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The effect of plant density and spraying with salicylic acid on the development, harvest, and chemical components of *Trigonella foenum-graecum* L. seeds

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Abstract

The investigation was carried out through the 2023-2024 growing season in one of the fields of Abi Al-Khasib District, Basrah Governorate, Iraq. The experiment aimed to study the effect of three plant densities on growing the fenugreek cv. local (61111, 81481, and 122222 ha^{-1}) and spraying fenugreek plants with three concentrations of salicylic acid (0, 20, and 40 mg L^{-1}) on the growth, yield, and chemical components of the seeds. The factorial investigation was taken out according to the complete randomized block design, with three replications for each treatment. The data were analyzed using the variance analysis and compared between the treatments means according to the least significant difference test at the probability level of 5%. The most important results obtained can be summarized as follows: The density of 61,111 plants ha^{-1} was significantly superior to the total number of leaves, the number of branches, the fresh weight of the plant, the dry weight of the plant, the total number of flowers, the number of seeds per pod, the total number of seeds, the weight of one thousand seeds, the seed yield per plant, the concentration of total soluble carbohydrates, the percentage of protein, a fat and volatile oil in the leaves reached 314.4 leaves, 17.72 branches, 48,089 g, 7.942 g, 115.22 flowers, 15.55 pod $seed^{-1}$, 1430.1 $seed^{-1}$, 10,694 g $seed^{-1}$, 15.296 g $plant^{-1}$, 76.69 $mg.g^{-1}$, 10.596.41 %, %, 3.592%, respectively. This treatment showed a significant decrease in height of the plant, the day numbers to flower opening, and total seed productivity of 61.34 cm, 69.0 days, and 0.934 tons ha^{-1}, respectively. The high density of 122,222 ha^{-1} plants led to a significant increase in the total seed yield, which amounted to 1.732 tons ha^{-1}. The plants sprayed with salicylic acid at 40 mg L^{-1} led to significantly increased plant height, several leaves, and branches, fresh and dry weight of the plant, the total number of flowers and pods, the weight of a thousand seeds, the concentration of total soluble carbohydrates, protein, fat and volatile oil in The seeds amounted to 64.87 cm, 334.8 leaves, 17.57 branches, 48.429 g, 7.942 g, 114.67 flowers 92.22 pods, 10.55 g, 74.14 mg g^{-1}, 23.91%, 10.529%, 3.528 %, respectively. The same concentration of salicylic acid reduced the number of days for flowers to open by 68.0 days. Whilst, the 20 mg L^{-1} concentration showed a significant increase in yield per plant and total productivity of 15.14 g, 1.320 tons ha^{-1}. The relations between the two investigation factors had a significant impact on most of the recorded characteristics.

Keywords: Dry weight; Plant density; Productivity; Soluble carbohydrates; Volatile oil.

Introduction .i

The fenugreek (*Trigonella foenum- graecum* L.) plant is one of the family Fabaceae plants. It is one of the important medicinal plants, as its seeds are rich in nutrients such as proteins, fats, carbohydrates, and vitamins (Makai et al, 1999), as well as containing many effective medical and pharmaceutical compounds, including the alkaloids trigonelline and coline (Qutb, 1981) which are used in the treatment of diabetes and lowering cholesterol. It is the treatment to prevent action in blood and is anti-bacterial (Petropoulos, 2002). It is also used in the treatment of inhibiting the growth of malignant

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tumors or preventing infection with them (Duham, 2001). The yield of fenugreek seeds and their chemical components is affected by many growth conditions, including plant density and spraying with plant hormones, including salicylic acid.

Determining the optimum plant density and the good distribution of plants in the field is one of the agricultural operations that have a direct impact on the growth and productivity of the crop. This is due to its great role in plant competition and its relationship to plant growth and the depletion of nutrients from the soil. As well as the effect of light needed to complete the process of photosynthesis and the accumulation of nutrients. Moosuvi et al (2013) indicated that when planting fenugreek with three plant densities (22, 33, and 66 plants m^2), the density of 22 plants m^2 was significantly superior in the pod numbers, and the seed yield of one plant. Tiwari et al (2016) studied six distances for planting fenugreek, which are 5x10 cm, 10x10 cm, 15x10 cm, 20x10 cm, 25 x10 cm, 30 x10 cm and found that the wide distance is 30x10 cm was shown a significant increase in height of the plant, the branch numbers and pods and total seed yield compared to the narrow distance of 5 x 10 cm and 10 x 10 cm. Al-Saidi (2017) observed that when planting fenugreek with distances of 10, 20, and 30 cm between plants and 25 cm between lines, the narrow distance of 10 cm was significantly exceeded in plant height and total seed yield. While the large distance of 30 cm significantly outperformed the number of branches, leaf numbers, plant fresh and dry weights, pod numbers, the thousand seed weights, and the seed yield of the plant.

Several studies also showed the effect of salicylic acid C_6H_4 $(OH)_2$ COOH (2.hydroxybenzioc) in improving the growth and harvest of many plants as a hormone from the phenol group that contributes to giving protection against biotic and abiotic stresses as well as regulating plant physiological processes Such as ion uptake, photosynthesis, thermoregulation of flowering, nitrate metabolism and ethylene production (Hayat et al, 2010)). Ramezani (2014) noted that soaking fenugreek seeds at 0.5 mmol salicylic acid concentrations caused a significant increase in the plant dry weight, the seed numbers in the pod and the seed harvest.

Abdul Hafeez (2019) obtained when spraying fenugreek plants at 300 mg L^{-1} salicylic acid concentration a significant increase in the pod numbers, seed weights per plant, total seed yield, the weight of 1000 seeds, and yield of fixed oils for both seasons of the experiment. Abdi (2020) revealed that spraying fenugreek plants in salicylic acid concentrations at 0, 0.5, 1, and 1.5 mmol under water stress conditions caused a significant increase in the pod numbers, the 1000 seed weights, protein, and total soluble sugars in seeds and total seed yield. Because of the importance of the medicinal fenugreek plant, this investigation was achieved to determine the finest plant density and its response to spraying in salicylic acid and their effect on vegetative growth, harvest, and the seed chemical components.

Materials and Methods .ii

The investigation was achieved during the winter season of 2023-2024in one of the fields of Abi Al-Khasib district, Basra governorate, in a mixture of clay soil. Table (1) indicates some chemical and physical properties of the soil.

Property	Value
pH	7.34
E.C.	12.7
Organic matter (g kg^{-1})	1.92
Calcium (mmol L^{-1})	12.58
Magnesium (mmol L^{-1})	11.65
Total nitrogen (g kg^{-1})	0.68
Uptake phosphours (mmol L^{-1})	0.72
Uptake potassium (mmol L^{-1})	1.87
Soil components	g kg^{-1}
Sand	27.0

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Silt	463.8
Clay	520.2
Texture	Silt clay

The investigation contained the influence of two factors, namely, three plant densities (61111, 81481, and 122222 plant ha^{-1}) resulting and the planting distance between plants was (20, 30, and 40 cm) and the distance between planting lines was 36 cm. Fenugreek plants, a local cultivar for the above treatments, were sprayed with salicylic acid at 0, 20, and 40 mg L^{-1} a month after sowing the seeds so that the number of factorial treatments was 9, and each of them was repeated three times. The experiment was implemented as a factorial according to a complete randomized block design based on Al-Rawi and Khalaf Allah (2000).

The soil was plowed by the flipper plow in an orthogonal manner. Then the soil was smoothed and leveled after plowing. Then decomposed animal manure (cow waste) was added to it at a rate of 24 tons ha^{-1}. Then the soil was divided into plots of length of 3.6 m and width of 1.2 m. Each plot was divided into ten sub-plots, the distance between one line and another was 0.36 m. The different treatments were distributed on the plots. The seeds were sown directly on 1/9, as five seeds were placed in one hole, they were coated with a light coating of peat moss, and after germination, and it was thinned to one plant/hole. Plants were sprayed with salicylic acid one month after planting and for one time. All agricultural service processes used to produce the harvest were carried out, including irrigation, weeding, and hoeing. Then the seeds were harvested for the period from 15/3 to 31/3.

Table 1: Decimal averages of temperature and relative humidity during the growing season of fenugreek in the field, Abi Al-Khasib District, Basrah, Iraq.

Date	Maximum Temperature (ºC)	Minimum Temperature (ºC)	Maximum Relative humidity (%)	Minimum Relative humidity (%)
1-10/9/ 2023	46.16	24.24	57.35	26.86
11-20/9/ 2023	45.76	23.16	57.86	27.16
21-30/9/ 2023	44.13	22.29	59.57	32.13
1-10/10/ 2023	41.12	19.95	68.28	35.65
11-20/10/ 2023	40.23	18.34	70.12	38.19
21-31/10/ 2023	39.65	17.46	71.97	37.25
1-10/11/ 2023	39.66	10.67	74.55	42.38
11-20/11/ 2023	31.56	7.26	73.29	45.17
21-30/11/ 2023	27.25	6.72	78.79	49.26
1-10/12/ 2023	23.17	5.89	83.28	54.85
11-20/12/ 2023	20.79	5.21	87.81	57.01
21-31/12/ 2023	19.24	4.43	89.98	59.87
1-10/1/ 2024	18.72	5.47	82.45	43.98
11-20/1/ 2024	17.25	4.15	85.62	41.81
21-31/1/ 2024	20.32	7.26	86.27	48.94
1-10/2/ 2024	21.37	8.32	88.34	43.17
11-20/2/ 2024	20.65	9.08	84.20	36.98
21-29/2/ 2024	23.68	12.87	79.98	32.63
1-10/3/ 2024	21.89	11.65	70.78	31.86
11-20/3/ 2024	23.18	14.76	67.54	27.75
21-31/3/ 2024	28.98	15.07	63.17	23.08

Meteorological Center, Basrah Governorate, Abu Al Khasib Station, Ministry of Agriculture, Iraq.

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Experimental data were possessed from five plants for each experimental unit at the growing season end and comprised plant height (cm), leaf numbers, branch numbers, fresh weight of the plant (g), dry weight of plant (g), number of flowers, number of days to bloom, number of pods, the seed numbers per pod, the thousand seed weights (g), the seed yield of one plant (g), the total productivity of seeds (tons ha^{-1}), the content of seeds of total soluble carbohydrates (mg g^{-1}), according to the method described by Dubois et al. (1956), the percentage of protein in the seeds (%) according to the method described by A.O.A.C. (2004), and the seed volatile oil percentage (%)according to the procedure illustrated by Guenther (1972).

The results were statistically analyzed according to the adopted design. The treatment means were compared utilizing the least significant difference test at the probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000) utilizing the Genstat program for statistical analysis.

iii. Results and Discussion

It is obvious in Table 3 that plant density had a significant impact on vegetative growth indicators. The low plant density 61,111 plants ha^{-1} was significantly superior to the increase in the leaf numbers, the branch numbers, and the plant fresh and dry weights compared to the high density 122,222 plants ha^{-1}, with an increase of 5.16, 18.68, 2.87, and 2.22 %, respectively. The density of 81,481 plants ha^{-1} was significantly superior to the plant height compared to the two densities 61111 and 122222 plants ha^{-1}, with an increase rate of 4.76, 7.11%, respectively. The increase may be due to reduced plant competition for water, light, and nutrients, which allowed the plants to grow well. These results agree with the results obtained by many researchers (Tiwari et al. 2016; Al-Saeedi, 2017).

It appears from the same table that spraying in salicylic acid caused an effective superiority in all the characteristics under study, achieving both concentrations of 20 and 40 mg L^{-1} an effective increase in plant height (10.09% and 12.84 %), the leaf numbers (13.67% and 18.30%), the branch numbers (19.72% and 20.75%), the fresh weight of the plant (2.31% and 3.60%), and the plant dry weight (2.44% and 2.22%), respectively.

The increase may be attributed to the physiological role of salicylic acid in improving and regulating physiological processes such as photosynthesis, nutrient absorption, hormonal balance, stomata movement, and an increase in enzymatic activity in plants, leading to an improvement in vegetative growth (Raskin, 1992). These findings are agreeing with the investigation by Ramezani et al. (2014).

The relationship between the two factors indicated a significant impact on plant height. As the density of 81,481 plants ha^{-1} that were sprayed with salicylic acid at 40 mg L^{-1} recorded the highest branch numbers and dry weight of the plant reaching 20.28 branches and 8.293 g, respectively. The plant density of 61,111 plants ha^{-1} that was not sprayed in salicylic acid recorded the lowest values, which were 11.34 branches and 7.467 g, respectively.

Table 4 shows that the low plant density of 61111 plants ha^{-1} caused a significant increase in the number of flowers compared to the two densities 81481 and 122222 plants ha^{-1} with an increase of 4.00 and 9.73%, respectively. This density treatment led to a decrease in the number of days to open flowers with a decrease of 12.16% and 11.53%, respectively. It led to a significant increase in the seed numbers per pod and the total seed numbers compared to the high density of 122,222 plants ha^{-1}, with an increase of 2.91 and 3.62%, respectively. The plant density did not show an effect significantly in the number of pods per plant. The moral superiority of the low plant density might be due to the lack of plant competition for water, nutrients, and growth. These findings are agreed with the results of other researchers (Mossavi et al., 2013; Tiwari et al., 2016; Al-Saeedi, 2017).

It appears from the same table that spraying in salicylic acid caused an effective increase in all studied characteristics. Both concentrations of 20 and 40 mg L^{-1} salicylic acid guided a significant increase compared to the control treatment in the number of flowers by 8.22 and 10.37%, the number of pods

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(1.70% and 1.46%), and the total number of seeds (6.24% and 3.74%), respectively. The 20 and 40 mg L^{-1} of salicylic acid guided a significant reduced in number of days for flowers to open, with a decrease of (16.63% and 16.82%), respectively. The concentration of 20 mg L^{-1} of salicylic acid was significantly superior to the increase in the seed numbers per pod compared to the control treatment by 4.4%. The moral superiority of spraying with salicylic acid may be attributed to its role in increasing the efficacy of the photosynthesis process and increasing the ion absorption (Hayat et al., 2010). Salicylic acid is also a growth regulator of flowering, which in turn leads to an increase in the emergence of flower buds (Cleland and Ajami, 1974). This result is in agreement with those obtained by Abdul and Hafeez (2010), and Ramezani et al. (2014).

The combination between the two factors showed a significant impact on the total flower numbers. The plant density of 61,111 plants ha^{-1} that were sprayed in salicylic acid at 40 mg L^{-1} was recorded the highest number of flowers was 122.67. While the plant density of 81,481 plants ha^{-1} that were not sprayed with salicylic acid was recorded the lowest number was 103.0 flowers. The plant density of 81,481 plants ha^{-1} that were sprayed in salicylic acid at 40 mg L^{-1} was significantly superior in reducing the days numbers for flower opening to 62.33 days, and the highest pod numbers was 93.67. While the plant density of 122,222 plants ha^{-1} that were not sprayed with salicylic acid recorded the most number of flowering days amounted to 81.67 days, and the plant density of 61,111 plants ha^{-1} that were not sprayed with this acid gave the least number of pods 90.33 pods.

Table 5 shows that the low plant density caused an effective increase in the 1000 seed weights and the plant seed harvest compared to the two plant densities 81481 and 122222 plants ha^{-1} and the percentage increase was (2.89% and 4.11%) and (3.93% and 7.92%), respectively. The high plant density caused an effective increase in the total seed productivity compared to the densities of 61111 and 81481 plants ha^{-1} with an increased rate of 85.43% and 48.15%, respectively. The reason for the increase in seed harvest per plant and the total seed harvest may be attributed to the increase in the number of plants per unit area. The results obtained are consistent with those of other studies (Moosavi et al., 2012; Al-Saeedi, 2017).

It is evident from the same table that the concentrations of salicylic acid spraying 20 and 40 mg L^{-1} caused an effective increase in the weight of 1000 seeds with an increase of 0.87% and 1.84%, the yield of one plant (7.23% and 5.73%) and the total productivity (6.10% and 4.44%) compared to the control treatment, respectively. The positive effect of salicylic acid in increasing the yield components may be due to the improvement of vegetative and flowering growth indicators and the number of pods and seeds (Tables 3 and 4) and its reflection on the increase in harvest. These findings are compatible with other findings acquired by Abdi (2020).

The combination between the two factors indicated a significant impact on the 1000 seed weights. As the plant density of 61111 plants ha^{-1} that were sprayed with a concentration of 40 mg L^{-1} was recorded the highest weight reached 10,877 g. The plant density of 122222 plants ha^{-1} that was not sprayed with salicylic acid was recorded at the lowest weight of 10.247 g. The plant density of 61111 plants ha^{-1} that were sprayed with salicylic acid at 20 mg L^{-1} was recorded the maximum yield of the plant was 15.90 g. The plant density of 81,481 ha^{-1} and unsprayed with salicylic acid recorded the lowest seed yield of 14.017 g.

Table 6 shows that the plant density of 61,111 plants ha^{-1} caused an effective increase in the total seed soluble carbohydrates and the total protein, fats, and volatile oil with an increase of (2.28%, and 16.05%), (4.64%, and 17.40%), (4.06% 9.70%), and (7.00 and 20.90)%, respectively, compared to the densities of 81481 and 122222 plants ha^{-1}. The cause for the increase may be back to the fact that the low density guided to an increase in the traits of vegetative and flowering growth (Tables 3 and 4) and an increase in the efficacy of the photosynthesis process and thus an increase in the accumulation of nutrients in the seeds.

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It appears from the same table that spraying with both concentrations of salicylic acid 20 and 40 mg L^{-1} induced a significant increase in the concentration of total seed-soluble carbohydrates and the percentage of protein, fat and volatile oil in the seeds with an increased rate of (10.87% and 12.11%), (24.85% and 28.61%), (8.06% and 10.04%), and (23.26% and 23.22%), respectively, compared to the control treatment. The moral superiority of salicylic acid may be attributed to its role in stimulating the vegetative and floral development of the plant (Tables 3 and 4) and increasing the nutrient absorption, thus increasing the efficacy of the photosynthesis process and increasing secondary metabolism products. These findings are consistent with the findings of other studies (Abdul-Hafeez, 2019; Abdi, 2020).

The combination between the two factors revealed a significant impact on the total seed soluble carbohydrates. The plant density of 61,111 plants ha^{-1} that was sprayed with salicylic acid at 20 mg L^{-1} was recorded as the highest concentration reaching 80.81 mg g^{-1}. The plant density of 122,222 plants ha^{-1} that were not sprayed with salicylic acid was recorded the lowest concentration was 64.38 mg g^{-1}. The plant density of 61111 plants ha^{-1} that was sprayed with salicylic acid at 40 mg L^{-1} has recorded the percentage of protein in the seeds reached 26.06%. The lowest percentage of total soluble carbohydrates in the seeds was in plant density of 81,481 plants ha^{-1} that was not sprayed with salicylic acid, reaching 18.34%.

iv. Conclusion

We conclude from this study that the best plant density of fenugreek that achieves high seed production is the density of 122222 plants ha^{-1}. The fenugreek plants that were sprayed with salicylic acid at 20 mg L^{-1} recorded the best growth and yield of the plant. Accordingly, we recommend adopting this density when growing fenugreek plants and treating them in salicylic acid at 20 mg L^{-1}.

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Table 3: Effect of plant density, spraying with salicylic acid, and the interaction between them on some vegetative growth indicators of fenugreek plant

Treatment			Plant height (cm)	Total leaf number per plant	Branch number per plant	Fresh weight (g)	Dry weight (g)
Plant density (plants ha^{-1})	61111		61.34	319.4	17.72	48.089	7.942
	81481		64.26	316.5	16.90	47.991	7.959
	122222		59.99	303.7	14.93	46.744	7.769
LSD p≥0.05			1.74	8.4	0.69	0.445	0.128
Salicylic acid (mg L^{-1})	0		57.46	283.0	14.55	46.744	7.769
	20		63.26	321.7	17.42	47.826	7.959
	40		64.87	334.8	17.57	48.429	7.942
LSD p≥0.05			1.74	8.4	0.69	0.445	0.128
The interaction between the plant density	61111	0	58.19	286.9	15.34	47.220	7.467
		20	63.33	330.3	17.52	48.267	8.067
		40	62.50	340.9	20.28	48.780	8.293
	81481	0	60.88	283.3	14.39	46.727	7.880
		20	58.04	326.9	18.80	48.323	7.960

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and Salicylic acid		40	63.52	339.2	17.49	48.923	8.037
	122222	0	56.16	278.9	13.91	45.763	7.557
		20	62.93	307.8	15.93	46.887	7.850
		40	60.85	324.2	14.94	47.583	7.900
LSD p≥0.05			0.292	NS	1.20	NS	0.221

Table 4: Effect of plant density, spraying with salicylic acid, and the interaction between them on some floral growth indicators and yield of fenugreek plant

Treatment			Total flower number per plant	Day number of opening flower	Pod number per plant	Seed number per pod	Seed number per plant
Plant density (plants ha^{-1})	61111		115.22	69.00	91.89	15.55	1430.1
	81481		110.78	78.56	92.33	15.33	1416.0
	122222		105.00	78.00	91.33	15.11	1380.1
LSD p≥0.05			2.17	1.52	NS	0.38	41.7
Salicylic acid (mg L^{-1})	0		103.89	79.44	90.89	15.00	1363.3
	20		112.44	68.11	92.44	15.66	1448.4
	40		114.67	68.00	92.22	15.33	1414.4
LSD p≥0.05			2.17	1.52	1.02	0.38	41.7
The interaction between the plant density and Salicylic acid	61111	0	105.00	78.00	90.33	15.00	1355.0
		20	118.00	64.33	93.00	16.00	1488.0
		40	122.67	64.67	92.33	15.66	1447.3
	81481	0	103.00	78.67	90.67	15.00	1360.0
		20	114.33	64.67	92.67	15.66	1452.0
		40	115.00	62.33	93.67	15.33	1436.0
	122222	0	103.67	81.67	91.67	15.00	1375.0
		20	105.00	75.33	91.67	15.33	1405.3
		40	106.33	77.00	90.67	15.00	1360.0
LSD p≥0.05			3.76	2.64	1.77	NS	NS

Table 5: Effect of plant density, spraying with salicylic acid, and the interaction between them on some yield components of fenugreek plant

Treatment			Weight of 1000 seeds (g)	Seed yield per plant (g)	Total productivity of seeds (tons ha^{-1})
Plant density (plants ha^{-1})	61111		10.694	15.296	0.934
	81481		10.393	14.717	1.199
	122222		10.271	14.173	1.732
LSD p≥0.05			0.065	0.455	0.064
Salicylic acid (mg L^{-1})	0		10.359	14.119	1.244
	20		10.450	15.140	1.320
	40		10.550	14.929	1.229
LSD p≥0.05			0.065	0.455	0.064
The interaction between the plant density and Salicylic acid	61111	0	10.520	14.253	0.871
		20	10.687	15.900	0.971
		40	10.877	15.740	0.961
	81481	0	10.310	14.017	1.142
		20	10.413	15.117	1.231
		40	10.457	15.017	1.223
	122222	0	10.247	14.087	1.721
		20	10.250	14.403	1.760

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		40	10.317	14.030	1.714
LSD p≥0.05			0.114	0.789	NS

Table 6: Effect of plant density, spraying with salicylic acid, and the interaction between them on some chemical components of fenugreek seeds

Treatment			Total soluble carbohydrate (%)	Total proteins (%)	Total fats (%)	Volatile oil (%)
Plant density (plants ha^{-1})	61111		76.69	23.41	10.596	3.592
	81481		70.82	22.37	10.182	3.357
	122222		66.08	19.94	9.659	2.971
LSD p≥0.05			1.67	0.67	0.310	0.193
Salicylic acid (mg L^{-1})	0		66.13	18.59	9.568	2.863
	20		73.32	23.21	10.340	3.529
	40		74.14	23.91	10.529	3.528
LSD p≥0.05			1.67	0.67	0.310	0.193
The interaction between the plant density and Salicylic acid	61111	0	68.55	18.66	9.897	3.070
		20	80.87	25.50	10.987	3.977
		40	80.66	26.06	10.903	3.730
	81481	0	65.45	18.34	9.987	3.007
		20	71.80	24.16	10.383	3.463
		40	75.21	24.60	10.477	3.600
	122222	0	64.29	18.76	9.120	2.513
		20	67.29	19.97	9.650	3.147
		40	66.57	21.08	10.207	3.253
LSD p≥0.05			2.90	1.17	NS	NS

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