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Effect of *Saccharomyces cerevisiae* and Tea Extract on Root Nodulation by *Rhizobium spp.* and Growth Parameters of *Vicia faba L.*

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Abstract: The experiment was conducted at the Field Crops Department Research Station, College of Agriculture, Samarra University, during the winter agricultural season 2024/2025, with implementation beginning on 12 November 2024. The experiment included three experimental treatments: yeast extract (R3), tea extract (R2), and the control treatment (R1). A local variety of broad bean was cultivated. This experiment was designed as a simple randomised complete block design (RCBD), with three replicates, resulting in a total of nine experimental units each unit contained four plants. This study aimed to evaluate the effects of yeast and tea extracts as organic fertilisers on the growth and anatomical characteristics of *fava beans*. Results showed that both biological treatments improved plant morphology compared to the control, with the tea extract yielding the highest stem length, number of leaves, and number of branches. The yeast extract, on the other hand, outperformed the control in the number of nodes. Anatomical analyses further revealed that both treatments increased stomatal number and index in the leaf epidermis, particularly the tea extract group. These improvements are attributed to the natural growth regulators and nutrients present in the extracts. Overall, the results confirm the potential of organic fertilisation in improving plant growth and leaf anatomy. The study recommends the use yeast and tea extracts as natural alternatives to synthetic fertilisers.

Keywords: *Saccharomyces cerevisiae*, *Rhizobium spp.*, *Vicia faba L.*, Tea Extract, Yeast Extract

Introduction

Over the past four decades, the world has witnessed a significant increase in population, leading to an increased demand for food, such as *Vicia faba L.* It holds an important position among food security crops in several countries [1]. This significance stems from the seeds' high protein content, which ranges from 25% to 40%, as well as their role as an energy source and their substantial crude fibre content, which ranges from 5% to 25% [2]. As a substitute to pricey animal proteins, this has made the crop one of the most significant low-cost food sources, particularly for the impoverished [3], [4]. To meet this demand, plant nutrition had to be improved to increase productivity [5], [6]. One of the most prominent solutions used to achieve this was commercial fertilisers, which facilitated crop production at relatively low costs through the implementation of high-intensity, high-productivity farming

systems, however, the excessive use of chemical fertilisers, despite its benefits in increasing agricultural yields, has led to the deterioration of soil health and the deterioration of environmental quality in general, the use of nitrogen (N) fertilisers has increased nine-fold [7]. In contrast, the use of phosphorus (P) fertilisers has more than quadrupled in recent years to meet growing food demand. Despite these increases, excessive fertilisers use and intensive agricultural methods have caused numerous environmental problems, including soil degradation, groundwater and surface water pollution, air pollution, decreased biodiversity, and ecosystem imbalance [8].

Biofertilisers, or microbial inoculants, are fertilisers that assist plants in fulfilling their nutritional requirements by the biological fixation of key elements. They also facilitate the breakdown process. This is achieved by introducing root-like bacteria that enhance plant development and supply nitrogen and phosphate [9]. These microbial preparations are carrier-based and contain live, beneficial microorganisms [10]. They are used on seeds or soil, facilitating enhanced plant development by improving nutrient absorption or stimulating the synthesis of growth hormones. Rhizobia are a genetically diverse and physiologically varied genus of bacteria that induce the development of nodules on the roots of leguminous plants. These bacteria live freely in the soil near the roots of legume plants until nodule formation occurs. Their ability to form symbiotic relationships with legumes is a hallmark of these bacteria. This relationship stimulates the formation of nodules on the roots, where the bacteria fix atmospheric nitrogen and provide it to plants as an essential nutrient [11], [12].

Researchers found that co-inoculation of *Saccharomyces cerevisiae* yeast and *Rhizobium* bacterium when plants were infected with *Rhizobium* bacteria in the presence of a culture filtrate hydrolysed from *S. cerevisiae*, similar results were seen. The tea extract increased the number of nodes, plant height, and dry weight of the plant *Vicia faba* L. planted in soil. Inoculation with live yeast cells, *Saccharomyces cerevisiae*, increased the number of nodes, dry weight of shoots, and biomass of roots in legumes [8]. The purpose of our study was to examine, for the first time, the effect of a recently developed biofertilizer on the development of fava bean plants. The fresh weights shoot lengths, and root lengths of treated and untreated plants were compared to evaluate growth. This study's central premise is that adding yeast and nitrogen-fixing *Rhizobium* bacteria as soil inoculants would increase the efficiency of nitrogen uptake, which will boost legume plant growth and production.

Materials and Methods

Experiment Site

The experiment was conducted at the Field Crops Department Research Station, College of Agriculture, Samarra University, during the 2024/2025 winter agricultural season, beginning on 12/11/2024. The experiment included two experimental treatments: yeast extract and tea extract. A local broad bean variety was grown. This experiment was conducted using a simple randomised complete block design (RCBD) with three replicates. Thus, the total number of experimental units was 9. Each experimental unit contained four plants. Analysis of variance (ANOVA) was performed using SPSS (version 24). The significance of differences between treatments was approximated using the least significant difference (LSD) test at the 0.05 level.

The seeds were planted on 11 December 2024, at a depth of 5 cm, at a rate of 3 seeds per hole, and then thinned to one plant two weeks after planting.

Treatments:

R1: Control treatment (no fertilisation)

R2: Fertilisation with *Saccharomyces cerevisiae* yeast extract (6 g/L)

R3: Fertilisation with tea extract (6 g/L)

Studied traits

Morphological characteristics

Stem length (cm), number of leaves per plant, number of branches per plant, number of nodes per plant, Leaf anatomical characteristics: upper leaf layer (number of stomata, stomatal index, stomatal frequency) / lower leaf layer (number of stomata, stomatal index, stomatal frequency).

Anatomical Study

The epidermis of fresh leaves collected from the field was studied immediately after fixation with Formalin-Acetic Acid-Alcohol (F.A.A.) solution and a mixture of Formalin-Acetic Acid-Alcohol as follows:

1. (90 ml) Ethyl alcohol (70%).
2. (5 ml) Glacial acetic acid.
3. (5ml) Formalin (37-40) %.

The fixation was done with (F.A.A) solution for 24 hours, then washed with 70% alcohol and kept at the same concentration until use. Then, the leaf was taken and placed in a sodium hypochlorite solution (artificial bleach) for 72 hours to obtain the upper and lower skins. It was focused on a fixed place (the middle of the leaf), approximately, so that it includes the middle vein and part of the blade and the edge. Peeling and stripping were employed to acquire the top and lower epidermis, utilising a dissecting blade and fine-tipped forceps. The prepared epidermis was subsequently placed in a Petri dish.

After that, the skin was placed in a Petri dish containing 1% Safranin stain, made with 70% ethyl alcohol, for two to five minutes. To remove excess discoloration, the skin was then repeatedly transferred to Petri plates filled with 70% ethyl alcohol. The skin was then prepared for analysis and research by being moved to a glass slide with a drop of glycerin, brushed with the skin, and covered with a coverslip. Measurements of the sizes and forms of normal skin cells on the top and lower surfaces were made after the samples were inspected. An ocular micrometer was used to measure them under a Motic-type compound microscope, and a Sony HD 16.1-megapixel digital camera was used to take pictures beneath the microscope.

With the modification, a method was used to remove the skin that is different from the old method (skinning method) from the tender plant leaves that were collected directly from the field. This is the Replica method, using a transparent nail polish material and transparent adhesive tape, where a colorless dye material containing (Cellulose acetate) was placed on the upper and lower surfaces of the leaf whose skin was to be removed, after a short period (1-3 minutes), this material dries, and then a transparent adhesive tape is placed over the transparent nail polish. The transparent adhesive tape is lifted in a specific manner, carrying with it a section of the plant part's skin to be examined. The transparent tape carrying the section is stuck to the glass slide. Thus, it is ready for microscopic examination, primarily to identify the hairs and stomata, as well as their frequency in each microscope field, under a magnification power of x40.

The diameter of the microscope field at a magnification power of x40 = 0.5 mm, i.e. its area is equal to 0.196 mm²). The frequency of hairs and stomata refers to the number of these structures per unit area. The stomatal index is calculated using the stomatal index equation. The stomatal index = {number of stomata / (number of stomata + number of normal epidermal cells per unit area)} × 100.

Results and Discussion

This study evaluated the effect of different organic fertilisers treatments (R3 tea extract and R2 yeast extract) on the morphological characteristics of the plants, as well as the R1 control sample. The results showed a big difference between the treatments (R1, R2, and R3) in most of the characteristics studied (stem length, number of leaves, number of branches, and number of nodes), which indicates that organic fertiliser helps improve the plant's growth and structure, as shown in Table 1 below.

Table 1. Effect of organic fertilization treatments on phenotypic traits

Treatments	Number of nodes	Number of branches	Number of leaves	stem length
R1	27	3	27.67	63
R2	47	3.33	44	79.66
R3	41	4.67	54	82

Table 2 illustrates the impacts of different organic fertilisation treatments (R3 and R2) on the plant's structure, with R1 serving as a comparison. The results showed a clear difference between the treatments (R1, R2, and R3) in several characteristics (number of stomata, the stomatal index, and the stomatal frequency) in both the top and bottom layers of the plant's leaves, suggesting that organic fertilisers improves the plant's structure.

Table 2. Effect of organic fertilization treatments on anatomical characteristics.

Treatments	The Upper Layer of The Leaf			Lower Leaf Layer		
	Stomatal Frequency	The Stomatal Evidence	Number of Stomata	Stomatal Frequency	The Stomatal Evidence	Number of Stomata
R1	15.23	28	80	20.38	36.83	120.33
R2	30.78	40.48	136	32.16	45.25	146.33
R3	36.08	48.45	188	35.13	44.72	159

Stem length

The current study found that using tea extract and *Saccharomyces cerevisiae* resulted in a noticeable increase in average stem length compared to the control group, with tea extract reaching the highest length of 82 cm and yeast extract averaging 79.66 cm. In contrast, the control group had the shortest length at 63 cm (Figure 1). The different letters used in the statistics show that there are important differences between the treatments. In comparison, the control treatment recorded the lowest value of 63 cm. The different statistical letters indicate significant differences between treatments. The treatments with extracts were marked with the letter (A), indicating their significant superiority over the control treatment, which was marked with the letter (B). These results indicate the effectiveness of homegrown organic fertilisers for enhancing vegetative plant growth with a positive effect on stem length.

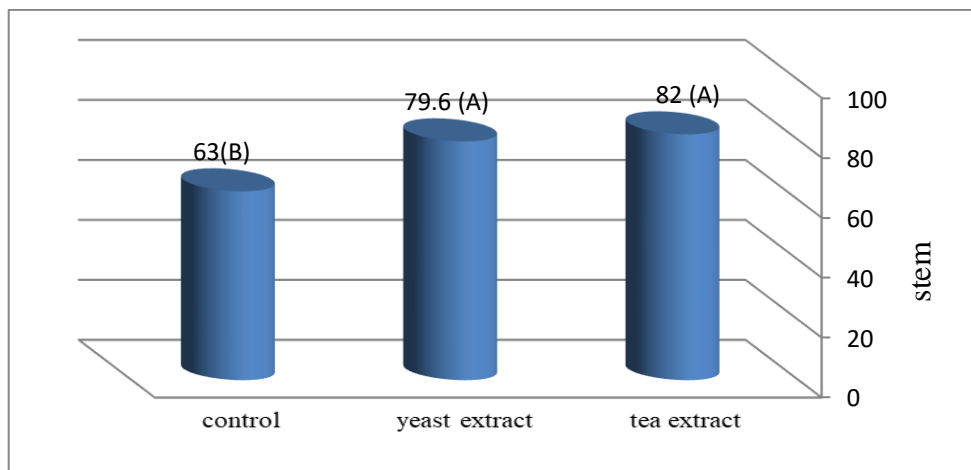


Figure 1. The effect of some biological treatments on the stem length of the plant compared to the control.

This improvement in stem length is attributed to the presence of growth-stimulating compounds in these fertilisers, such as auxins and cytokinin's, which contribute to stimulating cell division and elongation and positively impact longitudinal growth [13]. Auxins play a pivotal role in increasing stem growth by promoting cell elongation in the meristematic regions. Some yeast species can dissolve insoluble inorganic phosphates, making them readily available to plants, which positively affects growth [14].

Number of leaves

The current study demonstrated that organic fertiliser treatments significantly increased leaf counts compared to control treatments. Tea extract recorded the highest leaf number, with an average of 54 leaves, and was marked with the letter (A), indicating its significant superiority over the other treatments. As for the yeast extract, it reached 44 leaves, marked with the letter (B), indicating that there was no significant difference between them, but they significantly outperformed the control treatment. The control treatment had the fewest leaves, averaging 27.67, and was labelled with the letter "C," as shown in figure 2, which shows that it grew less well than the other treatments.

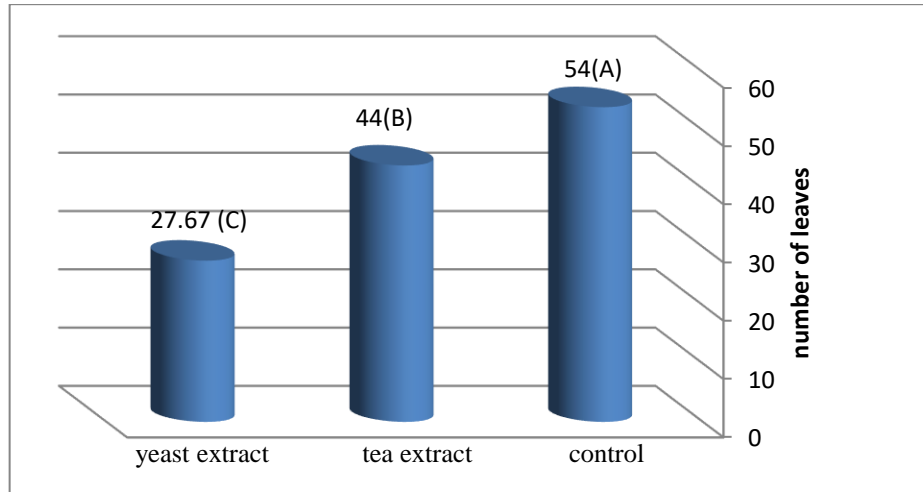


Figure 2. The effect of some biological treatments on the number of plant leaves compared to the control.

This increase in the number of leaves is due to fertilisers containing organic compounds and natural growth regulators, such as auxins and cytokinin's, which encourage cell division and boost the plant's activities, resulting in more leaves.

Number of branches

The current results, as shown in Figure 3, showed that the different biological treatments significantly increased the number of plant branches compared to the control treatment. The tea extract treatment recorded the highest number of branches, reaching 4.67. This number was followed by the yeast extract, which recorded 3.33 branches, while the control treatment (without addition) recorded the lowest value, 3.0 branches. Despite this numerical disparity, the statistical letter (A) common to all treatments indicates that there were no apparent significant differences between them.

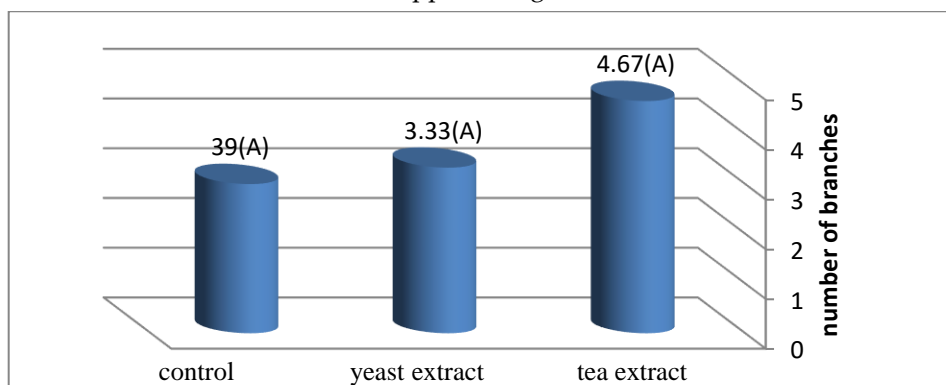


Figure 3. The effect of some biological treatments on the number of plant branches compared to the control.

The improved branching in the biological treatments is attributed to the extracts containing natural nutrients [15], plant growth regulators, and amino acids, which stimulate cell division and encourage lateral plant growth. These treatments also stimulated microbial activity in the soil and enhanced nutrient absorption, leading to improved vegetative characteristics of the plant. Several studies have indicated that organic extracts can increase the number of branches [16]. They demonstrated that yeast extracts contain growth-stimulating substances, including vitamins and plant hormones [17]. Endophytic yeast makes indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA), which help plants grow better. They observed a significant increase in plant growth by measuring root and shoot lengths, as well as dry weight, and levels of IAA and IPYA within the plant.

Number of nodes

The current results, as shown in Figure 4, indicate that the biological treatments led to an increase in the number of nodes in the plant compared to the control treatment, with the yeast extract recording the highest value of 47. This result was followed by the tea extract treatment, which recorded 41 nodes. In contrast, the lowest value was recorded in the control treatment, which recorded 27 nodes. However, the statistical letters (A, B, and C) between all treatments indicate the presence of significant differences between them.

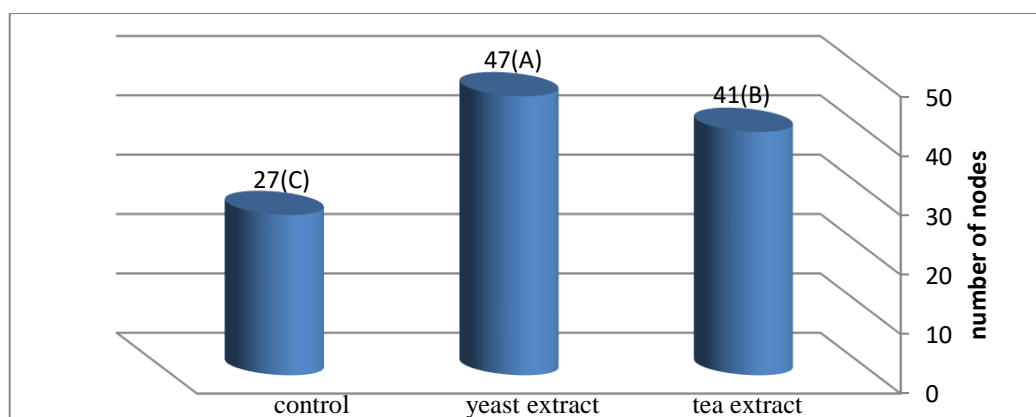


Figure 4. The effect of some biological treatments on the number of nodes in the plant compared to the control.

The current study demonstrated that treating plants with yeast extract is beneficial because the yeast *Saccharomyces cerevisiae* aids in rhizobium growth, thereby enhancing plant growth. Research has shown that yeast can collaborate with other beneficial bacteria in nature, and this partnership may enhance nitrogen fixation in various types of rhizobia [18].

Saccharomyces spp., not only affect the growth of plants and microbes but also play a role in the formation of soil aggregates and the maintenance of its structure. This phenomenon may be another reason for the increased growth observed in treated plants [19]. The study showed that specific yeast species, apart from *Saccharomyces cerevisiae*, synthesis carotenoids, which are important components of plant nutrition and serve as precursors to vitamin A. Naz et al. concluded that the identical yeast strain can synthesis polyamines (spermine and spermidine) that enhance cell proliferation in roots. A multitude of yeast species not only facilitates accelerated development but also plays a crucial role in the mineralization of organic materials and the dissipation of carbon and energy within the soil. A multitude of yeast species have been associated with functions in the natural nitrogen and Sulphur cycles in soil [14], [20], [21].

Anatomical characteristics of the leaf

The upper layer of the leaf

Number of stomata in the upper layer of the leaf

The current results showed that the biological treatments had a significant effect in increasing the number of stomata in the upper layer of the leaf compared to the standard treatment (control). The

tea extract recorded the highest number of stomata (188 A), followed by the yeast extract treatment (136 B). In contrast, the standard treatment recorded the lowest number of stomata (80°C). These results show that using biological treatments, especially tea extract, helped improve leaf growth and boost the plant's activity, as indicated by the higher number of stomata, a crucial sign of the plant's ability to photosynthesis and remain healthy.

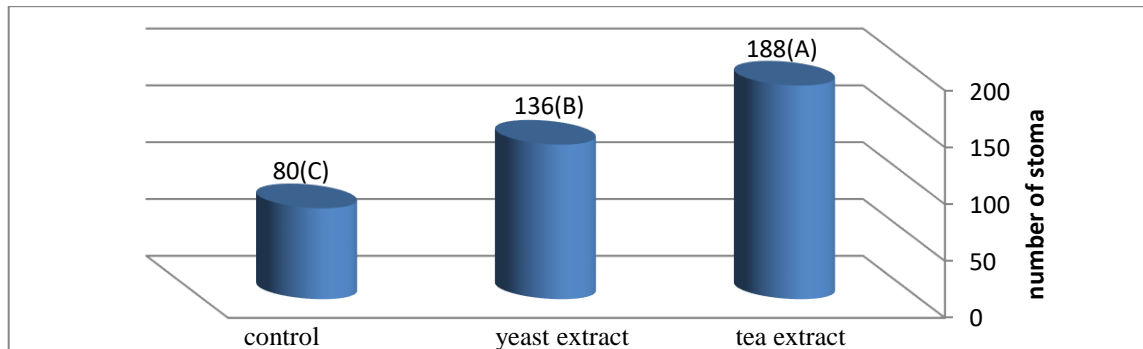


Figure 5. The effect of some biological treatments on the number of stomata in the upper layer of the leaf compared to the control.

These results align with those of Khan et al. [22], indicating that tea extract contains substances that promote plant growth, such as natural plant hormones (gibberellins, cytokinin's, and auxins), which stimulate cell division and the development of new tissue, ultimately leading to increased stomatal density. A study by Liu et al. [23], showed that organic fertilisers enhance soil fertility and increases the availability of nutrients, which is reflected in improved plant growth and increased anatomical structures in leaves, including stomata.

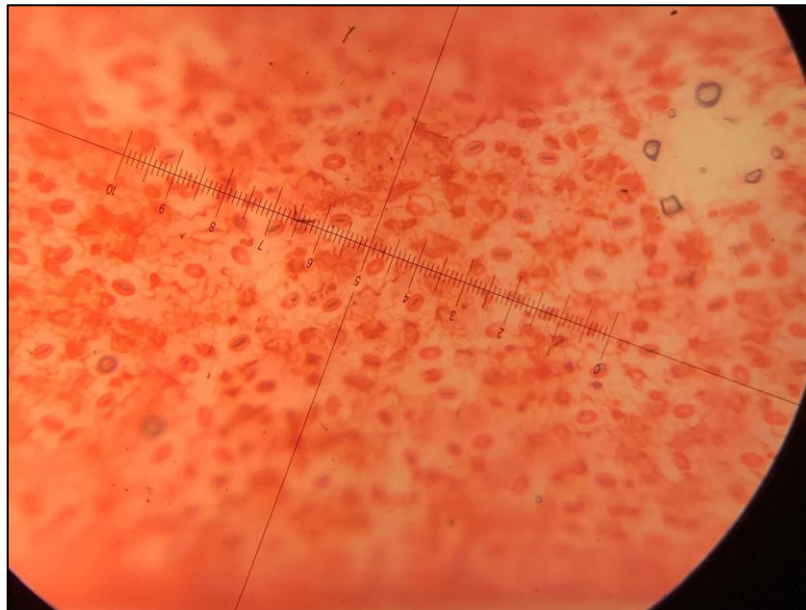


Figure 6. Shows the number of stomata in the upper layer of the leaf under a microscope.

4-2-1-2 The stomatal guide in the upper layer of the leaf

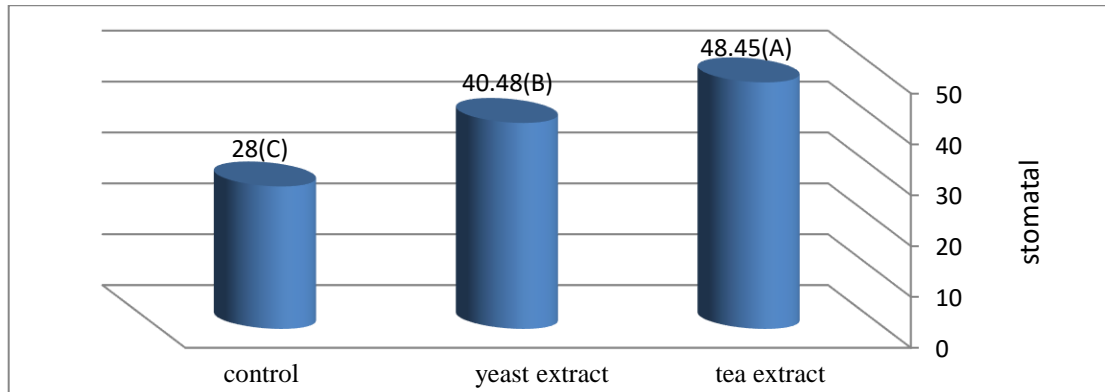


Figure 7. The effect of some biological treatments on the stomatal index in the upper layer of the leaf compared to the control.

4-2-1-3 The frequency of the gap in the upper layer of the leaf

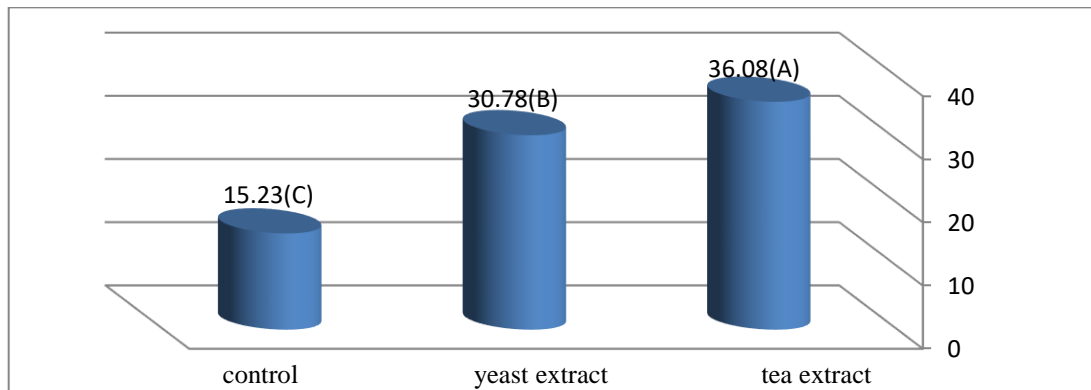


Figure 8. The effect of some biological treatments on the frequency of stomata in the upper layer of the leaf compared to the control.

4-2-2 The lower layer of the leaf

4-2-2-1 Number of stomata on the lower leaf layer

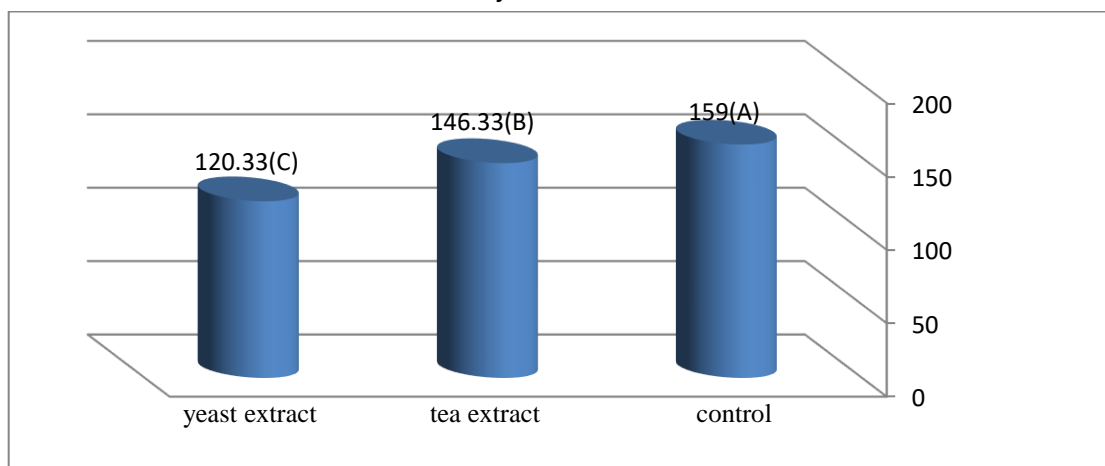


Figure 9. The effect of some biological treatments on the number of stomata in the lower layer of the leaf compared to the control.

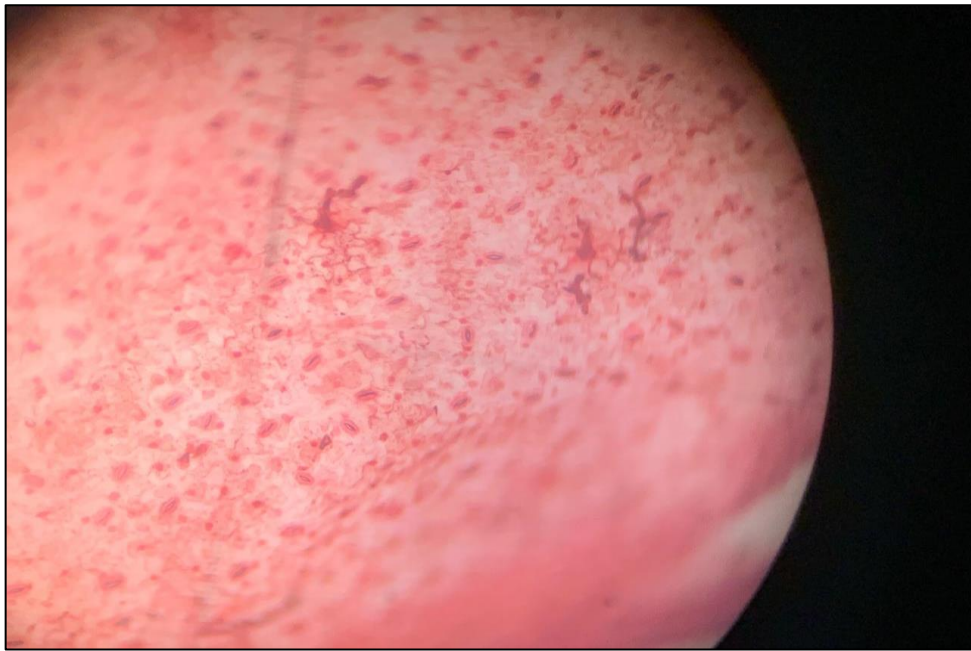


Figure 10. Shows the number of stomata in the lower layer of the leaf microscopically.

4-2-2-2 The stomatal guide of the lower leaf layer

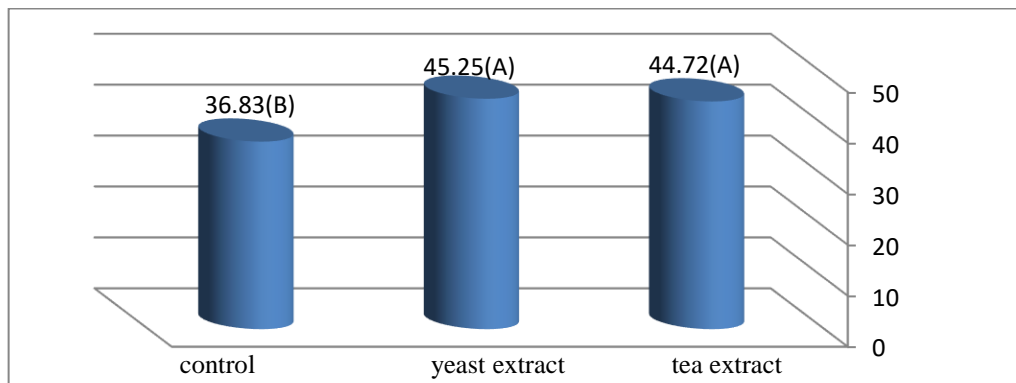


Figure 11. The effect of some biological treatments on the stomatal index in the lower layer of the leaf compared to the control.

4-2-2-3 The frequency of the lower leaf lay

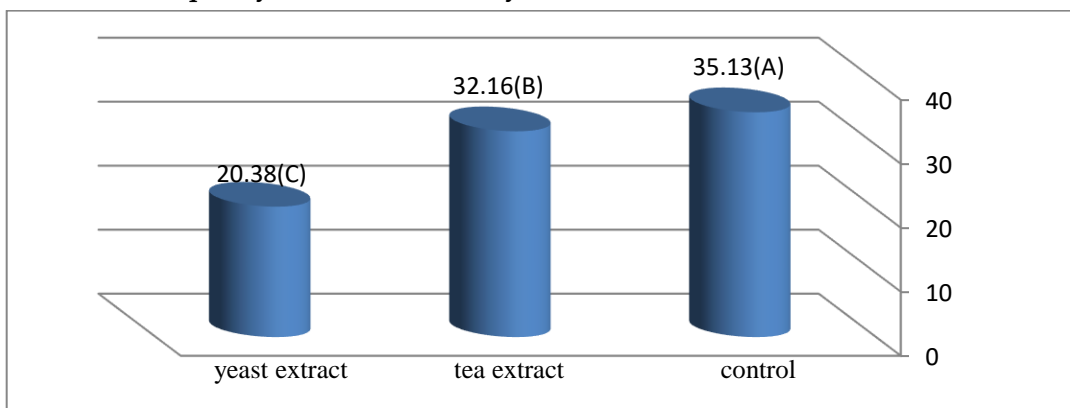


Figure 12. The effect of some biological treatments on the frequency of stomata in the lower layer of the leaf compared to the control.

When we examine the results from Figures 5, 7, 8, 9, 11, and 12 together, it is clear that the biological treatments, particularly the tea extract, consistently improve various signs related to the stomata, including the number of stomata, stomatal index, and stomatal frequency. The tea extract showed the best results in all three signs, indicating that it is very effective in increasing the number of stomata and ensuring they are evenly distributed. The tea extract showed the best results in all three signs, indicating its effectiveness in boosting the formation of stomata and ensuring they are evenly distributed. The tea extract recorded the highest values in all three indicators, indicating its high effectiveness in enhancing the formation of stomata and the regularity of their distribution.

These results indicate that increasing the number of stomata is associated with an increase in both the stomatal index and stomatal frequency, reflecting an overall improvement in the leaf's ability to perform its physiological functions. These results support previous studies, such as Khan et al. (2009) and Liu et al [24], on the effects of bio extracts on stimulating plant physiological and anatomical growth, as well as the importance of organic fertilisation in supporting leaf tissue growth and stomatal formation.

Conclusion

The current study demonstrated that treatment with tea and yeast extract resulted in a significant increase in the average length, number of leaves, number of branches, and number of nodes compared to the control samples. The tea extract yielded the highest values in increasing stem length, number of leaves, and number of branches compared to yeast. As for *Saccharomyces cerevisiae*, it yielded the highest value for increasing the number of nodes compared to the tea extract.

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