J. Plant Production, Mansoura Univ., Vol. 6 (8): 1395 - 1404, 2015

EFFECT OF ASCORBIC, HUMIC AND NICOTINIC ACIDS ON CERTAIN BIOCHEMICL CONSTITUENTS IN FENUGREEK (*Trigonella foenum-graecum L.*)

Mohamed, Z.A.; M.F.El_Banna and A.S.A.M. Shabara
Agric. Bot. Dept., Faculty of Agric., Mansoura University , 35516, El-Mansoura, Egypt

ABSTRACT

Two field experiments were conducted to evaluate the effects of ascorbic (AsA), humic (HA) and nicotinic (NA) acid on certain biochemical constituents that influence seed quality of Fenugreek (*Trigonella foenum graecum*, L.). Results indicated that presoaking or presoaking plus spraying in by AsA, HA and NA increased flavonoids ,alkaloids ,trigonelline ,protein ,total carbohydrates and oil percentage in seeds . With all tested metabolic inducers (MI), the enhancing effect was more pronounced at its higher adopted level and when it was applied as a combined presoaking plus a foliar spray treatment. Total alkaloid percentage was highest in response to HA at 3000 mg/l whereas trigonelline percentage recorded highest value in response to AsA at 200 mg/l. On the other hand, total phenols percentage was generally decreased with exogenous application of all MI , and the decreased was more evident at the higher level of either AsA, HA or NA . Based on the obtained results, it could be concluded that metabolism of Fenugreek *(Trigonella foenum graecum*, L.) could be modulated toward the accumulation of beneficial components by exogenous application of AsA, HA, and NA .

INTRODUCTION

Fenugreek (*Trigonella foenum graecum,L.*) is known as one of the oldest medicinal plants recognized in recorded history (Acharya *et al.*,2008). Fenugreek leaves and seeds are consumed in different countries around all the world for different purposes, the most prominent of which are its medicinal uses. Medicinally, it is used as anti-diabetic, lowering blood sugar and cholesterol levels, anticancer, and antimicrobial (Mehrafarin *et al.*, 2011). Fenugreek above-ground organs contain several bioactive Compounds including flavonoids, phenols and alkaloids, the most important of which is trigonelline (Snehlota and Payal, 2012). Trigonelline have several physiological effect the most notably of which is its hypoglycemic effect (Neveen *et al.*, 2007; Abd_Elmawla and Osman, 2011). Secondary metabolites biosynthesis and accumulation could be enhanced through application of plant growth regulators (Danesh-Talab et al., 2014) or certain elicitors (Abd_Elmawla and Osman ,2011). Benzylaminopurine (Ortuno et al. , 1998), 2-1-napthyl acetic acid (Alagukannan and Vijaykumar,1999), and both GA3 and NAA applied at various concentrations (Danesh-Talab et al.,2014) have been reported to have enhancing effects on certain Fenugreek seeds metabolites. However little is known about the effects of ascorbic and humic acids on the biosynthesis and accumulation of secondary metabolites in Fenugreek .Elicitation and precursor feeding are two common approaches for enhancing secondary metabolites biosynthesis and

accumulation. *Methyl Jasmonate* (MJ) was used as an elicitor for trigonelline induction in Fenugreek 's cell suspension cultures (Abd_Elmawla and Osman, 2011). They reported a 2-fold increase in trigonelline percentage in MJ-elicited cultures. In addition, precursor feeding with Nicotinic acid (NA) was found to enhance trigonelline content in callus cultures of *Moringa Oleifera* (Mathur and Kamal,2012.).On the other hand, the effectiveness of precursor feeding with NA *in vivo* is less investigated and poorly understood. Therefore the present investigation was conducted to assess the effects of *in vivo* application of ascorbic, humic and nicotinic acids on key secondary metabolites as well as major seed constituents of Fenugreek.

MATERIALS AND METHODS

Two field experiments were established during the two growth season 2012 /2013 and 2013 / 2014. in the soaking treatment, seeds ware soaked in solution of AsA and NA either at 100 or 200 mg/L as well as in HA either at 1500 or 3000 mg / L for 5 h. Seeds soaked in distilled water for the same period represent the control treatment. Soaked seeds were inoculated with *Rhizobium sp* before sowing at 15 cm apart in 2x3 m plots. In the combined soaking plus spraying treatment, plants were also received two foliar applications with the same metabolic inducers (MIs) and at the same level used in soaking. The first foliar spray was 30 days after sowing (DAS) whereas the second was 45 DAS. Except for treatment with either MI, all other tillage practices were followed as recommended by The Ministry of Agriculture Egypt. The experiment was laid out in a randomized complete block design. At the end of the growing season, seeds samples were collected to determine certain biochemical constituents. Table (1) shows Physical and chemical analyses of the soil used in the experiment.

Table (1): Physical and chemical analyses of the experimental soils

PH 1:1	EC at 25 °C	Anion and Cation meq/100 g soil								Size distribution %				Texture
										Sand				
		$Co_3^{=}$	$So_4^{=}$	HCO_3^{-}	Cl^{-}	Ca^{++}	Mg^{++}	K^{+}	Na^{+}	Fine	Coarse	Silt	Clay	
8.33	1.10	--	0.789	0.28	0.54	0.40	0.65	.007·	.40·	34.45	1.85	13.6	53	Clay loam

Determination of total carbohydrate:

A 5 g seed sample was extracted by ethanol 80 % and kept overnight at room temperature. The ethanolic extract was used for the determination of total carbohydrates using anthrone method.The developed green color was measured spectrophotometrically at 630 nm according to Sadasivam and Manickam (1996).

Determination of total alkaloids:

Total alkaloids was determined using the method that described by Aziz et al.(2005) by soxhlet , ten gram of the powdered sample seeds was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator, under reduced pressure at 40c .dry residue repeat by 150 mL of chloroform and acidify by HCL15% pH3, it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 mL of chloroform, basify by the NaHCO٣ 5% pH 9 and lit it during 15 minutes in the room temperature. The chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue is the total alkaloid.

Determination of total phenols:

The powdered seeds (2 g) was extracted with methanol, at room temperature overnight. The methanol extracts were combined and concentrated under reduced pressure on a rotary evaporator. Total phenolic percentage of each plants extract was determined with the Folinciocalteus reagent (FCR) acc0rding to Slinkard and Singleton, (1977). Each sample (0.5ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na2CO3 (7.5%,v.v) solution. The absorbance was then measured at 765 mn after incubation at 30 C for 90 minute .Results were expressed as Gallic acid equivalent (mg Gallic acid / g dried extract).

Determination of flavonoid:

The total flavonoid percentage of seed extract was determined by a colorimetric method as described in by (Zhissen et al., 1999). Each sample (0.5ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%). After 6 minutes, 0.15 ml of aluminum chloride (AlCl3) solution (10%) was added and allowed to stand for 6 minute, then 2 ml of NaOH solution (4%) was added to the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was then determined at 510 nm versus prepared water blank.

Determination of protein:

Percentage of Protein was calculated by multiplying the percentage of total nitrogen by the factor of 6.25 (A.O.A.C.,1980).

%Protein = % Nitrogen x 6.25

It could be mention that chemical determination were made only in the first season.

Determination of Trigonellin:

By Seed percentage of the active ingredient were taken 100 grams of seeds of each experimental unit and underwent grinding and sieving operations since taking 80 grams of seeds crushed in order to remove fat and processed for the purpose of the extraction process for vehicles. After Alkaloids remove fat from seeds according to the method (Wanger *et al.*, 1984) Then it was separated and purified compounds to take 40 grams of residue seeds were extracted defatted Alkaloids and purified according to the method (Tugrul and Ozer, 1985).

Determination of Fixed oil :

At harvesting time anthocyanin percentage was determined in air – dried Fenugreek according to the method descried by Du and Francis(1973).The percentage of fixed oil in seeds was determined according to the method mentioned by A.O.A.C (1980).

Statistical analysis:

The obtained data were subject to statistic of variance according to Gomez and Gomez(1984).The treatment means were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

Effect of AsA:

Ascorbic acid applied at 200 mg /l increased significantly all studied seed biochemical characters whereas decreased total phenols (Fig 4). At 100 mg/l, AsA increased total flavonoids (Figure 3), trigonelline (Fig 1), total carbohydrates and oil percentages (Table 2) whereas did not significantly affect other studied characters. At both AsA levels, its application as a combined presoaking plus foliar spray treatment was superior to its application as a presoaking treatment only. Highest alkaloids and trigonelline percentages were obtained in case of the interaction between the combined application methods with AsA at 200 mg/l.

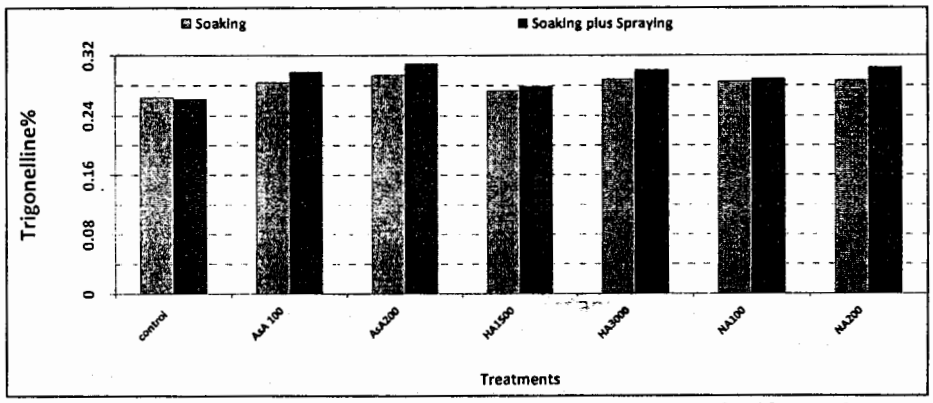

Figure (1) effects of exogenous application with ascorbic (AsA), humic (HA) and nicotinic (NA) acids on Trigonelline percentage in Fenugreek seeds.

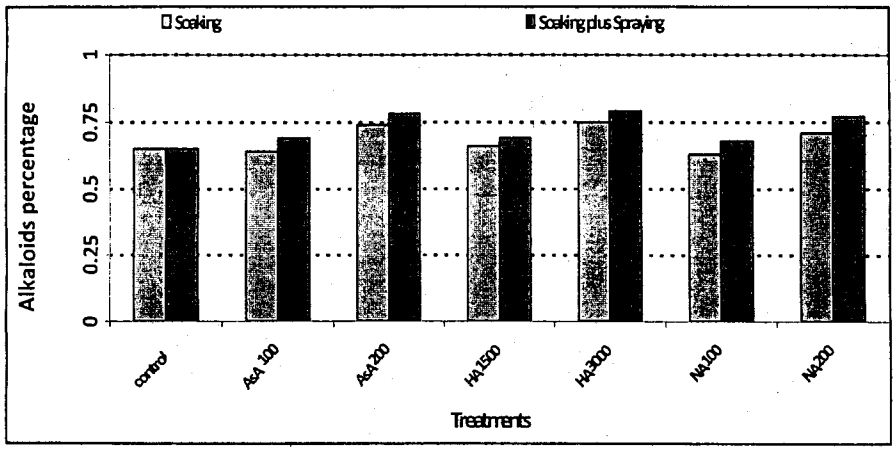

Figure (2) effects of exogenous application with ascorbic (AsA), humic (HA) and nicotinic (NA) acids on alkaloids percentage in Fenugreek seeds.

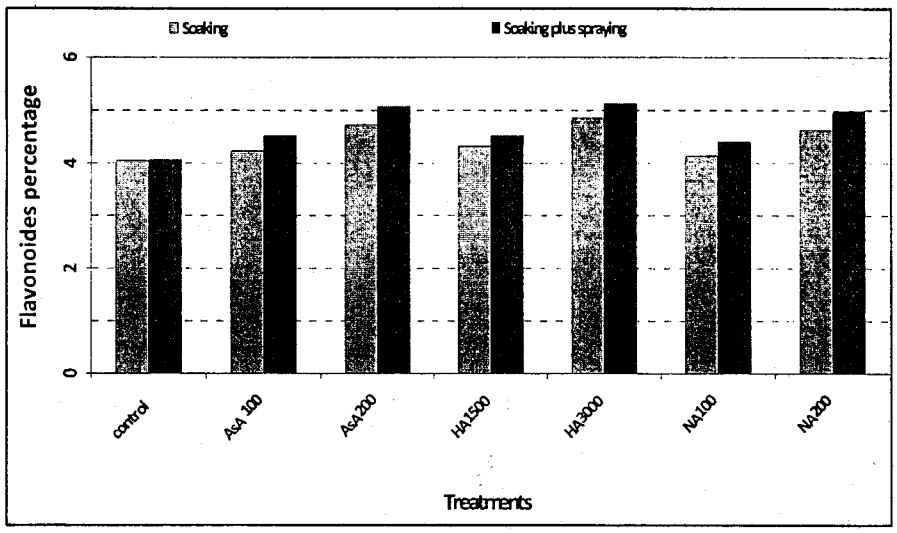

Figure (3) effects of exogenous application with ascorbic (AsA), humic (HA) and nicotinic (NA) acids on flavonoides percentage in Fenugreek seeds.

AsA is involved in a wide array of important functions in plant growth and metabolism. It has a fundamental role as defense antioxidant as well as essential roles in photoprotection, regulation of photosynthesis and growth (El-Lethy *et al.*, 2011). As primary and secondary plant metabolism are closely interconnected, it may be concluded that AsA–induced Flavonoids, alkaloids and trigonelline percentages May be as a consequence to its stimulating effects on plant primary metabolism /metabolites (Table 2). This conclusion is substantiated with the results of previous investigations in which

AsA exogenous application was reported to enhance photosynthetic activity and various plant primary metabolites in *Lycopersicon Essulentum* (El-sayed and Elsayed, 2013). In addition, AsA application was found to increase total phenols and total flavonoids in two different genotype of maize (Salama et al., 2013), as well as various secondary metabolites in *Pelargonium graveolens* (EL-Lethy *et al.*, 2011) and *Jasminum grandiflorum* (Eid *et al.*, 2010) .

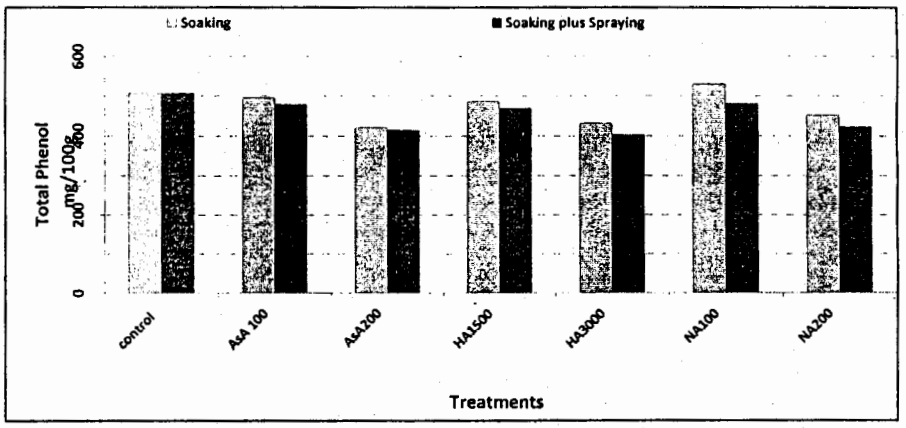

Figure (4) effects of exogenous application with ascorbic (AsA), humic (HA) and nicotinic (NA) acid on Total phenol in Fenugreek seeds.

Effects of HA:

HA applied at 3000 mg/lincreased all recorded parameters. On the other hand, total phenols percentage was decreased (figure 4).At its lower adopted level (1500 mgl/l), HA increased total flavonoids, trigonelline, total carbohydrates and oil percentage (Table 2). Whereas others parameters were not significantly affected. Higher increment levels were recorded when HA was applied as presoaking in conjunction with a foliar spray treatment. When considering the interaction between HA levels and application methods, the interaction between HA at 3000mg/L and the combined method of application was of superior effect. (Figures 1-4, Table 2)

HA was previously reported to both directly and indirectly affect plant's biochemical processes (Yong et al,2004; Rady,2012).HA was reported to positively influence net photosynthesis along with enhancing chlorophyll percentage and stomatal conductance (Tahira *et al*,2013).In addition, the activity of two key photosynthetic enzymes, phosphoenolpyruvate carboxylase and ribulose-1,5- bis phosphate carboxylase was enhanced in response to HA treatment (Zhang *et al* .,2014). Total sugars, total free amino acids and protein concentrations were increased in snap bean treated with HA (Hanafy Ahmed *et al.*, 2010). In their study, total phenols percentage was also increased in response to HA, in contrary to the results of the present investigation. This could be explained by lower HA concentration used in their study, being 2000 mgl-1. Inhibition of urease activity by humic acid was reported by Vaughan and Ord, (1991) which may reduce N loss by volatilization, thereby increase N availability to plants which enhance plant

biochemical processes in which N is involved. Trevisan *et al.* (2010) concluded a HA- dependent activation of plant biochemical processes and enzymes synthesis as well as auxin-like activities of HA which resulted in increased root mass, photochemical efficiency and antioxidant levels. In addition, Zhang and Ervin (2004) found that exogenous application of HA increased endogenous cytokinin and auxin levels, which may improve crop productivity .

Treatment		Protein %			T. Carbohydrates %			Fixed oil %		
		S	Sf	M(A)	S	Sf	M(A)	S	Sf	M(A)
Control		23.53	23.63	23.58	48.07	49.42	48.75	6.05	6.43	6.24
AsA	100 mg/l	23.77	24.24	24.00	53.14	52.29	52.71	7.95	8.19	8.07
	200 mg/l	24.62	24.94	24.78	53.43	53.14	53.29	7.75	8.25	8.00
HA	1500 mg/l	24.62	24.94	24.78	53.43	53.14	53.29	7.75	8.25	8.00
	3000 mg/l	23.94	24.31	24.13	50.04	52.44	51.23	7.82	7.78	7.80
NA	100 mg/l	24.71	25.01	24.86	52.42	53.41	52.90	7.82	7.80	7.81
	200 mg/l	23.71	24.09	23.90	51.50	52.48	51.98	6.92	7.14	7.03
M (B)		24.16	24.43		51.70	52.10		7.32	7.60	
LSD 0.05	A	0.81			2.22			0.61		
	B	0.43			1.19			0.32		
	A*B	1.15			3.13			0.85		

Table (2) effects of exogenous application with ascorbic (AsA), humic (HA) and nicotinic (NA) acid on Protein % , total carbohydrates % and Fixed oil %. In seed

Effect of Nicotinic acid (NA):

NA at both levels, increased trigonelline , total carbohydrates and oil percentage .On the other hand, total flavonoids, total alkaloids and protein percentage were increased only when NA was applied at 200 mg/l (Figures 1-4, Table 2). Total phenols percentage was decreased in response to the higher level, whereas it was not affected in response to the lower level NA. Application of NA as a combined presoaking plus spraying treatment was more effective in inducing secondary metabolites accumulation in fenugreek seeds .Nevertheless, more decrease in total phenols percentage was recorded due to the combined treatment (Figure 4). NA and NAD are chemically related (Ali, 2002), so similar effects of both on plant growth and metabolism is envisaged. Both NA and NAD increased ricinine alkaloids and oil percentage in *Ricinus communis* plants grown under salinity stress (Ali,2002). He concluded that adequate supply of NAD through NA

application is essential for normal plant growth and development.This may explain the positive effects of NA exogenous application on the recorded biochemical constitutes. Trigonelline serves as storage form of NA (Blaim and wanner,1960; c.f. Mathus and Kamal, 2012).So, trigonelline accumulation due to NA application recorded in the present investigation may be explained on the ground that Nicotinic acid acted as a precursor in the biosynthesis of trigonelline in Fenugreek. This conclusion is supported by the result of previous studies in which trigonelline formation was increased in NA-supplemented tissue cultures of *Trigonella foenum-graecum* (Khanna and Jain,1972), Allium cepa (Khanna *et al.*,1989) and *Moringa olifera* (Mathur and Kamal,2012).

REFERENCES

Abbas,T., Ahmed, S., Ashraf , M ., Shohid, M.A .and Abbas, S. (2012). Effect of humic acid application at different growth stage of kinnow mandarin *(Citrus reticulata blanco*) on the boses of physio –biochemical and reproductive responses . Acod-J- Biotechn ., 1:14-20

Abd-El maewla ,A.M.A.and Osman,H.E.H.(2011).Elicitation of Trigonelline and a-hydroxy isoleucine with hypoglycemic activity in cell suspention caltures of Trigonella foenum graecum l, The open conference proceeding journal ,2 :80-87.

Acharya, S.H., Tbomas , J . E., Basu, S. K. (2008). Fenugreek asn alferative crop for semianid regonis of North America. Crop-Sci., 48: 841-853.

Alagukannan, G. and Vijaykumar, M. (1999). Effect of plant growth substances on yield attributing parameters, yield and quality in fenugreek (Trigonella foenumgraecum L.). S. Ind. Hort. 47(1-6): 130-133.

Ali ,R.M. (2002).effect of nicotinic acid and nictinamide adenine dinucleotide on growth and percentage of oil ,glycerol and ricinine alkaloids of salinity stressed *ricinus communis L* .Phyton ,42 (2):269-277.

A.O.A.C. (1980). Official Methods of Analysis " Association of Official Agricultural Chemists, 13 th Ed. Washington, D.C., USA

Aziz Bouchelta , Boughdad, A. and Blenzar, A.(2005) Effects biocides des alcaloides ,des saponines et des flavonoides extraits de Capsicum frutescens L. (Solanaceae) sur Bemisiatabaci (Gennadius)(Homoptera: Aleyrodidae). Biotechnol.Agron.Soc.Environ 9(4):259-269.

Blaim,K.and Wanner,H.(1960). Presence of trigonelline in seed and seedling of *Melilotus officinales.* Naturwissench, 48:331-332-11.

Donesh – Talab, S , Mehroforin , A.; Lobbafi, M., Qavomi, N., Qoderi, A. and Naghdi Bodi, H .(2014). Response of Fenugreek (*Trigonella foenum-graecum*) to exogenous application of plant growth regulators (PGRS). Trakia J. Sci., 2:142-148.

DU, C. T. and Francis, F. J. (1973), Aanthocyanins of roselle *Hibiscus sabdariffa, L.* Journal of Food Science, 38: 810–812.

Eid,R.A.,Taha,S.and Ibrahim,S.M.M.(2010).Physiological properties studies on essential oil *of_ Jasminum grandiflorum L.*as affected by some vitamins . Ozean J. Appl. Sci., 3: 87-96.

El-Letby,Saffaa R., Ayad,Hosnaa S.and Reda,Fatma (2011). Effect riboflavin, ascorbic acid and dry yeast on vegetative growth, essential oil pottern and antioxidant activity of (*Pelargonium graveolens L.*)-Amer-Euras.J. Agric of Environ. Sci, 10:781-786.

El-Sayed,Hameda and El-Sayed,A.(2013).Exogenous application of ascorbic acid for Improve Germination, Growth, Water Relations, Organic and Inorganc Components in Tomato*(L-ycopersicon esculentum Mill.)* plant under salt-stress.New York Science Tournal 201:6(10):123-139.

Gomez, K.A. and A.A.(1984). Statistical procedures for Agriculture Research. John Wiley and Sons, Inc. New York.

Hanafy Ahmed, A-H., Nesiem , M.R., Heewdy , A.M and Sallam , H.El-S.(2010). Effect of simulation compounds on growth, yield and chemical composition of snap bean plants grown under calcareous soil conditions .J-Amer- Sci-, 6:552-569.

Khanna,P. and Tain ,S.C.(1972).Effect of nicotinic acid on growth and production of trigonelline by *Trigonello foenum graecum L.*tissue cultres .Indian J. Exp.Biol,10:248-249.

Khanna,P;Sharma,A.;Kaushik,P.and Chaturvedi,P.(1989). Trigonelline from three plant species in vivo and in vitro tissue culutres .Invest.New Drug, 26:334-336.

Mehrafarin ,A. ; Reza zadeh ,s.H.; Haghdi, B-H.;Hoor mohammdi , G-H.; Zand,E.and Qaderi ,A-(2011) .J-F medicinal platoy 10:6-20.

Mothur ,M.and Kamal,R.(2012). Studies on trigonelline from *Moringa olifera* and its in vitro regulation by feeding precursor in cell cultres .Brazilian J. phormacognosy, 22; 994-1001.

Neveen S.M.A Turky(2007) physiological studies on snap bean plant M.Sci.Thesis ,Fac .AGRIC.,Cairo univ.,Egypt.

Ortuno A., R. Oncina, J. M. Botia and Delrio, 1998. Distribution and changes of diosgenin during development of *Trigonella foenum-groecum* plants Modulation by benzlamino purine. Food Chem., 63:51-54.

Rady , A.A.(2012). A nove organo – mineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil. South afr.J. Bot., 81:8-14.

Sadasivam,S. and A. Manickam,(1996).Biochemical Methods,2nd Ed. New age inter .India.

Salama, ZeinabA., El-Fouly M-M. And Gaafor,A-A.(2013). Mitigation of the adverse effect of salinity through stimulation some secondary metabolites and antioxidant enzymes of metbanolic extract of maize cultivars by exogenous ascorbic acid. J. food, Agric of Environ., 11:1328 - 1335.

Slinkard, J. and Singleton, V.L.(1977) Total phonel analysis: automation and comparison with manual methods .AM.J.Enol. Viticult; 28:49-55.

Snehlota,H.S.and payol ,D.R.(2012). International Journal of current pharmaceutical review and research, 2:169-187.

Trevisem,S.;Francioso, 0.;Quaggiotti,S.and Nardi,S.(2010).Humic substances biological activity at the plant- soil interface .plant Signal. Behov. 5:1-9 Tugrul, L. and Ozer, A. (1985). Possibilities for the use of *Trigonella foenum-graecum* L. seeds as a crude drug in Turkey. Acta pharmaceutica Turcia. 27: 14-16

Vauhion,s.p.and Grd, B.G (1991). Influence of natural and synthetic humic substances on the activity of urease .J. soil sci.,42:17-23. Wagner, H ., Bladt, S. and Zgainski, E. M. (1984). Plant Drug Analysis: A Thin Layer Chromatography Atlas. Translated by scott, Th. A. Springer-Verlage, Berlin, Heidelberg. New York,. Tokyo, PP: 51-54.

Yong, H.L.,Hseu, Y.T.,Lu,F.J.,jLin,E. and Lai , J.s. (2004). Humic acid induces apoptosis in human Premyelo cytic lekem ia HL-60 cells .life Sc., 75 :1817-1831.

Zhang, X. and Ervin, E. H. (2004). Cytokinin – cotaining seaweed and humic acid extract associated with creeping bent gross leaf cytokinins and drought resistance. Crop Sci., 44:1737- 1745 .

Zhishen, J.;Mengcheng, T. and Jianming, W.The determination of flavonoid percentages in mulberry and their scavenging effects on doi.org/10.1016/S0308-8146(98)00102-2.

Zhong ,L.; Loi, J.; Gao, M. and Ashraf , M (2014). Exogenous glycine betaine and humic acid improve growth, nitrogen status, photosynthesis and antioxidant defense system and confer tolerance to nitrogen stress in maize seeding .J. plant interactions, 9:159- 166.

تأثير حمض الاسكوربك والهيوميك والنيكوتينك على محتوى بعض الصفات الكيميائيه لنبات الحلبه.

زين العابدين عبد الحميد محمد ، مصطفى فؤاد البنا و أمير صادق عباس محمد شباره

قسم النبات الزراعى – كليه الزراعه – جامعه المنصوره

تم اجراء تجربتين حقليتين بهدف تقييم تأثيرالمعامله بحمض الاسكوربيك والهيوميك و النيكوتينك علي بعض الصفات الكيميائيه التي تؤثر علي جودة بذور الحلبه واوضحت النتائج أن كل من حمض الاسكوربك والهيوميك والنيكوتينك ادى الى زياده نسبه كل من الفلافونيدات و القلويدات والتريجونيللين والبروتين و الكربوهيدرات ومحتوي الزيت في البذور. وقد اشارت النتائج ان اعلى النسب ظهرت عندما ت تمت المعامله بالنقع اولا ثم الرش. حيث سجلت اعلى قيمه للقلويدات عند تطبيق الهيوميك بتركيز ٣جم/لتر بينما كان محتوى التريجونيللين اعلى قيمه له عند استعمال الاسكوربك بتركيز ٢٠٠ ملجم/لتر ومن ناحيه اخري كان هناك نقص ملحوظ فى محتوى الفينول عند كل المعاملات . وطبقا للنتائج المتحصل عليها، يمكن الإستنتاج بان التمثيل الغذائى لنبات الحلبه يمكن تحسينه تجاه تراكم بعض المكونات المفيده فى البذور وذلك من خلال تطبيق بعض معاملات النقع والرش لكل من حمض الاسكوربك والهيوميك والنيكوتينك.