

EFFECT OF BIO-ENZYME ON BIOCHEMICAL OF BEETROOT SEEDLINGDivya B. sadhu*¹, Jyoti Chauhan*², Bharat Maitreya*³

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ABSTRACT

The effect of bio-enzyme on biochemical of beetroot was determined. Plant were treated with different concentration (Control , 2% , 4% , 6% , 8% , 10%) of bio-enzyme. An assess the effect of bio-enzyme on biochemical such as Phenol , Protein , Starch , Reducing sugar , Total sugar content of beetroot seedling. Highest amount of Phenol , Protein and Starch were found 2% treated beetroot seedling. Highest amount of Reducing sugar and Total sugar found in 8% treated beetroot seedling.

Key word : Beetroot seedling , Bio-enzyme , Biochemical

I. INTRODUCTION

Bio-enzyme is natural liquid which provide nutrition to plant. Fruit and vegetable waste fermented and made a nutritive liquid which called the bio-enzyme. Yeast , jaggery , peel or waste of fruit , vegetable , flower and water is a raw material to make bio-enzyme. After several weeks the bio-enzyme is ready to use. There is various type of bio-enzyme is available in market. Bio-enzyme also known as Garbage enzyme.

Citrus Bio enzyme , Marigold & Pomegranate Bio enzyme , Banana peel Bio enzyme , Rose Bio enzyme , Aloe vera Bio enzyme , Hibiscus Bio enzyme , Henna Bio enzyme , Lemon grass Bio enzyme , Tamarind Bio enzyme , Neem Bio enzyme , Basil leaves Bio enzyme , Albizia amara Bio enzyme.

Garbage Enzyme is the name given to the remedy that was used to form it successfully developed in 2006 by Dr. Rosukon Poompanvong using organic solid waste. Dr. Rosukon Poompanvong is credited with the original development of bio-enzyme. (Novianti et al, 2021; Sethi et al., 2021).

I. METHOD

- 1) Soil preparation-** Soil and fertilizer was respectively mixed ratio of 3:1 and six pots of was equally filled with prepared soil mixture.
- 2) Plant growing-** Seed of beetroot was grown in six pots and equal amount of water was sprinkled in each pots.
- 3) Bio-enzyme treatment-** Citrus and Banana bio-enzyme was used to treat the growing seed of beetroot. One of grown beetroot seedling was pot not treated with bio-enzyme called as Control remains five pots treated with respective different concentration of 2% , 4% , 6% , 8% , 10% bio-enzyme and after 8 days of time interval treatment was repeated and also labeled the pots.
- 4) Biochemical test-** After 16 days leaves of control and respectively treated seedling with concentration of bio-enzyme 2% , 4% , 6% , 8% , 10% collected of used for estimation of Phenol , Protein , Starch , Reducing sugar and Total sugar.
- 5) Estimation of Phenol-** Take 100 mg fresh leaves of beetroot seedling. Grind with 10 ml 80% boiling ethanol. Centrifuge at 5000 rpm for 10 min and take supernatant I in other test tube. Now, Add 10 ml 80% ethanol in residue and again centrifuge at 5000 rpm for 10 min. take supernatant II now mix supernatant I & II and Discard residue. Take 1 ml aliquot and 1 ml 20% Na₂CO₃. Add 0.5 ml 1 N Folin-Ciocalteu reagent. now boil it in water bath for 10 min and cool it. after that make the final volume of 20 ml with Distilled water. Check Absorbance at 660 nm. (Bray et al , 1954).
- 6) Estimation of Protein-** Take 1 gm fresh leaves of beetroot seedling. Grind it in 10 ml phosphate buffer. Now centrifuge at 10,000 rpm for 15 min. Take 0.2 ml supernatant and discard residue. Add 5 ml Bradford reagent. Now check absorbance at 595 nm. (Bradford , 1976).
- 7) Estimation of Starch-** Take 100 mg fresh leaves of beetroot seedling. Grind it with 10 ml 80% boiling ethanol. Centrifuge at 5000 rpm for 10 min and discard supernatant. Add 10 ml 80% ethanol in residue and centrifuge at 5000 rpm for 10 min. discard the supernatant. Put residue in water bath for drying. Now add 5 ml water and 6.5 ml 52% HClO₄. Put it in 0 ° C for 20 min. now centrifuge for 10 min. take this

supernatant I in other test tube. Now add 5 ml distill water , add 6.5 ml 52% HClO₄. Now centrifuge it. Take supernatant II and mix it with supernatant I, discard residue. Make 100 ml of final volume by adding D.W. add 0.2 ml aliquot. Add water make volume upto 1 ml. now add 4 ml anthron. Boiling for 8 min and cool rapidly. Green color appearance. Check absorbance at 630 nm. (Hedge et al , 1962).

8) Estimation of Reducing sugar- Take 100 mg fresh leaves of beetroot seedling. Grind it with 10 ml 80% boiling ethanol. Centrifuge at 5000 rpm for 10 min and Take supernatant I in other test tube. Add 10 ml 80% ethanol in residue. Again centrifuge at 5000 rpm for 10 min. take supernatant II in other test tube and mix both supernatant I & II , discard residue. Now Take 1 ml aliquot. Add 1 ml nelson somgiyi reagent. Boil at 100 ° C for 10 min. add 1 ml arsenomolybdate. Check absorbance at 680 nm. (somogayi , 1952).

9) Estimation of Total sugar- Take 100 mg fresh leaves of beetroot seedling. Grind it with 10 ml 80% boiling ethanol. Centrifuge at 5000 rpm for 10 min. take supernatant I in other test tube. Add 10 ml 80% ethanol in residue. Centrifuge at 5000 rpm for 10 min. take supernatant II in other test tube and mix both supernatant I & II , discard residue. Now add 1 ml aliquot. Add 1 ml 1 N H₂SO₄. Incubate it for 30 min at 49 ° C. add 1-2 drop methyl red indicator. Add 1 M NaOH dropwise color change(pink to yellow). Now add 1 ml nelson somgiyi reagent and incubate it for 20 min in waterbath. Add 1 ml arsenomolybdate. Make final volume of 20 ml with distilled water. Check absorbance at 620 nm. (Nelson , 1944).

II. RESULT AND DISCUSSION

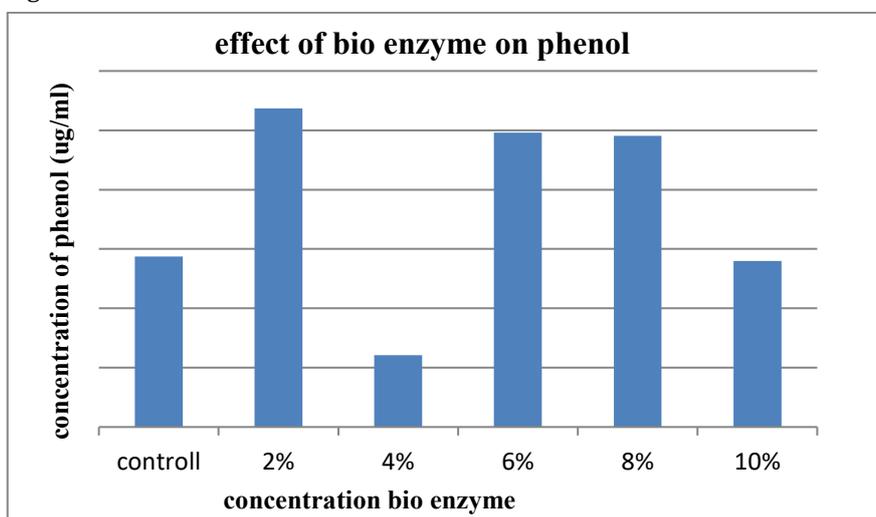
After 16 days , highest plant growth was found in bio-enzyme treated plant than control plant. Estimate various biochemicals in them

➤ Estimation of phenol

➤ **Table 1 :** concentration and absorbance of phenol in control and treated leaves

sample	concentration (ug/ml)	absorbance 660 nm
control	2.871	0.163
2%	5.369	0.303
4%	1.212	0.07
6%	4.959	0.28
8%	4.905	0.277
10%	2.8	0.159

Table 1 show the highest amount of phenol 5.3699 ug/ml was reported 2% treated leaves with bio-enzyme which is as greater than untreated plant seedling after that decrease 4.9595 and 4.9059 respectively in 6% and 8% treated seedling.



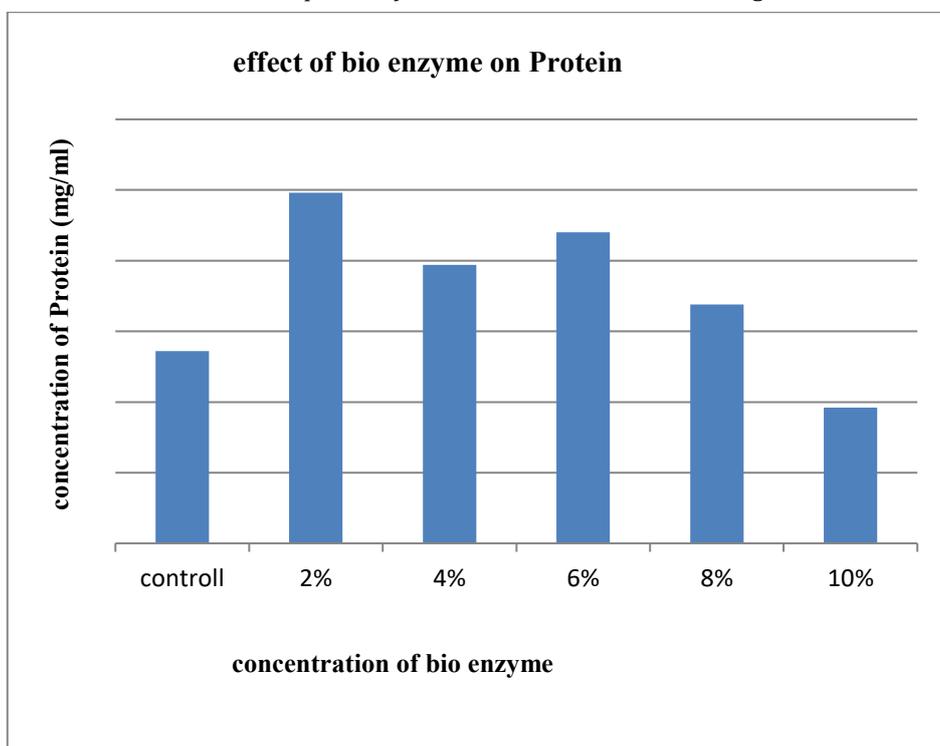
Graph 1: effect of bio-enzyme on phenolic content of leaves of beetroot seedling

➤ Estimation of protein

Table 2 : concentration and absorbance of protein in control and treated leaves

sample	concentration (mg/ml)	absorbance 595 nm
control	0.278	1.228
2%	0.289	1.587
4%	0.284	1.424
6%	0.287	1.498
8%	0.281	1.334
10%	0.274	1.1

Table 2 show the highest amount protein of 0.2898 mg/ml was reported in 2% treated leaves with bio-enzyme after that decrease 0.287 and 0.2847 respectively in 6% and 4% treated seedling



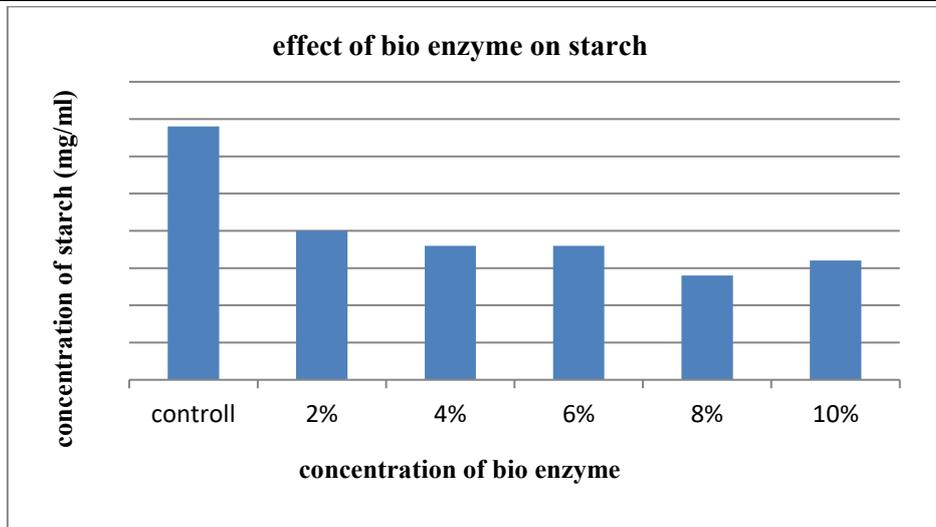
Graph 2 : effect of bio-enzyme on protein content of leaves of beetroot seedling

➤ Estimation of starch

Table 3 : concentration and absorbance of starch in control and treated leaves

sample	concentration (mg/ml)	absorbance 630 nm
control	0.099	0.091
2%	0.098	0.065
4%	0.097	0.062
6%	0.097	0.062
8%	0.097	0.054
10%	0.097	0.058

Table 3 show highest amount of 0.099 mg/ml was reported in control. Then gradually decrease 0.098 , 0.097 , 0.097 respectively in 2% , 4% , 6% and lowest was found in 8% with 0.097 mg/ml.



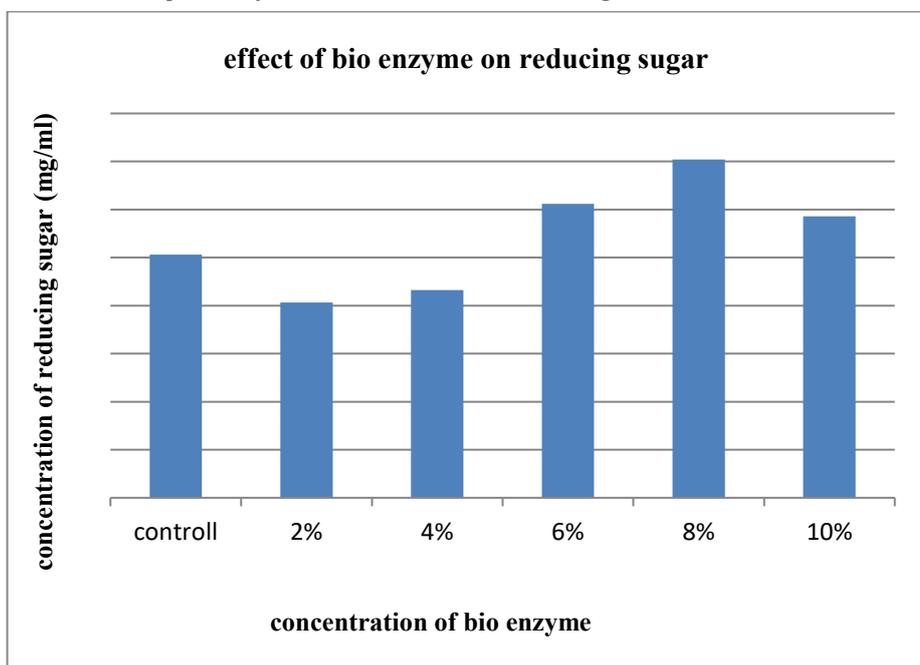
Graph 3 : effect of bio-enzyme on starch content of leaves of beetroot seedling

➤ **Estimation of reducing sugar**

Table 4 : concentration and absorbance of reducing sugar in control and treated leave

sample	concentration (mg/ml)	absorbance 620 nm
control	0.253	0.367
2%	0.203	0.317
4%	0.216	0.33
6%	0.306	0.42
8%	0.352	0.466
10%	0.293	0.407

Table 4 show highest amount of 0.352 mg/ml was reported in 8% treated leaves with bio-enzyme after that decrease 0.306 and 0.293 respectively 6% and 10% treated seedling.



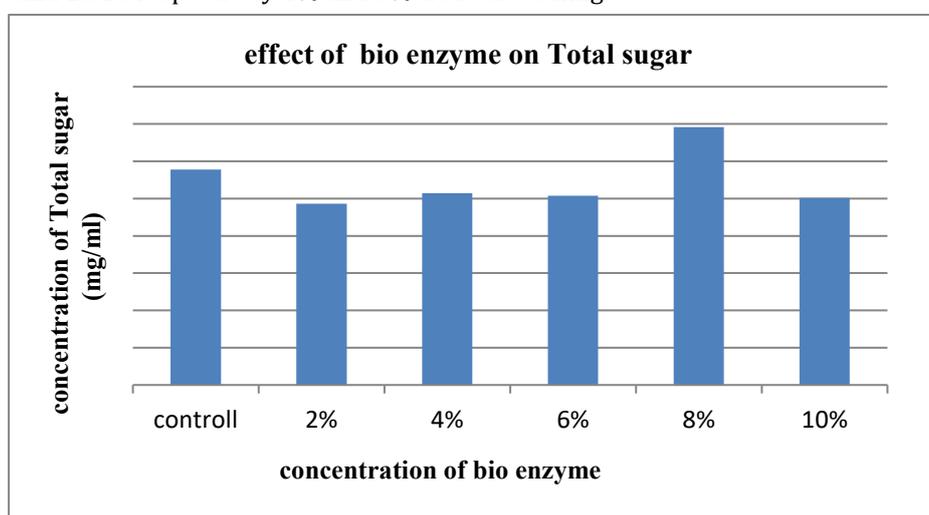
Graph 4 : effect of bio-enzyme on reducing sugar content of leaves of beetroot seedling

➤ Estimation of total sugar

Table 5 : concentration and absorbance of total sugar in control and treated leave

sample	concentration (mg/ml)	absorbance 620 nm
control	1.156	0.176
2%	0.972	0.147
4%	1.029	0.156
6%	1.016	0.154
8%	1.384	0.212
10%	1.003	0.152

Table 5 show highest amount of 1.384 mg/ml was reported in 8% treated leaves with bio-enzyme after that decrease 1.029 and 1.016 respectively 4% and 6% treated seedling.



Graph 5 : effect of bio-enzyme on total sugar content of leaves of beetroot seedling

III. CONCLUSION

A present experiment conclude that beetroot treated with bio-enzyme shows more concentration than untreated control. In beetroot seedling the phenol and protein shows the higher concentration in 2%. In estimation of starch shows the higher concentration in control. reducing sugar and total sugar content higher in 8% concentration of bio-enzyme in beetroot seedling.

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