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Effect of potassium humate on some anatomical traits of wheat roots under the influence of salt stress.

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Abstract:

In the labs of the Department of Life Sciences, College of Education for Girls, University of Anbar, a lab experiment was conducted to investigate a some anatomical traits of wheat plant roots under salt stress, potassium humate was added in certain amounts. Irrigation using river water was the first treatment, irrigation using saline water with 2500 mg/l was the second. Irrigation with 100% potassium humate and 2500 mg/l of salt water was the third treatment. Irrigation with 2500 mg/l of salt water and 75% potassium humate constituted the fourth treatment. Irrigation with 50% potassium humate and salt water was the Fifth treatment. The findings demonstrated that, in the face of salt stress, administering potassium humate improved the properties of the roots and brought the parenchymal cells back into equilibrium. It was also observed that a higher concentration of salt caused the exterior walls to bend and certain cells to distort, as well as a reduction in the properties that were being researched. The results of the study demonstrated that the treatments under investigation differed significantly from one another. The third treatment had the largest diameter of epidermal tissue cells, measuring 25,500 micrometers, while the second saline treatment had the lowest average length of epidermal cells, measuring 19,500 micrometers. The third treatment also had the largest average width of epidermal cells, measuring 24,667 micrometers, while the second treatment had the lowest width. It was 16.167 micrometers for epidermal cells. According to the data, the first treatment had the largest average thickness of cortical cells, measuring 131.667 micrometers, while the salt treatment had the lowest average thickness, measuring 85.833 micrometers. In every therapy under investigation, the vascular cylinder took on a circular configuration, and the tissues were arranged as alternating bundles between the phloem and xylem. The first treatment had the greatest average bark thickness, measuring 81.667 micrometers, while the fifth treatment had the lowest average thickness, measuring 41,333 micrometers. With an average length of 75,833 micrometers for the first treatment and 21,500 micrometers for the salt treatment, respectively, the first treatment had the longest wood cell length on average. According to the data, the first treatment's wood cell width rate was 62.833 micrometers, while the fifth treatment's rate was 40.167 micrometers.

Keywords: wheat, salt stress, potassium humate, epidermis, bark, wood .

تأثير هيومات البوتاسيوم في بعض الصفات التشريحية لجذور القمح تحت تأثير الاجهاد الملحي

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مستخلص:

نفذت تجربة مختبرية في مختبرات كلية التربية للبنات جامعة الانبار/ قسم علوم الحياة. لدراسة بعض الصفات التشريحية لجذور نبات القمح المضاف اليها هيومات البوتاسيوم بنسب معينة تحت ظروف الاجهاد الملحي. كانت المعاملة الاولى هي الري بمياه النهر والمعاملة الثانية هي الري بمياه ملحية 2500 ملغم/ لتر. والمعاملة الثالثة الري بمياه مالحة 2500ملغم / لتر مضافاً اليها ٪100من هيومات البوتاسيوم والمعاملة الرابعة الري بمياه مالحة 2500ملغم مضافا اليها ٪75 من هيومات البوتاسيوم والمعاملة الخامسة الري بمياه مالحة مضافا اليها ٪50 من هيومات البوتاسيوم. اظهرت النتائج أن إضافة هيومات البوتاسيوم ادى الى تحسين صفات الجذور وإعادة التوازن للخلايا البرنكيمية بوجود الإجهاد الملحي كما لوحظ ان زيادة التركيز الملحي ادى الى انخفاض في الصفات المدروسة وتشوه في بعض الخلايا وتعرج الجدران الخارجية. بينت الدراسة وجود فروق معنوية بين المعاملات المدروسة، وإن أعلى قطر لطول خلايا نسيج البشرة كان للمعاملة الثالثة 25.500 مايكرومتر أما أقل معدل لطول خلايا البشرة كان للعاملة الملحية الثانية19.500 مايكرومتر ، أما اعلى معدل لعرض خلايا البشرة فكان للمعاملة الثالثة إذ بلغت 24.667 مايكرومتر وأقل عرض لخلايا البشرة 16.167 مايكرومتر ، واظهرت النتائج ان اعلى معدل سمك لخلايا القشرة كان للمعاملة الأولى131.667 مايكرومتر واقل معدل سمك لخلايا القشرة كان للمعاملة الملحية 85.833 مايكرومتر وظهرت الأسطوانة الوعائية بشكل دائري في جميع المعاملات المدروسة وكانت الانسجة بشكل حزم متبادلة بين الخشب واللحاء . وكان اعلى معدل سمك للحاء هو للمعاملة الاولى حيث بلغ 81.667 مايكرومتر واقل معدل سمك هو للمعاملة الخامسة 41.333 مايكرومتر وإن اعلى معدل لطول خلايا الخشب هو للمعاملة الاولى وهو 75.833 مايكرومتر بينما اقل معدل لطول خلايا الخشب هو للمعاملة الملحية 21.500 مايكرومتر. واظهرت النتائج ان اعلى معدل لعرض خلايا الخشب هو للمعاملة الاولى 62.833 مايكرومتر وأقل معدل هو للمعاملة الخامسة 40.167 مايكرومتر.

الكلمات المفتاحية: القمح والاجهاد الملحي وهيومات البوتاسيوم ، البشرة ، اللحاء ، الخشب .

Introduction:

Wheat, scientifically known as *Triticum astivum*, is a critically essential crop that plays a vital role in food production and sustenance. It has a prominent position in Iraq as a leading winter crop, both in terms of planted area and yield. Iraq is recognized as one of the pioneering nations in the cultivation of wheat, owing to the presence of favorable conditions for its growth. However, the current level of output is regarded insufficient. Salinity poses a significant challenge to agriculture and agricultural development in several places worldwide, including Iraq, particularly in arid and semi-arid zones. Salinity arises from the build-up of salts caused by dryness and high temperature (Elsahookie & Al-Khafajy, 2014)(Al-khafaji, 2020). Ahmed et al., 2022In his investigation on the physiological changes in seed vitality and strength, found that higher concentrations of sodium chloride in the germination media result in a decline in the germination rate. At high concentrations, germination may be totally blocked. Irrigation with salt water results in the buildup of salts in the soil around the roots, which eventually hampers plant growth by increasing the osmotic pressure of the soil solution. This, in turn, reduces the roots' ability to take in water(Sharma et al., 2013) .

Iraq is seeing a decrease in wheat production. The decline in water levels can be attributed to the failure to implement proper management techniques, insufficient water releases from the Tigris and Euphrates rivers, limited rainfall, resulting in high salinity levels in the rivers, and the degradation of irrigation water quality due to pollution (Zidan et al., 1990). Potassium plays a crucial role in wheat's ability to tolerate salinity by mitigating the negative effects caused by certain ions like sodium. It achieves this by increasing the weight of the dry matter in the plant, improving the nutritional balance between the soil and the plant, and enabling the plant to maintain osmotic pressure. Potassium is the most crucial positively charged ion due to its essential physiological and metabolic roles. Potassium has a crucial role in several essential physiological processes in plants, including regulating the osmotic potential of plant cells and controlling stomatal movement, which in turn affects transpiration(Babana et al., 2016).

The germination stage is crucial in crop production since researchers often depend on it to determine the species and the level of resistance to different environmental conditions. Due of the significance of wheat, scientists have focused on this plant species and conducted study on its morphology, physiology, and its interaction with its growing environment. And the magnitude of its impact on it. Salinity is one of the elements that influence the determination of production and yield . One of the present challenges that pose a danger to plant biodiversity, diminish production effectiveness, and result in phenotypic abnormalities at different development stages is well acknowledged . The reference is from(Gholizadeh et al., 2014). Research indicates that salt stress has a distinct impact on the structure of wood tissue, phloem tissue, and the quantity of vascular bundles. The anatomical property of rising salinity leads to a rise in salt tolerance in the variety..The user's text is . (Olaetxea et al., 2016). Additionally, it was shown that salt induces alterations in the cell wall's anatomy and affects the epidermis and cortex of wheat plant roots . Considering the information provided, this research was conducted on a specific kind of bread wheat called Triticum astivum L, namely the Wafiyah cultivar. The study aimed to learn about the role of potassium humate in reducing the impact of salt stress and to determine the best level of potassium for the anatomical qualities of wheat roots..

Materials and methods:

Anatomical features:

A research was undertaken at the Graduate Studies Laboratory - Department of Life Sciences / College of Education for Girls - Anbar University to examine the anatomical traits of wheat plant roots growing under salt stress. The study consisted of five different treatments. The first treatment, T1, was the use of river water for irrigation(control treatment). The second treatment, T2, involved the use of salt water with a concentration of 2500 mg/l for irrigation. The third treatment, T3, is unspecified. The irrigation treatments consisted of using salt water with different concentrations of potassium humate. Treatment T4 involved irrigation with salt water at a concentration of 2500 mg/L along with 75% potassium humate. Treatment T5 involved irrigation with salt water along with

50% potassium humate. The potassium humate used in these treatments was obtained in solid form from local markets and was added at a rate of five grams per 100 liters, as recommended. According to the research, the proportions were allocated as follows: 5 grams for the third treatment, 3.75 grams for the fourth treatment, and 2.500 grams for the fifth treatment. The seeds were sown in a germination facility, following the prescribed conditions for grain germination, at a temperature range from 20 to 25 degrees Celsius. Each treatment was replicated three times, with three replicates per treatment. The water used was the one to which the concentrations were applied. A total of 1 liter of water was used, into which the salt concentrations were combined with humates. The seeds were sown. Each package includes 24 seeds. The seeds are planted in glass sand, which has been sterilized, cleaned from contaminants, sifted with 8 mm diameter sieve, and sterilized in an oven at a temperature of 250 degrees Celsius.

For each experimental unit, random samples of wheat plants were chosen. The physiological analysis for the study involved collecting fresh samples directly from the laboratory. These samples were then fixed with a solution of formalin acetic acid alcohol for 24 hours at room temperature. Afterward, they were washed with ethylol at a concentration of 70%. To eliminate any remaining remnants of the fixative solution, it was thereafter preserved in alcohol of the same concentration and kept in the refrigerator until it was used To prepare of anatomical sections of plant components(International & Latimer, 2012) . These sections were then examined to investigate the following characteristics:

1- Average width of root epidermal cells (µm)

2- Average length of root epidermal cells (µm)

3- Average thickness of root cortex cells (micrometres)

4- Average thickness of phloem tissue of roots (micrometres)

5- Average width of xylem cells in the vascular cylinder (micrometers)

6- Average length of xylem cells in the vascular cylinder (micrometer)

Statistical analysis:

The data was analyzed using a completely randomized design (C.R.D.), and the significant differences between the arithmetic means were tested using the least significant difference between the means (LSD) test at a significance level of 5%. The data was analyzed using the statistical program SPSS, and Excel was also used to display the results.

Results and Discussion

Anatomical characteristics of wheat plant Triticum astivum

Characteristics of the transverse section of the root:

Analysis of tissue cross-sections obtained from the first development of root hairs, as shown in Figures (1,2,3,4,5) revealed that the root section in the first treatment had a circular form, with no alterations seen in its exterior or internal structure. The epidermal layer exhibited regularity, followed by another layer of epidermis. The cortex is followed by the vascular cylinder. These findings suggest that the root cells were not affected by water and all observed attributes were favorable in the control treatment. The second treatment exhibited a semi-circular and winding growth pattern, but experienced stunting at the outer edges of the root due to salt stress. This stress led to cell damage caused by an increase in osmotic pressure, resulting in a difference in potential between the interior and exterior of the cell. Consequently, the cells shrunk and the parenchymal tissue was affected, leading to an imbalance due to the lack of support. Externally. The cells further experienced distortion and thickening of their walls. The user's text is(Majeed et al., 2014).The user's text is (Seleiman et al., 2022) Regarding the third treatment, it became evident that potassium effectively healed the damage and reinstated the equilibrium among the components. This was evident from the regularity of the wall and the healthy state of both the skin cells and the cortical cells.The user's text is(Anwar, 2016). The fourth treatment, like the others, was impacted by salinity and had a semi-oval shape. However, the application of humates restored equilibrium among the cell components, enabling it to reestablish its capacity to withstand adverse conditions. Potassium humate induced salt stress, resulting in the alteration of the outer epidermal layer (Anwar, 2016) and (Shafi et al., 2020). Regarding the sixth treatment, it sustained significant damage and had stunted walls. This suggests that the introduction of humates did not establish a condition of equilibrium between the cellular components and the additional salt, resulting in root damage caused by the salt(Begum et al., 2022)their severity and frequency are predicted to rise in the near future. Therefore, in the present study we investigated the mechanisms underlying plant responses to drought (5, 10 and 15% polyethylene glycol, PEG-6000 and (Olaetxea et al., 2016). Based on the observed structural properties of wheat plants, it seems that using 100% potassium humate is the most effective approach for dealing with salt stress.

Upon anatomical examination and histological tracing from the external to the internal layers, it was seen that each cross section was impacted in the following manner:

The epidermis: In all treatments, the epidermis was seen as a contiguous arrangement of cells. The hair layer was examined and the root hairs were clearly visible in the treatments that were analyzed. The epidermal cells had an enlarged form.

tables (1) and (2) demonstrate highly significant differences at a 1% level of significance between the study treatments. Specifically, the third treatment had the highest average width of epidermal cells at 24.667 micrometers, while the fourth treatment had the lowest average width at 16.167 micrometers. This is due to its high concentration of potassium humate. Potassium functions by enhancing the activation of crucial enzymes in the plant, including those that promote the production of carbohydrates and protein, and helps in combating salt stress(Anwar, 2016). The third treatment exhibited the largest rate of epidermal cell length, measuring 25,500 micrometers, whilst the lowest rate was not specified. Lengthy. The second treatment was the application of salt at a concentration of 19,500 micrometers. This is caused by a decrease in the uptake of water by the plant's roots owing to the elevated osmotic pressure of the surrounding medium caused by a high concentration of salts. The increased uptake of sodium hampers the assimilation of other essential nutrients as a result of the root cells losing their capacity to regulate ion absorption and engaging in competition for nutrients. The presence of absorption sites in the root is in accordance with the discovered by (Roy et al., 2014)(Shende et al., 2020).

Table (1) Average width of wheat plant root epidermis cells (micrometers)

Treatment	Mean	S.E.
River water	18.167	0.441
Salt (2500 mg/L)	22.667	1.453
Salt + 100% Potassium humates	24.667	1.481
Salt + 75% Potassium humates	16.167	0.726
Salt + 50% Potassium humates	17.667	0.167
LSD 5%	3.209	-
C.V.	8.767	-

Table (2) shows the dimensions of the epidermal cells of the roots of the wheat plant (length) micrometers

Treatment	Mean	S.E.
River water	24.833	1.364
Salt (2500 mg/L)	19.500	0.289
Salt + 100% Potassium humates	25.500	1.041
Salt + 75% Potassium humates	21.667	0.833
Salt + 50% Potassium humates	23.667	0.726
LSD 5%	2.943	-
C.V.	6.933	-

Cortex:

The shapes of the shell cells varied in the studied treatments, from spherical to polygonal. Table (3) shows that there are highly significant differences at the 1% level between the study treatments, as it is noted that the highest rate of shell thickness was in the first treatment, 131.667 micrometers, for the water treatment, and the lowest rate was for the salt treatment. 85.833 micrometers. This is due to the reduction in cell size and the decrease in the rate of cell division when the salt concentration in the roots increases. These results agreed with what was mentioned by (Majeed et al., 2014).

The Endodermis region also appeared clearly in the treatments, and this region was characterized by the presence of oval-shaped cells. This region is distinguished by the presence of stripe-shaped deposition surrounding the radial walls called the Gasparian strip, which acts as an adhesive for the endoderm cells (inner epidermis). Therefore, the passage of water from the cortex to The vascular cylinder takes place through special cells called Passage Cells, which are thin-walled cells devoid of suberin. We also saw damage to the cortex cells in the third and fifth treatments. The deformation was clear in the initial rows of cortex cells(Zhani et al., 2012)(EL Sabagh et al., 2021)

Table (3) shows the thickness of the cortex cells of wheat plants in micrometers

Treatment	Mean	S.E.
River water	131.667	1.666
Salt (2500 mg/L)	85.833	2.205
Salt + 100% Potassium humates	123.500	0.764
Salt + 75% Potassium humates	95.333	5.783
Salt + 50% Potassium humates	125.667	2.963
LSD 5%	10.138	-
C.V.	4.895	-

Vascular cylinder:

In all the treatments examined, the cylinder exhibited a circular shape, with the tissues arranged as alternating bundles between the wood and the phloem along the same diameter. The phloem was positioned on the outer side, while the wood was positioned on the inner side. Tables 4, 5, and 6 demonstrate that there are statistically significant variations at a significance level of 1% across the research treatments in the vascular cylinder. The analyzed data revealed that the treatment including water had the greatest average bark thickness, measuring 81.667 micrometers. The mean bark thickness for the sixth treatment was 41.334. The first treatment had the longest average length of wood cells, measuring 75.833 micrometers, while the salt treatment had the lowest average length of wood cells, measuring 21.500 micrometers. The reason for this is that salt hampers crucial biological processes, which in turn leads to a decrease in root development owing to a drop in the number of dividing cells. This is consistent with previous findings in root tip meristems(Babana et al., 2016)(Shafi et al., 2020). The first treatment had the greatest xylem cell width rate of 62.833 micrometers, while the fifth treatment had the lowest rate of 40.167 micrometers. The xylem cell width rate of the fifth treatment was not substantially different from the rates of the other treatments.

Table (4) shows the thickness of the phloem tissue of wheat plant roots in micrometers

Treatment	Mean	S.E.
River water	81.667	1.667
Salt (2500 mg/L)	42.500	2.500
Salt + 100% Potassium humates	70.167	2.892
Salt + 75% Potassium humates	43.333	0.833
Salt + 50% Potassium humates	41.333	0.882
LSD 5%	6.199	-
C.V.	6.029	-

Table (5) shows the width of xylem cells in the vascular cylinder (width) micrometer

Treatment	Mean	S.E.
River water	62.833	1.481
Salt (2500 mg/L)	45.667	2.333
Salt + 100% Potassium humates	44.167	0.601
Salt + 75% Potassium humates	42.500	0.866
Salt + 50% Potassium humates	40.167	0.726
LSD 5%	4.348	-
C.V.	5.013	-

Table (6) shows the average length of xylem cells in the vascular cylinder, micrometers

Treatment	Mean	1 S.E.
River water	75.833	0.601
Salt (2500 mg/L)	21.500	0.764
Salt + 100% Potassium humates	58.833	1.093
Salt + 75% Potassium humates	54.200	0.757
Salt + 50% Potassium humates	51.000	0.866
LSD 5%	2.656	-
C.V.	2.757	-

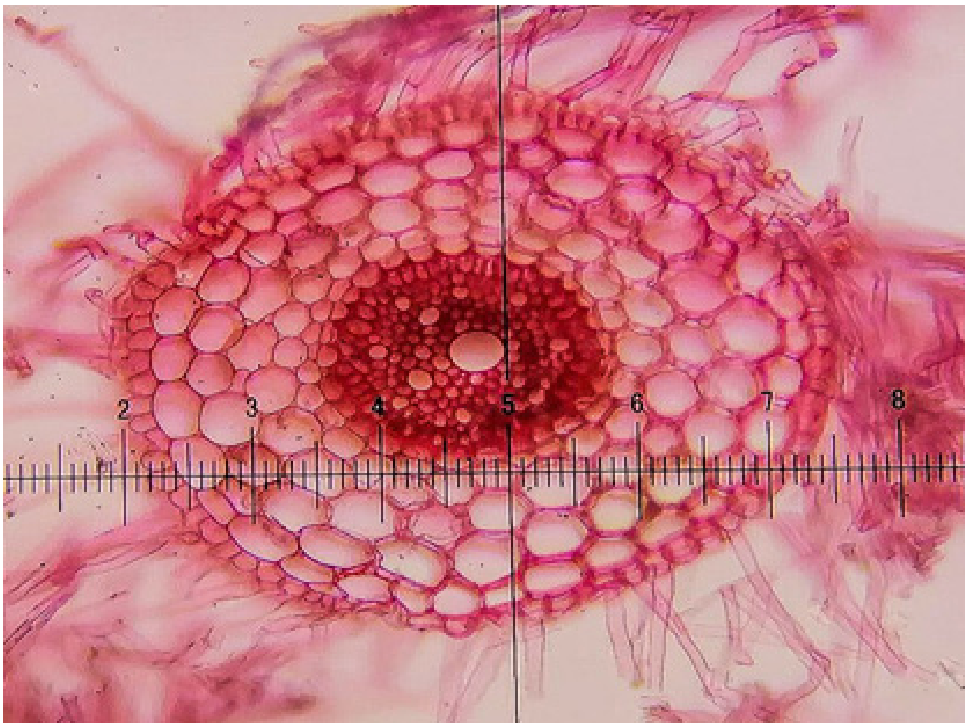

Figure (1) a cross-section of the root showing the epidermal cells and cortex tissue for the first treatment (water) 44x

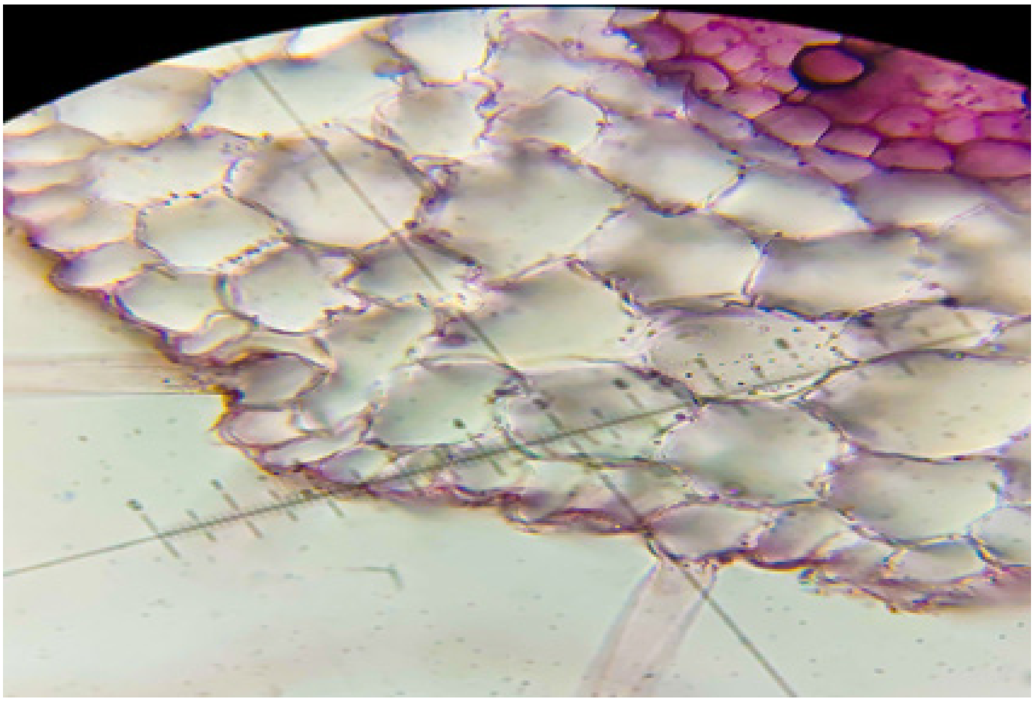

Figure (2) A cross-section of the root showing the epidermal cells and cortex tissue for the second treatment (salt)400x.

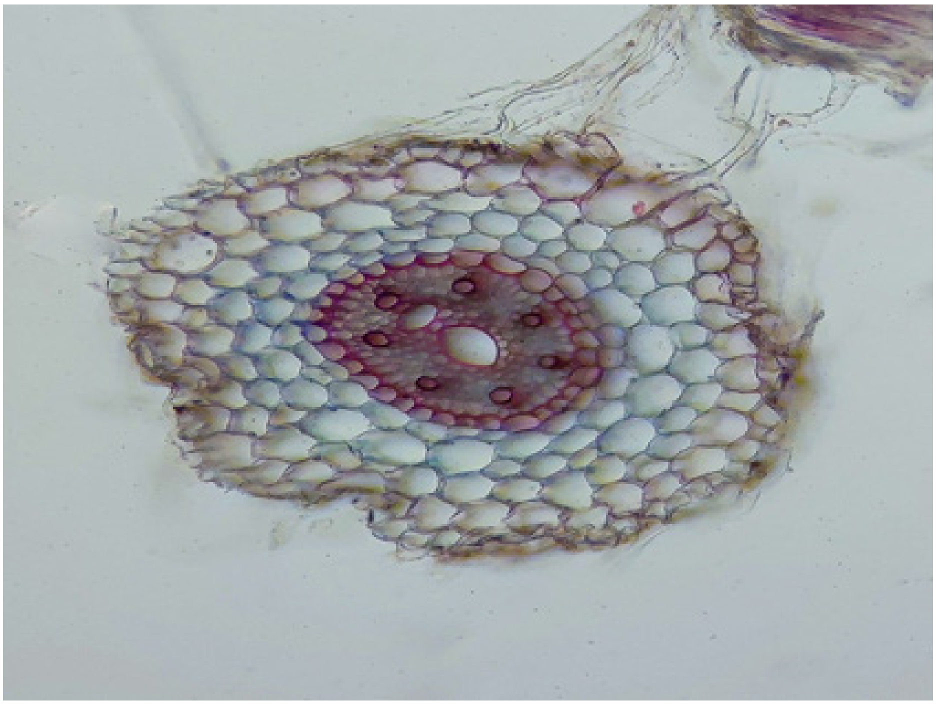

Figure (3/a) A cross-section of the root showing the epidermal cells and cortex tissue for the third treatment (salt + 100% potassium humate)400x

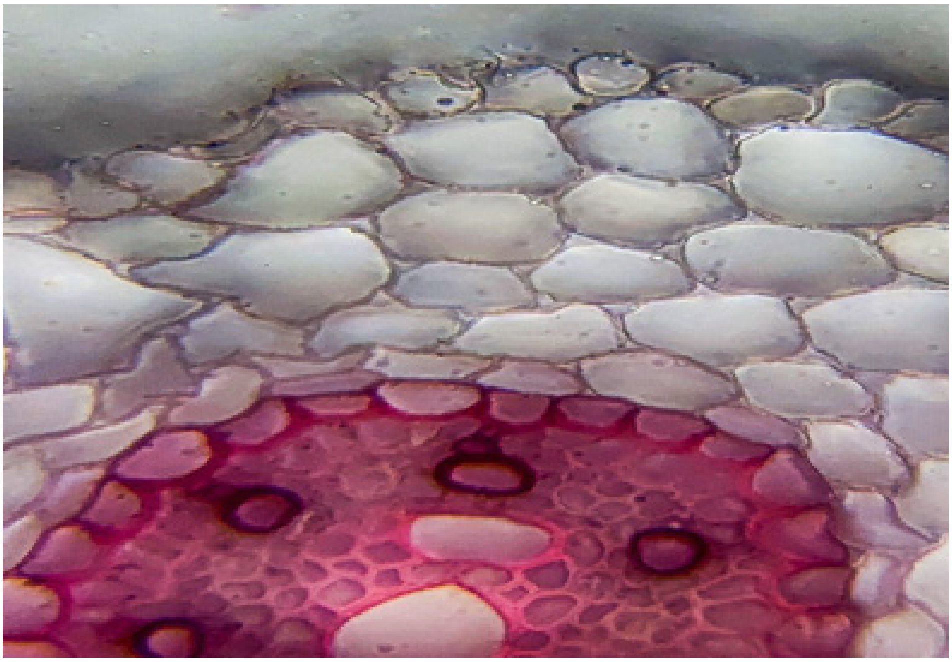

Figure (3/b) a cross-section of the root showing the epidermal cells and cortex tissue for the third treatment (salt + 100% potassium humate)400x

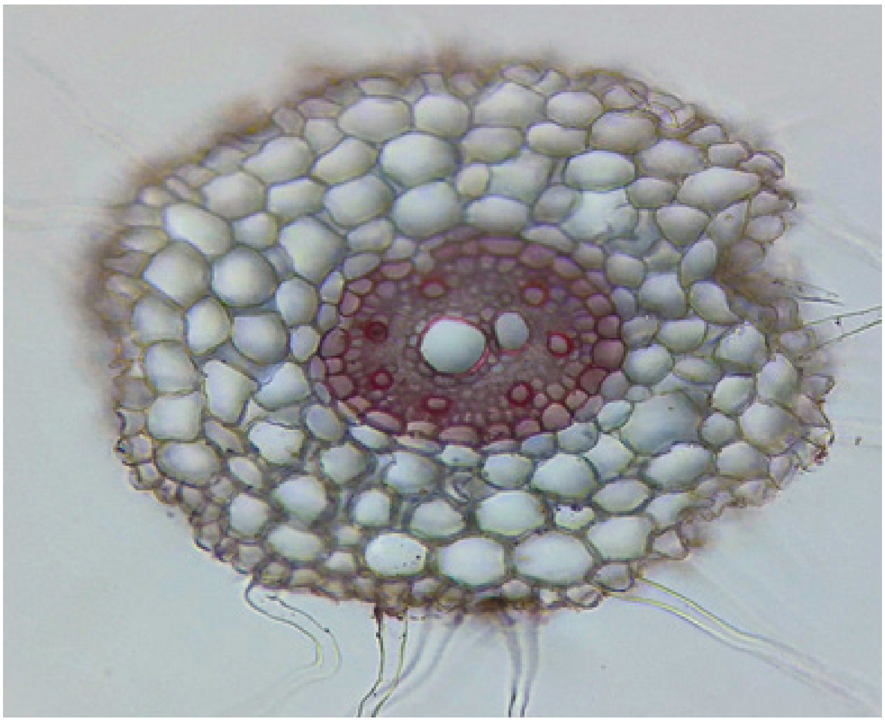

Figure (4) A cross-section of the root showing the epidermal cells
and cortex tissue for the fourth treatment (salt + 75% potassium humate)400x

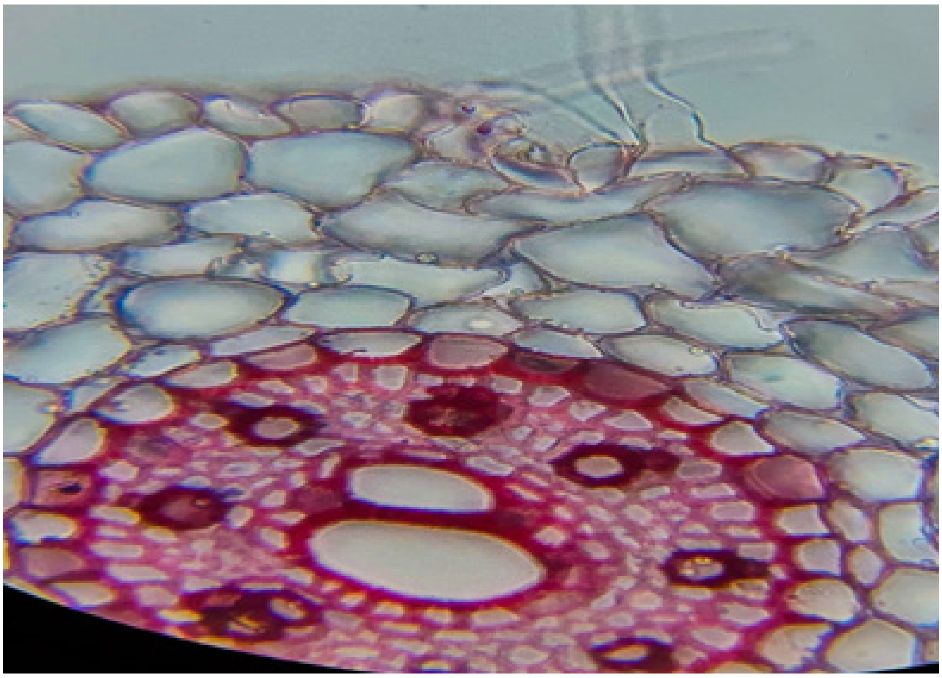

Figure (5): A cross-section of the root showing the epidermal cells
and cortex tissue for the fifth treatment (salt + 50% potassium humate)400x

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