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RESEARCH PAPER

Effect of two organic fertilizers on some anatomical features of *Pinus pinea L.* seedlings grown in field and lathe house

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A B S T R A C T:

Pinus pinea L. cultivated in open field and lath house treated with 0, 12g. plant^{-1} local compost and 9 g.plant^{-1} Bamboo biochar was studied by using Factorial Complete Randomized Design and carried out during 4th October-2020 to 5th May-2021. Cultivation of the seedlings in open field without fertilizers gave best values of epidermis, mesophyll and vascular bundle thickness (11.333, 39.000 and 55.000um respectively), the highest value of hypoderm thickness (14.000um) was recorded from combination of open field condition and Bamboo biochare fertilizer. While, the best stomata density was with application of Bamboo biochar under lath house condition (113.667.mm-2). Epidermis thickness (23.667 um) was obtained from the treatment of 12(g. plant^{-1}) local compost and lath house condition. However, the treatment of 12g. Plant^{-1} local compost gave the best vascular bundle thickness (176.667um) and the beast result of cortex thickness (91.000 um) was in the treatment of open field combined with 9 g.plant^{-1} Bamboo biochar.

Keywords: *Pinus pinea L.* seedlings; organic fertilizers; cultivation conditions; needle and stem anatomy.
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1.INTRODUCTION :

Pinus pinea L. which is well known as Stone pine is related to (Pinaceae) family, it is one of the most pivotal nut species in the world, its seed gives the high culinary value and nutrition with increasing demand (Loewe *et al*., 2017). It has a valuable environmental and economic value due to its edible seeds in the Mediterranean Basin (Pasalodos et al., 2016). Natural forests defiantly have been preserved of the impact of bio-fertilizers, organic manures and chemical fertilizers are being promoted to achieve supportable productivity from agro-forestry and forests (Lu *et al*., 2014).

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The solid product (Biochar) is derivative from waste biomass pyrolysis such as forestry production agricultural wastes (Lu *et al*., 2014).

It has an important role in soil fertility for this reason it became a promoting practice as way of soil alteration (Tarin *et al.*, 2018). Recently, researchers revealed that biochar in the absence of oxygen environment can the plant nutrients, soil carbon content and enhancing plant growth. Biochar derived from waste of animal and plant (Sun *et al*., 2016).
Shade houses (as Lath houses) afford many agricultural advantages such as protecting plant from high temperature, high light and decreasing moisture stress (Hartman *et al.*, 2002). A

time-honored cultural practice is utilizing shade house for decreasing nursery heats and sun light, in nursery; shade is mainly use (Dumroese *et al*., 2005).

The thin-walled epithelial cell and resin ducts Pinus can functionally separate from all other coniferous (Patel, 1971). Needles of Pine are hemispherical or triangular borne in fascicles. The single -needle are responding abnormal development, injury, unusual neutrinos they are circular in cross section. Needle pine in shade compare with sun grown needles hold a higher chlorophyll content and thinner with low stomata density (Pallardy, 2008).

Luomala *et al*. (2005) studied nutrient concentrations, anatomy and stomatal density of (Pinus sylvestris L.) Scots pine at high temperature in covered-top chamber the needles were minor density of stomata, in abaxial side had thinner mesophyll of needle, with thinner vascular cylinder. According to Äinen *et al.* (2007) Co_2 concentration and high temperature have impact on anatomy of 20-year Scot pine wood. High Co_2 reducing number of resin canal per mm², while high temperature reduces rays, number and tracheid per mm² of cross-section area. High temperature was impacted on xylem anatomy closed the primary wood more than elevated Co_2. The mesophyll area was stable. Scot pine needles lower stomata density did not reduce water uses. Nonetheless reducing water by high temperature. High nutrients in soils of Scots pine needle gave an important result of anatomical features, needles that grown on the top of hill declines with the age which leads to shortage of nutrient and water in its soil, otherwise needles of Scot pine that grown in slant hill have the highest proportion of xylem, mesophyll, and sclerenchyma in cross-section in the soil with high nutrients (Lukjanova and Mandre, 2008). De Muñiz et al. (2014) studied the effect of Charcoal that prepared at carbonization temperatures of 500, 600 and 700 °C to evaluate the potential use of *Pinus taeda* and *Pinus elliottii* needles for energy. Evaluation of histological sections of the needles identify the species; endoderm cells are a single homogeneous form, stomata present on every surface along with a beak-like feature, the hypodermis is two layers of cells on average and two fibro-vascular bundles. Estimating changes of anatomy in both shade and sun needles of Norway spruce trees 20-year-old that exposed to drought stress artifactually (Gebauer *et al*., 2015). The Position of canopy vital for structure of needles. Compare to shade needles, sun needles had higher effective value in case of anatomical characters such as (tracheid lumen area, number of tracheids in hydraulic conductivity, and needle area cross sections), trachieds of sun needles 1.7 times more than shade needles, its dimeter larger when it exposed to control stress because of larger and numerous tracheids, lumen area in sun needles per needles cross sections was larger than shade needles. Dörken and Lepetit (2018) revealed several morphological, physiological, and anatomical differences of shade and sun needles of *Abies alba.* Larger sun leaves with a palisade parenchyma a hypodermis with more stomata. However, leaves in shade show the dimorphism leaf. Compare to shade leaves, sun leaves are longer and thicker with stomata density nearly 1/4 higher compared to shade leaves. Galdina and Khazova (2019) studied anatomical traits of needles and identify anatomical variability of Scots pine planted in the zone of deciduous forests, southern forest-steppe and dry steppe. The study has showed that the assimilative and conductive tissues was thinner in dry steppe than conductive tissue in zone of deciduous forests, diameter of vascular bundles, thickness of folded mesophyll, and regularly reduced in dry steppe and had the higher value. The changes depending on the growing environment conditions (Galdina and Khazova, 2019).

The purpose of this study is to evaluate the effects of two organic fertilizers (local compost and Bamboo Biochar) needle and stem anatomical features of (*Pinus pinea L.*) seedling.

2-Materials and methods

2-1-Description of the experiments

The study was carried out during September 15th, 2020 to June 15th, 2021 in the Directorate of Forest and Pasture–Ministry of Agriculture and water resources–Kurdistan Region –Iraq. Two organic fertilizers (local compost and bamboo biochar) were used to study their effects on some anatomical features of needles and stems of *Pinus pinea* L. seedlings grown in two conditions; open field and lath house (50% shade). Seedlings of 20 months old were grown in black plastic bags (10*30cm) filed with silt clay soil (each bag 6 kg ± 0.2). Some physical and chemical properties of the experiments soil are shown in table (1). The metrological data during study period were recorded in table (2).

The experiment was designed according to Factorial Complete Randomized Design. For anatomical study local compost was used at the rates of 0 and 12 g.plant^{-1}soil (recommended by research center/ ministry of Agriculture and water resources/Kurdistan region-Iraq), and bamboo biochar was used at the rates of 0 and 9 g. plant^{-1} soil (as recommended by the producer company), in open field and lath house conditions. The two fertilizers were added to the soil at two times 15 /10 /2020 and 15 /3 /2021).

Table (1): Some physical and chemical properties of the soil of both experiments. *

pH	EC dS m^{-1}	O. M. gm kg^{-1}	PSD%			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	SO_4^{2-}	N %	P	Fe	Zn	Cu	B	Mn
			Clay	Silt	Sand	mmol$_c$ L^{-1}								mg kg^{-1}					
7.9	1.60	9.20	44.30	49.30	6.40	6.28	3.80	4.08	0.14	1.28	4.23	8.10	0.20	7.80	8.40	3.20	1.10	4.85	2.30
			Silt Clay soil																

PSD (Particle-Size Distribution), EC (Electrical Conductivity), O. M. (Organic Matter).
***Central research in Khabat- Erbil**

Table (2): The metrological data during study period. *

Years	Month	Average temperature °C		Average air humidity %
		maximum	minimum	
2020	October	36.19	9.42	20.72
	November	29.87	3.98	60.14
	December	21.69	-3.25	65.90
2021	January	20.18	-4.42	57.07
	February	23.93	-3.15	60.33
	march	28.43	-0.32	52.70
	April	37.50	4.85	29.65
	may	42.33	14.47	18.50
	June	45.48	16.18	13.86

*** Agricultural Research Center, Ministry of Agriculture and water resources - Kurdistan Region, Erbil, Iraq**

2-2- Description of the two studied fertilizers

The local compost was prepared by Agricultural Research Center, Ministry of Agriculture and water resources (Kurdistan region, Erbil, Iraq) that contain 15% poultry wastes and 85% Wheat straw), 1.52% total nitrogen, 0.42% P_2O_5 and 0.55% K_2O with 8.3 pH and 2.03 ds m^{-1} electric conductivity as analysis in the agricultural research center, ministry of agriculture and water resources Kurdistan region, Erbil, Iraq.

Bamboo biochar is a bio-organic fertilizer that contain; organic matter ≥ 45%, beneficial soil organisms' ≥20 million.g^{-1}, amino acids ≥ 5%, humic acid ≥ 10% total nitrogen + P_2O_5 + K_2O ≥ 5% (Seek Global Leader of Carbon fertilizers)

2-3- Anatomical measurements

Freshly collected material was cutie in two small pieces and then fixed in FAA (100 ml FAA contain 90 ml 70% ethanol + 5 ml acetic acid + 5ml formaldehyde solution 37%). The second step was the classical paraffin technique; pieces of samples after FAA treatment have been dehydrated using a series concentration of alcohol after that the samples were cleared by xylene for 3-4 hrs. Later, they were unfiltered in a mixture of xylene and paraffin for 30 min., and transferred into pure paraffin wax for overnight at 60°C. Paraffin blocks were made and sections were prepared with the thickness of 8 µm using the rotary microtome then stained by safranin and fast green or light green, the samples were viewed under a light microscope with camera attached, using Image Analysis Software (Najmaddin and Mahmood, 2016 and Dörken *et al*., 2017).

For stomata density measurement; the needles were bleached with a 4 % sodium hypochlorite solution which macerates the mesophyll, and vascular and epidermal tissue were brushed off. The remaining cuticle was then stained with saffranin and mounted in glycerin jelly on a microscopic slide. Stomatal density was measured as the number of stomata per square millimetre of leaf area (Kouwenberg *et al.,* 2004).

2-4 Statistical analysis

The experiment was conducted using analysis of variance (ANOVA). The means compared by Duncan's Multiple Range test and the treatments were considered significantly different at the 5% significance level (Al-Rawi and Khalaf-Allah,1980). The statistical analysis of the data was performed using SPSS V17.0 software.

3- Results

3-1 Needle anatomy

Results of cultivation condition (open field and lath house), organic fertilizers (local compost and bamboo biochar) and their interaction effects on epidermis, hypoderm, mesophyll and vascular bundle thickness in addition to stomata density of *Pinus pinea L*. needles were display in tables (3, 4 and 5 respectively). The obtained data (table 3) shows that the cultivation conditions had significant effect only on vascular bundle thickness and stomata density, when the open field gave the more effective result of vascular bundle thickness (45.000um), and the more stomata density (84.333.mm^{-2}) was recorded under 50% shade (lath house).

However, table (4) shows significant effects of studied organic fertilizers on mesophyll thickness and stomata density, the most significant value of the mesophyll (32.333.mm^{-2}) was obtained in the control without significant differences with compost treatment. While, it was observed highest significant increase in stomata density with Bamboo biochar application (94.333um) and local compost treatment gave the lowest value (49.500 um) compared to the treatment.

While, table (5) clarify that cultivation of the seedlings in open field without fertilizers gave best significant results of epidermis, mesophyll and vascular bundle thickness (11.333, 39.000 and 55.000um respectively), the highest value of hypoderm thickness (14.000um) was recorded from combination of open field condition and Bamboo biochare fertilizer, and the best stomata density was with application of Bamboo biochar under lath house condition (113.667.mm^{-2}).

Cross section of *Pinus pinea L.* needles clarify the studied anatomical characteristics of two treatments (figure, 1).

Table (3): Effect of cultivation condition on anatomical features of *Pinus pinea L*. needles. *

Cultivation conditions	Epidermis	Hypodermis	Mesophyll	Vascular bundle	Stomata density **(.mm-^{2})**
	Thickness **(um)**				
Open filed	8.444^{a}	12.222^{a}	30.333^{a}	45.000^{a}	60.778^{b}
Lath house	7.444^{a}	12.222^{a}	18.667^{a}	31.111^{b}	84.333^{a}

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

Table (4): Effect of local compost and Bamboo biochar on anatomical features of *Pinus pinea L.* needles. *

Organic fertilizer	Epidermis	Hypodermis	Mesophyll	Vascular bundle	Stomata density **(.mm-^{2})**
	Thickness **(um)**				
0 **(g.** plant^{-1}**)**	9.167^{a}	12.500^{a}	32.333^{a}	44.000^{a}	73.833^{b}
12 **(g.** plant^{1}**)** Loc. compost	6.667^{a}	11.000^{a}	23.167^{ab}	33.500^{a}	49.500^{c}
9 **(g.** plant^{1}**)**	8.000^{a}	13.167^{a}	18.000^{b}	36.667^{a}	94.333^{a}

Bam. biochar					

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

Table (5): interaction effect of cultivation condition and local compost and bamboo biochar on anatomical features of *Pinus pinea L.* needles. *

Cultivation condition	Organic fertilizer	Epidermis	Hypodermis	Mesophyll	Vascular bundle	Stomata density
		Thickness **(um)**				**(.mm-^{2})**
Open field	0 **(g.** plant^{1}**)**	11.333^{a}	13.333^{ab}	39.000^{a}	55.000^{a}	55.667^{c}
	12 **(g.** plant^{-1}**)** Loc. compost	7.000^{ab}	9.333^{b}	32.667^{ab}	33.667^{bc}	51.667^{c}
	9 **(g.** plant^{-1}**)** Bam. biochar	7.000^{ab}	14.000^{a}	19.333^{b}	46.333^{ab}	75.000b^{c}
Lath house	0 **(g.** plant^{1}**)**	7.000^{ab}	11.667^{ab}	25.667^{ab}	33.000^{bc}	92.000^{ab}
	12 **(g.** plant^{-1}**)** Loc. compost	6.333^{b}	12.667^{ab}	13.667^{b}	33.333^{bc}	47.333^{c}
	9 **(g.** plant^{-1}**)** Bam. biochar	9.000^{ab}	12.333^{ab}	16.667^{b}	27.000^{c}	113.667^{a}

*Values within each column followed with the same latter are significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

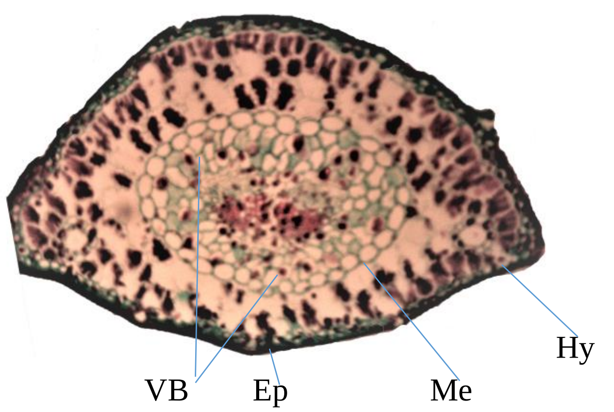

Figure (1): Cross-sectional diagram of a of *Pinus pinea L.* needles illustrating the different characteristics measured of the control treatment (10X):

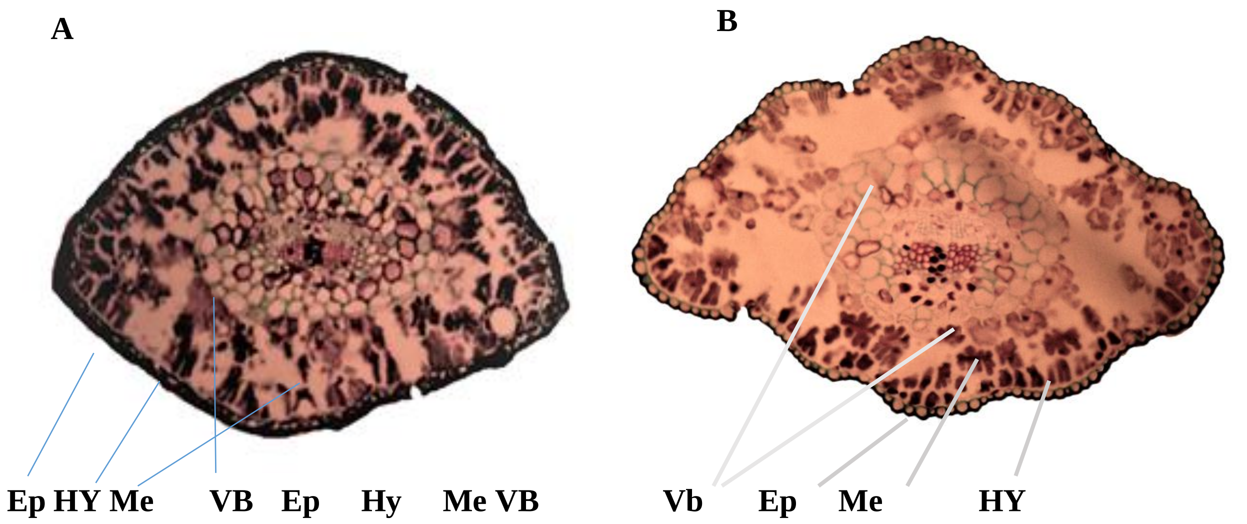

Figure (2): Cross-sectional diagram of a of *Pinus pinea L.* needles illustrating the different characteristics measured of two treatments (10X):

A- Control of open field.

B- Interaction of (9g. plant^{-1}) Bamboo biochar and lath house condition.

(Ep: Epidermis, Hy: Hypodermis, Me: Mesophyll, VB: Vascular Bubdle)

3-2 Stem Anatomy

Epidermis and hypodermis thickness of *Pinus pinea L.* stem, had no significant effects according two cultivation conditions, while the field condition had significant increase on vascular bundle and cortex thickness (155.556 and 87.556um respectively) over lath house condition, table (6)

There was no significant influence of the organic fertilizers on epidermis, hypoderm and vascular bundle thickness are shown in table (7), otherwise in the same table shows positive effect on cortex thickness, the application of (9g. plant^{-1}) Bamboo biochar was recorded highest result (86.833 um).

The interaction effect of cultivation conditions and studied organic fertilizers on studied parameters were shown in table (8). There were no significant differences between the means of hypodermis thickness, but highest significant result of epidermis thickness (23.667 um) was obtained in the treatment of (12g. plant^{-1}) local compost and lath house condition, and the best result of vascular bundle thickness (176.667um) was in the field condition with 12g. plant^{-1}local compost treatment. However, the best effective result of cortex thickness (107.333 um) was in the treatment of open field combined with 9g. plant^{-1}Bamboo biochar.

Cross section of *Pinus pinea L.* stems showing studied anatomical characteristics of two treatments (figure, 2).

Table (6): Effect of cultivation condition on anatomical features of *Pinus pinea L.* stems. *

Cultivation condition	Epidermis	Hypodermis	Vascular bundle	Cortex
	thickness **(um)**			
Open filed	17.778^{a}	45.778^{a}	155.556^{a}	87.556^{a}
Lath house	21.111^{a}	35.333^{a}	116.889^{b}	68.444^{b}

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

Table (7): Effect of local compost and bamboo biochar on anatomical features of *Pinus pinea L.* stems. *

Organic fertilizer	Epidermis	hypodermis	Vascular bundle	cortex
	thickness **(um)**			

0 (**g.** plant^{-1}**)**	17.500^{a}	37.333^{a}	120.000^{a}	78.167^{ab}
12(**g.** plant^{-1}**)** loc.compost	23.167^{a}	38.833^{a}	147.000^{a}	69.000^{b}
9 **(g.** plant^{-1}**)** Bam. biochar	17.667^{a}	45.500^{a}	141.667^{a}	86.833^{a}

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

Table (8): interaction effect of cultivation condition and local compost and bamboo biochar on anatomical features of *Pinus pinea L.* stems. *

Cultivation condition	Local compost	Epidermis	Hypodermis	Vascular bundle	cortex
		Thickness(um)			
Open filed	0	13.000^{b}	41.333^{a}	127.667^{ab}	91.000^{ab}
	12**(g.** plant^{-1}**)** loc.compost	22.667^{ab}	38.667^{a}	176.667^{a}	64.333^{c}
	9 **(g.** plant^{-1}**)** Bam.biochar	17.667^{ab}	57.333^{a}	162.333^{ab}	107.333^{a}
Lath house	0	22.000^{ab}	33.333^{a}	112.333^{b}	65.333^{c}
	12**(g.** plant^{-1}**)** loc.compost	23.667^{a}	39.000^{a}	117.333^{b}	73.667^{bc}
	9 **(g.** plant^{-1}**)** Bam.biochar	17.667^{ab}	33.667^{a}	121.000^{b}	66.333^{c}

*Values within each column followed with the same latter are significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

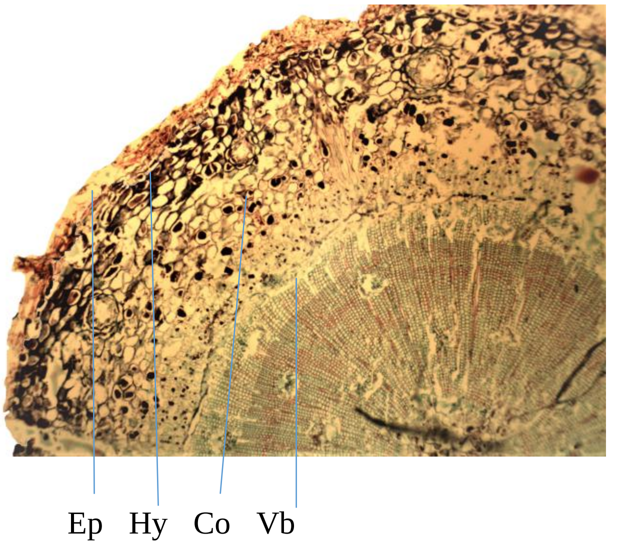

Figure (2): Cross-sectional diagram of a of *Pinus pinea L.* stems illustrating the different characteristics measured of control treatment (4X):

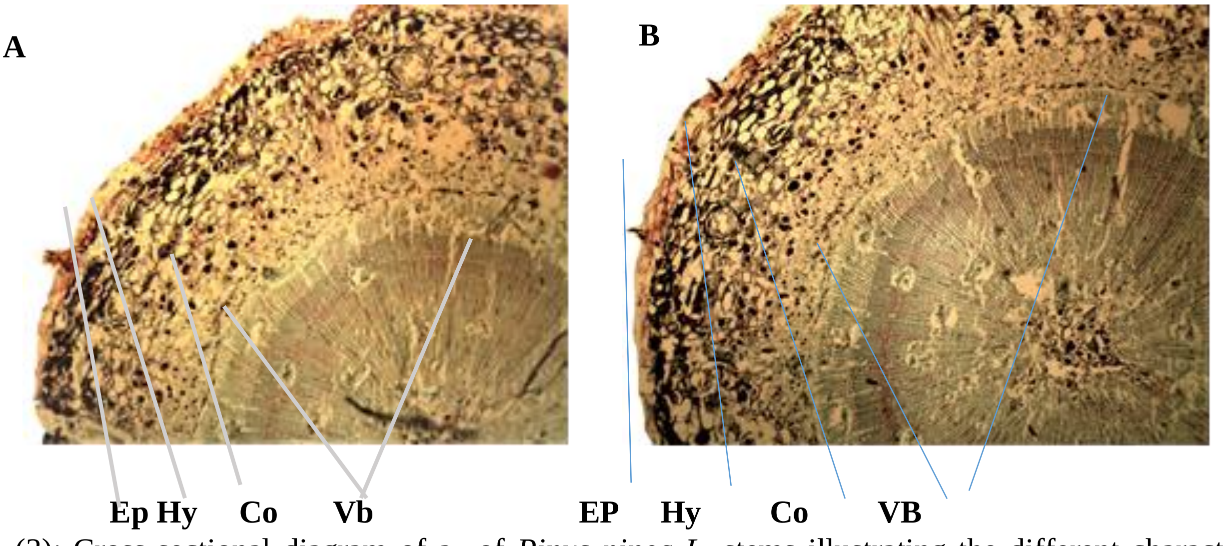

Figure (3): Cross-sectional diagram of a of *Pinus pinea L.* stems illustrating the different characteristics measured of two treatments (4X):
A- Interaction of 12(g. plant^{-1}) local compost and lath house condition.
B- Interaction of 9 (g. plant^{-1}) Bamboo biochar and open field condition.
(Ep: Epidermis, Hy: Hypodermis, Co: Cortex, VB: Vascular Bubdle).

5- Discussion

Experiment results revealed that needle and stem anatomy of the seedlings of *Pinus pinea L.* responded differently to studied factors. In general, lath house condition and Bamboo biochar gave less response.

Results confirmed the relation between increasing of stomata density in the needles epidermis and 50% shading. It is not agreed with Kardiman and Ræbild (2018), they found that stomata density generally in angiosperms was higher in the sun compared with shade ones, and it's varied between the species in response to the sun. Stomata density affects gas exchange, stomata conductance and instantaneous water-use efficiency (Woodward and Bazzaz, 1988). Recent research has shown that signals from older leaves can influence the development of stomata on the younger leaves, in that way, if the environmental conditions to which the older leaves are exposed change, then the younger leaves can increase or decrease their stomata density, this physiological adaptation make the plant cope with the environmental changing (Petrova, 2012).

Stomata density was highly influenced by (9g. plant^{-1}) Bamboo biochar application but under lath house condition. Otherwise, needles of the seedling grown under open field condition without fertilizers gave most significant results of epidermis, mesophyll and vascular bundle thickness, and with (9g. plant^{-1}) Bamboo biochar for hypodermis thickness, these results are similar to those obtained by Gebauer *et al.* (2015). Norway spruce trees, they found that sun needles had significantly higher values than shade needles for all anatomical traits that they studied, and may be due to that the maximum relative photosynthetic electron transport rates were significantly higher in sun leaves than in shade leaves, thus caused increasing of photosynthesis capacity (Dörken and Lepetit, 2018).

The highest thickness of vascular bundle and cortex in the stem obtained under open field condition with application of (12g. plant^{-1}) local compost and (9 g. plant^{-1}) Bamboo biochar respectively. While, epidermis thickness was increase significantly under lath house condition (50% shade) combined with of (12g. plant^{-1}) local compost. The organic fertilizer is essential to produce best features of potted plants, composts are high in organic matter and nutrients (Farrell and Jones, 2010). The quality of organic fertilizers given material influenced by the environmental conditions under which the plants grew and enhance the availability of N, P, K and other essential nutrients which play an important role in growth and development of plant (Palm *et al*., 2001).

6- Conclusion

The possible conclusion based on our results can be as follows:

1- Generally, needles of the seedling grown in open field without organic fertilizers exhibited more effective results of studied anatomical features (epidermis, mesophyll and vascular bundle thickness) than those grown in lath house. However, stomata density was more under lath house condition with Bamboo biochar.

2- Regarding to stem anatomy, application of organic fertilizers (especially local compost) was more efficient on the thickness of vascular bundle and epidermis. While, the open field had superiority effect over lath house in most studied parameters.

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