International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 06, 2020
ISSN: 1475-7192

Foliar Spraying With Protein Hydrolysates Preparation From Shrimp by-products Improves Growth, Yield And Quality Of Lettuce (Lactuca sativa L.) Grown Under Salinity Conditions

[1]Haider M.H.Al-Malieky, [2]Awatif N. Jerry

ABSTRACT--*The experiment was carried out during the winter season 2019-2018 in Al-Hartha district/Basrah Governorateto study the effects of shrimp by-product prepared by Alcalase and Flavourzyme enzyme as a foliar application on lettuce (Lactuca sativa L.) growth, yield and quality grown under salinity conditions. Shrimp by-product protein hydrolysates were spray-applied (3 and 6 ml. L^{-1}) additional to control treatment. Randomized Complete Block Design (R.C.B.D.) was used as factorial experiment .Treatment means were compared by using Least Significant Differences (L.S.D.) at a probability of 0.05. The results showed that spraying with protein hydrolysates had a significant effect on most studied characters compared to control treatment. Results showed that foliar application with (T2) significantly increased the total leaves number, leaves area, shoot fresh weight, total yield, total phenols , total soluble solids (TSS %),vitamin C dry mater, K^+ and K^+/ Na^+ content in addition were recorded significantly decrease of nitrate, Na^+,CL^- and Ion leakage contents, while foliar application with (T4) significantly increased in the plant height. The result showed that spraying six times (S6) were significant increases in these growth characters comparing with four sprays (S4), while decreased nitrate , Na^+,CL^- and Ion leakage contents when spraying six times(S6). The interaction between treatments and spraying number show significantly increased in some characters, the (T2 S6) had the highest value shoot fresh weight 1036.0 g and total yield 24.311* tone.donum^{-1} .

Keywords*-- Shrimp by-products, protein hydrolysates, salinity, yield, quality, lettuce*

I. INTRODUCTION

Lettuce (*Lactuca sativa* L.) belongs to Asteraceae family, it is one of the most important vegetables in Iraq and other countries as nutritive source of proteins, oils, fibers and minerals such as phosphorous, potassium, zinc and vitamins such as thiamine, riboflavin, niacin, folic acid, B6, C, D vitamins (USDA, 2019). Lettuce leaf extracts can control certain types particularly leukemia cells and breast cancer cells of cancer (Gridling *et al*., 2010).

Salinity is one of the most important abiotic stress factor that affect all vegetable crops, especially in semi-arid and arid regions (Munns and Tester, 2008) because of small rainfall. Biostimulants hold a great promise for

[1] Agriculture Directorate of Basrah,Ministry of Agriculture,Iraq, haidermajd6@gmail.com

[2]Horticulture and Landscape Design Dpt., College of Agric., Basrah –Iraq, a_n_jerry@yahoo.com.

Received: 22 Feb 2019 | Revised: 13Mar 2019 | Accepted: 05 Apr 2020

the future of agriculture. These environmental friendly and natural substances promote vegetative growth and tolerance of plants to abiotic stresses such as soil salinization, water deficit and thermal stress, nutrition and crop quality (Chojnacka *et al*.,2015).

Protein hydrolysates (PHs) are an important group of plant biostimulants that have received increasing attention in recent years because of their positive effects on crop performance and contribution to agroecological sustainability.

Protein hydrolysates are 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from by-products from animal or plant origins protein sources using partial hydrolysis' by chemical (acid and alkaline hydrolysis) or enzymatic hydrolysis of agroindustrial. (Schaafsma, 2009; Colla *et al*.,2015).

Several beneficial effects of plant-derived protein hydrolysates (PHs) on plants including increase nutrient uptake and assimilation and increasing tolerance against abiotic stress (Cavani *et al*., 2006). The shrimp by-products contain valuable proteins 39% on the dry weight (Holanda and Netto, 2006) .

Therefore, hydrolysis of shrimp by-products protein would be a proper strategy for economic profit. Several studies have shown the importance of Protein hydrolysate application of in improving growth and production of vegetable crops. (Koukounaras *et al*., 2013) found that Greenhouse applications of an animal- derived PHs 'Siapton' hydrolysate enhanced both plant height as well as number of flowers per plant in tomato (*Solanum lycopersicum* L.).

Botta (2012) observed that lettuce plants treated with an animal-based protein hydrolysed at 3 ml. L^{-1} had a higher fresh and dry weight compared with the control. Xu and Mou (2017) found that the application of fish-derived bio stimulants increased the lettuce leaf number per plant and shoot dry weight.

The aim of this study was to assess the effects of protein hydrolysate from shrimp by-products prepared by enzyme hydrolysis using Alcalase and Flavourzyme applied in different concentration and doses on lettuce growth, yield and quality grown salt stress conditions.

II. MATERIALS AND METHODS

The experiment was carried out during the winter season 2019-2018 in Al-Hartha district / Basrah Governorate to study the effects of shrimp by-products protein hydrolysate repared by Alcalase and Flavourzyme enzyme as a foliar application on growth, yield and quality of the lettuce plant grown under salinity conditions.

The physical and chemical characterization of field soil and irrigation water are shown in table (1). Lettuce seeds were used (Fajr), the seeds were sowing in 209 holes Styrofoam trays at 20/9/2018 in media consisting of peatmoss. The seedlings were transplanted to field after 30 days from sowing.The soil field preparing and divided into lines in 3.75 m of length and distance between them 0.75 m .The planting was on both sides of the line, 25 cm distance between plants, density plants 23466 plant. $donum^{-1}$, each treatment had 3 replicate.

Table 1: Soil physic-chemical characteristics and irrigation water

soil characteristics	
E.C. ($ds.m^{-1)}$	10.22
PH	7.69

Total nitrogen (g.kg^{-1})	0.75
Phosphorus (mg.kg^{-1}).	2.46
Potassium (mg.kg^{-1})	15.51
Organic matter (%)	1.64
soil structure	
Sand (%)	11
Silt (%)	69
Clay (%)	20
Texture Class	silty clay
irrigation water	
E.C. (ds.m$^{-1)}$	6.48
PH	7.39

Preparation of shrimp by-products protein hydrolysates (PHs).

The hydrolysis processes for shrimp by-products were carried out using method (Chotikachinda *et. al*.,2018 ; Klomklao and Benjakul ,2018). Shrimp by-products were purchased from Basrha markets. The by-products washed with water and dried at 40 °C .Then, they were ground, the fat was remove from powder according to (Qi *et al*., 2015). The powder shrimp by-products were mixed with water at ratio 1:6w/v.The mixed were heated at 85°C for 10 min (Guerad *et al*., 2002). The shrimp by-products were hydrolysate using Alcalase and Flavourzyme obtained from company Sigma-aldrach under optimal conditions of each: Alcalase (PH 8, 50°C) Flavourzyme (PH 7, 50°C), time 7hour. Enzyme and substrate ratio of 0.1 %. The hydrolysis degree was monitored during the process according to Holye and Merat (1994) and the reaction was stopped by inactivating the enzyme (90°C /10 min). The hydrolysis was centrifuged 4000 rpm for 20 min and concentration by Rotary evaporator. The study included ten treatments which were the combination of five treatments are: control (T0), hydrolysate using Alcalase T1: 3ml.l^{-1}, T2:6 ml.l^{-1} and Flavourzyme T3: 3ml.l^{-1} and T4: 6 ml.l^{-1},with S4: spraying four times and S6: spraying six times. The treated lettuce plants were sprayed four and six times during growing. Treatments were applied starting at 20 days after transplant at 10 day intervals. Head lettuce was harvested at marketable stage. Harvesting was carried out from 10/1 to 1/3/2018 Plant height, total leaf number per plant, leaf area, shoot fresh weight and total yield were recorded. Total phenols were estimated by method Butsat and Siriamorpum (2016) gallic acid was used as standard and the results expressed as mg/g dw. The nitrate was determined according to Cataldo, *et al*., (1975). Total soluble solids TSS determined as described by (A.O.A.C., 2012).Vitamin C was according to Dinesh *et al*. (2015).Chloride ion (CL^{-}) was estimated using method Sezey (2018). The Na^{+} and K^{+} determination as described by Page *et al*. (1982). Ion leakage was estimated by Jassim (1988).

III. CHARACTERIZATION OF SHRIMP BY-PRODUCTS PROTEIN HYDROLYSATES

Total amino acids in shrimp by-products protein hydrolysates were determined by using High Performance Liquid Chromatography (HPLC), according to Levin and Grushka (1985). Organic matter was determined by using method Ben-Dor and Banin (1989)

Table 2: Total amino acids and organic matter in shrimp by-products protein hydrolysate (%) using enzymes Alcalase and Flavourzyme

Amino acid			
Alcalase		Flavourzyme	
Asp 1.60	Trp 3.02	Asp 1.21	Trp 5.21
Glu 16.51	Val 2.71	Glu 15.30	Val 2.76
Gly 29.02	Met 3.17	Gly 26.98	Met 0.72
Ser 12.14	Cys 0.3	Ser 13.24	Cys 2.27
Ala 7.19	Lys 4.94	Ala 8.95	Lys 1.80
His 2.40	Arg 1.51	His 1.86	Arg 0.61
Thr 1.47	Ile 0.77	Thr 1.12	Ile 0.98
Pro 9.51	Leu 3.45	Pro 9.10	Leu 3.18
Phe 0.16		Phe 2.63	
Total amino acids 99.87		ATotal amino acids 97.92	
Total organic matter 90.18		Total organic matter 89.52	

IV. STATISTICAL ANALYSIS

The complete randomized block design was used factorial experiment with three replications for each parameter. The treatment means were compared with (L.S.D.) test at 0.05.The data were calculated using the program SPSS.

V. RESULTS AND DISCUSSION

Table (3) show that the foliar spraying by protein hydrolysate prepared from shrimp by-products by Alcalase and Flavourzyme enzymes significantly increased, The total leaf number 67.08 leaf.plant^{-1}, leaf area 540.8cm^2, shoot fresh weight 981.3 g and total yield 23.027 tone. dounum^{-1},these results were obtained from sprayed (T2) treatment. Significant increases were found in height plant 41.07 cm, when sprayed (T4) compared to other treatments , while (T0) treatment which had the lowest number of value 24.88 cm height plant, total leaf number 39.95 leaf. plant $^{-1}$, leaf area 214.9 cm^2, shoot fresh weight 452.0 g and total yield 10.606 tone. dounum^{-1}. The results showed that spraying six times(S6) were significant increases that all characteristics compared with spraying four times (S4).

The interaction between treatments and spraying number show significantly increased in some vegetative traits ,the (T2 S6) had the highest value in leaf area 576.9 cm^2, shoot fresh weight 1036.0 g and total yield 24.311 tone. $dounum^{-1}$, while the (T0S4) treatment had the lowest value leaf area 211.3 cm^2, shoot fresh weight 437.6 g andtotalyield10.267tone.$dounum^{-1}$

Table 3: Effect of foliar application of shrimp by-products protein hydrolysates and number of sprays on some vegetative growth characters of lettuce .

Treatments		Plant height (cm)	Total leaf Number	Leaf area (cm^2. $plant^{-1}$)	Shoot fresh weight (g)	Total yield (tone. $dounum^{-1}$)
Shrimp by-products protein hydrolysates ($ml.l^{-1}$)						
*T0		24.88	39.95	214.9	452.0	10.606
T1		36.03	56.73	394.6	718.4	16.858
T2		36.95	67.08	540.8	981.3	23.027
T3		30.27	50.15	333.1	546.3	12.820
T4		41.07	59.47	436.5	768.4	18.033
LSD 0.05		1.508	1.076	8.61	17.08	0.401
Number of sprays						
S4		32.61	53.68	364.3	670.9	15.745
S6		35.07	55.67	403.6	715.6	16.792
LSD 0.05		0.517	0.369	2.95	5.86	0.138
Shrimp by-products protein hydrolysates × Number of sprays						
T0	S4	24.13	39.77	211.3	437.6	10.267
	S6	25.63	40.13	218.4	466.4	10.944
T1	S4	35.83	55.97	380.3	711.5	16.696
	S6	36.23	57.50	408.8	725.3	17.021
T2	S4	36.00	66.10	504.7	926.5	21.743
	S6	37.90	68.07	576.9	1036.0	24.311
T3	S4	27.00	48.00	312.5	524.5	12.308
	S6	33.53	52.30	353.8	568.2	13.333
T4	S4	40.07	58.57	412.9	754.8	17.713
	S6	42.07	60.37	460.0	782.1	18.353
LSD 0.05		N.S	N.S	12.18	24.16	0.567

*T0: control (untreaeed) T1: Protein hydrolysate 3 $ml.l^{-1}$ prepared by Alcalase T2: Protein hydrolysate prepared by Alcalase at 6 $ml.l^{-1}$, T3: Protein hydrolysate prepared by Flavourzyme at 3$m.l^{-1}$, T4: Protein hydrolysate prepared by Flavourzyme at 6$ml.l^{-1}$

The results were agreed with observe Ertani *et al*. (2009) found that foliar application of protein hydrolysate improved vegetative growth of lettuce plants. Also, Xu and Mou (2017) observed the protein hydrolysate treated

lettuce plants significantly increased vegetative growth .The positive effect of protein hydrolysate low molecular weight peptides and amino acids containing on plant growth parameters could be stimulation nitrogen uptake and increased leaf N contents plant biomass, which treated with protein hydrolysate (Colla *et al*., 2014,2015) The regulation of nitrogen uptake by enzymes involved the N assimilation processes and regulation the enzymes activity of tricarboxylic acid cycle (TCA) (Colla *et al*.,2015a; Nardi *et al*., 2016). Protein hydrolysates are stimulating carbon, nitrogen and hormone metabolism of plant (Colla *et al*., 2017). The foliar application of protein hydrolysates contains low molecular weight peptides and amino acids, can induce plants defense responses, thus increasing plant tolerance to aviary of abiotic stress.

Table (4**)** shows asignificant difference among the treatments, the spraying (T2) treatment recorded highest total phenols, TSS, vitamin C and dry matter content 6.80 $mg.g^{-1}$, 5.53%, 12.89 $mg.100g^{-1}$and 8.59% respectively ,while (T2) had the lowest nitrate content 0.256 $mg.g^{-1}$.Significant increases were found in the total phenols, TSS ,vitamin C and dry matter content 5.95 $mg.g^{-1}$, 4.60 % , 10.89 $mg.100g^{-1}$ and 7.26 % respectively, when sprayed (T4), while (T4) had lowest value of nitrate 0.300 $mg.g^{-1}$,when compared with (T0) treatment which gave the lowest value total phenols , TSS ,vitamin C and dry matter content 3.42 $mg.g^{-1}$, 2.92% , 5.61 $mg.100g^{-1}$and 4.94% respectively, while (T0) gave the highest value of nitrate 0.394$mg.g^{-1}$.

Observe from results the spraying six times (S6)significantly influenced of total phenol, TSS, vitamin C and dry matter content compared with four times(S4), while decreased nitrate content ,when spraying six times(S6).

The interaction between studying factors, shows significantly increased in vitamin C and dry matter content, it is noticeable that treatment of (T2 S6) produced the vitamin C and dry matter content 14.18 $mg.100g^{-1}$and 9.46% respectively, except the nitrate content 0.251 $mg.g^{-1}$ was decreased, while (T0S4) treatment which had the lowest value of vitamin C and dry matter content 5.42 $mg.100g^{-1}$ and 4.81 % respectively , and gave the highest value of nitrate 0.391$mg.g^{-1}$.

Similar results were observed by Rouphael *et al*.(2018) found increased in total phenols ,TSS, vitamin C and dry matter plants contents , when foliar sprayed with biostimulant Trainer®. Tsouraltzis *et al*. (2014) observed the protein hydrolysate treatment reduced the nitrate content in lettuce by 29% .,While, Abdelgawad *et al*. (2018) they obtained the biostimulant "Super biomin" treatment could enhance the total soluble solid content in lettuce plants. Increased total phenols content caused by PHs contains the highest value of tryptophan, phenylalanine and tyrosine these amino acids are mainly synthesized of cinnamic acid ,which was used for synthesis of phenols (Colla *et al*., 2017). While, The decrease nitrate contents might be caused the role of amino acids on nitrate uptake and regulation of several process and metabolic pathways of plant nitrogen (Liu *et al*., 2008 a,b). Increased nitrogen supply increased the vitamin C content in some plants (Yang *et al*., 2017).

Table 4: Effect of foliar application of shrimp by-products protein hydrolysates and number of sprays on some quality characters of lettuce.

Ttreatments	Total phenol ($mg.g^{-1}$)dw.	Nitrate($mg.g^{-1}$) Fw.	Total soluble solid (TSS)%	Vitamin C ($mg.100g^{-1}$)	Dry matter (%)
	Shrimp by-products protein hydrolysates ($ml.l^{-1}$)				

T0	3.42	0.394	2.92	5.61	4.94
T1	5.43	0.322	4.30	10.07	6.71
T2	6.80	0.256	5.53	12.89	8.59
T3	4.48	0.370	4.03	9.36	6.24
T4	5.95	0.300	4.60	10.89	7.26
LSD 0.05	0.347	0.004	0.288	0.257	0.137
	Number of sprays				

S4		5.08	0.331	4.11	9.22	6.29
S6		5.35	0.325	4.44	10.31	7.20
LSD 0.05		0.119	0.001	0.099	0.088	0.047
Shrimp by-products protein hydrolysates× Number of sprays						
T0	S4	3.20	0.391	2.70	5.42	4.81
	S6	3.63	0.396	3.13	5.81	5.06
T1	S4	5.30	0.324	4.10	9.70	6.47
	S6	5.57	0.319	4.50	10.43	6.96
T2	S4	6.67	0.260	5.37	11.59	7.73
	S6	6.93	0.251	5.70	14.18	9.46
T3	S4	4.37	0.375	3.97	9.08	6.06
	S6	4.60	0.365	4.10	9.63	6.86
T4	S4	5.87	0.306	4.43	10.29	6.42
	S6	6.03	0.295	4.77	11.50	7.67
LSD 0.05		N.S	0.006	N.S	0.365	0.193

Results in table (5) shows that significant differences were generated by both protein hydrolysates. The lowest value Na^+ 0.390% , CL^- 3.37 % and ion leakage 4.06% were obtained by using sprayed (T2) treatment ,while observed significant increase in K^+ value 2.249% and K^+/Na^+ ratio 5.769% .The significant decreased were recorded in Na^+ 0.465% , CL^- 4.11%, and ion leakage 4.60 % in addition increased K^+ value 1.949 % and K^+/Na^+ ratio 4.190% ,when sprayed (T4) ,while (T0) treatment had the highest value of Na^+ 0.627%, CL^- 5.08% , ion leakage 6.19% and lowest of K^+ 0.993% and K^+/Na^+ ratio 1.583%.

Observe from results the spraying six times(S6) significantly influenced of K^+ content and K^+/Na^+ ratio compared with four times (S4), while decreased Na^+, CL^- and ion leakage content,when spraying six times(S6). The interaction between treatments and spraying number shows significantly affected in most studying characteristics. The (T2S6) treatment had the lowest value 0.382% Na^+ and ion leakage 3.90% and found increased in K^+ content compared with (T0S4) had the highest value in Na^+ 0.626 % and ion leakage 6.17% ,and gave lowest value in K^+.

Table 5: Effect of foliar application of shrimp by-products protein hydrolysates and number of sprays on Na^+, K^+, CL^- and ion leakage leaves of lettuce.

Received: 22 Feb 2019 | Revised: 13Mar 2019 | Accepted: 05 Apr 2020

Treatments		Na^+ (%)	K^+ (%)	CL^- (%)	K^+/Na^+ (%)	Ion leakage (%)
Shrimp by-products protein hydrolysates ($ml.l^{-1}$)						
T0		0.627	0.993	5.08	1.583	6.19
T1		0.477	1.587	4.29	3.330	5.14
T2		0.390	2.249	3.37	5.769	4.06
T3		0.561	1.218	4.64	2.175	5.76
T4		0.465	1.949	4.11	4.190	4.60
LSD 0.05		0.005	0.023	0.073	0.079	0.038
Number of sprays						
S4		0.509	1.572	4.33	3.301	5.27
S6		0.498	1.626	4.27	3.518	5.03
LSD 0.05		0.002	0.008	0.025	0.027	0.013
Shrimp by-products protein hydrolysates× Number of sprays						
T0	S4	0.626	0.990	5.05	1.582	6.17
	S6	0.628	0.995	5.11	1.584	6.22
T1	S4	0.484	1.556	4.32	3.217	5.31
	S6	0.469	1.617	4.27	3.444	4.97
T2	S4	0.398	2.227	3.41	5.593	4.22
	S6	0.382	2.271	3.34	5.945	3.90
T3	S4	0.570	1.196	4.70	2.098	5.91
	S6	0.551	1.241	4.58	2.251	5.62
T4	S4	0.471	1.893	4.17	4.014	4.74
	S6	0.460	2.006	4.05	4.366	4.45
LSD 0.05		0.007	0.033	N.S	0.113	0.054

The application of PHs improved salinity tolerance of lettuce plant increased the mineral uptake such as K^+content and higher K^+/Na^+ ratio, while, Na^+, CL^- and ion leakage was decreased in the leaves. In this work, PHs improved the lettuce plants to maintaining the osmotic potential and reduced uptake of cellular Na^+, CL^-. Also, PHs caused the decline in the ion leakage by changing membrane potential, this led to membranes lowest ion leakage. The results were agreed with Koleška *et al*. (2017) they used biostimulant Viva® on tomato plants and with Rouphael *et al*. (2018) when they used foliar application with biostimulant Trainer® on lettuce plants.

In present study the protein hydrolysates preparation from shrimp by-products contains highest percentage of amino acids (glutamic acid, glycine, serine, valine, proline, tryptophan and tyrosine) organic matter and may be content low molecular weight peptides, that accordingly effect plant growth.

VI. CONCLUSIONS

The protein hydrolysate derived from shrimp by-product using either Alcalase or Flavourzyme was effective improving the growth and yield of lettuce under salinity conditions and might have potential to be used for sustainable production of lettuce.

Received: 22 Feb 2019 | Revised: 13Mar 2019 | Accepted: 05 Apr 2020

REFERENCES

1. Abdelgawad , K. F.; Mhmoud, A.A. and Mohamed , H.F. (2018). Foliar spraying with some biostimulants improves growth , chemical constituents , and yield of head lettuce plant .Middle East Journal of Agriculture , 07:1268-1277.
2. AOAC. (2012). Association of Official Analytical Chemistry. 19. ed. Gaithersburg, :3000 p.
3. Ben-Dor, E. and Banin, A. (1989). Determination of organic matter content in arid zone soils using a simple "Loss-on-Ignition" method. Communications in Soil. Science and Plant Analysis, 20: 1675-1695.
4. Botta, A. (2012). Enhancing plant tolerance to temperature stress with amino acids: An approach to their mode of action. Acta Hortic., 1009: 29-36.
5. Butsat, S. and Siriamornpun, S. (2016). Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of Amomum chinense C. International Food Research Journal, 23(1): 180-187.
6. Cataldo,D.A.; Maroon,M.and Schrader,L.E. (1975). Rapid colorimetric determination of nitrate in plant-tissue by nitration of salicylic-acid. Communications in Soil Science and Plant Analysis, 6 (1): 71-80.
7. Cavani, L.; Ter-Halle, A.; Richar, D. C. and Ciavatta, C. (2006). Photosensitizing properties of protein hydrolysate-based fertilizers. J. Agric. Food Chem., 54: 9160– 9167.
8. Chojnacka, K.; Michalak, I.; Dmytryk, A.; Wilk, R. and Gorecki, H. (2015). Innovative natural plant growth biostimulants, in Fertilizer Technology: II Biofertilizer, eds Sinha, S.; Pant, K. K.; Bajpai, S. and Govil, J. N., editors. (Houston, TX: Studium Press LLC) :451–489.
9. Chotikachinda, R.; Tantikitti, C.; Benjakul, S. and Rustad, T. (2018). Tuna viscera hydrolysate products prepared by different enzyme preparations improve the feed intake and growth of Asian seabass, Latescalcarifer, fed total fishmeal replacement diets. Songklanakarin J. Sci. Technol., 40 (1), 167-177.
10. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini,L.; Canaguier, R. and Rouphael, Y. (2015). Protein hydrolysates as biostimulants in horticulture. Scientia Horticulturae, 196: 28-38.
11. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani,A.; Lucini,L. and Canaguier, R. (2015a). Protein hydrolysates as biostimulants in horticulture. Sci.Hortic., 96:28-38.
12. Colla, G.; Rouphael, Y.; Canaguier, R.; Svecova, E. and Cardarelli, M. (2014). Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Frontiers in Plant Science, 5 (448).
13. Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R. and Rouphael, Y. (2017). Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. Frontiers in Plant Science, 8 (2202).
14. Dinesh, B.; Yadav, B.; Reddy, R.D.; Padma, A.S. and Sukumaran, M.K. (2015). Determination of Ascorbic Acid Content in Some Indian Spices.International Journal of Current Microbiology and Applied Sciences, 4 (8): 864-868.
15. Ertani, A.; Cavani, L.; Pizzeghello, D.; Brandellero, E.; Altissimo, A. and Ciavatta, C. (2009). Biostimulant activities of two protein hydrolysates on the growth and nitrogen metabolism in maize seedlings. J. Plant. Nutr. Soil Sci.,172 :237-244.

Received: 22 Feb 2019 | Revised: 13Mar 2019 | Accepted: 05 Apr 2020

16. Gridling, M.; Popescu, B.; Kopp, B.; Wagner, K. H.; Krenn, L. and Krupitza, G. (2010). Anti-leukaemic effects of two extract types of *Lactuca sativa* correlate with the activation of Chk2, induction of p21, down regulation of cyclin D1 and acetylation of alpha-tubulin. Oncol. Rep., 23 (4): 1145-1151.
17. Guerard, F.; Guimas, L. and Binet, A. (2002). Production of tuna waste hydrolysates by a commercial neutral protease preparation. Journal of Molecular Catalysis B: Enzymatic, 19: 489-498.
18. Holanda, H.D. and Netto, F.M. (2006). Recovery of components from shrimp (*Xiphopenaeus kroyeri*) processing waste by enzymatic hydrolysis. Journel Food Science, 71(5) :298-303.
19. Hoyle,N.T. and Merrit ,J.H.(1994).Quality of fish protein hydrolysate from herring (*Clupea harengus*).J.Food Sci.,59:76-79.
20. Jasim, A.M.(1988). Studies on chilling injury of cucumber (*Cucumis sativus* L.) during storage. Ph.D Thesis, Kansas State University , Ks.USA.
21. Koleška ,I.; Hasanagic, D.; Todorovie, V.; Murtić, S. ;Klokie, I.; Paradikovie,N. and Kukavica, B. (2017). Biostimulant prevents yield loss and reduces oxidative damage in tomato plant grown on reduced NPK nutrition. Journal of Plant Interactions, 12 (1):209-218.
22. Klomklao1, S. and Benjakul, S. (2018). Protein hydrolysates prepared from the viscera of skipjack tuna (*Katsuwonus pelmamis*): antioxidative activity and functional properties. Turkish Journal of Fisheries and Aquatic Sciences, 18: 69-79.
23. Koukounaras, A.; Tsouvaltzis,P. and Siomos, A. (2013). Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. Journal of Food Agriculture and Environment, 11 (2): 644-648.
24. Levin, S. and Grushka, E.(1985). Reversed-phase liquid chromatographic separation of amino acids with aqueous mobile phases containing copper ions and alkylsulfonates. Anal. Chem., 57:1830-1835.
25. Liu, X.Q.; Chen, H.Y.; Ni, Q.X. and Lee,K.S. (2008a). Evaluation of the role of mixed amino acids in nitrate uptake and assimilation in leafy radish by using ^{15}N – labeled nitrate. Agric. Sci. Chin., 7: 1196-1202.
26. Liu, X.Q.; Ko, K.Y.; Kim, S.H. and Lee, K.S. (2008b). Effect of amino acid fertilization on nitrate assimilation of leafy radish and soil chemical properties in high nitrate soil. Commun. Soil Sci. Plant Anal., 39: 269-281.
27. Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651-681.
28. Nardi,S.; Pizzeghello,D.; Shiavon ,M.and Ertani , A.(2016). Plant biostimulants :Physiological responses induced by protein hydrolyzed –based products and humic substances in plant metabolism. Sci.Agric.,73:18-23.
29. Page,A.L.; Miller, R.H. and Keeney, D.R. (1982). Methods soil Analysis Parts,2nd ed. Madison,Wisconson.USA:1159.
30. Qi,J.; Yokoyama,W.; Masamba,K.G.; Majeed,H.; Zhong,F. and Li, Y.(2015). Structural and physicochemical properties of insoluble rice bran fiberieffect of acid- base induced modifications.J. RSC.Adv., (5):79915-79923.
31. Rouphael, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.C.; Bonini, P. and Colla, G. (2018). Plant-and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. Journal Agronomy, 8 (126).
32. Schaafsma, G. (2009). Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. Eur J Clin Nutr., 63 (10): 1161–1168.

33. Sezey, M. (2018).Validation of Mohr titration method to determine salt (NaCl) in olive and olive brine.M.SC.Thesis. Department of Food Engineering. Near East University. Nicosia. P:57
34. Tsouvaltzis, P.; Koukounaras, A. and Siomos, A.S. (2014). Application of amino acids improves lettuce crop uniformity and inhibits nitrate accumulation induced by the supplemental inorganic nitrogen fertilization. Int. J. Agric. Biol.,16: 951-955.
35. United States Department of Agriculture (USDA) (2019).National nutrient database for standard reference release. Available Online at:https://ndb.nal.usda.gov/ndb/foods/show/3001
36. Xu, C. and Mou, B. (2017). Drench application of fish–derived protein hydrolsates affects lettuce growth, chlorophyll content and gas exchange. HortTechnology, 27 (4): 539-543.
37. Yang, X.; Cui,X.; Zhao, L.; Guo, D.; Feng, L. ; Wei,S. ; Zhao, C. and Huang, D. (2017). Exogenous glycine nitrogen enhances accumulation of glycosylated flavonoids and antioxidant activity in lettuce (*Lactuca sativa* L.). Frontiers in Plant Science, 8 (2098).