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RESEARCH ARTICLE

Effect of Citric Acid Application on Anatomical and Physiological Properties of the Vegetative Parts of a Species of Tamarindus Indica Cultivated in Iraq

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Abstract

The present study included details of the anatomical properties of the vegetable parts of the stem, the cultured leaf in Iraq for some species of *Tamarindus indica*. The study examined the stomata index and the rate of both the length and width of the complex stomata and the thickness of the cuticle, the parenchymal and sclerenchymal tissue, phloem and xylem. It was taken the parts mentioned and measured after the treatment of the plant with citric acid and measured for the treated species with citric acid and untreated. The study showed that there is a clear variation in the properties above.

Introduction

The tamarind, orradb or haomer (*Tamarindus indica*) returns to Fabaceae family. It is tree, evergreen plant and a rapidly growing with a height of about three metres and its leaves a compound yellow-coloured, the fruits being pods, native to tropical Africa and known since time in Sudan, Egypt and India are also said to be home to the island of Madagascar and East Africa.

Tamarind contains between 16-18% acids including citric acid, malic acid, potassium citrate and mineral salts such as phosphorus, magnesium, iron, manganese, calcium, sodium and chlorine, as well as the tamarind contains vitamin B_3, essential oils, fats and carbohydrate substances. The recent studies have proved tamarind contains antibiotics that are capable of inhibition many different bacterial strains that are harmful to humans, besides its benefits as a laxative, antihistamine, dampening, and antipyretic, so some pharmaceutical companies classify the aquatic extract of tamarind fruit into baby medications. The citric acid is an organic acid in the Krebs cycle (TAC) which is the most efficient and effective in releasing energy and occurs in mitochondria with the presence of oxygen. This acid consists of revealing Acetyl Co A with oxaloacetate and releasing Co A, which is part of the aerobic reactions involving glycolysis, Krebs cycle and electronic transport system ETS [2].Citric acid contributes to the formation of all compounds and components that contribute to the construction of the tissues and composition of plant parts such as proteins, carbohydrates and fats, as well as to the cytoplasm, cytochrome, phytochrome and photosynthetic dyes [3].

Materials and Methods

The experiment was done in the glasshouse of the Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), Baghdad University during the growth season 2017-2018 using pots capacity 7 kg soil for each pot. The soil was taken from the botanical garden and was prepared and initialized then the soil weight was placed in each pot. The seeds of tamarind were planted at date 10/9/2017, with 10 seeds per pot, irrigated primary with water to 50% of the field capacity. After two weeks of germination, the plants were reduced to five in each pots, with all the agricultural processes of irrigation and deforestation were performed.

The foliar application of citric acid at a concentration of 50 mg. L^{-1} at date 10/10/2017 in the early morning using a hand-grenade and a week after the first workshop, the second workshop was applied at date 17/10/2017, with the same concentration. The parts of the type cultivated under the study of the treatment and untreated of citric acid were taken after reaching the flowering stage and beginning the formation fruits. The samples were cut by 2-3 cm for a stem of the middle and the leaves were cut by 1 cm^2 at the center of the leaves [4].

The fixation was carried out as the samples were fixed in F.A.A formalin acetic acid solution with 90 ml of alcohol at 70% and 5% formalin, 5% acetic acid and for 24 hours at the laboratory temperature. After that performed the clearing and filtration processes and then completed the known stages of anatomy using a rotary advice (Rotary microtome) and a thickness of 6 to 12 micromometer, the sections were stained with safranin and fast green, depending on the method of Johansen (1940) [5].

The sections were studied and photographed under the Meijtechne microscope, as well as studied the epidermis of stem and leave from the soft samples of the species under study. The epidermis of the stem and leaf were stripping off by hand and placed on a clean glass slide then added a drop of glycerin after that put the cover slide gently and thus became ready for examination and study [6].

Results and Discussion

Stem

All data of the transverse section and quantitative properties are included in Table 1 and Fig. 1. The stem shape appeared circle solid composed from the outside to the inside of the following tissues:

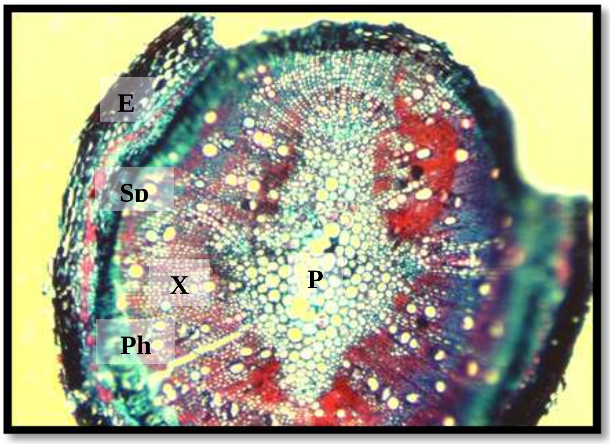
- Periderm: The torn epidermis was replaced and it was blurred. It have three layers cork, secondary cork cambium and cortex, the epidermis thickness of the species treatment with citric acid 29 micromometer, while the epidermis of the untreated plant 18 micromometer. We did not notice the complex stomata in the epidermis of the stems, which indicates the dependence of the plant on the complex stomata in the leaf.
- Cortex: followed by the epidermis, the tissue of the cortex appeared consisting of polygonal parenchyma cells that were 4-6 layers, and the rate of the parenchyma tissue layer that treated with citric acid was recorded 101 micromometer and the untreated with citric acid 56.5 micromometer. Then the sclerenchyma tissue cells, fibre (2-3) layers and the thickness rate of the treated sclerenchyma tissue with citric acid was recorded 35 micromometer and untreated with citric acid 23 micromometers.
- Vascular cylinder: The cortex tissue is followed by a layer of polygonal parenchyma cells represented by the pericycle, then the phloem tissue that appeared circular on a complete cylinder consisting of sieve tubes, companion cells, fibers and phloem parenchyma, where the treated plant was shown to be different thickness in citric acid 29.5 micromometers, while the untreated plant with citric acid was 62.5 micromometer.

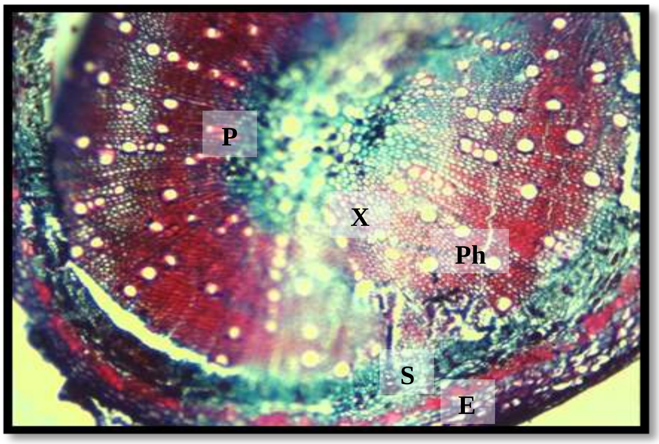
The phloem was followed by xylem, which appeared in a diffuse form of polygonal vessels as the tracheid, fibers and xylem parenchyma were shown and also appeared a difference in the treated plant thickness which recorded 215 micromometers, while the untreated plant record 165 micromometers.This is consistent with Shaheen and Abdl El-al, who reported that the treated plants with organic acids like citric acid plays an important role in improving the physiological properties of okra [7] and sweat pepper [8], therefore this was reflected in anatomical properties.

Table 1: The quantitative properties of the transverse section for *Tamarindus indica* stems that treated and untreated with citric acid, measured at micromometers

	Epidermis	Parenchyma tissue	Sclerenchyma tissue	Phloem	Xylem
	30-28	102-100	36-34	95-90	230-200

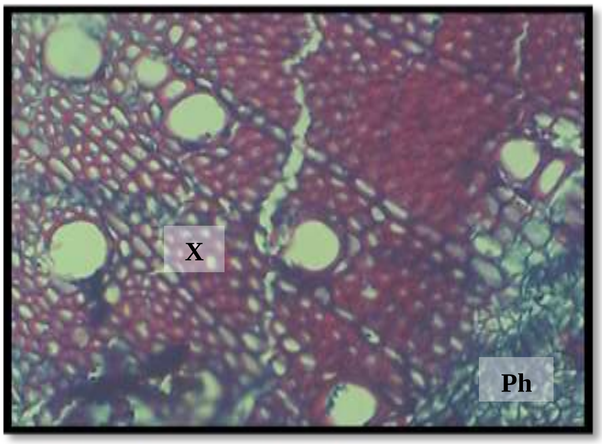
Treated with citric acid	(29)	(101)	(35)	(92.5)	(215)
Untreated with citric acid	20-16 (18)	58-55 (56.5)	26-20 (23)	65-60 (62.5)	170-160 (165)

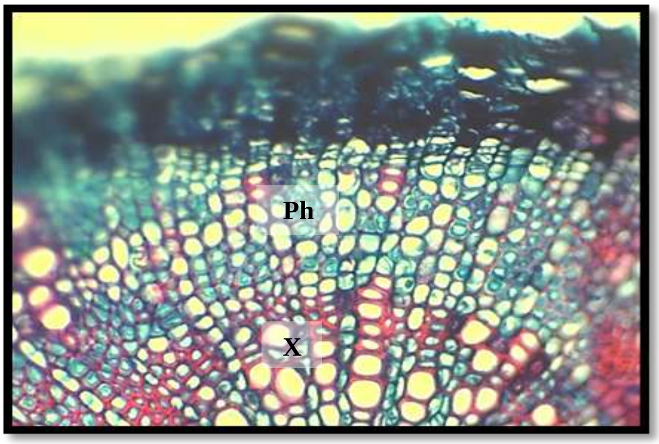




Treated plant 10x

Untreated plant 10x





Treated plant 40x

Untreated plant 40x

Figure 1: Transverse section of *Tamarindus indica* stems that treated and untreated with citric acid. Epidermis (E); Sclerenchyma (S); Pith (P); Phloem (Ph); Xylem (X)

Leaves

The surface view of the epidermis: All data that belong to surface view of the leaf epidermis were shown in Table (2), Figure (2): The epidermis of the plant treated and the untreated of citric acid for the *Tamarindus indica* plant appeared simple, uniseriate inner tangential and the outer tangential in both the upper and lower surfaces with walls undulating and it was more undulating in the plant that treated with citric acid.

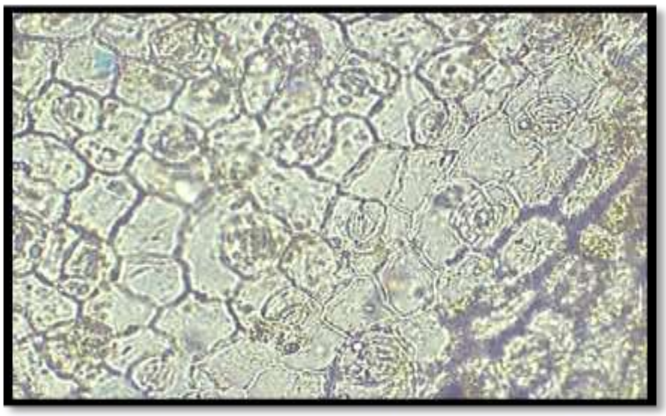
The complex stomata found on aboxial and adoxial surfaces of leaf so it is called amphistomata (present of stomata on the two surfaces) and the density of the stomata was higher prevalent on adoxial surface of the leaf. The actinocytic complex stomata found on adoxial surface, while the type of the complex stomata anisocytic in aboxial surface of both treated and untreated plants of citric acid as shown in Fig. 2.The lower values of stomata index for aboxial and adoxial surface of the treated and untreated plant species 24, while the highest value of the stomata index of treated and untreated plants 27.

As well as the forms of the stomata and the guards cells of both surfaces in the plants under study, appeared in an elliptical form and the guards cells have a kidney shape. The dimensions of the stomatal complex of the plants under the study showed variation, as the aboxial surface of the leaves, the average length of the stomatal complex is 25 micromometers at a minimum in the untreated plant and 35.5 micromometer as a maximum in the treated plants. As for the width of the stomatal complex on the same surface, it reached 17.5 micromometers at a minimum in the untreated plants and 29.5

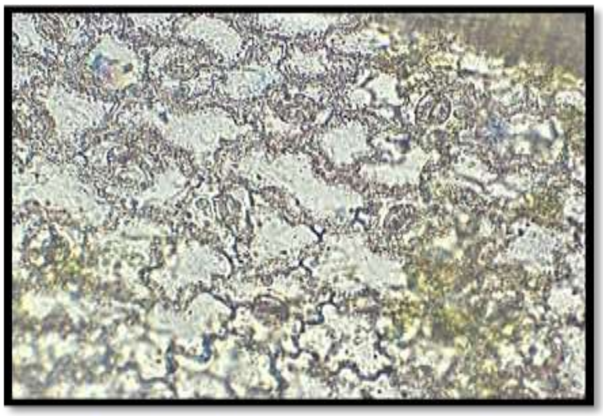
micromometers in the treated plant. While the dimension of stomatal complex on the adoxial surface of the leaves, the average length was 29 micromometers at the minimum in the untreated plant and 39 at the maximum of the treated plant, with a minimum of 21 micromometers width in the untreated plant and a maximum of 29 micromometers in the treated plant. This is consistent with stated [9] that citric acid helps regulate the pH of the stomatum and therefore regulates their opening and closing process. The aglandular trichromes appeared in the epidermis surface of the treated and untreated studied plant with citric acid. The types of these trichromes were uniseral with a circular to quadricular base and a sharp and hooked end.

Table 2: The quantitative properties of the stomatal complex and index for leaf epidermis under study to *Tamarindus indica* measured at micromometers

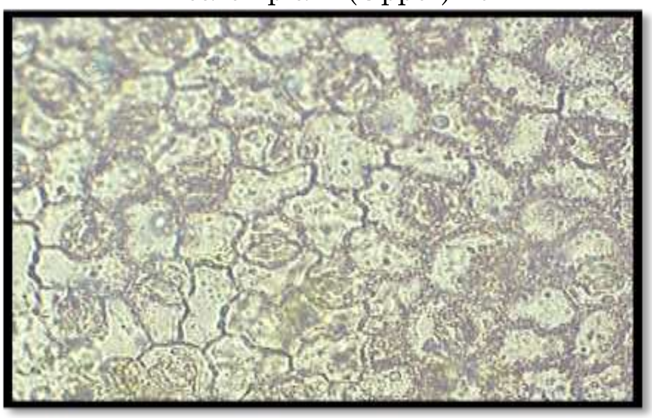
Plants	Stomatal complex of aboxial surface		Stomatal index	Stomatal complex of adoxial surface		Stomatal index
	length	width		length	width	
The tamarind plant treated with citric acid	39-32 (35.5)	32-27 (29.5)	27	40-38 (39)	30-28 (29)	42
The tamarind plant untreated with citric acid	26-24 (25)	19-16 (17.5)	24	31-27 (29)	22-20 (21)	35



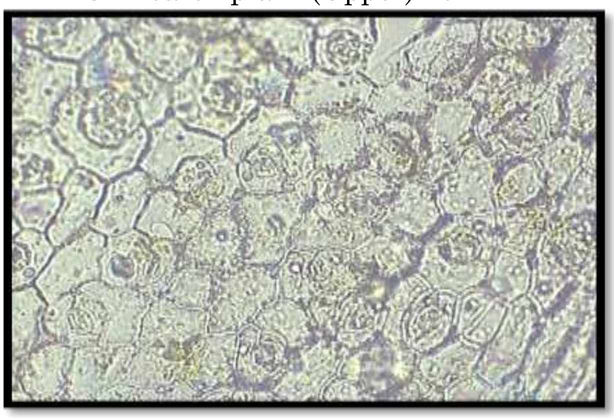
Treated plant (Upper) 40x



Untreated plant (Upper) 40x



Treated plant (Lower) 40x



Untreated plant (Lower) 40x

Figure 2: The stomatal complex in the upper and lower epidermis of *Tamarindus indica* that treated and untreated with citric acid

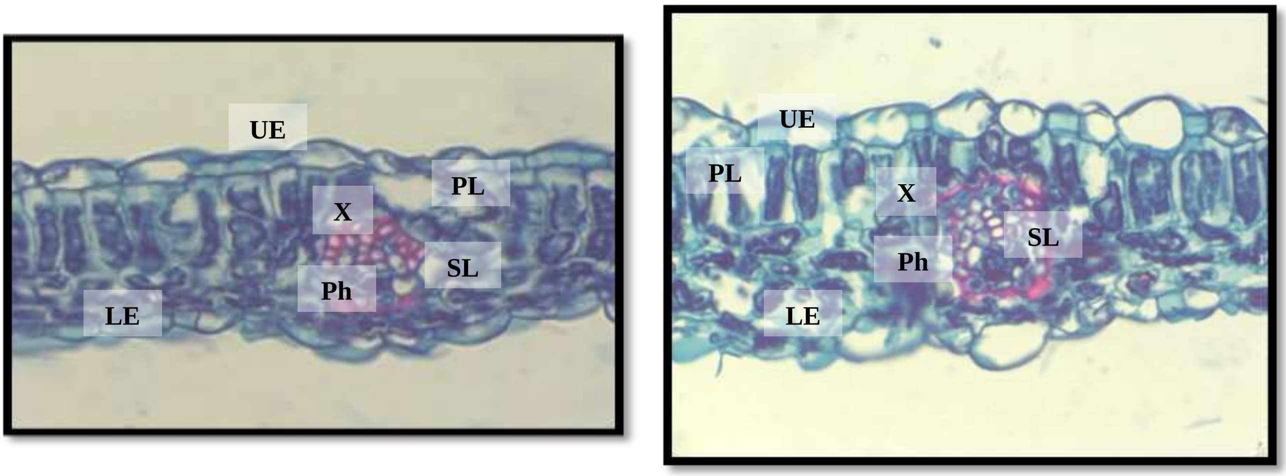
The vertical section of lamina: All data related to the shape of the leaf are included in Table (3) and Figure (3).

Appeared from top to bottom consisting of the following:

Epidermis: The epidermis was characterised by upper epidermis and lower epidermis but they were almost identical to simple, uniseriate which consisted of a single row of cells with quadricular shapes to rectangular and stacked with each other and free of chlorophyll except guard cell permeation of the epidermis layer of the stomatal complex. The walls of the epidermis cells appeared in surface view as undulat and the average thickness rate of the upper epidermis 13 micromometers at minimum in untreated plant and a maximum of 15 micromometers in the treated plant, while the rate of the lower epidermis thickness of 16.5 micromometers as a minimum in treated plants and 15 micromometers as maximum in untreated plant. The upper and lower epidermis covered a thin and smooth cuticle layer and was almost identical in both upper and lower epidermis and ranged from 4.5 to 6 micromometers in both treated and untreated plants.

Table 3: The quantitative properties of vertical section for lamina under study to *Tamarindus indica* measured at micromometers

	Upper epidermis	Lower epidermis	Palisade layer	Spongy layer	Phloem	Xylem
The tamarind plant treated with citric acid	17-13 (15)	18-15 (16.5)	40-38 (39)	30-26 (28)	28-26 (27)	32-30 (31)
The tamarind plant untreated with citric acid	14-12 (13)	17-13 (15)	36-34 (35)	25-20 (22.5)	22-18 (20)	29-26 (27.5)



Treated plant 40x Untreated plant 40x

Figure 3: Vertical section of *Tamarindus indica* lamina that treated and untreated with citric acid. Upper epidermis (UE); Lower epidermis (LE); Spongy layer (SL); Palisade layer (PL); Phloem (Ph); Xylem (X)

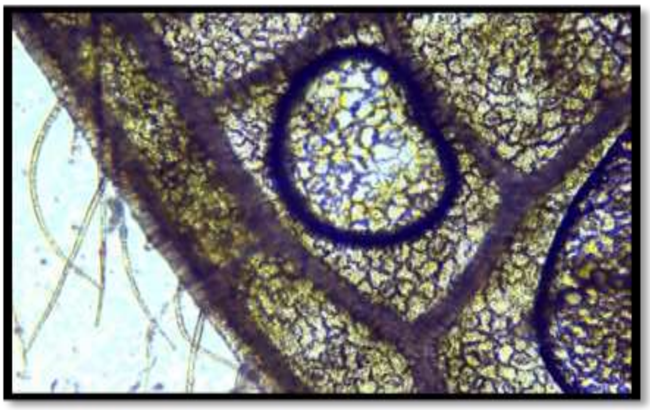
Mesophyll Tissue

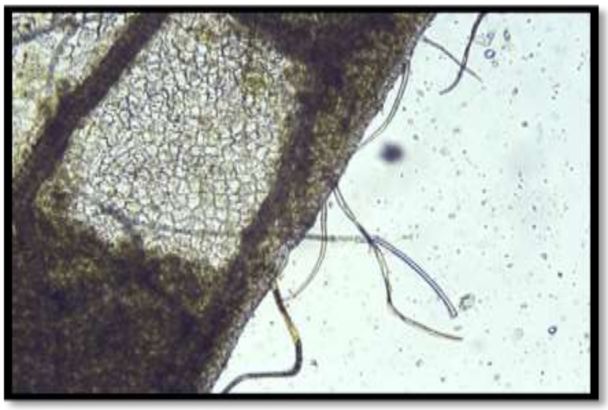
Epidermis: It appeared confined between the upper and lower epidermis of the leaf tissue. Palisade tissue consists from one layer beneath the upper epidermis and the sponge tissue, also from one layer and this conforms to what is mentioned [10]. The palisade tissue filled more area of sponge tissue in the lamina as well as palisade tissue consisting of a single layer of elongated cells with very narrow spacing.

The spongy tissue is a disjointed form of loose, irregular cells interspersed with a wide interspace and, in general, palisade tissue thickness of 35 micromometers at the untreated and 39 micromometers as maximum in the treated plant. While the thickness of the sponge tissue recorded a minimum of 22.5 micromometers in the untreated plant and a maximum of 28 micromometers in the treated plant.Vascular tissue: The vascular tissues appeared to consist of a single central vascular bundle that permeates the lamina and showed a variation in its shape as it appeared semi-oval in the untreated plant, when it took its semi-oval shape wider so that it took roughly the rectangular shape in the treated plant. This amplitude as a result of increased phloem and xylem thickness through citric acid effect.

Generally, the vascular bundle consists of phloem and xylem, and the last appeared in the upper epidermis area while the phloem appeared in the lower epidermis area, which consists of the sieve tubes, the accompanying cells, parenchyma and the phloem fibers. The shape of the phloem appeared in both treated and untreated plants as mass of oval cells too.

The wood consists of polyhedral vessels and has been heterogeneous in the number of units (3-4) in untreated plant and formed (6-7) rows per unit, while (5-6) units in the treated plants and formed (8-9) as a row in one unit as well as the vessels formed the xylem of the trichromes, fibers and xylem parenchyma. It also showed a difference in the thickness of the phloem tissue, with a minimum of 20 micromometers in untreated plant, 27 micromometers in the treated plant, and a minimum of 27.5 micrometer thickness in the untreated plant of 31 micromometers in the treated plant. The difference in thickness and length of the anatomical properties considered indicates the effect of the physiological properties on them, through the effect of citric acid. The citric acid improves the anatomical and physiological properties of the tamarind plant, which is a buffer solution in the plant cell that prevents the sudden change in the concentration of hydrogen ion (pH), which affects the metabolic activities of the plant and the presence of these solutions resist cells sudden change in pH [11], which is an organic acid in the Krebs cycle that consists of the interaction oxaloacetate (OAA) with acetyl-CoA to form citrate and be in turn isocitrate. In the Krebs cycle, all the important cellular components are formed to synthesis plant organs and continue to grow, such as proteins, nucleic acids, fats, steroids and hormones, etc [2, 12].





Treated plant 40x Untreated plant 40x

Figure 4: A glandular trichromes of *Tamarindus indica* plant that treated and untreated with citric acid

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