

Studies On Effect Of Bio-Enzymes On Growth And Biochemical Parameters Of Cicer Arietinum (L.) And Vigna Radiata (L.) Wilczk

Deep Bhimani, Bhavna Singh*, Hitesh Solanki

Department of Botany, Bioinformatics and Climate Change Impact Management, Gujarat University, Ahmedabad-380 009, Gujarat, India.

ABSTRACT

The present study was conducted to evaluate the effect of bio-enzyme on the growth and biochemical parameters of *Cicer arietinum* and *Vigna radiata*. Bio-enzyme was prepared from different plant sources and applied at varying concentrations (20%, 30%, 40%, and 50%) under controlled pot culture conditions. Growth parameters such as root and shoot length were recorded, and biochemical parameters including chlorophyll, protein, starch, reducing sugar, total sugar, and phenol content were estimated using standard methods. The results revealed that bio-enzyme treatment significantly influenced both plant growth and biochemical composition. Maximum growth was observed at 40% concentration in *Cicer arietinum*, whereas *Vigna radiata* showed optimum growth at 20–30%. Chlorophyll content showed improvement at lower concentrations but declined at higher concentrations. Protein content increased with increasing bio-enzyme concentration, reaching maximum at 50% in *Cicer arietinum* and 40% in *Vigna radiata*. Starch and phenol content were highest at moderate concentrations (30–40%), while reducing sugar content decreased progressively with increasing concentration. Total sugar content was found to be highest at lower to moderate concentrations and declined at higher levels. Overall, the study indicates that bio-enzyme acts as an effective plant growth promoter, with moderate concentrations showing the most beneficial effects, while higher concentrations may exhibit inhibitory effects on growth and biochemical parameters.

Keywords: Bio-enzyme, Plant growth, Biochemical parameters.

INTRODUCTION

Bio-enzyme is a natural liquid formulation obtained from the fermentation of organic waste materials such as fruit peels, vegetable residues, jaggery, and water. It is also commonly known as eco-enzyme or garbage enzyme due to its method of preparation. This concept was first introduced by Rosukon Poompanvong in 2006 using organic solid waste (Novianti & Muliarta, 2021). Bio-enzymes act in a way similar to natural enzymes, as they help in breaking down complex organic substances into simpler forms within a shorter time. These formulations are produced through the activity of microorganisms such as bacteria and yeast during fermentation, resulting in a solution that typically has a dark appearance and a vinegar-like odour (Arun & Sivashanmugam, 2015; Rundta *et al.*, 2022). Different types of bio-enzymes such as Terrazyme, Eco-enzyme, and Permazyme have been

reported, which mainly differ in their source materials but show similar functional properties (Lakra *et al.*, 2022; Sethi *et al.*, 2021).

The composition of bio-enzymes is generally complex and includes enzymes, organic acids, proteins, and other bioactive compounds. Studies have shown that different bio-enzyme formulations may contain enzymes like amylase, protease, and lipase, which are responsible for the breakdown of carbohydrates, proteins, and fats, respectively (Pooni *et al.*, 2021). In addition to enzymes, these formulations may also contain minerals such as calcium, magnesium, sodium, and potassium, which are important for plant growth. Some formulations also include non-ionic surfactants and protein-based compounds that help in reducing surface tension and improving the interaction between soil particles and water (AbouKhadra *et al.*, 2018; Marasteanu *et al.*,

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2005). However, due to the variation in raw materials and fermentation conditions, the exact composition of bio-enzymes can vary and is often difficult to determine precisely (Mekonnen *et al.*, 2020).

In recent years, bio-enzymes have gained attention in agriculture as an eco-friendly alternative to chemical fertilizers. Continuous use of chemical fertilizers has been reported to negatively affect soil health by causing nutrient imbalance, soil acidification, and reduction in microbial diversity (Ismail *et al.*, 2024; Fadlilla *et al.*, 2023). In contrast, bio-enzymes prepared from organic waste materials help in improving soil quality by enhancing nutrient cycling and maintaining soil pH. These formulations contain beneficial microorganisms and organic acids that support soil fertility and long-term agricultural productivity (Guo *et al.*, 2010; Rasit & Kuan, 2018).

MATERIAL AND METHOD

Preparation of bio-enzyme:

Bio-enzymes are usually prepared through a fermentation process that involves mixing organic waste, a carbohydrate source such as jaggery, and water in a specific ratio, commonly 1:3:10. The mixture is kept in a closed container under controlled conditions and allowed to ferment for a period of 30 to 90 days. During this time, microbial activity leads to the breakdown of organic materials and the formation of enzyme-rich liquid (Arun & Sivashanmugam, 2015). In addition to the conventional fermentation method, a rapid extraction method known as aqueous maceration is also used, where plant materials are soaked in water for a few days to obtain crude enzyme extracts. This method is useful for short-term experiments and preliminary studies, as it provides a quicker alternative to long-term fermentation (Lezoul *et al.*, 2020; Sasidharan *et al.*, 2010).

Morphological growth measurement:

Root and shoot lengths were measured to assess the growth of the plants. The measurements were taken using a scale after carefully uprooting the plants. Root length was measured from the collar region to the root tip, while shoot length was measured from the collar to the shoot tip. The values were expressed in cm. A

similar method using a ruler has been reported in seedling growth studies (Nethra, 2013).

Standard method for Biochemical parameters:

Chlorophyll estimation:

Chlorophyll content was estimated by the method of Daniel Arnon (1949). Fresh plant material (500 mg) was homogenized in 10 ml of 80% acetone using a mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 15 minutes, and the supernatant was collected. Absorbance of the extract was recorded at 663 nm, 645 nm, and 652 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll were calculated using standard equations and expressed based on the fresh weight basis.

Protein estimation:

Protein content was determined by the method of Marion Bradford (1976). Fresh plant material (1 g) was homogenized in 10 ml of phosphate buffer (pH 7.2) and centrifuged at 10,000 rpm for 15 minutes. Then 0.2 ml of the supernatant was taken and mixed with 5 ml of Bradford reagent. The mixture was allowed to develop colour and absorbance was measured at 595 nm using a spectrophotometer. Bovine serum albumin (BSA) was used as standard and protein content was determined from the standard curve and expressed as mg/ml.

Starch estimation:

Starch content was estimated by Anthrone method (Mc Cready, 1950). Fresh plant material (100 mg) was homogenized with 10 ml of 80% boiling ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the residue was again extracted with 10 ml of 80% ethanol, followed by centrifugation at 5000 rpm for 10 minutes. The final residue was dried in a water bath, then, 5 ml of distilled water and 6.5 ml of 52% perchloric acid were added and the mixture was kept at 0°C for 20 minutes. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. The residue was again treated with 5 ml distilled water and 6.5 ml perchloric acid, centrifuged, and both supernatants were combined. The final volume was made up to 100 ml with distilled water. Then 0.2 ml

aliquot was taken and made up to 1 ml with distilled water, followed by addition of 4 ml anthrone reagent. The mixture was heated in a boiling water bath for about 8 minutes and then cooled. Absorbance was recorded at 630 nm. Starch content was calculated using a glucose standard curve.

Reducing sugar estimation:

Reducing sugar content was estimated by the method of M. Somogyi (1952). Fresh plant material (100 mg) was homogenized in 10 ml of 80% boiling ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the residue was again extracted with 10 ml of 80% ethanol followed by centrifugation. Both supernatants were combined. Then, 1 ml of aliquot was taken and 1 ml alkaline copper tartrate (Nelson reagent) was added. The mixture was heated in a boiling water bath (100°C) for 20 minutes and then cooled. Then, 1 ml of arsenomolybdate reagent was added and mixed well. The absorbance was measured at 620 nm using a spectrophotometer. The reducing sugar content was determined using a standard glucose curve.

Total sugar estimation:

Total sugar content was estimated using the method described by Nelson N. (1944). Fresh plant material (100 mg) was homogenized with 10 ml of 80% boiling ethanol and centrifuged at 5000 rpm for 10 minutes. The extraction was repeated with 10 ml ethanol and the supernatants were combined. An aliquot of 1 ml extract was mixed with 1 ml of 1N sulphuric acid and incubated at 49–54°C for 30 minutes. After cooling, 1–2 drops of methyl red

indicator were added and the solution was give pink colour. Then add 1N sodium hydroxide drop wise until the colour changed from pink to yellow. Then, 1 ml of alkaline copper tartrate reagent was added and the mixture was heated in a boiling water bath for 20 minutes. After cooling, 1 ml of arsenomolybdate reagent was added and the final volume was made up to 20 ml using distilled water. The absorbance was recorded at 620 nm, and total sugar content was determined using a glucose standard curve.

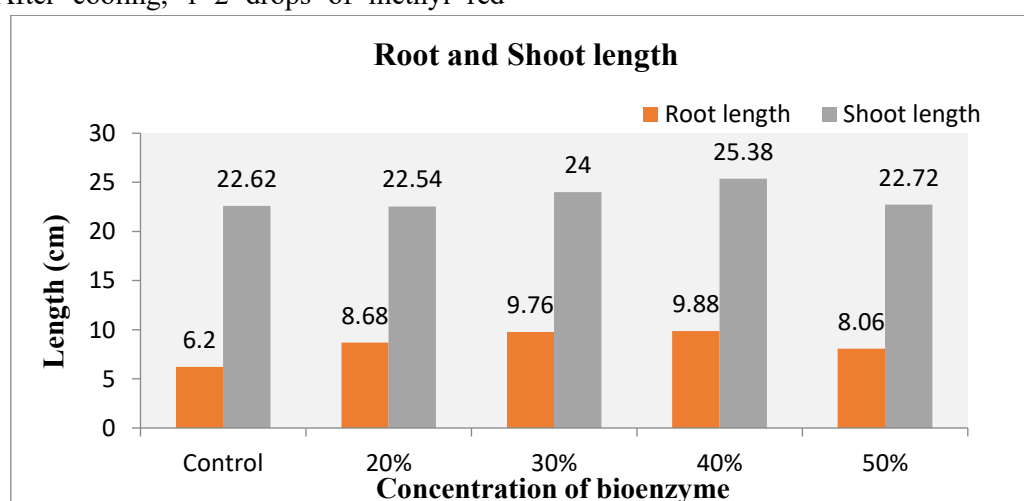
Phenol estimation:

Total phenolic content was estimated using the method of Bray and Thorpe (1954). Fresh plant material (100 mg) was homogenized with 10 ml of 80% ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the residue was re-extracted with 10 ml of 80% ethanol followed by centrifugation. Both supernatants were mixed. An aliquot of 1 ml extract was taken and mixed with 1 ml of 20% sodium carbonate solution. Then add 0.5 ml of diluted Folin–Ciocalteu reagent (1N). The mixture was heated in a boiling water bath for 10 minutes, cooled, and the final volume was made up to 20 ml with distilled water. The solution was filtered if necessary, and absorbance was measured at 630 nm. Phenolic content was determined using a standard calibration curve.

RESULTS

1. Effect of bio-enzyme on root and shoot length:

Plant 1: *Cicer arietinum*

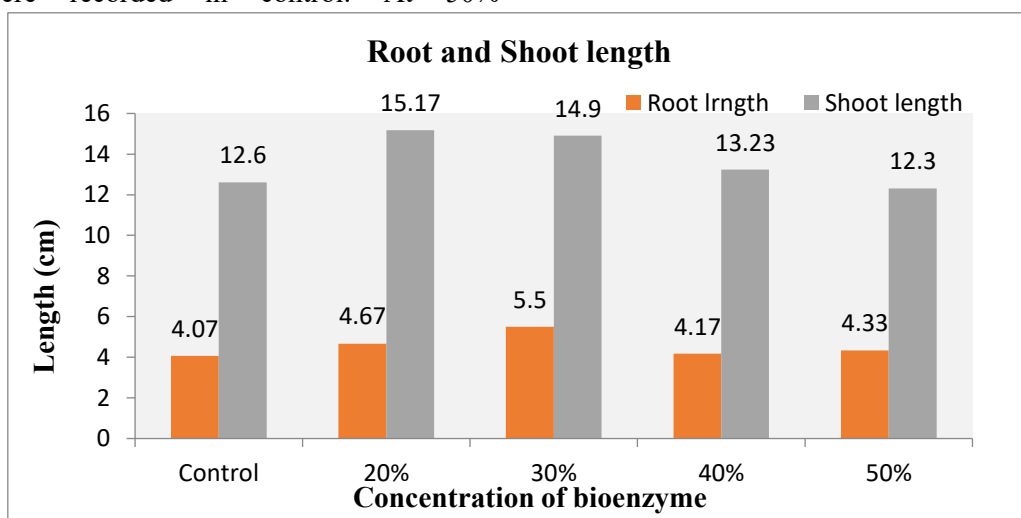


Graph 1: Effect of bio-enzyme on root and shoot length of *cicer arietinum*

Both root and shoot length increased with increasing concentration of bio-enzyme up to 40%. Maximum root (9.88 cm) and shoot length (25.38 cm) were observed at 40% concentration, while minimum values were recorded in control. At 50%

concentration, a slight decrease in both root and shoot length was observed.

Plant 2: *Vigna radiata*



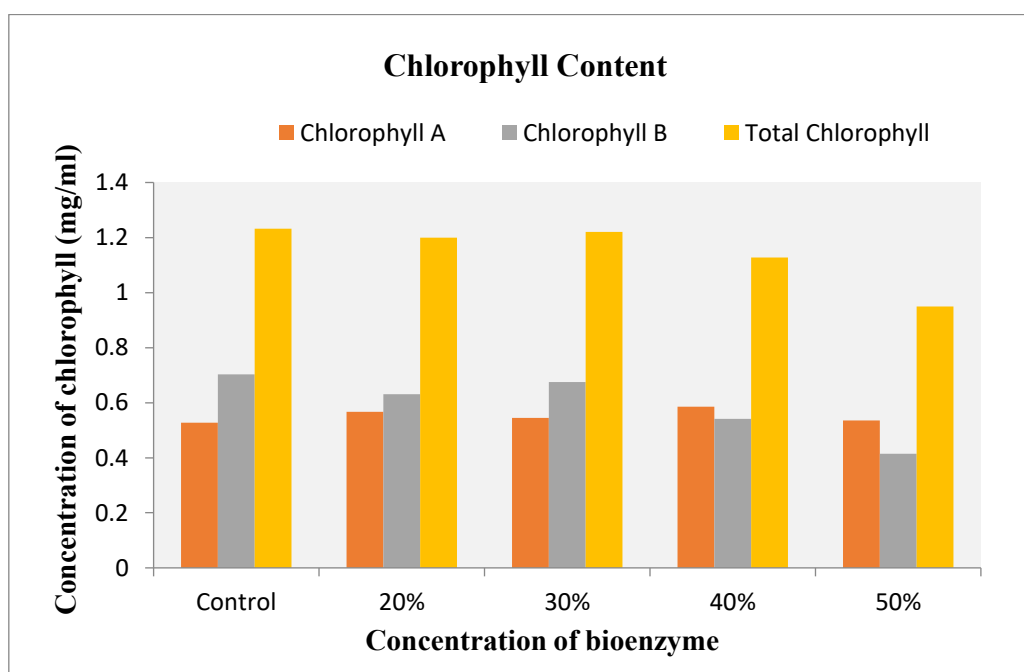
Graph 2: Effect of bio-enzyme on root and shoot length of *Vigna radiata*.

Both root and shoot length increased with increasing concentration of bio-enzyme up to 20–30%. Maximum root length (5.5 cm) was observed at 30%, while maximum shoot length (15.17 cm) was recorded at 20% concentration. At higher

concentrations (40% and 50%), a gradual decrease in both root and shoot length was observed.

2. Effect of bio-enzyme on chlorophyll content:

Plant 1: *Cicer arietinum*



Graph 3: Effect of bio-enzyme on chlorophyll content of *cicer arietinum*.

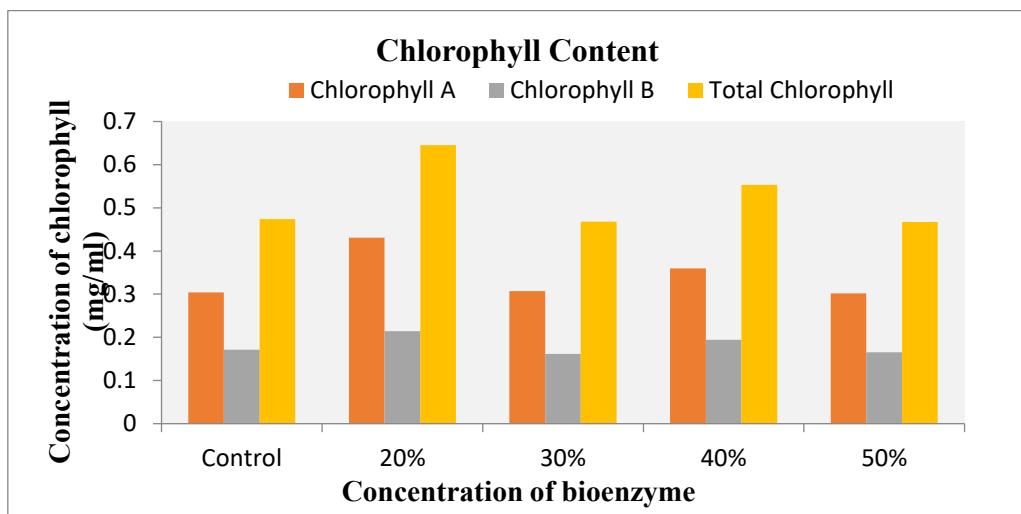
The highest chlorophyll a content was observed in 40% treatment (0.585 mg/ml). The lowest chlorophyll a was recorded in control (0.527 mg/ml). The highest

chlorophyll b content was found in the control sample (0.703 mg/ml). The lowest chlorophyll b was observed in 50% treatment (0.414 mg/ml). The total

chlorophyll content was maximum in control (1.232 mg/ml). The minimum total chlorophyll was observed at 50% treatment (0.950 mg/ml). Moderate increase in chlorophyll a was seen at 40%, but overall higher

concentrations (especially 50%) reduced total chlorophyll content.

Plant 2: *Vigna radiata*



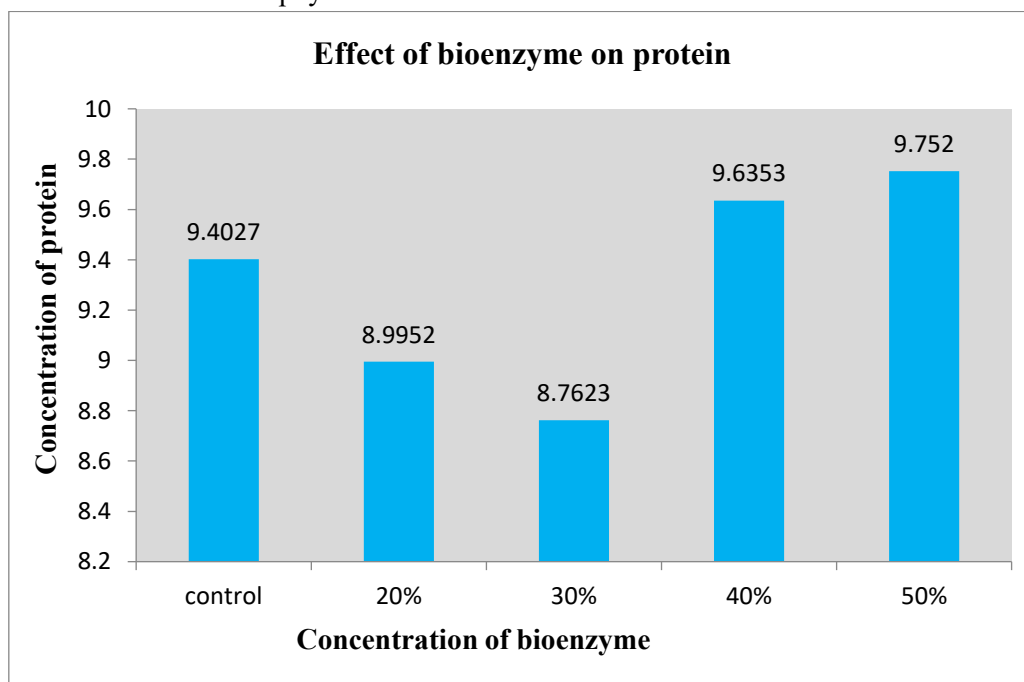
Graph 4: Effect of bio-enzyme on chlorophyll content of *Vigna radiata*.

The highest chlorophyll a content was observed at 20% treatment (0.431 mg/ml). The lowest chlorophyll a was found at 50% treatment (0.302 mg/ml). The highest chlorophyll b content was recorded at 20% treatment (0.214 mg/ml). The lowest chlorophyll b was observed at 30% treatment (0.162 mg/ml). The total chlorophyll was maximum at 20% (0.645 mg/ml). The minimum total chlorophyll was observed

at 50% (0.467 mg/ml). 20% concentration showed the best result for chlorophyll content, while higher concentrations (especially 50%) showed a decline.

3. Effect of bio-enzyme on protein content:

Plant 1: *Cicer arietinum*

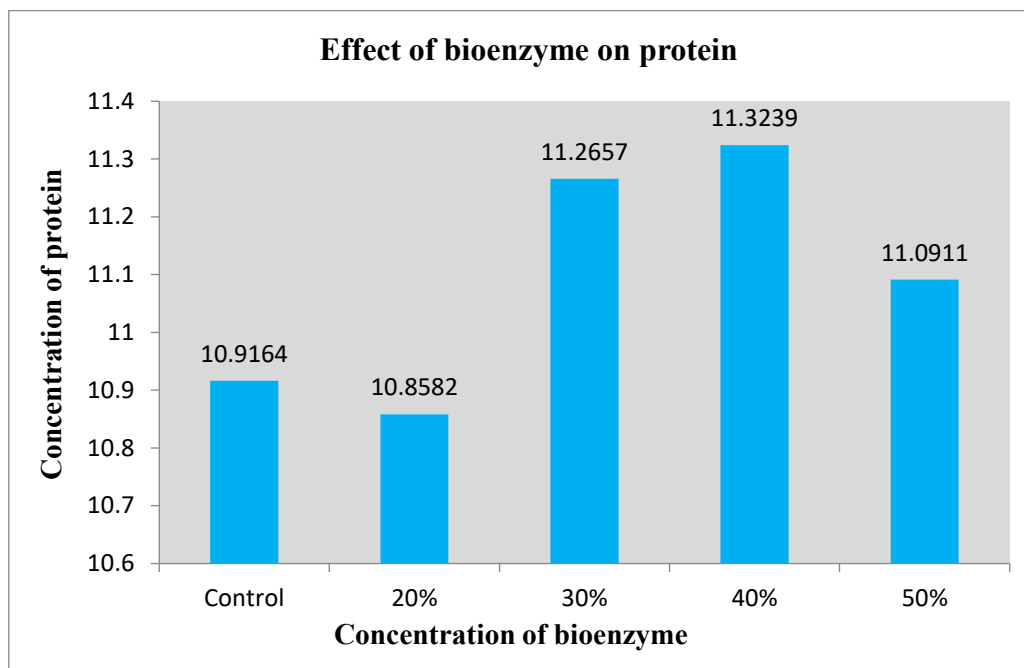


Graph 5: Effect of bio-enzyme on protein content of *cicer arietinum*

The results indicate that protein content increased in treated samples compared to control, with the highest concentration observed at 50% bio-enzyme treatment (9.7520 mg/ml). This suggests that higher bio-enzyme

concentration enhances protein accumulation in *Cicer arietinum*.

Plant 2: *Vigna radiata*

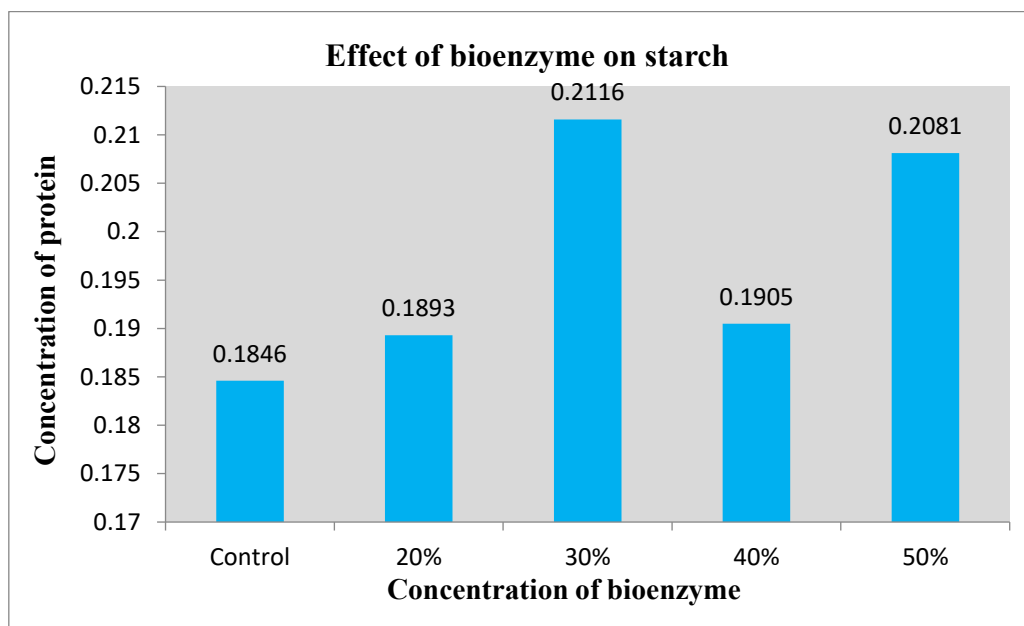


Graph 6: Effect of bio-enzyme on protein content of *Vigna radiata*.

The protein content was found to increase with bio-enzyme treatment, reaching a maximum at 40% concentration (11.3239 mg/ml), followed by a slight decrease at 50%. This indicates that moderate bio-enzyme concentration is more effective for protein enhancement in *Vigna radiata*.

4. Effect of bio-enzyme on starch content:

Plant 1: *Cicer arietinum*

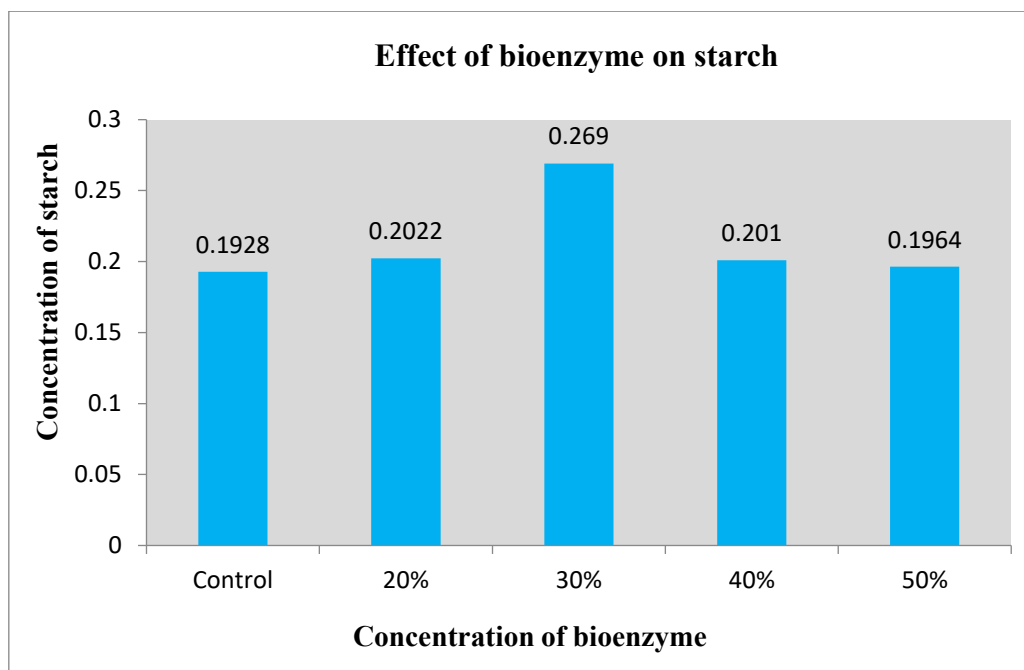


Graph 7: Effect of bio-enzyme on starch content of *cicer arietinum*.

The results indicate that starch content increased in treated samples compared to control, with the highest concentration observed at 30% bio enzyme treatment (0.2116 mg/ml). The lowest starch content was recorded in the control (0.1846 mg/ml). At higher concentrations (40% and 50%), the starch content

showed a slight decline compared to 30%. This suggests that moderate bio enzyme concentration is more effective in enhancing starch accumulation in *Cicer arietinum*.

Plant 2: *Vigna radiata*



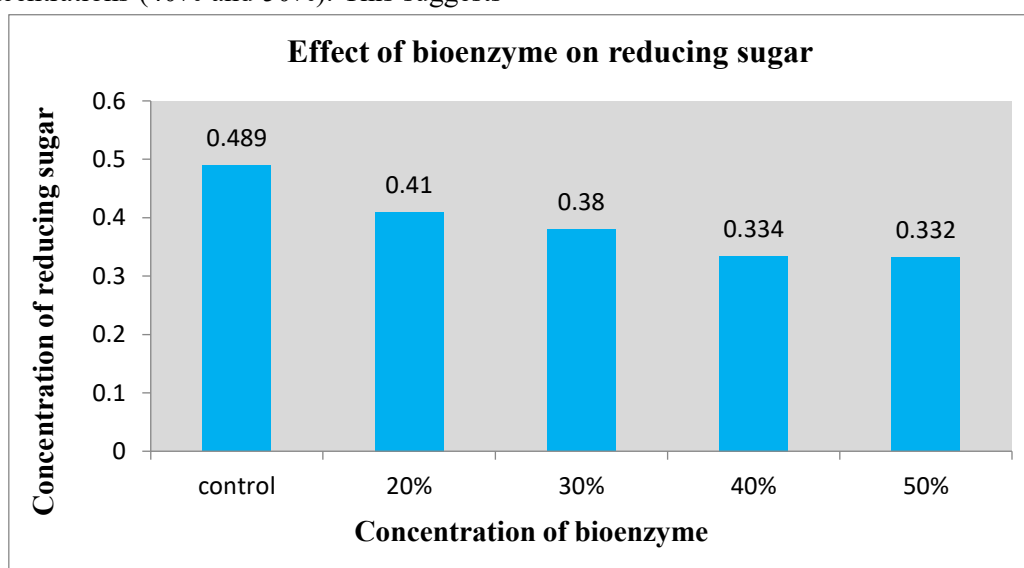
Graph 8: Effect of bio-enzyme on starch content of *Vigna radiata*.

The results indicate that starch content varied with bio-enzyme treatment, with the highest concentration observed at 30% treatment (0.2690 mg/ml). The lowest starch content was recorded in the control sample (0.1928 mg/ml). The starch content increased from 20% to 30% treatment and then decreased at higher concentrations (40% and 50%). This suggests

that moderate bio-enzyme concentration (30%) is most effective for enhancing starch accumulation in *Vigna radiata*.

5. Effect of bio-enzyme on reducing sugar content:

Plant 1: *Cicer arietinum*

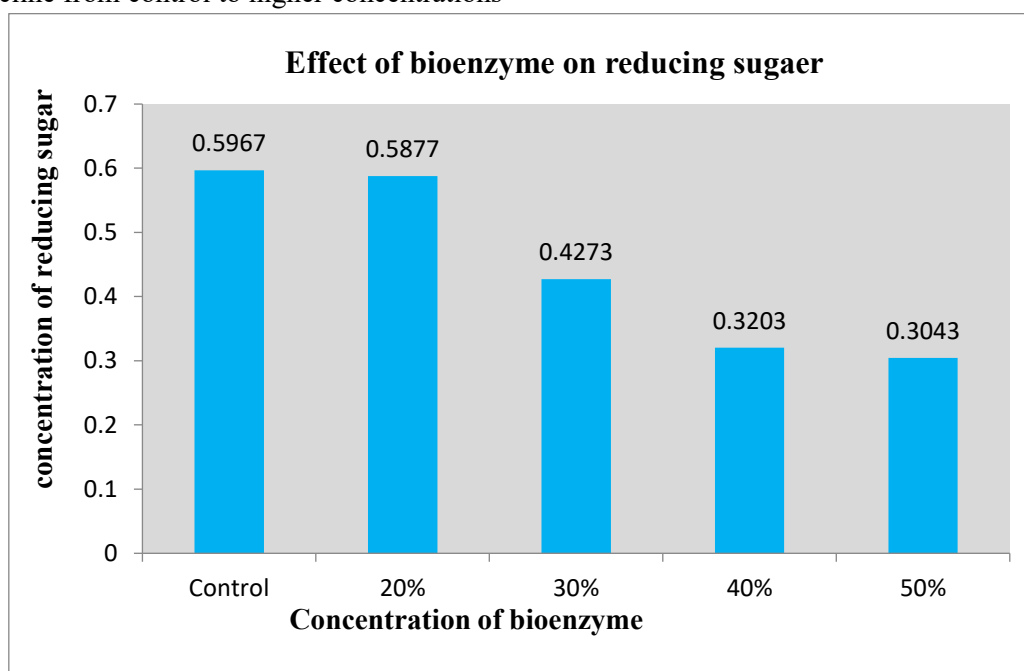


Graph 9: Effect of bio-enzyme on reducing sugar content of *cicer arietinum*.

The results indicate that reducing sugar content was highest in the control sample (0.4897 mg/ml) and decreased progressively with increasing bio-enzyme concentration. The lowest reducing sugar content was observed at 50% treatment (0.332 mg/ml). This gradual decline from control to higher concentrations

suggests that bio-enzyme treatment reduces reducing sugar accumulation in *Cicer arietinum*, with a more pronounced effect at higher concentrations.

Plant 2: *Vigna radiata*



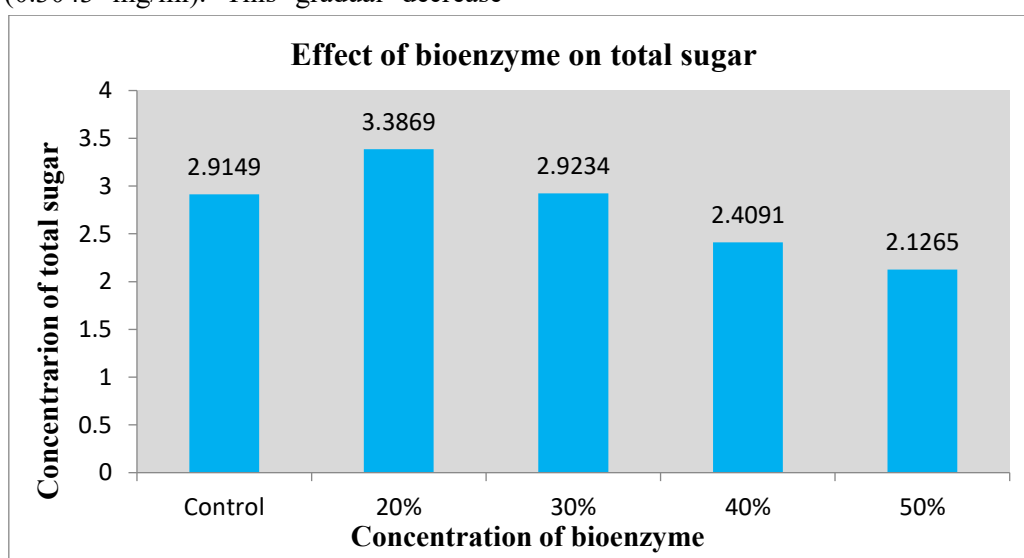
Graph 10: Effect of bio-enzyme on reducing sugar content of *Vigna radiata*.

The results indicate that reducing sugar content was highest in the control sample (0.5967 mg/ml) and showed a slight decrease at 20% treatment (0.5877 mg/ml). A significant decline in reducing sugar content was observed at higher bio-enzyme concentrations, with the lowest value recorded at 50% treatment (0.3043 mg/ml). This gradual decrease

suggests that increasing bio-enzyme concentration reduces reducing sugar accumulation in *Vigna radiata*.

6. Effect of bio-enzyme on total sugar content:

Plant 1: *Cicer arietinum*

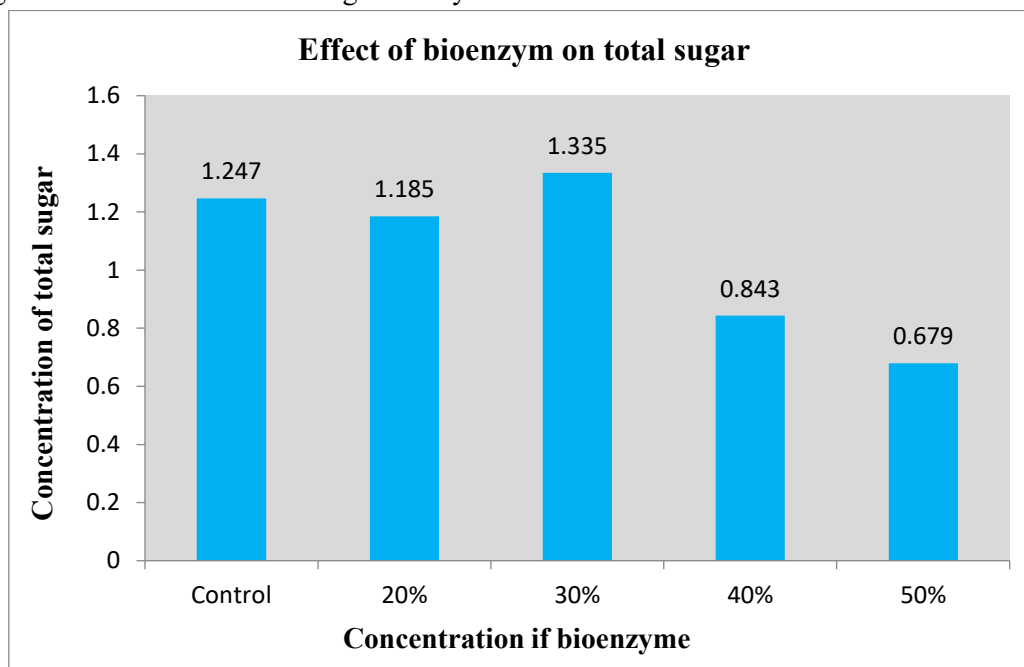


Graph 11: Effect of bio-enzyme on total sugar content of *cicer arietinum*.

The results indicate that total sugar content increased in treated samples compared to control, with the highest concentration observed at 20% bio-enzyme treatment (3.3869 mg/ml). The lowest total sugar content was recorded at 50% treatment (2.1265 mg/ml). After 20% treatment, the total sugar content showed a gradual decline with increasing bio-enzyme

concentration (30%, 40%, and 50%). This suggests that lower bio-enzyme concentration is more effective in enhancing total sugar accumulation in *Cicer arietinum*.

Plant 2: *Vigna radiata*



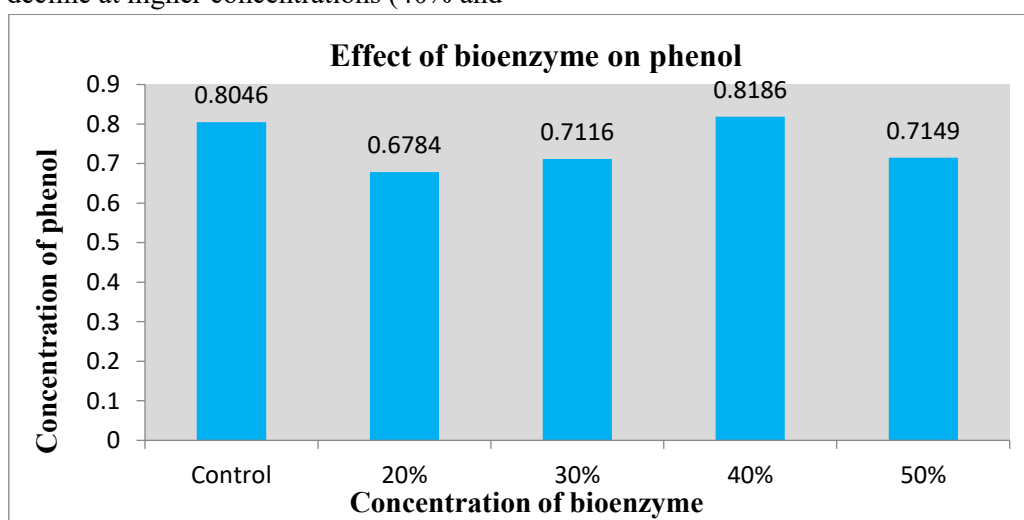
Graph 12: Effect of bio-enzyme on reducing sugar content of *Vigna radiata*.

The results indicate that total sugar content varied with bio-enzyme treatment, with the highest concentration observed at 30% treatment (1.335 mg/ml). The lowest total sugar content was recorded at 50% treatment (0.679 mg/ml). The total sugar content slightly decreased at 20% compared to control, increased at 30%, and then showed a significant decline at higher concentrations (40% and

50%). This suggests that moderate bio-enzyme concentration (30%) is most effective for enhancing total sugar accumulation in *Vigna radiata*.

7. Effect of bio-enzyme on phenol content:

Plant 1: *Cicer arietinum*

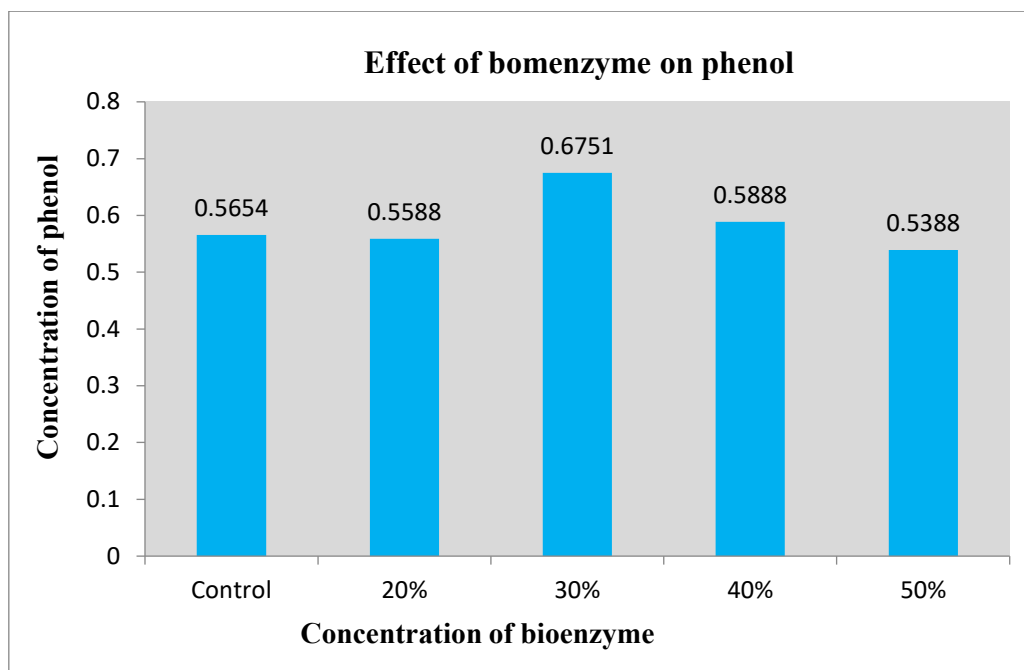


Graph 13: Effect of bio-enzyme on phenol content of *cicer arietinum*.

The results indicate that phenol content varied with bio-enzyme treatment, with the highest concentration observed at 40% treatment (0.8186 mg/ml). The lowest phenol content was recorded at 20% treatment (0.6784 mg/ml). The phenol content decreased at 20%, slightly increased at 30%, reached maximum at

40%, and then showed a slight decline at 50%. This suggests that moderate bio-enzyme concentration (40%) is most effective in enhancing phenol accumulation in *Cicer arietinum*.

Plant 2: *Vigna radiata*



Graph 14: Effect of bio-enzyme on phenol content of *Vigna radiata*.

The results indicate that phenol content varied with bio-enzyme treatment, with the highest concentration observed at 30% treatment (0.6751 mg/ml). The lowest phenol content was recorded at 50% treatment (0.5388 mg/ml). The phenol content showed a slight decrease at 20%, increased to a maximum at 30%, and then gradually declined at higher concentrations (40% and 50%). This suggests that moderate bio-enzyme concentration (30%) is most effective in enhancing phenol accumulation in *Vigna radiata*.

DISCUSSION

Bio-enzymes play a significant role in enhancing plant growth and biochemical processes by improving nutrient availability and stimulating metabolic activities within plants. Their application has been reported to enhance photosynthesis, increase chlorophyll content, and improve carbon fixation efficiency, thereby supporting overall plant productivity (du Jardin, 2015; Rouphael & Colla, 2020). In addition, bio-enzymes promote nitrogen metabolism and enzymatic activity, which contribute to increased protein synthesis and biomass

accumulation (Calvo *et al.*, 2014). They are also associated with the accumulation of important biochemical components such as soluble proteins and photosynthetic pigments, leading to improved metabolic efficiency even under nutrient-limited conditions (Rouphael & Colla, 2020). Furthermore, bio-enzymes strengthen the antioxidant defence system by enhancing the activity of enzymes such as peroxidase, catalase, and superoxide dismutase, which help in reducing oxidative stress in plants. This improved antioxidant capacity enables plants to better tolerate abiotic stresses such as drought and salinity, ultimately contributing to improved growth and resilience (Halpern *et al.*, 2015; Yakhin *et al.*, 2017).

Bio-enzymes act as biological activators that enhance soil fertility by accelerating the decomposition of organic matter and promoting nutrient mineralisation, thereby increasing the availability of essential nutrients in the soil (Burns *et al.*, 2013). They also improve soil structure, enhance water-holding capacity, and stimulate beneficial microbial activity in the rhizosphere, which supports better plant growth and overall soil functionality (Lal, 2015; Barea *et al.*,

2005). In addition to their role in soil improvement, bio-enzymes have been explored as eco-friendly agents for pest and disease management. The organic acids and bioactive metabolites produced during fermentation exhibit antimicrobial properties that help reduce the incidence of plant pathogens (Ricke, 2003). These compounds inhibit harmful microorganisms by lowering environmental pH and disrupting microbial cell membranes (Theron and Lues, 2011). Furthermore, bio-enzyme application can enhance plant defence responses through mechanisms such as induced systemic resistance, thereby improving plant protection against biotic stress (Pieterse *et al.*, 2014; Hoitink and Boehm, 1999).

In the present study, the effect of bio-enzyme on the growth and biochemical content of plants was investigated using *Vigna radiata* and *Cicer arietinum*. Bio-enzyme was prepared from different plant materials using the soaking method, where selected plant parts such as Aloe vera leaves, banana peels, fenugreek seeds, sweet potato tubers, and rice seeds were washed, processed, and soaked in water to obtain crude enzyme extracts. Bio-enzyme obtained from these five different plant sources was used to prepare a mixed bio-enzyme solution. Different concentrations of the mixture, namely 20%, 30%, 40%, and 50%, were prepared along with a control treatment.

The experiment was carried out using a pot culture method, where soil and compost were mixed in a 3:1 ratio and filled into pots. Seeds of *Vigna radiata* and *Cicer arietinum* were soaked, selected, and sown in prepared pots under controlled conditions. Bio-enzyme treatments were applied at regular intervals during the experimental period to study their effect on plant growth.

After completion of the growth period, biochemical analysis of plant samples was carried out to evaluate the effect of different bio-enzyme concentrations. Parameters such as chlorophyll content, protein, total sugar, reducing sugar, starch, and phenol were estimated to compare control and treated plants and to assess the influence of bio-enzyme on plant growth and biochemical composition..

CONCLUSION

The present study clearly demonstrates that bio-enzyme has a significant effect on both growth and biochemical parameters of *Cicer arietinum* and *Vigna radiata*. The results indicate that bio-enzyme enhances plant growth, as observed by increased root and shoot length at optimal concentrations. However, the effect is concentration-dependent, and higher concentrations (50%) showed a reduction in growth in both plant species.

Biochemical analysis revealed that bio-enzyme positively influenced protein, starch, total sugar, and phenol content, particularly at moderate concentrations (30–40%). Protein content showed a continuous increase with concentration in *Cicer arietinum*, whereas *Vigna radiata* showed maximum protein content at 40%. Starch and phenol accumulation were also highest at moderate concentrations, indicating improved metabolic activity. In contrast, reducing sugar content decreased with increasing bio-enzyme concentration, suggesting its utilization in metabolic processes.

Chlorophyll content showed variable responses, with better performance at lower concentrations and decline at higher concentrations, indicating possible stress effects at higher bio-enzyme levels. From the overall findings, it can be concluded that moderate concentrations of bio-enzyme (20–40%) are most effective in promoting plant growth and enhancing biochemical constituents, while higher concentrations may have inhibitory effects. Therefore, bio-enzyme can be considered a promising eco-friendly alternative for improving plant growth and productivity in sustainable agriculture.

REFERENCES

1. AbouKhadra, A., Zidan, A. F., & Gaber, Y. (2018). Experimental evaluation of strength characteristics of different Egyptian soils using enzymatic stabilizers. *Cogent Engineering*, 5(1), 1517577.
2. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Spinacia oleracea*. *Plant Physiology*, 24(1), 1–15.
3. Arun, C., & Sivashanmugam, P. J. P. S. (2015). Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of

- industrial waste activated sludge. *Process Safety and Environmental Protection*, 94, 471-478.
4. Barea, J. M., Pozo, M. J., Azcón, R., & Azcón-Aguilar, C. (2005). Microbial co-operation in the rhizosphere. *Journal of experimental botany*, 56(417), 1761-1778.
5. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254.
6. Bray, H. G., & Thorpe, W. V. (1954). Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis*, 1, 27-52.
7. Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., ... & Zoppini, A. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil biology and biochemistry*, 58, 216-234.
8. Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and soil*, 383(1), 3-41.
9. Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia horticulturae*, 196, 3-14.
10. Fadlilla, T., Budiastuti, M. S., & Rosariastuti, M. R. (2023). Potential of fruit and vegetable waste as eco-enzyme fertilizer for plants. *Jurnal Penelitian Pendidikan IPA*, 9(4), 2191-2200.
11. Guo, J. H., Liu, X. J., Zhang, Y., Shen, J. L., Han, W. X., Zhang, W. F., ... & Zhang, F. S. (2010). Significant acidification in major Chinese croplands. *science*, 327(5968), 1008-1010.
12. Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. *Advances in agronomy*, 130, 141-174.
13. Hoitink, H. A. J., & Boehm, M. J. (1999). Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual review of phytopathology*, 37(1), 427-446.
14. Ismail, A. Y., Nainggolan, M. F., Aminudin, S., Siahaan, R. Y., Dzulfannazhir, F., & Sofyan, H. N. (2024). Characterization of chemical composition of eco-enzyme derived from banana, orange, and pineapple pineapple peels. *Brazilian Journal of Biology*, 84, e286961.
15. Lakra, P., Saini, S. K., & Saini, A. (2022). Synthesis, Physio-Chemical Analysis and Applications of Bio-Enzymes Based on Fruit and Vegetable Peels. *Journal of Emerging Technologies and Innovative Research*, 9(9), a670-a680
16. Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability*, 7(5), 5875-5895.
17. Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules*, 25(20), 4672.
18. Marasteanu, M. O., Hozalski, R. M., Clyne, T. R., & Velasquez, R. (2005). Preliminary laboratory investigation of enzyme solutions as a soil stabilizer.
19. McCready, R. M., Guggolz, J., Silviera, V., & Owens, H. S. (1950). Determination of starch and amylose in vegetables. *Analytical chemistry*, 22(9), 1156-1158.
20. Mekonnen, E., Kebede, A., Tafesse, T., & Tafesse, M. (2020). Application of microbial bioenzymes in soil stabilization. *International journal of microbiology*, 2020(1), 1725482.
21. Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem*, 153(2), 375-380.
22. Nethra, N. S. (2013). Effect of oxadiargyl on seed germination and early seedling growth of sunflower and maize [Doctoral dissertation, University of Mysore]. Shodhganga.
23. Novianti, A., & Muliarta, I. N. (2021). Eco-Enzym Based on Household Organic Waste as Multi-Purpose Liquid. *Agriwar journal*, 1(1), 12-17.
24. Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., & Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual review of phytopathology*, 52(1), 347-375.
25. Pooni, J., Robert, D., Gunasekara, C., Giustozzi, F., & Setunge, S. (2021). Mechanism of enzyme stabilization for expansive soils using mechanical and microstructural investigation. *International Journal of Geomechanics*, 21(10), 04021191.
26. Rasit, N., & Kuan, L. C. (2018). Investigation on the influence of different organic wastes on the

- preparation of eco-enzyme. *International Journal of Engineering & Technology*, 7(4.28), 350–353.
27. Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry science*, 82(4), 632-639.
 28. Rouphael, Y., & Colla, G. (2020). Biostimulants in agriculture. *Frontiers in plant science*, 11, 40.
 29. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African journal of traditional, complementary and alternative medicines*, 8(1).
 30. Sethi, S. K., Soni, K., Dhingra, N., & Narula, G. B. (2021). Bringing lab to our home: Bio-enzyme and its multiutility in everyday life. *International Research Journal of Engineering and Technology (IRJET)*, 8(3), 1461-1476.
 31. Somogyi, M. (1952). Notes on sugar determination. *Journal of biological chemistry*, 195(1), 19-23.
 32. Theron, M. M., & Lues, J. R. (2010). *Organic acids and food preservation*. CRC press.
 33. Yakhin, O. I., Lubyantsev, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Frontiers in plant science*, 7, 20-49.

HOW TO CITE: Deep Bhimani, Bhavna Singh*, Hitesh Solanki, Studies On Effect Of Bio-Enzymes On Growth And Biochemical Parameters Of *Cicer Arietinum* (L.) And *Vigna Radiata* (L.) Wilczk, *Int. J. Sci. R. Tech.*, 2026, 3 (4), 950-962. <https://doi.org/10.5281/zenodo.19754439>