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Effect thefoliar spraying of Humiforte on three cultivated varieties *Sorghum bicolor* L.by studying the anatomical characteristics of stem

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Abstract: *This experiment was carried at the farm of College of Agriculture - The university of Baghdad during the summer season of 2016, to study the effect of foliar application of Humiforte at different concentrations (T0 = control (sprayed with water only), T1= 3 L/h, T2=5 L/h T3= 7 L/h and compare among three cultivated varieties of Sorghum bicolorunder foliarsprayed by Humiforte substance in two different dates by anatomical characteristics of stem epidermis and stem cross section.*
Study of this samples was by light microscope (LM) and scanning electron microscope (SEM) was obvious that sprayed the plants by 5 L/h of Humiforte on the Giza led to diffused the prismatic crystals in the epidermis cells also appeared unicellular and uniseriate trichomes in the first and second sprayed, likewise after the second spraying by 7 L/h of Humiforte obtained the increase the diameter of the stem in Ingath and Rabeh while decreasing the diameter of the stem in Giza.
Key words*: Sorghum bicolor,anatomical characteristics, Humiforte, Varieties.*

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I. Introduction

The white maize (*Sorghum bicolor* L. Moench) from Poaceae family is one of the world's major crops, it is distinguished from other crops by abundance product if where they have the appropriate environment and known for its extreme heat, drought and soil salinity(Al-Tamimi, 1968).

Dibb(1983) refer that the white maize is mainly used in the preparation of animal feed, where they represent about 50% of poultry feed, and also used as food for humans in many countries of the world especially the densely populated countries such as India and China where the largest areas of the world are grown from this crop in these countries.Also, Bukantis (1980)mention that the white corn plants (leaves and stem) are used as the feed for animals either directly or indirectly and the sugar is extracted from the stem of some varieties because they are containing sweet juice

The stomata of stem epidermis were Gramineae-Cyperaceae type, this type is characterized by havingtwo subsidiary cell and two guard cell was dumb-bell shape, the stomata complex is surrounded by two ordinary epidermal cells, the anticlinal wall undulate because presence of the primary pit fields which gave the walls a rosary beads, so all monocots stem is featured as circular shape in cross section and the epidermis consists of single row of stacked cells that are covered by a layer of cuticle,after epidermis tissuethere are located the ground meristem which consists of two layers are supported sclerenchyma cells and many layers of polygonal and unequal diameters parenchyma cells are including narrow ordinary schizogenous intercellular space (Esau, 2006).

The vascular bundle scattered in the center of stem, closed and collateral (xylem and phloem are located on the one radius) the bundles are small and close to one another near the epidermis layer while being large and separate when located closer to the center of stem, the xylem is contained limited number of vessels arranged as Y or V shapes or may call endoarch (the metaxylem outside the bundle while the protoxylem is on the inside), some of the protoxylem vessels is tearing to form xylem cavity (Schizo-lysigenous intercellular spaces),usually the bundles are surrounded by one or more fibers known as bundle sheath fiber (Esau, 2006).

Anglesio*et al*. (1971) indicates that the current trend is the expansion of cultivars and short-stemmed hybrid breeds (green and fodder) which tolerate difficult conditions such as drought, heat intensity, poor soil fertility and increased salinity.

One of the major problems in agriculture is biotic stresses which inhibit plants from realizing their full genetic potential and limits food production also sorghum crops usually obverse drought stress during the

flowering and grain filling period, in addition, the water deficit, which reduces grain yield dramatically (Mutegi*et al*., 2010).

Today, many of farmers are using the chemical compound to stimulate the growth of most economic crops like Humiforte compound, Humiforte is a high-tech soluble liquid nutrient, with rapid absorption via leaves or roots and a high concentration of free amino acids and biologically active oligopeptides, especially recommended for shock treatments (Slavik, 2005), such studies are the study of (Mostafa*et al*., 2010) on a tea crop by using Humiforte formulations to appreciate the quality and productivity of it, also the same researcher using study the effect of foliar spraying by Humiforte on wheat yield under drought of Ardabil region.

The present research was aimed to document and compare among three cultivated varieties of *Sorghum* under foliarsprayed by Humiforte substance in two different dates by anatomical characteristics of stem epidermis and stem cross section also this research is considering a new study to Iraq and the sources are not available or too little.

II. Materials and Methods

1. Collecting the samples:

Three varieties of *Sorghum bicolor* (Giza, Ingath and Rabeh) was carried at the farm of College of Agriculture - The university of Baghdad during the summer season of 2016, and has been harvested directly from the field during the flowering stage after twotreatment with spray by Humiforte compound at different concentration (3, 5 and 7) L h^{-1}, in addition, the control sample were sprayed by used tap water.

2. Preservation the samples:

The fresh samples of the stem are kept in formalin acetic acid (FAA) which was prepared according to (Johanson, 1940) for 24-48 hours then preserved in 70% alcohol until the date of experiments.

3. Study of stem epidermis:

Peeling the epidermis of the stem was done by mechanical scraping using the razor blade, followed by washing with distilled water and putting in 10% KOH, then passed through alcohol series for 10-15 minute, then stained in 1% Safranin for 30-45 minute. Excess stain was washed off with distilled water, dehydrated by Alcohol series (70, 95, and 100%) and cleared by pure Xylene at 10 minutes. Finally, the epidermal samples were put on slides and mounted by cover slides with Dextrin Plasticizer Xylene (D.P.X) this method followed as (Foster, 1977).

4. Study the sectioning samples of stem:

Fresh plant samples of stems were sectioned using hand sectioning method follows (Hutchinson, 1954) as:

Stems of the selected plant were cut into small pieces of a length between (5-7) cm. Segments were sectioned into thin pieces by a razor blade and treated with 0.5% Sodium Hypochlorite for 5 min. to remove the chlorophyll pigments, then all the plant samples were passed through of treatments the following:

1. Stained by 1% safranin in alcohol for (1-2) h.
2. Washing with 70% alcohol to remove the excess pigment.
3. 90% alcohol for 5 mins.
4. 95% alcohol for 2 mins.
5. Absolute alcohol for 2 mins.
6. Xylene + absolute alcohol (1:1) for 2 mins.
7. Xylene for 2 mints.

Finally, the samples were placed on the slides and mounted the cover slides by (D.P.X).

All permanent slides were examined by Olympus BH2 light microscope and scanning electron microscope (SEM)then photographed using Olympus CH3 camera.

Statistical analysis of the data was performed according to the variance analysis, and the averages were compared with the least significant difference at the probability level of 0.05 (5%) (Steel and Torrie, 1981).

III. Result and Dissection

1. Study of stem epidermis

The type Gramineae-Cyperaceae of stomata, in the stem, is considered the specialized feature to the Poaceae family. This type is characterized by having two subsidiary cell and the shape of guard cell was dumb-bell shape so the stomata complex is surrounded by two ordinary epidermal cells and the anticlinal wall was undulate because of presence the primary pit fields which gave the rosary beads of walls.

When the plants sprayed by different concentrations of Humiforte at first and second time not observed any changes in the stem epidermis except few changes showed in the size of guard cell, while spraying the plants in the second time by 5 L h^{-1} observed that the epidermis of Giza variety diffused the prismatic crystals in the cells and around the guard cells (Fig. 1 and 2).

2. Study of stem cross section

Sorghum stem distinct in those varieties (Giza, Ingath and Rabeh) of the control as a circular shape in cross section, the epidermis consists of a single row of stacked cells that are covered by a layer of cuticle and interspersed with stomata, after epidermis tissue located the ground meristem which consists of two layers supported sclerenchyma cells and many layers of polygonal and unequal diameters parenchyma cells including narrowordinary schizogenous intercellular space and the vascular bundles scattered in it.The vascular bundles ovule shaped in the cross section, the protoxylemand metaxylem ring in shape (Fig. 3, 4, 5, and 6) (Essu, 2006;Metcalfe, 1960).

Anatomical characters of *Sorghum* stems treatedby Humiforteappear in Fig. 7, 8, 9 and 10.

When treated the stem of *Sorghum* varieties (Giza, Ingath and Rabeh) by Humiforte (3, 5 and 7) L h^{-1} concentrations during the first and second spraying can show the results of the analysis of variance in Table (1) indicate a significant effect on the stem diameter of different varieties, concentrations, number of sprays and binary interaction of varieties and concentrations. Binary interference between concentrations, sprays, varieties, sprays and triple interference there were no significant differences between them.

It was observed from Table (1) that the plants of the Rabah recorded the highest average diameter of the stem was 1831.5 µm and a significant increase of 10 and 119.5 for the two varieties Giza and Ingath sequentially. The results of Table (1) show a significant increase in the average stem diameter by increasing the concentration of the Humiforte. The highest concentration of Humiforte was 7 L h^{-1} the highest mean of stem diameter reached to 1847.7 µm with an increase 0.74, 1.51 and 2.48% for concentrations 5, 3 and 0 L h^{-1}respectively, while the comparison treatment recorded low mean average 1803.0 µm. The increase in stem diameter can be attributed to leaf spraying of mixture micronutrients, mainly due to the increase in all internal tissues of the xylem and phloem, this result is consistent with (Eisa and Ali, 2014; Ali *et al.*, 2009; Mohamed and El-Yazal, 2004).

The same table showed a significant interfere of the stem diameter between the different varieties under the study and the different concentrations of the biological catalyst. The combination of Rabeh recorded in the high concentration 7 L h^{-1} the highest mean of this characteristic was 1979.0 µm while Ingath recorded low mean reached to 1665.0 µm in 0 concentrations.

The results of the table (2) showed that there were significant differences between the varieties and the number of spray in the thickness of the stem epidermis. While the concentrations and bilateral and trilateral interference were not significant differences.

The results of Table (2) indicate that Rabeh recorded highest thickness of epidermis reached to 3.14 µm while Rabah recorded lowest thickness of epidermis reached to 2.83 µm, the increase in thickness of stem epidermis may be due to the increased stem diameter synchronous with increase in the thickness of the epidermis, This synchronization between these two qualities indicates the efficiency of the plant to confrontation of environmental conditions where this layer considered a protective layer to the stem, the resultagree with (Crittendon, 1966).

It is clear from Table (2) that the number of sprays has significantly affected the thickness of the stem epidermis, where the second spray recorded the highest average reached to of 3.13 µm, while the first spray recorded the lowest average 2.91 µm, this may be due to an increase in the number of sprays, which in turn increases nutrients to help the plant resistance to environmental conditions (Elfving*et al.*, 2003).

Table (3) showed that the varieties, the concentrations of Humiforte, number of sprays and the bilateral interaction between the varieties and concentrations had a significant effect on the vascular bundle thickness.

Where we note from Table (3) superiority Inghath in the highest average of vascular bundle thickness reached to 82.24 µm, while Giza recorded lowest average 60.71 µm, this is due the plants need to produce more xylem vessels to withdraw large quantities of water and nutrients from the soil due to stem diameter decrease (Kofidis*et al.*, 2008).

The table also showed significant differences between the different concentrations of Humiforte in the thickness of the vascular bundle, where the concentration 7 L h^{-1} recorded highest mean of the vascular bundles thickness reached to 74.19 ?m with a significant increase of 2.06, 4.04 and 5.46 for concentrations 5, 3 and 0 L h^{-1} respectively, while the comparison treatment recorded a lower average 68.73 ?m, the observed increase in the thickness of vascular bundles in the main stem is due to the effect of leaf spraying with a mixture micronutrients, mainly affected by increase in the number of xylem and phloem rows in the vascular cylinder, this is consistent with (Eisa and Ali, 2014; Ali *et al.,* 2009).

Table (3) shows that there is a significant effect of the number of spray in the thickness of the vascular bundle, where the second spray significantly exceeded of the vascular bundle thickness 73.04 ?m, while the first spray recorded a lower average of 69.56 ?m.

As for the binary interference, the results of Table (3) showed that the effect of the interaction between the varieties and the concentrations of the Humiforte was significant in this feature, where the thickness of the vascular bundle of Ingath and Rabehincreased with the increase of the Humiforte

concentrations, the highest mean value at 7 L h^{-1} which was 89.97 µm in Ingath unlike Giza which resulted a decrease in the thickness of the vascular bundle in the same concentration reached to 54.10 µm.

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Table -1.Effect of spray by humifort in the stem diameter of the white maize.

Concentration **NO. spray + varieties**		**Concentration of humifort L h^{-1}**				**No. spray× varieties**
		0	3	5	7	
First spray	**Ingath**	1660.0	1712.0	1726.0	1733.0	1707.7
	Giza	1820.0	1800.0	1812.0	1825.0	1814.2
	Rabeh	1915.0	1935.0	1950.0	1970.0	1942.5
Second spray	**Ingath**	1670.0	1725.0	1730.0	1740.0	1716.2
	Giza	1830.3	1810.0	1820.0	1830.0	1822.6
	Rabeh	1923.0	1939.0	1967.0	1988.0	1954.2
Probability 5%		**n.s**				**n.s**
						No. spray
Concentration × No. spray	**First spray**	1798.3	1815.7	1829.3	1842.7	1821.5
	Second	1807.8	1824.7	1839.0	1852.7	1831.0

	spray					
Probability 5%		**n.s**				**4.24**
						varieties
Concentration × varieties	**Ingath**	1665.0	1718.5	1728.0	1736.5	1712.0
	Giza	1825.1	1805.0	1816.0	1827.5	1821.5
	Rabeh	1919.0	1937.0	1958.5	1979.0	1831.5
Probability 5%		**13.02**				**5.20**
Concentration		**1803.0**	**1820.2**	**1834.2**	**1847.7**	
Probability 5%		**11.19**				

Table-2. Effect of spray by humifort in the stem epidermis of the white maize.

Concentration NO. spray + varieties		**Concentration of humifort L h^{-1}**				**No. spray× varieties**
		0	3	5	7	
First spray	**Ingath**	2.70	2.73	2.77	2.93	2.78
	Giza	2.83	2.90	2.87	2.90	2.88
	Rabeh	3.93	2.90	3.17	3.30	3.08
Second spray	**Ingath**	2.80	2.83	2.93	2.93	2.88
	Giza	3.20	3.33	3.40	3.40	3.33
	Rabeh	3.13	3.06	3.33	3.27	3.20
Probability 5%		**n.s**				**n.s**
						No. spray
Concentration × No. spray	**First spray**	2.82	2.84	2.93	3.04	2.91
	Second spray	3.04	3.08	3.22	3.20	3.13
Probability 5%		**n.s**				**0.14**
						varieties
Concentration × varieties	**Ingath**	2.75	2.78	2.85	2.93	2.83
	Giza	3.02	3.12	3.13	3.15	3.10
	Rabeh	3.03	2.98	3.25	3.28	3.14
Probability 5%						**0.18**
Concentration		2.93	2.96	3.07	3.12	
Probability 5%		**n.s**				

Table -3. Effect of spray by humifort in the vascular bundle thickness of the white maize.

Concentration NO. spray + varieties		**Concentration of humifort L h^{-1}**				**No. spray× varieties**
		0	3	5	7	
First spray	**Ingath**	71.51	77.10	86.19	89.47	81.07
	Giza	66.96	60.77	52.20	53.10	58.26
	Rabeh	62.40	67.50	70.05	77.50	69.36
Second spray	**Ingath**	75.54	79.10	88.53	90.47	83.41
	Giza	70.43	66.89	60.27	55.10	63.17
	Rabeh	65.53	69.53	75.53	79.53	72.53
Probability 5%		**n.s**				**n.s**
						No. spray
Concentration × No. spray	**First spray**	66.96	68.46	69.48	73.36	69.56
	Second spray	70.50	71.84	74.78	75.03	73.04
Probability 5%		**n.s**				**1.38**
						varieties

Concentration × varieties	**Ingath**	73.52	78.10	87.36	89.97	82.24
	Giza	68.70	63.83	56.23	54.10	60.71
	Rabeh	63.97	68.52	72.79	78.52	70.95
Probability 5%		**3.29**				**1.70**
Concentration		68.73	70.15	72.13	74.19	
Probability 5%		**2.14**				

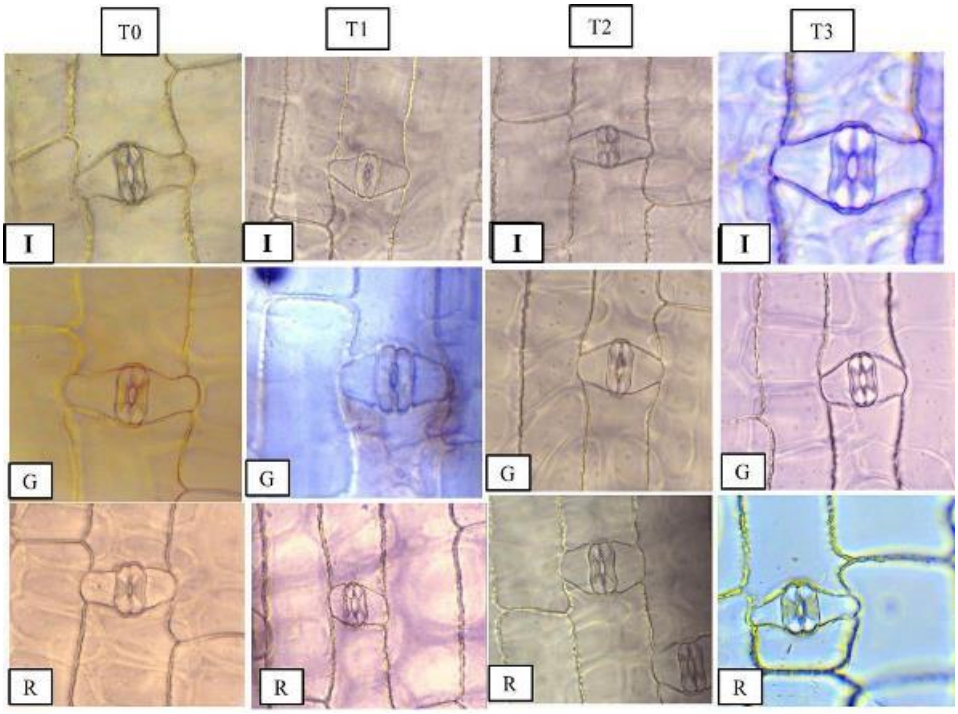


Fig 1: Shape of stem epidermis by (LM) to show the stomata complex after the first spray where is: T0: control, T1: 3 L h^{-1}con., T2: 5 L h^{-1}con., T3: 7 L h^{-1}con., I: Ingath, G: Giza and R: Rabeh.

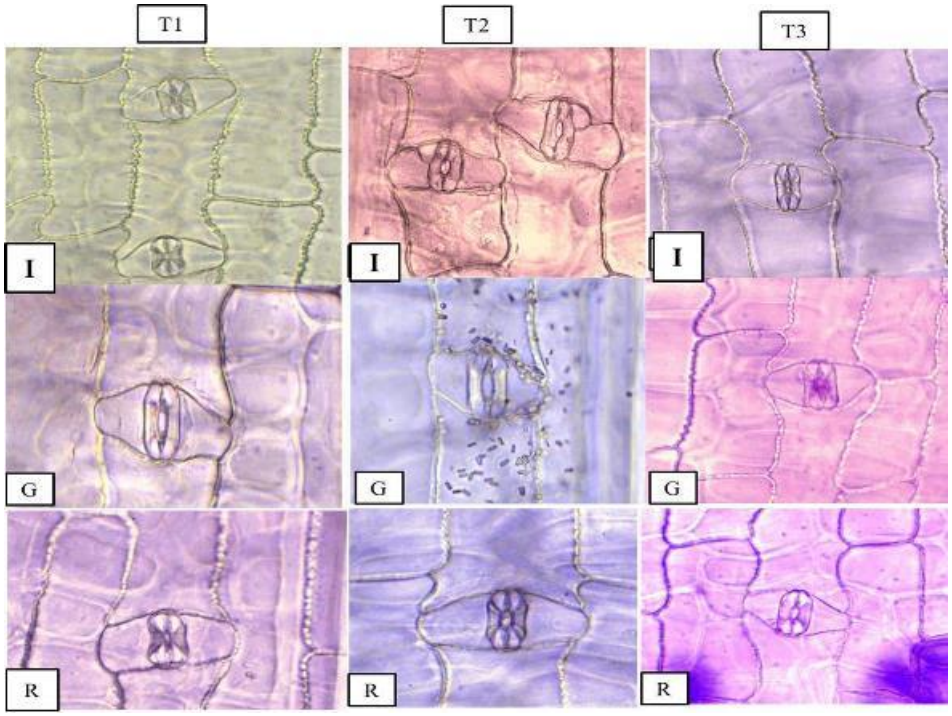


Fig2: Shape of stem epidermis by (LM) to show the stomata complex after the second spray where is: T0: control, T1: 3 L h^{-1}con., T2: 5 L h^{-1}con., T3: 7 L h^{-1}con., I: Ingath, G: Giza and R: Rabeh.

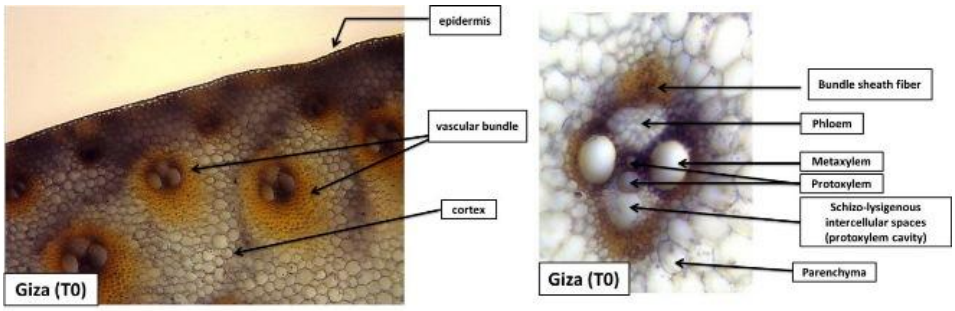


Fig3: Cross section of stem (control) in the Giza varieties by (LM) where is: T0: control.

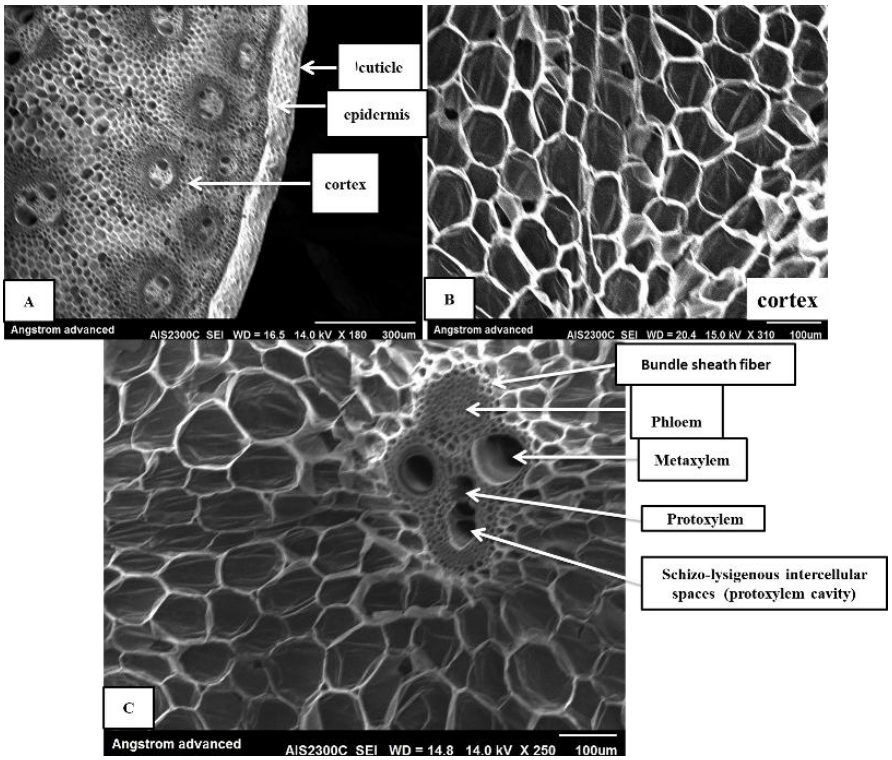


Fig4: Cross section of stem (control) in the Giza varieties by (SEM) where is: A: appear the cuticle and epidermis of stem, B: appear the cortex of stem and C: appear the vascular bundle.

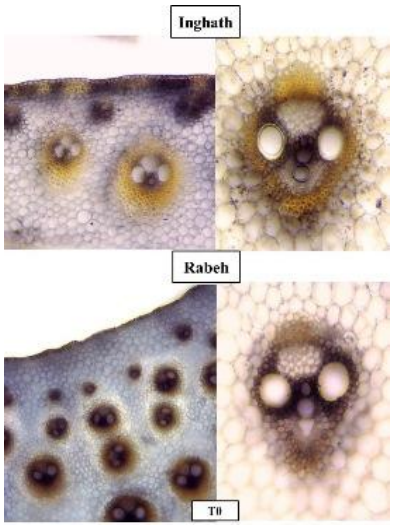


Fig5: Cross section of stem (control) in the Inghath and Rabeh varieties by (LM) where is: T0: control.

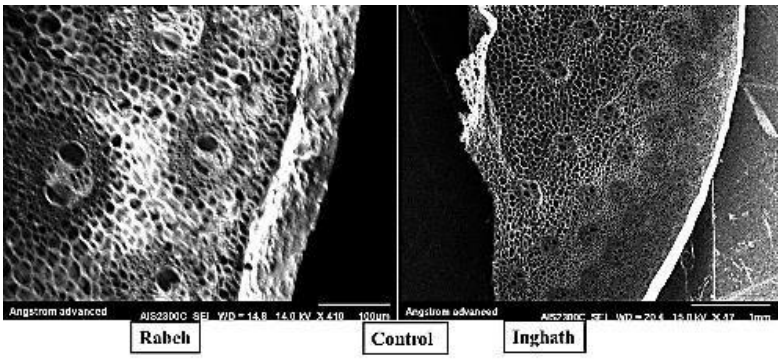
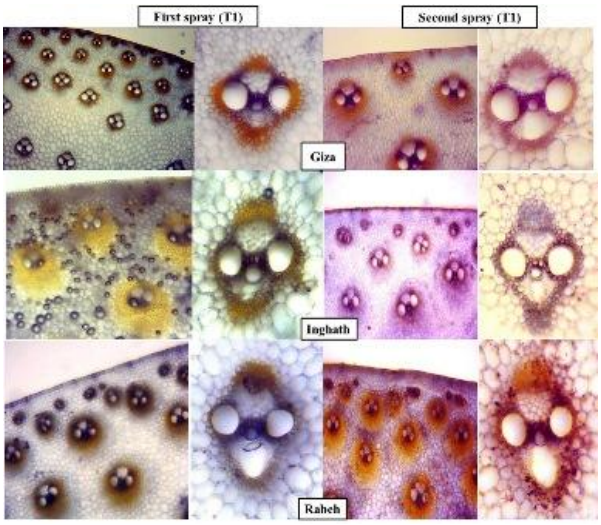


Fig6: Cross section of stem (control) in the Inghath and Rabeh varieties by (SEM)



Figu7: Cross section of stem in the (Giza, Ingath and Rabeh)varieties at first and second spray where is: T1: 3 L h^{-1}concentration.

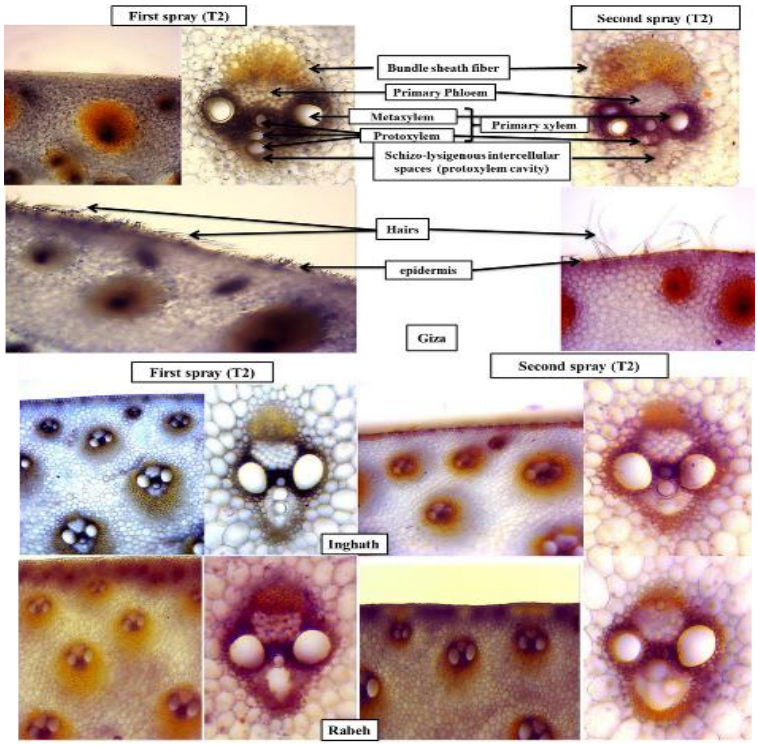


Fig8: Cross section of stem in the (Giza, Ingath and Rabeh)varieties by (LM)at first and second spray where is: T2: 5 L h^{-1}concentration.

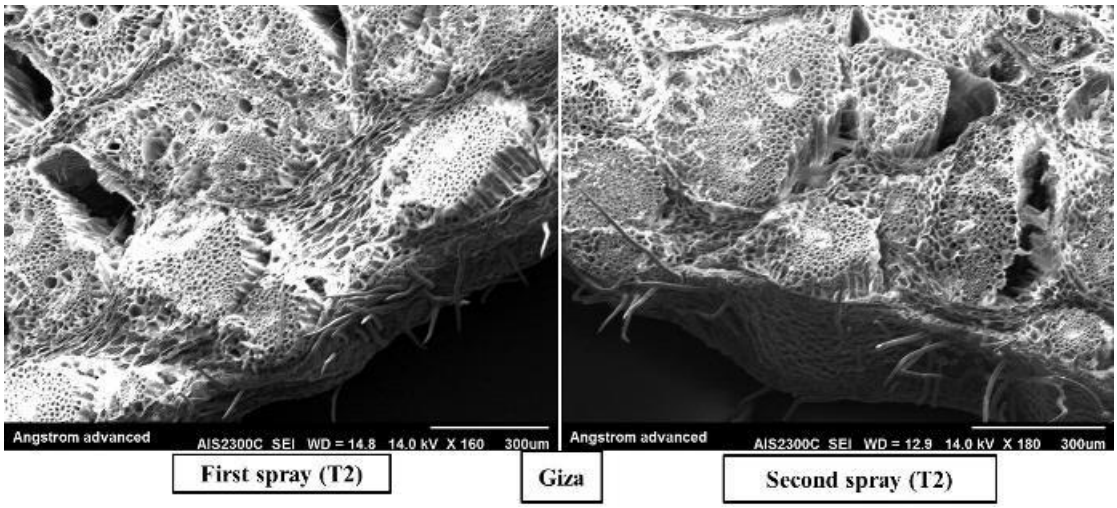


Fig9: Cross section of stem in the Gizavarieties by (SEM) at first and second spray where is: T2: 5 L h^{-1}concentration.

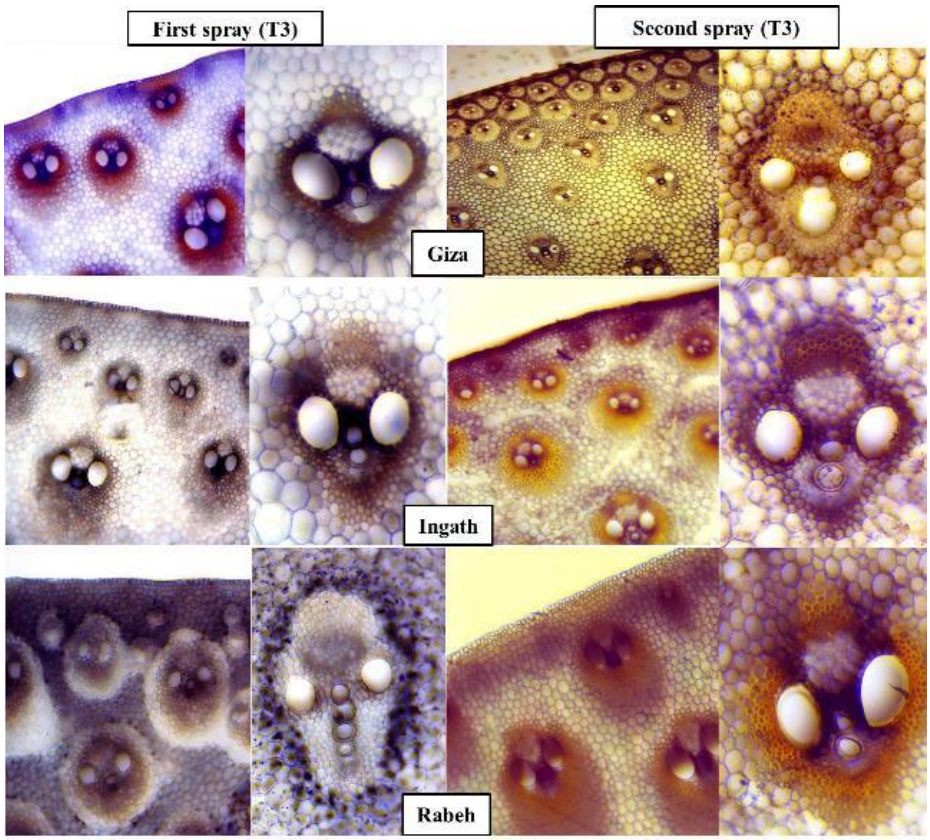


Fig10: Cross section of stem in the (Giza, Ingath and Rabeh)varieties by (LM) at first and second spray where is: T3: 7 L h^{-1}concentration.

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