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Effect of Foliar Fertilizer and Some Growth Regulators on Vegetative and Anatomical Characters of Dill (*Anethum graveolens* L.)

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Abstract: This experiment was conducted to study the effect of foliar fertilizer (Oligo green HF), Gibberellin and Naphthelene acetic acid and their interaction on some vegetative and anatomical Characters of Dill (*Anethum graveolens* L.) cv. local. The foliar fertilizer, (contains Fe, Zn, Mn, Cu and B), was used at rates of 0, 50, 100 and 150mg/L while the GA3 was used at concentrations of 250 and 500mg/L and NAA at concentrations of 600 and 1000mg/L. The results show that foliar fertilizer and both growth regulators increased plant height, stem diameter and shoot dry weight in proportion to increase in the concentrations used. Number of branches did not affected by the application of growth regulators but they increased significantly by the foliar fertilizer treatments. Also, the lower concentrations of the two growth regulators show no effect on number of leaves while all rates of the foliar fertilizer increased the number significantly. All rates of foliar fertilizer caused significant increase in cortex thickness, number and thickness of vascular bundles and vascular units diameter. Also, all growth regulators concentrations increased significantly cortex thickness but they have no effect on vascular bundles number. Vascular bundles thickness and vascular units diameter decreased significantly under the effect of both growth regulators. Pith thickness decreased significantly as the foliar fertilizer rates increased but it increased significantly due to the use of the growth regulators. Interaction between foliar fertilizer and gibberellin or naphthalene acetic acid had significant effect on most characters studied. It can be concluded that all characters studied are positively affected by factors under consideration.

Key words: Cortex thickness · Growth regulators · Micronutrients · Pith thickness · Vascular bundles

INTRODUCTION

Dill (*Anethum graveolens* L.) is an annual, aromatic herb plant belongs to umbelliferae family. It is cultivated in central and southern regions of Iraq as a winter crop. Dill plant is native to India and it was spread later to Mediterranean and Europe and then to America and Japan [1]. The plant has a potential importance as a medicinal herb that contains volatile oils such as B-camphene, α-pinene, anethole, lonone, umbelliferone and carvone [2, 3].

Foliar fertilizer is a mean of increasing agricultural production due to the rapid absorption of nutrients by plant. Micronutrients are needed in small quantities for normal plant growth and development [4-6] and to proceed biological processes such as photosynthesis, respiration, synthesis of chlorophyll and stimulation of many enzymes [7-10]. Plant growth regulators are used widely to improve plant performance. Gibberellic acid is one of those growth regulators that has positive effect on plant growth through the effect on cell division and elongation [11-13]. Naphthalene acetic acid is also used to improve plant growth [14, 15]. Anatomical characteristics of stems such as cortex thickness and vascular bundles diameter have been used as criteria for species characterization [16]. Several investigators study the effect of micronutrients and some growth regulators on the anatomaical characters of several plants and they found a pronounce effects on thickness of epidermis and cortex and number and diameter of vascular bundles [17-19].

The aim of the present study was to investigate the effect of foliar application of some micronutrients, Gibberellin and Naphthalene acetic acid on some vegetative and anatomical characters of Dill plant (*Anethum* graveolens L.).

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MATERIALS AND METHODS

This experiment was carried out during the growing season of 2009-2010 in the fields of college of agriculture/ Al-Qadisiya University to study the effect of foliar fertilizer and two growth regulators, Gibberellin and Naphthalene acetic acid, on some growth and anatomical characters of Dill (*Anethum graveolens* L.) cv. Local. The soil used for cultivation characterized as silty clay with, PH 7.8, EC 2.7 and 1.08% organic matter. The foliar fertilizer (Oligo green HF, Green HAS Company) contains; Fe 5%, Zn 2%, Mn 2%, Cu 1% and B 0.5%. The foliar fertilizer was used at rates of 0, 50, 100 and 150mg/L. Gibberellin (Flagro Comp.) was used at concentrations of 250 and 500mg/L while Naphthalene acetic acid (Green River Comp) was used at concentrations; 600 and 1000mg/L in addition to control (spray with distilled water only). Seeds of dill were planted at 15/10/2009 on 3m long rows with 75cm between them. The total area of the experimental unit was 9m^2. Seeds were planted at a rate of 5-6 seeds per cavity and at a distance of 25cm in between. After two weeks, seedlings were thinned to only two. At 16/11/2009, the two growth regulators and the foliar fertilizer were sprayed at the early morning. Spray was repeated one month after the first spray. All agricultural practices were done as usual. Vegetative characters of plants such as; plant height, stem diameter, number of branches per plant, leaves number and shoot dry weight were taken on three plants of each replicates at 13/1/2010. Three plants were chosen randomly at the beginning of blooming at 28/1/2010 for anatomical study.

Preparation of Samples: Cross sections of the middle part of the stem were taken. The fresh samples were preserved in glass tubes containing ethyl alcohol at a concentration of 70% to keep them fresh until use. Hand sectioning was used to prepare thin sections. Sections were transferred carefully by a brush into clean slides containing drops of Safranin dye and left for a period of 5-7 minutes. Then, stained sections were transferred to other slides containing drops of glycerin for immersion. Samples were examined under light microscopy and measurements were taken in micrometer using Ocular Micrometer. Thickness of cortex, vascular bundles, pith and vascular units diameter and number of vascular bundles were recorded. Sections were filmed using lucida camera, wild type, erected on microscope under the force of (X40).

Experiment was designed as a factorial experiment with two factors (4X5) in a completely randomized design with three replicates. Means were compared using LSD at 5%.

RESULTS

Results of Table (1) clearly show that all treatments have significant effect on plant height. All rates of foliar fertilizer used increased plant height significantly. The tallest plants were achieved at the rate of 150mg/L (82.27cm) while the shortest (70.76cm) were at the control. For growth regulators, an obvious increase in plant height was observed in correspondence to the increase in concentrations of the two growth regulators used. Gibberellin is more effective on plant height than naphthalene acetic acid. The highest concentration of gibberellin gives the tallest plants (84.83cm) compare to 79.68cm for the highest concentration of naphthalene and 62.92cm for the control. Interaction between foliar fertilizer and the two growth regulators shows significant effect. Combination of 150mg/L of the foliar fertilizer with 500mg/L of gibberellin gives the tallest plants (93.33cm). In general, combination of 500mg/L with all rates of foliar fertilizer gives tallest plants compare to other combinations.

With regard to stem diameter, it was clear that foliar fertilizer and the two growth regulators used caused significant increase in diameter (Table 2). Foliar fertilizer at 150mg/L records the highest stem diameter (8.74mm) compare to 5.40mm for the control. Also, the highest value of the diameter is achieved at 500mg/L of GA3 (7.75mm) and at 1000mg/L of NAA (7.81mm). For the interaction, it is shown that the highest two diameters (9.57 and 9.67mm) were reached at the combinations of foliar fertilizer at 150mg/L with 500mg/L of GA3 or 1000mg/L of NAA, respectively. The least stem diameter was at the control (4.33mm). All foliar fertilizer rates increased significantly the number of branches (Table 3). The percent Increase over the control was 26.25, 56.56 and 95.94% at the fertilizer rates of 50,100, And 150mg/L, respectively. However, the use of the two growth regulators has no effect on number of branches. For the interaction, the highest number of branches was obtained at the combination of 150mg/L of the foliar fertilizer along with all concentrations of both growth regulators.

Number of leaves increases in proportional manner with the increase in the rate of fertilizer used (Table 4). Plants sprayed with 150mg/L of the fertilizer recorded the highest number of leaves per plant (33.67) which was significantly differ from other treatments. For the effect of the two growth regulators, it was noted that the lowest concentration of both did not cause significant increase in leaves number, while the highest concentration of both caused significant increase. However, the higher concentrations did not differ significantly from each other in their effect. The highest number of leaves per plant

Table 1: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on plant height (cm) of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	55.67	74.00	79.67	70.00	74.00	70.76
50	59.67	77.33	80.00	73.42	78.70	73.82
100	64.67	79.60	86.33	74.61	82.30	77.50
150	71.67	83.66	93.33	79.00	83.70	82.27
Mean	62.92	78.65	84.83	74.26	79.68	

LSD $p<0.05$ foliar fertilizer growth regulators interaction
2.17 2.42 4.72

Table 2: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on stem diameter (mm) of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	4.33	5.00	6.10	5.33	6.23	5.40
50	5.00	6.02	7.33	6.67	7.33	6.47
100	7.00	7.53	8.01	7.67	8.00	7.64
150	8.00	8.13	9.57	8.33	9.67	8.74
Mean	6.08	6.67	7.75	7.00	7.81	

LSD $p<0.05$ foliar fertilizer growth regulators interaction
0.63 0.78 1.43

Table 3: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on number of branches of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	5.15	5.17	5.19	5.18	5.20	5.18
50	6.58	6.64	6.67	6.38	6.44	6.54
100	8.15	8.18	8.20	7.95	8.08	8.11
150	10.22	10.21	10.23	9.97	10.12	10.15
Mean	7.52	7.55	7.57	7.37	7.46	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 0.46 N.S 0.71

Table 4: Effect of foliar fertilizer (FF) and growth regulators(GR) concentrations and their interaction on leaves number of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	19.33	20.33	23.33	19.30	23.30	21.12
50	21.33	24.33	28.33	21.00	28.30	24.66
100	23.00	27.00	36.00	23.70	36.00	29.14
150	28.33	30.00	41.00	29.30	39.70	33.67
Mean	23.00	25.42	32.17	23.33	31.83	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 2.21 2.95 3.62

Table 5: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on percent dry weight of shoot of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	13.12	13.82	14.82	13.12	13.86	13.75
50	12.95	14.82	16.31	13.83	14.98	14.58
100	15.40	17.13	19.95	17.12	17.98	17.52
150	18.31	19.33	21.17	18.67	20.12	19.52
Mean	14.95	16.28	18.06	15.69	16.74	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 0.42 0.48 0.94

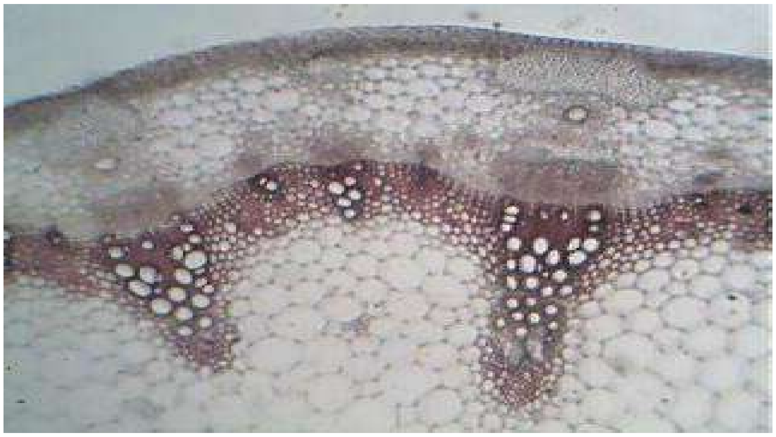

Fig. 1: Transverse cross-section of Dill stem (X40).

(32.1) was obtained at the treatment of GA3 at 500mg/L followed by 1000mg/L treatment (31.83). For the interaction, it was found that the use of the highest rate of the fertilizer with either GA3 at 500mg/L or NAA at 1000mg/L gave the highest number of leaves per plant (41.00 and 39.70, respectively). Results in table 5 show that as the foliar fertilizer rate increases, the dry weight increases also. It is noted also that all concentrations of growth regulators increased the percent dry weight. GA3 at 500mg/L gave the highest percent (18.06%). However, the two growth regulators did not differ from each other in their effect when they used at lower concentration. The combination of foliar fertilizer at 150mg/L and gibberellin at 500mg/L was superior in its effect. This combination gave the highest percent (21.17%) followed by the combination between the foliar fertilizer at 150mg/L and NAA at 1000mg/L (20.12%). Also, it was clear that all combinations that contain the higher concentration of foliar fertilizer and higher concentration of GA3 or NAA were the most effective which indicates the positive response of plant to such higher concentrations.

Stem cross-section reveals several distinct zones (Figure 1) starting from the cuticle layer, the outer layer, that surrounds a single row of cubic shape of epidermal cells. The epidermis is followed by layers of cortex which consist of paranchyma tissue. Cortex can be distinguished into two distinct zones; the outer zone is located directly under the epidermis and it is characterized by having chlorenchyma and an ample amount of chloroplasts. The inner zone is composed of several rows of an ordinary parenchyma cells with thin cell walls. Also, the cortex contains an oil ducts which are located directly underneath the collenchymaus tissue. Following the cortex there is the vascular cylinder which consists of vascular bundles. The vascular bundles are collateral. They are composed from soft area represents the phloem, then vascular cambium as a very narrow region and the xylem. Xylem tissue consists of vessels and tracheids. Vessels are arranged as rows that consist of several circles or ovule vascular units. The units become smaller in size whenever they headed towards the pith. The pith contains large storage parenchymaus cells. Parenchyma cells that are nearby the wood become smaller in compare to that near the cavity. It is also noted in the stem cross-sections under view that the cavity occupies the center of the stem.

Results of Table (6) show clearly that all foliar fertilizer rates increased significantly the cortex thickness. The highest thickness (385.84µm) was achieved at 150mg/L of the fertilizer while the lowest thickness was at the control treatment (277.48µm). Also, both growth regulators had significant effect on cortex thickness with the highest concentration of both gave the highest thickness. For the interaction, the combination of the highest rate of foliar fertilizer along with either gibberellin or naphthalene acetic acid at their higher concentrations gave the highest cortex thickness (415.12 and 420.10µm respectively).

Vascular bundles number and thickness were increased significantly in proportional to the increase in rates of foliar fertilizer (Table 7 and 8). The 150mg/L rate gave the highest number and thickness of the vascular bundles (18.80 and 684.01µm, respectively). In contrast, the two growth regulators had no effect on number of vascular bundles in the vascular cylinder, while they decrease the thickness at all concentrations especially the higher ones. Combination of foliar fertilizer at 150mg/L with 0mg/L of the growth regulators gave the highest number of vascular bundles (18.85).

Table 6: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on cortex thickness (μm) of stem of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	224.11	261.31	316.05	263.95	322.00	277.48
50	249.27	312.53	337.72	325.07	339.93	312.90
100	298.81	353.56	388.19	371.18	400.02	362.35
150	322.33	381.33	415.12	390.31	420.10	385.84
Mean	273.63	327.18	364.27	337.63	370.51	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 10.03 10.81 13.88

Table 7: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on number of vascular bundles Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	12.55	12.53	12.49	12.59	12.57	12.55
50	14.13	14.10	14.09	14.21	14.19	14.14
100	17.17	17.15	17.12	17.23	17.21	17.18
150	18.85	18.84	18.82	18.76	18.74	18.80
Mean	15.68	15.66	15.63	15.70	15.68	

LSD $p<0.055\%$ foliar fertilizer growth regulators interaction 0.53 N.S 0.83

Table 8: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on vascular bundle thickness (μm) of stem of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	438.01	408.16	365.55	420.05	382.13	402.78
50	520.39	488.70	458.13	490.11	478.00	487.07
100	642.22	601.41	574.00	610.33	570.19	599.63
150	725.13	685.30	661.15	688.57	659.92	684.01
Mean	581.44	545.89	514.71	552.27	522.56	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 12.27 15.63 19.18

Table 9: Effect of foliar fertilizer (FF) and growth regulators (GR) concentions and their interaction on on diameter of vascular units (mm) of stem of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	51.83	46.67	38.93	48.65	40.21	45.26
50	54.33	50.82	45.30	50.93	48.37	49.95
100	58.81	52.31	48.32	54.35	50.86	52.93
150	62.66	56.71	54.21	58.03	56.11	57.54
Mean	56.91	51.63	46.69	52.99	48.89	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 1.13 1.84 2.16

Table 10: Effect of foliar fertilizer (FF) and growth regulators (GR) concentrations and their interaction on pith thickness (mm) of stem of Dill *(Anethum graveolens* L.)

	GR Conc.(mg/L)					
FF Conc.(mg/L)	(0)	GA3 (250)	GA3 (500)	NAA (600)	NAA (1000)	mean
0	781.27	894.18	917.21	899.87	937.21	886.07
50	723.33	787.46	819.71	795.88	823.13	789.90
100	653.85	711.89	792.32.	730.81	797.79	737.33
150	584.98	618.73	667.80	631.44	679.90	636.57
Mean	685.86	753.22	799.26	764.50	809.51	

LSD $p<0.05$ foliar fertilizer growth regulators interaction 11.28 13.67 26.15

The lowest number was obtained at 0mg/L of the fertilizer plus GA3 at 500mg/L (12.49). Treatments of foliar fertilizer at 150mg/L regardless of the growth regulators used gave the thicker vascular bundles while the lowest thickness was obtained using the combination of 0mg/L of the foliar fertilizer with gibberellin or naphthalene at their higher concentration. It was evident that all rates of the foliar fertilizer increased significantly the diameter of the vascular bundles units (Table 9). However, all concentrations of both growth regulators decreased the diameter, whereas the 500mg/L of GA3 recorded the lowest diameter (46.69 µm). The combination of foliar fertilizer of150mg/L plus 0mg/L of the growth regulators recorded the larger diameter of the vascular bundles units, while the combination of 0mg/L of the fertilizer plus 500mg/L of GA3 recorded the lowest diameter.

Results in table (10) show that the increase in rates of foliar fertilizer was accompanied with a decrease in pith thickness. The 150mg/L recorded the least thickness (636.57mm) compare to the highest thickness (886.07m) for control. All growth regulators treatments increase pith thickness and the increase in concentration of each caused an increase in thickness. The interaction between the two factors had significant effects. Combination of foliar fertilizer at 0mg/L along with NAA at its higher concentration gave the highest pith thickness and combination of 0mg/L of the fertilizer along with 500mg/L comes after. The combination of 150mg/L of the fertilizer along with 0mg/L of the growth regulators gave the least pith thickness (584.98µm) which was significantly less than other combinations.

DISCUSSION

The positive effect of foliar fertilizer in present study on plant height and growth in general may be due to the role of micronutrients in the fertilizer. It is known that iron has a direct role in increasing chlorophyll content and synthesis of cytochrome and ferridoxin [20]. Zinc has positive effect in the synthesis of tryptophan, a precursor of IAA, that necessary for cell elongation [21, 22]. Our results come in agreement with the results of [23] on *Trachspermum ammi*; [24], on *Tagetes erecta;* and [25] on Ruta plant. Also, the obvious increase in plant height due to the use of the two growth regulators may be a attributed to the stimulatory effect of the growth regulators on cell division and elongation at the sub-apical meristem [26]. Similar results reported by [27-30]. With regard to stem diameter, it has been stated that the regulatory effect of growth regulators on cell division and elongation at the stem radial direction resulted in an increase in stem diameter [31]. This was noticed in our results (Table 2) and agreed with earlier findings by [32], on pumpkin and [33] on mustard. However, other investigators did not notice any significant effect of some growth regulators on stem diameter on other plants [34-36].

Some micronutrients such as manganese has a hormonal regulation role in plant. Manganese may activate IAA-oxidase and this would regulate the auxin content in plant tissues and in turn breaks the apical dominance and increases branching [31]. In addition, microelements have indirect role in increasing plant content of carbohydrates and this may have stimulated the lateral buds to grow and differentiate [37]. This positive effect was obvious in the current results at which all foliar fertilizer rates had positive effect on number of branches (Table 3). Other investigators [26, 38, 39] stated same results on other plants. However, the two growth regulators show no effect on number of branches. This comes in agreement with the results of [16] on henna and [40] on chilli. On the other hand, [30] found an increase in number of branches of black cumim due to the use of GA. [41] mentioned that the positive effect of micronutrients on leaves number of *Antharium andreanum* may be due to their stimulatory effects on carbohydrates synthesis and hormonal regulation and, as a result, growth in general. Our results agreed with that statement in which number of leaves increases in proportional manner with the increase in the rate of fertilizer used (Table 4). Also, it was noted that the lowest concentration of both growth regulators did not cause significant increase in leaves number which may indicate a suboptimal concentration. These results are in agreement with the earlier results of [30]. The increase in dry weight that was seen in this study (Table 5) may be a result of the stimulation of metabolism and then an increase in the accumulation of dry matter. In addition, the increase in vegetative growth due to the fertilizer would increase in photosynthesis rate and in turn the accumulation of carbohydrates and, as a result the dry matter. This result comes in accordance with the results of of [39] on *Sinapis alba*; [26] on plantago and [25] on Ruta plant. The present results also show that spray with GA3 or NAA cause an increase in the dry matter of shoot and this may be correlated with the increase in vegetative growth as mentioned earlier by [42].

Microelements may play a role, as cofactors, in activation of several enzymes such as those involved in photosynthesis. This would reflect in increasing the accumulation of carbohydratein cells [43]. Also, it is known that growth regulators activates some enzymes in addition to de novo synthesis of proteins which all contribute to stimulate growth [44, 45]. The increase in cortical thickness as a result of the treatments shown in this experiment (table 6) may be due to the effect on the size of the parenchyma cells because of the stimulation in the whole growth. These results come in agreement with the results of [46] on potatoes stem that sprayed with gibberellin and auxin at different concentrations.

Vascular bundles number and thickness are increased significantly as the rates of foliar fertilizer increased (Table 7 and 8). The increase in size of vascular bundles due to the fertilizer may be as a result of the enhancement of the activity of cambium to form and differentiate new vascular bundles. The result is in agreement with the result of [47] who was found an increase in stem diameter of treated Dill plant with some Microelements and also with the results of [17] on sweet fennel. In contrast, the two growth regulators had no effect on number of vascular bundles in the vascular cylinder, while they decrease the thickness at all concentrations especially the higher ones. This may be due to the negative effect on the diameter of vascular units without the effect on the activity of cambium. [35] mentioned that the decrease in stem diameter of soybean treated with gibberellin was due to the decrease in size of vascular cylinder layer. Also, our result comes in agreement of the results of [54] when they study the effect of gibberellin on leaves of sunflower. A negative effect of foliar fertilizer in decreasing pith thickness was noted (Table 10), this may be due to increase in size of vascular cylinder that occupies larger area of stem diameter in the expense of pith region. Also, the results show that the increase in rates of foliar fertilizer was accompanied with a decrease in pith thickness. However, all growth regulators treatments increase pith thickness and this may explain the positive effect of those promoters in increasing cell division and elongation.

CONCLUSION

It was obvious that foliar fertilizer and both growth regulators have positive effect on most vegetative characters studied especially at higher concentrations. Foliar fertilizer caused significant increase in cortex thickness, number and thickness of vascular bundles and vascular units diameter. Pith thickness decreased due to the use of foliar fertilizer but it increased due to the use of growth regulators. Interaction between the two factors under study had significant effect on most vegetative and anatomical characters.

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