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RESEARCH ARTICLE

INFLUENCE OF FOLIAR SPRAY BY ALGAE EXTRACT AND AMINO ACID ON BOTANICAL CHARACTERS AND SEED CHEMICAL COMPOSITION OF COMMON BEAN PLANT (*PHASEOLUS VULGARIS* L.)

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Abstract

This study was carried out during two consecutive seasons 2017 and 2018, on common bean plants (*Phaseolus vulgaris* L. cv. "Giza 6") at Zawiat Rizyyn village, El- Menoufiya Governorate, Egypt. In order to investigate the effect of algae extract (1ml/l), amino acid at the concentrations of (4, 6 and 8 ml/l) and the mixture of 1ml/l algae extract with 6 ml/l amino acid as a foliar application on growth, yield, anatomical structure and seeds chemical composition on the common bean plant. Results indicated that spraying the plants with the mixture significantly increased plant height, number of branches/plant, number of compound leaves/plant and plant productivity compared to the untreated plants. Also, this treatment showed promoted remarkable effect on the anatomical structure of the main stem and blade compared to the untreated plants. Generally, the maximum significant increase in any of the studied characters was detected when spraying the plants with the mixture between 1ml/l algae extract with 6 ml/l amino acid. Spraying plants with this mixture also increased the percentages of; nitrogen, phosphor, potassium, calcium and magnesium in seeds, in addition to total crude protein and total carbohydrates percentage. Results of analyzing the amino acids in treated seeds showed high concentration of Glutamic acid; moderate of Leucine, Lysine, Phenylalanine, Tyrosine, Arginine, Alanine and Valine and low of Tryptophan.

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Introduction:-

Common bean (*Phaseolus vulgaris* L.) is one of the most important seed food legumes in the world (Zhang *et al.*, 2008). It contains a source of dietary, fibers calories, proteins, minerals and vitamins for millions of people in countries worldwide (Shehata *et al.*, 2011). Egypt ranked the 6th in world production with an average production of about 270.8 thousand tons and is the 10th largest exporter of beans worldwide, with an average of exports about 11,000 tons, equivalent to 4% of total global crop exports (FAO, 2017).

Seaweed are known to cause many beneficial effects on plants as they contain growth promoting hormones; *i.e.* IAA, IBA and Cytokinins, trace elements of Fe, Cu, Zn, Co, Mo, Mn, and Ni, vitamins and amino acids (Khan *et*

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al., 2009). Seaweed extracts are biodegradable, non-toxic, non-polluting extract and nonhazardous to humans, animals and birds (Dhargalkar and Pereira, 2005). More than 15 million tons of seaweed products are used annually as nutrient supplements and biostimulants in agriculture and horticulture crop production; application of seaweed extracts enhances seed germination and seedling vigour (Economou *et al.*, 2007). Seaweed extract could serve as an alternative biofertilizer as it is eco-friendly, cheapest, deliver substantial economic and environmental benefits to farmers (Kumar and Sahoo 2011). Exogenous application of seaweed extract has already been shown to enhance plant growth, yield and its quality (Abou El-Yazied *et al.*, 2012).

Amino acids as organic nitrogenous compounds are well known to stimulate cell growth and acting as buffers maintaining favorable pH value within the plant cell as well as synthesizing other organic compounds (Opik and Rolfe 2005). Amino acids are fundamental ingredients in the process of protein synthesis, formation of plant tissue and chlorophyll synthesis. Similar effect and findings about amino acids were indicated by Abo Sedera *et al.*, (2010) and El-Desouky *et al.*, (2011).

Thereby, due to the high economic value of common bean plants, (*Phaseolus vulgaris* L.) the present study aimed to evaluate the promoter effects of alga extract and amino acids to increase growth and seed yield parameters as well as chemical constituents of common bean cv. "Giza 6".

Materials and Methods:-

Field experiment was conducted at Zawiat Riziyn village (Private Farm), Menouf, El- Menoufiya Governorate, through the two successive seasons of 2017/2018 to study the impact of foliar application with algae extract, amino acid and the mixture between them on growth and seed yield parameters, anatomical structure, as well as chemical constituents of common bean cv. "Giza 6".

Seeds of common bean were secured from Sars El-Layan Research Station, El-Menoufiya Governorate, Egypt. The algae species *Spirulina platensis* was used to produce extract at the Algae Production Station of the National Research Centre (NRC, Dokki, Cairo). Macro nutrients of the used alga extract are N (10%), P (18%), K (10%), Ca (0.4%), S (2.1%), organic matter (16.7%) in addition to micro nutrients; Mg (0.1%), B (0.12%), Zn (1.7%), Fe (1.32%), Mn (1.1%) and Cu (0.22%). A commercial amino acid was used, contains N (3%), organic matter (20%), free amino acid (10%), Mg (1.0%), Fe (1.5%), Zn (1.0 %) and Boron (3.87 ppm) with pH 6-7 and specific gravity 1.25 g/ml.

Common bean seeds were sown on 2nd September in both seasons. After 25 days from sowing, the plants were thinned to one plant/hill. The experiment layout was randomized complete block design of three replicates. Each replicate was comprised of six plots, each plot representing one treatment. The plot including 6 rows of 2m in length and 70cm in between, with 15cm hill spacing. Three seeds grown per hill. The treatments were; tap water "control"; alga extract at 1.0 ml/l; amino acids at 4.0 ml/l, 6.0 ml/l and 8.0 ml/l; mixture of alga extract at 1ml/l with amino acid at 6.0 ml/l. The treatments were applied twice; at 30 and 45 days after sowing. Random samples were taken from each plot at 60 days after sowing to record the morphological characters. At harvest (90 days), samples were randomly taken from each pot to determine the yield characters. All plants received recommended dose of NPK fertilizers

Recording of data

1-Morphological characters

- a- Plant height (cm)
- b- Number of branches/plant
- c- Number of leaves/plant

2- Yield characters

- d- Number of pods/plant
- e- Number of seeds/pod
- f- Weight of 100 seed (g).
- g- Seed yield/plant (g).

3- Anatomical studies

A microscopical study was carried out to investigate the anatomical structure of the stem, represented by the 4th internodes counted from the plant tip and the lamina of the terminal leaflet of the corresponding compound leaf at the age of 60 days, according to Nassar and El-Sahhar (1998).

4- Chemical analysis of seeds

a. Mineral elements content

At the harvest time in both seasons, samples from the mature dried seeds were subjected to determine; total nitrogen (N) and multiplied it with 6.25 to get the crude protein in seeds. Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) were determined and calculated as percentage of dry weight according to (A.O.A.C., 1999), at Faculty of Agriculture, Cairo University Research Park (CURP).

b. Determination of amino acids

Amino acids were extracted according to the methods described by Csomos and Simon-Sarkadi (2002) and Shalabia (2011) and measured using Amino Acid Analyzer (AAA 400 INGOS Ltd) at Faculty of Agriculture, Cairo University Research Park (CURP).

5- Statistical analysis

All collected data were subjected to statistical analysis as proposed by Gomez and Gomez (1984) and means were compared by LSD at 5% level of probability.

Results And Discussion:-

1- Morphological and yield characters:

Foliar spray with all tested concentrations of amino acids and algae extract showed obvious effect on all vegetative growth and seed yield characters of common bean plants. The only exception was the number of seeds/pod which did not affected by any of the three tested concentrations of amino acids or algae extract in the two growing seasons. It is clear from Table (1) that the highest values of plant height, number of branches/plant and number of compound leaves/plant were recorded when plants sprayed with the mixture of alga extract at 1.0 ml/l, with amino acid at 6.0 ml/l. The maximum increases recorded were; 40.6, 54.3; 82.0, 49.2; and 36.4, 36.7% for plant height, number of branches and number of leaves more than the control plants, in 1st and 2nd seasons, respectively.

Data presented in Table (2) showed that the maximum significant increase in number of pods/plant, specific weight of 100 seeds/plant and seeds yield/ plant were achieved at 1ml/l alga extract mixed with 6 ml/l amino acid, being 66.7, 24.1 and 73.4 % more than control, respectively in the 1st season. The same trend was observed in the 2nd season, whereas the percentages of increases were 58.8, 16.0 and 77.5 % for the same characters number. Latique *et al.*, (2013) reported that seaweed liquid fertilizer effect the physiological and biochemical parameters of *Phaseolus vulgaris* L. var *Paulista* under hydroponic system. Also, Raverkar *et al.*, (2016) mentioned that foliar application of seaweed stimulate yield, nodulation and nutritional quality in *Vigna radiata* L. in legumes. Kocira *et al.*, (2013) found that *Ecklonia maxima* extract have a positive effect on yield characters of white bean (*Phaseolus vulgaris* L.). El Kaoua *et al.*, (2013) found that the seaweed extract application at lower concentration enhanced the vegetative growth on *Salvia officinalis*. Salama and Yousef 2015 found that foliar application of amino acids or seaweed

Table 1: Effect of algae extract, amino acid and their combination on the morphological characters of common bean plants during the two seasons; 2017 and 2018

Treatments	Morphological characters					
	Plant height (cm).		No. of branches /plant		No. compound leaves	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Control	44.10	46.00	5.00	6.70	11.00	11.70
1 ml/l. algae extract	48.00	51.00	7.00	8.50	14.00	15.50
4 ml/ l. amino acid	49.04	50.00	6.03	7.20	12.00	13.60
6 ml/ l. amino acid	57.00	62.00	8.00	9.20	14.00	15.10
8 ml/ l. amino acid	51.00	55.00	6.00	7.10	13.00	14.50
6 ml/ l. amino acid +1 ml/ l algae extract	62.00	71.00	9.10	10.00	15.00	16.00
LSD at 0.05	1.16	1.21	0.19	0.21	0.39	0.42

Table 2: Effect of algae extract, amino acid and their combination on yield characters of common bean plants during the two seasons; 2017 and 2018

Treatments	No. of pods/plant		No. of seeds/pod		weight of 100 seeds(g)		Seed yield (g)/plant	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd Season
Control	9.00	10.70	2.33	2.40	42.17	42.50	12.99	13.91
1 ml/l. algae extract	13.00	14.00	2.33	2.50	44.06	45.70	13.36	15.99
4 ml/ l. amino acid	11.00	12.10	3.00	3.00	43.97	44.00	14.51	16.10
6 ml/ l. amino acid	14.00	14.70	3.00	3.00	50.10	51.00	21.04	22.49
8 ml/ l. amino acid	13.00	14.10	3.00	3.00	46.04	46.10	17.95	19.50
6 ml/ l. amino acid +1 ml/ l algae extract	15.00	17.00	3.00	3.00	52.33	49.30	2.53	24.69
LSD at 0.05	1.79	1.60	Ns	ns	0.93	0.86	1.36	1.42

extract enhanced the vegetative characters and yield components on *Ocimum sanctum* L. **Salama et al., (2016)** mentioned that the seaweed extract at the concentration of 1 ml/l exhibited significant promotive effect on all investigated morphological and yield characters of *Stevia rebaudiana* Bertoni. **Boghdady et al., (2016)** found that the seaweed extract at 1 ml/l induced significant promoting effect on vegetative growth and yield characters of *Cicer arietinum* L.

2- Chemical analysis of seeds:

a- Mineral elements content

Data presented in Table (3) influence the effect of different concentrations of amino acid, algae extract and the mixture between them as foliar application on mineral component (Nitrogen, Phosphor, Potassium, Calcium and Magnesium) as well crude protein and total carbohydrate in seeds of common bean plants. Spraying plants with mixture of 1ml/l algae extract and 6ml/l amino acid increased percentage of nitrogen, phosphor, potassium, calcium and magnesium in seeds by 85.2, 44.2, 17.4, 7.0 and 33.9%, respectively as compared with control. As well, using amino acid at 6 ml/l mixed with 1 ml/l algae extract resulting in increased total crude protein and total carbohydrates percentage in seeds by 82.3 and 14.3%, respectively compared with control.

These results are in harmony with those obtained by **Pise and Sabale (2010)**. They observed that total nitrogen in fenugreek increased at the low to high concentrations of seaweeds. Also **Nour et al., (2010)** mentioned that foliar spray with seaweed extracts on tomato, significantly increased N, P, K and protein percentages. **Rathore et al., (2009)** on soybean mentioned that foliar applications of different concentrations of seaweed extract improved nutrient uptake (N, P, K and S%). **Zodape et al., (2008)** found that okra plants sprayed with liquid seaweed fertilizers showed a significant increase of carbohydrate and protein contents. Also, significant increases in mineral content of chickpea seeds were found by using 1 ml/l seaweed extract (**Boghdady et al., 2016**).

b- Amino acids Content

Analysis of amino acids in common bean seeds detected nine types. These types were divided into essential amino acids *i.e.*, Tryptophan, Leucine, Lysine, Phenylalanine and Valine, and non essential amino acids; *i.e.*, Tyrosine, Arginine, Alanine, Glutamic acid (Table 4). The common bean seeds contain high concentration of Glutamic acid; moderate concentration of Leucine, Lysine, Phenylalanine, Tyrosine, Arginine, Alanine and Valine, and low concentration of Tryptophan.

Regarding to spraying with various concentrations of amino acids or algae extract on the amino acids content of common bean seeds, the obtained results showed that foliar spraying with mixture of 1 ml/l algae extract with 6 ml/l amino acids caused a marked increase in the concentration of Tryptophan, Phenylalanine, Tyrosine, Alanine and Valine comparing with the control plants. The increases percentage in the amino acids content reached about 154.5% in Tryptophan, 17.6% in Phenylalanine, 46.4% in Tyrosine, 13.1% in Alanine and 20.0% in Valine compared with untreated plants. Leucine acid recorded the highest value in the control plants. As for Lysine, the highest increase was observed in plants treated with 4 ml/l amino acid. It can be observed that the highest increase in Arginine and Glutamic acid were found in plants sprayed with algae extract and amino acid at 8 ml/l, respectively.

Table 3: The percentages of some mineral elements, crude protein and total carbohydrates in seeds of common bean plant cv. Giza 6 as affected by different concentrations of amino acid and algae extract and their combination in the 2nd growing season

Treatments	N%	P%	K%	Ca%	Mg%	Crude protein%	Total carbohydrates%
Control	0.027	0.113	0.46	0.85	0.062	0.17	4.2
1 ml/l. algae extract	0.047	0.144	0.54	0.86	0.081	0.29	4.5
4 ml/ l. amino acid	0.043	0.135	0.52	0.88	0.066	0.27	4.5
6 ml/ l. amino acid	0.045	0.147	0.49	0.86	0.074	0.28	4.4
8 ml/ l. amino acid	0.047	0.145	0.53	0.87	0.075	0.29	4.6
6 ml/ l. amino acid +1 ml/ l algae extract	0.050	0.163	0.54	0.91	0.082	0.31	4.8

Table 4: The concentrations of amino acid in common bean seed cv. Giza 6 as affected by different concentrations of amino acid and algae extract and their combination in the 2nd growing season

Treatments	Essential amino acid					Non essential amino acid			
	Tryptophan	Leucine	Lysine	Phenylalanine	Valine	Arginine	Alanine	Glutamic	Tyrosine
Control	0.0011	0.0078	0.0057	0.0051	0.0050	0.0060	0.0038	0.0151	0.0028
1 ml/l. algae extract	0.0020	0.0049	0.0044	0.0058	0.0057	0.0068	0.0040	0.0180	0.0039
4 ml/ l. amino acid	0.0014	0.0067	0.0061	0.0055	0.0047	0.0064	0.0036	0.0178	0.0033
6 ml/ l. amino acid	0.0013	0.0064	0.0060	0.0054	0.0042	0.0062	0.0034	0.0175	0.0035
8 ml/ l. amino acid	0.0018	0.0053	0.0050	0.0058	0.0055	0.0065	0.0039	0.0181	0.0037
6 ml/ l. amino acid+1 ml/ l algae extract	0.0028	0.0050	0.0048	0.0060	0.0060	0.0066	0.0043	0.0177	0.0041

In this respect, **Kalidass et al., (2010)** indicated that seaweed liquid extract has positive effect on the content of free amino acids in *Brassica nigra*. **Pise and Sable, 2010** found that the seaweed liquid extract has promoting effect on the content of free amino acids in *Trigonella foenumgraecum*. **Lingakumar et al., (2006)** reported that the liquid algae extract when added to the soil bed promoted amino acid content of *Phaseolus mungo*, *Zea mays* and *Cyamopsis tetragonoloba*. Also, **Lingakumar et al., (2002)** on *Zea mays* and *Phaseolus mungo*, observed that application of seaweed extract showed positive response on the total amino acid content. Foliar spraying with 1 ml/l seaweed extract caused a marked increase in the concentration of all essential and non essential amino acids in chickpea seeds comparing with the untreated control plants (**Boghdady et al., 2016**).

3- Anatomical studies

a- Stem anatomy

It is obvious from Table (5) and Figure (1) that foliar application with 1ml/l algae extract mixed with 6 ml/l amino acid increased the main stem diameter of common bean plants by 12.33% more than control. Worthy to mention that, increasing stem diameter could be attributed to the prominent increases in most of the included tissues; cortex thickness by 11.48%, phloem by 34.6% and xylem tissues by 11.66% more than control. Also, vessel diameter was increased over the control by 12.50% due to foliar spraying with 1ml/l algae extract mixed with 6 ml/l amino acid. Nevertheless, pith diameter in treated stems was decreased by 8.16% less than that of the control.

In this respect, **Salama and Yousef (2015)** recorded that foliar applications with seaweed extract at concentration of 1.5 ml/l, increased the main stem diameter, cortex, phloem and xylem tissues more than those of the control, and a decrement in pith thickness was observed less than control. As well as **Intedhar and Majeed (2015)** on two cultivars of pepper indicated that all seaweed extract concentrations used increased significantly the cortex thickness, the concentration of 6 ml/l gave the higher vascular cylinder thickness. In this concern, **Sabh and Shallah (2008)** studied stem cross sections of bean plants treated with seaweed extract and found that an increase in the thickness of; epidermis, cortex, paranchyma cells and pith size with increasing concentrations of extract used in

comparison to non-treated plants. **El-Desouky *et al.*, (2011)** and **Akladious and Abbas (2013)** mentioned that the use of a mixture of amino acids at different concentrations led to an increase in stem diameter of tomato as a result of increase in the thickness of epidermis and cortex as well as thickness of xylem, especially the number of vessels, compared to non-treated plants.

Table 5: Anatomical measurements (μ) of different tissues of 4th terminal leaflet lamina of common bean plant cv. Giza 6 aged 60 days as affected by mixture of 1 ml /l algae extract and 6 ml/l amino acid.

Measurements	Control	1 ml/l algae extract with 6 ml/l amino acid.	\pm Control
Stem diameter	2715	3050	+ 12.33
Cortex thickness	148	165	+11.48
Phloem tissue thickness	78	105	+34.61
Xylem tissue thickness	240	268	+11.66
Vessel diameter	80	90	+12.50
Pith diameter	1470	1350	-8.16

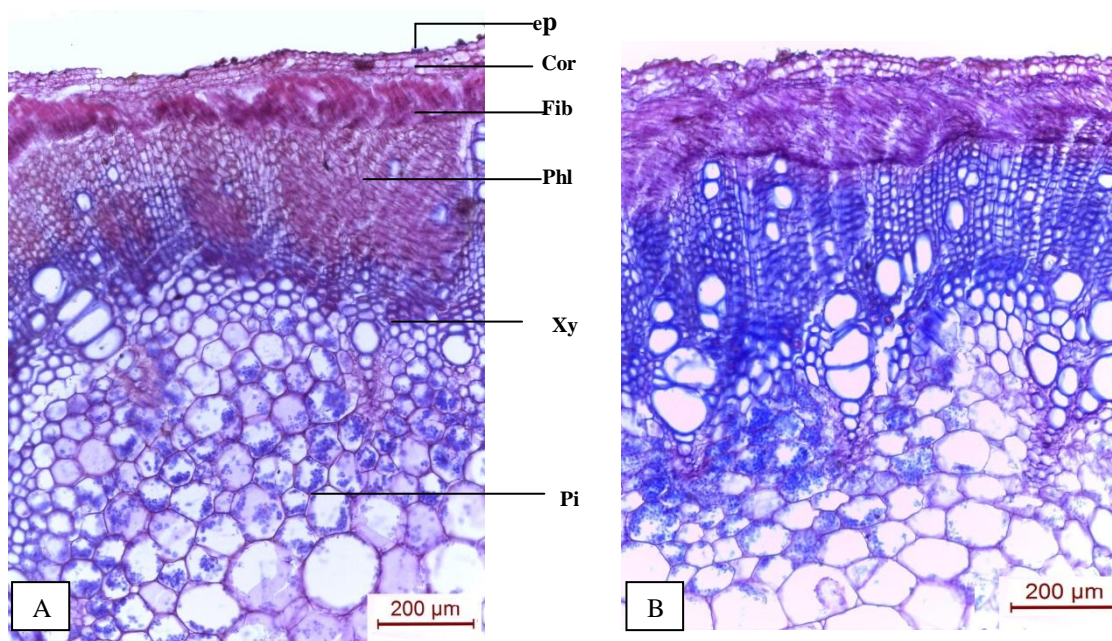


Fig. 1. Transverse sections through the 4th internode of common bean plants at age of 60 days.

A. control plants

B. plants as affected by foliar spray with 6 ml/l amino acid mixed with 1ml/l algae extract

Details: ep, epidermis; cor, cortex; fib, fibers; ph, phloem; xy, xylem and pi, pith. (X100)

b- Leaf anatomy

Data presented in Table (6) and Figure (2) indicated that spraying 1ml/l algae extract mixed with 6 ml/l amino acid on common bean cv. Giza 6 gave the highest increased in thickness of both midvein and lamina by 8.64 and 21.38% more control, respectively. The promotive effect of spraying 1ml/l algae extract mixed with 6 ml/l amino acid on leaf thickness due to an increase in palisade and spongy tissues by 8.91 and 70% compared to the control; respectively. Likewise, the vascular bundle of the midvein was increased in size as a result of spraying 1ml/l algae extract mixed with 6 ml/l amino acid. The increment was 38.75% in length and it was 4.22% in width more than the control. In this respect, **Salama and Yousef (2015)** stated that spraying basil plant with seaweed extract at 1.5 ml/l increased thickness of both midvein and lamina of leaf and the increase in lamina thickness was accompanied with increments in thickness of palisade and spongy tissues compared with the control. Likewise, the main vascular bundle of the midvein was increased in length and width, moreover, xylem vessels increased in diameter more than the control.

Table 6: Anatomical measurements (μ) of different tissues of median portion of 4th internode of common bean plant cv. Giza 6 aged 60 days as affected by mixture of 1 ml/l algae extract and 6 ml/l amino acid.

Measurements	Control	1 ml/l algae extract with 6 ml/l amino acid.	\pm Control
Midvein thickness	1643	1785	+8.64
Lamina thickness	173	210	+21.38
Palisade tissue thickness	101	110	+8.91
Spongy tissue thickness	50	85	+70.00
Midvein bundle dimensions			
Length	400	555	+38.75
Width	355	370	+4.22

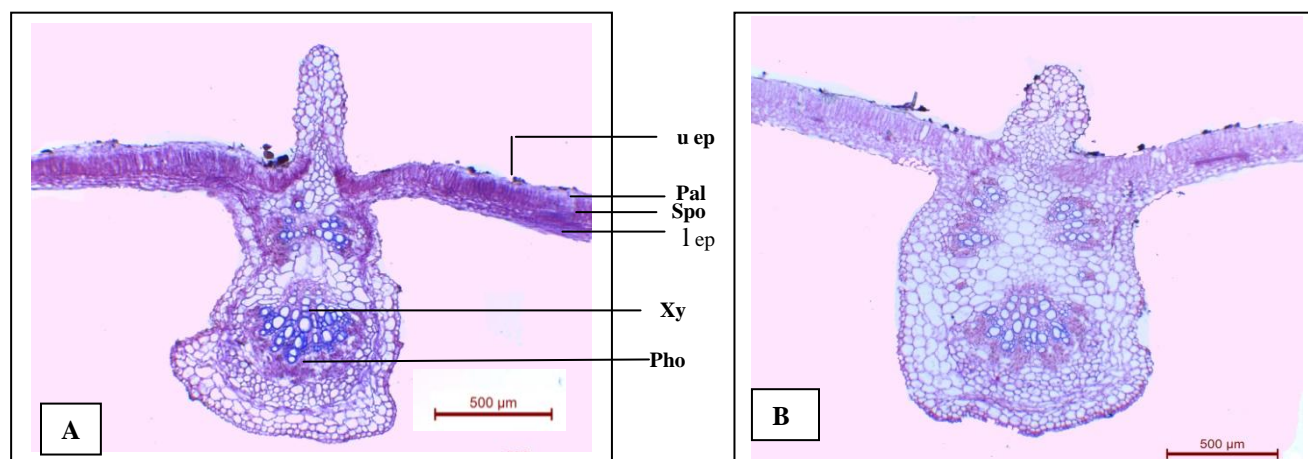


Fig. 2. Transverse sections through the blade of terminal leaflet of the compound leaf of common bean plants at age of 60 days.

A. control plants

B. plants as affected by foliar spray with 6 ml/l amino acid mixed with 1 ml/l algae extract.

Details: u ep, upper epidermis; pal, palisade tissue; spo, spongy tissue; l ep, lower epidermis; xy, xylem and Ph, phloem. (X 40)

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