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EFFECT OF ASAHI STAR AND YEAST EXTRACT ON VEGETATIVE GROWTH CHARACTERS OF LEAFY LETTUCE (*LACTUCA SATIVA L.*)

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Abstract

A field experiment was conducted out during the period of 27th October - 2018 to 19th April – 2019 to study the effect of Asahi star bio-stimulator and yeast extract bio-fertilizer on the vegetative growth of leafy lettuce. The experiment was laid out in F-CRD in four replicates, included sixteen treatments foliar spray by four levels of Asahi star (0, 2.5, 5 and 7.5 ml/20 l) and two levels of yeast extract (0 and 3 g/l) as foliar spray and rhizosphare application. The results revealed that the studied parameters were not responded significantly to foliar spray of Asahi star and yeast extract of both applications except the negative effect of Asahi star on head diameter. The combination of Asahi star and yeast extract foliar application gave the best values of number of leaves, head diameter and TSS was in the treatment of 2.5ml/20l Asahi star without yeast extract. However, the combination between Asahi star foliar spray and adding the yeast extract to the rhizosphare caused highest significant value only on leaf chlorophyll content (12.90 spad) and was obtained in 7.5ml/20l Asahi star with 3ml/l^{-1} yeast extract interaction treatment, and the best head diameter (18.25cm) was measured with 3ml/l yeast extract without using Asahi star, the level of (3ml/l) yeast extract in both applications caused significant increase in leaf chlorophyll content (12.90 spad). In the triple combination of studied factors the best results of longest leaf, plant height and plant fresh weight (16.55cm, 20.05cm and 73.52g respectively) were obtained from the interaction treatment of 2. 5ml/20l Asahi star with 3ml/l yeast extract foliar spray without rhizosphare application.
***Keyword*:** Asahi Star, Yeast, leafy Lettuce growth

Introduction

Lettuce (*Lactuca sativa* L.) is the needful leafy vegetable, cultivated in open fields and under control environment. It is utilized as fresh vegetable in relatively large amount. Its leaves contain 95% of water with low energy value (Dzida *et al*., 2012). The defiance of plant production these days is to increasing the request for food resources of plant source by the world are rapidly increasing population (Przybysz, *et al*., 2014**).** So some bio- technique is required to improve these plant products and reducing the hazard effect of mineral fertilization on human health. The use of the biostimulators are required, they are groups of comparatively new products (natural or synthetic consists of organic and inorganic components) of different formulations that affected positively on plant's vital processes, they are containing biologically active substances exist naturally in low amounts in a plant that modifying metabolism which lead to getting the best yield (Przybysz *et al*., 2010 and Calvo *et al*., 2014).

It was reported from many investigations about the beneficial stimulatory role of nitrophenolate compounds and yeast during plant growth and development. Asahi Star, known as Atonik, Chapperone (USA) or Asahi SL (Poland) classed under the group used as biostimulators and Plant Growth Regulators contains the following active ingredients: sodium p-nitrophenolate, sodium o-nitrophenolate, sodium 5-nitroguaiacolate, and these phenolic compounds may play a central role in secondary metabolism, defense mechanisms, mechanical support, and allelopathy (Doederlein *et al*., 2005). This group affected positively on plant biochemistry, physiology, morphology and yield and yield components (Przybysz *et al*., 2010). Yeast (*Saccharomyces cerevisiae*) extract is a natural bio-substance used in soil fertilization or as foliar application on the shoots of vegetable crops (El-Ghamriny *et al.,* 1999). It is contain high levels of content of nutrient elements and existence of stimulative growth regulator compounds like auxins, gibberellins and cytokinins, that stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll (Glick, 1995 and Amer, 2004), it is rich source of sugars, proteins, vitamins, enzymes, amino acids and minerals (Saker *et al*., 2015).

More recent researches demonstrate the advantage of nitrophenolic compounds to enhance several processes to control the growth, development and productivity of the plants. Przybysz *et al*. (2014) noted that Atonik had stimulatory effect on oilseed rape, cucumber and *A. thaliana* (as amodel plant) fresh weight, dry matter and yield production leaf area, chlorophyll content, greater intensity of photosynthesis, moreover relative water content was unchanged in Atonik-treated plants as well as the chemical composition of cucumber fruits was changed; soluble solids and phosphorus were increased, differently the content of nitrates and calcium were decline. Kocira, *et al.* (2015) found that the application of Asahi SL solution on common bean advantageously influenced on the number and the weight of seeds and the number of pods. Significant increase in total sugars, reducing sucrose content of potato tubers were demonstrated by an application of Asahi SL biostimulant compared with control under field condition (Zarzecka and Gugała, 2018).

Some other studies were evaluated the yeast effectiveness to enhancement the plant growth and development. Results obtained by Abd El-Motty *et al.* (2010) showed that spraying of Keitte mango trees with algae 2% and yeast at 0.2% once at full bloom stage was improved fruit set, fruit retention, yield (as fruits number and weight),and improved fruit quality represented by fruit length, fruit width, fruit weight, pulp/fruit percentage and enhanced total soluble solids and reduced weight of peel and

seed and fruit drop, moreover it was improved nitrogen, potassium and boron contents in the leaves compared with control (untreated). Fawzy *et al.* (2010) referred to that foliar spray of Snap bean plants with 4 g/l yeast gave the maximum number of leaves compared with other treatments. In addition, the highest values of leaf dry weight, pods weight, total yield and yield quality (represented by ascorbic acid and T.S.S content). Khalil and Ismael (2010) treated plants with yeast at rate of 8 g/L by different ways (as a foliar, soil and foliar plus soil treatments) resulting in an increase in yield and yield attributes of lupinus plants. Marzauk *et al*. (2014) revealed that the highest values of (*Vicia faba* L.) growth parameters as plant length, number of leaves and branches/plant and fresh and dry weights of leaves, branches and whole plant moreover the highest total pod yield and nitrogen and protein % in seeds were recorded when sprayed with 6 ml/l yeast extract. The results of Nassar *et al*. (2015) demonstrated that foliar application with yeast extract at (2,4 and 8 g /L.) increased significantly morphological characters of vegetative growth and yield of fresh herb per Basil plant (*Ocimum basilicum* L.) at full blooming stage without significant differences among the three mentioned concentrations. Spraying of cucumber plant with 20 g/l yeast and 2.4 g/l royal jelly increasing the vegetative characters, yield components, total yield and fruit quality in two seasons. Use 20 g/l yeast and 2.4 g/l royal jelly gave the best results of fruit yield, the increment were 109.40% and 145.22% in the first and second seasons, respectively when compared with the control (Nassef and El-Aref, 2016). Foliar spraying of neem plants (*Azadirachta indica* A. Juss.) with yeast extract significantly increased growth parameters (plants height, stem and root fresh and dry weights) and enhanced total chlorophyll a, phosphorus, potassium and total soluble sugars content, chlorophyll b, total carotenoids, total chlorophyll content, Nitrogen content, total soluble phenols and flavonoids leave content (Taha *et al.,* 2016)

To avoid the environmental risks of Chemical fertilizers their replacement with biostimulants and biofertilizers is required, so the purpose of this study was to estimate the effects of two biostimulators (Asahi Star and yeast) on vegetative growth of leafy lettuce.

Methods and Materials

The experiments were carried out on leafy lettuce plant grown in field conditions during 27th October - 2018 to 19th April - 2019. Lettuce seeds AGGREGATE cultivar was obtained from CHIA TAI co., Bankok, Thiland, with 98% germination. In 27th October seed were sown at 27th October - 2018 in the trays filled with peat moss from Pokon Naturado BV, Veenendaal, Holland, with pH of 5.2-6.2, contain NPK about (14: 16:18) and 50% organic matter. At the stage of three true leaves uniform seedlings were selected to transplant in polyethylene black bags (17 cm in width and 22cm in height), some chemical and physical properties of the soil used in the experiment is present in the table 1. The metrological data during the experimental period are shown in table 2.

Preparation of treatment solutions of Asahi Star

Asahi Star solution (from ASAHI chemical MFG company, LTD., Japan) contains 1g.l^{-1} sodium 5-nitroguaiacolate ($NaC_7H_6NO_4$), 2g.l^{-1} sodium ortho - nitrophenolate ($NaC_6H_4NO_3$) and 3g.l^{-1} sodium para-nitrophenolate ($NaC_6H_4NO_3$). The foliar spray solutions at the levels (0, 2.5, 5 and 7.5ml.20 l^{-1} distilled water) were prepared (depending on the instruction manual of the related company).

Preparation of yeast extract solutions

Dry yeast (*Saccharomyces cerevisiae*) at the weight of 3g with 6g sucrose were dissolved in 1000ml distilled water and left for 48 hr in the dark place to obtained the concentration of 3 g.l^{-1}, the prepared solutions were sprayed on the lettuce seedling and added to the soil in seedlings rhizosphare according to their treatments. Chemical analysis of activated yeast as recorded by (Nassef and El-Aref, 2016) contain amino acids (mg/100g dry weight).; Arginine (1.99), Histidine (2.63), Isoleiucine (2.31), Leucine (3.09), Lysine (2.95), (Methionine 0.72), Phrnylalanine (2.01), Theronine (2.09), Tryptophan (0.45), Valine (2.19), Glutamic acid(2.00), Serine(1.59), Aspartic acid (1.33), Praline (1.53), Tyrosine(1.49) and carbohydrates (23.20). Moreover it is contain a significant amount of vitamins; Thiamin, Riboflavin, Nicotinic acid, Pantothenic acid, Biotin, Pyridoxine, Folic acid, Cobalamin, and enzymes like; Oxidase, Peroxidase, Catalase. Some minerals P_2O_5, K_2O, MgO, CaO, SiO_2, SO_2, NaCl, Fe, Ba, Co, Pb, Mn, Sn and Zn are available in it.

Experimental design Statistical analysis

The experiment was established in a Factorial Complete randomized design (F-CRD), in four replicates. The experiment included sixteen treatments, foliar spray to the drip point by four levels of Asahi star (0, 2.5, 5 and 7.5ml.20 l^{-1}) and two levels of yeast extract (0 and 3 g.l^{-1}), the plants were sprayed twice (one month interval) started on 17th December, and treating the seedlings rhizosphare with 10 ml of yeast extract (0 and 3 g.l^{-1}), the control treatment sprayed with distilled water.

Finally, data the data were submitted to analysis of variance, the means compared by least significant differences (L.S.D.) at probability level ($P \leq 0.05$) significant using. (SAS institute, 2005).

The experiment parameters

At the end of the experiment (19th April – 2019) the data collected for all plants. Studied parameters included: longest matured leaf, number of leaves, plant height, head diameter and fresh weight of vegetative growth. Moreover the Leaf chlorophyll content was estimated using a portable SPAD 502 meter (Minolta, Japan), for each replicate the mean of three readings from mature leaves were take out (Incesu *et al*, 2015). However Total Soluble Solid (TSS%) of mature leaves were measured by using an ATC-1E hand-held Refractometer (Shahmaleki *et al.,* 2014).

Results and Discussion

Figure 1, 2 and 3 shows that statistical analysis of collected data of lettuce head parameters were not responded significantly to foliar spray of Asahi star and yeast extract by both foliar spray and rhizosphare applications except the negative effect of Asahi star on head diameter.

About nitrophenolates plant growth regulators similar results of soybean pod and seed losses were obtained by Arioglu (1988), Tukamuhabwa *et al*. (2002) and Gulluoglu *et al*. (2006) by using Atonik growth regulator, moreover no significant differences in lint yield were observed between the treatments and the

control, the results of Doederlein *et al.* (2005) on cotton yield and quality and Bynum *et al*. (2007) and the research of Zarzecka and Gugała (2018) when they demonstrated that sucrose content in potato tubers was cultivar-related not using of biostimulants (Kelpak and Asahi SL).

Bilateral interference tables are not encouraging. Table (3) displays the interaction effect of Asahi star and yeast extract foliar application; the results indicated that there are significant differences in number of leaves, head diameter and TSS (%). Negative effects were obtained in number of leaves and head diameter when the highest values (42.33 and 17.50 cm respectively) were recorded from control treatment. While, the best result of TSS (4.90%) was achieved by 2.5ml. $20l^{-1}$ Asahi star without yeast extract. However, the combination between Asahi star foliar spray and adding the yeast extract to the rhizosphare region of lettuce plants caused significant effects only on leaf chlorophyll content; the highest value (12.90 spad) was obtained in 7. 5ml.$20l^{-1}$ Asahi star with 3ml.l^{-1} yeast extract interaction treatment. While, the best head diameter (18.25cm) was measured with 3ml.l^{-1} yeast extract without using Asahi star (table 4). Illustrated results in table 5 shows that the yeast extract (3ml.l^{-1}) in both applications caused highest significant increase in leaf chlorophyll content (12.90 spad), whereas the other parameters had no response to the combination of the two types of application of yeast extract.

The number of leaves and TSS% were not affected by treatments of triple combinations of the three studied factors, however longest leaf, plant height, head diameter, plant fresh weight and chlorophyll content were respond significantly (table 6). The best results of longest leaf, plant height and plant fresh weight (16.55cm, 20.05cm and 73.52g respectively) were obtained from the interaction treatment of 2. 5ml.$20l^{-1}$ Asahi star with 3ml.l^{-1} yeast extract foliar spray. Using of 3ml.l^{-1} of yeast extract in both applications without Asahi star increased the head diameter and chlorophyll content. The effect of yeast extract was more efficiency than Asahi star on growth parameters. The enhancement effect of yeast extract might be attributed to its influence on metabolism, biological activity and photosynthetic pigments and enzyme activity which in turn encourage vegetative growth. It is acting as a source of plant growth hormones, carbohydrates, amino acids and vitamins (Taha *et al.*, 2016). In addition yeast extract have a role in increasing the levels of endogenous hormones in treated plants when contain the highest values of cytokinins in the leaves, yeast is a natural source of cytokinins and has stimulatory effects on bean plants which could be involve by cell division and cell elongation (Amer, 2004). Yeast has also protective and stimulatory effect because of to its content that enriched with the sources of phyto-hormones especially cytokinins, vitamins, enzymes, amino acids and minerals (El-Tohamy *et al*., 2008, Nassef and El-Aref, 2016 and Thalooth *et al*., 2019).

Conclusion

Based on the previous results it can be concluded that the application of Asahi star and yeast extract (foliage spray and rhizosphare) had no significant role in growth parameters while Asahi star play a negative effect on head diameter. Regarding the interaction between two factors had no encouraging responses; the interaction of Asahi star with yeast extract foliar application caused significant increase in TSS%, and its combination with rhizosphare application of yeast extract cause of an increase in chlorophyll content. Concerning the triple interaction of studied factors was varied in their effects, head diameter and chlorophyll content increased by using yeast extract in the combination of the two application methods without Asahi star, but the longest leaf, plant height and fresh weight were recorded with the interaction of low concentration of Asahi star with foliage spray of yeast extract. The roles of Asahi star and yeast extract still are not well understood on lettuce growth. Asahi star and Yeast extract, which is environmentally friendly, nutritious, and favourable to use and worth to try. To achieve more dependable applicability further studies are recommended with different concentrations in a wide range of these two bio-stimulator and fertilizer instead of chemical fertilizer.

Table 1 : Some physical and chemical properties of the soil used in the study*.

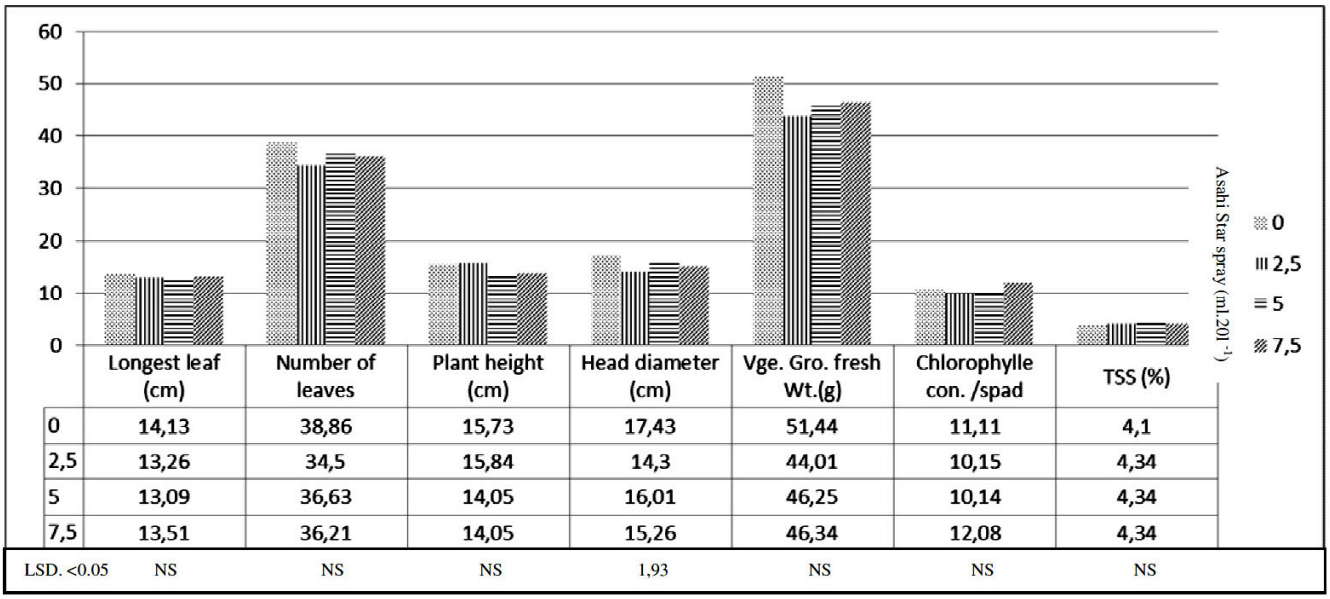
Properties	Field Soil
pH	7.79
Cat ion exchange capacity (mol.kg $^{-1}$)	19.68
Organic matter (g. kg $^{-1}$)	16.5
Total $CaCO_3$ (g. kg $^{-1}$)	270
K^+ (m.mol.l^{-1})	0.72
Mg^+ (m.mol.l^{-1})	0.80
Bulk density (µg.m^{-3})	1.3
Silt (%)	70
Sand (%)	25
Clay (%)	5
Soil texture	Sandy Loam

*Laboratory of Soil and Water Department/ Collage of Agriculture / Salahaddin University.

Table 2 : The metrological data during the study periods*:

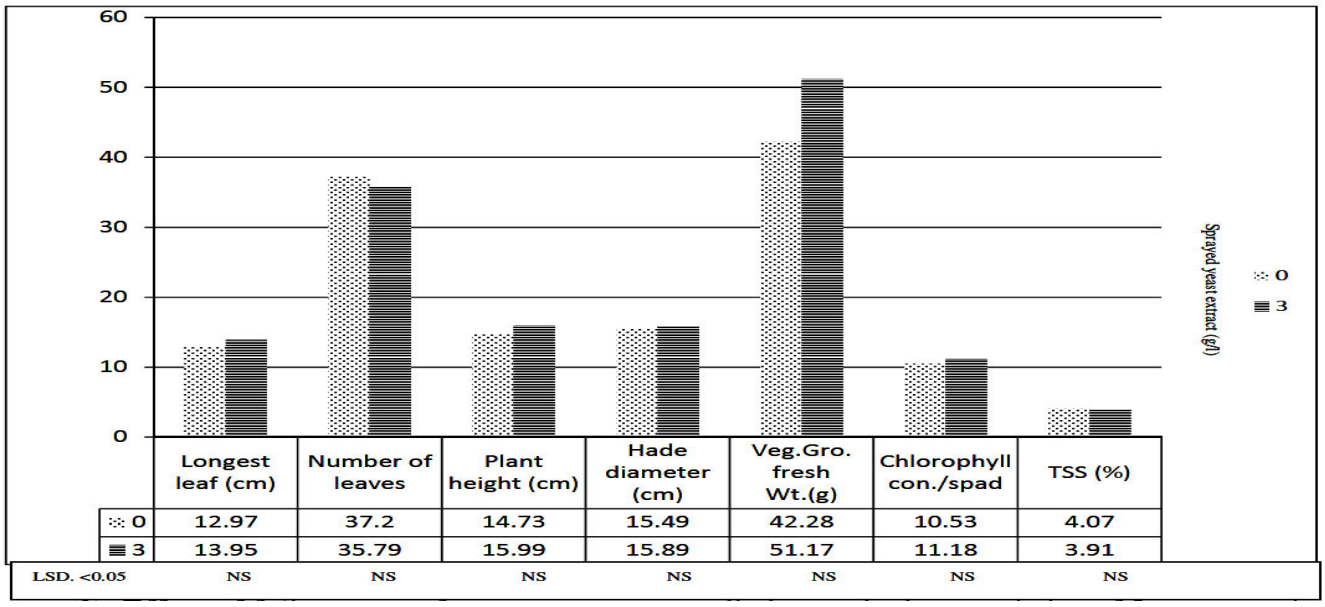
Years	Months	Average air temp. °C		Average relative Hum. %		Total rain(mm)
		Min.	Max.	Min.	Max.	
2018	October	11.4	38.7	26.81	94.00	22.6
	November	12.06	28.2	45.31	98.3	113.5
	December	8.07	14.74	58.48	91.64	160.8
2019	January	5.03	13.11	49.32	86.24	96.3
	February	5.82	15.04	45.77	85.12	42.4
	March	7.52	16.16	50.81	88.08	215.9
	April	10.28	21.34	41.68	86.51	125.7

*Agriculture research center Erbil, Ministry of agriculture of Kurdistan region of Iraq.



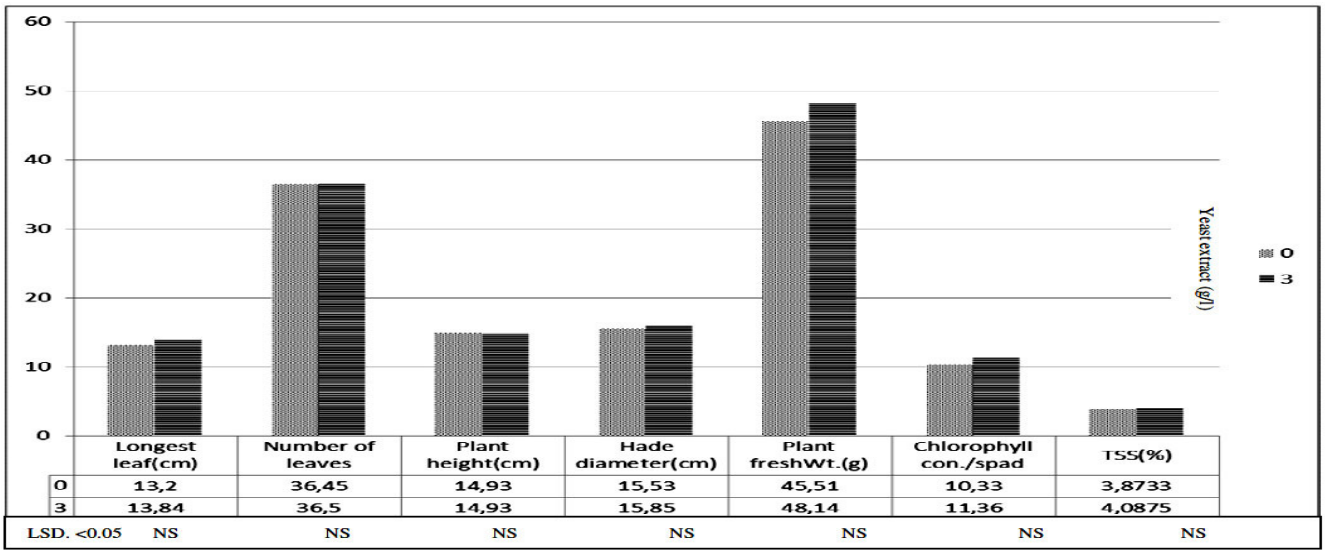
	Longest leaf (cm)	Number of leaves	Plant height (cm)	Head diameter (cm)	Vge. Gro. fresh Wt.(g)	Chlorophylle con. /spad	TSS (%)
0	14,13	38,86	15,73	17,43	51,44	11,11	4,1
2,5	13,26	34,5	15,84	14,3	44,01	10,15	4,34
5	13,09	36,63	14,05	16,01	46,25	10,14	4,34
7,5	13,51	36,21	14,05	15,26	46,34	12,08	4,34
LSD. <0.05	NS	NS	NS	1,93	NS	NS	NS

Fig. 1 : Effect of Asahi Star as foliar spray on studied growth characteristics of *Lactuca sativa L.*



	Longest leaf (cm)	Number of leaves	Plant height (cm)	Hade diameter (cm)	Veg.Gro. fresh Wt.(g)	Chlorophyll con./spad	TSS (%)
0	12.97	37.2	14.73	15.49	42.28	10.53	4.07
3	13.95	35.79	15.99	15.89	51.17	11.18	3.91
LSD. <0.05	NS	NS	NS	NS	NS	NS	NS

Fig. 2 : Effect of foliar spray of yeast extract on studied growth characteristics of *Lactuca sativa* L.



	Longest leaf(cm)	Number of leaves	Plant height(cm)	Hade diameter(cm)	Plant freshWt.(g)	Chlorophyll con./spad	TSS(%)
0	13,2	36,45	14,93	15,53	45,51	10,33	3,8733
3	13,84	36,5	14,93	15,85	48,14	11,36	4,0875
LSD. <0.05	NS	NS	NS	NS	NS	NS	NS

Fig. 3 : Effect of yeast extract (adding to the rhizosphare) on studied growth characteristics of *Lactuca sativa L.*

Table 3 : Interaction effects of Asahi Star and yeast extract as foliar spray on studied growth characteristics of *Lactuca sativa L.*

Asahi Star spray (ml.20l $^{-1}$)	yeast extract spray (g.l $^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Hade diameter (cm)	Plant fresh Wt.(g)	Chlorophyll con. /spad	TSS (%)
0	0	13.60	42.33	14.67	17.50	47.01	10.700	3.87
	3	14.53	36.25	16.53	17.38	54.77	11.43	4.28
2.5	0	12.50	33.75	14.98	13.63	35.76	10.78	4.90
	3	14.03	35.25	16.70	14.98	52.27	9.53	3.78
5	0	12.53	37.75	13.18	16.58	47.01	8.25	3.68
	3	13.65	35.50	14.93	15.45	45.49	12.03	3.58
7.5	0	13.43	36.25	16.08	14.75	40.51	12.43	3.78
	3	13.60	36.17	15.83	15.77	52.17	11.73	4.00
L.S.D.<0.05		N S	8.36	N S	2.58	N S	N S	1.29

Table 4 : Interaction effects of Asahi Star and yeast extract (adding to the rhizosphare) on studied growth characteristics of *Lactuca sativa* L.

Asahi Star (ml.20l $^{-1}$)	yeast extract add to rhizosphare (g.l $^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Hade diameter (cm)	Plant fresh Wt.(g)	Chlorophyll con. /spad	TSS (%)
0	0	13.33	35.33	15.37	16.33	44.34	9.40	3.83
	3	14.73	41.50	16.00	18.25	56.77	12.40	4.30
2.5	0	13.50	35.50	16.65	14.00	48.51	12.45	4.18
	3	13.03	33.50	15.03	14.60	39.51	7.85	4.18
5	0	12.28	38.50	16.65	16.48	45.24	8.00	3.78
	3	13.90	34.75	15.88	15.55	47.26	12.28	3.48
7.5	0	13.32	36.17	15.60	15.52	43.67	11.25	3.70
	3	13.70	36.25	16.30	15.00	49.01	12.90	4.08
L.S.D.<0.05		N S	N S	N S	2.56	N S	4.47	N S

Table 5 : Interaction effects of yeast extract as foliar spray and adding to the rhizosphare on studied growth characteristics of *Lactuca sativa* L.

Yeast extract spray (g.l $^{-1}$)	yeast extract add to rhizosphare (g.l $^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Hade diameter (cm)	Plant fresh Wt.(g)	Chlorophyll con. /spad	TSS (%)
0	0	12.46	36.57	14.46	15.40	38.01	11.34	4.13
	3	13.43	37.75	14.96	15.56	46.01	9.81	4.01
3	0	13.65	36.33	15.35	15.65	52.08	9.45	3.65
	3	14.25	35.25	16.64	16.14	50.27	12.90	4.16
L.S.D.<0.05		N S	N S	N S	N S	N S	3.28	N S

Table 6 : Interaction effects of Asahi Star and yeast extract as foliar spray and adding to the rhizosphare on studied growth characteristics of *Lactuca sativa* L.

Asahi Star spray (ml.20l $^{-1}$)	yeast extract spray (g.l $^{-1}$)	yeast extract add to rhizosphare (g.l $^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Hade diameter (cm)	Plant fresh Wt.(g)	Chlorophyll con. /spad	TSS (%)
0	0	0	14.20	36.00	17.60	16.80	43.01	13.80	3.20
		3	13.30	45.50	13.20	17.85	49.01	9.15	4.20
	3	0	12.90	35.00	14.25	16.10	45.01	7.20	4.15
		3	16.15	37.50	18.80	18.65	64.52	15.65	4.40
2.5	0	0	10.45	33.50	13.25	12.50	23.51	12.20	5.15
		3	14.55	34.00	16.70	14.75	48.02	9.35	4.65
	3	0	16.55	37.50	20.05	15.50	73.52	12.70	3.20
		3	11.50	33.00	13.35	14.45	31.01	6.35	4.35
5	0	0	12.55	40.50	11.60	17.85	51.01	7.30	4.00
		3	12.50	35.00	14.75	15.30	43.01	9.20	3.35
	3	0	12.00	36.50	12.85	15.10	39.46	8.70	3.55
		3	15.30	34.50	17.00	15.80	51.52	15.35	3.60
7.5	0	0	13.50	36.00	16.95	15.15	37.01	13.30	3.70
		3	13.35	36.50	15.20	14.35	44.01	11.55	3.85
	3	0	13.14	36.34	14.25	15.89	50.33	9.20	3.70
		3	14.05	36.00	17.40	15.65	54.02	14.25	4.30
L.S.D.<0.05			5.47	N S	7.71	3.91	32.21	4.47	N S

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