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Effect of Foliar Spray of Bioenzyme and Fruit Pulp Extract on Growth, Photosynthetic Pigments, and Oxidative Stress Parameters in Tomato (*Solanum lycopersicum*)

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Abstract: Considering the growing worldwide population, there's a pressing need to augment food production. To address this challenge, efforts are underway to enhance crop yields. Scientists prefer biological components over chemicals to boost productivity, mainly due to their eco-friendly attributes and minimal harm to organisms. This study involved the application of two different products: bioenzyme, a fermented substance, and extracts from orange, grapes, and pomegranate fruit pulp. These were applied through foliar spraying on tomato plant leaves every 15 days post-sowing. The investigation encompassed evaluations of growth parameters, photosynthetic pigments, and oxidative stress parameters. The study's outcomes unveiled noteworthy findings regarding tomato plants. All dilutions of bioenzyme and fruit pulp extract prompted significant height increments. Particularly, the treatment involving 1ml per litre of fruit pulp showcased a highly significant increase in the leaf area compared to the control group. Changes in photosynthetic pigments and oxidative stress parameters were significant across all treatments following the second spray. The most favourable outcomes for tomato plants emerged after two applications, using various dilutions of bioenzyme and fruit pulp extract. Nevertheless, increased frequency in applications resulted in a decline across all evaluated parameters. This decline could potentially be attributed to excessive use of bioenzyme or fruit pulp extract. Thus, optimal results can be achieved with only two sprays every 15th day.

Key Words **– Chlorophyll, Leaf area, Malondialdehyde (MDA), Plant height, Peroxidase.**

I. INTRODUCTION

The garbage made of fruit and vegetable waste on fermentation produces a very nutritive solution rich in vitamins and minerals. In 2006, Dr. Rosukon Poompanvong, a researcher from Thailand, successfully developed a sophisticated solution using organic solid waste and named it "Garbage Enzyme." So, the initial creation of Bio-enzyme can be attributed to Dr. Rosukon Poompanvong (Sethi et al., 2021; Novianti and Muliarta, 2021). The solution presents itself as a murky brown liquid, accompanied by an aroma reminiscent of vinegar (Rungta et al., 2022). Bioenzyme, known by various names such as Eco-enzyme, Garbage enzyme, Terrazyme, Fruit enzyme, Flower enzyme, and more, are an alternate designation for these organic catalysts (Khadka et al., 2019; Singh et al., 2019). The bio-enzyme samples have exhibited cost-effectiveness, antimicrobial properties, and eco-friendliness. Bioenzyme, a liquid enzyme, is derived naturally and possesses non-toxic, non-flammable, and non-corrosive characteristics. Its application enhances the engineering

properties of soil, resulting in improved soil compaction densities and increased stability. Additionally, bioenzyme aids in the conversion of certain waste materials into valuable substances, offering an economical and easily accessible solution to society. The end product derived from this process proves to be entirely useful and beneficial (Dhavale et al., 2020).

Fruit pulp extracts may also increase the availability of nutrients for plants especially micronutrients (Al-Hamdany et al, 2011). Banana, orange, and pomegranate having important macronutrients, amend plants with N and Mg (El-Serafy and El-Sheshtawy, 2020). El-Serafy et al., 2021 discovered the potential of banana, orange, and pomegranate peels to enhance phenols, flavonoids, and DPPH activity while reducing H_2O_2 and Malondialdehyde (MDA) in Schefera plant leaves. Heat-shock proteins were induced, improving heat tolerance. Orange peels at 16g/pot proved the most effective, outperforming other peels. Tomatoes are currently an important food component globally, in fact the second largest vegetable both in terms of production and consumption (FAO, 2016). Moreover, processed tomato products like soup, paste, concentrate, juice, and ketchup (Bergougnoux, 2014) make a beneficial contribution to human health through the constituents found within these items. The increasing popularity of tomatoes has led to the emergence of new varieties, and throughout the 20th century, the tomato industry has progressively expanded to offer a wider range of tomato products (Naika et al., 2005).

Numerous studies have reported that tomatoes are rich in vitamins, pro-vitamins, minerals (such as potassium), and various secondary metabolites including lycopene, flavonoids, phytosterols, and polyphenols, making them nutritionally valuable (Luthria et al., 2006). Research by Levy and Sharon, 2004 indicated that tomatoes and tomato-based products serve as the primary source, contributing to 85% of human lycopene intake. Tomatoes contain a variety of terpenoids, with lycopene being one of them. Lycopene, a prominent terpenoid present in tomatoes, constitutes the predominant carotenoid within this fruit. Its significance extends to the realm of human health maintenance, encompassing crucial roles in mitigating the risk of chronic ailments like cancer, heart disease, and various others. (Setyorini, 2021). This finding explains why tomatoes are frequently utilized in functional food products and occasionally considered functional foods themselves (Shi and Mayer, 2000; Viskelis et al., 2005; Canene-Adams et al., 2005). Tomato cultivation is versatile and can thrive in various climatic conditions. It can be carried out both outdoors, in fields, and indoors, under controlled environments. By employing artificial lighting, heaters, and fertigation techniques, superior-quality tomatoes can be produced (Oda and Saito, 2006).

II. MATERIAL AND METHODS

The study was conducted in Department of Biochemistry, Govt. Holkar Science College, Indore. The crop selected for the study was Tomato (*Solanum lycopersicum*) - variety Hybrid tomato Ansal. The seeds were treated with 0.1% mercuric chloride solution for 5 minutes to remove any fungal growth, followed by washing 4-5 times with distilled water. After surface sterilization, 5 seeds placed in a Petri dish containing 75 gm of soil and kept for germination in the dark. The seedlings of tomato were transferred to pots of 15cm x 20 cm size at 8 cm apart from each other. After 45 days, the seedlings were transferred to the field having a randomized block design and each block represents a single treatment.

Two products were used, Bioenzyme provided by Soil and Soul Foundations, Bangalore (Karnataka) and fruit pulp extract prepared from orange, grapes, and pomegranate in the ratio of 1:1:1. Three different dilution of each product was used as follows: -

Name of Treatment	Treatment given
T1	Untreated plants
T2	Bioenzyme - 1ml per liter
T3	Bioenzyme - 2ml per liter
T4	Bioenzyme - 3ml per liter
T5	Fruit pulp extract- 1ml per liter
T6	Fruit pulp extract- 2ml per liter
T7	Fruit pulp extract- 3ml per liter

Treatments were given in the form of foliar spray. The first treatment was given after 30 days of sowing, and a total of 4 treatments were given at the interval of 15 days. Growth, photosynthetic pigments, and oxidative stress parameters were studied in the plants of tomato after 15 days of 2^{nd} and 4^{th} spray.

Shoot length measurement- The lengths of seedlings were measured using a standard centimeter scale.

Leaf area measurement- It was measured using the Millimeters graph paper method given by Pandey and Singh, 2011.

No. of leaves per plant

Chlorophyll and Carotenoid estimation- Chlorophyll was estimated according to the method given by Lichtenthaler and Welbum. (1983). Leaf samples were extracted with 80% acetone. the absorbance of the extracts was measured at 646 and 663 nm and 470 nm for spectrophotometric determination of chlorophyll a, chlorophyll b, and carotenoid contents and calculated using the following formula: -

Chlorophyll a in µg/g tissue = 12.21 (A_{663}) – 2.81 (A_{646})

Chlorophyll b in µg/g tissue = 20.13 (A_{646}) – 5.03 (A_{663})

Carotenoid in µg/g tissue = [1000 (A_{470}) – 3.27 (chl a) – 104 (chl b)] / 229

MDA (Malondialdehyde) estimation - MDA is a decomposition product of peroxidized polyunsaturated fatty acid component of membrane lipid. The level of lipid peroxidation was measured by estimating MDA. For spectrophotometric analysis of MDA, the plant leaf was homogenized with 10% TCA, and the homogenate was mixed with TBA reagent as the reactive material and the absorbance was measured at 532nm and the extinction coefficient of 155 mM^{-1} $cm^{-1}$was used following the method of Heath and Packer, (1968).

Peroxidase activity- The activity of the enzyme was assayed using o-dianisidine as a hydrogen donor and H_2O_2 as an electron acceptor. The rate of formation of yellow-orange colored dehydrogenation product was a measure of peroxidase activity and assayed spectrophotometrically at 430 nm. Expression of the specific activity of enzyme as units/min/mg protein or per g weight of sample considering one unit of enzyme as an increase in OD by 1.0 under standard conditions was done according to the method given according to Summer et al., 1943.

III. RESULTS AND DISCUSSION

Results are expressed as mean ± SD and the P-value was calculated to test significant difference.

Table 1. Effect of different dilutions of bioenzyme and fruit pulp extract on growth parameters of *Solanum lycopersicum* (Tomato).

Treatments	Height (in cm)	Number of leaves	Leaf area (in cm^2)
T1	25.66±3.3	19±6.65	10.83±2.05
T2	$39±2.94^{**}$ (51.98)	$25±0.82^{ns}$ (31.57)	$11.83±3.66^{ns}$ (9.23)
T3	$44±1.63^{**}$ (71.47)	$28±0.94^{ns}$ (47.36)	$11±2.04^{ns}$ (1.56)
T4	$39±5.35^{**}$ (51.98)	$27±1.25^{ns}$ (42.10)	$11.17±1.43^{ns}$ (3.13)
T5	$44±2.94^{**}$ (71.47)	$27±0.94^{ns}$ (42.10)	$21±3.24^{**}$ (93.90)
T6	$41±3.74^{**}$ (59.78)	$28±0.82^{ns}$ (47.36)	$16.17±3.01^{ns}$ (49.30)
T7	$43±0.82^{***}$ (67.57)	$29±0.94^{ns}$ (52.63)	$19.5±3.94^{*}$ (80.05)

- *Indicates significant, **highly significant, ***extremely significant, ^{ns} non-significant, parenthesis indicates the percent change

Growth parameters- In the current study, there was an extremely significant increase observed in plant height among those treated with 3ml per liter of fruit pulp, measuring 43±0.82, and a highly significant height increase across all bioenzyme and fruit pulp treatments, compared to the control group while non-significant change was found in no. of leaves with all the treatments. In the case of leaf area, a highly significant increase was found in plants treated with 2ml per liter of fruit pulp which is 21±3.24, and found non-significant with all other treatments as compared to the control. In 2015, Sau et al. observed an increase in plant height and number of leaves per branch following the application of bioenzyme, indicating a positive influence on plant growth. This effect could be attributed to the improved nutrient uptake by the plants and the quicker movement of plant metabolites within the plant canopy. Additionally, pulp extracts might augment nutrient availability for plants, particularly micronutrients that play a role in enzymatic activities related to both cell division and elongation (Al-Hamdany et al., 2011). According to Gonzalez et al., 2010 the enlargement of leaf size was primarily attributed to an increase in the number of cells. In a study conducted by Manna et al., 2012 the enhanced growth resulting from bioenzyme application is linked to the presence of cytokinins and auxin precursors which increases the cell division, cell enlargement and results in rapid vegetative growth.

Table 2. Effect of different dilutions of bioenzyme and fruit pulp extract on pigments in leaves of *Solanum lycopersicum* (Tomato).

Treatments	Chlorophyll a (in µg/g)		Chlorophyll b (in µg/g)		Carotenoid (in µg/g)	
	After 15 days of 2nd spray	After 15 days 4th spray	After 15 days of 2nd spray	After 15 days 4th spray	After 15 days of 2nd spray	After 15 days 4th spray
T1	101.71±0.19	31.48±10.46	27.10±0.07	49.76±16.71	24.35±0.10	40.26±6.98
T2	104.3±0.229*** (2.54)	70.09±11.05* (122.64)	27.58±0.09*** (1.77)	110.12±22.64* (121.30)	25.80±0.08*** (5.95)	43.53±2.02ns (8.12)
T3	109.5473±0.23*** (7.69)	64.60±10.93* (105.20)	28.81±0.10*** (6.30)	106.57±18.92* (114.16)	25.30±0.07*** (3.90)	42.20±1.44ns (4.81)
T4	114.73±0.26*** (12.80)	71.76±4.44** (127.95)	32.46±0.18*** (19.77)	117.41±6.39** (135.95)	25.80±0.07*** (5.95)	40.50±0.78ns (0.59)
T5	104.73±0.41*** (2.96)	61.82±3.64** (96.37)	25.76±0.08*** (-4.94)	97.34±5.69** (95.61)	24.61±0.11*** (1.06)	44.68±1.54ns (10.97)
T6	120.4±0.24*** (18.37)	72.73±3.25** (131.03)	32.65±0.19*** (20.47)	115.36±13.48** (131.83)	24.84±0.08* (2.01)	41.96±3.02ns (4.22)
T7	119.6±0.16*** (17.58)	58.48±3.46* (85.76)	30.51±0.08*** (12.58)	95.43±4.92* (91.78)	23.77±0.11**(-2.38)	42.34±2.17ns (5.16)

- *Indicates significant, **highly significant, ***extremely significant, ns non-significant, parenthesis indicates the percent change

Photosynthetic pigments- In the present study, after 15 days of the second spray, there was an extremely significant increase in the levels of chlorophyll a, chlorophyll b, and carotenoid observed across all treatments as compared to the control and after 15 days of 4th spray a significant rise was noted in the content of chlorophyll a and b, while the change in carotenoid content was not statistically significant as compared to control. The tomato plants that received foliar fertilizer displayed slightly elevated levels of leaf chlorophyll and this rise in chlorophyll content is likely linked to the presence of auxin, as previous studies have indicated its role in promoting chlorophyll synthesis in leaves (Ayala-Silva et al., 2004; Ofosu-Anim et al., 2007).
However, a different pattern emerged where chlorophyll a showed a decrease, while chlorophyll b and carotenoid exhibited an increased concentration even in the control plants moving to the 15-day mark following the 4th spray. According to the study by Ofosu-Anim et al., 2007, it is suggested that increased concentrations of Biozyme could potentially lead to a reduction in the chlorophyll content of leaves. As the concentration of Biozyme increased, there was an observable decrease in the levels of total soluble sugars. This decrease in chlorophyll levels, associated with the rising hormone concentrations, could explain the observed changes. Mitra and Mandal (2012) identified an increase in the total chlorophyll content of rice on substitution of chemical fertilizers by bioenzyme. Essential macronutrients present in banana, orange, and pomegranate fruit pulp facilitate nitrogen (N) and magnesium (Mg) supply crucial for chlorophyll synthesis. (El-Serafy and El-Sheshtawy, 2020). Ascorbic acid present in orange peel extract alleviate water deficit condition and significantly enhanced chlorophylls, carotenoids, and pigments in quinoa plants (El-Bassiouny et al. 2016).

Table 3. Effect of different dilutions of bioenzyme and fruit pulp extract on oxidative stress parameters in leaves of *Solanum lycopersicum* (Tomato).

Treatments	MDA (in µmoles/gm)		Peroxidase activity (in unit/min/gm)	
	After 15 days of 2nd spray	After 15 days of 4th spray	After 15 days of 2nd spray	After 15 days of 4th spray
T1	6.45±0.52	40.25±6.44	2.02±1.077	7.24±2.24
T2	11.11±0.79^{ns} (72.24)	35.95±5.66 ^{ns} (-10.68)	2.36±1.997^{ns} (16.83)	7.30±0.32^{ns} (0.82)
T3	6.3±0.52** (-2.32)	38.53±10.60 ^{ns} (-4.27)	3.36±1.644* (66.33)	9.42±1.7^{ns} (30.11)
T4	3.1±2.25*** (-51.93)	33.54±2.24^{ns} (-16.67)	2.34±0.591^{ns} (15.84)	7.08±0.39^{ns} (-2.20)
T5	16±3.14*** (148.06)	36.98±6.15 ^{ns} (-8.12)	1.00±0.211^{ns} (-50.49)	3.86±0.38^{ns} (-46.68)
T6	3.77±0.52*** (-41.55)	32.17±4.20 ^{ns} (-20.07)	0.74±0.242^{ns} (-63.36)	6.32±1.65^{ns} (-12.70)
T7	19.1±5.16*** (196.12)	36.47±2.08 ^{ns} (-9.39)	2.36±1.179^{ns} (16.83)	7.08±1.87^{ns} (-2.20)

- *Indicates significant, **highly significant, ***extremely significant, ^{ns}non-significant, parenthesis indicates the percent change

Oxidative stress parameters-

Hasanuzzaman et al., 2014 noted that when plants are exposed to harsh outdoor conditions, they generate an abundance of free radicals, increasing the production of reactive oxygen species (ROS). This heightened ROS production can potentially trigger cell death by encouraging processes like lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, and the initiation of programmed cell death. In the control group, the constrained availability of nutrients in the soil might result in nutrient deficiency, further amplifying the production of reactive oxygen species (ROS). In the present study extremely significant decrease in MDA content was observed in plants treated with 3 ml per liter of bioenzyme and 2 ml per liter of fruit pulp as compared to control after 15 days of 2nd spray while found to increase with further treatments. A drop in lipid peroxidation, indicated by reduced MDA content, signifies heightened plant resilience to varying environmental conditions. Bioenzyme supplementation meets nutrient requirements, counteracting deficiency stress and consequently lowering ROS production and MDA content. According to Wu et al., 2022 Peroxidase (POD) is a highly active enzyme found in plants and plays a pivotal role in the enzymatic defence system against stress. It's closely associated with key physiological activities in tomato plants, including the conversion of toxic hydrogen peroxide (H_2O_2) into harmless water (H_2O) via activity adjustments. In the present study, Peroxidase activity was found significant only in the plants treated with 2ml per liter of the bioenzyme after 15 days of 2nd spray as compared to the control, and non-significant change was observed with the further treatments. Ranieri et al., 2001 reported elevated levels of hydrogen peroxide (H_2O_2) as iron deficiency effects in sunflower plants may be due to decreased levels of various peroxidase isoenzymes. Iron is one of the micronutrients present in bioenzyme (Sethi et al., 2021) and in fruit pulp (Sethi et al., 2021) may be the reason behind the increased peroxidase activity.

Fig 1 Treatment of tomato with different dilutions of bioenzyme

Fig 2 Treatment of tomato with different dilutions of fruit pulp extract

IV. Conclusion

To address food and hunger challenges, we should adopt sustainable agriculture, ensuring a stable food supply while safeguarding our planet for future generations, and promoting a fairer, more sustainable world. The essential nutrients such as nitrogen, magnesium, vitamin C, flavonoids, stilbenes, and antioxidants contribute to plant growth and provide protection against biotic and abiotic stress. Bioenzyme, a fermented product and fruit pulp extract, derived from fruits like grapes, pomegranate, and orange, can increase the availability of nutrients to plants. The present study shows the positive effect of different dilutions of the bioenzyme on leaf area while both bioenzyme and fruit pulp extract shows positive results on height, photosynthetic pigments. Oxidative stress marker MDA was decreased with supplementation of bioenzyme in leaves of tomato plant while the peroxidase activity was enhanced with both the use of bioenzyme and fruit pulp extract. In tomato plants all dilutions of bioenzyme and fruit pulp extract showed best results after two treatments but with the frequent treatments, there was a decrease in all the studied parameters which may be due excess application of bioenzyme or fruit pulp extract. According to observation the plants treated with 2ml per liter of bioenzyme and 1 ml per liter of fruit pulp may be the best dilution to get good results. Overall, the treatments with different dilutions of bioenzyme and fruit pulp extract in agriculture could be given to improve crop productivity and address the challenge of feeding a growing population. By utilizing these natural and environmentally friendly alternatives, we can promote sustainable farming practices and contribute to food security and environmental conservation. Further research and application of these techniques are necessary to fully understand their potential and optimize their use in agricultural systems.

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