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RESEARCH ARTICLE

Assessing the effects of some bio-stimulants on soil properties, physiological, and biochemical responses of cowpea plants under water deficit conditions

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Abstract

Humic acid (HA) and potassium silicate (PS) are bio-stimulants that can improve cowpea growth and yield under water deficit conditions. This study aimed to investigate the mechanism of using 500 mg L^{-1} HA and 500 mg L^{-1} PS in improving the *Vigna unguiculata*, cvs. Sudany and Dokii 331 growth and yield under water deficit conditions. The experiment was conducted in the split-split plot with three replications. during two successive growing seasons and irrigation intervals, treatments (5, 10 and 15 days (100, 80 and 50% irrigation requirements) were assigned to the main plots, cowpea genotypes (Sudany and Dokii331) were assigned to the sub-plots, and bio-stimulants (Control (distilled water), HA and PS at 500 mg L^{-1}) were assigned to the sub- subplot. In this study, the application of 500 mg L^{-1} HA and 500 mg L^{-1} PS significantly increased plant height, root length, leaf area, number of branches, fresh and dry weights per plant, pod length, number of pods per plant, weight of 100 seeds, and seed yield per plant, especially in the Sudany cultivar. PS also increased electrode leakage (EL) and relative water content (RWC), increasing plant water stress tolerance. HA improved soil properties, including organic matter, total N, available P and K, and dry matter, decreasing calcium carbonate content, salinity, and pH. Overall, the study found that PS and HA can improve cowpea growth and yield under water deficit conditions. Still, PS is more effective for the Sudany cultivar and HA is more effective for soil improvement.

Keywords Cowpea genotypes; Drought; Electrode leakage; Potassium humate, Potassium silicate; Soil characteristics

Introduction

The sustainability of plant production and global food security face a significant threat from both biotic and abiotic stresses, particularly considering current climate changes. Among the abiotic stresses, drought stress is receiving increased attention as it negatively affects plant growth and development, leading to a substantial reduction in plant biomass and production. This, in turn, contributes to global food insecurity (Seleiman et al. 2021). Cowpea is a significant legume crop in Egypt known for its drought resistance and high nutritional value for forage. It is also a crucial crop in newly cultivated lands. However, its productivity is largely restricted due to water scarcity and inadequate soil fertility (El-Sobky and Hassan 2021). Crop growth and productivity in arid and semi-arid regions are negatively impacted by drought. As the demand for irrigation water increases, alternative sources are being explored. While seawater salinity was once deemed unsuitable for irrigation, it can now be utilized to cultivate crops given specific circumstances (Sadak et al. 2015). Reducing the amount of irrigation water supplied to plants hurts numerous physiological processes, including leaf water potential, photosynthesis activity, and nutrient absorption and translocation, ultimately leading to a decline in plant growth (Sivakumar and Shaw 1998). Water stress has been shown to

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decrease leaf area, shoot dry weight, and the number of leaves per plant (Turk and Hall 2005). Additionally, although it may increase the protein percentage of grains (Wien et al. 1979). It can reduce seed yield, the number of seeds per pod, and the weight of 1000 grains in cowpea plants exposed to irrigation cut during flowering, pod formation, and seed filling stages (Rezaee and Haghighi 2009). Choudhury et al. (2011) reported a decrease in the 1000-seed weight of various bean genotypes under drought stress, which may be attributed to the decrease in seed filling. Furthermore, increasing the irrigation interval can also lead to a reduction in seed yield due to a decrease in the number of pods per plant and 100 dry seed weight (Khedri and Mojaddam, 2014).

The sustainability of the soil ecosystem faces additional challenges due to the persistent and detrimental effects of drought stress (Geng et al. 2014). Drought stress can harm soil properties by significantly reducing saturated hydraulic conductivity, total porosity, and concentrations of total soil organic carbon and N (Bodner et al. 2013; Zhang et al. 2019). The decrease in aboveground biomass during drought stress is attributed to the reduction in porosity, leading to compaction and fewer pore spaces, resulting in the reduction of soil organic matter and soil structure (Wu et al. 2016). Drought is considered one of the worst environmental factors that affect plant productivity. Water constitutes a significant portion of the fresh biomass of the plant body, and it is essential for many physiological processes, including growth, development, and metabolism (Abbasi et al. 2010; Brodersen 2019). Drought is regarded as the primary environmental stressor for various plants, especially in drought-prone areas, and is considered the single greatest threat to future global food security and the cause of significant past famines (Anjum et al. 2011; Okorie et al. 2019; Okorie et al. 2020). In recent years, humic acid-based products have been included in crop cultivation to improve soil properties, plant growth, and other agronomic aspects. Humic acids can improve the biological, chemical, and physical characteristics of the soil, including its ability to store water, cation exchange capacity, pH, carbon content, enzyme activity, and nutrient cycling and availability (Ampong et al. 2022; Bhatt and Singh 2022). The hydrophilic properties of humic acids draw hydrogen ions, increasing the soil's ability to store water and enhance its structural integrity (Fahramand et al. 2014).

Silicon applications have demonstrated an increase in drought tolerance in rice (Chen et al. 2011; Ming et al. 2012; Geng et al. 2018), sorghum (Hattori et al. 2005; Ahmed et al. 2014), wheat (Gong et al. 2005; Ma et al. 2016; Ahmed et al. 2016), tomato (Elsadek et al. 2019), and soybean (Shen et al. 2010), despite Si not being considered an essential plant element (Epstein 2009). The beneficial effects of Si fertilization under water-restricted conditions include the maintenance of high relative water content (Kaya et al. 2006), increased water potential through osmotic adjustment (Gong et al. 2005; Shen et al. 2010; Chen et al. 2011; Ming et al. 2012), decreased electrolyte leakage (Agarie et al. 1998; Shen et al. 2010), and modification of proline levels. Additionally, Si may improve the antioxidant defense system against drought conditions. This research aimed to investigate whether the application of HA and PS improves soil properties, and physiological and biochemical responses to water deficit stress at various growth stages in two cowpea cultivars, over 21 days from planting to harvesting**.**

Materials and Methods

Site, Plant Materials and Experimental procedure

The study was conducted at the Agricultural Experimental Farm, Faculty of Agriculture, South Valley University, Qena Governorate, Egypt. The location is (latitude 26° 11' 22.2" N and longitude 32° 44' 25.5" E), and it sits 81 meters above sea level. The experiment was carried out over two consecutive summer seasons, in 2019 and 2020. The physical and chemical properties (Table 1) of the experimental soil (prior to the start of the experiment) were assessed as Jackson (1973). Cowpea cultivars were chosen according to their drought tolerance (Sudany cv), and sensitivity (Dokki 331 cv), as previously reported by El-Shaieny (2017). Seeds were planted on 15th March 2019 and 2020. The experimental design was split-split plot with three replicates.

The main plots were allocated for 3 irrigation intervals treatments (5 days (normal irrigation (NI)), 10 days (Medium stress (MS)) and 15 days severe stress (SS)); two cowpea cultivars (Dokki 331 and Sudany) were arranged in subplots and 3 bio-stimulators treatments were distributed at random in sub–sub plots to obtain 18 treatments. The irrigation treatments in the main plot were separated by five meters to avoid horizontal soil water movement. The sub-plot rows were 3 meters long, with 60 cm between rows and 20 cm between plants. The different water-stress regimes were applied 3 weeks after plants emerged, however, the control plants were irrigated every 5 days as well as the medium-stress was irrigated every 10 days and severe-stress plants were irrigated every 15 days. The furrows were small, parallel channels that were used to carry water to the plants for surface irrigation. Both foliar spraying of (PS) and soil application of (HA) were carried out at 500 mg L-1 and distilled water in the control treatment. All other recommended agronomic practices were followed to obtain optimum yield.

Table 1 Physical and chemical properties of the soil used in this experiment.

Texture	Sand	Silt	Clay	pH (1:2.5)	EC (dS m^{-1})	Calcium Carbonate (%)	Organic Matter (%)	Total N (%)	Available P (mg/ kg)	Available K (mg /kg)
Sandy loam	72	21	7	8.19	0.75	9.98	0.74	0.015	4.41	114

Soil and Plant Analysis

Soil samples were collected from the experimental field at the beginning time (before planting) and at 70 days from planting, three soil samples (0-15 cm) were taken from each plot air-dried passed through a 2-mm sieve and kept for some physical and chemical properties analyses. Soil texture was determined using the micro-pipette method (Shirazi and Boersma 1984). The modified Walkley and Black method was used to determine the amount of soil organic carbon (USDA 1996). Total calcium carbonate ($CaCo_3$) was determined using a Schreiber calcimeter according to Jackson (1973). Soil pH was measured in 1:2.5 (soil: water) suspensions using a glass electrode (Jackson 1973). Total soluble salts were determined in the 1:5 soil-to-water extract by measuring the electrical conductivity (Jackson 1973). Using the micro Kjeldahl method, total N was determined (Black et al. 1965). The available phosphorus in soil was determined spectrophotometric using the Olsen method as described by Olsen (1954). The available potassium was determined by flame photometer method according to Jackson (1973). Additionally, plant samples were taken from each treatment after 70 days from cowpea seeds sowing, wet weight was determined, then samples were oven-dried at 70 °C for 72 hrs and recorded the plant dry weight. samples were digested with the acid mixture of sulphuric acid and hydrogen peroxide according to Lowther (1980), determine nitrogen, Phosphorus, and potassium content according to Jackson (1973).

Morphological Traits Measurements

The following measurements were done at harvest time for each treatment: plant height (cm), root length (cm), number of branches per plant, number of leaves per plant, fresh and dry weights (g), and. Leaf area (cm).

Yield and Quality Traits Measurements

At harvesting, five plants from each treatment were chosen to estimate pod length, (cm), number of pods per plant, 100-seeds weight (g) and seed yield per plant (kg).

Physiological Traits Measurements

The relative Amount of Chlorophyll Content was determined using chlorophyll Meter (Minolta SPAD- 502 meter, Tokyo, Japan). Fifteen readings per plant (five leaves per plant) were taken from the widest portion of the leaf lamina, to avoid major veins. The samples were selected with concerning the heterogeneities within each sample point. The selected leaves were clean, dry, green, and free of signs of disease or damage. Then the values were averaged as SPAD units. The water status of the plants was determined by measuring relative water content (RWC) according to Loutfy et al. (2012).

$$RWC= ((FW-DW)/(TW-DW)) \times 100$$

Where FW is the fresh weight, DW is the dried weight and TW is the turgid weight of tissue after being soaked in water for 12 h at room temperature. Cell membrane stability was measured by electrode leakage (EL) from plants using the method described by Soliman and El-Shaieny (2014). The sampled plants were cut into discs 2 mm in diameter. The discs were rinsed three times with distilled water and 10-15 discs were put in a test tube containing 6 ml distilled water. The test tubes were agitated on a shaker for about 1h and conductivity (C1) of the solution was measured with a conductivity meter (Cyberscan100; Iuchi, Tokyo, Japan). Seedling discs then were heated in an oven at 70 to 80°C for 1h, and the conductivity of the solution containing the dead tissue (C2) was measured after the tubes had cooled down to room temperature and had been agitated on a shaker for 1 h. The relative ion leakage was calculated as (C1/C2)×100.

Statistical Analysis

All obtained data were statistically analyzed with the technique of analysis of variance (ANOVA) by using SAS computer software package SAS 9.1 program software, (SAS 2004). Also, the Least Significant of Difference (LSD) method was used to test the differences between treatment means at a 5% level of probability as described by (Gomez and Gomez, 1984).

Results

Performance of cowpea cultivars under irrigation intervals

Data in Table 2 and Fig. 1 indicated that irrigation treatments had significant on all studied traits of cowpea in two seasons. It could be inferred that full water levels at irrigation intervals treatments 5 days (NI) produced the maximum values of all studied traits in both seasons with no significant differences among irrigation intervals treatments NI, MS and SS at both seasons for number branches per plant and relative chlorophyll content (SPAD) in the 1st and 2nd season, respectively. On the contrary, the lowest values of all vegetative and yield previously mentioned parameters were recorded under severe stress (SS) where, the decreases in total yield (average the two seasons) were about 10.9 and 7.2% due to irrigation with 80 % IR as well as 32.4 and 10.1% due to irrigation under (MS) than full irrigation (NI) in Dokki 331 and Sudany cvs, respectively. Severe drought stress (SS) caused a reduction in all studied plant growth characters ranged from 14.4 and 14.7% (number of branches per plant) to 42.5 (root length) and 45.1% (number of leaves per plant) in Sudany and Dokki 331 cvs, respectively.

Effect of cowpea cultivars:

Data in Table 2 indicate that the highest values of plant height (55.85 and 55.75), fresh weight g (316.48 and 316.37 g), dry weight g (71.7 and 72.22 g), leaf area (76.8 and 77.22 cm^2), pod length (12.92 and 13.17), 100-seed weight (15 and 15.1 g), seed yield per plant (42.81 and 42.39 g) and relative water content (80.81 and 80 77) were recorded in Dokki 331 cultivar, while the shortest of these traits was recorded in Sudany cultivar in the first and second seasons, respectively. Also, the highest chlorophyll content (77.98 SPAD) and root length (22.97 cm) were recorded in the Dokki 331 cultivar in 1^{st} and 2^{nd} seasons, respectively.

Table 2. Vegetative growth, physiological, yield traits of cowpea as affected by irrigation regime during two seasons.

		PH (cm)	RL (cm)	NB/P	NL/P	FW (g)	DW (g)	LA (cm)	EL	PL (cm)	No. P	100-SW (g)	SY/P (g)	Ch (Spad)	RWC
1st season															
Dokki	NI	65.8	20.3	3.2	20.0	369.5	81.7	85.5	26.3	14.2	51.2	16.2	50.1	79.7	84.9
	MS	55.8	17.8	2.8	16.0	324.7	71.8	78.2	16.3	12.7	47.5	14.7	44.4	76.8	82.8
	SS	46.0	11.4	2.7	10.9	255.3	61.7	66.7	11.8	12.0	38.7	14.1	34.0	77.4	74.8
	mean	55.9	16.5	2.9	15.6	316.5	71.7	76.8	18.1	12.9	45.8	15.0	42.8	78.0	80.8
Sudany	NI	57.9	24.7	4.4	33.8	196.4	54.1	57.0	34.7	11.0	57.6	11.7	44.0	82.0	82.6
	MS	51.5	20.8	4.1	31.2	183.8	48.8	52.5	27.0	10.6	52.3	11.4	40.8	67.6	81.2
	SS	36.0	14.5	3.8	25.9	166.3	41.3	40.8	14.2	10.5	44.2	10.5	39.5	69.1	76.8
	Mean	48.4	20.0	4.1	30.3	182.2	48.1	50.1	25.3	10.7	51.4	11.2	41.4	72.9	80.2
2nd season															
Dokki	NI	64.3	23.2	3.2	20.7	375.0	81.6	85.9	26.0	14.7	54.9	16.3	49.5	71.7	84.7
	MS	56.4	22.9	2.9	16.2	322.4	73.0	78.4	17.7	12.7	47.8	14.8	44.3	75.0	82.8
	SS	46.6	22.8	2.8	11.5	251.7	62.1	67.3	12.2	12.1	38.6	14.2	33.3	65.3	74.7
	Mean	55.7	23.0	3.0	16.1	316.4	72.2	77.2	18.6	13.2	47.1	15.1	42.4	70.7	80.8
Sudany	NI	56.2	24.5	4.5	34.3	199.8	54.7	57.7	35.5	11.4	57.7	11.7	44.3	80.7	81.6
	MS	51.9	24.1	4.1	32.0	183.0	50.0	52.7	27.5	10.9	52.3	11.6	41.2	75.0	82.2
	SS	36.6	13.8	3.8	26.0	167.7	42.0	41.3	14.5	10.6	44.2	10.6	39.9	67.2	76.4
	mean	48.2	20.8	4.1	30.8	183.5	48.9	50.6	25.8	11.0	51.4	11.3	41.8	74.3	80.1

PH, Plant height, RL, Root length, NB/P, Number of branches per plant, NL, Number of leaves, FW, Fresh weight, DW, Dry weight, EL, Electrode leakage, PL, Pod length, No. P, Number of pods per plant, 100-SW, 100 seeds weight, SY/P, Seed yield per plant, CH, Chlorophyll, RWC, Relative water content.

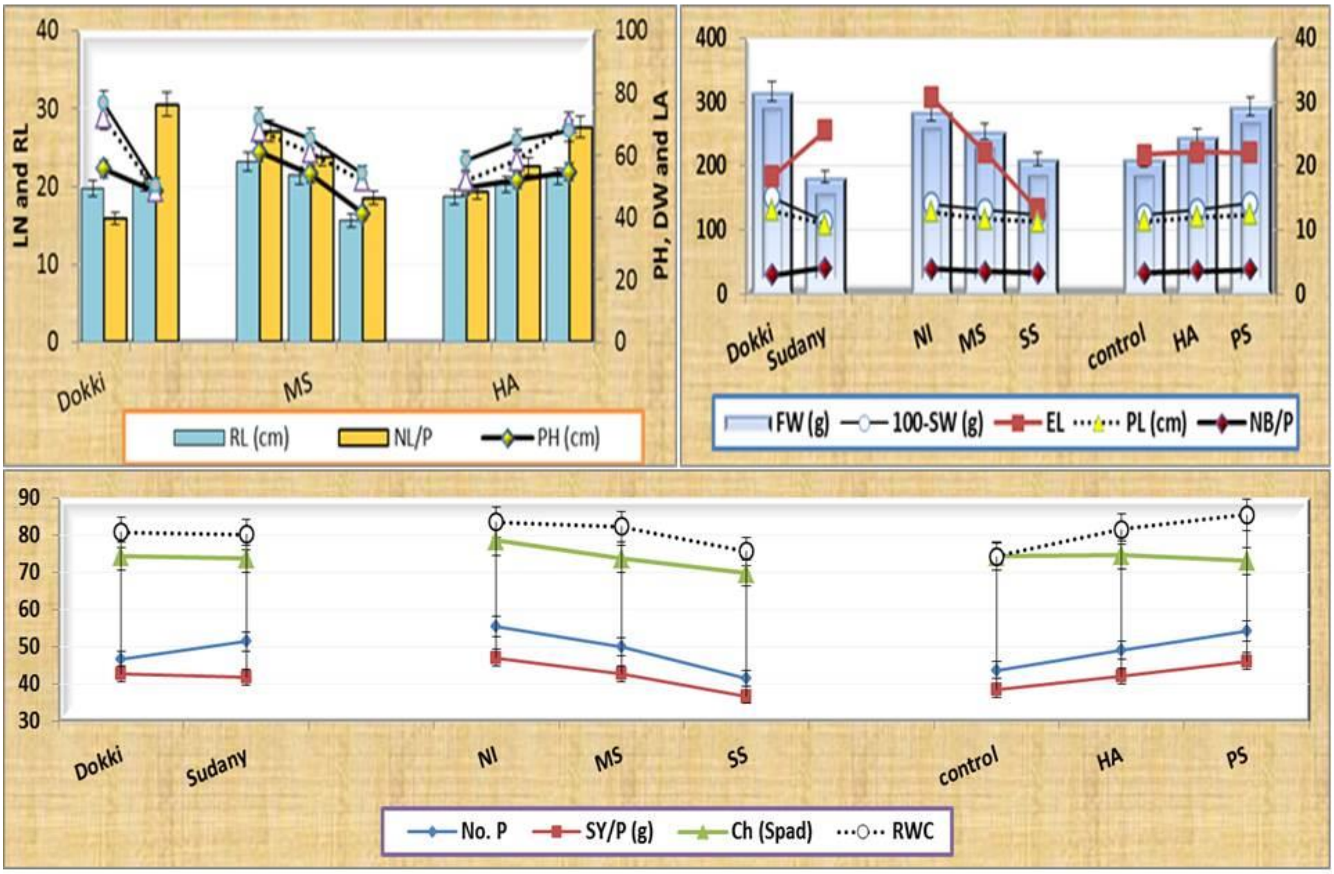

Fig.1 Vegetative growth, yield traits of cowpea as affected by genotypes, irrigation regime and stimulators individually (average two seasons).

Effect of stimulators

Application of HA or PS significantly increased all vegetative and pod traits in both seasons except electrode leakage and chlorophyll (SPAD) which significantly decreased in 2nd season Table 3 and Fig. 2 PS treatment exhibited the highest value for all abovementioned traits followed by HA except the two traits of EL and chlorophyll (SPAD) in 2nd season with no significant differences between PS and HA in root length and pod length in 1st and 2nd season, respectively. However, the increment in total yield (average of two seasons) was about 21.1 and 19.9% due to PS application as well as 9.5 and 9.7% due to HA application than corresponding control of Dokki 331 and Sudany cvs, respectively. Potassium silicate (PS) caused an increment in all studied plant growth characters ranging from 5.7%, plant height to 79.9 number of branches per plant in Dokki 331 cv., and from 6.2% fresh weight to 49.9% dry weight in Sudany cv. Fig.3.

Water stress and stimulators interaction

The interaction between irrigation treatments and PS or HA treatments was significant for all studied traits except plant height in both seasons and chlorophyll in the second season Fig. 4 the highest mean values of all vegetative, physiological, qualitative, and quantitative yield traits were obtained under normal irrigation and silicon treatment in both seasons except EL and chlorophyll content (SPAD) of the 2nd one, followed by the treatment of NI x HA for most traits. Opposite, the lowest values of all studied traits were obtained under severe stress (SS) and control (NI) treatment in the first and second seasons. However, the increment in most traits was more in SS levels compared to MS or NI levels interacted with stimulants Figs.

5&6 data in Fig.7 shows the increment in number of pods (16.9% and 30.1%) and total yield (11.1% and 21.1%) (average the two seasons) due to HA and PS, respectively than the corresponding control.

Stress Tolerance Indices:

Results of Fig. 8 showed that the highest seed-yielding treatments under normal irrigation (NI) were PS (50.958 g) whereas HA had 47.175 g on average of both cultivars. However, under water stress (SS), both PS (40.1 g) and HA (36.798 g) treatments had the highest seed yield with increments of 11.1 and 21.1%, respectively over the average control of both cultivars. Meanwhile, the drought stress Control (distilled water) treatment gave the lowest values (33.113 g) with the highest (22.6%) reduction percentage compared with the well-watered-plants control (distilled water) treatment (42.798 g).

According to the MP index, the highest value of MP was recorded by PS × Sudany interaction treatment (45.73 g) as the average of both normal and 50% water deficit conditions, whereas the least values (37.93 g) were expressed by control × Sudany treatment Table 3. As shown in Fig. 9 (HA or PS) × Sudany treatments recorded the highest Dt (>1, drought tolerance) as well as the lowest SI <0.5 Susceptibility index) and Reduction % as compared with Dokki 331 cultivar suggesting a more stress tolerance mechanism.

Table 3. Vegetative growth, physiological, yield and related traits of cowpea as affected by stimulators during two seasons.

	PH (cm)	RL (cm)	NB/P	NL/P	FW (g)	DW (g)	LA (cm)	EL	PL (cm)	No. P	100-SW (g)	SY/P (g)	Ch (Spad)	RWC
	1st season													
control	49.7	15.7	3.3	19.1	209.2	51.7	58.2	21.2	11.2	42.8	12.2	38.3	69.9	74.4
HA	52.1	18.6	3.5	22.4	245.2	58.0	64.6	21.8	11.9	48.8	13.1	42.0	75.8	81.6
PS	54.7	20.4	3.7	27.4	293.6	69.9	67.5	22.1	12.3	54.1	14.0	46.1	80.6	85.6
LSD	1.1	0.5	0.0	0.4	1.4	0.4	0.3	0.1	0.8	0.2	0.1	0.1	0.6	0.2
	2nd season													
control	49.5	21.6	3.3	19.6	210.3	52.2	58.6	22.2	11.6	44.5	12.3	38.3	78.6	74.3
HA	51.9	21.9	3.6	22.8	246.6	58.8	65.1	22.5	12.1	49.0	13.1	41.9	73.4	81.5
PS	54.5	22.1	3.8	27.9	292.9	70.7	67.9	22.0	12.5	54.2	14.1	46.2	65.4	85.5
LSD	1.0	0.3	0.0	0.2	0.9	0.4	0.2	0.0	0.0	2.8	0.0	0.1	6.7	0.2

PH, Plant height, RL, Root length, NB/P, Number of branches per plant, NL, Number of leaves, FW, Fresh weight, DW, Dry weight, EL, Electrode leakage, PL, Pod length, No. P, Number of pods per plant, 100-SW, 100 seeds weight, SY/P, Seed yield per plant, CH, Chlorophyll, RWC, Relative water content.

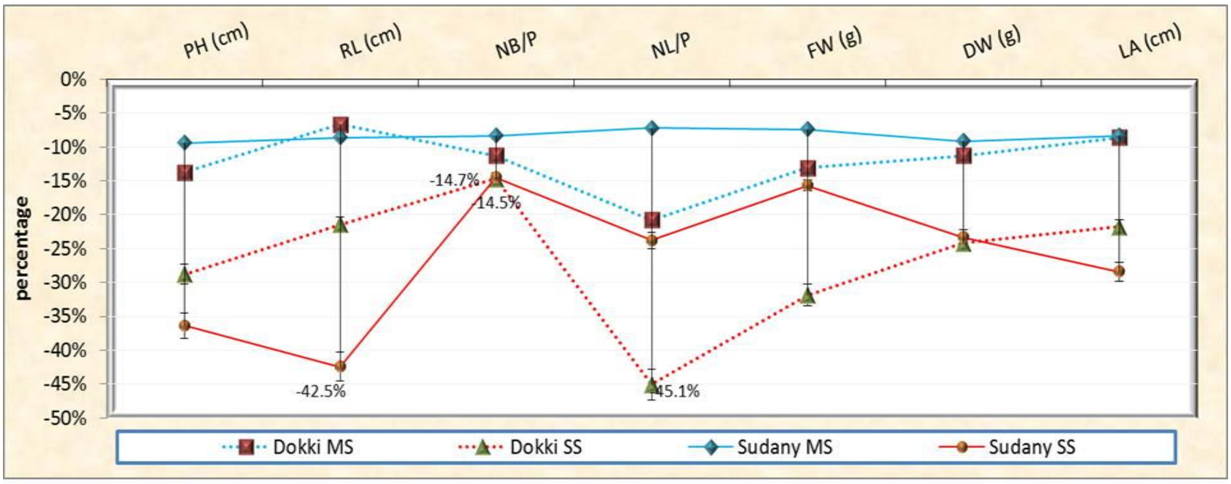

Fig.2 Reduction % of vegetative traits of cowpea as affected by irrigation regime (average two seasons).

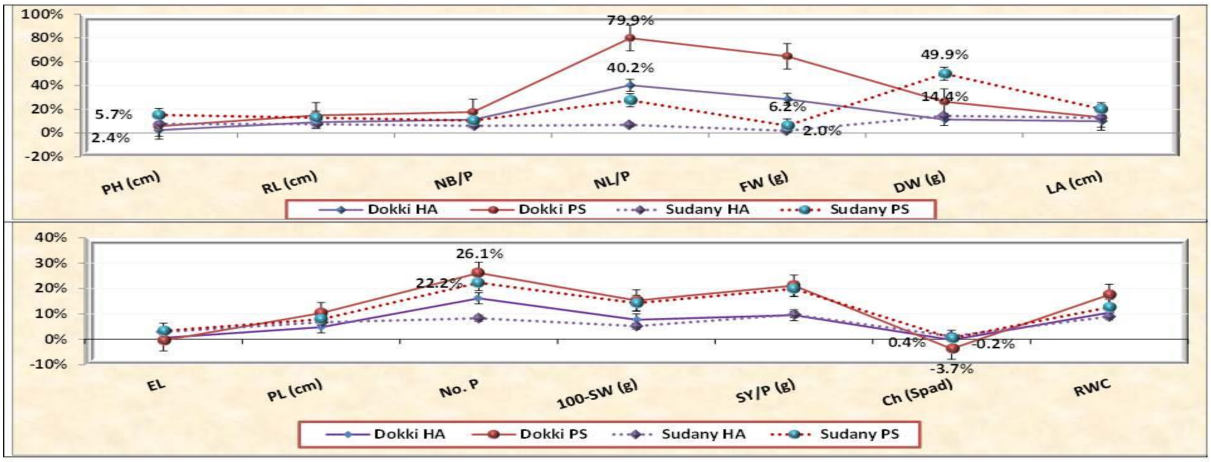

Fig. 3: Increment % of Vegetative growth, yield and related traits of cowpea as affected by stimulators × cultivars in average of both seasons

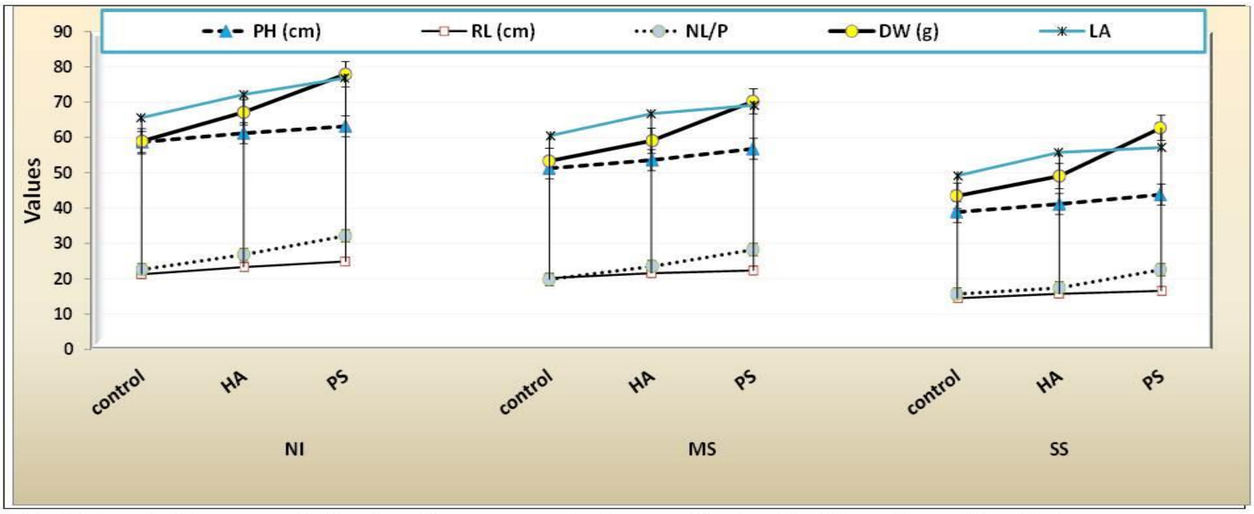

Fig.4 Vegetative growth traits of cowpea plants as affected by the interaction between stimulants and irrigation regime (average both seasons).

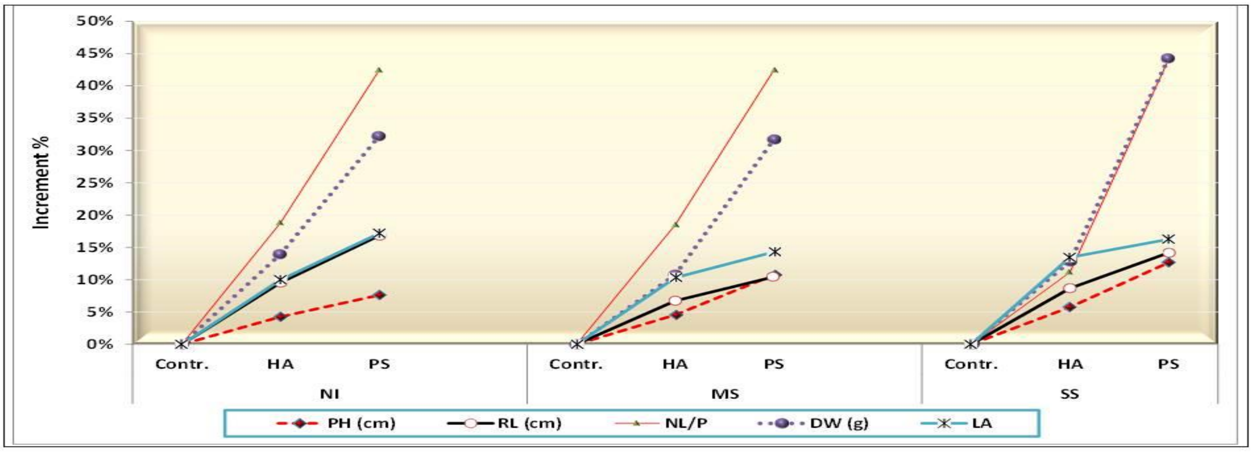

Fig.5: Increment of some vegetative growth traits of cowpea plants as affected by the interaction between stimulants and irrigation regime (average both seasons).

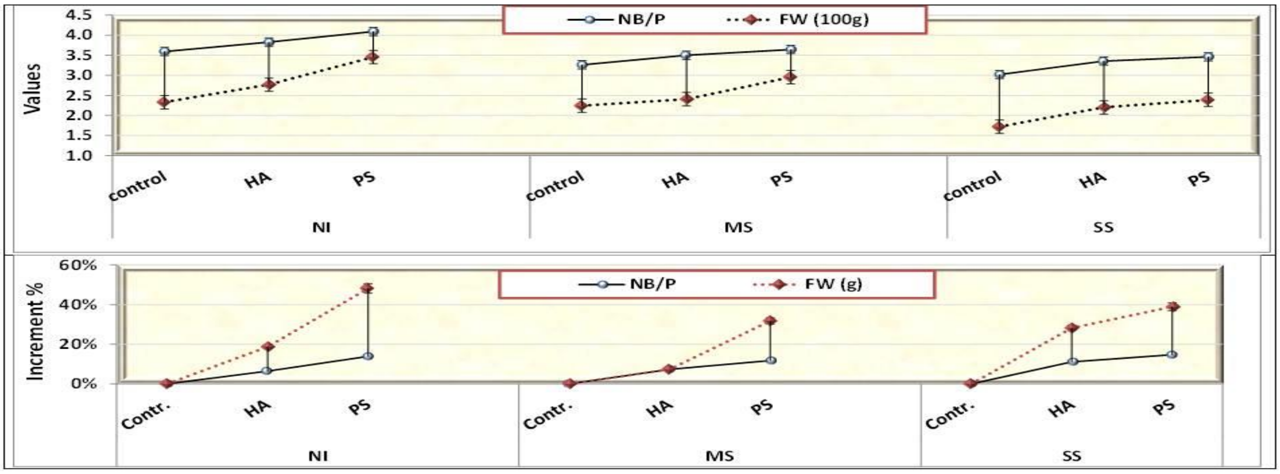

Fig.6: NB/P and FW of Vegetative growth traits of cowpea plants as affected by the interaction between stimulants and irrigation regime (average both seasons).

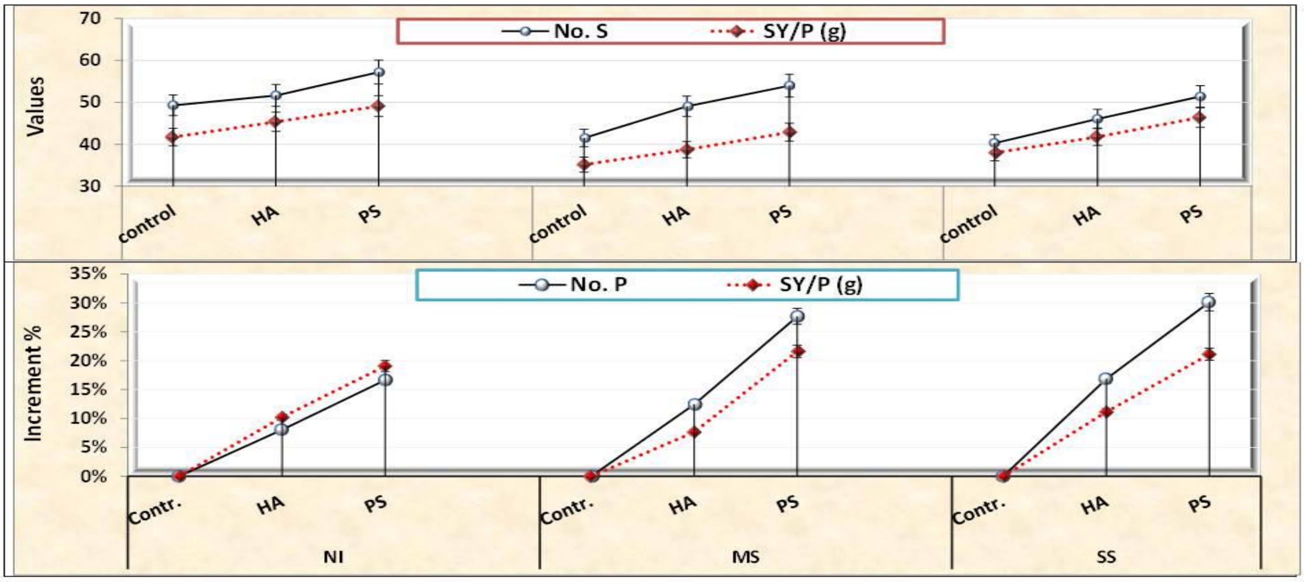

Fig.7: No.P and SY/P of cowpea plants as affected by the interaction between stimulants and irrigation regime (average both seasons).

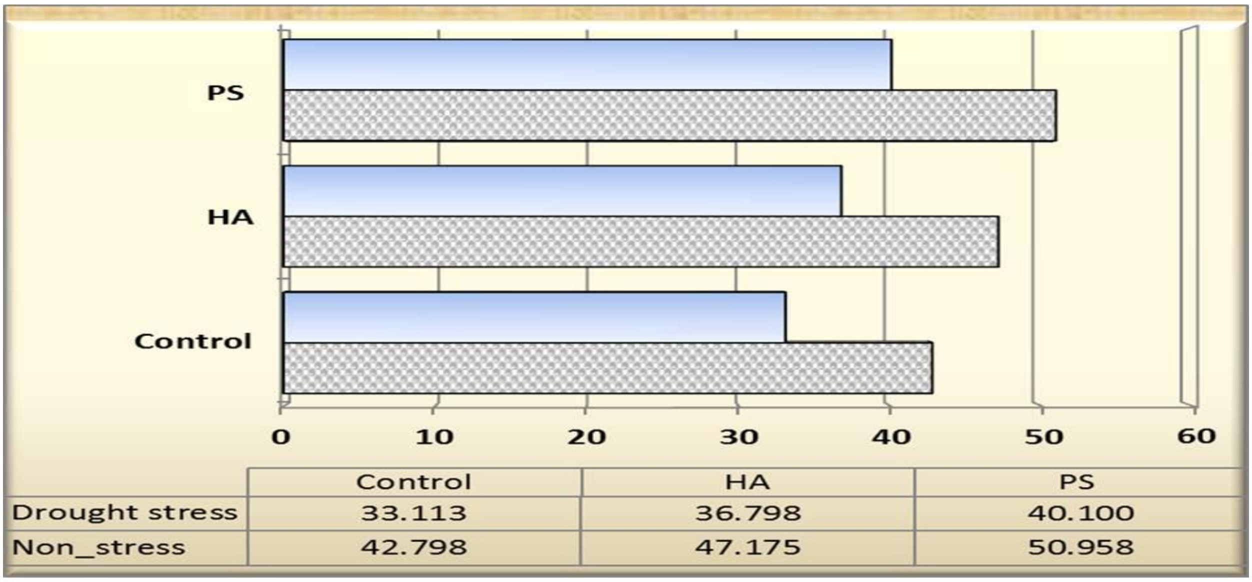

Fig. 8: Seed yield of cowpea as affected by stimulants applications (average two cultivars).

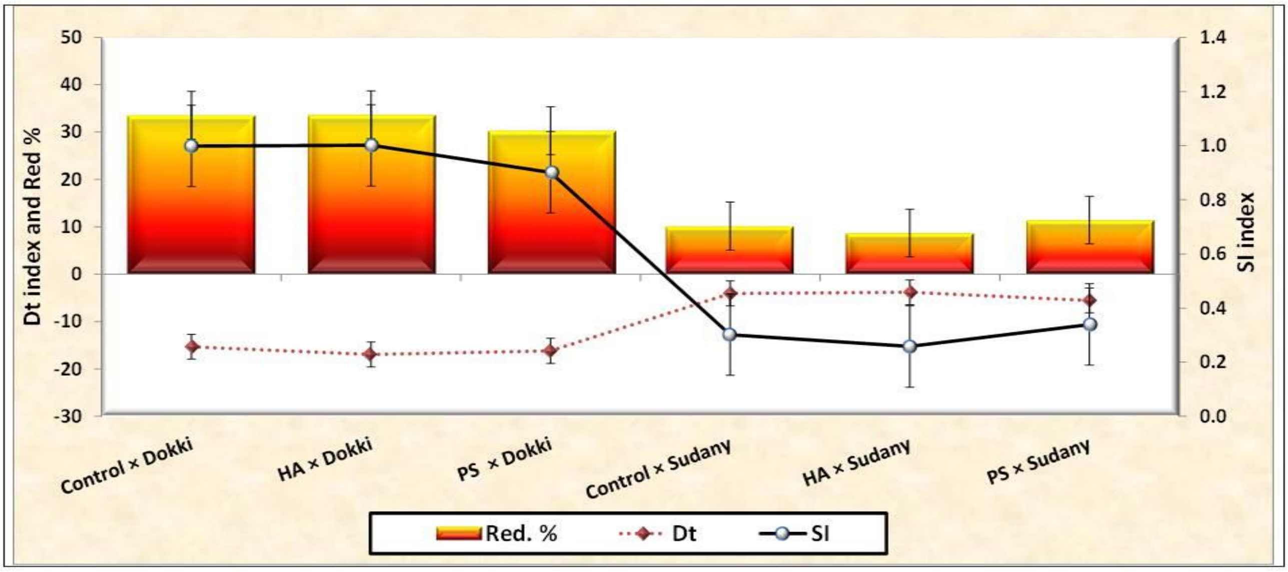

Fig. 9 Drought tolerance indices and yield under non-stress and stress conditions

Effect of water deficit and application with stimulants on some soil properties.

The changes in the soil salinity (EC), soil PH, organic matter, calcium carbonate ($CaCO_3$%) content, total nitrogen and availability of P and K induced by the investigated treatments are presented in Table 4.

Soil salinity

The data in Table 4 Show that, after the first and second seasons, the soil's salinity significantly increased with an increase in the interval between watering. After the first season, the highest soil salinity values of 2.61, 2.96 and 2.81 dSm^{-1} were found with the application of distilled water (C), HA and PS, respectively, under severe stress for Dokki331 cultivar, 2.63, 2.92 and 2.83 dSm^{-1} for Sudany cultivar, with no significant differences between the effects of the two

Table 4. Effect of water regime and application with bio-stimulants on soil properties of two cowpea cultivars in both seasons.

Treatments			2020							2021						
cultivars	water deficit	BS	pH (1:2.5)	EC dS/m	O.M (%)	$CaCO_3$ (%)	Total N (%)	Available p (mg/kg)	Available K(mg/kg)	pH (1:2.5)	EC dS/m	O.M (%)	$CaCO_3$ (%)	Total N (%)	Available p (mg/kg)	Available K(mg/kg)
Dokki331	**NI**	**DA**	7.99	1.68	0.90	8.04	0.043	78.19	162.52	8.12	1.34	0.85	7.25	0.040	66.19	129.33
		HA	7.64	1.90	1.05	7.25	0.049	58.24	262.45	7.77	1.91	1.01	6.04	0.049	46.24	235.78
		PS	7.77	1.80	0.88	7.94	0.042	80.19	267.12	8.08	1.74	0.78	6.92	0.037	68.19	243.79
	MS	**DA**	7.83	2.00	0.98	8.25	0.046	72.92	159.95	8.03	2.11	0.91	8.02	0.043	60.92	133.28
		HA	7.80	2.15	1.11	7.79	0.052	52.12	228.79	7.91	2.22	1.02	6.63	0.047	52.12	218.79
		PS	7.81	2.09	0.97	7.88	0.046	68.79	270.89	8.13	2.16	0.86	7.46	0.041	70.01	260.89
	SS	**DA**	8.01	2.61	0.87	8.05	0.041	70.10	183.24	8.04	2.76	0.94	7.53	0.045	72.38	163.24
		HA	7.94	2.96	1.14	7.75	0.054	67.72	198.85	7.98	3.09	1.03	6.27	0.048	56.10	178.85
		PS	7.98	2.81	0.87	8.10	0.041	71.24	239.91	8.11	2.79	0.79	7.53	0.037	67.24	219.91
Sudany	**NI**	**DA**	7.88	1.65	0.96	7.94	0.045	78.10	162.7	8.08	1.37	0.91	7.61	0.043	62.77	132.70
		HA	7.55	1.93	1.12	7.30	0.053	58.00	260.81	7.88	1.90	1.04	6.93	0.049	46.00	237.48
		PS	7.76	1.79	0.89	7.93	0.042	79.37	265.62	7.99	1.74	0.79	7.68	0.037	67.37	232.29
	MS	**DA**	7.93	1.99	0.96	8.04	0.045	70.44	162.7	8.03	2.13	0.86	7.92	0.041	58.44	129.37
		HA	7.84	2.08	1.07	7.79	0.050	50.44	233.22	7.99	2.35	0.98	6.81	0.046	53.77	223.22
		PS	7.84	2.12	0.92	7.88	0.043	73.77	262.29	8.12	2.26	0.82	7.68	0.039	75.99	252.29
	SS	**DA**	8.12	2.63	0.84	8.07	0.040	75.62	181.81	8.05	2.86	0.91	7.48	0.043	74.78	165.14
		HA	7.88	2.92	1.18	7.56	0.056	64.38	199.07	7.98	3.15	1.07	6.88	0.050	55.59	183.74
		PS	7.94	2.83	0.91	8.16	0.043	73.11	241.55	8.12	3.05	0.82	7.46	0.039	70.44	224.88
LSD 0.05 C*WD*BS			0.085	0.08	0.07	0.17	0.0034	8.11	7.15	0.085	0.085	0.083	0.44	0.004	6.56	12.38

EC, Electronic conductivity, O.M, organic matter, cultivars. Moreover, after second season, soil salinity values of 2.76, 3.03 and 2.79 dSm^{-1} were found with the application of C, HA, and PS, respectively, under severe drought stress at Dokki331 cultivar and 2.86, 3.13 and 3.05 dSm^{-1} were found with the application of C, HA, and PS, respectively, under severe drought stress at Sudany cultivar. Over average of both seasons and cultivars, HA and PS increased EC by 11.6% and 5.71% compared to the control Figs. 10&11.

Soil pH

The results in Table 4 clearly that, the soil pH increased with an increase in the interval between watering's after the first and second seasons. After the first season the highest soil pH values of 7.94, 8.14 and 7.98 were found with application of Control, HA, and PS, respectively, under severe drought stress for Dokki331 cultivar and 7.93, 8.12 and 7.94 for Sudany cv. Moreover, after second seasons, soil pH values of 8.04, 8.17 and 8.11 for Dokki331 cultivar and 8.05, 8.17 and 8.12 for Sudany cv., were found with application of Control, HA, and PS, respectively, under severe drought stress. Under severe stress, PS and HA caused 0.22 and 1.37% PH reduction over average of both cultivars Fig. 10, however, the decrease with HA x Dokki331 cv. (1.92%) was more than HA x Sudany cv. (0.81) as shown in Fig. 11.

Organic matter and calcium carbonate contents.

Data presented in Table 4 show that, after the first and second seasons, drought stress treatments did not show clear effect on the organic matter and calcium carbonate contents in the soil. On the other hand, HA treatment recorded the highest values of organic matter under different drought treatments compared with other treatments. the soil organic matter content as an affected by application of HA after the first season, reached 1.05, 1.11 and 1.14% with Dokki331 cultivar, whereas 1.12, 1.07 and 1.18 % with Sudany cv for NI, MS, and SS treatments, respectively. Moreover, after the second, the soil organic matter content was 1.01, 1.02 and 1.07% with Dokki 331 cv. and it was 1.04, 0.98 and 1.07 % with Sudany cv. for NI, MS, and SS treatments, respectively. Over an average of both seasons, under severe stress, HA increased O.M by 28.57% and 19.89% in the case of Sudany and Dokki 331cvs, respectively, whereas PS caused 8.29% (Dokki331 cv) and 1.14% (Sudany cv.) decreasing O.M compared to the control Fig. 11 in addition to, calcium carbonate contents decreased by 10.01% (Dokki331cv) and 7.14% (Sudany cv) as affected by the application of HA in average of both seasons Fig.11 it's recorded the lowest values of calcium carbonate compared with other treatments under different drought treatments. After the first season, the soil calcium carbonate contents as affected by the application of potassium humate were 6.25, 5.79 and 5.75 % for NI, MS, and SS treatments, respectively with Dokki331 cv., and were 6.30, 5.79 and 5.56 % for NI, MS, and SS treatments, respectively with Sudany cv. Moreover, after the second season, were 6.04, 5.63 and 5.27 % for NI, MS, and SS treatments, respectively under with Dokki331 cv. and were 6.61, 5.70 and 5.15 % for NI, MS, and SS treatments, respectively under Sudany cv.

Total N, Available P and K in soil:

Data in Table 4 indicated that, after the first and second seasons, drought treatments have not clear effect on total N, Available P and K in soil. While HA treatments recorded the highest values of total N compared with other treatments under different drought treatments. Soil total N content as an affected by application of HA after the first season, reached 0.049, 0.052 and 0.054% for NI, MS, and SS treatments, respectively with Dokki 331 cv, and recorded 0.053, 0.050 and 0.056 % for NI, MS, and SS treatments, respectively with Sudany cv. Moreover, after the second, were 0.049, 0.047 and 0.048% for NI, MS, and SS treatments, respectively with Dokki331 cv and 0.049, 0.046 and 0.050 % for NI, MS, and SS treatments, respectively with Sudany cv. On the other hand, the data in Table 4 and Fig.11, also, show that the available P values decrease as affected by the application of HA compared to PS application. Humic acid (HA) treatments recorded

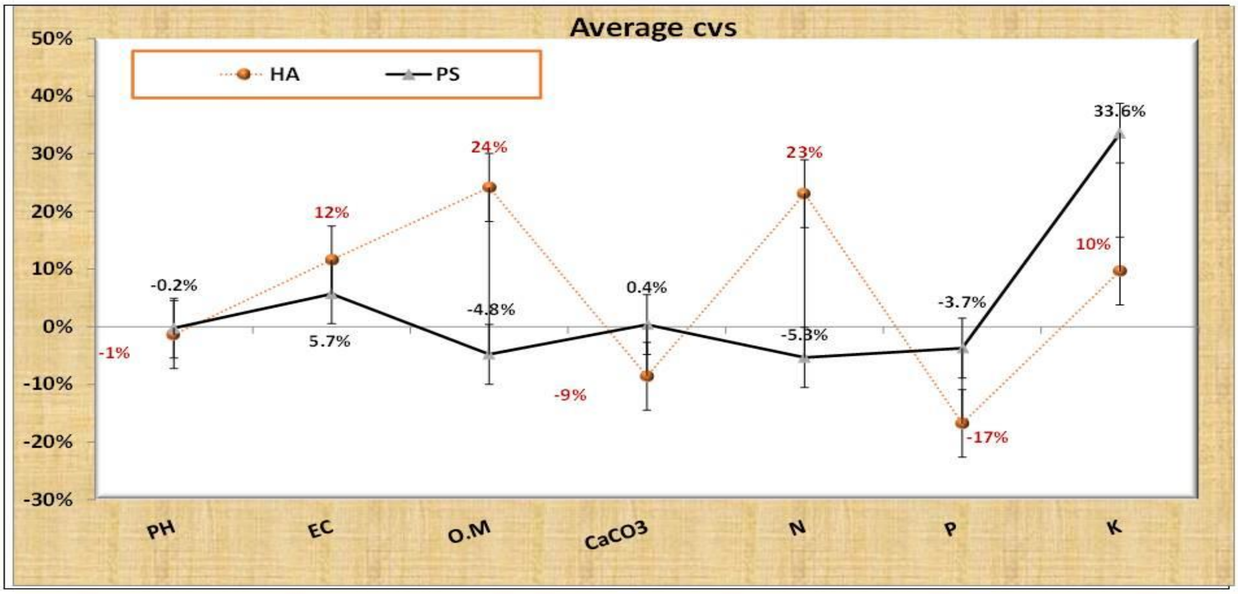

Fig.10: Changes percentage of PH, EC, O.M, and CaCO3 compared to control as affected by stimulators × average cultivars under severe drought stress.

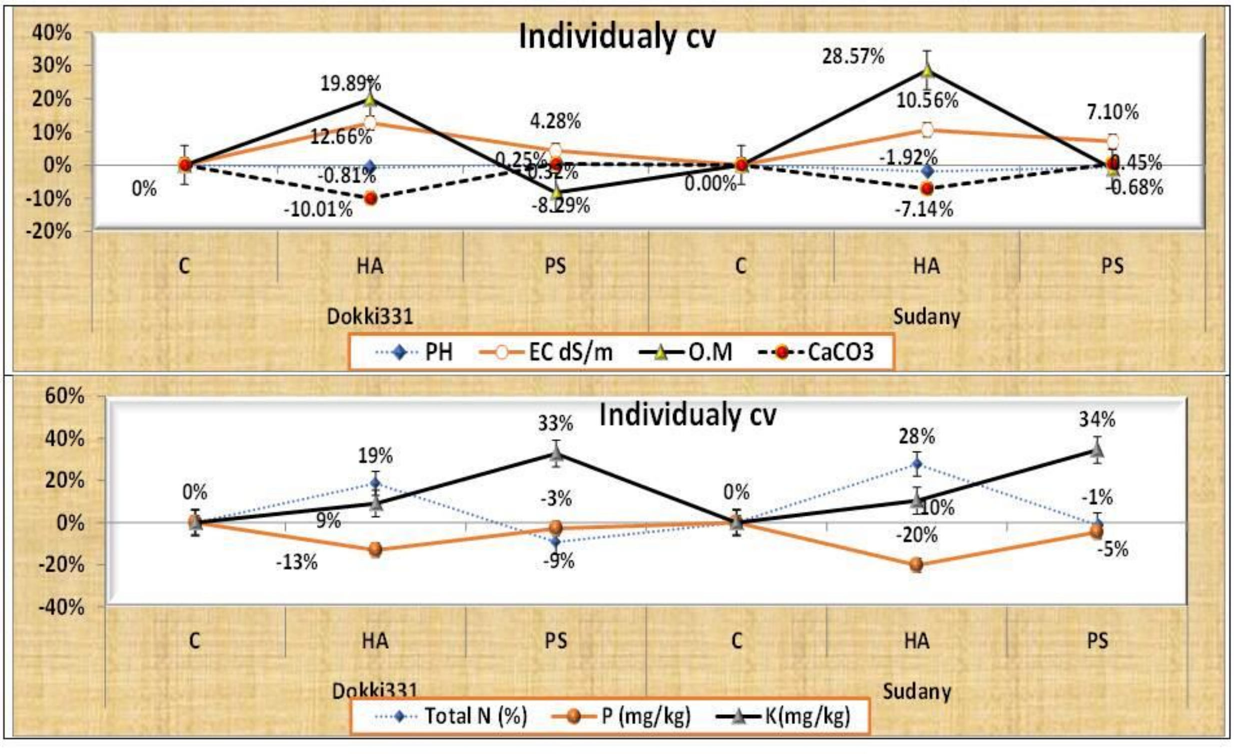

Fig. 11: Changes percentage of PH, EC, O.M, and CaCO3 (UP) as well as N, P and K in soils compared to control as affected by stimulators × individualy cultivar under severe drought stress

the lowest values of available P compared with PS treatments under different drought treatments. After the first season, available phosphorous contents as affected by the application of HA were 58.24, 52.12 and 67.72 mg kg-1 for NI, MS, and SS treatments, respectively with Dokki331, and 58.00, 50.44 and 64.38 mg kg-1 for NI, MS, and SS treatments, respectively with Sudany cv. Moreover, after the second season, were 46.24, 52.12 and 56.10 mg kg-1 with Dokki331 cv. and were 46.00, 58.44 and 55.59 mg kg-1 with Sudany cv. for NI, MS, and SS treatments, respectively.

Effect of bio-stimulants application on dry matter and N, P and K concentration in plants.

Dry matter

The effect of treatments on dry matter of cowpea cultivars is presented in Table 5 the data show that, after the first and second seasons, drought treatments under severe stress have negative effect on the dry matter of plants compared to other treatments. Drought treatments under severe stress recorded the lowest values of dry matter compared with other drought treatments under different the bio-stimulants applied. Additionally, the data in Table 5 show that, in most cases Sudany cv. Recorded highest values of dry matter compared with Dokki331 cv. Moreover, PS and HA treatments recorded the highest values of dry matter of plants compared with control treatment under different drought treatments. The dry matter content as affected by the application of PS and HA after the first season, reached 23.24, 21.36 and 15.73% for HA and 21.7, 21.71 and 15.62 % for PS under NI, MS, and SS, respectively with Dokki331 cv, and recorded 22.93, 22.76 and 16.31% for HA and 24.41, 21.63 and 16.79% for PS under NI, MS, and SS treatments, respectively with Sudany cv. Moreover, after the second season, the dry matter content was 20.75, 17.34 and 15.56 % for humic acid and 24.09, 19.12 and 17.50% for PS under NI, MS, and SS treatments, respectively with Dokki331 cv. and were 24.44, 21.61 and 16.04% for HA and 24.66, 21.94 and 16.35 % under NI, MS, and SS treatments, respectively with Sudany cv.

N, P and K concentrations in plants

Data in Table 5 show that, after the first and second seasons, drought treatments have not a clear effect on concentration of N, P and K in plants. On other hand, bio-stimulants treatments (HA and PS) recorded the highest values of concentration of N, P and K in plants compared with control treatment, under different drought treatments. Additionally, the data in Table 5 illustrate that, under different drought treatments, HA treatment recorded the highest values of concentration of N, P and K in plants compared with PS treatment, after the first season, the concentration of N, P and K in plants as an affected by application of potassium humate , reached 4.12, 5.04 and 4.24 % for N , 0.33, 0.48 and 0.45% for P and 1.55, 1.67 and 1.53 % for K , for NI, MS and SS treatments, respectively with Dokki331 cv, and recorded, 4.30, 5.77 and 4.93% for N , 0.44, 0.53 and 0.51 % for P and 1.68, 2.38 and 1.79 % for K , for NI, MS and SS treatments, respectively with Sudany cv. after the second season, were 4.38, 6.45 and 5.05% for N, 0.51, 0.78 and 0.62% for P and 1.78, 2.14 and 1.63 % for K, for NI, MS and SS treatments, respectively with Dokki331 cv, and 5.06, 5.47 and 5.35 % for N , 0.56, 0.78 and 0.73 for P and 2.01, 2.19 and 1.81% for K, for NI, MS and SS treatments, respectively with Sudany cv. Moreover, in most cases, under different drought and bio-stimulants treatments with

Table 5. Effect of water regime and bio-stimulants application on dray matter and N, P and K concentration of two cowpea cultivars in both seasons.

Treatments			2020				2021			
Cultivars	water deficit	BS	dry matter (%)	N (%)	P (%)	K (%)	dry matter (%)	N (%)	P (%)	K (%)
Dokki331	NI	DA	17.41	3.89	0.25	1.17	22.25	3.37	0.34	1.42
		HA	23.24	4.12	0.33	1.55	20.75	4.38	0.51	1.78
		PS	21.70	3.75	0.32	1.54	24.09	4.47	0.57	1.91
	MS	DA	18.30	3.95	0.24	1.33	16.74	3.40	0.32	1.32
		HA	21.36	5.04	0.48	1.67	17.34	6.45	0.78	2.14
		PS	21.71	4.13	0.42	1.63	19.12	4.65	0.76	1.96
	SS	DA	12.45	4.44	0.23	1.07	15.21	3.84	0.39	1.09
		HA	15.73	4.24	0.45	1.53	15.56	5.05	0.62	1.63
		PS	15.62	4.11	0.41	1.35	17.50	4.38	0.63	1.62
Sudany	NI	DA	18.48	3.81	0.24	1.11	17.48	3.16	0.38	1.40
		HA	22.93	4.30	0.44	1.68	24.44	5.06	0.56	2.01
		PS	24.41	4.16	0.33	1.62	24.66	4.59	0.54	1.52
	MS	DA	18.59	3.48	0.25	1.57	18.06	3.41	0.31	1.39
		HA	22.76	5.77	0.53	2.38	21.61	5.47	0.78	2.19
		PS	21.63	4.19	0.45	1.65	21.94	4.97	0.76	2.17
	SS	DA	12.49	3.39	0.22	1.04	13.72	3.83	0.37	1.06
		HA	16.31	4.93	0.51	1.79	16.04	5.35	0.73	1.81
		PS	16.79	4.76	0.48	1.67	16.35	4.93	0.65	1.66
LSD 0.05 C*WD*BS			0.55	0.26	0.054	0.23	0.75	0.24	0.061	0.076

Sudany cv recorded highest values of the concentration of N, P and K in plants compared with Dokki 331 cv.

Discussion

Drought stress is one of the environmental factors that can reduce plant growth and productivity. The current study found that the growth parameters of two cowpea cultivars were significantly decreased under drought conditions as in Fig. 2 and this may be due to the water stress causing losses in tissue water which led to a reduction in the turgor pressure in the cells, thereby inhibition enlargement and cell division as concluded by Hsiao and Acevedo (1974). Water stress causes an increase in ABA/CYT ratio, which in turn decreases plant growth (Marschner 1995), whereas, under sufficient water conditions, there was a decrease in ABA and an increase in CYT, GA, and IAA reflecting good growth and dry matter content.

The increment in water supply led to an increase in the soil moisture content and caused no suffering of plants to get their water requirements, where primarily irrigation improves leaf growth which in turn increases the net assimilation of organic nutrients and subsequently plant growth and yield. Again, low water supply content decreases root growth and inhibits leaf elongation rate associated with an increase in ABA concentration in leaves (Smith and Dale 1988) and decreases CYT production and export (Atkin et al. 1973) and also, harmful impact of drought could be due to that drought negatively affects cell division and elongation, delay cellular growth, reduces photosynthetic rate, and finally this in turn affect the growth and yield of plants (Hafez et al. 2020). The increasing yield by increasing irrigation level might be due to the increase of total chlorophyll content; in addition, it might be due to the increment of leaf transpiration, which correlates with the increasing water supply. Consequently, this might have a positive effect on yield via the enhancing gas exchange and photosynthesis process (Foti et al. 1995). Thus, to achieve high yielding of pods, the soil water content should be no lower than 50% of the maximum available water in the root zone, particularly during the flowering period (Harris 1978).

The differences between cowpea cultivars could be due to their genetic constitutions and their interaction with the environmental factors prevailing during development. El-Shaieny (2017) concluded that the Sudany cultivar was more tolerant of water stress compared with other studied cultivars in both seasons. Plants have evolved a variety of physiological and biochemical mechanisms to cope with or tolerate stress, such as drought stress, which is a common environmental challenge. Application of HA or PS significantly increased all vegetative and pod traits in both seasons except EL and Chl Table 3 and Fig. 1 These results indicate the role of silicon and humic acid in alleviating the adverse effects of water stress and results in a significant increment of growth, yield and its components (Hattori et al. 2005; El-Sayed et al. 2018). The further developing impact of potassium silicate on vegetative development attributes might be because of the helpful impact of potassium and silicon, where potassium assumes a significant part in osmoregulation, photosynthesis, happening, stomatal opening and shutting, protein synthesis, and acclimatization are changed over into sink organs and chemicals framed. (Mengel and Kirkby 2001; Cakmak 2005; Milford and Johnston 2007). Additionally, silicon successfully enhances the architecture of the plant, revealing more upright leaves that capture more solar light, increasing photosynthetic efficiency and chlorophyll content (Braga et al.

2009), In addition to its role in plant tolerance, it is linked to a number of stressors. Moghith et al. (2020) reported that the maximum growth parameters of the chia plant (*Salvia hispanica* L.) were found to be preferable for cultivation under potassium silicate spray at 2000 ppm and saline water irrigation at (0.68 dSm-1). It also maintained higher water status, leaf water potential, and relative water contents (Ali et al. 2013).

Treatments that enjoyed high seed yield under normal and stressed irrigation conditions, had high values of MP index (Farshadfar and Sutka, 2002). Shirazi et al. (2009) stated that the higher yield in the non-stress condition resulted in an increase in the MP index and cannot be considered a valid indicator for identifying treatments that reduce the effect of stress.

As for soil properties, according to Bodner et al. (2013), Wu et al. (2016) and Zhang et al. (2019), drought stress had a detrimental impact on the soil's hydrological characteristics; it decreased overall porosity and hydraulic conductivity while increasing bulk density in the topsoil layer, leading to accelerate the accumulation of salts in the soil and slows salt loss by leaching from soil with irrigation water. Additionally, the soil salinity increased when humic acid was used compared to other treatments Fig. 10 this may be attributed to, humic acid lowering the soil's pH, which increases the solubility of the salts in the soil like calcium carbonate of the soil, with the released carbonate anion leading to increases in the pH value at the end of the first and second seasons Table 4 and Fig. 10 Dinçsoy and Sönmez (2019) found that soil's pH decreased by humic acid. Farrag and Bakr (2021) conclude that organic amendments had effects on the increases in the dissolution of the soil salts. It could be attributed to the emerging organic acids from organic matter decomposition that accelerates the solubility of salts in the soil and also, to the impact of drought stress on decreased soil porosity and soil hydraulic conductivity (Bodner et al. 2013; Wu et al., 2016; Zhang et al. 2019). This helps in accelerating the accumulation of salts in the soil including alkaline ions such as carbonate and bicarbonate ions, which help to increase the pH value of the soil.

Results of Organic matter traits as presented in Table 4 and Fig.10 are consistent with Zaky et al. (2006); Mahmoud et al (2011) and Dinçsoy and Sönmez (2019). Abbasi et al. (2010) and Brodersen et al. (2019) concluded that one of the worst environmental factors that affects plant productivity is drought including a variety of elements related to plant development, growth, and metabolism (Corso et al. 2020). The primary elements influencing how plants react to drought include growth stages, age, plant species, and the intensity and duration of the drought (Gray and Brady 2016). The mechanism by which plants fight drought also varies between plant species (Osakabe et al. 2014; Bielach and Hrtyan 2017).

These results are consistent with (Bhatt and Singh 2022) who found that, Different crops' agronomic performance, including plant height, spread, dry matter accumulation, crop growth rate, relative growth rate, nodule count, nodule dry weight, nutrient content, yield components, yield, and quality, could be considerably impacted by the application of humic acid.

Delfine et al. (2005)found that humic compounds had a stimulating effect represented in increased of plant growth and higher absorption of macronutrients like nitrogen, phosphorus, potassium, and magnesium. According to several studies, humic acids are the primary fractions of humic substances and the most active parts of organic matter in soil and compost. Humic acids work on mechanisms related to cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, and enzyme activity in order to boost plant growth and, subsequently, yield (Nardi 2002; Chen 2004; Fahramand et al 2014). Moreover, Humic acid was generally helpful to the shoot and root growth of plants, according to studies on the effects of humic compounds on plant growth. Additionally, the presence of humic molecules increased the impact of nitrogen, phosphorus, and potassium fertilization on plants (Fahramand et al. 2014). Humic acid (HA) plays a critical role in improving the properties of soil, such as the aggregation and the soil's ability to hold water, its cation exchange capacity (CEC), and macronutrient availability (Ampong et al. 2022). Also (Stevenson 1994, Zaky et al. 2006 and Mahmoud et al. 2011) found that significant increases in soil nitrogen were seen after applying humic acid to the soil. This increase can be attributed to improved soil nutrient retention and increased soil microbial activity, which helps to transform organic nutrients into mineral form. In addition, available soil K increased with the application of HA and potassium silicate compared to water treatment. This could be because the used compounds contain potassium within their chemical composition, so they are considered a source of potassium addition to the soil. On the other hand, decreased of available phosphorus concentration in soil can be due to; the soil containing a high percentage of calcium carbonate Table 4 and the effect of humic lowers the soil's pH (Dinçsoy and Sönmez 2019). This promotes the dissolution of calcium carbonate, which releases carbonate anion and raises pH levels. The rise in the concentration of soluble calcium under high pH circumstances leads to the production of insoluble triple calcium phosphate, which may account for the decrease in the concentration of available phosphorous. This result agrees with (Leytem and Mikkelsen 2005; Farrag and Bakr 2023).

Conclusion

Soil application of 500 mg L^{-1} HA and spraying 500 mg L^{-1} PS significantly increased plant growth plant height, root length, leaf area, number of branches, fresh and dry weights per plant, pod length, number of pods per plant, 100-seed weight, and seed yield per plant, especially in the Sudany cultivar. PS also increased electrode leakage (EL) and relative water content (RWC), which are indicators of plant water stress tolerance. Drought treatments do have not a clear effect on soil organic matter, calcium carbonate content, total N, available P and available K in soil, as well as dry matter and concentration of N, P and K in plants. while the soil pH and soil salinity increased, as well as a decrease plant dry matter with an increase in the interval between watering’s Moreover, soil organic matter, soil salinity, soil pH and concentration of N, P and K in plants increased. While soil calcium carbonate content decreased as an affected of the application of HA more than other bio-stimulant treatments. Additionally, in most cases, Sudany cv recorded the highest values of dry matter and concentration of N, P and K compared with Dokki 331 cv. under different drought and bio stimulant treatments.

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