DJAS
Damietta Journal of Agricultural Sciences
مجلة دمياط للعلوم الزراعية

Damietta Journal of Agricultural Sciences

Volume 2, Issue I, 2023

Damietta Journal of Agricultural Sciences
http://publication.du.edu.eg/journal/ojs302design/index.php/agr/index
ISSN: 2812-5347(Print)- 2812-5355 (Online)

Improving Growth, Productivity and Anatomical Features of Wheat Using Licorice root Extract.

Wanas, A.L. [1] and Bazeed, Zeinab.H.*[1]
[1].Department of Agriculture botany, Faculty of Agriculture, Damietta University, Egypt.
Email address: ahmedwanas@du.edu.eg
*Corresponding author: zeinabbazeed@du.edu.eg

Key words:
Wheat
licorice root extract
Growth
Anatomy
Yield
Sustainability

Accepted 8/11/2022

ABSTRACT

The present study was conducted to evaluate the effect of licorice root extract (LRE) as a biostimulants on the growth and productivity in relation to anatomical Features of wheat plants during the 2018/19 and 2019/20 seasons. Normally, LRE was used with different concentrations (of 2.5, 5, and 10 g l^{-1} as a grain soaking and then foliar spraying on 45-day-old seedlings). The results indicated that aapplication of LRE significantly increased root size, plant height, root and shoot dry weights, tillers and leaves number as well as leaves area $plant^{-1}$. Meanwhile decreased root/shoot ratio and leaf area ratio. Additionally, LRE treatments significantly increased the concentration of photosynthetic pigments in the flag leaf, NPK, crude protein, and total carbohydrates in the shoots compared to untreated plants. Anatomically, LRE treatments increased the thickness of stem wall tissues, epidermis, ground tissue, and the number of vascular bundles of the wheat stems as well as the thickness of flag leaf mid-vein and lamina, mesophyll, phloem, and xylem tissues relative to untreated plants. These anatomical alterations are consistent with the higher grain yield and its components and quality (N, P, K, protein and carbohydrates). Generally, application of 5 g l^{-1} LRE was the most effective concentration compared to the control and the other LRE treatments during both seasons. It may be concluded that using LRE at a concentration of 5 gl^{-1} as an organic, eco-friendly treatment to improve growth and productivity of wheat and reduce the risks of using synthetic growth regulators.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's largest and most important human food crop. Therefore, continuous wheat improvement is an imperative strategy for feeding a constantly growing human population. In 2020, the local production of bread wheat was about 9.10 million tons, while consumption reached about 21.98 million tons, a self-sufficiency rate of 41.4% **(CAPMAS, 2020)**. This would threaten Egyptian food security and put further pressure on the agricultural trade balance, due to the over increase in the annual wheat import, resulting in the inability to achieve development objectives **(Kandil and Mohamed, 2019)**. So great efforts have been carried out to minimize this gap by improving growth and productivity of wheat using different agricultural practices such as growth regulators **(Barányiová and Klem, 2016; Zhang, *et al.*, 2019)** and mineral nutrients **(Allam, 2005; HoliK, *et al.*, 2018)**.

Recently, there has been vast interest in using organic enhancers as a sustainable and eco-friendly strategy for improving plant productivity **(Povero *et al.*, 2016; Wanas *et al.*, 2018; Desoky *et al.*, 2019; Wanas & Khamis, 2021)**. Licorice root extract (LRE) represents a potent growth enhancers due to their richness in growth-promoting substances, osmoprotectants, antioxidants, and nutrients, which are paramount for improving plant growth and productivity **(Wanas *et al.*, 2018; Desoky *et al.* 2019; Wanas & Khamis 2021)**. Licorice (*Glycyrrhiza glabra* L.) is a traditional medicinal herb that belongs Fabaceae family and grows in various parts of the world **(Ross, 2001)**. **Wanas *et al.*, (2018)** reported that LRE applied as a seed pre-sowing treatment then as a foliar spray on cold-stressed squash caused a significant increase in the stem length and diameter, leaves number $plant^{-1}$, roots, stem and leaves dry weights, total leaf area $plant^{-1}$ and the leaf concentration of photosynthetic pigments, mineral nutrients and total soluble sugars in compared to those of untreated plants.

Therefore, the investigation aimed to study the effect of Egyptian LRE on the vegetative growth, some biochemical trials, anatomical characteristics, and yield components of wheat plant.

MATERIAL AND METHODS

1- Experiment design:

Two pot trials were conducted at the Experimental Station, Faculty of Agriculture, Damietta University during two successive winter seasons of 2018/19 and 2019/20 to investigate the role of LRE on some growth aspects, chemical constituents, anatomical features and yield attributes of wheat (*Triticum aestivum* L.). Wheat grains cv. Misr1 was purchased from the Directorate of Agriculture, Damietta, Egypt. The experiment included four treatments as follows:

T_1: Distilled water (control).
T_2: LRE at a concentration of 2.5 g l^{-1}.
T_3: LRE at a concentration of 5 g l^{-1}.
T_4: LRE at a concentration of 10 g l^{-1}.

The assigned concentrations of LRE and the distilled water were applied as grain-soaking for 8 hours, directly before planting and then foliar spraying on the plants grown up on 45th day after planting (DAP). Pots of 30 cm diameter were filled with a 10 kg mixture of clay and sand (1:1 v/v), then pre-planting treated grains with different assigned treatments were planted (24 pots for each treatment and 3 grains pot^{-1}) on the 20th of November for the 2018/19 and 2019/20 seasons. After three weeks from planting, seedlings were thinned to one per pot. The experimental design was a randomized complete-block design with four replicates, each with six pots. In both seasons, irrigation with tap water and other agricultural practices of cultivated wheat were followed up.

2- **Preparation of LRE:**

Egyptian licorice roots were purchased from a local market in Damietta, Egypt. Stock licorice root extract was prepared according to the method described by **Almehemdi *et al.* (2011)**. One hundred grams of licorice root powder was added to 200 ml distilled water in a dark bottle and kept inside an incubator at 50˚ C for 24 hours, then filtered by Buchner fennel. The final volume reached one litre by distilled water. The stock solution (100 g l^{-1}) was kept in a refrigerator at a temperature of 5 ± 2 ˚C until the preparation of desired concentrations. Chemical analysis of Egyptian LRE was previously recorded **(Desoky *et al.* 2019)** and indicated in Table (1).

3- Sampling and collecting data:

3.1-Vegetative growth characteristics:

Eight plants from each treatment were randomly taken at 100th DAP in both seasons to record some growth parameters such as roots size (cm^3),

roots weight (g), plant height (cm), number of tillers, shoot (main stem + tillers) dry weight (g), and number of leaves plant^{-1}. The roots' size was determined according to the proposition of Hanson and Churchill (1968). To get the root system as complete as possible, the plant pot is tilted while a gentle stream of water is carefully poured over the soil surface until the soil is completely displaced from the roots. The root system is then dried with paper towels, and placed in a volumetric flask with a known volume of water, and the increased volume of water is measured to indicate the volume of the root system.

Table 1. Minerals and antioxidants content of Egyptian LRE on dry mass basis (Desoky *et al.*, 2019).

Component	Values	Unit
1. Antioxidants and osmoprotectants:		
Free proline	36	g kg^{-1} DM
Soluble sugars	148	
Glutathione (GSH)	30.2	
Ascorbic acid (AsA; Vit.	41.0	mg kg^{-1} DM
Selenium (Se)	0.90	
2. Phytohormones		
Total auxins	4.2	mg kg^{-1} DM
Total gibberellins	5.2	
Zeatin-type cytokinin	4.1	
3. Mineral nutrients:		
Nitrogen (N)	20.2	g kg^{-1} DM
Phosphorus (P)	21.3	
Potassium (K)	47.2	
Calcium (Ca)	2.20	
Magnesium (Mg)	3.80	
Sulfur (S)	2.40	
Iron (Fe)	0.94	
Manganese (Mn)	0.62	
Zinc (Zn)	0.21	
Cupper (Cu)	0.02	

Leaf area plant^{-1} (cm^2) was determined using the disk method as described by **Derieux *et al.* (1973)**.

The obtained data concerning the plant biomass (plant dry matter) and leaf area plant^{-1} were used for computing some important growth indices such as:

a) Root/ shoot ratio:

The root/shoot ratio of a plant expresses the ratio between the dry matter accumulated in a plant's roots and shoots. It was computed using the following formula:

$$Root/\,Shoot\,ratio = \frac{Root\ dry\ weight\ \ \text{Plant}^{-1}}{Shoot\ dry\ weight\ Plant^{-1}}$$

b) Leaf area ratio (LAR):

The leaf area ratio (LAR) plant^{-1} expresses the ratio between leaf area plant-1 and total plant biomass, or LAR indicates leaf area formed per unit of plant biomass and expressed in cm^2 g^{-1} of plant dry weight. It was calculated at 100 DAP according to the formula of **Radford (1967)**.

$$LAR\ \ (\boldsymbol{cm^2 g^{-1}}) = \frac{leaf\ area\ Plant^{-1}\ \ (cm^2\,)}{Plant\ dry\ weight}$$

3.2- Photosynthetic pigments:

Chlorophyll a, b and carotenoid levels were determined in the flag leaf on the 100th DAP in both seasons using the spectrophotometric method recommended by **Lichtenthaler (1987)** and calculated as mg g^{-1} fresh weight (FW).

3.3- Anatomical study:

According to the observed differences in the morphological characteristics of wheat plants due to treatments in the first season, a comparative anatomical study on the main stem and flag leaf of treated plants compared with those of the control was microscopically done during the second season.

Specimens (1 cm long) from the middle part of the terminal internode of the main stem and the flag leaf blade were taken on the 100th DAP. The specimens were killed and fixed for at least 48 hours in FAA (10 ml formalin, 5 ml glacial acetic acid, and 85 ml ethyl alcohol 70%). Samples were then washed in ethanol (50%), dehydrated in a normal butyl alcohol series, and embedded in paraffin wax at the melting point of 56 °C. By using a rotary microtome, sections were cut at a thickness of 20 µ, double-stained with crystal violet-erythrosine, cleared in xylene, and mounted in Canada balsam **(Nassar & El-Sahhar, 1998)**. The prepared sections were microscopically examined.

3.4-Chemical analysis of shoots and grains:

Samples of representative shoots on the 100th DAP and of mature grains at harvest date, i.e., 165th DAP were taken from 4 replicates of each treatment and dried at 70 ˚C until constant weight. The dried shoots and grains were ground to a fine powder and then kept in paper sachets at the laboratory temperature (25 ˚C) for determination of the following chemical constituents in both shoots and grains:

3.4.1- Total carbohydrate:

Total carbohydrate concentration was determined using the phenol-sulphuric acid method **(Dubois *et al.*, 1956)** and calculated as mg g^{-1} D.W.

3.4.2- Total nitrogen (N) and crude protein:

Total N was determined by the wet digestion of the dried shoots and grain powders using the Kjeldahl method as described by Horneck and **Miller (1998)**. A sample of 0.5 g from the representative dry matter was digested with sulphuric and perchloric acid. Distillation was carried out with 40% NaOH and ammonia was received in a 4% boric acid solution. The distillates were then titrated against 0.02 N HCl using the mixed methyl red bromocressol green and then N concentration was calculated as mg g^{-1} DW. The total nitrogen concentration in wheat shoots and grains was multiplied by a conversion factor of 5.75 to calculate the crude protein concentration in wheat shoots and grains **(A.O.A.C., 1990)**.

3.3.4.3- Phosphorus (P, mg g^{-1} D.W.):

Phosphorus concentration was determined by "Vanadomolybdo phosphoric acid" yellow color method. Digestion of samples was done by tri acid mixture **(Jackson, 1973)**.

3.3.4.4- Potassium (K, mg g^{-1} D.W.):

Using the Flam photometer model Carl-Zeiss, potassium was determined according to the **Horneck and Hanson (1998)** method procedure.

3.5- Yield characteristics:

Eight plants per each treatment were randomly taken at harvest time (165th DAP) to record the number of spikes $plant^{-1}$, main spike length (cm), number and weight (g) of grains main $spike^{-1}$, 1000 grain weight (g), total grain yield (g) $plant^{-1}$ and straw yield (g) $plant^{-1}$. The relative grain yield of the treated plants was calculated as a percentage of the control yield**.**

4- Statistical analyses

Data of vegetative growth, yield, and chemical composition were subjected to proper statistical analysis of variance, according to **Snedecor & Cochran (1989)**. Using the least significant differences test (LSD), differences were compared among treatment means at level of 5% of probability.

RESULTS AND DISCUSSION

4.1- Growth parameters:

Data in Table 2 show the effect of LRE at 2.5, 5, and 10 g l^{-1} (grain-soaking plus foliar spraying) on some wheat plant growth criteria. The results showed that LRE concentrations significantly increased most growth criteria of wheat plants expressed as roots size, roots and shoot dry weights, plant height tiller, and leaf numbers $plant^{-1}$ and leaf area $plant^{-1}$ at 100th DAP. In contrast, a significant reduction in the root/shoot ratio and LAR occurred with all applied treatments of LRE compared to untreated plants during both seasons. The highest values of roots size, roots and shoot dry weights, plant height, number of both tillers and leaves $plant^{-1}$, and leaf area $plant^{-1}$, and the lowest values of root/shoot ratio and leaf area ratio were obtained by 5 g l^{-1}, followed by 10 g l^{-1} and then 2.5 g l^{-1} of LRE, respectively. Consistent results were reported by **Belal (2018)**, **Wanas *et al.* (2018)** on squash, and **Wanas & Khamis (2021)** on strawberry.

The vigorous growth of wheat plant obtained via LRE treatments may be attributed to its possess plant-performance-enhancing capabilities due to its richness in antioxidants and osmoprotectants, which are very important for improving plant growth and productivity **(Belal, 2018; Wanas *et al.*, 2018; Desoky *et al.*, 2019)**. LRE also contains some nutrients which paramount for plant growth like phosphorus, potassium, magnesium, iron, and zinc **(Moses *et al.*, 2002; Desoky *et al.*, 2019)**. For enistance, phosphorus is involved in ATP synthesis and the formation of RNA and phospholipids.

Table 2: Effect of LRE treatments on some growth criteria of wheat plant at 100 DAP during 2018/19 and 2019/20 seasons.

Characters / Treatments		Roots size plant^{-1} (cm^3)	Roots DW plant^{-1} (g)	Plant height (cm)	Tillers No plant^{-1}	Leaves No. plant^{-1}	Shoots DW plant^{-1} (g)	Root / Shoot ratio	Total DW plant^{-1}	Leaf area plant^{-1} (cm^2)	LAR Cm^2 g^{1-} plant DW
Season 2018/2019											
Control		44.50	6.88	77.75	4.25	29.00	29.90	0.23	36.78	828.30	22.52
LRE	2.5 g l^{-1}	51.25	8.67	88.38	6.00	35.25	41.28	0.21	49.95	1010.48	20.23
	5 g l^{-1}	58.80	9.33	96.50	7.00	36.13	46.66	020	55.99	1064.94	19.02
	10 g l^{-1}	57.60	9.28	92.13	6.75	35.50	45.20	0.21	54.48	1056.22	19.38
LSD at 0.05		2.06	0.36	3.19	0.49	1.76	1.37	0.01	1.46	56.36	0.48
Season 2019/2020											
Control		39.65	5.35	71.25	4.00	26.75	26.73	0.21	32.08	786.56	18.86
LRE	2.5 g l^{-1}	48.25	7.34	83.00	6.25	31.75	40.79	0.18	48.13	869.64	18.07
	5 g l^{-1}	52.50	7.56	90.25	6.75	33.50	44.48	0.17	52.04	923.86	17.75
	10 g l^{-1}	50.80	7.45	88.63	6.50	32.75	42.87	0.17	50.32	903.33	17.95
LSD at 0.05		1.17	0.21	3.88	0.63	1.46	1.12	0.01	0.51	49.38	0.28

Abbreviations: LRE = licorice root extract, DW = Dry weight, DAP = Days after planting, No. = number and LAR = leaf area ratio.

Hence, it directly enhances and regulates many biosynthesis processes required for plant growth, e.g., carbohydrate and sugar formation, nucleic acid, enzymes, and hormones **(Yelenosky, 1985; El-Dengawy *et al.*, 2017; Wanas *et al.*, 2018)**. LRE also contains significant amounts of auxins, gibberellins (GAs), and zeatin (**Desoky *et al.*, 2019**). Gibberellins and auxins promote and regulate several plant responses, especially cell division and elongation (**Wanas *et al.*, 1998; Moses *et al.*, 2002**). Zeatin is the most prevalent cytokinin in plants (**Hwang *et al.*, 2012**), which are well known to stimulate and regulate many growth aspects such as cell division, differentiation, root formation, and branching, protect the cells from the aging effects of reactive oxygen species and help in nutrients assimilation (**Chen, 1997; Hwang *et al.*, 2012**).

4.2-Photosynthetic pigments:

The results in Table 3 show that, compared to untreated plants, LRE treatments significantly increased the concentrations of photosynthetic pigments expressed as chlorophyll a, b, and carotenoids in the wheat flag leaf in both seasons. The highest concentration of chlorophyll a or b was obtained due to LRE at 5 g l^{-1}, whereas the highest increment in carotenoid level was gained by LRE treatment at 10 g l^{-1}. Also, results showed that chlorophyll a was the most responded pigment to all used LRE treatments. The percentage increase in chlorophyll "a" concentration was 77.86, 97.05and 92.62% over the control value in the first season and 55.56, 90.08, and 71.42% over the control value in the second season with LRE at 2.5, 5 and 10 g l^{-1},

Table 3: Effect of LRE treatments on the photosynthetic pigments level (mg g^{-1} FW) in wheat flag leaf at 100th DAP during 2018/19 and 2019/20seasons.

Pigments / Treatments		Chlorophyll a		Chlorophyll b		Carotenoids	
		$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Season 2018/2019							
Control		2.71	0.00	1.63	0.00	1.84	0.00
LRE	2.5 g l^{-1}	4.82	+77.86	2.10	+28.83	2.41	+30.98
	5 g l^{-1}	5.34	+97.05	2.28	+39.88	2.64	+43.48
	10 g l^{-1}	5.22	+92.62	2.06	+26.38	2.72	+47.83
LSD at .05		0.36		0.18		0.27	
Season 2019/2020							
Control		2.52	0.00	1.52	0.00	1.71	0.00
LRE	2.5 g l^{-1}	3.92	+55.56	1.89	+24.34	2.15	+25.73
	5 g l^{-1}	4.79	+90.08	2.05	+34.87	2.36	+38.01
	10 g l^{-1}	4.32	+71.43	1.97	+29.61	2.42	+41.52
LSD at 0.05		0.43		0.11		0.18	

Abbreviations: LRE = licorice root extract, FW= Fresh weight, DAP = Days after planting, ± % = ± % relative to the control values.

respectively. The present results were confirmed by the findings of **EL-Dengawy *et al.* (2017), Wanas *et al.* (2018), Merwad (2020), Wanas and Khamis (2021)** .

The advantageous effect of LRE on photosynthetic pigments level could be attributed to its richness in growth-promoting hormones such as GAs and zeatin (**Desoky *et al.*, 2019**). GAs and cytokinins are well known to be induced the biosynthesis of chloroplast pigments in many plants (**Fletcher and Arnold, 1986; Chen, 1997; Wanas and Khamis, 2021**).

3- Chemical constituents of wheat shoots:

As shown in Table 4 the three assigned LRE treatments significantly increased the concentration of each N, P, K, crude protein, and total carbohydrates in the shoots of treated wheat plants at 100 DAP during both seasons compared to those of control plants. The highest percentage increases in N, P, K, crude protein and total carbohydrates levels obtained by LRE at 5 g l^{-1} were respectively 23.48, 27.27, 18.88, 23.48, and 15.41% during the first season and 22.93, 40.00, 16.18, 22.93 and 14.66 % during the second season. These results are in agreement with the findings of **Belal (2018) Wanas *et al*. (2018), Desoky *et al.* (2019) and Wanas & Khamis (2021).**

The LRE-promoting effect on the chemical constituents of wheat shoot could be considered a direct effect of this organic extract on stimulating mineral absorption through the vigorous root system of the treated plants (Table 2). Additionally, the increase in leaf area (Table 2), photosynthetic pigments (Table 3), and increased accumulation of dry matter in the shoots all indicate the LRE-enhancing effect on photosynthesis efficiency. More photoassimilates are being produced as a result, which improves the translocation of minerals from roots to shoots (**Wanas, 2007; Belal, 2018**).

Table 4: Effect of LRE treatments on NPK and crude protein and total carbohydrate level (mg g^{-1} DW) in wheat shoots at 100th DAP during 2018/19 and 2019/20 seasons.

Chemical constituents		N		P		K		Crude protein		Total carbohydrates	
		$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Season 2018/2019											
Control		27.60	0.00	1.10	0.00	28.44	0.00	158.70	0.00	508.17	0.00
LRE	2.5 g l^{-1}	30.57	+10.76	1.30	+18.18	30.71	+7.98	175.78	+10.76	543.05	+6.86
	5 g l^{-1}	34.08	+23.48	1.40	+27.27	33.81	+18.88	195.96	+23.48	586.48	+15.41
	10 g l^{-1}	33.83	+22.57	1.40	+27.27	31.72	+11.53	194.52	+22.57	566.38	+11.45
LSD at 0.05		1.23	-	0.09	-	0.36	-	2.11	-	2.36	-
Season 2019/2020											
Control		26.60	0.00	1.00	0.00	26.52	0.00	152.95	0.00	489.14	0.00
LRE	2.5 g l^{-1}	29.40	+10.53	1.20	+20.00	29.78	+12.29	169.05	+10.53	514.14	+5.11
	5 g l^{-1}	32.70	+22.93	1.40	+40.00	30.81	+16.18	188.03	+22.93	560.86	+14.66
	10 g l^{-1}	32.42	+21.88	1.30	+30.00	30.72	+15.84	186.42	+21.88	560.32	+14.55
LSD at 0.05		0.87	-	0.09	-	0.28	-	1.78	-	3.06	-

Abbreviations: LRE = licorice root extract, DAP = Days after planting, DW = Dry weight, ± % = ± % relative to the control values.

4-**Anatomical studies:**

4.1- Anatomy of the main stem:

As shown in Table 5 and Fig. 1, the three applied LRE treatments positively affected many anatomical features of the main stem terminal internode (carrying the main spike). The diameter of the whole stem section was increased by 14.41, 26.13 and 20.41% by LRE at 2.5, 5, and 10 g l^{-1}, respectively. The increment in stem diameter was mainly due to the increase in hollow ground tissue diameter and stem wall thickness. For instance, with the same treatments in the same order, stem wall thickness increased by 25.06, 34.35, and 28.03% over the control value (100%). These results showed that the LRE treatment at 5 g l^{-1} was the most effective in this regard. The increment in the stem wall thickness was due to the increase in the thickness of its comprising tissues, i.e., epidermis, chlorenchyma tissue beneath the epidermis, peripheral sclerenchyma, and parenchymatous ground tissue, as well as the number and dimensions (thickness and width) of the vascular bundles comparing with those of the control. Moreover, the increment of the vascular bundle thickness was accompanied by an increase in the thickness of its tissue components, i.e., phloem, xylem, and bundle sheath. Increases reached the highest value with the treatment of 5 g l^{-1} LRE.

Table 5: Effect of LRE treatments on the anatomical features of the main stem terminal internode of wheat at 100th DAP during the 2019/2020 season.

Measurements (µ) & counts / Treatments			Diameter of whole section	Diameter of hollow ground tissue	Thick. of stem wall	Thick. of epidermis	Thick. of chlorenchyma beneath the epidermis	Thick. of peripheral fibers	Thick. of parenchymatous ground tissue	No of vascular bundles	Length of the largest vascular bundle	Width of the largest vascular bundle	Thick. of the bundle sheath	Thick. of phloem tissue	Thick. of xylem tissue
Control		$\bar{X}$	2234.79	1384.95	424.92	12.84	78.47	11.93	321.68	49.00	146.20	112.88	12.77	43.15	77.51
LRE	2.5 g l^{-1}	$\bar{X}$	2556.76	1493.98	531.39	13.34	94.15	13.64	410.26	52.00	157.02	113.73	13.86	46.24	83.06
		± %	+14.41	+7.87	+25.06	+3.89	+19.98	+14.33	+27.54	+6.12	+7.40	+0.75	+8.54	+7.16	+7.16
	5 g l^{-1}	$\bar{X}$	2818.81	1677.01	570.91	14.93	97.86	16.87	441.24	53.00	160.29	114.68	14.81	47.18	83.49
		± %	+26.13	+21.09	+34.36	+16.28	+24.71	+41.41	+37.17	+8.16	+9.64	+1.59	+15.97	+9.34	+7.72
	10 g l^{-1}	$\bar{X}$	2688.65	1600.61	544.02	14.58	95.16	14.28	420.00	52.00	159.30	114.45	14.44	46.90	83.52
		± %	+20.31	15.57	+28.03	+13.55	+21.27	+19.70	+30.56	+6.12	+8.96	+1.39	+13.08	8.69	+7.75

Abbreviations: LRE = licorice root extract, DAP = Days after planting, ± % = ± % relative to the control values.

Table 6: Effect of LRE treatments on the anatomical features of the flag leaf of wheat at 100th DAP during the 2019/2020 season.

Measurements (µ) & counts / Treatments			Thick .of mid-vein	Thick. of uppermost sclerenchyma tissue	Thick. of lowermost sclerenchyma tissue	Thick. of the main v.b.	Width of the main v. b. sheath	Thick. of bundle sheath	Thick. of phloem tissue	Thick. of xylem tissue	Thick. of lamina	Thick. of upper epidermis	Thick. of lower epidermis	Thick. of mesophyll tissue
Control		$\bar{X}$	298.93	116.78	47.90	105.59	106.07	10.83	35.42	48.51	218.97	15.65	13.01	190.31
LRE	2.5 g l^{-1}	$\bar{X}$	318.18	123.90	50.57	113.29	119.70	11.00	36.46	54.83	235.19	16.69	13.73	204.77
		±%	+6.44	+6.10	+5.57	+7.29	+12.85	+1.57	+2.94	+13.03	+7.41	+6.65	+5.53	+7.60
	5 g l^{-1}	$\bar{X}$	362.62	146.93	59.90	124.84	130.85	11.93	38.52	68.47	252.86	17.13	13.82	211.91
		±%	+21.31	+25.82	+25.05	+18.23	+23.36	+10.16	+8.75	+41.15	+15.48	+9.46	+6.23	+11.35
	10 g l^{-1}	$\bar{X}$	346.25	143.28	56.12	120.75	125.86	11.63	38.14	64.46	239.28	16.88	13.62	208.78
		±%	+15.83	+22.69	+17.16	+14.36	+18.66	+7.39	+7.68	+32.88	+9.28	+7.86	+4.69	+9.71

Abbreviations: LRE = licorice root extract, DAP = Days after planting, Thick. = thickness, No = number v.b. = vascular bundle ± % = ± % relative to the control values.

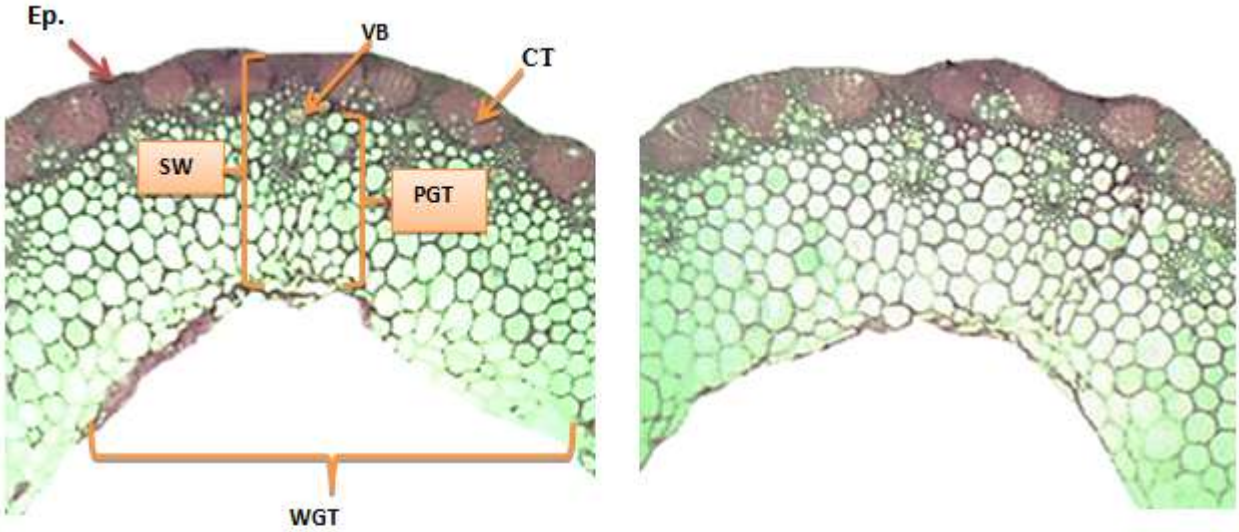

Fig. 1: Transverse sections (100 X) through the through the middle part of the main stem terminal internode of wheat plant.

Abbreviations: Ep. = Epidermis, CT = Chlorenchyma tissue, WGT = Whole ground tissue = PGT= Parenchymatous ground tissue, VB+ Vascular bundle.

Control **LRE at 5 g l^{-1}**

4.2 -Anatomy of the flag leaf:

As displayed in Table 6 and Fig. 2, the three assigned LRE treatments positively affected different studied anatomical features of the flag leaf. Since the mid-vein thickness was increased over the control value by 6.44, 21.31, and 15.83% by LRE treatments at 2.5, 5, and 10 g l^{-1}, respectively. The increment of the mid vein thickness was due to the increase in the thickness of uppermost and lowermost sclerenchyma tissues and the main vascular bundle. The thickness of vascular bundle reached its highest value (124.84µ), representing 118.23 % of the control value (105.59µ) with LRE treatment at 5 g l^{-1}. Also, the width of this bundle was increased with the applied LRE treatments to reach its maximum (123.36% of the control value) with the same treatment, i.e., LRE at 5 g l^{-1}. In addition, the increment in the vascular bundle thickness was due to the increase in the thickness of its constituent's tissues, i.e., phloem, xylem and bundle sheath.

The lamina thickness was also increased with all applied LRE treatments and reached its highest percentage increase with the LRE treatment at 5 g l^{-1} (15.48 %). The increment of lamina thickness was mainly due to the increases in the thickness of its upper and lower epidermis as well as mesophyll tissue. The highest increment in the mesophyll tissue thickness was obtained by LRE at 5 g l^{-1} (11.35 %) of the control value. Consistent results were reported by **El-Dengawy *et al.* (2017), Wanas *et al.* (2018), and Wanas & Khamis (2021).**

The positive alternations in all studied anatomical traits of stem terminal internode and flag leaf blade by all applied LRE treatments during the heading stage are of great interest. The alterations included the thickness of photosynthates creator (mesophyll tissue) and their passage (phloem tissue) as well as the thickness of different raw materials passage (xylem tissue), which means that the applied extracts improved translocation and caused more raw materials to be absorbed and reached leaves and other sinks (developed grains) and more photosynthates to be allocated and partitioned to other plant parts leading to vigorous growth and enhancement of heading, hence increment the final grain and straw yields.

Other studies have confirmed the essentiality of increasing the cross-sectional area of phloem and xylem tissues accompanied by creating more photosynthates and absorbing more mineral nutrients for improving the growth and productivity of some economic plants (**Wanas, 2006 & 2007; Belal, 2018; Wanas *et al.*, 2018; Wanas & Khamis,2021**).

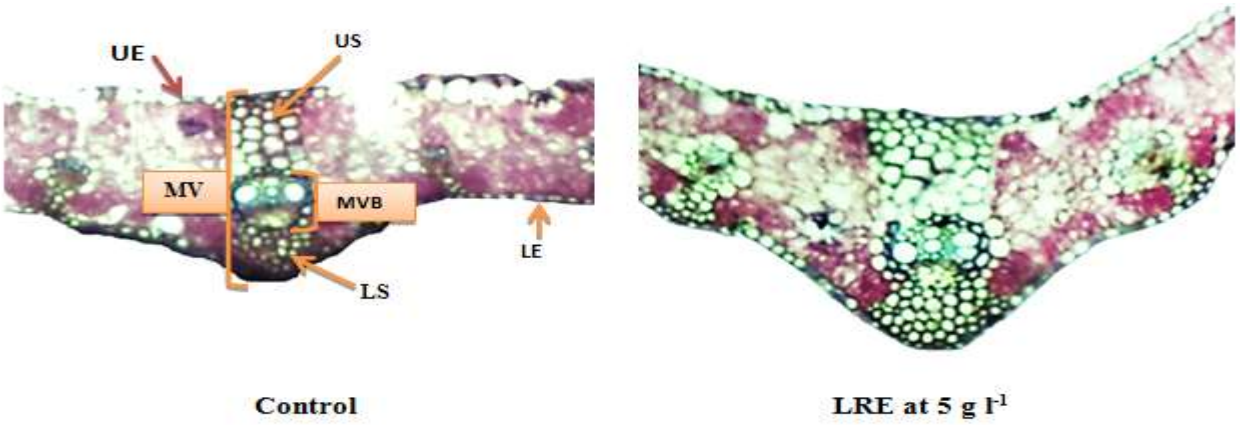

Fig. 2: Transverse sections (150 X) through the middle part of the wheat flag leaf blade. Abbreviations: MV= Mid-vein, MVB= Main vascular bundle, LP = Lower epidermis, UE = Upper epidermis, US = Uppermost sclerenchyma, LS = Lowermost sclerenchyma

5- Yield characteristics

Data presented in Table 7 reveal that the total grain yield plant^{-1} was significantly increased by LRE treatments compared to the untreated plants during both seasons. The highest percentage increase in grain yield of 43.38% and 53.77% was recorded with LRE treatment at 5 g l^{-1} in the first and second seasons, respectively. The total grain yield increment was mainly due to the increase in its contributing traits, i.e., the number of fertile tillers and spikes plant^{-1}, spike length, number and weight of grains spike^{-1}, and weight of 1000 grains as well. Additionally, the applied LRE treatments significantly increased the straw yield (g)

Table 7: Yield characteristics of wheat plants as affected by LRE treatments during 2018/19 and 2019/20 seasons.

Characteristics / Treatments		N0.of spikes/plant	Length of main spike (cm)	No .of grains main spike^{-1}	Weight of grains main spike^{-1} (g)	Grain yield plant^{-1} (g)	Straw yield plant (g)	Weight of 1000 -grain (g)	*Relative grain yield (%)
Season 2018/2019									
Control		3.75	14.66	34.00	1.64	4.68	7.90	50.33	100.00
LRE	2.5 g l^{-1}	5.88	14.78	35.50	1.95	5.92	13.38	60.90	126.50
	5 g l^{-1}	6.75	15.00	37.75	2.11	6.71	13.74	71.10	143.38
	10 g l^{-1}	6.00	15.25	36.25	2.05	6.58	13.56	69.05	140.60
LSD at 0.05		0.54	0.15	0.48	0.18	0.57	0.83	1.11	-
Season 2019/2020									
Control		3.50	14.50	33.25	1.47	4.24	8.50	50.26	100.00
LRE	2.5 g l^{-1}	5.75	14.54	34.50	1.70	5.75	13.20	60.75	135.61
	5 g l^{-1}	6.25	15.00	35.68	2.00	6.52	13.52	70.00	153.77
	10 g l^{-1}	5.75	15.50	35.25	1.95	6.44	13.40	68.80	151.89
LSD at 0.05		0.42	0.21	0.72	0.15	0.52	1.21	1.25	-

Abbreviations: LRE = licorice root extract, DAP = Days after planting.
***Relative grain yield was calculated as a percentage of the control grain yield.**

plant^{-1} in both seasons. This increase was mainly due to the increment in both tiller number and shoot dry weight plant^{-1} (Table 2).

The improvement of grain yield plant^{-1} by using LRE treatments was mainly due to its capacity to enhance the tillers number, leaf area, dry matter accumulation (Table 2), photosynthetic pigments (Table 3), NPK, protein, and carbohydrate levels (Table 4), as well as the positive alterations existed in the anatomical features of both stem terminal internode and flag leaf blade (Tables 5 & 6; Figs. 1 & 2). Besides, improving grain weight may be due to the partitioning of assimilates and/or mobilization of soluble stem reserves towards developing grains for increasing grain size because of increased cytokinin levels (**Dietrich *et al.*, 1995**). The application of cytokinins-rich LRE might contribute to enhanced sink capacity to fulfill heading resulting in improved wheat yield. The increase in grain weight due to increasing the endogenous level of cytokinins was reported by **Wanas (1998 & 2007)**, indicating the increase in the grain weight was due to more supplements of assimilates towards developing grains. These results and interpretations go along with those of **Belal (2018), Wanas *et al.* (2018), Merwad (2020) and Wanas & Khamis (2021)**.

6- Chemical constituents of wheat grains:

Data presented in Table 8 indicate that the applied LRE treatments, i.e., 2.5, 5, and 10 g l^{-1} significantly increased the concentration of N, P, K, crude protein, and total carbohydrates in wheat grains compared with the control in both seasons. Increases reached their highest values with the LRE treatment at 5 g l^{-1} followed by 10 g l^{-1}, and 2.5 g l^{-1} of LRE. The highest percentage increase in N, P, K, crude protein, and total carbohydrates concentrations obtained by LRE treatment at 5 g l^{-1} was respectively, 21.44, 15.79, 14.15, 21.44, and 13.42% in the first season and 18.51, 47.74, 10.82, 18.51 and 12.00 in the second season.

Table 8: Effect of LRE treatments on some chemical constituents (mg g^{-1} DW) of wheat grains during the 2018/19 and 2019/20 seasons.

Determinations / Treatments		N		P		K		Crude protein		Total carbohydrates	
		$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Season 2018/2019											
Control		32.60	0.00	1.90	0.00	38.10	0.00	187.45	0.00	579.96	0.00
LRE	2.5 g l^{-1}	35.86	+10.00	2.10	+10.53	40.30	+5.77	206.20	+10.00	614.66	+5.98
	5 g l^{-1}	39.59	+21.44	2.20	+15.79	43.49	+14.15	227.64	+21.44	657.80	+13.42
	10 g l^{-1}	37.82	+16.01	2.18	+14.74	42.38	+11.23	217.47	+16.01	639.56	+10.28
LSD at 0.05		1.52	-	0.11	-	0.36	-	3.33	-	4.83	-
Season 2019/2020											
Control		31.60	0.00	1.55	0.00	36.80	0.00	181.70	0.00	568.93	0.00
LRE	2.5 g l^{-1}	34.16	+8.10	1.80	+16.13	39.58	+7.55	196.42	+8.10	593.32	+4.29
	5 g l^{-1}	37.45	+18.51	2.29	+47.74	40.78	+10.82	215.34	+18.51	637.20	+12.00
	10 g l^{-1}	36.76	+16.33	2.22	+43.23	39.98	+8.64	211.37	+16.33	626.80	+10.17
LSD at 0.05		1.18	-	0.16	-	0.23	-	2.87	-	4.69	-

Abbreviations: LRE = licorice root extract, DW= Dry weight ± % = ± % relative to the control values.

The present results are in harmony with the findings of **Belal (2018), Wanas *et al.* (2018), Desoky *et al.* (2019), Merwad (2020), and Wanas & Khamis (2021)**. In this respect, Wanas *et al.* (2018) mentioned that LRE applied as integrative treatments (i.e., as seed-soaking and foliar spray) significantly increased the fruit content of total soluble sugars, N, P, K, crude protein, and total carbohydrates in cold-stressed squash.

Increasing the grain content of total carbohydrates could be indicated by improving wheat growth concerning efficient photosynthesis and improving the translocation of their products and nutrients to the developing grains affected by the LRE treatments. Thereby, the obtained grain yield by these treatments was of good quality.

CONCLUSION

Therefore, the present study strongly recommends the use of LRE at 5 g/l^{-1} as an organic growth enhancer or biostimulant for all food crops to achieve the highest yield with high sanitary quality and to avoid all warnings concerning the excessive use of synthetic growth regulators and mineral fertilizers for saving the environment and human health.

FUNDING:

This research did not receive any funding.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Wanas, A.L. and Bazeed, Zeinab.H. developed the concept of the manuscript. Wanas wrote the manuscript. All authors checked and confirmed the final revised manuscript.

REFERENCES

A.O.A.C. 1990. Official Methods of Analysis, 15th Ed., Association of Official Analytical Chemists, Washington DC, USA.

Allam, S.A. 2005. Growth and productivity performance of some wheat cultivars under various nitrogen fertilization levels. J. Agric. Sci., Mans. Univ., 30(4): 1871-1880.

Almehmedi, A.F; Nasralla, A.Y. and Anna, S. 2011. Effect of licorice, fenugreek extracts and GA_3 on yield of caraway (*Carum carvi* L.). Iraqi J. Desert Studies, 3 (1): 27-42.

Barányiová, L. and Klem, K. 2016. Effect of application of growth regulators on the physiological and yield parameters of winter wheat under water deficit. Plant Soil Environ., 62 (3): 114-120.

Belal, H.O.A. 2018. Physiological studies on squash (*Cucurbita pepo* L.) plants grown under cold stress conditions. MSc. thesis, Fac. Agric., Damietta Univ.

CAPMAS. 2020. Central Agency for Public Mobilization and Statistics.

Chen, C.M. 1997. Cytokinin biosynthesis and interconversion. Physiol. Plant., 101: 665-673.

Derieux, M.; Kerrest, R. and Montalon, Y. 1973. Etude de la sulface foliare et de L' activite photosynthetique chez qulques hybrides de mais. Ann. Amelior Plantes, 23: 95 – 107.

Desoky, E.M.; Elrys, A.S. and Rady, M.M. 2019. Licorice root extract boosts *Capsicum annuum* L. production and reduces fruit contamination on a heavy metals-contaminated saline soil. International Letters of Natural Sciences, Sci. Press Ltd., Switzerland, 73, pp 1-16.

Dietrich, J.T.; Kaminek, V.; Belvins, D.G.; Reinbett, T.M. and Morris, R.D. 1995. Changes in cytokinins and cytokinin oxidase activity in developing maize kernel and the effects of exogenous cytokinin on kernel development. Plant Physiol. Biochem., 33: 327–336.

Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebens, P.A. and Smith, F. 1956. Colorimetric method for determination sugars and related substances. Anal. Chem., 28: 350 – 356.

El-Dengawy, E.F.; Wanas, A.L. and Farrag, M.H.M. 2017. Improvement of rooting efficiency and vegetative growth in date palm offshoots by Licorice root extract and auxins mixture applications. J. Plant Production, Mansura Univ., 8 (7): 789-796.

Fletcher, R.A. and Arnold, V. 1986. Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol. Plant., 66: 197-201.

Hanson, H.C. and Churchill, E.D. 1968. The plant community. 3rd Ed., Reinhold Pub. Co., New York, pp: 108-111.

HoliK, L.; Hlisnikovsky, L. and Kunzova, E. 2018. The effect of mineral fertilizers and farmyard manure on winter wheat grain yield and grain quality. Plant Soil Environ., 64 (10): 491–497.

Horneck, D.A. and Hanson, D. 1998. Determination of potassium and sodium by Flame spectrophotometry. In: Handbook of Reference Methods for Plant Analysis. Kalra, Y.P. (Ed.): 153-155.

Horneck, D.A. and Miller, R.O. 1998. Determination of total nitrogen in plant tissue. In: Handbook of Reference Methods for Plant Analysis. Kalra, Y.P. (Ed.): 75-83.

Hwang, I.; Sheen, J. and Muller, B. 2012. Cytokinin signaling networks. Annual Review of Plant Biology, 63, 353-380.

Jackson, L.M. 1973. Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Kandil, S.A.M. and Mohamed, F.H. 2019. An economic study of the food gap from wheat crop in Egypt. Egy. J. Agric. Eco., Agric. Club, Dokki, Cairo, 29(2):463-472.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In methods in Enzymology. 148:350-382. .

Merwad, A.M.A. 2020. Mitigation of salinity stress effects on growth, yield and nutrient uptake of wheat by application of organic extracts. Communications in Soil Science and Plant Analysis, 51(9):1150-1160. https://doi.org/10.1080/00103624.2020.1751188

Moses, T.N.A.; Wheeb, W.; Al- Hadithy, Z. and Ellewy, A.N. 2002. Studying some components of the local Licorice (*Glycyrrhiza glabra*, L.) roots powder. J. Agric. Sci. Iraqi. 34(4): 30-38.

Nassar, M.A. and El-Sahhar, K.F. 1998. Botanical preparations and microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, pp. 219 (In Arabic).

Povero, G.; Mejia, J.F.; Di Tommaso, D.; Piaggesi, A. and Warrior, P. 2016. A Systematic approach to

discover and characterize natural plant biostimulants. Front. in Plant Sci., 7. https://doi.org/10.3389/fpls.2016.00435

Radford, P.J. 1967. Growth analysis formulac- their use and abuse. Crop Science, 7: 171-175.

Ross, A.I. 2001. Medicinal plants of the world. Volume. 2: Chemical constituents traditional and modern medicinal uses. Human Press, Springer Sience +Buisness Media ,LLC:191-221.

Snedecor, G.W. and Cochran, W.G. 1989. Statistical Methods 8th Ed., Iowa State Univ. Press., Ames. Iowa, USA.

Wanas, A.L. 2006. Response of squash plants grown in winter season to some natural extracts and antioxidants. Ann. Agric. Sci., Moshtohor, 44(4): 1571-1591.

Wanas, A.L. 2007. Effect of some natural extracts and benzyladenine on growth and productivity of wheat plants. J. Agric. Sci. Mansura Univ., 32 (2): 1011-1029.

Wanas, A.L. and Khamis, M.I. 2021. Effect of garlic and licorice extracts on vegetative growth and leaf anatomy of strawberry plants cultivated in different growing media. Scientific Journal for Damietta Faculty of Science 11 (1): 89-102.

Wanas, A.L. El-Desouky, S.A. and Khedr, Z.M. 1998. Utilization of some natural plant extracts of garlic and yeast) as seed-soaked materials to squash (*Cucurbita pepo*, L.). II-Effects on the histological features and the endogenous hormones. Ann. Agric. Sci., Moshtohor, Zagazig Univ., 35(2): 855-878.

Wanas, A.L.; Serag, M.S.; Abd Elhamied, A.S. and Abd Elaziz H.O.2018. Effect of some natural treatments on vegetative growth and leaf chemical composition of squash plants growing under cold conditions. J. Plant Production, Mansoura Univ., 9 (6): 543– 551.

Yelenosky, G. 1985. Extension growth and cold hardening of young 'Valencia' orange trees sprayed with AMO-1618. Plant Growth Regulation. 3, 47-53. DOI. 10.1007/BF00123545.

Zhang, W.; Huang; Z. Xu, K.; Liu, L.; Zeng, Y.; Ma, S. and Fan, Y. (2019). The effect of plant growth regulators on recovery of wheat physiological and yield-related characteristics at booting stage following chilling stress. Acta Physiologiae Plantarum, 41(8): 1-10

تحسين النمو والانتاجية والصفات التشريحية للقمح باستخدام مستخلص جذورالعرقسوس

أحمد لطفى ونس [1]، زينب هشام بازيد*[1].

1قسم النبات الزراعى ، كلية الزراعة، جامعة دمياط، مصر.

أجريت هذه الدراسة لتقييم تأثير مستخلص جذور العرقسوس (LRE) كمصدر عضوى غنى بالعديد من المواد المحسنة للنمو على نمو وإنتاجية وعلاقته بالخصائص التشريحية لنباتات القمح خلال موسمي 19/2018 و 20/2019. وفى هذه الدراسة تم استخدام مستخلص جذور العرقسوس بثلاثة تركيزات 2.5 و 5 و 10 جم لتر$^{-1}$ ، تم تطبيقه كل تركيز منها كمعاملة نقع للحبوب قبل الزراعة ثم كرش ورقى على النباتات الناتجة فى عمر ٤٥ يوم بعد الزراعة. وقد أوضحت النتائج المتحصل عليها حدوث تحسن واضح فى نمو نباتات القمح صنف مصر ١ بواسطة معاملات مستخلص العرقسوس. أشارت النتائج إلى وجود زيادة معنوية في حجم المجموع الجذرى، وارتفاع النبات ، والأوزان الجافة للجذور والمجموع الخضرى وكذلك عدد الأشطاء والأوراق ومساحة الأوراق الكلية نبات$^{-1}$ بواسطة جميع المعاملات المستخدمة من مستخلص جذور العرقسوس. وفي المقابل، حدث نقص معنوى في نسبة المجموع الجذرى/ المجموع الخضرى ومعدل مساحة الورقة مع كل المعاملات. إلى جانب ذلك، سببت جميع المعاملات زيادة فى تركيز صبغات التمثيل الضوئى في ورقة العلم، وكذلك تركيز كل من NPK والبروتين الخام والكربوهيدرات الكلية في المجموع الخضرى مقارنة بالنباتات غير المعاملة. ومن الناحية التشريحية، حدثت زيادات واضحة فى سمك جدار الساق والأنسجة المكونة له، أي البشرة والنسيج الأساسى وعدد الحزم الوعائية. بالإضافة إلى حدوث زيادة في سمك العرق الوسطى لورقة العلم وسمك النصل والأنسجة المكونة لهما مع جميع معاملات مستخلص العرقسوس. وهذه التغيرات التشريحية الإيجابية، متناسقة مع النمو القوي الذي تم الحصول عليه وتفسر الانتاجية العالية من الحبوب التى تم تسجيلها مع معاملات مستخلص العرقسوس. حيث أدت جميع المعاملات إلى حدوث زيادة معنوية في محصول الحبوب والصفات المساهمة فيه مقارنة بالنباتات غير المعاملة. كان محصول الحبوب الذي تم الحصول عليه ذو جودة عالية بسبب التأثيرات المحسنة لمعاملات مستخلص العرقسوس على تعديل علاقة المصدر بالمخزن (الحبوب) بما يتماشى مع زيادة توجيه المغذيات ونواتج البناء الضوئى نحو الحبوب النامية. لذلك احتوت الحبوب الناضجة على تركيزات أعلى من النيتروجين والفوسفور والبوتاسيوم والبروتين الخام والكربوهيدرات الكلية مقارنة بالنباتات غير المعاملة. علاوة على ذلك ، أظهرت النتائج أن معاملة مستخلص العرقسوس بتركيز 5 جم لتر$^{-1}$ كانت هى الأكثر فاعلية.

لذلك، توصي الدراسة باستخدام مستخلص العرقسوس و بتركيز 5جم لتر$^{-1}$كمحسن نمو عضوي لتحسين نمو وانتاجية القمح وتقليل مخاطر استخدام منظمات النمو الصناعية.

DJAS., Volume (2), Issue (I), 2023