

## EFFECT OF GROWTH STIMULANT SUBSTANCES ON THE PRODUCTIVITY AND QUALITY OF SOME EGYPTIAN CLOVER (*TRIFOLIUM ALEXANDRINUM*, L.) VARIETIES

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**ABSTRACT:** Two field experiments were carried out at the Experimental Farm, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt during 2015/2016 and 2016/2017 seasons to investigate the effect of some plant stimulant substances, i.e control, ascorbic acid (1 g/L), citric acid (1 g/L), humic acid (2 g/L) and salicylic acid (0.5 g/L) as foliar application on vegetative growth performance (plant height, number of leaves and stems/m<sup>2</sup>, leaf area/m<sup>2</sup> and total plant dry weight/m<sup>2</sup>), photosynthetic pigments (total chlorophyll), crude protein % in the plant, forage yield (fresh and dry forage /fed) and seed yield and its components (number of heads/m<sup>2</sup>, number of seeds/head, 1000-seed weight, seed weight/head and seed yield/fed) of some Egyptian clover varieties (Serw1, Sakha 4, Helaly, Gemmeiza 1 and Giza 6). The data indicated that Helaly variety surpassed the other varieties in most growth characters (plant height, number of leaves/m<sup>2</sup>, leaf area/m<sup>2</sup> and total dry weight/m<sup>2</sup>), total chlorophyll, crude protein%, fresh and dry forage yields/fed and seed production (number of seeds/head, seed weight/head and seed yield /fed). However, Gemmeiza 1 variety surpassed the other varieties in number of stems/m<sup>2</sup> and number of heads/m<sup>2</sup>. On the other hand, Serw1 variety gave the highest value of 1000-seed weight in both seasons. Foliar application of humic acid (HA) was superior to the other rest stimulant substances in most growth and forage yield characters. However, Salicylic acid (SA) produced generally the highest values of CP% and seed yield and its components. On the other hand, unsprayed clover plants (control) gave the lowest values of characters studied. The interactions between Egyptian clover varieties and stimulant substances were found to be significant for most characters under study. It could be concluded that Helaly variety treated with HA being the most effective combined treatment for maximizing forage yield/fed, while the same variety treated with SA produced the highest values of seed yield/fed of Egyptian clover under the experiment conditions. Seasonal fresh and dry forage yields /fed were significantly and positively correlated with most characters studied except number of stems/m<sup>2</sup>, number of seeds/head and seed weight/head in the first season and 1000-seed weight in the two seasons. Also, seed yield/fed was significantly positively associated with all characters studied except 1000-seed weight in both seasons.

**Key words:** Egyptian clover, varieties, stimulant substances, Forage production and quality.

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### INTRODUCTION

Egyptian clover (*Trifolium alexandrinum*, L.), namely also (berseem), is one of the best annual legume crops for winter forage production. The advantage of the clover crop lies in its multi-cut type (up to 4 cuts), long duration of fresh yield availability (November to May), high fresh forage yield (35-40 ton/fed), good forage quality (17-20% crude protein) and high palatability for livestock feed. Berseem forage is superior to

the grasses forage crops in protein and mineral contents (Laghari *et al.*, 2000). It plays vital role in soil improvement as well as excellent plant for suppressing and controlling weeds. Also, it is can be environmental friendly crop because lower or no pesticides are used. In Egypt, the cultivated area devoted to Egyptian clover is about 1.624 million fed. (0.228 million fed for catch crop, 1.298 million fed for permanent crop and 0.0977 million fed for seed

production) produced about 42 million ton fresh forage and about 27669 ton seeds (Statistics of Ministry of Agriculture and land reclamation Economic Affairs Sector, Egypt 2015). Therefore, it is necessary to increase the productivity of Egyptian clover to meet the increasing demand for animal feed.

A great deal of attention has been given to raise clover productivity by using the most suitable agricultural practices such as growing high yielding varieties and pay attention to spraying with plant stimulant substances. Egyptian clover varieties are much differed in their needs for climatic conditions and nutrition and consequently differed in their growth behavior. In this respect, previous studies found high variations among the Egyptian clover varieties in their growth characters (Tarrad and Zayed, 2009 and Hussein *et al.*, 2012), forage production (Abdel-Galil *et al.*, 2007 and Ahmed and Fateh, 2016) and seed production (El-Nouby *et al.*, 2008).

Recently, many attempts have been done for using some new stimulant substances such as ascorbic acid (AA), citric acid (CA), humic acid (HA) and salicylic acid (SA) for maximizing the forage and seed production of Egyptian clover. Exogenous application of AA (called vitamin C) has been very effective in improving plant growth and development by altering oxidative defense system, phytohormone signaling, cell expansion, ion transports and other related processes under stress or non-stress conditions (Darvishan *et al.*, 2013). It plays an important role in electron transport system and regulates plant growth and development (El-Kobisy *et al.*, 2005). Moreover, Barth *et al.* (2006) found also that AA played a great effect on physiological processes such as cell division, plant growth and the biosynthesis of cell wall, metabolites and phytohormones, renovation of chloroplast and mitochondrial membranes. Application of CA to plants significantly increased internal CA concentration in plant

tissues and improved plant growth and stress tolerance during exposure to saline stress conditions (Sun and Hong, 2011). HA improved plant physiological processes by enhancing the availability of major and minor nutrients as well as enhancing the vitamins, amino acids, and also auxin and cytokinin contents of the plants (Vanitha and Mohandass, 2014). Moreover, Chen and Aviad (1990) found that HA compounds have various biochemical effects either at cell wall, membrane level or in the cytoplasm, including increased photosynthesis and respiration rates in plants, enhanced protein synthesis and plant hormone-like activity and enhancing uptake of macronutrients (N, P and S). SA has important role in many plant physiological processes such as photosynthesis, transpiration, nutrient uptake, and chlorophyll and protein synthesis, endogenous signaling and mediating in plant defense mechanisms against biotic and abiotic stresses (Wahid *et al.*, 2007). Moreover, Hayat *et al.* (2010) told that SA played an important role in retards ethylene synthesis, stimulate photosynthetic machinery, increases the chlorophyll content and increase grain yield in cereals and legumes crops under high temperature stress condition.

Therefore, the objective of this investigation is to study the effect of foliar application of some growth stimulant substances on vegetative growth, photosynthetic pigments, quality and productivity of forage and seed yield of some Egyptian clover varieties for finding a way to increase its productivity and quality.

## **MATERIALS AND METHODS**

Two field experiments were carried out at the Experimental Farm, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt (latitude 30°31'42"N, longitude 31°04'08"E) to study the effect of some plant stimulant substances on vegetative growth,

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photosynthetic pigments, protein content and forage and seed yields of some Egyptian clover (*Trifolium alexandrinum*, L.) varieties during 2015/2016 and 2016/2017 seasons. Each experiment included twenty five treatments which were the combination of five varieties (Serw 1, Sakha 4, Helaly, Giza 6 and Gemmeiza 1) and five plant stimulants substances, i.e control (tap water), ascorbic acid (at a rate of 1 g/L) citric acid (at a rate of 1 g/L), humic acid (at a rate of 2 g/L) and salicylic acid (at a rate of 0.5 g/L).

The treatments were arranged at random in a split plot design with three replications. The main plots were allocated for Egyptian clover varieties. While, the stimulants substances were assigned at random in the sub-plots. The tested varieties seeds were obtained annually from the Forage Crops Research Section, Field Crops Research Institute, ARC, Giza, Egypt. The tested stimulant substances were obtained from El-Gomhouria Company, Cairo, Egypt. They were foliar applied twice at 25 and 35 days after sowing "DAS" before the first cut and repeated at 10 and 20 days after each cut. The volume of water used in each foliar application amounted to 200 L water / fed. The characteristics of the tested stimulant substances are shown in Table (1-a).

Seeds of Egyptian clover varieties were sown on 12<sup>th</sup> and 8<sup>th</sup> October in the first and second season, respectively by hand broadcasting at a rate of 25 kg seeds / fed after flooding the field with water. The preceding sequence crops before the present investigation are berseem followed by maize in the first season and berseem followed by fallow in the second season. The area of each experimental plot was 9 m<sup>2</sup> (3 m X 3 m). During soil preparation, phosphatic fertilizers was added as the rate of 15 kg P<sub>2</sub>O<sub>5</sub> /fed in the form of calcium superphosphate (15% P<sub>2</sub>O<sub>5</sub>). After plant emergence, 15 kg N /fed was added to plants through the first irrigation (10 DAS) in the form of ammonium nitrate (33.5 % N).

The normal cultural practices of growing Egyptian clover plants under Menoufia government were applied as recommended. Four cuts were done at 55,100,140 and 175 DAS for first, second, third and fourth cuts, i.e. 55, 45, 40 and 35 days intervals between cuts, respectively during the both growing seasons. After 4<sup>th</sup> cut, the plants were lefted in each experimental plot to produce the seeds. Experimental soil samples of the investigation location were randomly collected before sowing from depths of 0–30 using an auger for estimating some mechanical and chemical properties of soil as described by Jackson (1973) and Chapman and Pratt (1978). The data of soil analysis are presented in Table (1-b).

### **Plant measurements**

- 1- Vegetative growth characters:** Four samples were taken randomly from each experimental plot at the four cuts to estimate plant height (cm), number of stems / m<sup>2</sup>, number of leaves / m<sup>2</sup>, leaf area / m<sup>2</sup> (cm<sup>2</sup>) "using disc method" and total dry weight / m<sup>2</sup> (g.).
- 2- Photosynthetic pigments:** Total chlorophyll (Chl. a +b) in leaves was estimated at each cut using SPAD meter.
- 3- Crude protein content:** Crude protein % in whole plant was calculated by multiplying N % x 6.25. Nitrogen percentage was determined by using the micro Kjeldahl method as described by A.O.A.C. (2000).
- 4- Forage yields:** The central area (2 x 2 m) of each plot was manually harvested to estimate the fresh and dry forage yields/fed (ton) as well as dry matter percentage (DM %).
- 5- Seed yield and its components:** At seed maturity stage (238 and 240 DAS in the first and second seasons, respectively), seed yield and its components, i.e. number of heads / m<sup>2</sup>, number of seeds / head, 1000-seed weight (g.), seed weight /head (g.) and seed yield / fed (kg) were estimated.

Table (1-a): The characteristics of the four tested stimulant substances.

Characters	Ascorbic acid (AA)	Citric acid (CA)	Humic acid (HA)	Salicylic acid (SA)
Appearance	White or slightly yellow powder	White crystalline powder	Dark brown to black powder	White to off-white crystalline powder
Chemical formula	$C_6H_8O_6$	$C_6H_8O_7$	$C_9H_9NO_6$	$C_7H_6O_3$
IUPAC name	(R)-3,4-dihydroxy-5-((S)-1,2-dihydroxyethyl)furan-2(5H)-one	2-Hydroxypropane-1,2,3-tricarboxylic acid	bicyclic [2.2.1] hept-5-ene-2,3 dicarboxylic	2-Hydroxybenzoic acid
Molecular Weight	176.12 g/mol	192.12 g/mol	226.139 g/mol	138.12 g/mol
Solvent solubility	Soluble in water and sparingly soluble in alcohol	Soluble in water and alcohol	Soluble in water and alkaline but insoluble in strong acids	Soluble in alcohol, ether, boiling water, chloroform, benzene and acetone
References	<a href="https://en.wikipedia.org/wiki/Vitamin_C">https://en.wikipedia.org/wiki/Vitamin_C</a>	<a href="https://en.wikipedia.org/wiki/Citric_acid">https://en.wikipedia.org/wiki/Citric_acid</a>	<a href="https://www.indiamart.com/proddetail/humic-acid-3688849248.html">https://www.indiamart.com/proddetail/humic-acid-3688849248.html</a> <a href="http://www.webqc.org/mmcalc.php">http://www.webqc.org/mmcalc.php</a>	<a href="https://en.wikipedia.org/wiki/Salicylic_acid">https://en.wikipedia.org/wiki/Salicylic_acid</a>

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**Table (1-b): Soil mechanical and chemical properties of the experimental site during 2015/2016 and 2016/2017 seasons.**

Soil properties	Mechanical analysis				Chemical analysis					
	Sand %	Silt %	Clay %	Texture	EC. (dS/m)	pH	O.M %	N (ppm)	P (ppm)	K (ppm)
2015/2016	21.29	40.16	38.55	Clay loam	0.73	7.5	1.79	29.28	9.37	305.23
2016/2017	21.34	41.85	36.81	Clay loam	0.71	7.4	1.81	25.62	8.74	274.56

**6- Phenotypic correlation:** Simple correlation coefficients between fresh forage, dry forage and seed yields/fed were calculated with each of plant height, number of leaves and stems/m<sup>2</sup>, leaf area/m<sup>2</sup>, total dry weight/ m<sup>2</sup>, total chlorophyll, crude protein%, fresh and dry forage yields/fed, number of heads/m<sup>2</sup>, number of seeds/head, 1000-seed weight, seed weight/head and seed yield/fed.

**Statistical analysis**

All measurements data during the two seasons were analyzed according to the methods described by Snedecor and Cochran (1980). Duncan's multiple range test (Duncan, 1955) was used to compare between the treatments mean at probability of 0.05. The mean values within each column followed by same letters are not significantly different. Statistical analysis was done using the CoStat package program, version 6.311 (Cohort software, USA).

**RESULTS AND DISCUSSION**

**1- Growth characters:**

The average performance data of the tested five Egyptian clover varieties (Serw 1, Sakha 4, Helaly, Giza 6 and Gemmeiza 1) for the vegetative growth characters studied, i.e. plant height, number of stems/m<sup>2</sup>, number of leaves/m<sup>2</sup>, leaf area /m<sup>2</sup> and total dry weight/m<sup>2</sup> at four cuts and their averages in 2015/2016 and 2016/2017 seasons are presented in Table (2). The data showed that the five tested clover varieties

significantly differed in their plant height in the four cuts and their averages in both seasons. As an average of the four cuts, Helaly variety had the significant tallest plants compared to the other tested varieties in both seasons but without significant differences with Gemmeiza 1 variety in the first season and with Serw1 and Gemmeiza1 varieties in the second season. However, Giza 6 and Sakha 4 varieties recorded the shortest plants in both seasons. With regard to the number of stems and leaves/m<sup>2</sup>, it can be noted that there are significant variation among the five tested clover varieties in the four cuts and its average in both seasons. The highest significant values of those characters were obtained by Gemmeiza1 variety (in the first season) and Gemmeiza 1 and Sakha 4 varieties (in the second season) for number of stems/m<sup>2</sup>, while by Helaly and Sakha 4 varieties (in the first season) and Helaly variety (in the second season) for number of leaves/m<sup>2</sup>. The data in Table (2) show also that there are significant differences among the tested Egyptian clover varieties in their leaf area/m<sup>2</sup> in the four cuts in the two seasons. As an average of the four cuts, Helaly variety produced the highest values. While, the lowest values were recorded by Giza 6 clover variety in both seasons. The superiority of Helaly variety in the leaf area/m<sup>2</sup> may be due to the raising in its number of leaves/m<sup>2</sup> as previously discussed. In addition, the data of total dry weight /m<sup>2</sup> showed that Helaly variety was significantly superior to the most tested

Table (2): Vegetative growth characters of some Egyptian clover varieties at four cuts during 2015/ 2016 and 2016/2017 seasons.

Characters	Varieties	2015/2016 season					2016/2017 season				
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Overall mean	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Overall mean
Plant height (cm)	Serw 1	65.66bc	70.23 b	76.40ab	86.25 a	74.64bc	60.44 a	78.77 a	86.75 ab	87.35 a	78.32 a
	Sakha 4	65.07 c	69.04 b	77.72 a	85.75 a	74.40bc	55.68 b	72.35 b	83.80bc	85.05bc	74.22 b
	Helaly	68.12 a	74.16 a	79.01 a	87.28 a	77.14 a	60.81 a	79.05 a	89.57a	87.52 a	79.23 a
	Giza 6	64.86 c	71.17ab	72.33 b	82.99 b	72.84 c	58.75ab	72.40 b	82.28 c	84.81 c	74.56 b
	Gemmeiza	67.20ab	72.17ab	76.42ab	86.09 a	75.47 ab	59.51 a	76.01 a	85.32 bc	87.05ab	76.97 a
	Gemmeiza	1204.05b	709.05b	793.05b	1051.95bc	939.53 c	1216.05bc	798.00 b	806.25 ab	952.05 ab	943.09 b
Number of stems / m <sup>2</sup>	Sakha 4	1405.95b	786.00ab	864.60b	1135.05a	1047.90b	1372.05ab	807.00 b	859.05 a	1015.05 a	1013.29 a
	Helaly	1308.00b	790.95ab	850.35b	1024.95c	993.56bc	1216.95bc	829.95 ab	798.60 b	1008.00 a	963.38 b
	Giza 6	1315.05b	778.95ab	799.65b	1041.00bc	983.66bc	1176.00c	748.05 c	778.80 b	886.95 b	897.45 c
	Gemmeiza	1726.95a	829.05a	981.15a	1071.00b	1152.04a	1497.00a	847.95 a	812.85 ab	973.05 a	1032.71 a
	Serw 1	6508.05c	3630.00b	3852.00b	6484.95bc	5118.75c	6112.05b	3723.00a	4108.05b	5260.95b	4801.01c
	Sakha 4	7231.05a	4063.05a	4519.05a	6643.05ab	5614.05a	6391.95ab	3865.95a	4167.00b	5863.05ab	5071.99b
Number of leaves / m <sup>2</sup>	Helaly	7413.00a	4095.00a	4549.95a	6826.05a	5721.00a	6775.05a	4005.00a	4504.05a	5926.05a	5302.54a
	Giza 6	6700.95bc	3826.05b	3571.05b	6369.00c	5116.76c	6079.05b	3186.00b	3778.95c	5257.05b	4575.26d
	Gemmeiza	7006.95ab	3841.95b	3955.95b	6529.95bc	5333.70b	6193.95b	3855.00a	4158.00b	5577.00ab	4945.99bc
	Serw 1	46239.60bc	48313.35b	57322.65ab	54756.60b	51658.05c	43251.15ab	41647.65bc	45762.75c	45806.85b	44117.10cd
	Sakha 4	54791.70ab	52405.35ab	62710.80ab	62729.70a	58159.39ab	42877.95ab	46617.75ab	50865.00b	50241.00a	47650.43b
	Helaly	57851.40a	54100.95a	66420.45a	64327.35a	60675.04a	46427.85a	48162.60a	57658.35a	52450.50a	51174.83a
Leaf area (cm <sup>2</sup> / m <sup>2</sup> )	Giza 6	43455.45c	48109.80b	51602.70b	54726.45b	49473.60c	39970.80b	37525.50c	45145.20c	45466.20b	42026.93d
	Gemmeiza	47552.40bc	51568.80ab	58192.95ab	59805.75ab	54279.98bc	42691.20b	46421.70ab	47748.15bc	48360.00ab	46305.26bc
	Serw 1	368.85bc	413.25b	486.60bc	663.45ab	483.04c	297.75a	406.05ab	503.40b	562.2cd	442.35c
	Sakha 4	428.55a	462.75a	531.9ab	687.90ab	527.78ab	302.85a	435.90a	535.05ab	644.55ab	479.59ab
	Helaly	442.05a	475.35a	572.40a	714.60a	551.10a	309.60a	445.65a	562.65a	668.25a	496.54a
	Giza 6	324.90c	402.45b	432.90c	645.45b	451.43d	276.75b	349.35b	427.35c	552.30d	401.44d
Total dry weight (g/ m <sup>2</sup> )	Gemmeiza	391.95ab	428.40b	497.40abc	687.75ab	501.38bc	299.70a	430.5a	522.15b	603.90bc	464.06bc



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varieties without significant differences with Sakha 4 variety. However, Giza 6 variety was significantly inferior to the other tested varieties for such trait. These results are fairly true generally at the four cuts and their average in both seasons. The superiority of Helaly variety in the total dry weight /m<sup>2</sup> may be attributed to the increase in its plant height and number of leaves /m<sup>2</sup> which caused an increase in the amount of metabolites and synthesized and this in turn increased the capacity of dry matter accumulation in the plant. In comparison among the four cuts, over all the tested varieties, it can be noticed that the values of plant height, leaf area/m<sup>2</sup> and total dry weight/m<sup>2</sup> were increased gradually and progressively from 1<sup>st</sup> cut up to later cut (4<sup>th</sup> cut). The increase in the those values in the late cut may be due to the suitable climatic conditions prevailing in such cut time as well as the increase in the dry matter percentage in the plant organs. In this concern, many investigators found variation among some (*Trifolium alexandrinum*, L.) varieties in their growth characters such as plant height (Sarhan and Abd EL-Maksoud, 2002; Yousef and Abdel-Mottaleb, 2009; Radwan *et al.*, 2014 and Mohamed, 2017), number of stems/plant (Al-Suhaibani and Al-Doss, 2004 and Radwan *et al.*, 2014), number of leaves/plant (Sarhan and Abd EL-Maksoud, 2002; Naeem *et al.*, 2006; Tarrad and Zayed, 2009; Ewis and Rajab, 2016 and Fatima and Mahmood, 2016), leaf area/plant (Chaudhary and Agrawal, 2013) and total dry weight/plant (Hussein *et al.*, 2012).

Table (3) included the data of growth characters studied as affected by the foliar application of four stimulant substances, i.e. ascorbic acid (AA), citric acid (CA), humic acid (HA) and salicylic acid (SA) compared to the foliar application of water as a control treatment during four cuts and their average in 2015/2016 and 2016/2017 seasons. The data show that plant height was significantly affected by the foliar application of the tested stimulant substances in the four cuts

and their average in the two growing seasons. The tallest plants were obtained by foliar application with HA in the four cuts in both seasons. As an average of the four cuts, foliar application of HA significantly increased plant height by 7.48 and 5.43% more the control treatment in the first and second seasons, respectively. Other tested stimulant substances, i.e. SA, CA and AS exhibited moderate values in a descending order in both seasons. In addition, the values of number of stems and leaves/m<sup>2</sup> were significantly increased by foliar application of the four stimulant substances as compared with the control treatment (spray with water) at all cuts studied and over their averages in both seasons. The foliar application of SA and HA produced the higher values of number of stems and leaves/m<sup>2</sup>, respectively than the rest stimulant substances, i.e. CA and AS in a descending order in both seasons. As an average of the four cuts, the increase percentage in the number of stems/m<sup>2</sup> due to the application of SA amounted to 10.42 and 11.82% as well as the increase percentage in the number of leaves/m<sup>2</sup> due to the application of HA amounted to 10.50 and 21.18% more than the control treatment in the first and second seasons, respectively. Moreover, the values of leaf area /m<sup>2</sup> were significantly increased by foliar application of the tested stimulant substance treatments compared to the control treatment in most cuts and their averages in both seasons. This increase amounted to 17.17, 12.68, 10.46 and 6.04% when the plants were sprayed with HA, SA, CA, and AS compared to the control treatment, respectively, as an average of the four cuts over the two seasons. From these results, it can be concluded that the stimulating effect of HA was more pronounced than the other tested stimulant substances for enhancing the leaf area/m<sup>2</sup> which may be due to the increase in the number of leaves/m<sup>2</sup> obtained by the application of HA as previously discussed.

Table (3): Vegetative growth characters of Egyptian clover as affected by some stimulant substances at four cuts during 2015/ 2016 and 2016/2017 seasons.

characters	Stimulant substances	2015/2016 season					2016/2017 season				
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Overall mean	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Overall mean
Plant height (cm)	Control	64.31 c	70.16 b	72.08 c	82.67 b	72.31 d	57.55 b	73.65 b	83.81 b	82.24 c	74.31 c
	Ascorbic acid	65.31 bc	69.76 b	76.27 b	85.28 b	74.16 c	57.67 b	75.91 ab	86.12 ab	85.85 b	76.39 b
	Citric acid	66.71 ab	70.29 b	77.16 ab	84.92 b	74.77 bc	58.95 b	74.13 b	85.47 ab	87.69 ab	76.56 b
	Humic acid	68.42 a	73.37 a	78.95 a	90.12 a	77.72 a	62.07 a	77.64 a	85.82 ab	87.87 ab	78.35 a
	Salicylic acid	66.19 b	73.19 a	77.42 ab	85.38 b	75.55 b	58.96 b	77.25 a	86.50 a	88.14 a	77.71 a
Number of stems / m <sup>2</sup>	Control	1356.00 a	697.05 b	788.70 b	1018.05 c	964.95 b	1110.00 d	786.00 b	821.70 a	925.05 b	910.69 c
	Ascorbic acid	1359.00 a	813.00 a	862.35 a	1044.00 bc	1019.59 ab	1245.00 c	781.95 b	789.75 a	970.95 b	946.91 b
	Citric acid	1380.00 a	798.00 a	863.55 a	1057.95 abc	1024.88 ab	1324.95 bc	802.95 ab	785.40 a	928.05 b	960.34 b
	Humic acid	1420.95 a	766.05 ab	882.15 a	1098.00 ab	1041.79 a	1342.05 b	829.05 a	842.55 a	1033.05 a	1011.68 a
	Salicylic acid	1444.05 a	820.05 a	892.05 a	1105.95 a	1065.53 a	1447.05 a	832.05 a	816.15 a	978.00 ab	1018.31 a
Number of leaves / m <sup>2</sup>	Control	6618.00c	3858.00a	3400.95c	6409.95b	5071.73 c	5157.00c	3532.95b	3958.95b	4972.95b	4405.46c
	Ascorbic acid	6733.95 bc	3865.05a	4105.95b	6504.00ab	5302.25 b	6148.05b	3601.95b	3961.05b	5710.95a	4855.50b
	Citric acid	7176.00ab	3901.05a	4096.05b	6583.95ab	5439.25ab	6165.00b	3609.00b	4240.95ab	5713.95a	4932.23b
	Humic acid	7257.00a	3895.05a	4546.05a	6720.00a	5604.53a	7044.00a	4264.05a	4300.05a	5746.95a	5338.76a
	Salicylic acid	7075.05abc	3930.75a	4299.00ab	6634.95ab	5484.94a	7038.00a	3627.00b	4255.05a	5739.00a	5164.76a
Leaf area (cm <sup>2</sup> / m <sup>2</sup> )	Control	46329.15b	46346.40b	51354.00b	58765.80abc	50698.84d	39825.90b	41794.65b	47739.75c	38285.85c	41911.54d
	Ascorbic acid	50802.15ab	45509.85b	61343.10a	54877.20c	53133.08c	43101.60ab	42005.70b	47855.85bc	46913.10b	44969.06c
	Citric acid	51332.85ab	53490.60a	58980.45a	57017.40bc	55205.33bc	45908.70a	42537.00b	50048.40ab	49336.65ab	46957.69bc
	Humic acid	52461.00a	55480.05a	62524.35a	63257.25a	58430.66a	43369.20a	50374.35a	51076.35a	54859.05a	49919.74a
	Salicylic acid	48965.55ab	53671.35a	62047.65a	62428.20ab	56778.19ab	43013.55ab	43663.65b	50458.80a	52929.90ab	47516.48b
Total dry weight (g/ m <sup>2</sup> )	Control	355.95b	395.10c	413.70b	642.60b	451.84d	268.80c	393.90b	476.70b	517.20c	414.15d
	Ascorbic acid	389.55ab	417.90bc	519.30a	660.15b	496.73c	288.75b	394.80b	502.80ab	592.95b	444.83c
	Citric acid	395.10a	441.90ab	519.00a	671.10ab	506.78bc	309.15a	402.45b	516.15a	606.45b	458.55b
	Humic acid	412.65a	466.50a	538.35a	712.20a	532.43a	309.45a	448.35a	531.30a	663.45a	488.14a
	Salicylic acid	403.20a	460.95a	530.85a	712.95a	526.99ab	310.50a	427.80ab	523.80a	651.00a	478.28a



## **Effect of growth stimulant substances on the productivity and quality .....**

Moreover, the foliar application of the tested stimulant substances had a significant stimulating effect on the total dry weight/m<sup>2</sup> as compared with control treatment in the four cuts and all their averages in the two seasons. The greatest values of total dry weight/m<sup>2</sup> were obtained by foliar application of HA followed by SA, CA and AS in a descending order in the four cuts and their averages in the two seasons. As an average of the four cuts, the foliar application of HA increased the total dry weight/m<sup>2</sup> by 17.83% and 17.86% in the first and second seasons, respectively more than the untreated plants (control treatment). The favorable effects of HA on the total dry matter/m<sup>2</sup> may be attributed to its stimulation on the growth characters, i.e. plant height as well as number of stems and leaves /m<sup>2</sup> as previously discussed. The superiority effect of HA obtained herein may be due to its role in enhancing the uptake of macronutrients such as N, P and S (Chen and Aviad, 1990) or micronutrients such as Fe, Zn, Cu, Mn (Chen *et al.*, 1999). Moreover, the superiority effect of SA may be due to its role in ion uptake and transport (Khan *et al.*, 2003), the regulation of plant growth and development (Horvath *et al.*, 2007) and enhancement of plant resistance against different biotic and abiotic stress through different mechanism (Kadioglu *et al.*, 2011). In this concern, many investigators found improving effects of some tested stimulant substances such as HA on plant height of clover (El-Hefny, 2010; Daur, 2014 and El-Maaz and Ismail, 2016), leaf area of cowpea (El-Hefny, 2010) and peas (Osman and Rady, 2012) and total dry weight of clover (Abbas *et al.*, 2013) and cowpea (El-Hefny, 2010) as well as AS on number of stems / clover plant (Kumar *et al.*, 2013 and Rani *et al.*, 2017).

The interaction between the tested varieties and stimulant substances declare that the differences among the interaction treatments were fluctuated in their significances from cut and/or season to

other. Therefore, the interaction effects will be discussed herein only for the average or total of the four cuts. The interactions between varieties and stimulant substances were found to be significant for plant height, number of stems / m<sup>2</sup> and total dry weights / m<sup>2</sup>, as an average of the four cuts, in the two growing seasons (Figure 1). On the other hand, the interactions between the two factors were not significant for number of leaves and leaf area/ m<sup>2</sup> indicating that each factor affected such traits independently, so, those data were excluded. The data illustrated in Figure (1) showed that foliar application of Helaly variety plants with HA was the more effective treatment in increasing plant height in the two seasons, where such treatment produced the tallest plants (82.56 and 82.09 cm) in the first and second seasons, respectively compared to the other tested treatments. On the other hand, the untreated plants of Giza 6 variety with any stimulant substances (control treatment) recorded the lowest values (70.75 and 72.15 cm) in the same respective seasons. Moreover, it is evident from the same figure that the number of stems /m<sup>2</sup> recorded the highest values (1213.75 and 1177.50 stems/m<sup>2</sup>) when the plants of Gemmeiza 1 variety were sprayed by SA in the first and second seasons, respectively. However, Serw 1 and Giza 6 varieties had their lowest values (776.00 and 825.38 stems /m<sup>2</sup>) in the first and second seasons, respectively when the plants were untreated with any stimulant substances (control treatment). The data of total dry weight show that the plants of Helaly which were sprayed with HA produced the greatest values (593.68 and 530.43 g/ m<sup>2</sup>) while, the lowest values (405.07 and 377.54 g/ m<sup>2</sup>) were obtained by the untreated plants of Serw 1 variety in both seasons. The increment percentage obtained by the best interaction treatment (Helaly + HA) amounted to 46.56 and 40.50 % more than the lowest one (untreated plants of Serw1 variety) in the first and second seasons, respectively.

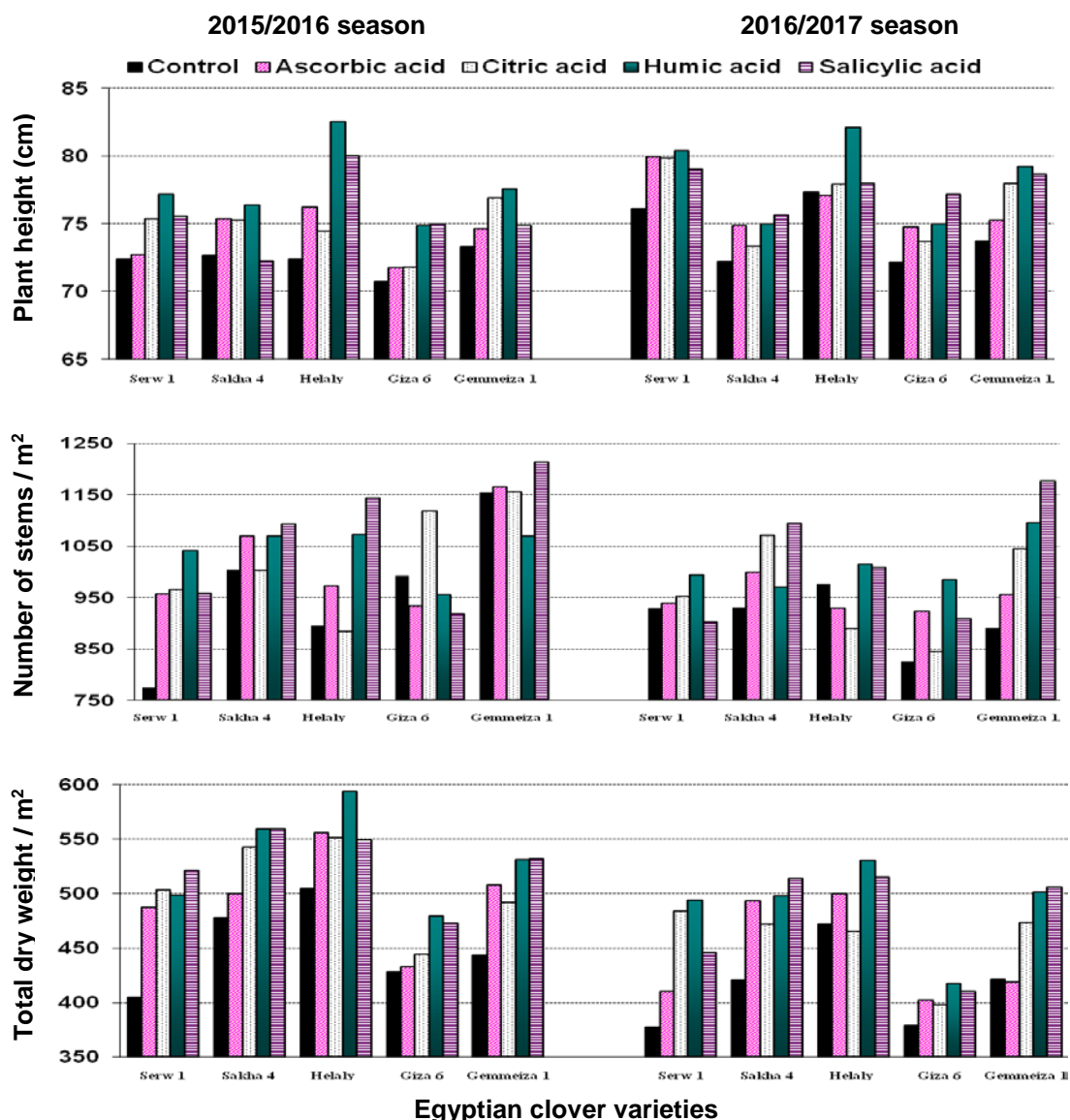


Figure (1): Effect of the interaction between Egyptian clover varieties and plant stimulant substances on some growth characters during 2015/2016 and 2016/2017 seasons.

## 2- Photosynthetic pigments:

The data in Table (4) showed that there are significant differences among the tested clover varieties for photosynthetic pigments (total chlorophyll) in the leaves at the first, second and third cut in the first season as well as at the second, third and fourth cuts in the second season. Helaly variety had higher significant values of total chlorophyll than the rest tested varieties which did not appeared any significant variation among them in this respect in both seasons. The

superiority of Helaly variety in the total chlorophyll might be owing to its superior in the number and area of leaves/m<sup>2</sup> as previously discussed. In this concern, Hussein *et al.* (2012) and Chaudhary and Agrawal (2013) found variation among some clover varieties in their contents of total chlorophyll in favour of the varieties which had more number of leaves.

The data showed in Table (5) that the foliar application of the four tested stimulant

Table (4): Total chlorophyll and crude protein content of some Egyptian clover varieties at four cuts during 2015/2016 and 2016/2017 seasons.

Characters	Varieties	2015/2016 season				Overall mean	2016/2017 season				Overall mean
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	
Total chlorophyll (SPAD value)	Serw. 1	44.99 b	47.41 c	51.77 ab	52.62 a	49.20 c	44.46 a	46.50 a	49.30 b	49.02 b	47.32 b
	Sakha 4	45.21 b	48.85 b	52.97 ab	52.68 a	49.93 b	44.46 a	45.99 ab	50.53 ab	51.04 ab	48.00 b
	Helaly	46.26 a	50.48 a	53.35 a	52.93 a	50.75 a	45.86 a	46.61 a	52.04 a	52.21 a	49.18 a
	Giza 6	44.64 b	48.72 b	51.05 b	52.09 a	49.12 c	44.44 a	45.67 b	50.53 ab	50.75 ab	47.84 b
	Gemmeiza 1	44.63 b	49.50 b	52.70 ab	52.27 a	49.78 b	44.23 a	46.22 a	50.54 ab	50.42 ab	47.85 b
	Serw. 1	18.27 b	17.95 b	18.02 a	16.24 c	17.62 d	18.47 b	18.59 a	16.89 b	16.08 c	17.51 c
Crude protein (%)	Sakha 4	19.53 a	18.94 ab	18.62 a	17.38 ab	18.62 ab	19.44 ab	18.79 a	17.44 ab	16.81 b	18.12 ab
	Helaly	19.52 a	19.29 a	19.33 a	17.93 a	19.02 a	19.71 a	19.31 a	17.92 a	17.64 a	18.65 a
	Giza 6	18.76 ab	18.97 ab	18.59 a	17.38 ab	18.43 bc	19.19 ab	18.77 a	17.45 ab	16.21 c	17.91 bc
	Gemmeiza 1	18.48 b	18.36 ab	18.15 a	16.94 b	17.98 cd	19.00 ab	18.73 a	17.21 ab	16.73 c	17.92 bc
	Serw. 1	18.27 b	17.95 b	18.02 a	16.24 c	17.62 d	18.47 b	18.59 a	16.89 b	16.08 c	17.51 c
	Sakha 4	19.53 a	18.94 ab	18.62 a	17.38 ab	18.62 ab	19.44 ab	18.79 a	17.44 ab	16.81 b	18.12 ab

Table (5): Total chlorophyll and crude protein content of Egyptian clover as affected by stimulant substances at four cuts during 2015/ 2016 and 2016/2017 seasons.

Characters	Stimulant substances	2015/2016 season				Overall mean	2016/2017 season				Overall mean
		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut		1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	
Total chlorophyll (SPAD value)	Control	44.48 b	49.17 a	50.38 b	48.32 b	48.09 b	43.42 c	44.14 c	48.45 b	48.04 c	46.01 c
	Ascorbic acid	44.89 b	48.64 a	52.73 a	53.38 a	49.91 a	45.05 ab	45.62 b	49.91 b	49.99 b	47.64 b
	Citric acid	45.07 ab	49.55 a	52.25 a	52.65 a	49.88 a	44.93 ab	46.92 a	49.75 b	50.09 b	47.92 b
	Humic acid	45.24 ab	48.16 a	53.47 a	54.20 a	50.27 a	44.53 b	47.41 a	52.31 a	52.73 a	49.24 a
	Salicylic acid	46.05 a	49.43 a	53.01 a	54.04 a	50.63 a	45.52 a	46.88 a	52.52 a	52.61 a	49.38 a
	Control	17.73 b	18.13 b	17.82 c	15.94 c	17.41 d	18.70 a	17.45 c	16.76 b	16.13 b	17.26 c
Crude protein (%)	Ascorbic acid	19.06 a	18.53 ab	18.06 bc	16.71 bc	18.09 c	19.04 ab	18.48 b	17.14 ab	15.98 b	17.66 b
	Citric acid	19.15 a	18.74 ab	18.05 bc	17.20 b	18.29 bc	19.19 ab	18.93 b	17.64 a	16.35 b	18.03 b
	Humic acid	19.21 a	18.88 ab	18.89 b	17.56 ab	18.64 b	19.36 a	19.59 a	17.63 a	17.29 a	18.47 a
	Salicylic acid	19.41 a	19.24 a	19.88 a	18.46 a	19.25 a	19.54 a	19.74 a	17.72 a	17.71 a	18.68 a
	Control	17.73 b	18.13 b	17.82 c	15.94 c	17.41 d	18.70 a	17.45 c	16.76 b	16.13 b	17.26 c
	Ascorbic acid	19.06 a	18.53 ab	18.06 bc	16.71 bc	18.09 c	19.04 ab	18.48 b	17.14 ab	15.98 b	17.66 b

substances caused an increase in the total chlorophyll in the leaves as compared with the control treatment in the four cuts and their averages in both season. This increase was more pronounced when the plants were sprayed with SA which amounted to 5.28 and 6.20% more than the untreated plants (control treatment), as an average of the four cuts in the first and second seasons, respectively. These increments may be due to the role of SA as antioxidant which led to prevent the rapid degradation of chlorophyll and consequently increased it in the cells. Moreover, it is clear from the results that there was a progressive increase in the values of total chlorophyll with increasing plant age up to the later cut (4<sup>th</sup> cut) when the plants were treated with the four tested stimulant substances, while the values of total chlorophyll in the untreated plants (control treatment) were increased gradually up to the 3<sup>rd</sup> cut only and then were declined at the cut (4<sup>th</sup>) in both seasons. This means that the plants treated with the tested stimulant substances enhanced the chlorophyll production and consequently delayed their maturity stage compared to

untreated plants which matured early. In this respect, many investigators found that chlorophyll content was increased by the application of some stimulant substances such as AA in Egyptian clover (Aly *et al.*, 2012), alfalfa (Arab and Ehsanpour, 2006 and Tarraf *et al.*, 2015) and lupine (El-Awadi *et al.*, 2016), SA in cowpea (Afshari *et al.*, 2013 and Umebese and Bankole, 2013) and HA in cowpea (Chatterjee and Choudhuri, 2012).

The interaction effect between Egyptian clover varieties and plant stimulant substances for total chlorophyll (overall mean of the four cuts in each season) are illustrated in Figure (2). The data show that Helaly and Sakha 4 varieties surpassed the other varieties in the total chlorophyll content when their plants were sprayed with CA or SA which produced the highest values (51.35) in the first season. Meanwhile, Helaly variety had the highest values of the total chlorophyll (50.81) when it was sprayed with SA in the second season. On the other side, the lowest values (46.80 and 44.73) were achieved by untreated plants of Serw1 variety in both growing seasons.

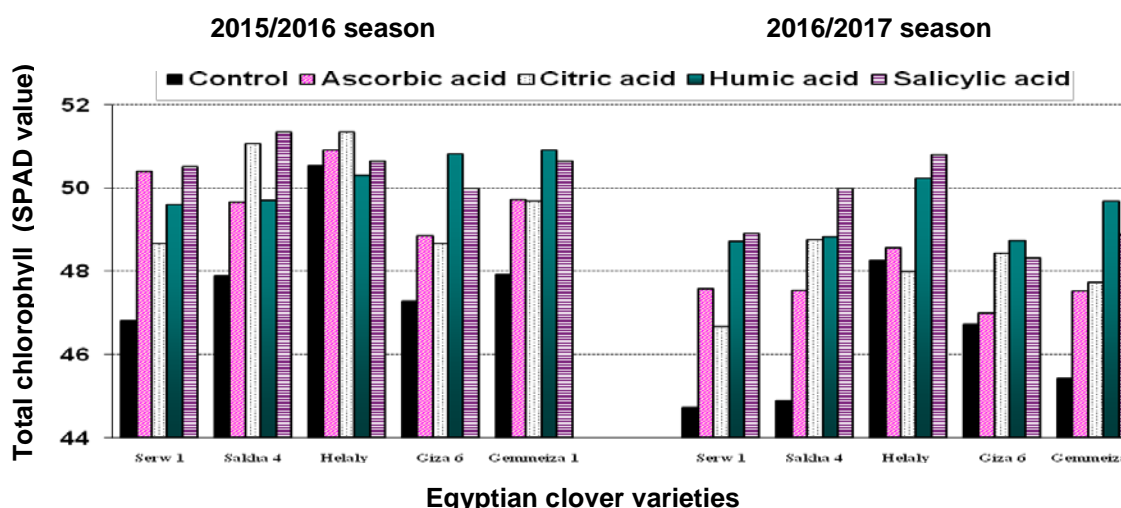


Figure (2): Effect of the interaction between Egyptian clover varieties and plant stimulant substances on total chlorophyll during 2015/2016 and 2016/2017 seasons.



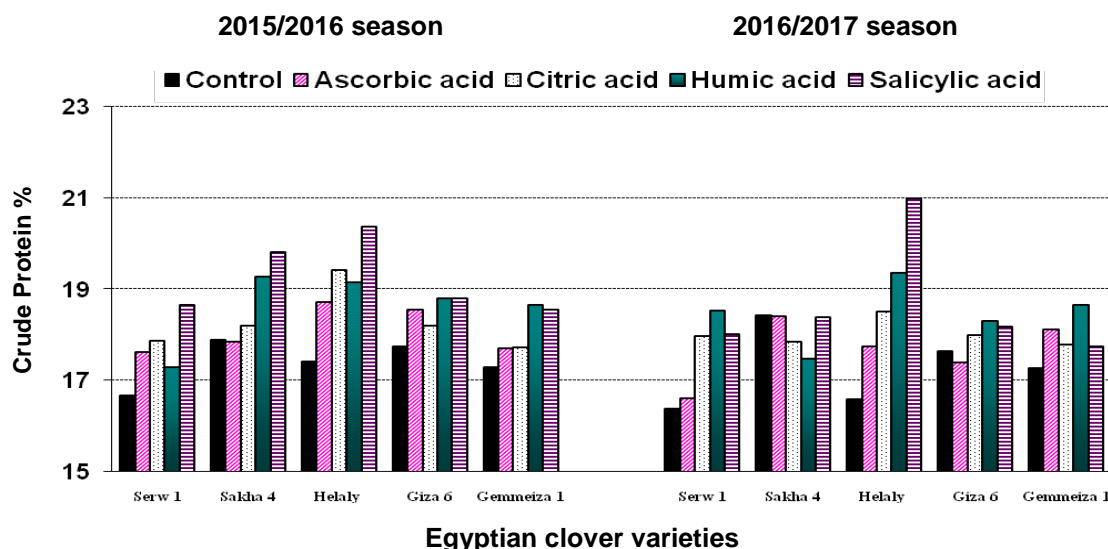
**3- Crude protein content:**

Data in Table (4) show the varietal differences among the five tested Egyptian clover varieties with regard to the crude protein percentage (CP %) in whole plant at four cuts and their averages in the two seasons. The varietal differences in the CP% of clover plants were generally significant during the most cuts and at their averages in both seasons. Helaly variety performed the highest values of CP % (19.017 and 18.645%) in the first and second seasons, respectively followed by Sakha4 variety without significant differences between them compared to the other tested varieties. On the other hand, it can be noticed that the values of CP% was decreased gradually from the first cut up to late cut (4<sup>th</sup> cut) as a mean of all tested clover varieties in the two seasons. In this concern, Seif and Saad (2014) found variation among some Egyptian clover varieties, i.e. Gemmeiza1, Sakha 4, Helaly, Giza 6 and Serw1 in their CP%.

The data in Table (5) showed that the differences among the stimulant substances

treatments were found to be significant for CP% in favour of SA which gave the highest values of CP % followed by HA, CA and AA in a descending order, while the untreated plants produced the lowest values. These results are mostly true in the four cuts and their average in the two seasons. In this concern, other investigators found enhancing in protein synthesis by the application of HA in common vetch (Saruhan *et al.*, 2011), SA in alfalfa (Morsy *et al.*, 2011) and AA in alfalfa (Tarraf *et al.*, 2015).

The interaction between the tested Egyptian clover varieties and foliar application of stimulant substances (the average of the four cuts) had significant effect on CP % in whole plants as shown in Figure (3). The data showed that the plants of Helaly variety that sprayed with SA produced the highest significant values of crude protein % (20.38 and 21.00 %) in 2015/2016 and 2016/2017 seasons, respectively. However, untreated plants of Serw variety produced the lowest significant values of CP % (16.68 and 16.38%) in the same respective seasons.



**Figure (3): Effect of the interaction between Egyptian clover varieties and plant stimulant substances on crude protein % during 2015/2016 and 2016/2017 seasons.**



#### 4- Forage yield:

The performances of the five tested clover varieties for fresh and dry forage yields /fed at each cut and their (seasonal yield) in both seasons are presented in Table (6). The data showed significant variation among the five varieties at different cuts and their total for fresh and dry forage yields /fed in the two seasons. It can be observed that the highest significant values of total fresh and dry forage yields/fed were obtained by Helaly variety followed by Sakha 4, Gemmeiza1, Serw1 and Giza 6 in a descending order, as an average of the two seasons. The superiority of Helaly variety in the forage production may be due to that it had more superiority in growth characters (plant height, number of leaves, leaf area and total dry weight /m<sup>2</sup>) than the other clover varieties as previously shown in Table (2). On the other hand, in comparison among the four cuts, the data show that the dry forage yield/fed was increased gradually and consistently from the first cut up to the late cut (4<sup>th</sup> cut) which produced the highest values in both seasons. The superiority of dry forage yield /fed in the 4<sup>th</sup> cut may be due to the higher dry matter % in such cut more than the other cuts as shown in Table (6). In this concern, many investigators found variation among different clover varieties in their fresh and dry production as reported by Ranjbar (2007), Ahmed and Fateh (2016) and Ewis and Rajab (2016). Moreover, Abd El-Naby and Sakr (2012) found that Helaly variety outyielded other clover varieties (Giza 6, Sakha 4, Gemmeiza 1 and Serw 1) in fresh and dry forage yields/fed.

The results presented in Table (7) showed that the foliar application of the tested stimulant substances caused significant increases in fresh and dry forage yields/fed either each cut or their total as compared with untreated plants (control treatment) in the two seasons. In comparison among the tested substances, it can be noted that the application of HA produced the highest significant values of total fresh and dry forage yields/fed followed

by SA, CA and AA in a descending order in both seasons. The superiority of forage production either as a fresh or dry by using the tested stimulant substances especially HA may be due to the increase in the values of growth characters, i.e plant height, number of leaves and total dry weight/m<sup>2</sup> as previously shown in Table (3). In comparison among the four cuts over all means of tested stimulant substances, it can be observed that the total dry forage yield/fed was gradually and progressively increased from the 1<sup>st</sup> cut up to the later cut (4<sup>th</sup> cut) which produced the highest production. These results could be explained on the basis of the dry matter% in the late cuts was more than that in the early ones as shown in Table (7). These findings are in harmony with those obtained by other researchers who reported that the fresh and dry forage production were improved by foliar application of some stimulant substances such as SA in alfalfa (Morsy *et al.*, 2011), AA in alfalfa (Tarraf *et al.*, 2015) and HA in Egyptian clover (Sultan *et al.*, 2016).

The total fresh and dry forage yields /fed were significantly responded to the interaction between Egyptian clover varieties and stimulant substances during the both seasons as graphically illustrated in Figure (4). However, dry matter percentage showed insignificant response to such interaction during both seasons, therefore this data were excluded. Helaly variety showed the best response, generally, when their plants were treated with all stimulant substances, especially with HA which gave the best productivity of total fresh forage /fed (50.25 and 40.36 ton/fed) followed by SA (45.67 and 39.13 ton/fed) in both seasons. The increase in the total fresh yield/fed observed herein by the best interaction treatment (Helaly + HA) was amounted to 37.86 and 76.48 % more than the lowest treatment (untreated Giza 6 variety) in the first and second seasons, respectively. The promoting effect of the interaction treatment (Helaly + HA) on fresh matter production may be due to the pronounced increase in plant height as previously discussed in Figure (1).

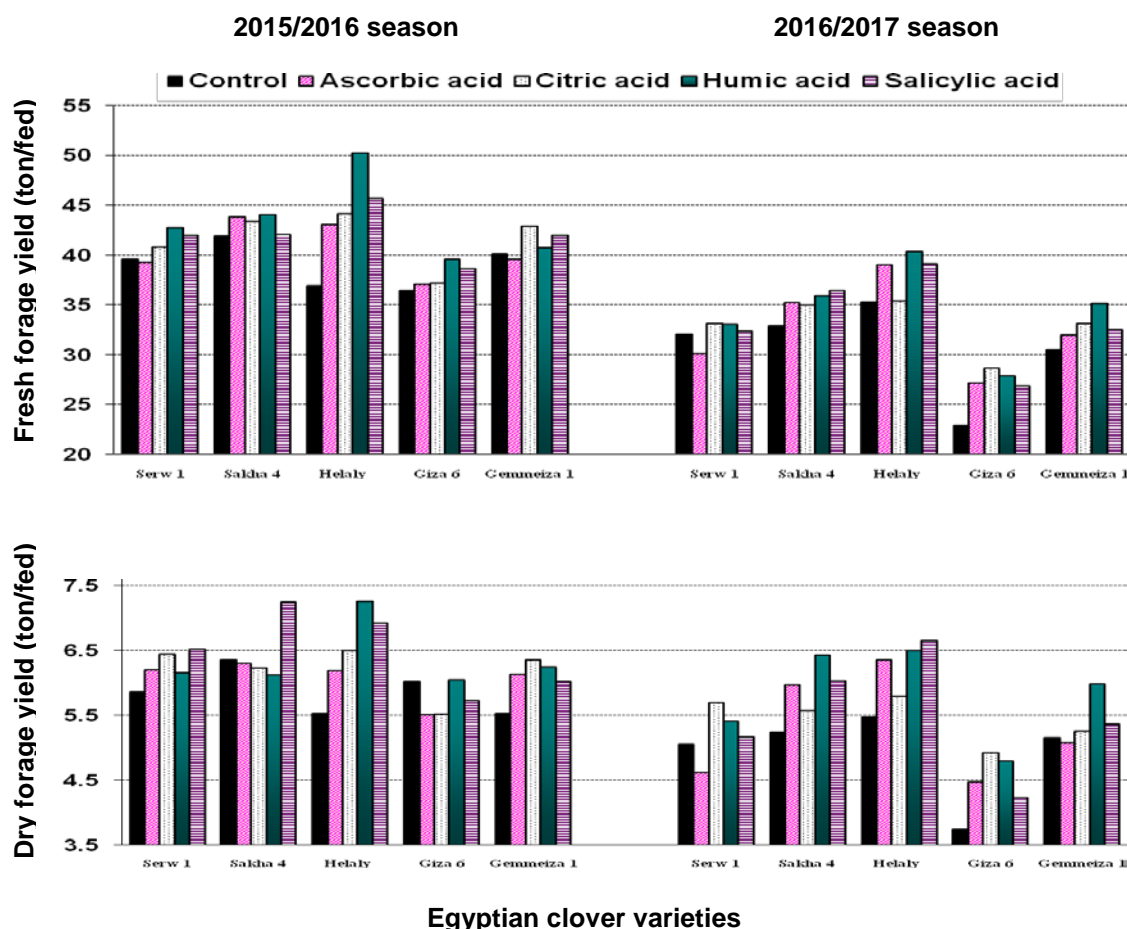
**Table (6): Forage yield / fed and dry matter % of some Egyptian clover varieties at four cuts during 2015/ 2016 and 2016/2017 seasons.**

Varieties	2015/2016 season				2016/2017 season				Total	Total
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut		
Fresh forage yield (ton/fed)										
Serw 1	11.438 b	8.849ab	9.154bc	11.432ab	40.873 b	8.616 b	7.444 b	7.907 b	8.174 b	32.141 c
Sakha 4	11.774ab	9.362 a	9.979ab	11.893 a	43.008ab	8.885 b	7.476 b	8.637ab	10.092 a	35.090 b
Helaly	11.831 a	9.600 a	10.483 a	12.086 a	44.000 a	9.869 a	8.529 a	9.350 a	10.096 a	37.844 a
Giza 6	11.040 c	8.004 b	8.505 c	10.235 b	37.784 c	7.935 c	5.867 c	5.875 c	7.009 b	26.686 d
Gemmeiza 1	11.572ab	8.929ab	9.096bc	11.444ab	41.041 b	8.651 b	7.457 b	8.085 b	8.455 b	32.648 c
Dry forage yield (ton/fed)										
Serw 1	1.228ab	1.389 a	1.581 b	2.066ab	6.264 b	1.133 c	1.195ab	1.357 a	1.502 b	5.187 b
Sakha 4	1.304 a	1.414 a	1.589 b	2.174 a	6.481 a	1.200 b	1.268 a	1.562 a	1.816 a	5.846 a
Helaly	1.315 a	1.450 a	1.759 a	1.987bc	6.511a	1.361 a	1.399 a	1.563 a	1.828 a	6.151 a
Giza 6	1.139 b	1.277 b	1.499 b	1.880 c	5.795 d	1.020 d	1.044 b	1.116 b	1.251 b	4.431 c
Gemmeiza 1	1.187ab	1.370 a	1.590 b	2.038abc	6.185 c	1.162bc	1.256 a	1.425 a	1.519 b	5.362 b
Dry matter (%)										
Serw 1	10.736 a	15.697 a	17.271 a	18.072 ab	-	13.150 ab	16.053 a	17.162 ab	18.375 a	-
Sakha 4	11.075 a	15.104 a	15.923 a	18.279 a	-	13.506 ab	16.961 a	18.085 ab	17.994 a	-
Helaly	11.115 a	15.104 a	16.780 a	16.440 b	-	13.791 a	16.403 a	16.717 b	18.106 a	-
Giza 6	10.317 a	15.955 a	17.625 a	18.368 a	-	12.854 b	17.794 a	18.996 a	17.848 a	-
Gemmeiza 1	10.258 a	15.343 a	17.480 a	17.808 ab	-	13.432 ab	16.843 a	17.625 ab	17.966 a	-

Table (7): Forage yield / fed and dry matter % of Egyptian clover as affected by some stimulant substances during 2015/ 2016 and 2016/2017 seasons.

Stimulant substances	2015/2016 season					2016/2017 season				
	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Total	1 <sup>st</sup> cut	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	Total
	Fresh forage yield (ton/fed)									
Control	10.841 c	8.744 b	8.558 b	10.834 b	38.977 d	8.680bc	6.880 c	7.076 c	8.087 b	30.723 c
Ascorbic acid	11.509 b	8.817ab	8.911 b	11.292 b	40.529 c	8.744bc	7.390ab	7.826 b	8.743ab	32.703 b
Citric acid	11.690ab	8.820ab	9.802 a	11.361 b	41.673bc	8.631 c	7.356 b	8.110ab	8.968 a	33.065 b
Humic acid	11.923 a	9.288 a	10.098 a	12.164 a	43.473 a	9.019 a	7.711 a	8.669 a	9.059 a	34.458 a
Salicylic acid	11.692ab	9.077ab	9.847 a	11.439 b	42.055ab	8.881ab	7.436ab	8.172ab	8.969a	33.458ab
	Dry forage yield (ton/fed)									
Control	1.140 b	1.323 c	1.485 b	1.929 b	5.877 d	1.152b	1.174 b	1.206 b	1.399 c	4.931 c
Ascorbic acid	1.248 a	1.366abc	1.536 b	1.934 b	6.084 cd	1.176b	1.192 b	1.393 a	1.535bc	5.296 b
Citric acid	1.238 a	1.358 bc	1.656 a	1.972 b	6.224 bc	1.161b	1.201 b	1.444 a	1.639ab	5.445 b
Humic acid	1.275 a	1.437 a	1.658 a	2.165 a	6.535 a	1.216 a	1.355 a	1.538 a	1.708 a	5.817 a
Salicylic acid	1.272 a	1.416 ab	1.676 a	2.146 a	6.510 ab	1.172b	1.241 b	1.442 a	1.634ab	5.489 b
	Dry matter (%)									
Control	10.516 a	15.130a	17.352a	17.805ab	-	13.272 a	17.064a	17.044 a	17.299 b	-
Ascorbic acid	10.844 a	15.493a	17.237a	17.127ab	-	13.449 a	16.130a	17.800 a	17.557 b	-
Citric acid	10.590 a	15.397a	16.894a	17.358ab	-	13.452 a	16.327a	17.805 a	18.276ab	-
Humic acid	10.694 a	15.472a	16.419a	17.798ab	-	13.483 a	17.572a	17.741 a	18.854 a	-
Salicylic acid	10.879 a	15.600a	17.020a	18.760 a	-	13.197 a	16.689a	17.646 a	18.218ab	-

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**Egyptian clover varieties**  
**Figure (4): Effect of the interaction between Egyptian clover varieties and plant stimulant substances on forage yield during 2015/2016 and 2016/2017 seasons.**

This means that such interaction treatment (Helaly + HA) was the most effective treatment for enhancing the fresh forage yield /fed. Regarding the total dry forage yield/fed in both seasons, the results in Figure (4) indicate that the plants of Helaly variety sprayed with HA in the first season and with AS in the second season produced the heaviest total dry forage yield/fed, i.e. 7.25 and 6.64 ton/fed, respectively as compared to the other interaction treatments. On the contrary, untreated plant of Gemmeiza 1 and Giza 6 varieties produced the lowest values of total dry forage yield/fed (5.52 and 3.74 ton in the first and second seasons, respectively).

**5- Seed yield and its components:**

Data presented in Table (8) showed that

significant variation could be detected among the five tested varieties in seed yield/fed and its components (number of heads /m<sup>2</sup>, number of seeds/head, 1000-seed weight and seed weight / head). With respect to the number of heads/m<sup>2</sup>, Gemmeiza 1 variety had the highest values in the two seasons followed by Helaly, Sakha 4 and Serw 1 varieties in the first season and by Helaly in the second season in a descending order without significant differences among them. On the other hand, Helaly variety produced higher significant values of number of seeds /head than the rest varieties in both seasons without significant differences with Giza 6 variety in the first season and Gemmeiza 1 variety in the second seasons. However, Serw 1 variety had lower values than the rest

varieties in both seasons. Reversely, Serw1 variety had the heavier significant values of 1000- seed weight than the rest varieties without significant differences with Helaly, Giza 6 and Gemmeiza 1 varieties in both seasons. The superiority of 1000- seed weight in Serw 1 variety may be attributed to the fewer number of seeds/head in such variety which led to a low competition among the seeds in each head and consequently allowed to increase the weight of seeds. With regard to seed weight /head, the data show that Helaly and Serw1 varieties were superior and inferior, respectively to the other tested clover varieties in both growing seasons. Since, the seed weight /head is a resultant to two components (number of seeds /head and 1000-seed weight), therefore it could be noticed that the superiority of Helaly variety in seed weight/head may be due to the increase in the number of seeds /head and not to the increase in 1000 -seed weight. Reversely, the inferiority of Serw1 variety in seed weight/head may be due to the reduction in the number of seeds/head and not to the

reduction in 1000-seed weight. In addition, the data of seed yield/fed showed that the differences among the five tested clover varieties were statistically confirmed in the two seasons as shown in Table (8). Helaly variety outyielded the rest varieties in seed yield /fed, but without significant differences with each of Gemmeiza1 and sakha4 varieties in the first season as well as with Gemmeiza1 in the second season. On the other hand, Giza 6 variety produced the lowest values of seed yield /fed in both seasons. The superiority of Helaly variety in the seed yield /fed may be generally due to the increase in the number of heads/m<sup>2</sup> as well as number and weight of seeds/head. In previously studies, El-Zanaty (2005b) reported that Egyptian clover varieties differed widely in number of heads/m<sup>2</sup>, number of seeds/head and 1000-seedweight and seed weight/10 heads. Moreover, Al-Suhaibani and Al-Doss (2004); El-Zanaty (2005a); Abd El-Naby and Sakr (2012) and Bakheit *et al.* (2012) found variation among some Egyptian clover varieties in their seed yield/unit area.

**Table (8): Seed yield and its components of some Egyptian clover varieties during 2015/2016 and 2016/2017 seasons.**

Varieties	Number of heads / m <sup>2</sup>	Number of seeds/head	1000-seed weight (g.)	Seed weight /head (g.)	Seed yield (kg/fed)
	2015/2016 season				
Serw 1	1590.40 a	42.98 c	2.719 a	0.0810 c	436.75 b
Sakha 4	1631.73 a	45.75 bc	2.561 b	0.0856 bc	489.17 a
Helaly	1633.60 a	51.79 a	2.627 ab	0.0966 a	499.55 a
Giza 6	1376.53 b	48.67 ab	2.665 ab	0.0909 bc	422.08 b
Gemmeiza 1	1681.07 a	45.75 bc	2.661 ab	0.0894 b	489.22 a
2016/2017 season					
Serw 1	983.73 bc	38.92 b	2.628 a	0.0774 c	276.04 b
Sakha 4	1070.93 bc	40.05 b	2.540 b	0.0802 bc	276.33 b
Helaly	1110.67 ab	44.40 a	2.575 ab	0.0950 a	351.69 a
Giza 6	956.00 c	38.94 b	2.574 ab	0.0863 bc	275.27 b
Gemmeiza 1	1201.33 a	44.35 a	2.571 ab	0.0842 b	321.03 a



## **Effect of growth stimulant substances on the productivity and quality .....**

The data in Table (9) indicate that the values of seed yield/fed, number of heads/m<sup>2</sup>, seed weight/head and its main components (number of seeds/head and 1000-seed weight) were significantly influenced by the foliar application of the tested stimulant substance treatments during the both seasons. The number of heads/m<sup>2</sup> was significantly increased by the foliar application of SA by 11.3% and 14.56% more than the control treatment in the first and second seasons, respectively. Moreover, it can be noted that the rest stimulant substances (AA, CA and HA) did not exert any significant differences among them but still they produced number of heads/m<sup>2</sup> more than the control treatment in the two seasons. In addition, it can be observed that the number of seeds/head were significantly increased when the plants were sprayed with the tested stimulant substances such as SA, HA and CA in a descending order, without significant differences among them, as compared with untreated plants (control treatment) which produced the lowest values in both seasons. The favorable effect of SA on the number of heads/m<sup>2</sup> and number of seeds/head may be due to its role in flowering stimulation in the plant as recorded by Kumar and Tayal (1982), Raskin (1992) and Singh and Usha (2003). Moreover, Kumar *et al.* (2013) reported that foliar application of SA at a rate of 50 mg/l increased number of heads/m<sup>2</sup> and number of seeds/head of Egyptian clover as compared with untreated plants. With regard to the results of 1000 - seed weight and seed weight /head, the obtained data showed pronouncing increase in their values when the tested stimulant substances were foliar applied in favor of HA which produced the higher increases than the other substances. Similar results were previously obtained by other researchers who reported that 1000 -seed weight of Egyptian clover was increased when the plants were sprayed with HA (El-Maaz and

Ismail, 2016) or SA (Rani *et al.*, 2017). In addition, the data of seed yield /fed indicate that there are significant increase when the clover plants were sprayed by any tested stimulant substances as compared with the control treatment in the two growing seasons. The increase in the values of seed yield /fed amounted to 21.89, 20.41, 13.98 and 3.31% in the first season and 21.54, 11.30, 9.56 and 4.28% in the second one for the plants supplied with SA, HA, CA and AA, respectively more than the unsupplied plants with those substances. This means that SA was more effective for increasing the seed yield/fed than the other tested substances. The superiority of SA in increasing seed yield/fed may be mainly due to the increase in the number of heads/m<sup>2</sup> and/or number of seeds/head as ascertained by the previous results obtained in the same Table (9). In this concern, other investigators reported that seed yield/unit area of clover was increased by the application of some stimulant substances such as SA (Kumar *et al.*, 2013 and Rani *et al.*, 2017) and HA (El-Maaz and Ismail, 2016).

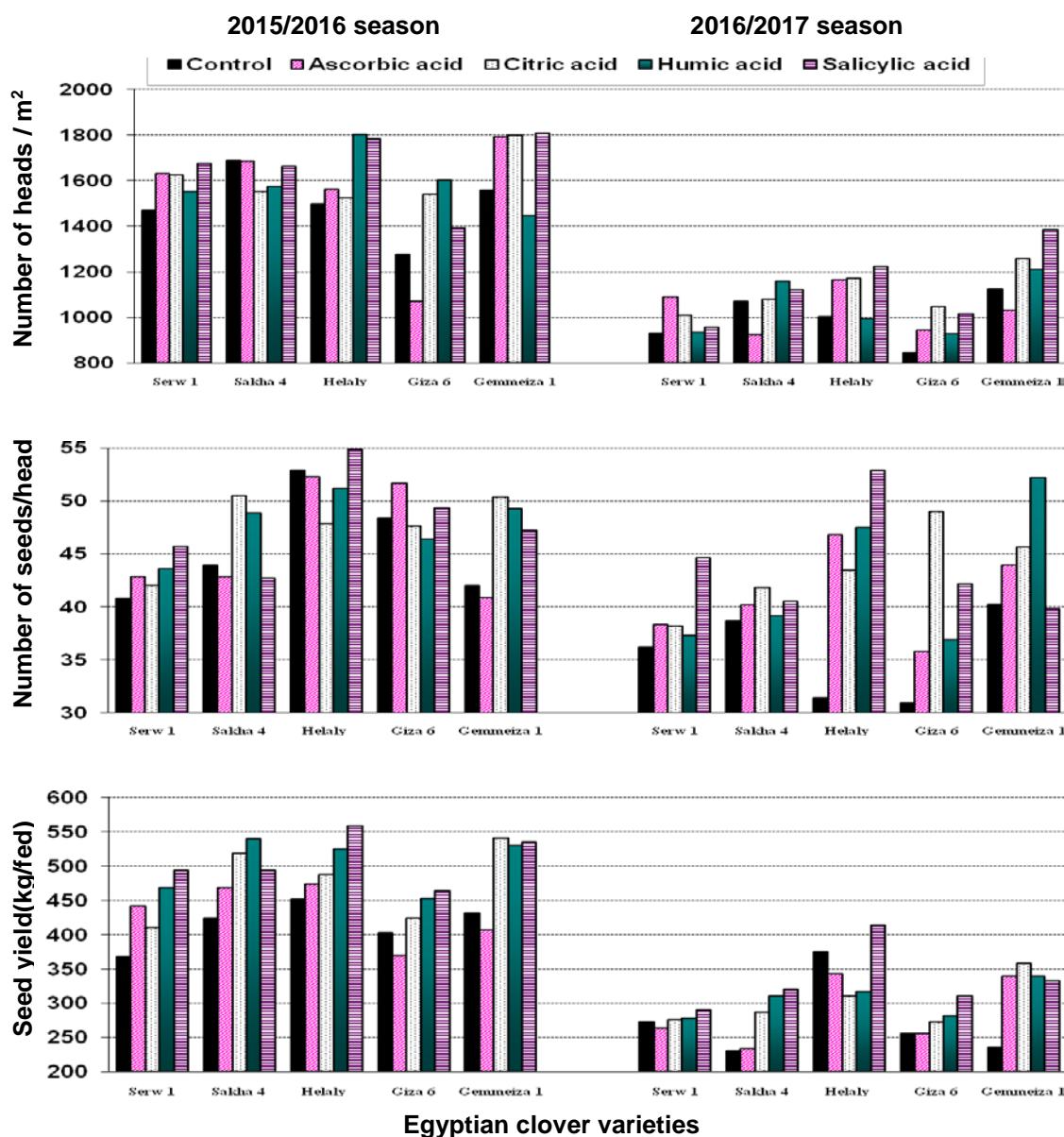
The interactions between Egyptian clover varieties and stimulant substances were found to be significant for number of head/m<sup>2</sup>, number of seeds/head and seed yield/fed in the two seasons (Figure 5). However, the interactions between the two factors for the other traits studied herein were not reached 5% level of significance, therefore the data were excluded. It is interesting to note that the most pronounced interaction treatments for increasing number of heads /m<sup>2</sup> was happened when the plants of Gemmeiza 1 variety were sprayed with SA (1808.00 and 1382.67 heads/m<sup>2</sup>) in the two seasons. However, the lowest significant values were obtained especially when the plants of Giza 6 variety were treated with AA (1070.67 heads/m<sup>2</sup>) in the first season and untreated with any stimulant substances (845.33 heads/m<sup>2</sup>) in the second season. Moreover, it is clear that the foliar application

of Helaly variety plants with SA exhibited the maximum numbers of seeds/ head (54.83 and 52.90 seeds/head) and seed yield/fed (558.93 and 413.60 kg/fed) in the first and second seasons respectively. From these results, it can be suggested that the

translocation of photoassimilate from the vegetative plant tissues to the seeds was much in Helaly variety when it was sprayed with SA which leads to promotion of storage capacity building.

**Table (9): Seed yield and its components of Egyptian clover as affected by some stimulant substances during 2015/ 2016 and 2016/2017 seasons.**

Stimulant substances	Number of heads / m <sup>2</sup>	Number of seeds/head	1000-seed weight (g.)	Seed weight /head (g.)	Seed yield (kg/fed)
	2015/2016 season				
Control	1497.87 c	45.61 c	2.600 c	0.0824 c	415.95 c
Ascorbic acid	1548.00 bc	45.87 bc	2.618 bc	0.0873 b	431.77 c
Citric acid	1607.47ab	47.65 ab	2.619 bc	0.0899 a	476.37 b
Humic acid	1595.47abc	47.86 a	2.709 a	0.0920 a	503.25 a
Salicylic acid	1664.53 a	47.94 a	2.685 bc	0.0914 a	509.44 a
2016/2017 season					
Control	994.67 c	35.47 c	2.533 b	0.0800 c	274.45 c
Ascorbic acid	1030.40 bc	40.98 b	2.571 ab	0.0817 c	286.21 bc
Citric acid	1113.60 ab	43.62 ab	2.567 ab	0.0823 c	300.68 b
Humic acid	1044.53 bc	42.60 ab	2.611 a	0.0928 a	305.45 b
Salicylic acid	1139.47 a	44.00 a	2.605 a	0.0865 b	333.58 a



**Figure (5): Effect of the interaction between Egyptian clover varieties and plant stimulant substances on seed yield and its components during 2015/2016 and 2016/2017 seasons.**

**6- Simple phenotypic correlation:**

The results in Table (10) indicated that there was a positive and significant correlation coefficient between fresh and dry forage yield/fed and each of plant height, number of leaves/m<sup>2</sup>, leaf area /m<sup>2</sup>, total dry weight/m<sup>2</sup>, total chlorophyll, crude protein %, number of heads/m<sup>2</sup> and seed yield /fed in both seasons. Moreover, the fresh forage yield/fed was positively and

highly significantly correlated with dry forage yield/fed in both seasons. On the other hand, the values of correlation coefficient between fresh and dry forage yield/fed and each of number of stems/m<sup>2</sup>, number of seeds/head, 1000 - seed weight and seed weight/head were not adequate enough to reach the 5 % level of significance in the first and / or second seasons. In addition, according to the mean values of correlation

coefficient (r) of the two seasons, it can be suggested that the most important effective characters for increasing fresh forage yield/ fed were dry forage yield/ fed (r = 0.754), total dry weight/ m<sup>2</sup> (r = 0.712), leaf area/ m<sup>2</sup> (r = 0.648) and plant height (r = 0.549) in a descending order as an average of the two seasons. Moreover, the most important effective characters for increasing dry forage yield/ fed were fresh forage yield/ fed (r = 0.754), total dry weight/ m<sup>2</sup> (r = 0.642), leaf area/ m<sup>2</sup> (r = 0.551) and number of leaves/ m<sup>2</sup> (r = 0.483) in a descending order as an average of the two seasons. In this concern Gaballah (2001) found positive and significant correlation between each of plant height, fresh and dry forage yields of Egyptian clover. Moreover, Radwan *et al* (2014) confirmed that high significant correlation was obtained between dry forage yield/ fed and each of plant height, number

of tillers and leaves / plant and leaf area of berseem.

The data in the same table show that seed yield / fed was positively and significantly correlated with each of plant height as well as number of stems, leaves, leaf area and total dry weight/ m<sup>2</sup>, total chlorophyll, crude protein % , fresh and dry forage yields/ fed , number of heads/ m<sup>2</sup> as well as number and weight of seeds/ head. However, insignificant correlation coefficient was found between seed yield / fed and 1000 seed weight in both seasons. Moreover, according to the values of correlation coefficient (r). It can be suggested that the seed yield components namely seed weight / head (r = 0.442), number of heads/ m<sup>2</sup> (r = 0.418) and number of seeds / head (r = 0.391) are the major contributors to seed yield/ fed in a descending order as an average of the two seasons.

**Table (10): Simple phenotypic correlation between each of fresh and dry forage and seed yields /fed with some characters studied during 2015/2016 and 2016/2017 seasons, (over all tested Egyptian clover varieties and plant stimulant substances).**

Characters	Fresh forage yield /fed		Dry forage yield /fed		Seed yield /fed	
	2015/2016	2016/2017	2015/2016	2016/2017	2015/2016	2016/2017
Plant height (cm)	0.682 **	0.417 **	0.376 **	0.329 **	0.609 **	0.338 **
Number of stems/m <sup>2</sup>	0.189 NS	0.383 **	0.097 NS	0.359 **	0.366 **	0.303 **
Number of leaves/m <sup>2</sup>	0.471 **	0.514 **	0.439 **	0.527 **	0.497 **	0.375 **
Leaf area (cm <sup>2</sup> /m <sup>2</sup> )	0.639 **	0.658 **	0.511 **	0.591 **	0.551 **	0.480 **
Total dry weight/m <sup>2</sup>	0.660 **	0.765 **	0.525 **	0.759 **	0.676 **	0.394 **
Total chlorophyll	0.269 *	0.402 **	0.321 **	0.421 **	0.493 **	0.504 **
Crude protein (%)	0.334 **	0.352 **	0.374 **	0.405 **	0.518 **	0.299 **
Number of heads/m <sup>2</sup>	0.491 **	0.330 **	0.410 **	0.380 **	0.402 **	0.435 **
Number of seeds/head	0.129 NS	0.373 **	-0.003 NS	0.352 **	0.390 **	0.392 **
1000-seed weight (g.)	0.005 NS	0.064 NS	-0.137 NS	0.077 NS	0.200 NS	0.178 NS
Seed weight/ head (g.)	0.115 NS	0.381 **	-0.066 NS	0.364 **	0.449 **	0.436 **
Fresh forage yield (ton/fed)	—	—	0.595 **	0.914 **	0.576 **	0.430 **
Dry forage yield (ton/fed)	0.595 **	0.914 **	—	—	0.325 **	0.377 **
Seed yield (kg/fed)	0.576 **	0.430 **	0.325 **	0.377 **	—	—

NS, \* and \*\* : Non significant, significant and highly significant at probability of 0.05 and 0.01, respectively. Critical r values are 0.225 and 0.294 at probability of 0.05 and 0.01, respectively.

## **Conclusion:**

From the abovementioned results, it can be concluded that the foliar application of Egyptian clover varieties with some stimulant substances (AA, CA, HA, SA) were found to be important to maximize their productivity especially when Helaly variety was sprayed with HA at a concentration of 2 g /L for forage yield or with SA at a concentration of 0.5 g /L for seed yield/ fed under the conditions of this experiment in Menoufia governorate.

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## **تأثير المواد المنشطة للنمو علي إنتاجية وجودة**

### **بعض أصناف البرسيم المصري**

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#### **الملخص العربي**

أجريت تجربتان حقليتان بالمزرعة البحثية لكلية الزراعة جامعة المنوفية بشبين الكوم بمصر خلال موسم 2016/2015، 2017/2016م لدراسة تأثير رش بعض المواد المنشطة للنمو وهي حمض الأسكوربيك (1جم/لتر)، حمض الستريك (1جم/لتر)، حمض الهيوميك (2جم/لتر) ، حمض الساليسيليك (0.5جم/لتر) بالإضافة الي معاملة الكنترول (الرش بماء الصنبور) وذلك علي صفات النمو الخضري (طول النبات ، عدد الأوراق والسيقان/م<sup>2</sup>، مساحة الأوراق /م<sup>2</sup>، وزن النبات الكلي الجاف/م<sup>2</sup>) صبغات التمثيل الضوئي (الكلوروفيل الكلي) ، النسبة المئوية للبروتين الخام ، محصول العلف الأخضر والجاف /فدان ومحصول البذور ومكوناته (عدد النورات /م<sup>2</sup> ، عدد البذور/ النورة ، وزن 1000 بذرة ، وزن البذور/ النورة ، محصول البذور/فدان) لخمس أصناف من البرسيم المصري (سرو1، سخا4، هلاي ، جيزة6، جميزة 1). ويمكن تلخيص أهم النتائج التي تم الحصول عليها على النحو التالي:

- 1- تفوق الصنف هلاي علي بقية الأصناف الأخرى في معظم صفات النمو الخضري (طول النبات، عدد الأوراق/م<sup>2</sup>، مساحة الأوراق/م<sup>2</sup>، وزن النبات الكلي الجاف/م<sup>2</sup>) وصبغات التمثيل الضوئي (الكلوروفيل الكلي) ، النسبة المئوية للبروتين الخام ، محصول العلف الكلي الأخضر والجاف/فدان ومحصول البذور ومكوناته (عدد البذور/ النورة ، وزن البذور/ النورة، محصول البذور/فدان). بينما تفوق الصنف جميزة 1 علي الأصناف الأخرى في عدد السيقان /م<sup>2</sup> وعدد النورات/م<sup>2</sup>. ومن ناحية أخرى فقد أعطي الصنف سرو1 أعلي وزن 1000 بذرة في كلا الموسمين.
- 2- أدى رش نباتات البرسيم المصري بحمض الهيوميك الى زيادة في معظم صفات النمو المدروسة ومحصول العلف للفدان. بينما أعطي الرش بحمض الساليسيليك أعلي قيم للنسبة المئوية للبروتين الخام ومحصول البذور للفدان ومكوناته مقارنة بباقي المواد المنشطة للنمو المختبرة. في حين أعطت نباتات البرسيم غير المعاملة بمنشطات النمو أقل قيم لمعظم الصفات المدروسة.
- 3- أظهر التفاعل بين أصناف البرسيم المصري ومنشطات النمو زيادة معنوية لمعظم الصفات تحت الدراسة حيث وجد أن رش نباتات الصنف هلاي بحمض الهيوميك كانت أكثر المعاملات تأثيراً في زيادة إنتاجية محصول العلف للفدان بينما أعطي رش نفس الصنف بحمض الساليسيليك أعلي إنتاجية لمحصول البذور للفدان وذلك تحت ظروف التجربة.
- 4- أظهرت تقديرات معامل الارتباط أن هناك علاقة موجبة وعالية المعنوية بين محصول العلف الأخضر والجاف للفدان مع معظم صفات النمو الخضري والكلوروفيل والبروتين الخام ومحصول البذرة للفدان ومكوناته في موسمي الزراعة عدداً عدد الفروع/م<sup>2</sup>، عدد ووزن البذور/النورة في الموسم الأول، وزن الألف بذرة في الموسم الثاني. هذا ويوجد أيضا ارتباط معنوي موجب عالي المعنوية بين محصول البذور للفدان ومعظم الصفات تحت الدراسة ماعدا وزن 1000 بذرة في كلا الموسمين.

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