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EFFECT OF SEED-SOAKING IN POLY ETHYLENE GLYCOL AND HUMIC ACID ON THE PRODUCTIVITY AND QUALITY OF FODDER BEET UNDER SOIL SALINITY CONDITIONS.

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ABSTRACT

Two field experiments were conducted at El-Serw Agricultural Experiment and Research Station, during two successive winter seasons 2011/2012 and 2012/ 2013, to study the effect of soaking seeds in solutions of poly ethylene glycol (PEG) and humic acid (HA) on growth, yield, and nutrients contents of fodder beet (*Beta vulgaris L. (vorochenger*) grown in newly reclaimed saline soils conditions.

Results showed positive effects of soaked seeds compared to the un-soaked seeds. At soaking time 12 h, the increases due to HA treatments were greater than those attributed to PEG. Soil pH was almost unchanged, the EC values were decreased by 35.5% and 37.99% while soil available NPK were increased by 28.44% and 33.65% for N; by 11.28% and 96.24% for P while K was increased by 13.14% and 18.5% for both PEG and HA treatments respectively compared with the control. The fresh and dry weights of root were increased by 133.3% and 136.86%, respectively, for PEG treatments. For HA treatments, the increases in the above mentioned characteristics amounted 155.26% and 161.63%, respectively. Root length and diameter were enhanced significantly. the increases in roots N content amounted 21.74 and 29.5%, P by 37.88 and 50%, K by 7.82 and 12.35% and Protein by 21.71 and 26.63% for PEG and HA, respectively. The leaves chlorophyll content (*Chl*) was increased by 84.43% and 156.51% and emergence by 36.36% and 42.15% while the proline decreased by 16.07% and 25.71% for PEG and HA, respectively.

Keywords: Seed-Soaking; Polymers; Emergence; Chlorophyll; Salinity Stress.

INTRODUCTION

The semiarid climate and the progressive land Salinization are severe problems affecting the seed germination and plant growth causing yield decrease in many crops. Under nutrient stress conditions, the plant accumulates reactive oxygen species (ROS) and results in oxidative burst inside the plant cells (Yang *et al*., 2011). Plants can cope with reduced nutrient availability conditions by trigger physiological responses to increase nutrient acquisition that may alter the whole plant morphology and metabolism.

One of the advanced methods for accelerating crop plant seed germination is the use of the technology of seed dehydration to increase the ability of seeds to germinate and grow under stress conditions. Seeds are subjected to low potential osmotic solutions of substances such as polyethylene glycol (PEG), KNO_3, NaCl, glycerol, and mannitol. The morphological structure of seed has hardly been related to salt tolerance and seed coat may have an important role in the ion exchange greater than simply as a physical protective barrier. Ethylene was synthesized by poly ethylene glycol (PEG)-treated plants. It was involved in the induction of early senescence processes characterized by synthesis of ROS, per-oxidation of membrane lipids and a decrease in chlorophyll content (Ben Hassine and Lutts, 2010; Mardani *et al*., 2013). Polyethylene glycol simulates osmotic stress effects, cannot enter seeds and does not cause the side effects produced by salts (Yasari et al., 2013). Higher EN biosynthesis can effectively mitigate salt stress evoked by the accumulation of high levels of Na in seed tissues. NaCl inhibited germination of *Stylosanthes* seeds, *Medicago sativa (L.), Astragalus adsurgens (Pall.)* and *Coronilla varia (L.)* through inhibition of EN biosynthesis (Wu *et al*., 2011; Silva *et al.,* 2014).

Ethylene-responsive element binding factors (ERFs) like CitERF, are a member of the gene family of plant transcription factors suggested to play an important role in improving tolerance to drought and salt stress. CitERF expression had induced continuously during the treatment by 10% PEG but it could be induced to high level at 1 h after the treatment at 4 °C or 250 mM NaCl and then declined continuously (Yang *et al*., 2011). Poly ethylene glycol as a water-soluble polymer of low toxicity used to create high osmotic pressure in biochemistry and bio-membrane technologies. It has a positive effect on the hydrolysis of different ligno-cellulose materials due its interaction with lignin. The addition of PEG 4000 at 1 g (g substrate), enzyme concentration and hydrolysis time could be reduced by approximately two thirds and one third, respectively, without loss in sugars yield (Ivetić *et al*., 2012).

EN could help plants to retain K in shoots and roots to improve salt tolerance. In a similar manner, Ca, Mg, Zn, Fe, Mn, Cu, Se can be involved. A complete understanding of the EN and nutrients interaction would provide new strategies for improving crop vigor and development under changing environment (Iqbal *et al.,* 2013).

The effects of different treatments of PEG on corn grains germination were studied. The maximum germination rate was achieved by priming for 12 hours with 5% PEG (Yasari *et al*., 2013). On the other hand, rate of wheat germination was improved when the seed soaked KNO_3 2% compared with PEG, KCL and water (Ajirloo *et al.,* 2013). The root length increased at lower concentration of PEG (1% and 5%) but decreased at higher PEG concentration. At 1% PEG concentration chlorophyll content increased compared to control. The results demonstrate a concentration dependent decline in the chlorophyll content with increasing concentration of PEG-6000 while proline content increased significantly. Decrease in the percentage and rate of germination and seedling growth by PEG stress is observed in *Senna occidentalis* and Zea maize **(Jain *et al*., 2013)**. Effect of PEG on the amount of *chlorophyll* a, *chlorophyll* b and the total leaf of sugar beet have been studied previously (Nagl *et al*., 2010; Fahim *et al*., 2014).

Humic substances constituting 65-75% of organic matter with major functional groups include carboxyl, phenolic hydroxyl, alcoholic hydroxyl and ketone. Humic acid (HA) application increase plant growth parameters under salinity condition depending on the crop species. It is safe, increased the uptake of P, K, Mg, Na, Cu and Zn, increased macro-nutrient contents and enhanced micro-nutrient contents of the plant organs by increasing the permeability of root cells membranes (Valdrighi *et al*., 1996). The seed-soaked in HA have been significantly reduced the damaging action of salinity on plant growth and enhanced the yield production of sugar beet under saline conditions (Eisa *et al*., 2012).

The present study aims to evaluate the effect of seed-soaking in poly ethylene glycol and humic acid on the productivity and quality of fodder beet under salinity conditions.

MATERIALS AND METHODS

Two field experiments were conducted at El-Serw Agricultural Experiment and Research Station, during two successive winter seasons 2011/2012 and 2012/ 2013. The aim is to study the effect of soaking seeds of fodder beet (*Beta vulgaris L. (vorochenger*) in solutions of PEG and HA on growth, yield, and nutrients contents of fodder beet grown in newly reclaimed saline soils conditions. Some physical and chemical properties of a representative soil sample used in the experiment were determined before preparation according to Jackson, (1973) and data are presented in Table 1. Soil particle size distribution was determined by the international Pipette method (Piper, 1950). Soil available N was extracted using 2N KCl solution and measured according to the modified Kjeldahel method. Available P was extracted by 0.5N sodium-bicarbonate and determined colorimetrically (Olsen and Sommers, 1982). Available K was determined using the Flame-Photometer (Chapman and Pratt, 1961).

Table (1): Some of the physical and chemical properties of the soil before planting.

Coarse sand (%)	**Fine sand (%)**	**Silt (%)**	**Clay (%)**	**Texture**		**OM (%)**		**$CaCO_3$ (%)**
3.71	48.33	14.29	33.67	Sandy clay loam		0.48		4.96
pH	EC	Cations (meq l^{-1})				Anions (meq l^{-1})		
(1: 2.5)	(dSm^{-1})	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
8.08	10.53	12.83	15.90	75.69	0.88	4.18	68.49	32.63
Macronutrients (mg kg^{-1})				Micronutrients (mg kg^{-1})				
N	P	K		Fe		Mn		Zn
36.79	3.92	183		2.39		1.20		0.81

Table (2):Humic analysis.

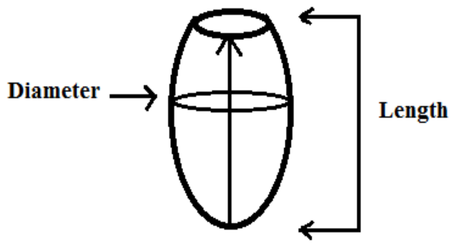
pH	EC (dSm^{-1})	O.M. (%)	Macronutrients (%)			Micronutrients (mg kg)		
			N	P	K	Fe	Mn	Zn
7.85	2.14	68.00	2.18	0.29	3.48	395	273	218

Planting and fertilization

The area of each plot was 50 m^2 (10 m length x 5 m width), with six ridges 50 cm apart, 3.5 m in length. Sowing during the 1^{st} and 2^{nd} seasons was on the 5^{th} of November 2011/2012 and 2012/2013, respectively. Seeds of (*Beta vulgaris L. (vorochenger*) were soaked in PEG-6000 or HA solution (3g PEG/L dist. H_2O - 20 L for 4 kg seed) for 6, 8, 12 hr before planting and sown in hills 25 cm apart (3-4 seeds per hill). Plants were thinned to two plants per hill after 30 days from planting, and then were thinned to one plant per hill after 45 days from planting. Nitrogen fertilization was used in the form of urea (46% N) at a rate of 100 kg N fed^{-1} and added in three equal doses after 21, 45 and 60 days. Potassium fertilizer as potassium sulphate (48% K_2O) was applied at a rate of 75 kg K_2O fed^{-1} in two doses after plant thinning and after 60 days from planting. Phosphorous fertilizer as calcium super phosphate was applied at a rate of 200 kg fed^{-1} (15.5% P_2O_5) during soil preparation. Other cultural practices were carried out as recommended.

Harvesting and sampling

On the day before harvesting, 5 plants of each plot were taken with the soil surrounding roots as plant and soil samples. The plants were get rid of surrounding soil layer, washed, divided into roots and shoots and weighed. An additional sample of shoots was taken for oven drying. Fodder beet was harvested on the 20^{th} and 12^{th} of May for the first and the second season, respectively. At harvesting (190 days from sowing date), ten plants were randomly taken from each plot and the growth characteristics; root length (cm), root diameter (cm) and weight of top (Mg fad^{-1}) were recorded (Illustrate 1.). The yield of each plot was recorded after obtaining all the plot plants which were roughly cleaned and weighed. Fresh yields of roots and tops were determined and samples were oven dried at 70 °C to a constant weight as dry matter content.



$$Diameter\ (d) = \frac{Circumference\ (C)}{\pi}$$

Illustrate 1. Root dimensions

Chemical analysis

Root tissues were wet digested by a mixture of $HClO_4$ and H_2SO_4 acids and their nutritional content was estimated as follows: N by semi micro Kjeldahl, P spectrophotometrically using stannous chloride reagent, K by the Flame photometer (Chapman and Pratt, 1961). Photosynthetic pigment (Chlorophyll a, b) in the fresh leaves and Proline content were estimated as described by Witham *et al.* (1971) and Bates *et al.,* (1973), respectively. Protein percentage of root and top were calculated by multiplying the N % by 6.25 (Hymowitz *et al*., 1972).

Statistical analysis

The experimental design was completely randomized blocks with three replicates. The obtained data were statistically analyzed using the Costat program and L.S.D. test at the probability levels of 5% (Gomez and Gomez, 1984). The discussed data is the mean value for both seasons. The increase or decrease percent (%) was calculated by dividing the difference between the specified treatment value and the control by the control.

RESULTS

Effect of PEG and HA treatments on soil

Data in Table (3) showed the effect of soaking seed in PEG or HA on some soil properties. Soil pH was almost unchanged. The EC values were decreased by 35.5% and 37.99% for both PEG and HA treatments respectively as the soaking time increased compared with the control. Similarly, soil available NPK for both seasons were increased. Maximum increase values were obtained for maximum soaking time (12 h). For PEG and HA respectively, available N was increased by 28.44% and 33.65%; P was increased by 11.28% and 96.24% while K was increased by 13.14% and 18.5%. The increases due to HA treatment were greater than that due to PEG.

Table 3: Some of the soil available nutrients as affected by different rates treatments

Treatments	Soaking time (h)	pH (1:2.5)		EC (dS m^{-1})		N (mg kg)		P (mg kg)		K (mg kg)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		8.06	8.03	8.37	7.66	38.25	40.89	3.97	4.01	185	188
PEG	6	8.04	8.01	6.10	5.81	43.85	43.98	4.25	4.32	200	208
	8	8.03	8.00	5.99	5.14	49.25	51.20	4.39	4.45	203	212
	12	8.01	7.98	5.38	4.96	50.76	50.99	4.42	4.46	208	214
	L.S.D.	ns		1.29		2.56		0.13		17.48	
HA	6	8.02	7.97	7.96	6.57	47.21	48.29	4.69	4.73	213	218
	8	8.01	7.95	5.57	5.04	52.19	53.06	4.72	7.78	218	220
	12	7.98	7.95	5.14	4.80	52.63	53.14	7.81	7.85	219	223
	L.S.D.	ns		1.98		1.92		3.98		8.72	

Effect of PEG and HA treatments on fodder beet growth characteristics

According to the growth characteristics data presented in Table (4), it can be said that the fodder beet growth have been enhanced by soaking the seeds in PEG and/or HA solutions. The soaking time 12 h gave the highest enhancement. Humic acid seemed to be more effective than PEG in enhancing the studied fodder beet growth characteristics. In respect to soaking time the increases in the weight of fresh root, weight of dry root, root length, root diameter and weight of top amounted 133.3, 136.86, 22.67, 66.16 and 75.48% respectively, for the PEG treatment. For the HA treatments, the increases in the above mentioned characteristics amounted 155.26, 161.63, 31.05, 70.69 and 86.97%, respectively.

Table 4: Fodder beet growth characteristics as affected by as affected by different rates of treatments

Treatments	Soaking time (h)	Fresh weight of root (Mg fed^{-1})*		Weight of dry root (Mg fed^{-1})		Root Length (cm)		Root Diameter (cm)		Weight of Top (Mg fed^{-1})	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		10.59	11.27	1.59	1.72	34.39	34.41	8.12	8.46	12.6	13.5
PEG	6	18.94	22.70	2.59	2.70	38.14	38.55	12.80	12.85	21.4	22.2
	8	22.39	25.63	3.25	3.60	42.10	42.16	13.64	13.74	22.2	22.8
	12	24.83	26.17	3.55	4.29	42.19	42.21	13.73	13.82	22.7	23.1
	L.S.D.	6.68		1.07		0.54		0.19		1.39	
HA	6	23.86	24.66	3.45	3.61	38.65	40.09	13.66	13.88	22.9	23.5
	8	25.59	27.29	3.75	3.88	44.97	45.00	14.05	14.10	23.4	24.0
	12	27.66	28.14	3.77	4.89	45.04	45.12	14.12	14.18	24.1	24.7
	L.S.D.	2.52		1.48		1.87		0.31		1.35	

*** 1 fed = 0.42 ha**

Fodder beet roots and top macro-nutrient and protein contents as affected by different treatments

Tables (5a&b) present the macro-nutrient and protein content of the fodder beet roots and tops at different treatments. As shown, the total NPK and protein % were increased with increasing soaking time up to 12 h for both PEG and HA. The average increase percent (%) in both seasons as follows:

(a) Roots; N: 22.6, 30.2 % – P: 37.88, 50% – K: 7.82, 12.35% – Protein: 21.71, 26.63% for PEG and HA, respectively, at 12 h soaking time.

b) Tops; N: 5.88, 17.23 % – P: 29.31, 43.1% – K: 6.78, 8.81% – Protein: 5.82, 16.63% for PEG and HA, respectively, at 12 h soaking time.

Table 5(a): Fodder beet roots and top roots macro-nutrient and protein content as affected by different rates of treatments (a) Roots.

Treatments	Soaking time (h)	N (%)		P (%)		K (%)		Protein (%)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		1.59	1.63	0.31	0.35	2.41	2.45	9.94	10.19
PEG	6	1.84	1.88	0.38	0.41	2.54	2.56	11.5	11.75
	8	1.93	1.94	0.41	0.43	2.58	2.60	12.06	12.13
	12	1.95	1.97	0.45	0.46	2.61	2.63	12.19	12.31
	L.S.D.	0.06		0.05		0.05		0.37	
HA	6	1.95	1.98	0.37	0.39	2.64	2.66	12.18	12.37
	8	2.03	2.06	0.44	0.47	2.68	2.71	12.69	12.87
	12	2.07	2.10	0.48	0.51	2.72	2.74	12.37	13.12
	L.S.D.	0.07		0.06		0.05		1.03	

Table 5(b): Top

Treatments	Soaking time (h)	N (%)		P (%)		K (%)		Protein (%)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		2.38	2.43	0.28	0.30	2.92	2.98	14.87	15.19
PEG	6	2.45	2.49	0.33	0.34	3.05	3.09	15.31	15.56
	8	2.49	2.53	0.35	0.37	3.09	3.12	15.56	15.81
	12	2.52	2.57	0.37	0.38	3.12	3.18	15.75	16.06
	L.S.D.	0.1		0.03		0.1		0.61	
HA	6	2.63	2.66	0.32	0.35	3.10	3.14	16.44	16.62
	8	2.75	2.77	0.37	0.38	3.15	3.18	17.18	17.31
	12	2.79	2.82	0.41	0.42	3.20	3.22	17.44	17.62
	L.S.D.	0.06		0.04		0.07		0.37	

Fodder beet leaves characteristics as affected by different treatments

At different treatments of PEG and HA, the content of *Chl*, proline and percent of emergence of leaves varied significantly (Table 6). As the soaking time increased the *Chl* content and emergence percent increased while the proline content decreased. At soaking time 12 h, the *Chl* content increased by 84.43% and 156.51% and emergence by 36.36% and 42.15% while the proline decreased by 16.07% and 25.71% for PEG and HA, respectively.

Table (6): Fodder beet leaves characteristics as affected by different rates of treatments

Treatments	Soaking time (h)	Chlorophyll (a+b) (mg/g. f.w.)		Proline (μmol/g. leaf tissue.)		Plant Emergence (%)	
		1st	2nd	1st	2nd	1st	2nd
Control		2.95	2.96	0.141	0.139	59	62
PEG	6	4.19	4.22	0.128	0.122	66	69
	8	5.37	5.39	0.121	0.117	75	78
	12	5.44	5.46	0.120	0.115	81	84
	L.S.D.	0.05		0.01		6.75	
HA	6	6.82	6.88	0.119	0.114	71	74
	8	7.10	7.14	0.112	0.109	79	82
	12	7.56	7.60	0.105	0.103	85	87
	L.S.D.	0.11		0.01		6.09	

DISCUSSION

Results of the present study indicated that the concentration of the macro-nutrients N, P and K in the available and total states is positively affected by soaking the fodder beet seeds in the PEG and HA solutions compared to the un-soaked seeds under salinity conditions. These results were in agreement with the results mentioned by Provenza *et al*., 2000. Additionally, the roots protein and leaves *Chl* content increased while the proline accumulation decreased. The fresh and dry weight of root as well as the root length and diameter were enhanced significantly. The soaking time appeared to have a great effect on the product characteristics as indicated previously (Yasari *et al*., 2013). A twelfth hour soaking time gave the highest values for the estimated parameters for the current study. The solutions of PEG and HA might have been affected the fodder beet seeds chemically and physiologically through creating a specific osmotic pressure atmosphere surrounding the seeds leading to a promoted growth indices (Jain *et al*., 2013). Humic acid treatments showed promising effects as stated earlier due to its bio-compatibility being the principle organic matter (Paksoy *et al*., 2010; Eisa *et al*., 2012; Jain *et al*., 2013).

In the present study, the effect of PEG and HA is expected to be reflected at the root distribution zone (RDZ) being the zone of contact with soil. Their chemical structure will often play an important control role within the soil matrix affecting the mobility and concentration of the soluble ions in the soil solution rather than the acid-base balance. At basic medium (soil pH 8.03 – 8.06 > 7), protons H^+ are less competing for the partially dissociated hydroxyl (–OH) functional groups of PEG and HA than other soluble cations. Another concept may be suggested, that is a thin layer of polymers coats seeds and/or soil particles forming a semi-permeable membrane which restricts soluble ions distribution and hence the osmotic pressure within the RDZ. Partitioning of labile species including soluble salts and nutrients may be highly controlled by the osmotic pressure created by the macromolecules, PEG and HA. Specific solute-membrane interactions may occur and in turn, the absorption of nutrients by the plant roots will be affected.

Photosynthetic pigments determine the physiological status of the plants. Chlorophyll is the molecule that acts as a photoreceptor. Entire pathway of *Chl* biosynthesis is operated in plastids by a complex set of reactions involving many intermediates. The change in the *Chl* provides further information about the process taking place in the photosynthetic apparatus. Increase in *Chl* content has been demonstrated in graminaceous chlorophyllic cell lines of grass *Bouteloua gracilis* exposed to different concentrations of PEG 8000 (PEG 6000 imposed water deficit has affected the activities of enzymes of *Chl* metabolism also). Salinity has been found to enhance the chlorophyllase activity in pigeonpea and *Gingellay* which results in lowering of chlorophyll content (Jain *et al*., 2013).

Proline accumulation under stress has been linked with its role as an osmolyte by contributing towards osmotic adjustment between cytoplasm and vacuoles. Because of its zwitter ionic and highly hydrophilic character it has a role as an osmoticum and acts as a compatible solute for plants subjected to low water potential and other environmental stresses. Increase in the free proline content during water stress condition due to PEG has been stated (Jain *et al*., 2013).

Finally, it could be concluded that presented soaking seeds of fodder beet to different time 8 and 12 h were effect on the productivity and quality of fodder beet crops under saline soil conditions.

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تأثير نقع البذور فى محاليل البولى إيثيلين جليكول وحمض الهيوميك على إنتاجية وجودة بنجر العلف تحت الظروف الملحية

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أجريت تجربتان حقليتان فى موسمى شتاء 2012/2011 و 2013/2012 فى محطة البحوث الزراعية بمنطقة السرو – دمياط - مصر وذلك لدراسة تأثير نقع البذور فى محلول من البولى إثيلين جليكول (PEG-6000) أو حمض الهيوميك (HA) على نمو وإنتاجية وجودة محصول بنجر العلف وكذلك محتواه من العناصر الغذائية تحت ظروف الأراضى الملحية حديثة الإستصلاح. إستخدم فى هذه الدراسة الصنف (*Beta vulgaris L. (vorochenger*) وهو من أصناف بنجر العلف متعددة الأجنة.

اوضحت النتائج ان حموضة التربة لم تتاثر معنويا وان النقص فى ملوحة التربة كان بنسبة 35.5 % و 37.99 % بينما زادت محتوى التربة من العناصر النتروجين والفوسفور والبوتاسيوم بنسبة 28.44 و 18.55% للنتروجين وكذلك 11.28 و 96.24 % للفوسفور و 13.14 و 18.50 % للبوتاسيوم وذلك لتاثير النتاتج عن كل من الايثلين جليكول وحمض الهيويك على التوالى.

بينت الدراسة التأثير الإيجابى لنقع البذور مقارنة بالبذور التى لم يتم نقعها , وكانت الزيادة نتيجة المعاملة بـHA أعلى من مثيلاتها المعاملة ب PEG . بالنسبة لمعاملات PEG كانت الزيادة فى الوزن الرطب للجذور بمقدار 133.3% والوزن الجاف 136.86% , بينما لمعاملات HA كانت الزيادة بمقدار 155.26% و 161.63% لكل من الوزن الرطب والجاف للجذور على التوالى .

كما بينت الدراسة التأثير الإيجابى على صفات النمو التى تمت دراستها حيث زاد محتوى الجذور من عناصر النيتروجين (N) بمقدار21.74% و 29.5% والفوسفور (P) 37.88 % و 50% والبوتاسيوم (K) 7.82% و 12.35% والبروتين 21.71% و 26.63% لكل من ال PEG و HA على التوالى , مقارنة بمعاملات المقارنة. كذلك زاد محتوى الأوراق من الكلوروفيل بمقدار 84.43 % و 156.51 % وإنخفض تراكم البرولين بمقدار 16.07% و 25.71% وذلك كأعلى قيم للمعاملات المذكورة على التوالى.