Effect of Foliar Spraying with Melatonin and Dry Yeast Extract on Vegetative Growth Indicators and Some Physical and Chemical Characteristics of Fig Seedlings (*Ficus carica* L.)

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Abstract

The experiment is carried out in a protected nursery at Al-Nasiriya city, Dhi Qar Governorate, Iraq, for a period of 8 weeks. The effect of foliar melatonin and dry yeast extract on the vegetative growth of seedlings and some physical and chemical properties of fig seedlings is investigated. The experiment is designed as a 4 × 3 factorial (0, 50, 100, and 150 mg L^{-1} melatonin and 0, 5, and 10 g L^{-1} dry yeast extract) in a randomized complete block design (R.C.B.D) with a 14-day interval between applications. The highest plant height (34.70 cm) and total chlorophyll content (2.69 mg gL^{-1} FW) are found with the combination of 150 mg L^{-1} melatonin and 10 g L^{-1} dry yeast extract. Therefore, enhanced vegetative growth, pigment concentration, and nutrient status are obtained by the combined foliar treatment.

Keywords: Biostimulants, Ficus carica, Melatonin, Seedling Vigor, Yeast Extract.

Introduction

The fig (*Ficus carica* L.) is an economically and nutritionally valuable fruit tree species that is cultivated in Mediterranean and Middle East countries. Orchard establishment and performance will be uniform with good quality seedlings [1].

Vegetative traits such as plant height, stem diameter, leaf number, leaf area and biomass allocation are used to assess seedling quality [2, 3] while chlorophyll and nutrient concentrations are used to assess photosynthetic capacity and nutritional status, respectively.

Thus, enhanced development of these characteristics is important for nursery care. Melatonin has been identified as a signaling molecule in plants and has been also shown to have beneficial impacts on growth, photosynthetic productivity, nutrient status, and abiotic stress tolerance [4] [5].

Dry yeast extract is regarded as a biologically derived biostimulant and its amino acids, vitamins, minerals, and growth stimulating compounds have been related to increase in vegetative growth and nutrient assimilation [6].

The synergic use of melatonin and DYE to fig seedlings, however, has not been documented to a sufficient extent. Thus, the responses of these treatments when applied alone and together to vegetative growth and some physical and chemical seedling properties are tested. The combination therapy is thought to have a more potent effect than either therapy alone [7,8].

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Material and Methods

Experimental site and conditions

The experiment was performed in a protected nursery under a hot Mediterranean-subtropical climate in Al-Nasiriya, Dhi Qar Governorate, Iraq with a mean daily temperature of 20 – 38 °C for experimental period and mean RH of 35 – 60%. The growing environment is described by daily meteorological data (air temperature, relative humidity and daily irradiation), which are recorded at a meteorological station in the vicinity of the experiment. Seedlings are cultivated under 50% shading net in the plastic greenhouse to prevent excessive irradiance, and natural ventilation and forced circulation fan are used to control excessive temperature. The pots are uniform plastic pots (15 cm diameter x 2.5 L capacity) filled with a standard substrate of peat moss: washed river sand: loamy soil (2:1:1, v/v/v) and stored on raised beds with a spacing of 30 cm between pots. The drip irrigation is delivered through an emitter system designed to deliver uniform irrigation, and watering is done to maintain substrate moisture near field capacity, but not waterlogged. Biotic stresses are kept to a minimum throughout the trial by application of routine nursery sanitation practices and integrated pest management.

Plant material

The cultivars used are widely cultivated in the local community and are propagated by hardwood cuttings from healthy and virus free mother plants of Ficus carica L. At the beginning of the treatments seedlings are an average of 22 ± 3 cm tall and 4.2 ± 0.5 cm in diameter in the same substrate and under the same irrigation regime, after having been acclimatised for 2 weeks in the greenhouse for the same substrate and irrigation regime. Only plants without uniform growth and damage are discarded prior to randomisation; light pruning is done to remove basal shoots and to standardise the initial architecture.

Experimental design

The experiment is arranged as a two-factor 4 × 3 factorial experiment in a randomized complete block design (R.C.B.D) with three replicates. Melatonin is applied at 0, 50, 100, and 150 mg L−1, and dry yeast extract is applied at 0, 5, and 10 g L−1. All 12 treatment combinations are included, with four seedlings per replicate. Pots are randomized within each block, and buffer rows are used to reduce spray drift.

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Preparation and application of treatments

Preparation of melatonin (N-acetyl-5-methoxytryptamine, analytical grade) stock solution: Melatonin powder is dissolved in a small amount of 95% ethanol (<1% v/v) and diluted with distilled water to desired concentration, and then a non ionic surfactant (Tween 20) is added to the solution at 0.05% (v/v) to enhance leaf wetting. Dry yeast extract is made by suspending the desired amount of commercial baker's yeast (Saccharomyces cerevisiae) in warm distilled water (40 °C) and letting it sit for 30 minutes with mild agitation before it is filtered through muslin cloth, making a uniform extract with the appropriate concentrations. Foliar applications are made with a hand-held compression sprayer that is calibrated to

apply about 50 mL per plant to the point of surface runoff. The first application is made when the seedlings grow to about 25 cm (2 weeks after acclimatization) and the remaining 2 applications are done at 14-day intervals (at 14 and 28 days). Evaporation and photo toxicity are minimized by performing applications in the early morning (07:00 - 09:00). Sprayers are cleaned between uses and are clearly labelled for each application to prevent cross-contamination and used at different application levels.

Measured physical characteristics

The vegetative growth and morphological parameters are measured at the end of the experiment (8 weeks after the first application) and an intermediate time-point (4 weeks) to measure the response dynamics. The height of the plant (cm) is determined by using a graduated ruler from the top of the substrate to the highest point of the shoots. Stem Diameter (mm) is recorded with a digital caliper, 1 cm above the surface of the substrate. The total number of leaves per plant is counted and the leaf area (cm2) of three representative fully expanded leaves per plant (middle canopy position) is measured with a portable leaf area meter (or three leaves per plant sample area estimated with a validated nondestructive leaf area formula of length × width × a correction coefficient). Root systems are washed gently in the harvest to remove the substrate and the length of the main root (cm) is measured; root volume (mL) is measured by water displacement. Shots and roots are measured fresh weight immediately after harvest with an analytical balance and oven dried at 70 °C until a constant weight (usually 48–72 h) for dry biomass determination. Total dry biomass and shoot/root ratio are determined.

Measured chemical characteristics

Leaves are harvested from the middle third of the tree canopy and analysed for pigment content and nutrients between 09:00 and 11:00, minimising diurnal variation. 0.5 g of fresh leaf tissue is extracted with 10 ml of 80% acetone; chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents are measured by absorbance measurement on a UV Vis spectrophotometer at 663, 645 and 470 nm, respectively, and calculated by standard equations and expressed on a fresh weight basis. For macronutrient analyses, composite leaf samples per replicate are oven-dried, ground, and then digested by Kjeldahl digestion for total nitrogen content, molybdenum-blue colorimetric digestion for phosphorus, and flame photometry for potassium content. Ca and Mg are measured by atomic absorption spectrophotometry and selected micronutrients (Fe, Zn, Mn and Cu) are determined after acid digestion. Another biochemical parameters such as total soluble sugars (anthrone method), proline content (ninhydrin assay) and total phenolics content (foline-ciocalteu method) are also determined according to the standard protocols. Chemical assays use proper blanks, standards and replicates and subsamples are kept at −20 °C if analysis is not done immediately.

Statistical analysis

Collected data is analysed using the two-way analysis of variance (ANOVA) for factorial R.C.B.D with blocks as the random effect and the level of melatonin and the level of yeast-extract as the fixed effects. Prior to analysis, normal and homogeneity of variance are tested. Mean separation at $p \leq 0.05$ is done using Tukey's honestly significant difference test. Data are reported as mean ± SEM and different letters are used to show significant differences in tables and figures.

Results and Discussion

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Results

The results of the experiments on the effect of melatonin and dry yeast extract applied to seedlings are summarized in this section. Data are presented and arranged to show the individual and interactive effects of these biostimulants on physical growth and chemical leaf profiles. Numerical results are reported as means and statistical significance is determined by two-way ANOVA and mean separation tests.

Physical growth characteristics

The results of the analysis of variance (ANOVA) for the most important physical growth parameters are summarized in Table 1. Both melatonin and application level of yeast had significant effects ($p < 0.05$) in all measured traits, as well as the interaction between these factors.

Table 1: ANOVA summary for physical growth characteristics of fig seedlings.

Source of Variation	Plant Height	Stem Diameter	Leaf Number	Shoot Dry Weight	Root Dry Weight
Melatonin (M)	*	*	*	*	*
Yeast (Y)	*	*	*	*	*
M × Y Interaction	*	*	*	*	*
*Significant at $p < 0.05$					

According to Figure 1, the combination of melatonin and yeast has a significant effect on plant height. The height of seedlings is measured, and the maximum is recorded as 34.70 cm in the seedlings treated with 150 mg/L melatonin and 10 g/L yeast, which is higher than that of the seedlings in the control group (18.13 cm). Likewise, these treatments have a positive effect on stem diameter as shown in Figure 2 with the greatest stem diameter of 49.27 mm resulting from the treatment of M150-Y10.

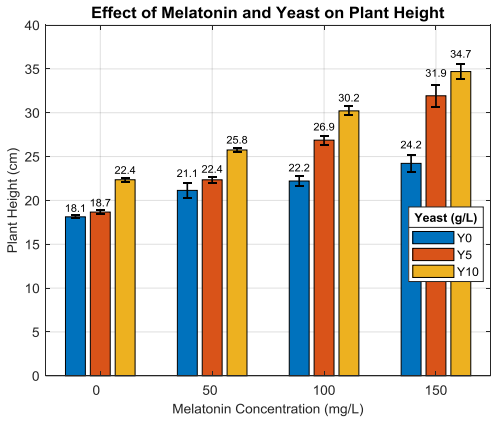


Figure 1: Effect of foliar-applied melatonin and dry yeast extract on the plant height (cm) of fig seedlings.

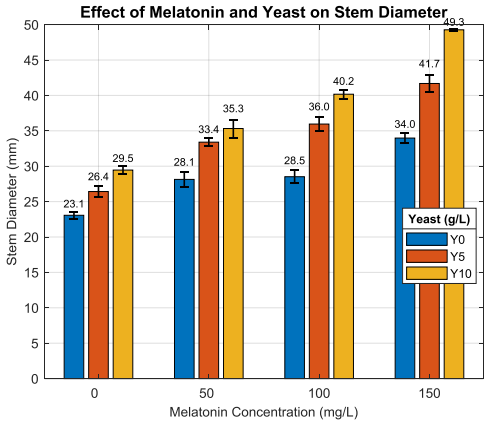


Figure 2: Effect of foliar-applied melatonin and dry yeast extract on the stem diameter (mm) of fig seedlings.

The foliar treatments also significantly enhance the total number of leaves and the total leaf area. In Figure 3 the number of leaves is indicated as 11.32 in the control and 23.07 in the superior treatment. As seen in Figure 4, the leaf area increases greatly, by up to 45.05 cm² in the highest dose of both substances. It seems that the response to both the biostimulants is linear with an increase in concentration.

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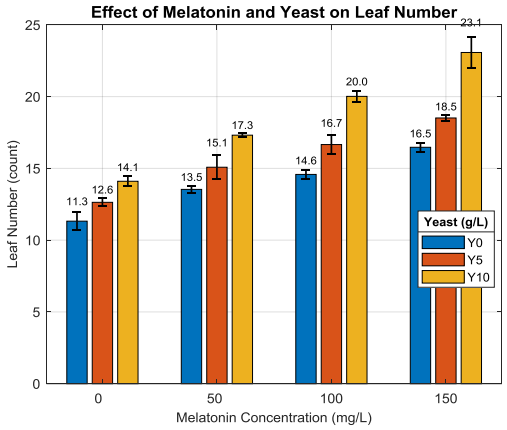


Figure 3: Effect of foliar-applied melatonin and dry yeast extract on the total leaf number per plant of fig seedlings.

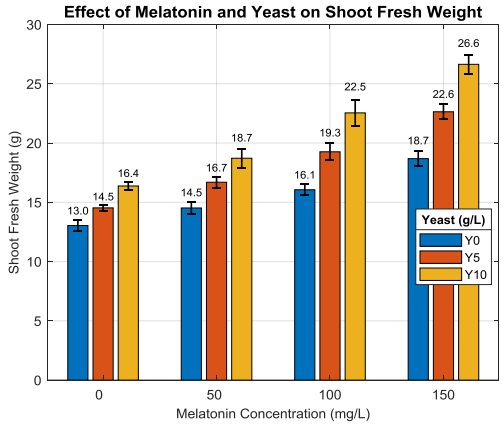


Figure 5: Effect of foliar-applied melatonin and dry yeast extract on the shoot fresh weight (g) of fig seedlings.

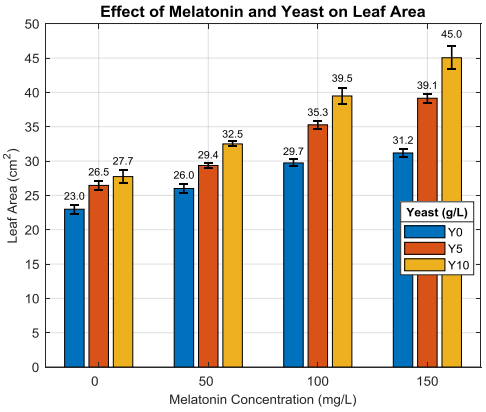


Figure 4: Effect of foliar-applied melatonin and dry yeast extract on the leaf area (cm²) of fig seedlings.

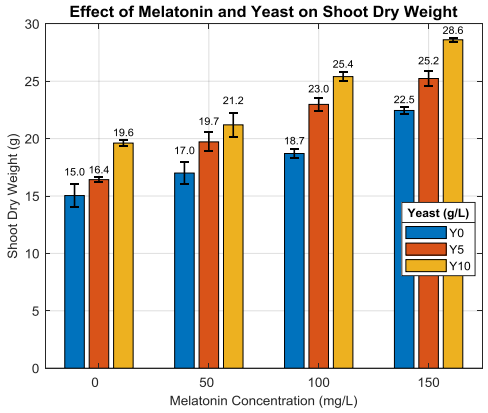


Figure 6: Effect of foliar-applied melatonin and dry yeast extract on the shoot dry weight (g) of fig seedlings.

A similar pattern is seen in the allocation of biomass (shoot and root weights). In Figures 5 and 6, shoot fresh and dry weights are shown to reach their peaks of 26.64 g and 28.61 g, respectively. Root development is also stimulated as seen in Figures 7 and 8, its fresh weight and dry weight are highest at 59.50 g and 38.35 g respectively. The data indicate that the treatment with the combination of two applications is significantly more effective than each one applied separately.

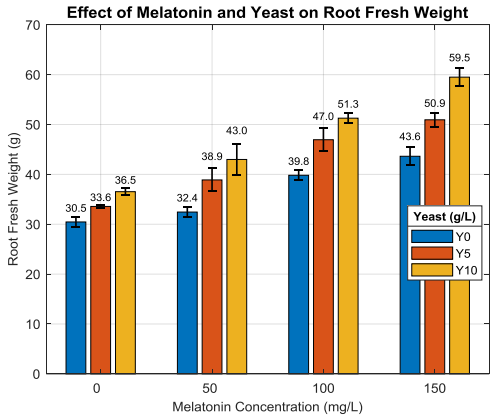


Figure 7: Effect of foliar-applied melatonin and dry yeast extract on the root fresh weight (g) of fig seedlings.

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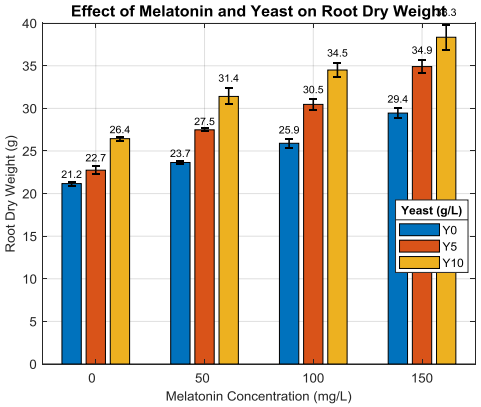


Figure 8: Effect of foliar-applied melatonin and dry yeast extract on the root dry weight (g) of fig seedlings.

Chemical characteristics of leaves

As shown in Figures 9 - 12, the pigment concentration in the leaf is highly affected by the treatment(s). The total chlorophyll content is found to be increased from 1.28 mg/g FW at control to 2.69 mg/g FW at M150-Y10, which shows that the photosynthetic capacity is increased. The same trend is observed in the case of chlorophyll a and b content (2.10 and 1.17 mg/g FW respectively) as displayed in the respective figures.

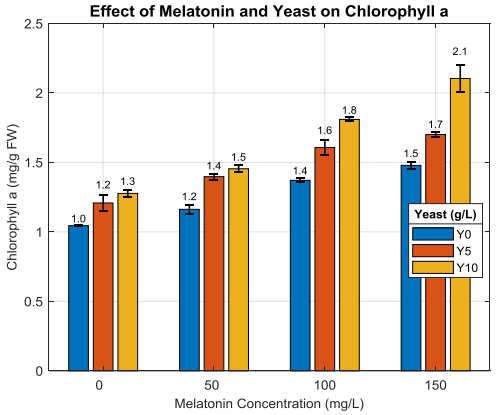


Figure 9: Effect of foliar-applied melatonin and dry yeast extract on leaf chlorophyll a content (mg/g FW) of fig seedlings.

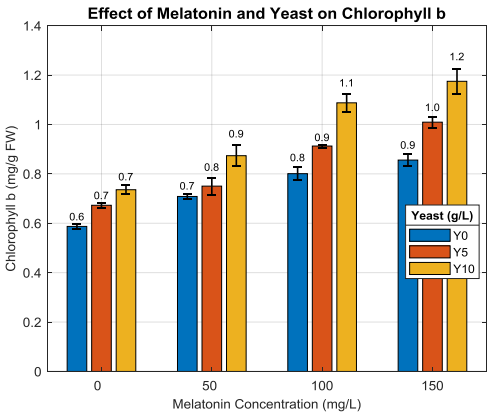


Figure 10: Effect of foliar-applied melatonin and dry yeast extract on leaf chlorophyll b content (mg/g FW) of fig seedlings.

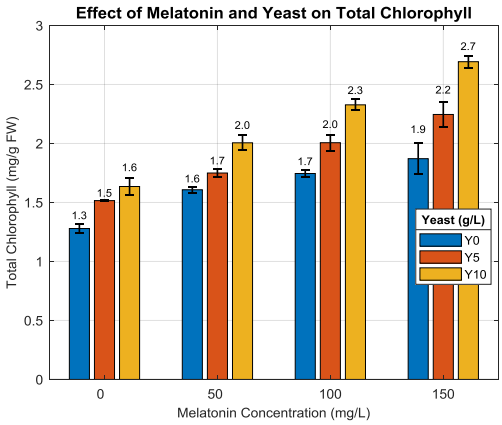


Figure 11: Effect of foliar-applied melatonin and dry yeast extract on total leaf chlorophyll content (mg/g FW) of fig seedlings.

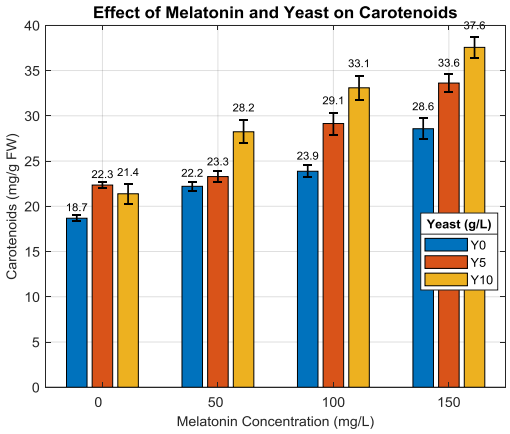


Figure 12: Effect of foliar-applied melatonin and dry yeast extract on leaf

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carotenoid content (mg/g FW) of fig seedlings.

Melatonin and yeast are also used to enhance nutrient accumulation patterns. Figure 13 demonstrates that leaf nitrogen (N) increased from 2.31% to 4.82% for the most effective treatment. Similar improvements in phosphorus (P) and potassium (K) levels are shown in Figures 14 and 15, where the improvements are 0.63% and 4.08% respectively. The result indicated that these biostimulants help the seedlings of fig trees to better uptake and assimilate nutrients.

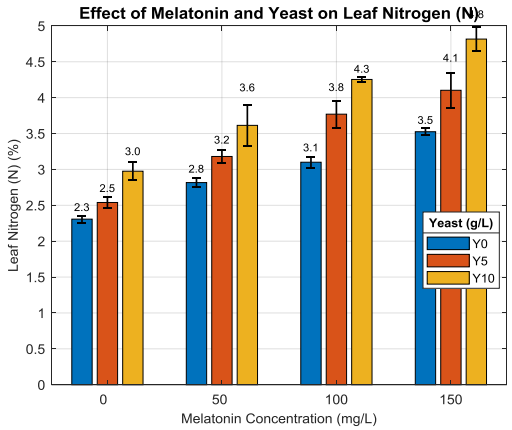


Figure 13: Effect of foliar-applied melatonin and dry yeast extract on leaf nitrogen (N) concentration (%) of fig seedlings.

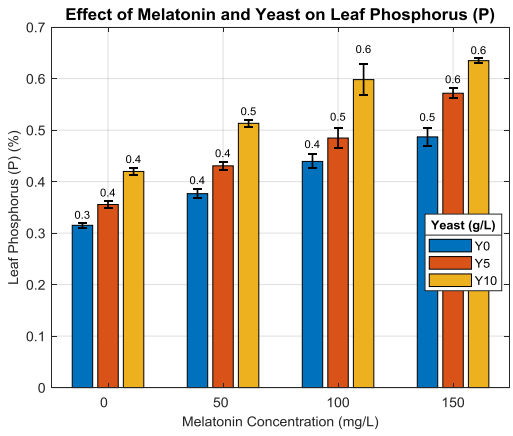


Figure 14: Effect of foliar-applied melatonin and dry yeast extract on leaf phosphorus (P) concentration (%) of fig seedlings.

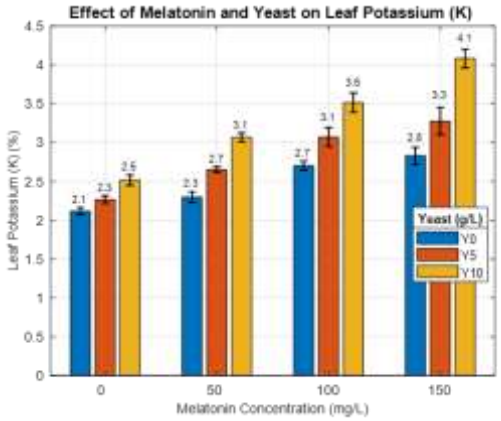


Figure 15: Effect of foliar-applied melatonin and dry yeast extract on leaf potassium (K) concentration (%) of fig seedlings.

The biochemical indicators of plant vigour and stress tolerance given are in Figures 16, 17 and 18. Increase in total soluble sugars to 32.27 mg/g DW is observed as shown in Figure 16 and the proline content increases to 27.28 µmol/g FW as seen in Figure 17. Additionally, the amount of total phenolics is markedly increased, reaching the highest value of 54.16 mg GAE/g DW in Figure 18. The increases are a result of a high metabolic response from the foliar biostimulants.

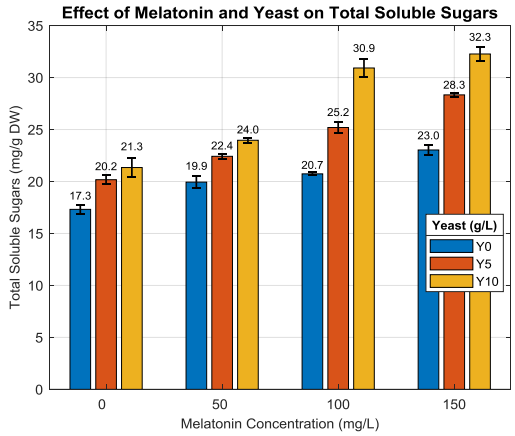


Figure 16: Effect of foliar-applied melatonin and dry yeast extract on total soluble sugar content (mg/g DW) of fig seedlings.

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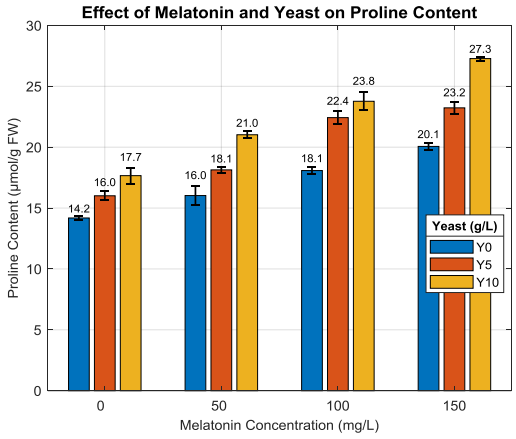


Figure 17: Effect of foliar-applied melatonin and dry yeast extract on leaf proline content (µmol/g FW) of fig seedlings.

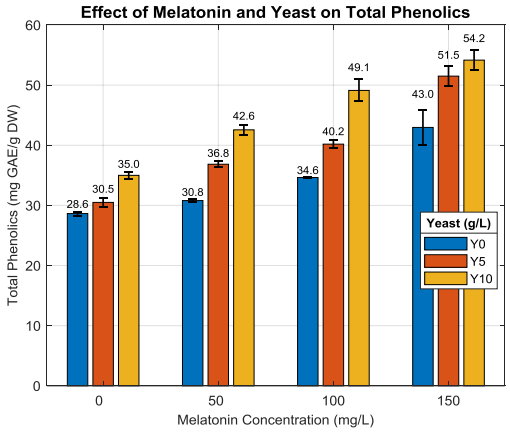


Figure 18: Effect of foliar-applied melatonin and dry yeast extract on total phenolic content (mg GAE/g DW) of fig seedlings.

Interaction effects and response patterns

There is a strong synergistic effect of melatonin with dry yeast extract on both physical and chemical characteristics. A greater response to elevated melatonin is found in the presence of higher levels of yeast. The M150+Y10 treatment is always determined as the most effective treatment and yields the greatest nutrient status, chlorophyll density and vigor of seedlings.

The trends shown in the data suggest that the dose increments to be consistently positive but not linear. Melatonin is very effective in enhancing all parameters as compared to the control, and this efficacy is almost doubled when yeast is added to the foliar program. This implies that the two substances act synergistically and are involved in different pathways to improve the overall physiological functions of fig seedlings.

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Discussion

In this section, the benefits obtained by foliar application of melatonin and dry yeast extract on the physical and chemical characteristics of the fig seedlings are discussed. The results are discussed based on physiological mechanisms, and also in relation to other literature available and in terms of implications for nursery management.

Interpretation of growth responses

The higher levels of plant height, stem diameter, leaf number, leaf area and biomass are due to the combined effect of the two biostimulants. Melatonin is said to control the growth, elongation, root system, and stress-related signaling of cells [4] and compounds from yeast are reported to stimulate cell growth and nutrient uptake [6]. This enhanced response from M150–Y10 is assumed to be a combination of the biostimulant effect of both materials (not one or the other) [7].

Interpretation of chemical and physiological changes

Photosynthetic and nutritional capability is enhanced by foliar treatments as shown by the increases in chlorophyll, carotenoids, and macronutrients in the leaves. Melatonin or naturally occurring biostimulants are found to improve the concentration of the pigments, nutrients

and metabolites involved in stress reactions in horticultural crops, as reported [4, 5, 6]. The build up of soluble sugars, proline and phenolics is also regarded as an index of increased metabolic adjustment and protective ability [4, 8].

Comparison with previous studies

The findings of the present study somewhat agree with the reports of melatonin's positive effects on vegetative performance and stress tolerance in woody and horticultural plants [4, 5]. They are also similar to the known effects of biologically sourced biostimulants on plant growth, nutrient uptake and sustainability [6, 7]. The synergistic effect noted in fig seedlings further confirmed that melatonin and dry yeast extract may be used in a single foliar programme in the conditions tested.

Practical implications for fig nurseries

According to the experimental results, the application of melatonin + 10 g/L dry yeast extract in the leaves of the fig tree is recommended in nurseries at 150 mg/L. This method of treatment is effective and carried out once every 14 days; high-quality seedlings with good physical and chemical characteristics are produced. Use of this biologically based program has several benefits for growers: taking the pressure off the need to use synthetic chemical fertilizers and encouraging more sustainable nursery practices. Therefore it is thought to be economically attractive and practical to use this approach for commercial use, as the cost of dry yeast extract is relatively low and melatonin is effective at low concentrations. This growing regime should yield seedlings better established and with higher survival rate when planted in the field.

Conclusion

The findings of this research demonstrate the significant effects of these seedling treatments: melatonin and dry yeast extract (DYE) on physical and chemical properties of fig seedlings. The results indicate that melatonin + yeast extract treatment in combination produces the maximum growth vigor, photosynthetic capacity and nutrient accumulation at all tested concentrations of melatonin. The results are presented as a nursery management recommendation to implement this integrated biostimulant program as an alternative that is both sustainable and cost-effective to synthetic fertilizers. The synergism that was identified between the two substances is emphasized as the main reason for better seedling quality. In future studies, it is recommended to test these treatments under a variety of field situations and with different cultivars of fig trees. Moreover, the long term effect of these biostimulants on fruit yield and fruit quality at later developmental stages are recognized as crucial for further research. The protective action of these substances against particular environmental stresses (salinity and drought) is further suggested to be studied in the future to further improve the stress resistance of these fig nurseries.

Limitations and future research

The results can be used for the specific nursery conditions tested. The result of the experiment may differ under other environments and genetic materials because the experiment is carried out at a particular site, with a particular season and cultivar of fig. Hence, treatments are suggested for testing under field conditions and with other cultivars. The long-term effects on fruit yield and quality and salinity or drought performance are also determined for future research.

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