

# NUTRIENT CONTENT OF BIOENZYME AND ITS EFFECT ON GROWTH AND PHYTOCHEMICAL COMPOSITION OF *PLECTRANTHUS AMBOINICUS*

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## ABSTRACT

A substitute for chemical fertilizers is the need of the hour. The objective of this study was to assess if bioenzymes which have been extensively studied for its potential as a cleaning agent, could be used as a biofertilizer. Bioenzyme was prepared using jaggery, Citrus limetta peel, and water in ratio 1:3:10 respectively with *Saccharomyces cerevisiae*. This study involved analysis of nutritive content of bio enzyme and effects of its use on growth parameters (shoot length, number of leaves) and phytochemical composition of leaves (Chlorophyll and flavonoids) of *Plectranthus amboinicus*. The results indicated that primary nutrients such as Nitrogen (300mg/L), Potassium(379mg/L), Phosphorous(69mg/L) and secondary nutrients such as Iron (1.33 mg/L), Copper (6.99mg/L) and Calcium(288mg/L) were in adequate amounts in the bioenzyme. Control and test groups with three saplings each were used to assess the potential of bioenzyme as fertilizer. The test group plants were grown in soil supplemented with bioenzyme and were also irrigated with diluted bioenzyme. Significant increase was observed in shoot length, number of leaves ( $p < 0.05$ ) and flavonoid content of leaves ( $p < 0.01$ ) in the test group compared to the control group. No statistically significant difference was observed in chlorophyll content.

## INTRODUCTION

The need for food sources is increasing in multiple folds due to burgeoning population. This has resulted in extensive use of chemical fertilizers leading to numerous ill effects. It is the need of the hour to find suitable alternatives to chemical fertilizers. Hence, organic fertilizers and biofertilizers are the focus of current research. Studies with organic manures and foliar fertilization with natural growth stimulants have demonstrated significant increases in growth parameters. (Kumar *et al.*, 2020) The combination of bio fertilizers – azotobacter and VAM along with foliar spray of 4% vermiwash significantly increased the growth and yield of Gerbera. (Neelima *et al.*, 2018)

In this context, this study was carried out to assess the potential of bioenzyme as a fertilizer. Bioenzymes, a fermented product prepared from jaggery, fruit peel, and water in ratio 1:3:10 respectively with *Saccharomyces cerevisiae*. (John and Singh., 2022) have been widely analysed for its role as cleaning agent. Biomass used during preparation of bio-enzyme has been separated for use as organic manure. (Muruganandam *et al.*, 2022). Survey of literature indicated that the potential of whole bio enzyme as a fertilizer has not been studied systematically. Hence in this pilot study, the nutrient content (primary and secondary) of bioenzyme and its role as a fertilizer was studied.

The plant chosen to study the potential of bioenzyme as fertilizer was *Plectranthus amboinicus* since it is an herb which grows easily and possesses medicinal properties. (Arumugam *et al.*, 2016) The effect of bioenzyme on growth parameters (shoot length and number of leaves) and phytochemical

composition (chlorophyll and flavonoids) of leaves was studied.

## MATERIALS AND METHODS

Bio-enzyme was prepared as reported in literature (John & Singh., 2022) using 1500 g of *Citrus limetta* fruit peels, 500g of jaggery and 5litres of water (ratio 3:1:10) with a slight modification. 1 teaspoon of *Saccharomyces cerevisiae* was added as inoculant instead of 3 teaspoons as mentioned in literature. The contents were mixed together, tightly closed and left undisturbed. The mixture was allowed to ferment for three weeks and bio-enzyme obtained was taken for study.

pH of bioenzyme was determined using pH meter. (Pye., 1962) Primary nutrients, Nitrogen was estimated by Kjeldahl method (Kjeldahl., 1883), phosphorous using ANSA reagent (Fiske and Subbarow., 1925) and potassium by Flame photometry (Pauline and Burkett., 1958) Iron was estimated using 2,2' dipyrindyl reagent (Ramsay., 1954) calcium by titrimetric method (Mishra *et al.*, 2017) and copper using sodium diethyl dithio carbamate. (Robinson., 1949) Estimation of tannins was done using modified Folin Dennis assay. (Sadasivam & Manickam., 1990) 6 gardening bags (18 x 12 x 9) and 12 kg of gardening soil were purchased from local nursery in Chennai. The experiment groups were control group and test group, each group having three saplings. The control group bags were filled with 2kgs of untreated soil each and test group bags were filled with 2kgs of soil treated with 5ml of bio-enzyme per bag. The bags were arranged in same location

uniformly. 6 saplings (5 inches) of the plant *Plectranthus amboinicus* with same number of leaves were taken from pre-existing well grown plant and planted in all six bags. Only tap water irrigation and no treatment was done for the control group, the test group was irrigated with solution prepared by mixing bio-enzyme and tap water in the ratio 1:5. Irrigation was done once in two days.

Shoot length and number of leaves were measured once in 10 days for 30 days. Parameters such as shoot length was measured from the base of the stem to topmost leaf using an inch tape and number of leaves was determined by counting. Leaves were taken for analysis after 30 days. Leaf extract for estimation of flavonoid was prepared by taking 0.5g of ground leaves in 50ml of distilled water. The extract was boiled for 10 minutes and filtered. Flavonoid was estimated by Aluminium chloride method. (Shanmugam *et al.*, 2010) Chlorophyll content was estimated by Arnon's method. (Arnon.,1949) 1g of leaves was extracted with 80% acetone and the absorbance was read at 663 and 645 nm.

The data obtained from test and control plants were analysed by student's t-test using data analysis pack (add-in) in Microsoft excel.

## RESULTS AND DISCUSSION

### ANALYSIS OF THE BIOENZYME

The pH and primary nutrients are important considerations to study the role of bioenzyme as a fertilizer. The results are presented in Table 1. The pH of the bioenzyme was found to be 4.20. It has been reported that good quality liquid organic fertilizers should normally have a pH less than 5. This is the standard for liquid organic fertilizer in Thailand. (Phibunwathanawong and Riddech, 2019). Nitrogen content of bioenzyme 300mg/L, is less than that found in traditional organic fertilizers but is comparable to the content in washed rice water which reportedly increased vegetable growth. (Nabayi Abba *et al.*,2021) Phosphorous content 69mg/L lies within the reported range in organic fertilizers prepared from molasses, distillery slop and sugarcane leaves. (Phibunwathanawong and Riddech, 2019). Potassium content of 379mg/L is comparable to potassium content in panchagavya which is used as foliar spray for plant growth. (Vala and Chavda., 2021). Results of secondary nutrients and tannin content is presented in Table 2. Iron content in the prepared bioenzyme is 1.33mg/L. Iron level of even 10mg/L in culture solution have been reported to cause toxicity. (Sahrawat.,2004) Hence this bioenzyme may be suitable for application in soil. Bioenzyme contains 288mg/L of calcium.

**Table 1: pH and Primary nutrient content of Bioenzyme**

pH	4.20
Nitrogen	300 mg/L
Phosphorous	69mg/L
Potassium	379 mg/L

**Table 2: Secondary Nutrients & Tannin content of Bioenzyme**

Iron	1.33mg/L
Calcium	288mg/L
Copper	6.99mg/L
Tannins	131mg/L

**Table 3: Average shoot length of control and test plants (*Plectranthus amboinicus*)**

Shoot Length (Inches)	Control	Test
Day1	5	5
Day10	5.96 ± 0.06	6.13 ± 0.40NS
Day20	7.26 ± 0.25	8.03 ± 0.45NS
Day30	9.46 ± 0.50	13 ± 1 *

n = 3, Value represent mean ± SD, \* = p < 0.05, NS – Not significant

**Table 4: Average number of leaves in control and test plants (*Plectranthus amboinicus*)**

No. of Leaves	Control	Test
Day 1	6	6
Day 10	8.33 ± 1.52	17.33 ± 3.05*
Day 20	16.33 ± 7.09	38 ± 8.11*
Day 30	22 ± 9.16	53.67 ± 4.73*

n = 3. Value represent mean ± SD, \* - p < 0.05

**Table 5: Chlorophyll and flavonoid content in leaves of control and test plants (*Plectranthus amboinicus*)**

	CONTROL	TEST
Chlorophyll Content	4.28 ± 1.29	4.84 ± 0.62 NS
Flavonoid Content	16.83 ± 0.76	37.5 ± 2.5**

n = 3, Value represent mean ± SD, NS – Not significant, \*\* - p < 0.01

Calcium levels starting from 8mg /L have been reported in different types of washed rice water which is reported to enhance growth of plants. (Nabayi Abba *et al.*,2021) Bioenzyme has 6.99mg/L of copper. It is reported that 0.03 ppm of copper is adequate for growth in hydroponics. (Dey *et al.*, 2023) Bioenzyme contains 131mg/L of tannin. Tannin enriched compost has been reported to reduce gaseous emission from composts particularly in subtropical conditions. (Jordan. *et al.*,2015) which provides an added advantage.

The prepared bioenzyme has pH, primary nutrients (Table 1) and secondary nutrients (Table 2) in the range reported in varied type of fertilizers thereby make it a suitable candidate to be studied for its potential as fertilizer.

### Use of Bioenzyme as growth enhancer

Average shoot length of control and test plants is depicted in Table3. Shoot length is an important parameter in monitoring growth. The shoot length was observed for a period of 30 days and it was noted that test plants showed a mildly greater increase in shoot length than the control plants initially but it was not statistically significant. Statistically significant difference was noted on the 30<sup>th</sup> day. The results are similar to earlier studies with fruit peels. It has been reported that fruit peels significantly increased the shoot height of *Solanum scabrum* plants. (Sakpere *et al.*,2018)

Average number of leaves in control and test plants is depicted in Table 4. Significant increase in number of leaves was observed in bioenzyme treated plants compared to control (p<0.05) when analysed using t-test from day 10. Similar results have been reported in earlier studies with fruit peels. Fruit peels have been reported to significantly increase the number of leaves in *Solanum scabrum* plants. (Sakpere *et al.*,2018). Chlorophyll and flavonoid content in control and test plants is presented in Table 5. The parameters were assessed on day 30. There is no statistically significant difference in chlorophyll content between control and bioenzyme treated plants though a mild increase is noted.

There is a statistically significant difference ( $p < 0.01$ ) between control and test plants in flavonoid content of leaves. Fruit peel supplementation has been reported to increase the chlorophyll and flavonoid content of leaves in *Schefflera arboricola* plants. Increase in flavonoids aids in protection against environmental stress. (El Sarafi *et al.*, 2023). It also increases the medicinal property of the leaves. In this study significant increase in flavonoids was noted but not chlorophyll. This difference may be attributed to the difference in nutrient content of the peel powder and bioenzyme.

This pilot study has provided preliminary evidence for effectiveness of whole bio-enzyme as a growth enhancer. Significant increase ( $p < 0.05$ ) in growth parameters like shoot length, number of leaves and flavonoid content ( $p < 0.01$ ) of the leaves were observed in plants which were grown with bioenzyme treatment. Large-scale studies along with analysis of additional growth parameters and phytochemicals in plant parts, in addition to studies with different plants are necessary to conclusively prove the efficacy of bio enzyme as a promising fertilizer.

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