 e-g	24.70±1.66 f-i	78.47±2.28 e-g
	CHT (2)	8.48±0.64 e-g	169.95±19.24 d-g	5.41±0.27 d-f	27.72±0.88 e-h	74.13±6.07 f-h

HA (1) = Humic acid at 300 ppm, HA (2) = Humic acid at 600 ppm, CHT (1) = Chitosan at 300 ppm, CHT (2) = Chitosan at 600 ppm. The mean ± standard error of three replicates is represented by each value. Using the Duncan multiple range test, means in a column with various letters show a significant difference for each variable at the 5% level.

lowering of growth parameters in response to water scarcity, which ultimately results in a decrease in vegetative biomass (Rouphael *et al*., 2012). The results of reducing growth parameters of *paspalum vaginatum* owing to water deficient are analogy with those obtained by previous authors (Cui *et al*., 2020; Katuwal *et al*., 2021; Errickson *et al*., 2023; Taleb *et al*., 2023; Porcelli *et al*., 2024; Hejl *et al*., 2024).

Data in Table 4 also showed that under each time between irrigations, in most cases spraying the plants with any dosage of HA or CHT caused a significant increase ($p<0.05$) in the tested growth traits in contrast to control plants (water-stressed pants without any HA or CHT treatments). The data also disclosed that in general with any one of the two tested bio-stimulators (HA or CHT), increasing the application rate resulted in steady increase in the recorded mean values compared to the control. Under the same HA or CHT level, HA was more effective for increasing studied growth parameters than CHT especially the highest concentration (600 ppm) since recorded the highest values in most cases. The results of boosting growth traits owing to CHT treatment are in sequence with the findings of prior reports (Dzung *et al*., 2011; Li *et al*., 2022; Cheng *et al*., 2024). While increasing the growth parameters due to HA treatments are in the same line of the findings of other researches (Shahin *et al*., 2015; El-Sayed *et al*., 2017; Abdou *et al*., 2020; Badran *et al*., 2023; Taher *et al*., 2023). In this regard El-Sayed *et al*. (2016) mentioned that application of HA has a beneficial impact on growth attributes of seashore paspalum turfgrass subjected to drought stress. The beneficial impact of HA on growth parameters under drought stress is likely due to its capacity to increase soil water retention, boost nutrient availability, and support the plant's physiological response to stress. Under of water deficient stress, HA has the ability to improve soil structure and water-holding capacity that makes it an essential soil conditioner. It also improves uptake of nutrients, giving plants the tools they need to survive adversity. Moreover, protect plants from oxidative damage brought on by drought, HA can increase antioxidant activity (Chen *et al*., 2022). The useful effect of CHT on growth parameters may be attributed to an augmentation in the availability, water uptake and vital nutrients by modifying the cellular osmotic pressure and reducing the free radical accumulation by increasing the antioxidant enzymes activity (Pirbalouti *et al*., 2017).

Chemical constituents

Total Chlorophylls and carbohydrates contents. The data shown in Table 5 visualized that total chlorophylls and carbohydrates contents were significantly impacted by watering intervals, bio-stimulators treatment and interaction effects. Data in Table 6 emphasized that within each level of HA or CHT, the recorded mean values were decreased in parallel with prolonged watering intervals from 2 to 5, 8 or 11 days. The reductions in tested components as a result of water scarcity stress are in harmony with the finding of earlier reports (Chai et *al*., 2010; Shahidi *et al*., 2017; Sharaf El-Din *et al*., 2017; Sheikh Mohammadi *et al*., 2017; Wang *et al*., 2017; Gholamian *et al*., 2019; Fariaszewska *et al*., 2020; Katuwal *et al*., 2021; Shen *et al*., 2024). Reducing chlorophylls and photosynthetic activity may indirectly resulted a decrease in the amount of carbohydrates. The decrease in total chlorophylls caused by water deficiency may be linked to increased production of reactive oxygen species, which causes oxidative stress, damage to chloroplast structure, and chlorophyll losses. Additionally, low water helps abscisic acid for stomatal closure, which could lead to a decrease in net photosynthesis and the accumulation of carbohydrates (Baccari *et al*., 2020).

The data in the same table elucidated that, within each irrigation frequency, the plants sprayed with any concentration of HA or CHT had significantly higher values of total chlorophylls and carbohydrates content than those of the control. Generally, increasing the application rate of any of HA or CHT caused steady increase in the recorded mean values compared to control, with superiority of HA especially the highest on (600 ppm) which registered the highest mean values of tested components. These results are similar to those obtained by earlier studies which reported that application of CHT caused increase in total chlorophylls or carbohydrates content (Dzung *et al*., 2011; Li *et al*., 2022; Cheng *et al*., 2024). Whereas, the noticeable

Table 5 - Mean square for the impact of irrigation frequency and bio-stimulators treatments and their interaction on some chemical constituents of *Paspalum vaginatum*

Traits	Source of variation						
	Treatment			Error		CV	
	Irrigation frequency (A)	Bio-stimulators (B)	(A × B)	(A)	(B)	(A)	(B)
Total chlorophylls content (SPAD)	37.930 **	39.226 **	0.418 *	0.672	0.328	2.32	1.77
Total carbohydrates (% DW)	19.123 **	23.345 **	0.442 *	0.117	0.298	3.94	8.02
N (% DW)	0.911 **	0.632 **	0.005 *	0.002	0.005	1.91	2.61
P (% DW)	0.012 **	0.009 **	0.002 *	0.001	0.002	2.23	3.01
K (% DW)	0.003 *	0.005 **	0.002 *	0.001	0.002	7.30	10.13
Proline (µ moles/g fresh matter)	24.53 ***	9.88 **	0.92 **	0.11	0.14	7.60	9.33
Total Phenols (mg/100 g DW)	0.817 *	1.664 **	0.128 *	0.147	0.207	8.47	11.44
CAT (units mg protien) in clippings	6.275 *	1.122 **	2.752 **	0.093	0.044	8.00	5.22
SOD (units mg-1 protein) in clippings	4.275 *	1.003 **	1.912 **	0.068	0.033	7.00	3.52
Ascorbate peroxidase (APX)	2.994 **	0.437 **	1.032 *	0.036	0.018	3286	1833

*, **, *** significant at P ≤ 0.05, P≤0.01, P≤0.001, respectively.

increase in tested components due to HA treatments are in harmony with previous studies (Shahin *et al*., 2015; El-Sayed *et al*., 2017; Taher *et al*., 2023). Additionally El-Sayed *et al*. (2016) mentioned that application of HA has a valuable effect on the tested components of seashore paspalum turfgrass subjected to drought stress. The valuable effect HA on increasing total chlorophyll and carbohydrate content is likely due to its ability to improve nutrient uptake and transport, leading to enhanced photosynthetic efficiency. Furthermore, HA can impact enzymatic activity, decrease pH, and stimulate the biosynthesis of essential compounds such as chlorophyll and carbohydrates (Shaabani *et al*., 2022).

N, P and K (% of dry matter). It is evident from data in Table 6 that three nutrients (N, P and K %) were significantly impacted by interval between irrigations, bio-stimulators treatment and interaction effects. The data in Table 6 showed that within each level of the two tested HA or CHT, in most instances extending interval between irrigations from 2 to 5, 8 or 11 days resulted in reduction in N, P and K % compared to the brief intervals (2 days). The present reduction in the tested nutrients owing to stress caused by a drought stress has been reported by earlier studies (Shen *et al*., 2024).

The negative impact of drought stress on absorption and the three nutrients' buildup in plants could be caused by prolonging the irrigation intervals resulted in low soil moisture content, which impacts the elements' solubility and plant absorption capacity, hence reducing their accumulation in plant tissues. Furthermore, the roots absorb fewer nutrients and accumulate them in clippings as a result of reduced transpiration rates, compromised active transport, and poor membrane permeability (Filipović, 2021).

The data in the same table also elucidated that inside each irrigation frequency, spraying the plants with any concentrations of HA or CHT resulted in higher values than those of control plants. In most

Table 6 - Total chlorophylls, total carbohydrates, N, P, and K% as influenced by the interactions between irrigation frequency and bio-stimulators treatments (mean of two seasons)

Irrigation frequency	Bio-stimulators	Total chlorophylls content (mg/g FW)	Total carbohydrates (% DW)	N (% DW)	P (% DW)	K (% DW)
2 days	Control	1.85±0.14 e-h	17.93±3 d-f	1.71±0.05 a-d	0.17±0.01 e-h	1.69±0.09 a-d
	HA (1)	2.66±0.04 ab	22.88±1.4 a-c	2.02±0.21 ab	0.21±0.01 b-d	1.95±0.04 ab
	HA (2)	2.84±0.18 a	24.18±1.2 a	2.14±0.08 a	0.27±0.02 a	2.07±0.27 a
	CHT (1)	2.44±0.10 bc	23.60±1.3 ab	1.97±0.02 a-c	0.20±0.01 b-e	1.92±0.02 ab
	CHT (2)	2.35±0.12 b-d	23.45±0.2 ab	2.0±0.32 a-c	0.20±0.02 b-d	1.98±0.16 ab
5 days	Control	1.62±0.03 hi	16.70±1.7 e-g	1.29±0.16 d-f	0.16±0.01 f-h	1.42±0.15 c-e
	HA (1)	2.23±0.06 cd	20.76±0.3 a-d	1.73±0.17 a-d	0.19±0.02 b-f	1.71±0.07 a-d
	HA (2)	2.33±0.14 b-d	21.93±0.6 a-d	1.71±0.21 a-d	0.22±0.03 b	1.79±0.12 a-c
	CHT (1)	2.01±0.27 d-g	19.52±2 b-f	1.68±0.05 a-e	0.17±0.01 d-h	1.66±0.21 a-d
	CHT (2)	2.16±0.14 c-f	19.18±0.7 c-f	1.82±0.16 a-c	0.18±0.02 d-g	1.7±0.17 a-d
8 days	Control	1.45±0.04 ij	16.24±1.8 fg	1.19±0.27 ef	0.14±0.01 h	1.32±0.07 de
	HA (1)	2.11±0.13 c-g	20.69±0.2 a-e	1.69±0.1 a-e	0.16±0.01 f-h	1.66±0.1 a-d
	HA (2)	2.20±0.06 c-e	21.35±0.6 a-d	1.64±0.29 b-e	0.21±0.02 bc	1.73±0.01 a-d
	CHT (1)	1.84±0.15 f-h	18.81±2 c-f	1.59±0.23 b-e	0.16±0.01 e-h	1.60±0.04 b-d
	CHT (2)	2.07±0.06 d-g	18.55±1.9 d-f	1.72±0.31 a-d	0.16±0.02 e-h	1.66±0.25 a-d
11 days	Control	1.17±0.09 j	12.92±1.6 g	1.09±0.14 f	0.08±0.03 i	1.16±0.1 e
	HA (1)	2.01±0.04 d-g	18.61±2.8 d-f	1.50±0.18 c-f	0.16±0.01 e-h	1.65±0.17 a-d
	HA (2)	2.04±0.12 d-g	19.85±2.4 b-f	1.60±0.26 b-e	0.20±0.02 b-e	1.62±0.19 b-d
	CHT (1)	1.63±0.17 hi	18.92±0.4 c-f	1.58±0.24 b-f	0.15±0.01 gh	1.68±0.17 a-d
	CHT (2)	1.80±0.14 g-i	19.57±0.7 b-f	1.77±0.22 a-d	0.18±0.02 c-g	1.70±0.18 a-d

HA (1) = Humic acid at 300 ppm, HA (2) = Humic acid at 600 ppm, CHT (1) = Chitosan at 300 ppm, CHT (2) = Chitosan at 600 ppm. The mean ± standard error of three replicates is represented by each value. Using the Duncan multiple range test, means in a column with various letters show a significant difference for each variable at the 5% level.

cases, HA was preferable in its effect than CHT. The obtained increases in N, P and K % owing to CHT treatments are similar to those obtained by prior researche (Abou dahab *et al*., 2023) while, the increase as a result of HA application are in accordance with the results of earlier workers (Shahin *et al*., 2015; Taher *et al*., 2023). HA can mitigate unfavorable impacts of drought stress on plants, specifically by rising the availability and uptake of essential nutrients like N, P and K. This is accomplished by a number of methods, such as better soil structure, better water retention, and more accessible nutrients (El-Damarawy *et al*., 2025).

Proline and phenols content. Results in figure 1 revealed that within each level of the two tested HA or CHT, proline and phenols content were increased concurrently with rising irrigation intervals from 2 to 5, 8 or 11 days. In plants exposed to various stressors, the amino acid proline is quite helpful. Proline serves three primary functions during stress: as a metal chelator, an antioxidant defense molecule, and a signaling molecule, in addition to being a great osmolyte (Hayat *et al*., 2012). Additionally, In order to defend against oxidative damage brought on by reactive oxygen species (ROS), plants adapt to water-deficient stress like drought by raising their phenolic content (Albergaria *et al*., 2020).The results of increasing proline or phenols content due to drought stress are concordant with those obtained by previous studies (Bandurska and Jozwiak, 2010; Salehi *et al*., 2014; Marimuthu and Murali, 2018; Gholamian *et al*., 2019; Fariaszewska *et al*., 2020; Katuwal *et al*., 2020).

Results in figure 1 also showed that within each irrigation frequency, in most instances the plants sprayed with any concentrations of HA or CHT had notably greater values of proline or phenols than those of control plants. At the same level as the two bio stimulators that were tested, HA was more effective than CHT. The present increases in proline or phenols content owing to CHT treatments are in line with those findings of prior author (Cheng *et al*., 2024). Moreover earlier report (El-Sayed *et al*., 2016) stated the valuable effect of HA on enhancing phenols content of seashore paspalum subjected to drought stress. HA has the ability to enhance the plant's defense mechanisms against stress. Particularly, HA can enhance the expression of nitrogen metabolism and proline metabolism genes, and also improve the plant's ability to uptake vital nutrients like iron and phosphorus, this improved nutritional status, thus lead to an increase in proline, which acts as an osmoprotectant and antioxidant under stress conditions (Bakry *et al*., 2014.)

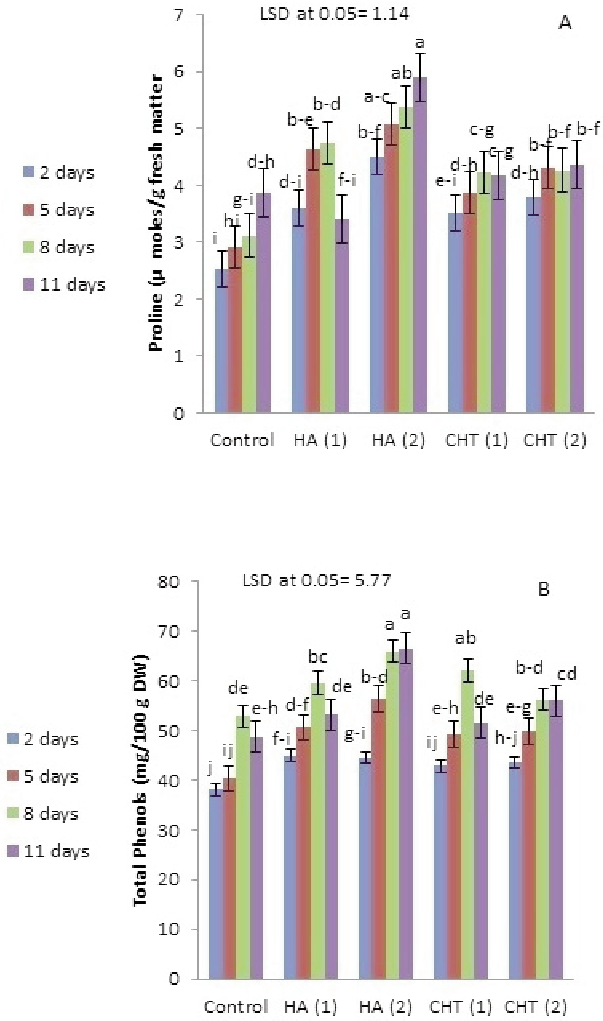

Fig. 1 - Proline content (A) and total phenols (B) as influenced by the interactions between irrigation frequency and bio-stimulators treatments (mean of two seasons). HA (1) = Humic acid at 300 ppm, HA (2) = Humic acid at 600 ppm, CHT (1) = chitosan at 300 ppm, CHT (2) = chitosan at 600 ppm. Column with different letters indicate a significant difference at 5% level. Three replicates' standard error (SE) is shown by vertical bars.

Enzyme activity. It is clear from data in figure 2 that inside each of the two levels of HA or CHT, CAT, APX and SOD content were enhanced steadily with extending irrigation intervals from 2 to 5, 8 or 11 days. Although, previous report (Shahidi *et al*., 2017) indicated decrease in the activities of CAT and SOD due to drought stress. The current results are consistent with those of (Bian and Jiang, 2009; Bandurska and Jozwiak, 2010; Salehi *et al*., 2014; Gholamian *et al*., 2019; Katuwal *et al*., 2020; Shen *et al*., 2024) who reported increase in CAT, APX or SOD due to water deficient stress. Under drought stress, raising the activity of antioxidant enzymes like CAT, SOD, and APX is a plant's defense mechanism to resist oxidative stress induced by the accumulation of reactive oxygen species (ROS). These enzymes function by scavenging ROS and stopping them from causing harm to the cellular constituents of the plant (Laxa *et al*., 2019).

Results in figure 2 also indicated that at each irrigation frequency, in most cases application of any concentrations of HA or CHT resulted in significant increase in CAT, APX and SOD compared to control plants (water-stressed pants without any biostimulator treatments). Under the same HA or CHT level, HA was Superior in its effect than CHT. Such results are in conformity with the findings of previous workers that mentioned increase in CAT, APX and SOD due to either CHT (Zhao *et al*., 2019; Liu *et al*., 2020; Almeida *et al*., 2020) or HA treatments (Ozfidan-Konakci *et al*., 2018). Enhanced nutrient uptake, better soil qualities, and a direct effect on plant metabolism are some of the possible causes of the increase in enzyme activity in plants under drought stress, especially when humic acid (HA) therapy is applied. HA may also alleviate the harm caused by reactive oxygen species (ROS) by triggering the plant's enzymatic defense mechanism against stress (Ozfidan-Konakci *et al*., 2018).

4. Conclusions

Drought stress had an unfavorable impact on growth traits, total Chlorophylls, carbohydrates and nutrient uptake while, increased proline, phenols content and Enzyme activity (CAT, APX and SOD). Foliar application of HA or CHT at higher concentrations increased growth traits, total chlorophylls, total carbohydrates, nutrient uptake, proline, phenols and enzyme activity. HA was generally superior in its effect than CH. Based on the outcome of the present research it can be inferred that, application of HA at 600 ppm can ameliorates the harmful impacts of drought stress on *Paspalum vaginatum*.

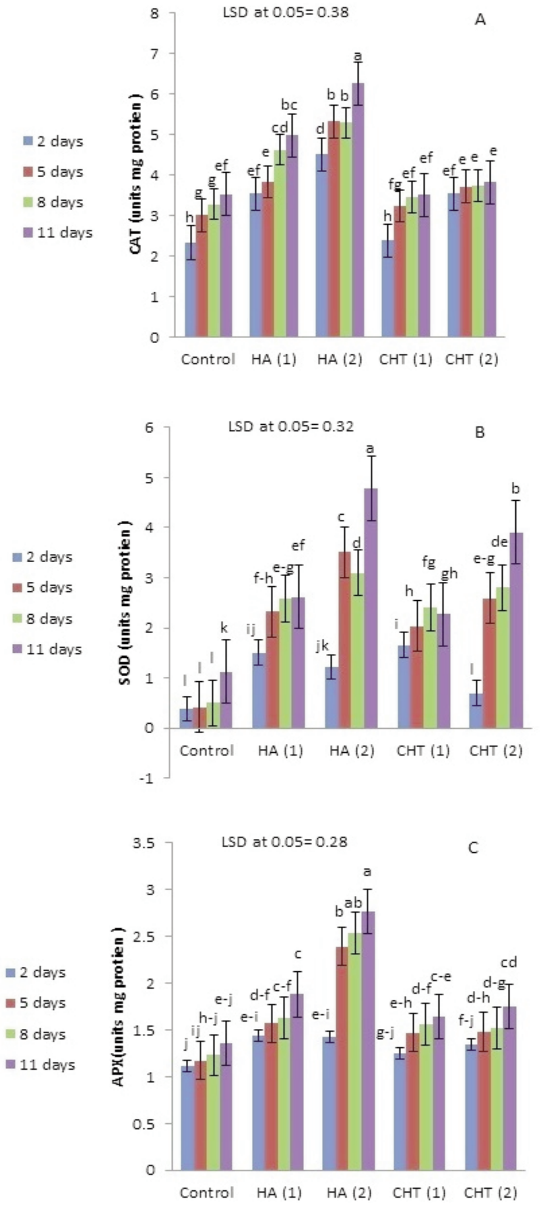

Fig. 2 - CAT (A), SOD (B) and APX (units mg protein) as influenced by the interactions between irrigation frequency and bio-stimulators treatments (mean of two seasons). HA (1) = Humic acid at 300 ppm, HA (2) = Humic acid at 600 ppm, CHT (1) = chitosan at 300 ppm, CHT (2) = chitosan at 600 ppm. Column with different letters indicate a significant difference at 5% level. Three replicates' standard error (SE) is shown by vertical bars.

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