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Effect of applying integrated organic and bacterial fertilization on the tissue growth of broad bean (*Vicia faba* L)

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Abstract

Background: Broad bean (*Vicia faba* L.) is an important legume crop valued for its high nutritional content and its role in improving soil fertility.

Objective: This study aimed to evaluate the effect of organic and bio-fertilization on improving the tissue growth of broad bean plants under field conditions.

Methods: The experiment was conducted at the Field Crops Research Station, College of Agriculture, University of Samarra, during the winter growing season of 2024/2025, starting on November 12, 2024. The study was arranged in a randomized complete block design (RCBD) with four treatments (R_0: Control treatment (no fertilization), R1 Fertilization with tea extract (6 g/L), R2 Fertilization with baker's yeast extract (6 g/L), R3: Fertilization with animal manure extract) with three replicates.

Results: Treatment R1 recorded the highest values: epidermis 9.07 µm, internode 49.33 µm, cortex 33.67 µm, 30 vascular bundles, and pith 120.33 µm, while the control (R_0) had lower values of 5.10, 24.33, 14.67 µm, 15 bundles, and 50.00 µm, respectively. Similarly, treatment R1 had the greatest impact on the structural properties of the leaves. Treatment R1 recorded the highest values, with the outer cuticle layer measuring 6.20 µm, the outer epidermis 11.77 µm, the mesophyll 79.67 µm, the mesospastic mesophyll 59.67 µm, the outer epidermis 7.00 µm, and the outer cuticle 4.08 µm. In contrast, the control group (R0) recorded lower values of 2.03, 3.93, 41.33, 29.67, 2.07, and 1.00 µm, respectively.

Conclusion: The results indicate that organic fertilization contributes to improving the anatomical structure of the plant by promoting cell division and increasing the efficiency of physiological processes, which is reflected in the overall growth of the plant.

Keywords: Organic Fertilization; Tea Extract; Yeast Extract; Tissue Growth; Broad Beans; Anatomical Characteristics

1. Introduction

Legumes are important economic crops due to their high nutritional value and their role in improving soil fertility through nitrogen fixation. Broad bean (*Vicia faba* L.) is a versatile legume that is used worldwide as a food and medicine, among other things. It is a common edible plant in the Middle East, the Mediterranean, China, Ethiopia and Northern Europe (Baranowska, 2024). The type and amount of fertilizer used will dramatically impact vegetative growth and plant structure. In particular, organic and biofertilizers are relatively more effective than conventional chemical fertilizers in enhancing physiological processes and internal tissues.

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Bio-extracts such as compost tea, yeast extract, and animal manure extract waste extracts are characterized by their content of organic compounds, amino acids, natural hormones, and trace minerals that promote vegetative growth, support anatomical structures, and improve the ability of plants to withstand environmental stresses (Haska *et al*., 2022; Pilla *et al*., 2023).

Organic fertilization is one of the most important sustainable agricultural practices aimed at improving soil fertility and promoting plant growth by supplying them with macro and micronutrients. It also plays a role in activating beneficial microorganisms that improve soil structure and mineral absorption (Singh *et al*., 2024). Organic fertilization also contributes to improving plant anatomical structures by increasing epidermal and cortex thickness and supporting vascular tissue formation, which positively impacts the plant's physiological efficiency and its ability to withstand environmental stresses (Haska *et al.*, 2022).

In addition, yeast extract (*Saccharomyces cerevisiae*) is considered one of the most important biofertilizers used to improve the vegetative and anatomical growth of plants, due to its content of bioactive compounds such as amino acids, vitamins (especially B vitamins), and natural plant hormones (cytokinins), as well as its role in promoting cell division and elongation (Singh *et al*., 2024). Studies have shown that fertilizing with yeast leads to increased leaf thickness, improved photosynthetic efficiency, and enhanced vascular tissue growth, which in turn improves growth and productivity (Pilla *et al*., 2023).

Thus, the present study is aimed to investigate the effect of organic fertilization (waste from tea extract and animal manure extract) and yeast biofertilizer on tissue growth of broad bean (*Vicia faba* L.) plants through studying anatomical properties of stem and leaf planted in field conditions to contribute with providing sustainable alternatives for traditional chemical fertilizer.

2. Materials and Methods

2.1. Study design

The experiment was conducted at the Field Crops Research Station, College of Agriculture, Samarra University, during the 2024/2025 winter growing season, starting on November 12, 2024. The experiment included four treatments: tea extract, yeast extract, animal manure extract, and a control. A local variety of broad bean was cultivated. This experiment was conducted as a simple experiment using a completely randomized book design (RCBD) with three replicates . Therefore, there were a total of 12 experimental units. Each experimental unit contained four plants. Analysis of variance (ANOVA) was performed using SPSS software (version 24). The significance of the differences between treatments was approximated using the least significant difference (LSD) test at a level of 0.05. The seeds were sown on November 12, 2024, at a depth of 5 cm, with a rate of 3 seeds per hill. They were then thinned to one plant per hill two weeks after sowing. The seeds were sown on 12/11/2024 at a depth of 5 cm, at a rate of 3 seeds per hole, then thinned to one plant two weeks after sowing.

2.1.1. Treatments

- **R0:** Control treatment (no fertilization)
- **R1**: Fertilization with tea extract (6 g/L)
- **R2:** Fertilization with baker's yeast extract (6 g/L)
- **R3:** Fertilization with animal manure extract

2.2. Studied traits

Anatomical characteristics of the plant

- **Stem:** Epidermis, Endodermis, Cortex, Vascular Bundles, Pith.
- **Leaf:** Upper Cuticle, Upper Epidermis, Palisade Mesophyll, Spongy Mesophyll, Lower Epidermis, Lower Cuticle.

2.3. Preparation of Cross Sections

Fresh plant samples collected from the field were used to prepare transverse sections using the hand sectioning method following (Al-Khafaji, 2004). Stem segments were cut from the middle region of the plant and thin transverse sections were obtained using a sharp dissecting blade. Leaf sections were also prepared from the middle part of the leaf under a dissecting microscope. The sections were transferred to sodium hypochlorite solution (0.5 mL NaOCl with 1 mL distilled water) for 5 min to remove chlorophyll, then stained with safranin prepared by dissolving 1 g of dye in 99 mL distilled water for 1–2 h. Excess stain was removed using 70% ethanol. The sections were subsequently passed through graded ethanol concentrations (90%, 95%, and absolute ethanol), cleared in a mixture of absolute ethanol and xylene (1:1), followed by pure xylene. Finally, the sections were mounted on glass slides using Canada balsam, covered with a cover slip, and examined under a light microscope (Olympus) and photographed using a digital camera.

2.4. Statistical analysis

Data obtained were subjected to statistical analysis in accordance with randomized complete block design (RCBD). Statistical shift among treatments was determined through an analysis of variance (ANOVA). Means were compared by the Least Significant Difference (LSD) test at probability level 0.05. The appropriate statistical software was used for all statistical analysis.

3. Results and discussion

The effect of organic fertilization treatments on the anatomical characteristics of the broad bean stem (*Vicia faba* L.) is shown in Table (1). In general, results showed significant genetic differences ($p < 0.05$) between treatments for all studied traits (R0, R1, R2 and R3).

Table 1 Effect of organic fertilization treatments on the anatomical characteristics of the stem

Treatments	Epidermis	Endodermis	Cortex	Vascular Bundles	Pith
R0	5.1000	24.333	14.667	15.000	50.000
R1	9.0667	49.333	33.667	30.000	120.333
R2	7.2000	41.333	24.667	22.000	100.333
R3	8.0333	44.333	31.333	24.667	110.000

3.1. Stem cross-sections

Differences in anatomical features among treatments were pronounced across these cross sections. These disparities serve as crucial indicators for identifying the optimal organic fertilization approach. The observed changes were related to the epidermis, perianth, cortex, vascular bundles and stem medulla (Table 1).

Thus, it was possible to verify the effect of the fertilization treatments on anatomical characteristics of the stem when compared with the control treatment (R0). The anatomical response of the plant to the biological treatments applied (tea extract R1, yeast extract R2 and cattle extract R3) showed a significant increase in the thickness of epidermis, pericycle; cortex; vascular bundles and pith that demonstrate beneficial effects by enhancing vegetative growth

3.1.1. Epidermis

Stem epidermal thicknesses showed a clear effect of fertilization treatments (Figure 1). The leaf epidermal thickness of the tea extract treatment (R1) had the highest value at 9.07 µm, followed by the cattle dung extract treatment (R3) at 8.03 µm and baker's yeast extract (R2) 7.20 µm while, control (R0) was found to be lowest at 5.10 µm.

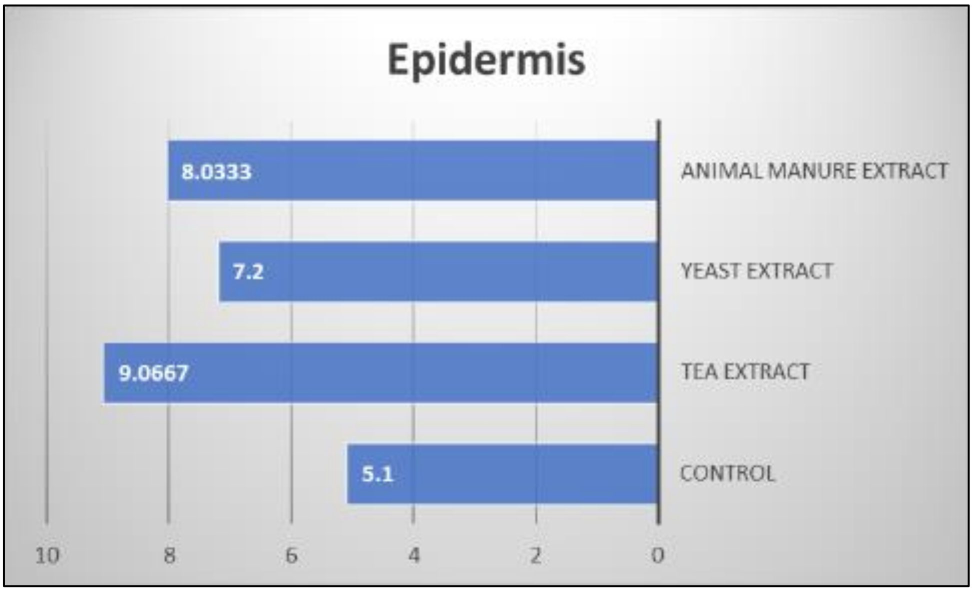

Figure 1 Effect of the treatments on skin thickness compared to the control.

Organic fertilizers, particularly plant-based and bioactive organic materials like tea, offer diverse macro- and micronutrient sources, in addition to bioactive compounds that are able to induce cell division and expansion of the epidermis (Al-Hlfie & Hussein, 2024). In the present study, the highest levels were obtained in R1 (tea extract); this improvement may be due to phenolic compounds, amino acids, and trace elements found in tea extract that lead to strengthen the outer layer and protect it from exterior conditions. (Herman *et al*., 2022). This finding is consistent with the results of (El-Sayed *et al*., 2018), which have shown that organic inputs resulting in a thicker protective tissues (epidermis and cortex) as compared with the unfertilized samples. Cow manure (in red beet [Beta vulgaris]) and worm-based compost (at least for fruits) significantly increased cuticle thickness compared to control plots receiving no such organic sources of nutrients, indicating the potential for organic nutrient provision to build tissue composition and structural toughness (Al-Hlfie & Hussein, 2024).

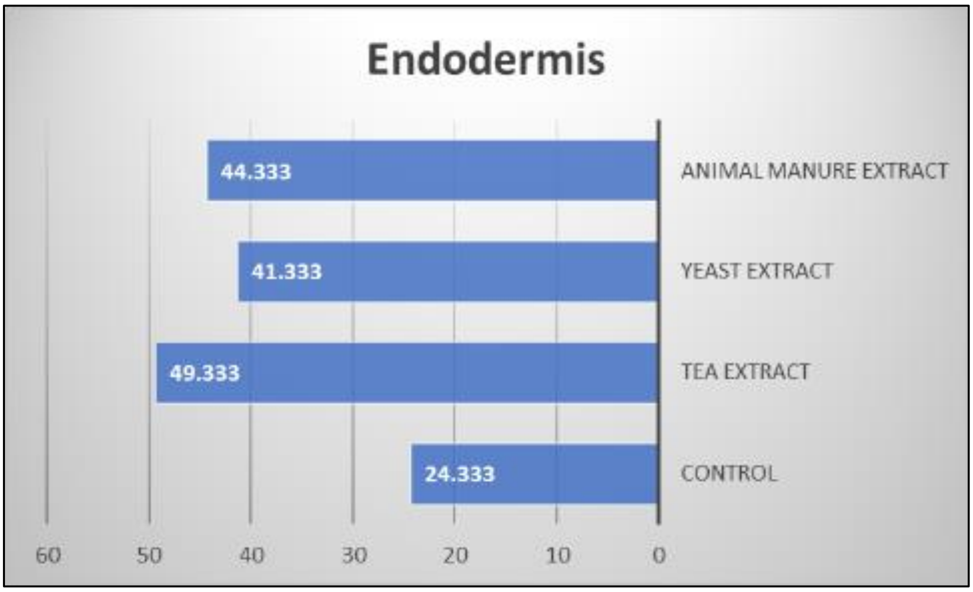

Figure 2 Effect of the parameters on the Endodermis compared to control.

3.1.2. Endodermis

The endodermis thickness in broad bean stems varied significantly among the fertilization treatments, with the tea extract treatment (R1) exhibiting the largest value (49.33 µm), followed by the cattle manure extract (R3, 44.33 µm), baker's yeast extract (R2, 41.33 µm), and the control (R0, 24.33 µm) (Figure 2).

The highest values were registered for endoderm developed treatments with the tea extract, The promotion of endodermal development underline how organic fertilization is essential to support structural and functional needed for transport in stem tissues (Lu *et al.*, 2025). The availability of several nutrients and organic compounds that enhance soil fertility from organic sources like tea extract and animal dried manure could lead to an increase in nutrient bioavailability, this can promote cell division and differentiation of supportive tissues especially the endodermis(Liu *et al.*, 2025). Organic materials are also known to increase soil organic matter and microbial activity, which promote nutrient cycling and thus plant uptake of important elements such as nitrogen, phosphorus, calcium and micronutrients all correlating with better anatomical tissue development when compared to unfertilized controls. (Xie *et al.*, 2025), organic extracts contain the organic acids and calcium and silica elements, which can trigger development of endoderm tissues helping to properly thicken the stem structure.

3.1.3. Cortex

The highest cortex thickness was observed with the tea extract treatment (33.667 µm), followed by the cattle extract (31.333 µm), while the control recorded the lowest thickness (14.667 µm), as shown in Figure 3.

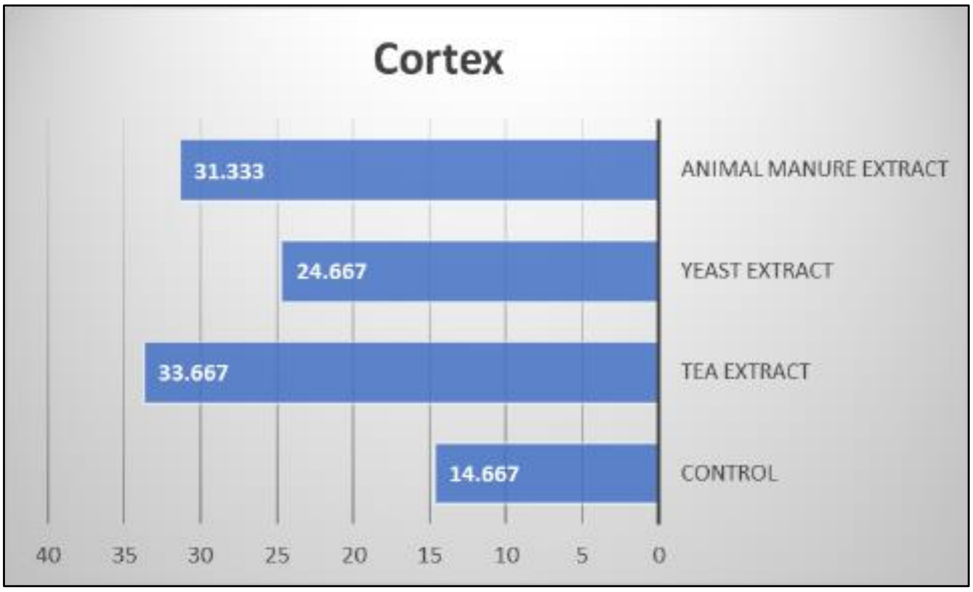

Figure 3 Effect of the treatments on Cortex compared to the control

This significant increase of the cortex thickness under organic fertilization specifically with tea extract can be attributed to phytohormones (cytokinins) and other growth-promoting organic compounds in applied extracts (Byan, 2014). Cytokinins are established regulators of cell division and differentiation; they promote mitotic activity in meristematic and parenchyma tissues, increasing cell number and expanding tissue layers (e.g. cortex). Many plant extracts contain small quantities of these phytohormones and a variety of in vitro studies have demonstrated the anatomical improvements due to exogenous application or release into the soil through organic amendments, including increased tissue development compared to controls unamended with fertilizer (Sosnowski *et al.*, 2023). El-Sayed et al. (2024) also indicated that bio-extracts rich in natural hormones such as cytokinins increase the volume of the cortex and the living tissues responsible for storage. Furthermore, plant extracts, such as tea, contain a variety of vitamins, amino acids, and micronutrients in their organic forms, enhancing nutrient absorption and metabolism, which directly benefits cell proliferation in supporting tissues. Organic inputs rich in bioactive compounds promote growth processes and help regulate endogenous hormonal balance, significantly contributing to the growth of cerebral cortex tissue (Bagchi *et al.*, 2016). Therefore, the beneficial effect of R1 on cortical thickness may be attributed to the combined enhancement of nutrient supply and hormone stimulation of cell division.

3.1.4. Vascular Bundles

The tea extract treatment (R1) showed the highest number of vascular bundles (30), followed by the (R3) animal manure extract (24,667), while the control (R0) had the lowest levels. as shown in Figure 4.

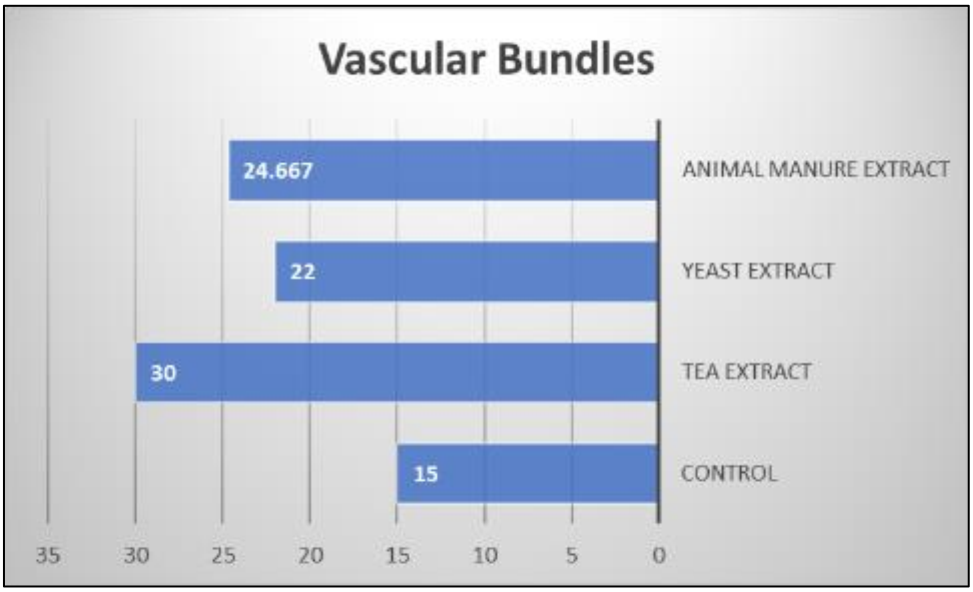

Figure 4 Effect of the treatments on the formation of vascular bundles compared to the control.

This indicates that organic treatments support increased growth of vascular tissues (xylem and phloem), thereby improving water transport and metabolic efficiency. Moreover, applications of soil biological activity-promoting organic- and bio-fertilizers have been associated with improved plant anatomical traits and growth performance (including vascular development). While we already mentioned physiological, agricultural aspects of organic fertilizers, other important components of it are the availability of different plant nutrients and stimulation of microbial activity that enhance nutrient uptake and plant utilization facilitating vascular tissue formation and differentiation (;Nassar *et al.*, 2011; Chang *et al.*, 2022) confirmed that yeast and animal extracts contain macro- and micronutrients that promote vascular tissue formation.

In addition, organic fertilizers promote more soil microbial activity and decomposition of organic matters which provide a slower and more efficient root uptake with gradual release of nutrients(Nassar *et al.*, 2011). As a result, this more efficient acquisition of nutrients stimulates the production of vascular cambium that produces more vascular strands, which increases the number of vascular bundles in plants and thus improving water and nutrient transport. (Byan, 2014) reported similar results in their analysis, that pointed out that vascular tissue differentiation is highly dependent on nutrient availability and hormonal signals controlling cambial cell division(I El-Shafey *et al.*, 2020).

3.1.5. Stem cavity (Pith)

The largest medulla volume was recorded in the tea extract (120.333 µm), followed by the cattle extract (110 µm), as shown in Figure 5, reflecting a high stimulation of internal cell growth. Khlaf *et al* (2024) and Mohamed *et al* (2023) explained this phenomenon by the presence of amino acids and sugars in the organic extracts, which act as an energy source for the internal tissues.

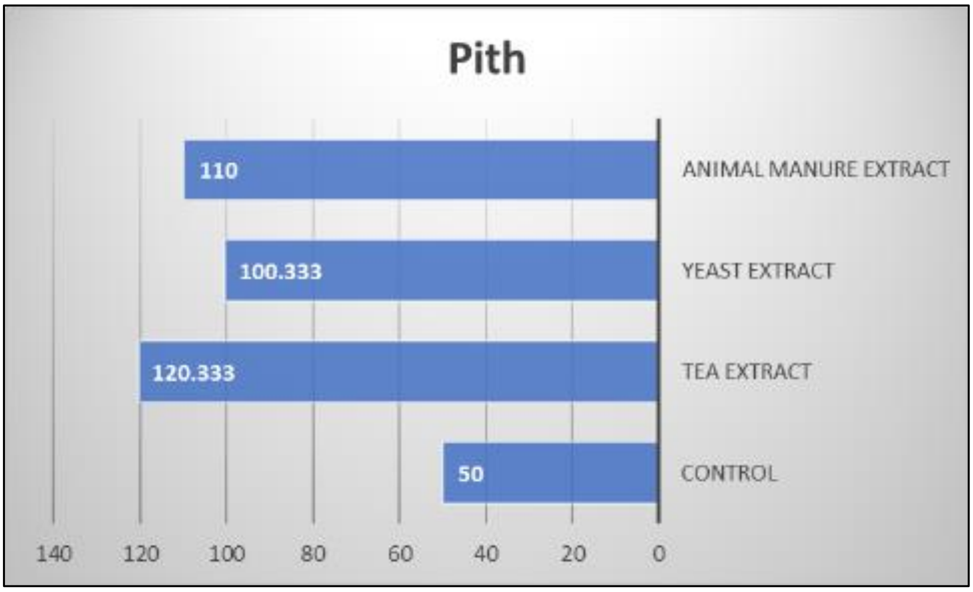

Figure 5 Effect of the treatments on the size of Stem cavity (Pith) compared to the control.

These results are consistent with recent studies that have confirmed the role of organic and bio-extracts in improving the anatomical characteristics of plants. Montesdeoca-Flores *et al*. (2024) found that biofertilization with natural extracts increases epidermal and cortex thickness and promotes vascular tissue formation. El-Sayed *et al*. (2024) also demonstrated that bio-extracts contribute to improving tissue strength and supporting the structural growth of plants under various environmental conditions.

3.2. Cross section in leaf

Table (2) shows the effect of organic fertilization treatments on the anatomical characteristics of the leaf. The results indicated statistically significant differences ($p < 0.05$) among the treatments, with treatment R1 recording the highest values for all studied traits compared with the control treatment (R0).

Table 2 Effect of organic fertilization treatments on the anatomical characteristics of the leaf

Treatments	Upper Cuticle	Upper Epidermis	Palisade Mesophyll	Spongy Mesophyll	Lower Epidermis	Lower Cuticle
0 R	2.0333	3.9333	41.333	29.667	2.0667	1.0000
R1	6.2000	11.7667	79.667	59.667	7.0000	4.0833
R2	3.1667	7.3333	61.667	41.333	4.2333	2.2333
R3	3.8667	8.1333	70.333	50.667	4.8333	2.9800

3.2.1. Upper Cuticle

The results showed significant differences in the upper cuticle thickness of broad bean leaves among the fertilization treatments. The highest thickness was recorded in R1 (6.20 µm), followed by R3 (3.87 µm), R2 (3.17 µm), while the control (R0) had the lowest value (2.03 µm). This indicates that organic fertilization, particularly with tea extract (R1), substantially increases the protective cuticle layer of leaves. (Figure 6).

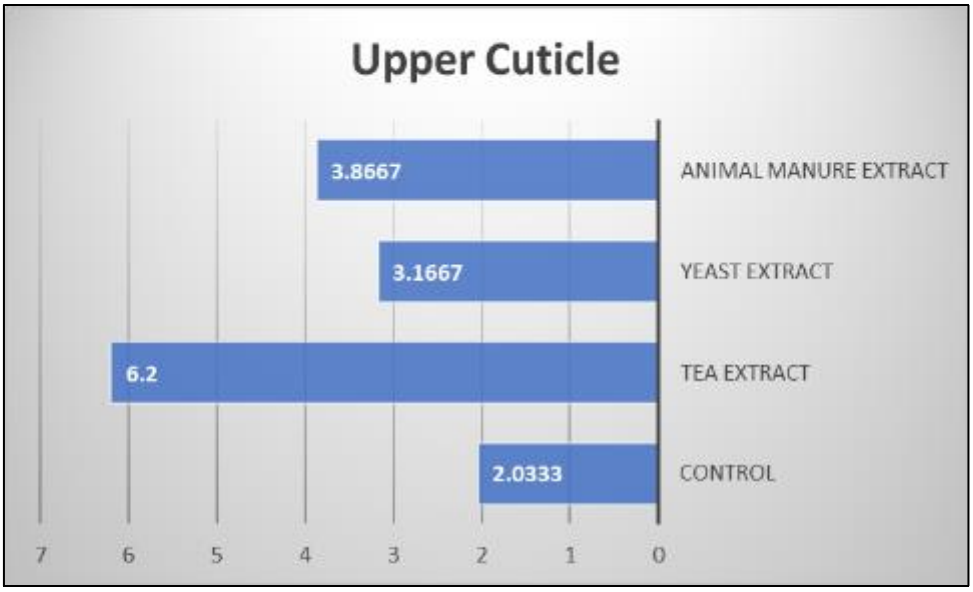

Figure 6 Effect of the treatments on the upper cuticle layer of the leaf blade compared to the control.

3.2.2. Upper Epidermis

The upper epidermis thickness of broad bean leaves varied significantly among the treatments. The highest value was observed in R1 (11.77 µm), followed by R3 (8.13 µm) and R2 (7.33 µm), while the control (R0) showed the lowest thickness (3.93 µm). These results indicate that organic fertilization, particularly with tea extract (R1), greatly enhances the protective epidermal layer of the leaves. (Figure 7).

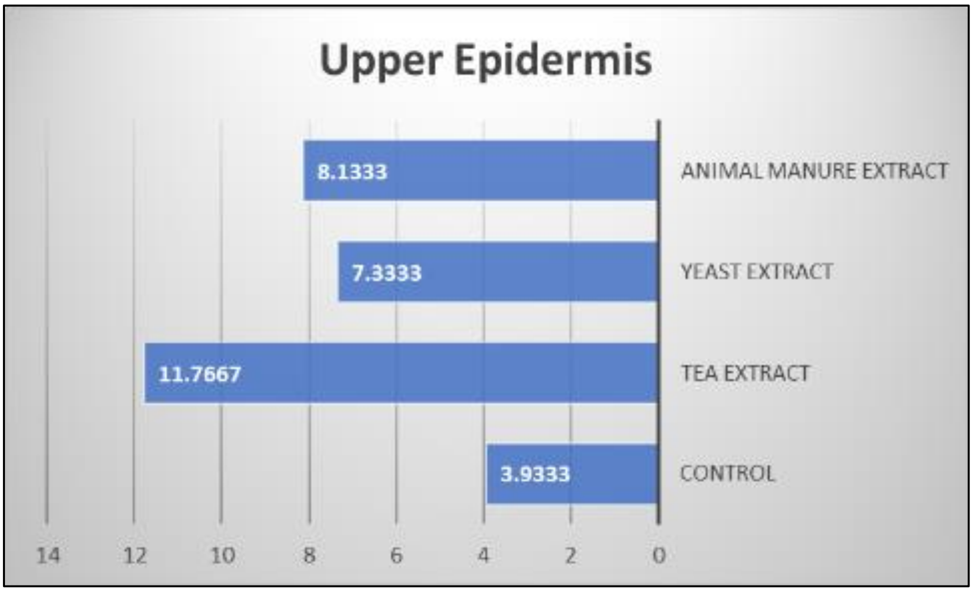

Figure 7 Effect of the treatments on the upper epidermis of the leaf compared to the control.

3.2.3. Palisade Mesophyll

The thickness of the palisade mesophyll in broad bean leaves showed significant variation among the treatments. Treatment R1 recorded the highest thickness (79.67 µm), followed by R3 (70.33 µm) and R2 (61.67 µm), while the control (R0) had the lowest value (41.33 µm). This indicates that organic fertilization, especially with tea extract (R1), markedly enhances the photosynthetic tissue of the leaves, potentially improving light capture and photosynthetic efficiency. (Figure 8).

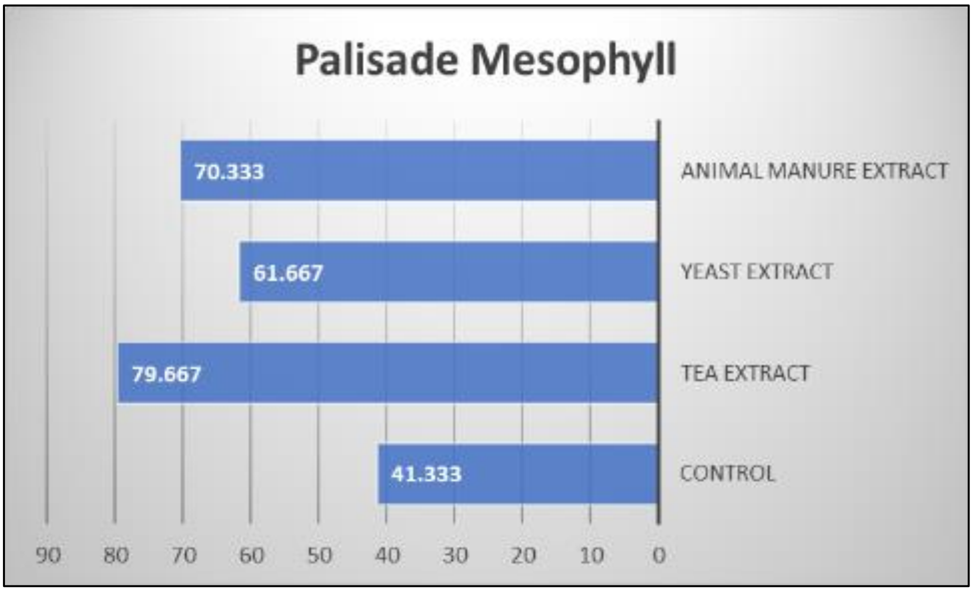

Figure 8 Effect of treatments on the vertical thickness of the leaf compared with the control.

3.2.4. Spongy Mesophyll

The spongy mesophyll thickness of broad bean leaves varied significantly among the treatments. The highest value was recorded in R1 (59.67 µm), followed by R3 (50.67 µm) and R2 (41.33 µm), while the control (R0) had the lowest thickness (29.67 µm). (Figure 9).

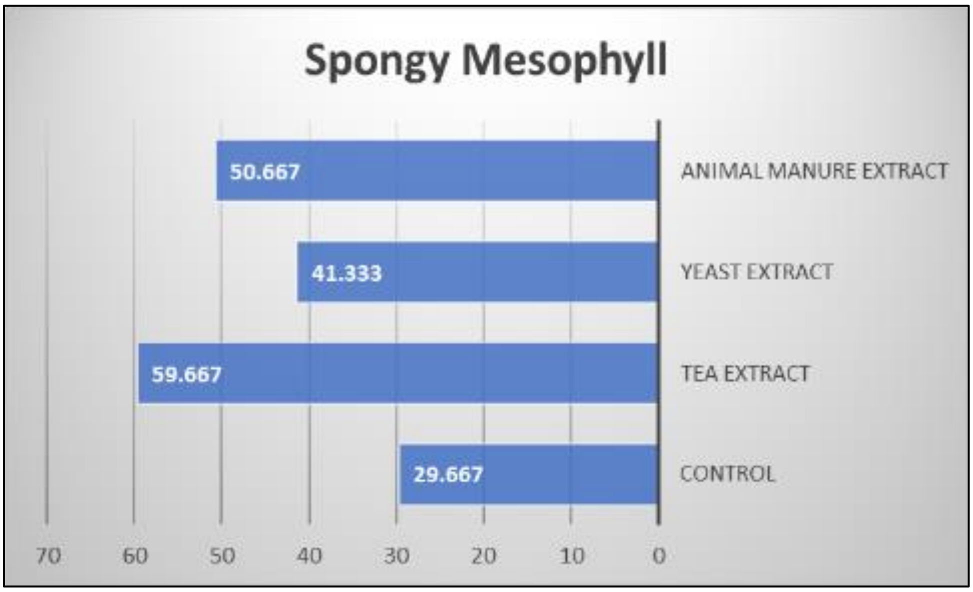

Figure 9 Effect of treatments on the radial layer of the leaf compared with the control.

3.2.5. Lower Epidermis

The lower epidermis thickness of broad bean leaves differed among the treatments. Treatment R1 recorded the highest thickness (7.00 µm), followed by R3 (4.83 µm) and R2 (4.23 µm), while the control (R0) had the lowest value (2.07 µm). These results show that organic fertilization, especially R1, substantially increases the lower epidermal layer of the leaves. (Figure 10).

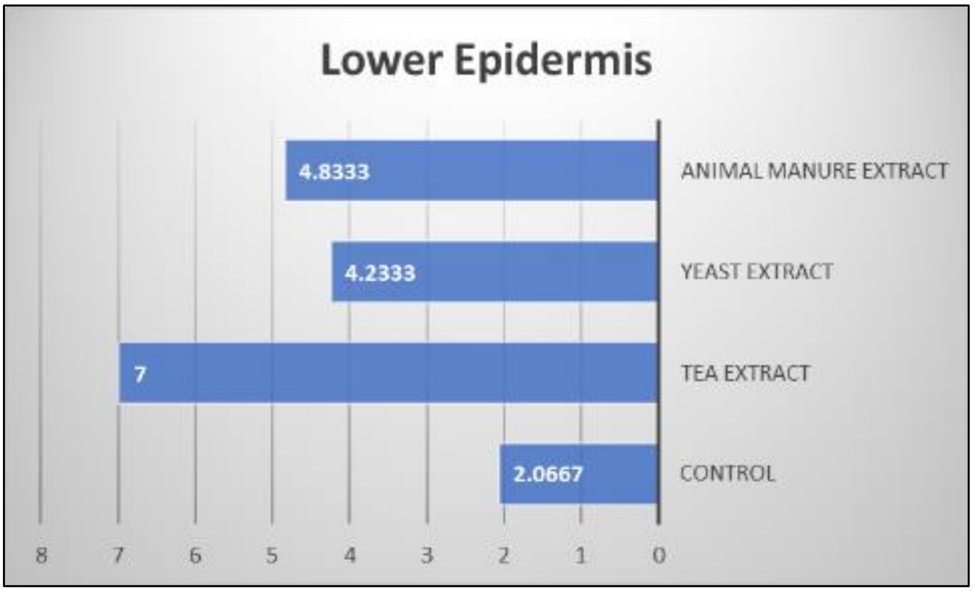

Figure 10 Effect of the treatments on the lower epidermis of the leaf blade compared to control.

3.2.6. Lower Cuticle

The lower cuticle thickness of broad bean leaves varied among the treatments. Treatment R1 showed the highest value (4.08 µm), followed by R3 (2.98 µm) and R2 (2.23 µm), while the control (R0) had the lowest thickness (1.00 µm). These results indicate that organic fertilization, particularly R1, effectively enhances the protective lower cuticle layer of the leaves. (Figure 11).

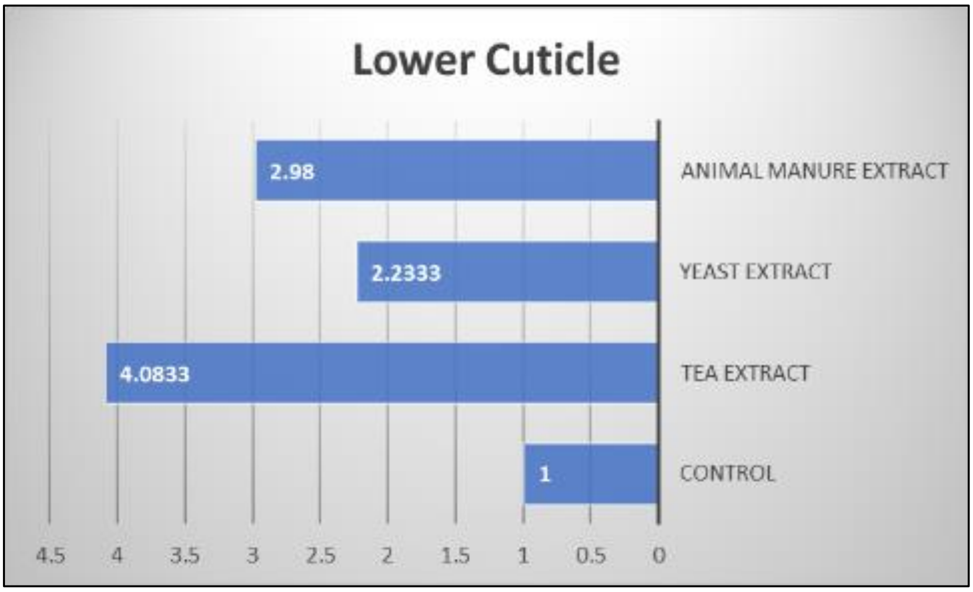

Figure 11 The effect of the treatments on the lower cuticle layer of the leaf blade compared to the control.

The results showed that the anatomical features of leaf blade from each treatment with organic fertilizer were significantly better than those of the control treatment, which could be attributed to the positive impact of organic fertilizer on physiological and anatomical growth in plants. Organic fertilizer treatment resulted in the development of a thicker upper cuticle and lower cuticle layers, which can effectively improve the ability to reduce transpiration water loss and protect the tissue status. This is attributed to the role of organic fertilizer in improving the plant's nutritional status and increasing the formation of waxy substances, thus helping the plant resist various environmental conditions(Čabilovski *et al*., 2023).

The **upper and lower epidermis** also showed increased thickness compared to the control, attributed to improved cell growth resulting from the balanced availability of macro and micronutrients from the organic fertilizer. The epidermis is a protective layer that contributes to regulating gas exchange; therefore, its anatomical improvement positively impacts the efficiency of vital processes within the leaf .They also showed that using organic fertilizer led to a significantly thicker palisade layer. This layer is mainly responsible for photosynthesis. This means that due to the greater number and larger size of palisade cells high in chloroplasts, light absorption for carbohydrate production is also improved. Additionally, the radial layer displayed superior thickness and cellular arrangement configurations that promoted the effectiveness of gas exchange in the leaf (Azizi Yeganeh *et al*., 2024). This presents an anatomical change that indicates the better physiological state of plant condition due to organic fertilizer treatment. This fertilizer enhances all soil characteristics and stimulates the flora (micro-organism) of soil that enhances its nutrient introduction. These results overall indicated that the use of organic fertilizer improved the anatomical structure of leaf blade with respect to control, resulting in enhanced utilization efficiency for photosynthesis, improved hydration capacity and growth, as well as adaptation to changing ecological conditions. These results are in line with Marschner (2012), which noted that better plant nutrition resulted in favorable anatomical stimulation of leaf tissues, particularly the photosynthetic layers. Taiz *et al.* (2017), reported that higher photosynthetic efficiency and transpiration regulation has been demonstrated by increasing palisade and epidermal thickness. Arancon *et al*. (2004) and Edwards *et al*. (2011) mentioned that the use of organic fertilizers not only improves properties of soil and availability of nutrients, but also positively affects plant cell growth by improving leaf anatomical structure. Similarly, Khalid *et al*. (2017) reported that the use of organic fertilizers increases cuticle and epidermal thickness, which allows plants to cope with various environmental stresses.

3 Conclusion

The study showed that integrated organic fertilization significantly improved the anatomical growth of bean stems and leaves. Fertilization with tea extract was the most effective treatment, promoting plant tissue growth and structural development, which may contribute to stronger, healthier plants and improved overall performance

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