



## Preparation Protein Hydrolysates from Fish by-product and Study Effect on Lettuce (*Lactuca sativa* L.) Growth, Yield, Quality and Enhanced Salt Tolerance

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**Abstract:** The experiment was carried out during the winter season 2019-2018 in Al-Hartha district, Basrah Governorate to study the effects of fish by-product prepared by Alcalase and Flavourzyme enzyme as a foliar application on lettuce (*Lactuca sativa* L.) yield and quality grown under salinity conditions. Fish by-product protein hydrolysate was spray-applied (3 and 6ml. L<sup>-1</sup>) additional to control treatment (spraying with water) referred to (T0-T4) at four (S4), six(S6) times after 20 days transplanting at 10-day intervals. 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, carbohydrate, proline, shoot fresh weight of plant, dry matter, total soluble solids (TSS %) and total yield additional to significantly decreased in nitrate contents, while foliar application with (T4) significantly increased in the plant height and stem height. The results showed that spraying six times (S6) were significant increases in these growth characters comparing with four sprays (S4). The interaction between treatments and spraying number show significantly increased in some characters, the (T2 S6) had the highest value shoot fresh weight 762.5g and total yield 17.899 *tone.donum*<sup>-1</sup>.

**Keywords:** Fish by-product, Protein hydrolysates, Salinity, Yield, Quality, Lettuce.

### Introduction

Lettuce (*Lactuca sativa* L.) belongs to Asteraceae family, it is one of the most important vegetables in Iraq and other countries. The plant shoot is source of proteins, oils, fibers and minerals such as phosphorus, 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 factors that affect all vegetable crops, especially in semi-arid and arid regions (Munns & Tester, 2008) because of small rainfall biostimulants hold a great promise for

the future of agriculture. These environmentally and friendly natural substances promote vegetative growth, and tolerance of plants to abiotic stresses such as soil salinity, water deficit, and thermal stress, nutrition and crop quality (Chojnacka *et al.*, 2015). Protein hydrolysates are an important group of plant biostimulants that have received growing attention in recent years because of their positive effects on crop performance and contribution to a groecological sustainability. Protein hydrolysates are mixtures of polypeptides, oligopeptides and amino acids that are manufactured from by-products of animals or plant origins protein sources using partial hydrolysis by chemical (acid and alkaline hydrolysis) or enzymatic hydrolysis of a groindustrial (Schaafsma, 2009; Colla *et al.*, 2015).

Several beneficial effects of plant-derived protein hydrolysates on plants including increased nutrient uptake and assimilation and increasing tolerance against abiotic stress (Cavani *et al.*, 2006). The fish by-products contain valuable proteins and essential amino acids. Therefore, hydrolysis of fish protein would be a proper strategy for economic profit (Wisuthiphaet & Kongruang, 2015).

Several studies have shown the importance of Protein hydrolysate application for improving the growth and production of vegetable crops. Koukounaras *et al.* (2013) found that applications of an animal-derived hydrolyzed protein Amino16® enhanced both plant height as well as the number of flowers per plant in tomato (*Solanum lycopersicum* L.). Xu & Mou (2017) found that the application of fish-derived biostimulants increased the lettuce leaf number from 22 to 28, shoot fresh and dry weight 59 to 89 g and 5.5 to 7.5 g .

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

## Materials & Methods

The experiment was carried out during the winter season 2019-2018 in Al-Hartha district, Basrah Governorate to study the effects of fish by-product protein hydrolysate prepared by Alcalase and Flavourzyme enzyme as a foliar application on growth, yield and quality of lettuce plant under salinity conditions. The chemical and physical characteristics of field soil and irrigation water used in the field reported in table (1). Lettuce cv. Fajr seeds were used, the seeds were cultivated in 209 holes Styrofoam trays at 20/9/2018 in media (peatmou). The seedlings were transplanted to field after 30 days from seed sowing. The soil field preparing and divided into ridge in 3.75 m of length and distance between them 0.75 m. The cultivation was on both sides of the line, 25 cm distance between plants, density plants 23466 plant. donum<sup>-1</sup>, each treatment had three replicate.

### Preparation of fish by-product protein hydrolysates:

The hydrolysis processes for fish by-products were carried out using method Chotikachinda *et al.* (2018); Klomklaol & Benjakul (2018). Fish by-products of Marine whitefin wolf-herring (*Chirocentrus nudus*) (head and tails) were purchased from Basrah markets. The by-products washed with water and dried at 40 °C. Then they were ground, the fat was

**Table (1): Chemical and physical properties of the soil and irrigation water used in field.**

Soil Chemical	
E.C. (ds.m <sup>-1</sup> )	10.22
pH	7.69
Total nitrogen (g.kg <sup>-1</sup> )	0.75
Phosphorus (mg.kg <sup>-1</sup> ).	2.46
Potassium (mg.kg <sup>-1</sup> )	15.51
Organic matter (%)	1.64
Soil Structure	
Sand (%)	11
Silt (%)	69
Clay (%)	20
Texture Class	Silty clay
Irrigation Water	
E.C. (ds.m <sup>-1</sup> )	6.48
pH	7.39

removed from powder according to Qi *et al.*, (2015). The fish by-products were mixed with water at ratio 1:6. The mixed was heated at 85°C for 10 min (Guerard *et al.*, 2002).

The fish by-products were hydrolysate using Alcalase and Flavourzyme under optimal conditions of each: Alcalase (pH 8, 50°C) and Flavourzyme (pH 7, 50°C) for 6 hours. Enzyme and substrate ratio of 0.1 %. The hydrolysis degree was monitored during the process, and the reaction was stopped by inactivating the enzyme (90°C /10 min). The hydrolysis was centrifuged at 4000rpm for 20 min and concentration by rotary evaporator. The study included ten treatments which were the combination of five treatments are control (T0), Tow concentrations from hydrolysate using Alcalase T1:3 ml.l<sup>-1</sup>, T2: 6 ml.l<sup>-1</sup> and Flavourzyme T3: 3 ml.l<sup>-1</sup> and T4:6 ml.l<sup>-1</sup>. Plants were sprayed with hydrolyzed protein treatments four times (S4) and six times (S6) during the growing season. Treatments were applied to start at 20 days after transplant at 10- day intervals. Head lettuce was harvested at the marketable stage.

Harvesting was carried out from 10/1 to 12/2/2018. Plant height(cm), stem height (cm), total leaf number, plant leaf area (cm<sup>2</sup>. plant<sup>-1</sup>), shoot fresh weight (g), dry matter (g) and total yield (tone. donum<sup>-1</sup>) were recorded at harvest. The nitrate was determined according to Cataldo *et al.* (1975) carbohydrate determined by using methods Dubois *et al.* (1956). Proline was determined using the method of Bates *et al.* (1973). and TSS determined as described by AOAC (2012).

#### **Characterization of fish by-products protein hydrolysates**

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

The Randomized Complete Block Design (R.C.B.D.) was used factorial experiment with three replications for each parameter.

**Table (2): Total amino acids and organic matter in fish by-product protein hydrolysates (%) using enzymes Alcalase and Flavourzyme.**

Amino acid %						
Alcalase			Flavourzyme			
Asp	1.48	Trp	3.73	Asp	1.51	
Glu	22.72	Val	7.42	Glu	20.70	
Gly	15.79	Met	1.07	Gly	14.13	
Ser	14.45	Cys	1.80	Ser	15.63	
Ala	5.18	Lys	0.72	Ala	5.05	
His	4.07	Arg	3.38	His	0.17	
Thr	0.05	Ile	3.71	Thr	4.89	
Pro	5.70	Leu	7.02	Pro	5.41	
Phe	1.46			Phe	3.56	
Total amino acids			99.75%	Total amino acids		96.73%
Total organic matter			87.2%	Total organic matter		84.07%

The treatment means were compared with (L.S.D.) test at 0.05. The data were analyzed by using the statistical program Genstat (version 7.2).

## Results & Discussion

Table (3) displayed that the T2 and T4 had a significant effects on vegetative growth. Plant height and stem height of T4 plants were 35.05 cm and 19.18 cm respectively. T0 treatment recorded the 24.88 and 11.34 cm respectively. Significant increases were found in the total leaf number and plant leaf area 58.87 and 424.94 cm<sup>2</sup>. plant<sup>-1</sup> respectively. of (T2) compared with T0 treatment had the lowest value of total leaf number 39.65 leave and leaves area 214.85 cm<sup>2</sup>. plant<sup>-1</sup>.

The results showed that spraying six times (S6) significantly increases growth parameters comparing with four sprays (S4).

The interaction between treatments and spraying numbers shows significantly increased in leaf area, the (T2 S6) had the highest leaf area value 445.70 cm<sup>2</sup>. plant<sup>-1</sup>,

## Statistical analysis

while the (T0S4) treatment had the lowest value 211.34 cm<sup>2</sup>. plant<sup>-1</sup>. The positive effect of protein hydrolysates low molecular weight peptides and amino acids stimulate plant growth parameters through stimulation nitrogen uptake and increased leaf N contents plant biomass (Colla *et al.*, 2014; 2015). As well as the regulation of nitrogen uptake and the enzymes activity of TCA cycle (Colla *et al.*, 2015; Nardi *et al.*, 2016). Protein hydrolysates are also stimulating plants carbon and nitrogen metabolism (Colla *et al.*, 2017).

The foliar application of protein hydrolysates can induce plant defense responses, thus increasing plant tolerance for various abiotic stresses. Also, Protein hydrolysates have chelating activities that reduce the impact of different stresses on plant growth (Colla *et al.*, 2014). In the present study, the protein hydrolysates preparation from fish by-products contains several amino acids such as Glutamic acid, Glycine,

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

Treatments	Plant height (cm)	Stem height(cm)	Total Leaf number	Leaf area (cm <sup>2</sup> . plant <sup>-1</sup> )	
Fish by-products protein hydrolysates (ml.l <sup>-1</sup> )					
*T0	24.88±0.60	11.34±0.43	39.65±0.20	214.85±2.87	
T1	29.42±1.10	17.30±1.00	50.50±0.95	337.09±17.79	
T2	30.72±1.31	17.80±1.38	58.87±0.65	424.94±16.95	
T3	27.43±0.62	14.56±0.51	46.33±1.63	296.44±20.83	
T4	35.05±0.36	19.18±1.53	53.03±1.57	360.88±21.09	
LSD 0.05	1.364	0.801	1.403	12.41	
Number of sprays					
S4	28.51±3.16	14.97±2.11	48.44±5.75	307.35±58.8	
S6	30.49±3.09	17.30±3.02	50.90±6.07	346.32±69.1	
LSD 0.05	0.862	0.507	0.887	7.85	
Fish by-products protein hydrolysates × Number of sprays					
T0	S4	24.13±0.60	10.83±0.22	39.40±0.74	211.34±1.45
	S6	25.63±0.34	11.89±0.40	39.90±0.32	218.37±0.67
T1	S4	28.07±0.71	16.40±0.34	49.33±1.08	315.30±8.62
	S6	30.77±1.42	18.86±0.38	51.66±1.08	358.88±8.96
T2	S4	29.10±0.60	16.10±0.39	58.06±0.68	404.17±6.53
	S6	32.33±0.57	19.49±0.70	59.67±0.40	445.70±3.19
T3	S4	26.67±1.08	13.93±0.28	44.33±1.08	270.92±14.91
	S6	28.20±0.60	15.20±0.37	48.33±1.08	321.96±6.90
T4	S4	34.60±0.86	17.30±0.36	51.10±0.60	335.04±4.25
	S6	35.50±0.64	21.06±0.89	54.96±0.67	386.71±5.60
LSD 0.05	N.S	N.S	N.S	17.56	

\*T0: Control (untreated) T1: Protein hydrolysate 3 ml.l<sup>-1</sup> prepared by Alcalase T2: Protein hydrolysate prepared by Alcalase at 6 ml.l<sup>-1</sup>, T3: Protein hydrolysate prepared by Flavourzyme at 3ml.l<sup>-1</sup>, T4: Protein hydrolysate prepared by Flavourzyme at 6ml.l<sup>-1</sup>, S4; Plants were sprayed with hydrolyzed protein treatments four times, S6; Plants were sprayed with hydrolyzed protein treatments six times. The significant at P <0.05 level. ± means Standard deviation (S.D.) ; n=3 and Not significant (N.S.) .

Serene, Alanine, Proline and tryptophan as in the table (2), which stimulate photosynthesis, hormone balance and vegetative growth (Zhao, 2014). The results agreed with those of Lucini *et al.* (2015), who found that foliar

application of protein hydrolysates improved the vegetative growth of lettuce plants. Also, Xu & Mou (2017) observed that the protein hydrolysates treated lettuce plants significantly increased vegetative growth.

Table (4) revealed a significant difference among the treatments, the spraying (T2) treatment recorded the highest carbohydrate and proline content 75.31 mg. g<sup>-1</sup> and 19.84 μmol.g<sup>-1</sup> respectively, while (T2) had the lowest nitrate content 0.309 mg.g<sup>-1</sup> compared with (T0) treatment which gave the lowest value carbohydrate and proline content 34.57 mg. g<sup>-1</sup> and 13.35 μmol.g<sup>-1</sup> respectively, while (T0) gave the highest value of nitrate 0.394 mg.g<sup>-1</sup>.

Spraying six times (S6) significantly increased carbohydrate, proline and

chlorophyll contents compared with four times (S4), while it decreased nitrate content. The interaction between treatments and spraying numbers shows significantly decreased in nitrate content, It is noticeable that treatment T2S6 produced lowest value of nitrate content 0.306 mg. g<sup>-1</sup> compared with (T0S4) treatment which had the highest value of nitrate 0.391mg.g<sup>-1</sup>. The protein hydrolysate used in this study contained percentage of glycine and proline (table 2) that may promotes the accumulation of carbohydrate,

**Table (4): Effect of foliar application of fish by-products protein hydrolysates and number of sprays on nitrate, carbohydrate and proline of lettuce leaves.**

Treatments	Nitrate (mg.g <sup>-1</sup> fw)	Carbohydrate (mg.g <sup>-1</sup> dw)	Proline (μmol.g <sup>-1</sup> dw)
Fish by-products protein hydrolysates (ml.l <sup>-1</sup> )			
T0	0.394±0.001	34.57±2.61	13.35±0.13
T1	0.357±0.004	56.20±2.58	17.09±0.21
T2	0.309±0.002	75.31±2.63	19.84±0.59
T3	0.377±0.003	46.69±1.85	16.01±0.45
T4	0.346±0.003	61.59±1.42	18.14±0.35
LSD 0.05	0.015	3.13	0.81
Number of sprays			
S4	0.359±0.02	52.15±12.6	16.46±1.84
S6	0.354±0.03	57.59±12.4	17.31±2.12
LSD 0.05	0.003	1.98	0.51
Fish by-products protein hydrolysates × Number of sprays			
T0	S4	0.391±0.002	31.37±0.84
	S6	0.396±0.001	37.78±2.39
T1	S4	0.362±0.003	53.04±1.68
	S6	0.351±0.003	59.36±1.58
T2	S4	0.312±0.04	72.08±0.27
	S6	0.306±0.003	78.54±2.15
T3	S4	0.381±0.002	44.42±3.45
	S6	0.373±0.002	48.96±1.26
T4	S4	0.350±0.001	59.85±0.26
	S6	0.342±0.003	63.33±1.94
LSD 0.05	0.002	N.S	N.S

regulate proline biosynthetic pathway enzymes. While, the decrease of nitrate contents might be caused by the role of amino acids on nitrate uptake the regulation of several processes and metabolic pathways of plant nitrogen such as nitrate and nitrite reduction and glutamic synthesis activities (Liu *et al.*, 2008 a, b).

Similar results were observed by Rouphael *et al.* (2017) who found an increase in the accumulation of proline of lettuce contents when foliar sprayed with protein hydrolysates "Trainer". Tsouvaltzis *et al.* (2014) observed that amino acids treatment reduced the nitrate content of lettuce by 29%, while Genc &

Atici (2019) obtained that the protein hydrolysate preparation from chicken feather treatment could enhance the free sugars contents in wheat plants.

Result in table (5) indicated significant difference among the treatments, The spraying T2 and T4 treatment influenced most studies characteristics, but the highest significant increased obtained from (T2) in shoot fresh weight 756.1 g, dry matter 7.09%, TSS 4.50% and total yield 17.747 ton. donum<sup>-1</sup> compared with (T0) treatment which gave the lowest value of shoot fresh weight 452.0 g, dry matter 4.94 %, TSS 2.92 % and total yield 10.606 tone. donum<sup>-1</sup>.

**Table (5): Effect of foliar application of fish by-products protein hydrolysates and number of sprays on yield and some quality parameters.**

Treatments	Shoot fresh weight (g)	Dry matter (%)	Total soluble solid (TSS) (%)	Total yield (tone. donum <sup>-1</sup> )	
Fish protein by-products hydrolysates (ml.l <sup>-1</sup> )					
T0	452.0±11.7	4.94±0.09	2.92±0.17	10.606±0.27	
T1	571.2±9.7	6.31±0.32	3.98±0.17	13.404±0.22	
T2	756.1±5.3	7.09±0.29	4.50±0.11	17.747±0.12	
T3	498.5±11.5	5.88±0.33	3.83±0.16	11.699±0.27	
T4	629.0±6.3	6.49±0.28	4.23±0.17	14.761±0.14	
LSD 0.05	14.1	0.216	0.294	0.331	
Number of sprays					
S4	570.5±97.5	5.81±0.58	3.69±0.50	13.387±2.56	
S6	592.3±94.2	6.47±0.72	4.08±0.47	13.900±2.21	
LSD 0.05	8.93	0.136	0.186	0.209	
Fish protein hydrolysates × Number of sprays					
T0	S4	437.6±3.5	4.81±0.15	2.70±0.14	10.267±0.08
	S6	466.4±6.8	5.06±0.11	3.13±0.17	10.944±0.15
T1	S4	559.3±14.7	5.91±0.19	3.77±0.17	13.123±0.34
	S6	583.2±10.8	6.71±0.09	4.20±0.21	13.685±0.25
T2	S4	749.8±11.2	6.73±0.06	4.37±0.10	17.595±0.26
	S6	762.5±8.3	7.46±0.06	4.63±0.31	17.899±0.19
T3	S4	484.4±6.7	5.47±0.33	3.63±0.11	11.368±0.16
	S6	512.6±1.5	6.28±0.03	4.03±0.11	12.030±0.03
T4	S4	621.3±6.4	6.14±0.02	4.00±0.10	14.580±0.15
	S6	636.8±2.8	6.83±0.02	4.43±0.11	14.942±0.06
LSD 0.05		19.97	0.305	N.S	0.468

The results showed that spraying six times (S6) significantly increases in characters compared with four sprays (S4). The interaction between treatments and spraying number shows significantly affected all studying characteristics except TSS. The (T2S6) had the highest values of shoot fresh weight 762.5g, dry matter 7.46%, TSS 4.63% and total yield 17.899 tone. donum<sup>-1</sup> compared with (T0S4) that had the lowest values 437.6 g, 4.81%, 2.70%, and 10.267 tone. donum<sup>-1</sup>.

Protein hydrolysates may positively affect plants due to amino acids (table 2) acting on an increase in vegetative growth. Such as leaf number and leaf area, photosynthesis, chlorophyll content, protein synthesis and mechanisms involved in abiotic stress resistance (Cavani *et al.*, 2006). As well as chelating effects and hormone-like activity (Colla *et al.*, 2014) . The results agree with the study of Xu & Mou (2017) on the application of fish-derived protein hydrolysates, which significantly increased fresh weight lettuce crop. Tarantino *et al.* (2015) observed higher dry matter in pepper, cauliflower and fennel crops when, treated with biostimulant "Siapton". Significant improvement in TSS was observed by Tsouvaltzis *et al.* (2014) when lettuce plants treated with amino acids. Polo & Mata (2018) observed increased tomato yield when peptone treated plants.

## Conclusions

The protein hydrolysate derived from fish by-product using either Alcalase or Flavourzyme was effective in improving the growth, yield and quality of lettuce under salinity conditions. protein hydrolysis applied at T2 and T4 treatment gave the highest value of shoot fresh weight and total yield compared with T0. Spraying six

times (S6) significantly increases in most parameters compared with four sprays (S4). protein hydrolysates is possible used for sustainable production of lettuce.

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