



# EFFECT OF BIO-ENZYME ON BIOCHEMICALS OF FENUGREEK LEAVES

Mayur A. Patel<sup>1</sup>, Jyoti Chauhan<sup>2</sup>, Bharat Maitreya<sup>3</sup>

Department of Botany, bioinformatics and climate change impacts management, Gujarat University, Ahmedabad-380009, Gujarat, India.

## ABSTRACT

Bio-enzyme have a multiple functionality like use as bio-fertilizer, reducing contamination of ground water and use as natural detergent. This paper's objective is to assess the effect of bio-enzyme on biochemical like total Protein content, total Phenol content, total Reducing sugar, Starch and chlorophyll content of fenugreek leaves. Treated fenugreek with 4% bio-enzyme shows, more concentration of biochemical than other dilution of bio-enzyme and control plants.

**Keyword:** Bio-enzyme, Biochemical, chlorophyll, Protein.

## INTRODUCTION

Vegetable waste is disposed of in excess of 21 tons annually India generates 450 gm of waste per person every day. People don't appear to care much about the environment in the modern world therefore that contaminate the air, water and soil. Fruit and vegetable waste is responsible for the majority of water and soil contamination. This waste's greenhouse gas emissions are another source of pollution. We must take the necessary steps as soon as possible to perfect nature's gift. Environment friendly items are made using enzymes. A natural remedy fruit and vegetable peel. Jaggery bio cleaner and water with the help of microorganisms like yeast and bacteria it also contains organic acids and mineral salts. The bio cleaner creates enzymes from benign that break down organic waste into small pieces. The cleaning abilities of bio-enzymes are extremely strong, secure, and specialized. Additionally, they use less expensive preparation techniques and are environmental friendly. This method of generating the bio-enzyme can be carried out in every household due to its ease of accessibility and minimum effort. It's not intended use human eating. The major goal is to examine the enzymatic activity of the bio-enzyme made from fruit and vegetable peels, as well as the effectiveness of the resulting biomass as bio-manure. By utilizing bio-enzyme this study helps to decrease the buildup of peel waste and produce a sustainable environment.

Inorganic fertilizers typically include the three essential plant nutrients nitrogen, phosphorus and potassium. A number of major negative consequences of inorganic fertilizer on farmland soil have been documented, including: soil infertility, contamination of groundwater (leaching), soil friability, mineral depletion, loss of essential minerals, reduced nutrient, killing of microorganisms, etc. The pH of soil is lowered by inorganic fertilizers with high nitrogen concentration, making top soil extremely acidic. Crop productivity is reported to be significantly reduced as an increased soil acidity. Natural fertilizers, such as bio-enzyme, which are full organic and act as a wholly natural alternative, can readily fix issues. (Patil, 2019) (Selvakumar, et al., 2017) (Thirumurugan, 2016) (Yadav, et al., 2014).

## METHOD

### (1) Soil preparation

Soil (75%) and cow dung (25%) respectively mixed in 3:1 ratio and 6 same sized pots was equally filled with prepared soil mixture.

### (2) Plant Growing

Fenugreek seed was soaked in water overnight at room temperature after that used for plant growing, equal numbers of soaked seed was grown in six pots and two days of time interval equal amount of water was sprinkled in each pots.

**(3) Bio-enzyme Treatment**

Bio-enzyme contains Buttermilk, jaggery, Green waste, Fresh cow dung and water was used for treatment of Fenugreek seeds. one pot was not treated with Bio-enzyme called as control but remains Five pots was treated with 2%,4%,6%,8%,10% bio-enzyme respectively at five days of time interval till 15 days and also labeled the pots.

**(4) Biochemical test**

After 15 days leaves of controlled, 2%,4%,6%,8%,10% treated with bio-enzyme respectively collected and used for Estimation of Protein, Reducing sugar, Starch, Chlorophyll and Phenol.

**(5) Estimation of phenol**

100mg leaves of control and treated plant with bio-enzyme respectively used to checked phenolic compound by Folin-Ciocalteu method. Now leaves crushed with 10ml of 80% ethanol and centrifuged of 5000-10000 rpm for 15 min. Take 1 ml Folin-Ciocalteu reagent followed by add 4ml distilled water, now add 1ml sodium carbonate and 3 ml Distilled water in them and take absorbance at 765nm. Various concentration of Gallic acid standard were used for preparing standard curve the total phenol in controlled and treated leaves was calculated using the calibration curve and result were expressed as Gallic acid equivalent/ gram of sample (GAE/g). (Patel, et al., 2021)

**(6) Estimation of Chlorophyll**

For chlorophyll estimation D.L. Arnon 1949 standard protocol was followed 1gm of leaves were taken respectively controlled and treated plants with bio-enzyme.

Now leaves were crushed with 10ml of 80% acetone. It was then centrifuged at 5000-10000 rpm for 15 min. Now supernatant transferred and make final volume 15ml with 80% acetone. The absorbance of the solution was read at 645nm and 663nm and chlorophyll a, chlorophyll b and total chlorophyll concentration were calculated using following equation. (Dash, et al., 2017) (Rajalakshmi, et al., 2015).

Chlorophyll a =  $12.7 \times \text{O.D. at } 663 \text{ nm} - 2.69 \times \text{O.D. at } 645 \text{ nm} \times v / 1000w$

Chlorophyll b =  $22.9 \times \text{O.D. at } 645 \text{ nm} - 4.68 \times \text{O.D. at } 663 \text{ nm} \times v / 1000w$

Total chlorophyll content =  $20.2 \times \text{O.D. at } 645 \text{ nm} + 8.02 \times \text{O.D. at } 663 \text{ nm} \times v / 100$

Where, V = total volume of the chlorophyll solution in ml, W = weight of the leaf extract in gm.

**(7) Estimation of Protein**

500mg leaves of controlled and treated with bio-enzyme was respectively used to estimate protein content by Bradford's method. Now leaves were homogenized with 10ml phosphate buffer and centrifuged it at 5000-10000rpm for 15min. take 1ml supernatant and add 4ml of Bradford reagent and take absorbance at 595nm. Various concentration of Bovine Serum Albumin as a standers was used for preparing a calibration in curve. The protein concentration in controlled and treated leaves was calculated using the calibration curve and result was express as mg/ml. (Bonjoch, et al., 2001).

**(8) Estimation of Starch**

From controlled and treated with bio-enzyme plant 100mg leaves was respectively collected and homogenized with 10ml of 80% ethanol and centrifuged at 5000-10000 rpm for 15 min and supernatant was discarded and residue was used for further estimation. In residue add 10ml of 80% ethanol and again centrifuged at 5000-10000 rpm for 15 min and supernatant was discarded and residue was dissolved in 10ml of 0.7% KOH and boiling to gelatinize for 40min at 90°C temperature.

Now take 1ml of supernatant and add 0.5ml of 20% Gallic acetic acid ,1ml citrate buffer followed by add 0.2 ml 10% I<sub>2</sub>KI and incubated at for 10 min. now add 0.3 ml Distilled Water and take absorbance at 600nm. Total starch in controlled and treated leaves was calculated using standard curve of starch. (Chinoy, 1939).

**(9) Estimation of reducing sugar**

1mg of leaves from controlled and treated with bio-enzyme respectively collected and crushed with 10ml of 80% ethanol and centrifuged at 5000-10000 rpm for 15 min. and take 1ml supernatant. now 1ml of Distilled Water and 1ml of alkaline copper tartrate add respectively boiled for 10min at 60°C temperature and cooled than add 1ml arsenomolybdate and take

absorbance at 620nm .various concentration of D-glucose was used for preparing a standard curve .and total reducing sugar in controlled treated leaves was calculated using reference curve of D-glucose. (Khatri, et al., 2020).

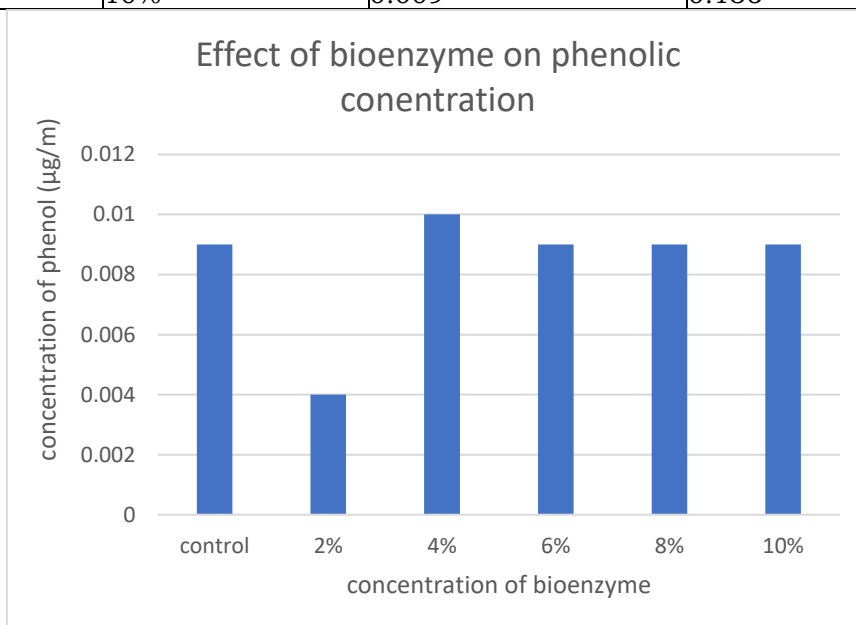
**RESULT AND DISCUSSION**

After 15 days, more plant growth was found in bio-enzyme treated plants than controlled fenugreek plant. Assessment of Total protein, Total Phenol, Total reducing sugar, starch and Total chlorophyll of leaves of Trigonella foenum-graecum L. were executed by the standard technique and the average value of triplicates were considered.

**Estimation of Phenol**

Table 1: Concentration and Absorbance of Phenol in control and treated leaves

Test tube no.	Sample	Concentration (µg/ml)	Absorbance at 756 nm
1	Control	0.009	0.137
2	2%	0.004	0.035
3	4%	0.010	0.142
4	6%	0.009	0.132
5	8%	0.009	0.127
6	10%	0.009	0.136



Graph1: Concentration of phenol in controlled and treated leaves

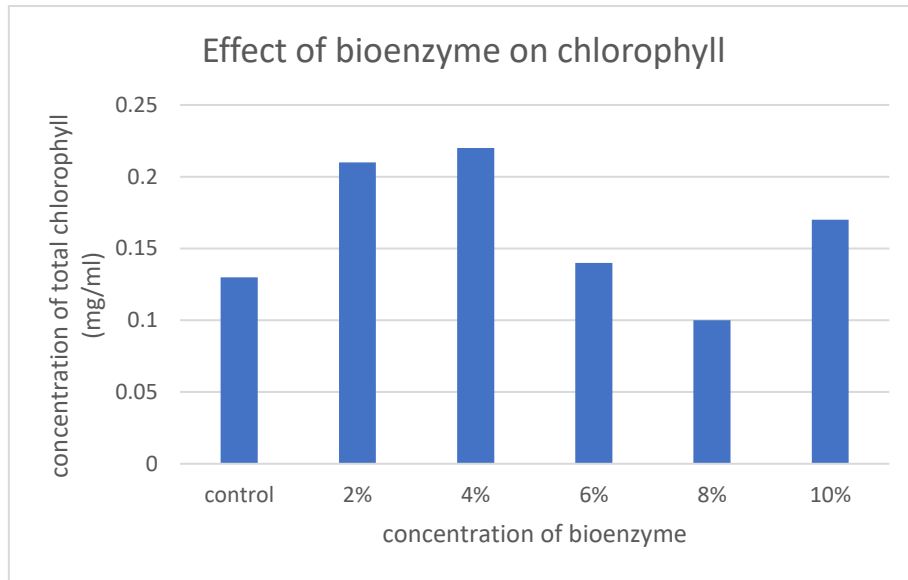
Table 1 and Graph 2 show the highest amount of total phenol 0.010µg/ml was reported in 4% treated leaves with bio-enzyme after that decrease 0.009,0.0009,0.009 respectively in 6%,8%.and 10% treated leaves with bio-enzyme that was similar to control leaves .leaves amount of total phenol 0.004 µg/ml found in 2% treated leaves.

**Estimation of chlorophyll**

Table 2: Absorbance of chlorophyll-a, chlorophyll-b and total chlorophyll in controlled and treatedleaves

Sample	Absorbance 663nm	Absorbance 645 nm	chlorophyll a (mg/ml)	chlorophyll b (mg/ml)	total chlorophyll (mg/ml)
Control	0.749	0.377	0.08	0.05	0.13
2%	1.225	0.571	0.14	0.07	0.21
4%	1.25	0.585	0.14	0.07	0.22
6%	0.813	0.401	0.09	0.05	0.14
8%	0.585	0.289	0.06	0.03	0.1
10%	0.58	0.383	0.06	0.06	0.17

Table 2 and Graph 2, shows the highest chlorophyll-a, chlorophyll-b, and total chlorophyll respectively 0.14mg/ml,0.070mg/ml and 0.22 mg/ml leaves reported in 4% treated leaves.



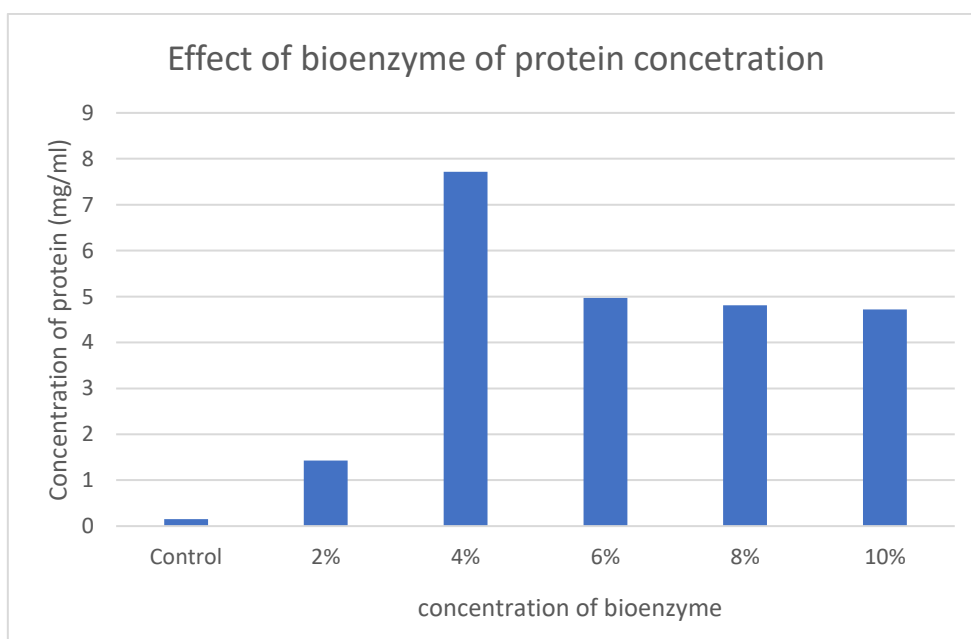
Graph2: Concertation of chlorophyll in controlled and treated leave

Table 2 and Graph 2, shows the highest chlorophyll-a, chlorophyll-b, and total chlorophyll respectively 0.14mg/ml,0.070mg/ml and 0.22 mg/ml leaves reported in 4% treated leaves.

**Estimation of Protein**

Table 3: Concentration and Absorbance of protein in control and treated leaves

Test tube No.	Sample	Concertation (mg/ml)	Absorbance at 595nm
1	Control	0.15	0.245
2	2%	1.43	0.285
3	4%	<b>7.72</b>	0.482
4	6%	4.97	0.396
5	8%	4.81	0.391
6	10%	4.72	0.388



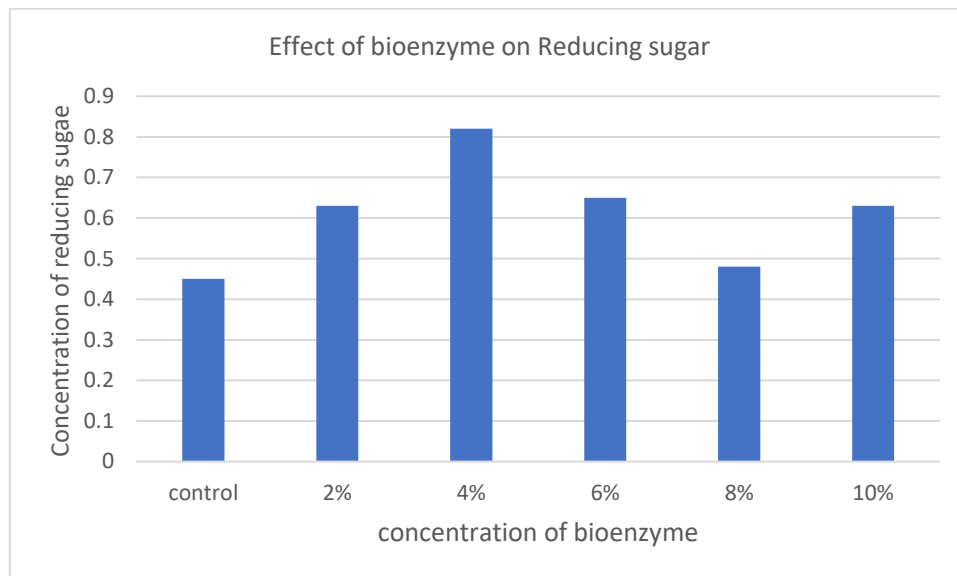
Graph3: Concentration of protein in controlled and treated leaves

Table 3 and Graph 3 shows, The concentration of total Protein was reported lowest 0.15 mg/ml in control leaves gradually increase 1.43mg/ml in 2% treated fenugreek leaves with bio-enzyme, highest amount of total protein 7.72 mg/ml found in 4% treated leaves with bio-enzyme .after that 6%, 8% and 10% treated leaves with bio-enzyme was respectively 4.97, 4.81, 4.72, mg/ml found.

### Estimation of reducing sugar

Table 4: Concentration and Absorbance of reducing sugar in control and treated leaves

Test tubeno.	Sample	Concertation(mg/ml)	Absorbance at 765 nm
1	Control	0.45	0.564
2	2%	0.63	0.748
3	4%	0.82	0.934
4	6%	0.65	0.767
5	8%	0.48	0.594
6	10%	0.63	0.747



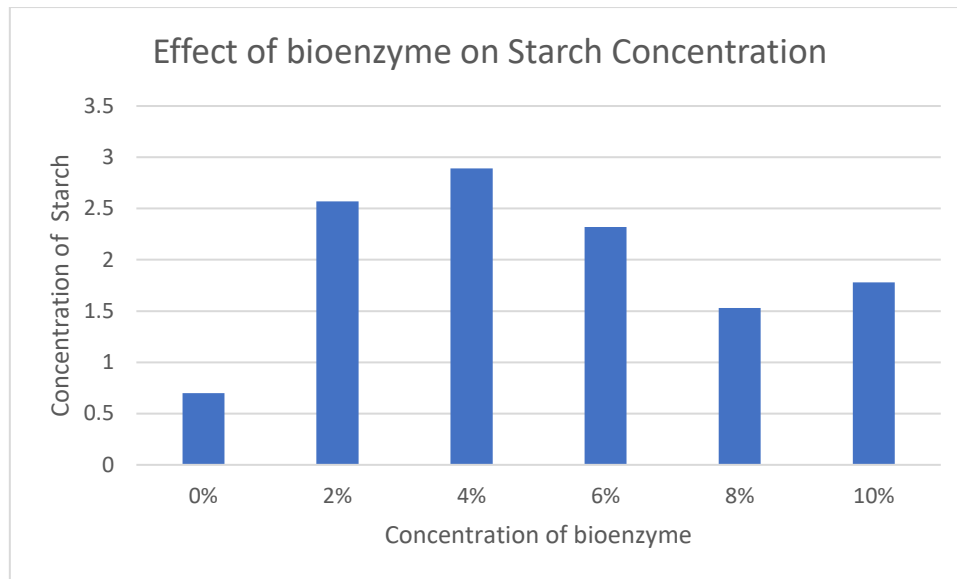
Graph 4: Concentration of reducing sugar in controlled and treated leaves

Table 4 and Graph 4 shows, The concentration of total Reducing sugar was reported lowest highest 0.82 in 4% treated leaves with bio-enzyme and found lowest in untreated plant control 0.45mg/ml after that respectively decreased 0.65,0.48,and 0.63 in 6%,8%,and 10% treated plants with bio-enzyme.

### Estimation of Starch

Table 5: Concentration and Absorbance of starch and controlled and treated leaves

Test tube no.	Sample	Concertation of Gallic acid (mg/ml)	Absorbance at 765 nm
1	Control	0.70	0.132
2	2%	2.57	0.231
3	4%	2.89	0.248
4	6%	2.32	0.218
5	8%	1.53	0.176
6	10%	1.78	0.189



Graph5: Concentration of starch and controlled and treated leaf

Table 5 and Graph5 shows, The concentration of Starch was also highest found 2.89,mg/ml in 4% treated leaves with bio-enzyme and lowest 0.70mg/ml in control leaves.2.32,1.53,1.78 mg/ml concentration of starch respectively found in 6%,8% and 10% treated leaves bio-enzyme which gradually decrease than 4% treated leaves with bio-enzyme

## CONCLUSION

A presented study concluded that, treated fenugreek plant with bio-enzyme shows more concentration of bio-enzyme than untreated control fenugreek plant. Treated fenugreek plant leaves with 4% bio-enzyme shows highest amount of Chlorophyll, Phenol, content Protein content, Reducing sugar and Starch out of other centered treated plants. So bio-enzyme use as natural alternative of bio-fertilizer.

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