

Physiological and Anatomical Study of Caraway (*Carumcarvi L.*) Plant Sprayed with Serine and Arginine

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Abstract

The study aimed to estimate the effects of two amino acids serine and arginine on Caraway (*Carumcarvi L.*) plant in the old green garden of College of Education for Pure Sciences Ibn AL-Haitham, Baghdad University, during the growing season 2016-2017. Complete randomized design (CRD) was used to conduct the experiment at two treatment (15, 30) mg.L⁻¹ for serine and two treatment (20 and 40) mg.L⁻¹ for arginine foliar spraying with three replicates, and control treatment sprayed with distilled water. Vegetative growth parameters of plants were estimated (plant height, dry weight, fertilizer relative efficiency and absolute rate growth), chemical contents as (content of nitrogen, phosphorus and potassium, protein and arginine concentration, and total chlorophyll) were determined, and seeds yield per plant were calculated. Results showed the response of growth parameters increased with the foliar spraying of both amino acids treatment as compared to control and highest interaction between the two factors was found between 30 mg.L⁻¹ serine and 40 mg.L⁻¹ arginine. As the anatomical feature the study appears that the foliar spray by amino acids (arginine and serine) and their interaction between Arginine 20 mg.L⁻¹ and Serine 15 mg.L⁻¹, led to increase of the epidermis thickness of stem and diameter of stem, also the amino acid led to increase the cortex thickness of stem about 80.2 μm and thickness of sclerenchyma layers significantly about 54.5 μm comparison with control treatment 40.7μm when treatment the plant with Arginine 20 mg.L⁻¹ and Serine 30 mg.L⁻¹, so the treatment led to decrease the vascular bundles thickness from the control treatment especially the treatment, consist from mixed with 40 mg.L⁻¹ Arginine and 30 mg.L⁻¹ Serine about 42.7 μm from the control treatment 109.1 μm.

Key words: Caraway plant, Physiology, Anatomy, Serine, Arginine.

Introduction

Caraway (*carumcarvi L.*) plant belong to family Apiaceae, it is a biennial herbaceous plants its height ranging from 1-1.5m with lateral branches, the fruits and its oils have several medical purpose as antispasmodic, carminative and as a tonic in the treatment of digestive disorders, it has economic and foodimportance, its widely used for flavoring bread, cake, pastry and cheese [1].

Amino acids are proteinogenic compounds, play an important role in building and synthesize the protein Also, have a high total of different metabolic pools were used to support the plant growth [2].so can improve the fertilizer absorption, increase uptake of nutrients and water, improve the photosynthesis and dry matter, also they increase the crop yield [3,4]. Refer to using the amino acid may be useful to minimize the amount of chemical fertilization.

The Serine consider amino acid which is forming part of the protein and participates in some of the essential processes in the plants like as biosynthesis of glycine, methionine, and cysteine and sphingolipids[5]. Serine is a good source of one-carbon which contribute carbon for purine and pyrimidine nucleotide bases [6]. It has a major role in root growth and participation in phospholipids biosynthesis which are essential for plant viability [7].

Serine has a role in plant mechanism and development of pollen [8] and plays as a metabolic signal for the regulation of photorespiration especially in the glycine to serine inter conversion reactions [9]. Nitrogen is a limiting resource for plant growth and agricultural productivity, since large amount of nitrogen are important in nucleic acid and protein synthesis.

Arginine has the highest nitrogen to Carbon ratio which makes it a major storage and transport form of nitrogen in plants [10], in addition to its role as an amino acid for protein synthesis and portent for polyamines and nitric oxide both of them involved all physiological and biochemical processes. Arginine plays important role in adaptation of plant to biotic and abiotic stresses [11].

The Amino acids like as glutamic acid, aspartic acid, proline, serine and arginine led to increased rooting percentage and number of roots per rooted [12]. Also may influence in the cell division, growth and development of somatic embryos and chlorophyll content [13]. Also [14] found the treatment plant *Ocimum sanctum* L. by some amino acid led to increase the thickness of both midvein and lamina of leaf, diameter of the stem, thickness of epidermis, cortex, phloem and xylem Therefore, this research aimed to study the effect of foliar spray by amino acids (arginine and serine) on yield characters, stem anatomy of *Carumcarvi* L.

Materials and Methods

Physiological study

Pot experiment was conducted in the old garden belong to Department of Biology, College of Education for pure Science Ibn AL-Haitham, during the growth seasons 2016-1017 to investigate the role of two amino acids serine with two concentrations (15,30) mg.L⁻¹ and Arginine with two concentrations (20.40)mg.L⁻¹ foliar spraying on caraway plant *carumcarvi* L.

The experiments were arranged in complete randomized design (CRD) with three replicates. Healthy seeds of caraway were sown in pots containing 10Kg soil in 23-10-2016 ,six plants per pots were remained after thinning, seedling were sprayed with serine and Arginine in 26-12-2016, the control plants were sprayed with distilled water .

Vegetative Growth Parameters

Two plant were carefully removed from each pot in 1-2-2017, plant height (cm) were measured using a meter scale then oven dried (65C°) for 72 hr., crushed and weighted to measured dry weight, and fertilizer relative efficiency according to the equation [15].

$$\text{Fertilizer relative of efficiency} = \frac{\text{Dry weight for treatment} - \text{Dry weight for control}}{\text{Dry weight for control}} \times 100$$

Absolute rate growth was evaluated according to the equation [16].

$$\text{Absolute rate growth} = \frac{W_2 - W_1}{T_2 - T_1}$$

W₁ = Dry weight at the first harvest

W₂ = Dry weight at the second harvest

T₁ = Date for the first harvest

T₂ = Date for the second harvest

Chemical Constituents

Dry shoot samples were crushed and digested according to [17], chemical analysis of nitrogen [18], phosphorus [19] and potassium [20] were estimated. Protein concentration was evaluated [21], arginine concentration [22], and total chlorophyll according to [23].

Plant Yield

At the harvest date 26th April 2017, two plants in each experimental pot were harvest, dry fruits of caraway were extracted from the influences and weighted then the seed yield per plants were calculated.

Anatomical Study

The fresh material of stem was putted in the formalin acetic acid (FAA) at 24-48h and

changed the solution by ethanol (70%). Fresh plant samples of stems were sectioned by using the hand sectioning method follows by [24] were the parts of stem sectioned into thin and small pieces (4-6 cm) by a razor blade then treated by 0.5% sodium hypochlorite for 5mint to remove the chlorophyll pigments. Finally, the samples of stem were placed on the slides and fixed by Olympus KRÜSS light microscope then photographed using DCM35 camera. The characters were studied, the epidermis thickness of stem, thickness of stem cortex, thickness of sclerenchyma layer, thickness of stem vascular bundle and stem diameter.

Statistically Data

The data were analyzed at (P<0.05). Genstat software was used to test of least significant

differences at 5% level of probability (LSD 5%) was used to compare calculated averages of characters[25,26].

Results and Discussion

Physiological Study

Present data shows that plants foliar sprayed with both amino acids were significantly affected, since the control plants recorded the lowest results as compared with the treated plants. The average mean values of plant height, dry weight, absolute growth rate,

fertilizer relative efficiency in Table (1) exhibited significant increasing with foliar spraying with serine from 0 to 30 mg.L⁻¹ (20.42 , 40.88 , 34.38, 134.75)% respectively. And spraying with arginine from 0 to 40 mg.L⁻¹ (15.54, 42.54, 57.89, 143. 59) % respectively. For the interaction the treatment 30 mg.L⁻¹ serine with 40 mg.L⁻¹ arginine scored the best values for plant height, dry weight and fertilizer relative efficiency, while the treatment 15 mg.L⁻¹ serine and 20 mg.L⁻¹ arginine gave the best value for absolute growth rate.

Table1: Effect of serine and arginine on some morphological parameters of caraway plant

| Serine (mg.L ⁻¹) | Arginine (mg.L ⁻¹) | | | | Arginine (mg.L ⁻¹) | | | |
|------------------------------|---|-------|-------|----------------|--|--------|--------|----------------|
| | 0 | 20 | 40 | mean of serine | 0 | 20 | 40 | mean of serine |
| | Plant height(cm) | | | | Dry weight(gm) | | | |
| 0 | 27.00 | 29.50 | 36.50 | 31.00 | 1.26 | 1.70 | 2.46 | 1.81 |
| 15 | 30.50 | 30.00 | 34.00 | 31.50 | 1.78 | 2.52 | 2.48 | 2.26 |
| 30 | 39.00 | 32.00 | 41.00 | 37.33 | 2.39 | 2.44 | 2.81 | 2.55 |
| Mean of arginine | 32.17 | 30.50 | 37.17 | | 1.81 | 2.22 | 2.58 | |
| LSD 0.05 | Serine concentration=3.30 Arginine concentration=3.30 Interaction=5.72 | | | | Serine concentration=0.14 Arginine concentration=0.14 Interaction=0.26 | | | |
| | Absolut growth rate(gm.day ⁻¹) | | | | Fertilizer relative efficiency(%) | | | |
| 0 | 0.034 | 0.072 | 0.086 | 0.064 | 0.00 | 36.03 | 97.16 | 44.40 |
| 15 | 0.063 | 0.102 | 0.093 | 0.084 | 42.78 | 101.68 | 107.48 | 83.98 |
| 30 | 0.075 | 0.085 | 0.091 | 0.084 | 92.47 | 95.42 | 124.80 | 104.23 |
| Mean of arginine | 0.057 | 0.086 | 0.090 | | 45.08 | 77.71 | 109.81 | |
| LSD 0.05 | Serine concentration=0.012 Arginine concentration=0.012 Interaction=0.020 | | | | Serine concentration=2.80 Arginine concentration=2.80 Interaction=4.86 | | | |

As shown in Table (2) serine application at its high level 30 mg. L⁻¹ resulted in highest increase in nitrogen, phosphorus and potassium content by (66.34, 63.15, and 57.94) % respectively, as compared to the control. While arginine application at the concentration 40 mg.L⁻¹ gave the significant increase in the means of the parameters as (57.63, 28.79, 39.82) %respectively.

The highest values for the interaction between both factors were found at the treatment 30 mg.L⁻¹ serine and 40 mg.L⁻¹ arginine and gave (92.73, 10.39) for nitrogen and phosphorus content. The treatment 30 mg.L⁻¹ serine with 20 mg.L⁻¹ arginine gave the best value for potassium content (59.05) in comparison with the control.

Table 2: Effect of serine and arginine on some macro elements content (mg.plant⁻¹) of caraway plant

| Serine (mg.L ⁻¹) | Arginine (mg.L ⁻¹) | | | | Arginine (mg.L ⁻¹) | | | |
|------------------------------|---|-------|-------|----------------|--|-------|-------|----------------|
| | 0 | 20 | 40 | mean of serine | 0 | 20 | 40 | mean of serine |
| | Nitrogen content | | | | Phosphorus content | | | |
| 0 | 26.57 | 51.08 | 78.31 | 51.99 | 3.90 | 5.78 | 8.82 | 6.16 |
| 15 | 56.80 | 85.43 | 80.85 | 74.36 | 8.37 | 9.83 | 8.93 | 9.04 |
| 30 | 76.42 | 90.30 | 92.73 | 86.48 | 9.51 | 10.26 | 10.39 | 10.05 |
| Mean of arginine | 53.28 | 75.60 | 83.97 | | 7.26 | 8.62 | 9.38 | |
| LSD 0.05 | Serine concentration=8.15 Arginine concentration=8.15 Interaction=14.19 | | | | Serine concentration=1.04 Arginine concentration=1.04 Interaction=1.80 | | | |
| | Potassium content | | | | | | | |
| 0 | 19.36 | 34.69 | 51.72 | 35.26 | | | | |
| 16 | 43.8 | 50.16 | 50.84 | 48.27 | | | | |
| 30 | 50.96 | 59.05 | 57.05 | 55.69 | | | | |
| Mean of arginine | 38.05 | 47.97 | 53.20 | | | | | |
| LSD 0.05 | Serine concentration=4.38 Arginine concentration=4.38 Interaction=7.58 | | | | | | | |

Result in Table(3) showed that some chemical compounds (protein and arginine concentration) had good rising by the highest concentration of serine (22.74, 22.71) % and the treatment had slight effect on total chlorophyll 6.90%. Also arginine with height concentration had good effect on the parameters and there were increase in protein, arginine concentration and total chlorophyll by about (19.02, 19.14, 10.84) % as compared with control. For the interaction the treatment 30 serine with 20 arginine gave a good values (23.12, 11.84, and 1.01)

respectively compared with unsprayed plants that amounted the lowest values. Results for yield component (wt. seeds. Plant⁻¹) Table (4) Indicated that foliar spraying with serine with height concentration 30 mg.L⁻¹ caused a significant increase in the parameter 17.68%, in the other side arginine with 40 mg.L⁻¹ showed a significant increase in wt. seeds. Plant-1 by about 22.23% .The interaction between both factors gave good value at the treatment 30 mg.L⁻¹ serine and arginine 40 mg.L⁻¹ and it was 2.56 gm.

Table 3: Effect of serine and arginine on some chemical compounds of caraway plant

| Serine (mg.L ⁻¹) | Arginine (mg.L ⁻¹) | | | | Arginine (mg.L ⁻¹) | | | |
|---|---|-------|-------|----------------|--|-------|-------|----------------|
| | 0 | 20 | 40 | mean of serine | 0 | 20 | 40 | mean of serine |
| | Protein % | | | | Arginine % | | | |
| 0 | 13.19 | 18.78 | 19.88 | 17.28 | 6.75 | 9.62 | 10.18 | 8.85 |
| 15 | 19.94 | 21.19 | 20.37 | 20.50 | 10.21 | 10.85 | 10.44 | 10.50 |
| 30 | 19.88 | 23.12 | 20.63 | 21.21 | 10.18 | 11.84 | 10.56 | 10.86 |
| Mean of arginine | 17.67 | 21.03 | 20.29 | | 9.04 | 10.77 | 10.39 | |
| LSD 0.05 | Serine concentration=1.03 Arginine concentration=1.03 Interaction=1.80 | | | | Serine concentration=0.53 Arginine concentration=0.53 Interaction=0.92 | | | |
| Total chlorophyll (mg.gm ⁻¹ fresh wt.) | | | | | | | | |
| 0 | 0.75 | 0.97 | 0.89 | 0.87 | | | | |
| 15 | 0.85 | 0.96 | 0.97 | 0.93 | | | | |
| 30 | 0.89 | 1.01 | 0.89 | 0.93 | | | | |
| Mean of arginine | 0.83 | 0.98 | 0.92 | | | | | |
| LSD 0.05 | Serine concentration=0.05 Arginine concentration=0.05 Interaction=0.088 | | | | | | | |

Table 4: Effect of serine and arginine on yield component (wt. seeds.Plant⁻¹gm) of caraway plant

| Serine (mg.L ⁻¹) | Arginine (mg.L ⁻¹) | | | |
|------------------------------|---|------|------|----------------|
| | 0 | 20 | 40 | mean of serine |
| 0 | 1.38 | 2.12 | 2.46 | 1.98 |
| 15 | 2.05 | 2.10 | 1.88 | 2.01 |
| 30 | 2.20 | 2.22 | 2.56 | 2.33 |
| Mean of arginine | 1.88 | 2.15 | 2.30 | |
| LSD 0.05 | Serine concentration = 0.15 Arginine concentration=0.15 Interaction = 0.26 | | | |

Spraying with amino acids caused increase in amino acids content, purine and pyrimidine which are the building block for nucleic acids synthesis, precursors for sucrose synthesis, polysaccharide phospholipids and energy sources, which they are very importance for metabolism process in the plant cell[27].Phosphoserine phosphatase (PSP)is an enzyme in serine biosynthesis pathway its expression in anther so its play a role in pollen development, and able to regulate the glycol tic flex, affecting the Krebs cycle and the biosynthesis of tryptophan, which in turn affect metabolism of sugar[28].Plant development and metabolism are dynamically regulated the day night cycle [29].

Light is very important factor for regulation the dial processes, carbon assimilation, becomes induced after onset of light [30]. Photorespiration also responds to light, many of photorespiration enzymes accumulate after the illumination [31].

Serine is a metabolic signal for regulation of photo respiratory pathway particularly at conversion step(glycine to Serine) [9]. Caraway plants were responded to foliar spray with both amino acids serine and arginine, since growth, yield and chemical constituents of the caraway plant were improved at the high concentration 30 mg.L⁻¹ for serine and 40 mg.L⁻¹ for arginine.

Anatomical study

In the present study the Table (5) showed the Arginine and Serine concentration and their interaction had a significant effect on the epidermis thickness of stem. the interaction between Arginine 20 mg.L⁻¹ and Serine 15 mg.L⁻¹ concentration led to increase of thickness of epidermis significantly about 7.3 μ m comparison with control treatment 4.7 μ m, the mixed between this two solutions due to increase the cells of epidermis because the important function of epidermis was protective and supports the plant shape and treating the plant by this amino acids gives the cells more protein to build the thickness walls, this results agree with [32] who found when treated the ross cutler by amino acid led to increase the cell number and protein percentage.

The Table (6) showed the Arginine and Serine concentration and their interaction had a significant effect on the cortex thickness of stem and the interaction between Arginine 20mg.L⁻¹ and Serine 30mg.L⁻¹ concentration led to increase of thickness of cortex significantly about 80.2 μ m comparison with control treatment 74.2 μ m. The cortex already consist from parenchyma and collenchyma tissue, collenchyma tissue gives support and protective the stem from the environmental conditions also the parenchyma availability tensile strength, that's mean the increase of this layers give support to the stem, this result agree with [33] who found the amino acid supply the plant tissue more portions to build the cells and tissue in the higher plants. The Table (7) shows the Arginine and Serine concentration and their interaction

had a significant effect on the sclerenchyma layers thickness of stem.

The same interaction between Arginine 20mg.L⁻¹ and Serine 30mg.L⁻¹ concentration led to increase of thickness of sclerenchyma layers significantly about 54.5 μ m comparison with control treatment 40.7 μ m, increase this layer also gives support and protective the stem from the environmental conditions like as the collenchyma tissue in the cortex. So the table (8) showed the Arginine and Serine concentration and their interaction had a significant effect on the vascular bundles thickness of stem.

The results shows that the all treatment led to decrease the thickness of vascular bundles from the control treatment especially the treatment consist from mixed with 40mg.L⁻¹ Arginine and 30mg.L⁻¹ Serine when reached to 42.7 μ m from the control treatment 109.1 μ m, that's mean the spraying the plant with high concentrations of amino acid reduced the size of vascular bundles, which in turn reduced the absorption of active substances and water from the soil.

Increase all the tissue the above mentioned led to effect on the diameter of stem when the Arginine and Serine concentration and their interaction had a significant, from Table (9) showed that the interaction between Arginine 20mg.L⁻¹ and Serine 15mg.L⁻¹ concentration led to increase of diameter of stem significantly comparison with control treatment, due to the mixed between the two solutions because all the internal tissue increase in the thickness so it's naturally reflected at the stem diameter.

Table 5: Effect of serine and arginine on thickness of epidermis of caraway plant

| | | Arginine (mg.L ⁻¹) | | |
|------------------------------|--|--------------------------------|-----|-----|
| Serine (mg.L ⁻¹) | | 0 | 20 | 40 |
| 0 | | 4.7 | 6.3 | 5.2 |
| 15 | | 5.5 | 7.3 | 5.4 |
| 30 | | 4.4 | 5.2 | 4.8 |
| LSD 0.05 | | 0.32 | | |

Table 6: Effect of serine and arginine on thickness of cortex of caraway plant

| | | Arginine (mg.L ⁻¹) | | |
|------------------------------|--|--------------------------------|------|------|
| Serine (mg.L ⁻¹) | | 0 | 20 | 40 |
| 0 | | 74.2 | 53.7 | 38.7 |
| 15 | | 52.9 | 32.9 | 62.4 |
| 30 | | 70.8 | 80.2 | 63.7 |
| LSD 0.05 | | 3.19 | | |

Table 7: Effect of serine and arginine on thickness of sclerenchyma layers of caraway plant

| Arginine (mg.L ⁻¹) | | | |
|--------------------------------|------|------|------|
| Serine (mg.L ⁻¹) | 0 | 20 | 40 |
| 0 | 40.7 | 26.0 | 32.0 |
| 15 | 36.9 | 45.3 | 39.2 |
| 30 | 39.0 | 54.5 | 39.0 |
| LSD 0.05 | 1.24 | | |

Table 8: Effect of serine and arginine on thickness of vascular bundles of caraway plant

| Arginine (mg.L ⁻¹) | | | |
|--------------------------------|-------|------|-------|
| Serine (mg.L ⁻¹) | 0 | 20 | 40 |
| 0 | 109.1 | 86.3 | 64.3 |
| 15 | 70.3 | 54.4 | 100.4 |
| 30 | 108.0 | 99.2 | 42.7 |
| LSD 0.05 | 2.03 | | |

Table 9: Effect of serine and arginine on stem diameter of caraway plant

| Arginine (mg.L ⁻¹) | | | |
|--------------------------------|-------|-------|-------|
| Serine (mg.L ⁻¹) | 0 | 20 | 40 |
| 0 | 526.7 | 375.7 | 454.9 |
| 15 | 463.3 | 585.4 | 526.7 |
| 30 | 515.1 | 529.9 | 453.7 |
| LSD 0.05 | 6.13 | | |

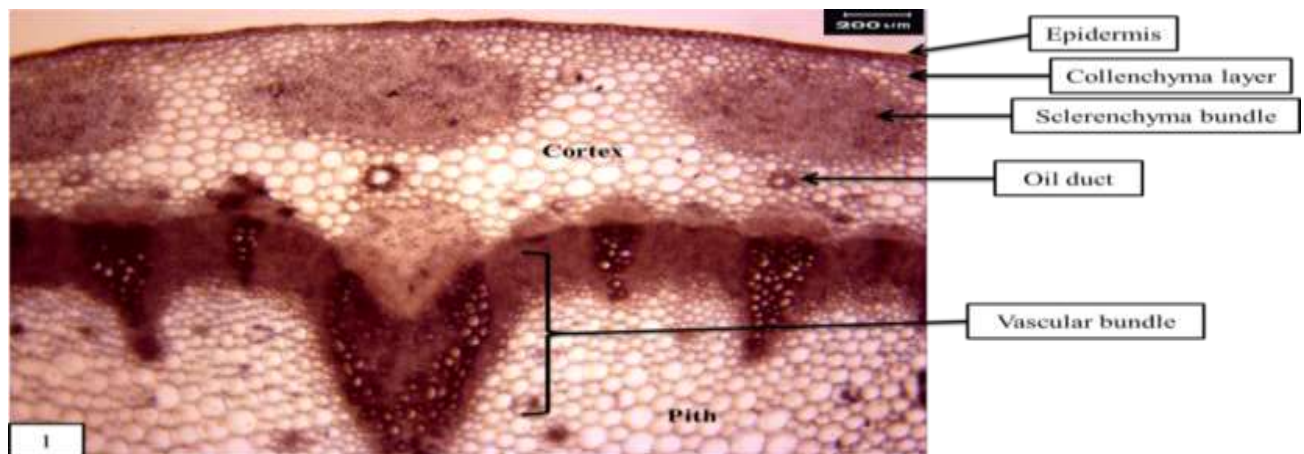


Figure 1: cross section of control treatment (0x0) stem of Caraway plant

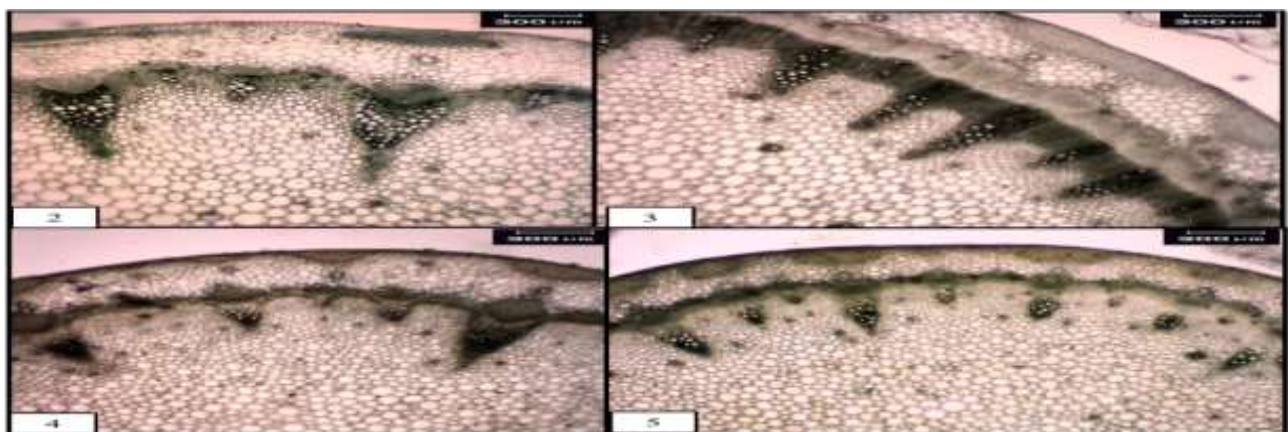


Figure 2: Cross section of stem of Caraway plant, 2: 20mg.L⁻¹ Arginine treatment, 3: 40mg.L⁻¹ Arginine treatment, 4: 15mg.L⁻¹ Serine treatment, 5: 15+20mg.L⁻¹ Arginine and Serine treatment

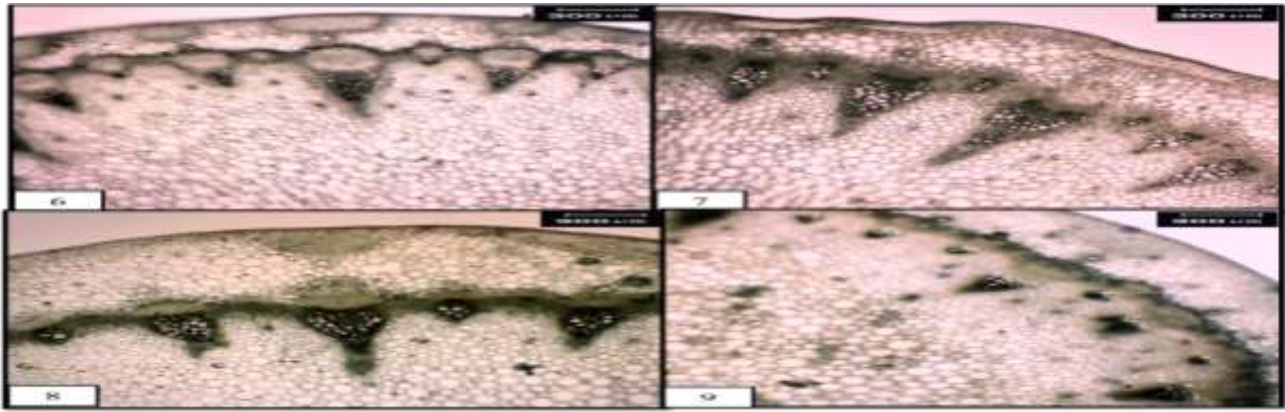


Figure 3: Cross section of stem of Caraway plant, 6: 15+40mg.L⁻¹ Arginine and Serine treatment, 7: 30mg.L⁻¹ Serine treatment, 8: 20+30% Arginine and Serine treatment, 9: 30+40mg.L⁻¹ Arginine and Serine treatment

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