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Growth Promoter: Bioenzyme A Kitchen Waste Product And Its Effects On Pea Plant Growth

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ABSTRACT :

This study explores the sustainable production of bio enzyme from citrus fruit/kitchen waste using a 4:1:9 ratio of jaggery, organic waste, and water, with yeast supplementation to accelerate anaerobic fermentation in bioreactors. The fermentation process spanned 60 days, yielding a liquid rich in proteases, amylases, and organic acids (pH 3.7-3.5) that enhances nutrient bioavailability. Four treatments were evaluated on Pisum sativum: bio enzyme application (at varying concentrations), inorganic fertilizers (urea and DAP), combined bio enzyme-fertilizer, and control. Results demonstrated comparable plant height, leaf count, and pod yield between bio enzyme and synthetic fertilizer groups, with bio enzyme-treated soil showing 25% higher organic matter and improved water retention.

Keywords- Bioenzyme, food waste, yeast fermentation, soil fertility, eco-friendly fertilizers

Introduction

The focus of study was on the conversion of kitchen waste into bioenzyme and the evaluation of their effects as growth promoters for pea plants (*Pisum sativum*). This initiative aligns with the college's goal of promoting eco-friendly practices and supporting local agricultural communities through innovative research and student involvement.

We collect and sort kitchen waste suitable for bioenzyme production (e.g., fruit peels, vegetable scraps) Preparing bioenzyme solutions using the standard 4:1:9 formula (Waste: Jaggery :Water).

Monitoring the fermentation process and maintaining records of temperature, pH, and fermentation time. Filtering and storing the developed bioenzyme solution for application. Setting up controlled
experiments to apply bioenzymes to pea plants and monitoring their growth.

OBJECTIVES

The main objectives of the study are:

- To develop a sustainable process for producing bioenzyme from citrus fruit and kitchen waste using a specific ratio (4:1:9) of jaggery, organic waste, and water, with yeast supplementation to accelerate anaerobic fermentation.
- To characterize the resulting bioenzyme, focusing on its enzymatic content (proteases, amylases) and organic acid profile, as well as its physicochemical properties such as pH.
- To evaluate the agronomic effectiveness of the produced bioenzyme on Pisum sativum (pea) by comparing plant growth parameters (height, leaf count, pod yield) under different treatments: bioenzyme at various concentrations, inorganic fertilizers (urea and DAP), combined bioenzyme-fertilizer, and control.
- To assess the impact of bioenzyme application on soil quality, specifically measuring changes in organic matter content and water retention compared to conventional fertilizer and control treatments.

- To compare the performance of bioenzyme with synthetic fertilizers, aiming to demonstrate the potential of bioenzyme as an eco-friendly alternative for sustainable agriculture and waste valorization.

Fig:- Different type of Waste Collected in a container

MATERIAL AND METHODOLOGY

BIOENZYME PRODUCTION

- **Materials**

In this study, bio-enzymes were prepared for the analysis. The bio-enzymes are fermented from peels of

- Banana, OrangePineapple, Naseberry ,Apple etc .

The materials used for preparation of enzymes are as follows-

- Jaggery (used at homes)
- Water (normal drinking water)
- Plastic container with a screw cap
- Fruit peels and vegetable peels (mentioned above)
- Yeast (available at local market)

Methodology- The present analysis and characterization can be divided into five main steps-

1. Preparation of Bio- Enzyme
2. Filtration of Bio-enzymes
3. Characterization of Bio-Enzymes
4. Bio – Chemical Analysis of bio - enzymes

- **Preparation of Bio-Enzyme :**
  - The peels of consumed fruits and vegetables at home were collected for the analysis.
  - The peels were further divided into smaller pieces to increase the surface area of the reaction.
  - Jaggery (10g), peels (40g) and water (90 mL) were taken in the ratio of 1: 4: 9 into an air tight plastic container and mixed thoroughly.
  - Then a pinch of yeast was added (This yeast used is baker's yeast or Saccharomyces cerevisiaes.
  - Gases will be produced in this process of fermentation.

- So, we choose plastic containers because they can expand otherwise glass bottles would have exploded. Then the containers were kept undisturbed at a safer place for 2 month for the fermentation reaction to proceed.
- Gases are required to be released at different time intervals from the containers.
- To the gases to be released, the lid of the plastic containers was opened once in a day for a minute and the lid was closed again.
- After some days the gases will considerably decrease and after one month a coloured liquid will be produced along with the small particles and some solid residue [9, 10]. The liquid part is the raw bio-enzymes and it is needed to be separated out by filtration.
- We can make bio-enzyme without yeast.
- Both bio enzyme with yeast and without yeast we can prepare and used.

➢ Observation Of Bio-enzymes

The Bio-enzyme is observed in every 10 days and stored for biochemical analysis.

➢ Filtration of Bio-enzymes

- Filtration of Bio-Enzymes was done after 2 months to obtain the raw liquid sample, filtered bio-enzyme solution was stored in the bottle.
- And the solid residue left at the last is collected and dried for the preparation of bio-compost. The characterization of the bio-enzymes was done with the liquid part collected.

➢ Characterization Of Bio –enzymes

The filtered liquid part is used for the characterization of bio - enzymes. Before finding usability of bio – enzymes it is necessary to find out the physical characterization of bio-enzymes. The parameters like odour etc The odour of bio–enzyme is ALCOHOLIC and colour ORANGE.

Bio-Chemical Analysis of bio-enzyme:

Preliminary tests for qualitative analysis of bio-enzymes were carried out in order to test the presence of different biochemical constituents. This was done because bio-enzymes are prepared by fermentation of fruit and vegetable peels, which shows that there should be presence of organic compounds. Alcohol and carboxylic group were already present in the sample because that was a fermentation process. The sample were tested for carbohydrates, metabolites, lipids, proteins with chemicals and solutions available in laboratory.

4.1 Identification of metabolites (Phytochemicals)

Tests were carried out to confirm the presence of flavonoids, phenols/ tannins, alkaloid, quinones and saponins, respectively.

- **Test 1- For Flavonoids** – (Alkaline reagent test) The 2 ml of sample was taken in a test tube and few drops of dilute 10% NaOH solution were added to it. Then dilute HCl was added to the solution and yellow colour formed and after addition of base it was changed to colourless.
- **Test 2 -For Phenols (Tannins)** – (Ferric chloride test Dilute 5% ferric chloride (FeCl3) was added to the 2 ml of the sample and the deep blue colour was noted.
- **Test- 3 - For Cardenolides-** (Keller test) Few drops of acetic acid were added to 2 ml of sample in a test tube and few drops of dilute 5% FeCl3 solution was added to it. Then conc. H2SO4 was added carefully to the walls of the test tube and formation of brown rings was done.
- **Test-4 - For Quinones**- (Acid test) Concentrated HCl was added to 2ml of sample till yellow precipitate was seen.

4.2 Identification of Carbohydrates

In the fermentation process jaggery was added to the solution, so carbohydrates should be present in the samples of bio-enzymes. To test the presence of carbohydrates two tests were performed and the reference was taken as market available sugar.

- **Molisch's test**- 1 ml of sample solution was added to α-naphthol and mixed well. Then conc. H2SO4 was added along the sides of the test tube to form the purple rings at the interface of the two layers.
- **Benedict's test** – This test was performed to check the presence of reducing sugars in the samples. Commercially available 2 ml Benedict's reagent (mixture of sodium citrate, sodium carbonate, and the pentahydrate of copper (II) sulphate) was added to the samples and was added to a water bath for 3-5 minutes. Change of clear blue to greenish blue or yellow-orange colour precipitate was seen.

4.3 Identification of Proteins

Yeast is used for making of solution of bio-enzymes, it produces enzymes to complete metabolic activities for growth and later on, it dies because of lack of nutrients but enzymes are left behind in the solution. As enzymes are complex proteins so the solution of bio-enzymes also contains proteins. Two tests were performed to confirm the presence of proteins and the reference was taken as Amul Full fat milk from the market.

Ninhydrin test- 1 ml of Ninhydrin solution was added to 1 ml of sample and was shaken and was kept on the water bath for 5-10 minutes till boiling. Then the dark purple to light purple colour was observed in all the samples.

(B) Plant Experiment Setup

- The Study Conducted in the Department of Biochemistry Govt. Holkar Science College, Indore. The Crop Selected for the Study was pea plant (*Pisum Sativa*m)
- Variety name-SVS 10.
- In this study four different treatment were administered to investigate their effect on-Pea Plant growth and development.

Experimental Groups

After germination, treatments are applied to each group every 15 days. Here's a detailed of each experimental group and the treatment protocol:

1. Control Group

- **Description:**
  Pea plants receive only water.
- **Treatment:**
  No fertilizer or Bioenzyme is applied. Plants are watered as needed.
- **Purpose:**
  Serves as a baseline to assess the natural growth of pea plants without any external nutrient input.

2. Fertilizer Group

- **Description:**
  Pea plants receive conventional inorganic fertilizers.
- **Treatment:**
  - Apply a mixture of DAP (Diammonium phosphate) and urea in a 1:1 ratio.
  - The fertilizer solution is prepared according to recommended agronomic doses.
  - Application is done as a soil drench at the base of each plant.
  - **Frequency:** Every 15 days after germination **37.**

- **Purpose:**
  To compare the effect of standard chemical fertilizers with bioenzyme and control.

3. Bioenzyme 5:100

- **Description:**
  Pea plants receive a lower dose of bioenzyme.
- **Treatment:**
  - Apply 5 ml of bioenzyme diluted in 100 ml of water per plant.
  - The bioenzyme is prepared from fermented kitchen or citrus waste.
  - Application is done as a soil drench at the base of each plant.
  - **Frequency:** Every 15 days after germination.
- **Purpose:**
  To evaluate the effect of a low dose of bioenzyme on plant growth and health.

4. Bioenzyme 10:100 -

- **Description:**
  Pea plants receive a higher dose of bioenzyme.
- **Treatment:**
  - Apply 10 ml of bioenzyme diluted in 100 ml of water per plant.
  - The bioenzyme is prepared as above.
  - Application is done as a soil drench at the base of each plant.
  - **Frequency:** Every 15 days after germination.
- **Purpose:**
  To assess if a higher dose of bioenzyme results in improved growth compared to the lower dose and other treatments.
-
- Treatment Schedule

Interval:
Repeat each treatment every 15 days throughout the experimental period.

This field experiment aims to evaluate the effect of standard chemical fertilizer and two different dilutions of bio enzyme (5:100 and 10:100) on the growth and yield of pea plants (Pisum sativum).

Field/Treatment Name	Treatment Description	Dilution/Rate	Application Method
Control	No fertilizer or bio enzyme	None	None
Fertilizer	Standard chemical fertilizer	As per agronomic guidelines	Soil application
Bioenzyme(5:100)	Bio enzyme dilution (5%)	5 mL in 100 mL water	Foliar spray/irrigation
Bioenzyme (10:100)	Bio enzyme dilution (10%)	10 mL in 100 mL water	Foliar spray/irrigation

Obesrvation Table :-

Parameter	**Frequency/Time**
Germination rate	**After 7–10 days**
Plant height	**Weekly**
Number of leaves	**Biweekly**
Flowering time	**At onset of flowering**
Number of pods/plant	**At maturity**
Yield per plant/plot	**At harvest**
Optional: Soil health	**Before and after experiment**

The table outlines when to measure key plant growth parameters during an experiment. Germination rate is checked after 7–10 days to assess seed sprouting. Plant height is measured weekly, while the number of leaves is counted every two weeks to track vegetative growth. Flowering time is recorded at the onset of flowering, and the number of pods per plant is noted at maturity. Yield per plant or plot is measured at harvest. Optionally, soil health can be assessed before and after the experiment to evaluate changes due to cultivation or treatments. This schedule ensures comprehensive monitoring of plant development.

OBSERVATIONS AND RESULTS

BIOENZYME -02

BIOENZYME -02 (WITH YEAST)									
S. NO	TEST	TEST NAME	DAY - 01	DAY - 02	DAY - 03	DAY - 04	DAY - 05	DAY - 06	DAY - 07
01	CARBOHYDRATE	MOLISCH TEST	Violet colour is ring obtained	Violet colour is ring obtained	Violet colour is ring obtained	Violet colour is ring obtained	Violet colour is ring obtained	Violet colour is ring obtained	Violet colour is ring obtained
02	CARBOHYDRATE(REDUCING SUGAR)	BENEDICT TEST	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained	Green , yellow and orange colour is obtained
03	PROTEIN	NINHDRIN TEST	Dark purple colour is obtained	Dark purple colour is obtained	Dark purple colour is obtained	Dark purple colour is obtained	Dark purple colour is obtained	Dark purple colour is obtained	Dark purple colour is obtained

04	Flavonoids	Alkaline reagent test	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)	Yellow colour is disappear after adding HCL (flavonoids is present)
05	Quinones	Acid test	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present	Yellow and red colour is obtained quinones present
06	Phenols(Tannis)	Ferric chloride test	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)	Deep blue colour obtained (tannis present)
07	Cardenelides	Keller test	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained(cardenelides present)	Brown colour ring obtained (cardenelides present)

Result and observation of pea plant

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

*Significant p-value < 0.05

**Highly Significant p-value <0.01

***Extremely Significant p-value < 0.001

Table 1 - Effect of Different treatment on Biochemical Parameters of Pea Protein(*Pisum satvium)*

S.NO.	Field name	Protein content in (mg/ml)
1	Control	0.011203±0.001162
2	Fertilizer	0.011542±0.005202
3	Bioenzyme 1	0.016831±0.005651
4	Bioenzyme 2	0.020898±0.003903

The control and fertilizer groups show nearly identical protein content with values of 0.011203 and 0.011542 mg/ml, respectively. This suggests that the application of chemical fertilizer does not significantly enhance protein content compared to the untreated control. The overlapping standard deviations further support the lack of substantial difference between these two groups.
In contrast, both bioenzyme treatments (B1 and B2) show a marked increase in protein content. Bioenzyme1 increases the protein content to 0.016831 mg/ml, while Bioenzyme2 achieves the highest value at 0.020898 mg/ml. The increase observed with bioenzyme treatments is substantial when compared to both the control and fertilizer groups. The double asterisks (**) next to the Bioenzyme 2 value may indicate statistical significance, suggesting that the increase is not due to random variation.

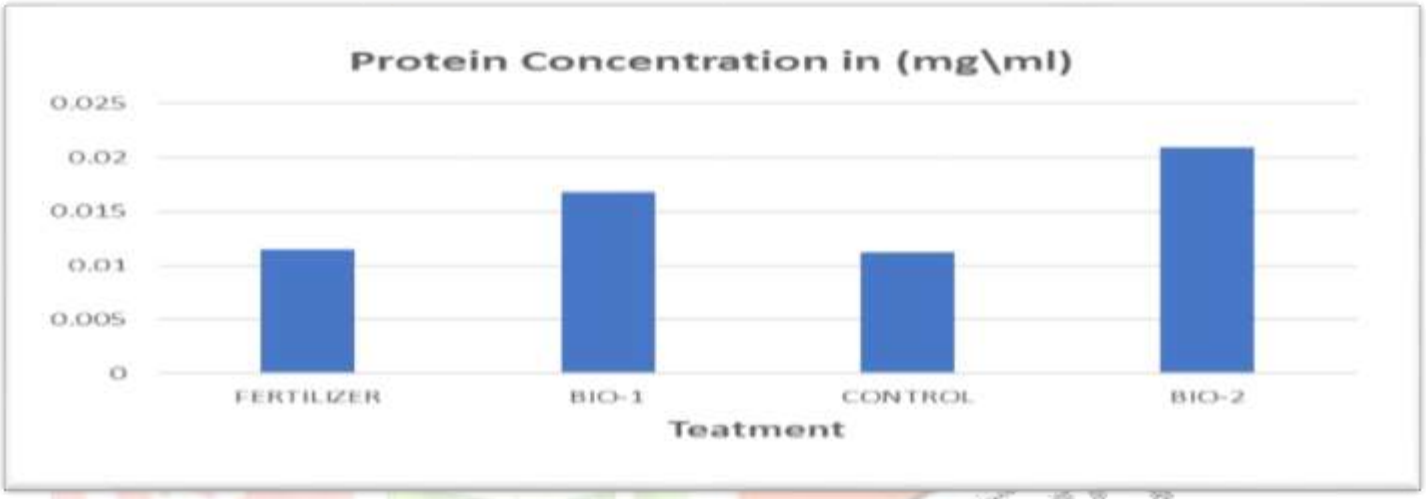

Fig: Protein content of Pea Plant

Table 2:- Effect of Different treatment on Physical Parameters of Pea (*Pisum satvium*)

s.no.	Field name	Week 1	Week 2	Week 3	Week 4	Week 5
1	control	12± 1.59	13.6 ± 1.14	16.8 ± 1.64	24 ± 2.73	25.4 ± 1.67
2	fertilizer	12.9 ± 1.94	17.9 ± 3	26.2 ± 2.77	28.8 ±3.03	31.4 ± 2.97
3	Bioenzyme 1	14.6 ± 3.91	14.6 ± 5.28	27. ± 4.81	29.2 ± 5.06	31.4 ± 4.67
4	Bioenzyme 2	12.5 ± 1.12	14.8 ± 1.79	21± 4.00	21.6 ± 2.96	24.4 ± 2.89

The table and bar graph illustrate the effect of different treatments (Control, Fertilizer, Bioenzyme1, and Bioenzyme2) on plant total length (in cm) over five weeks.

- **Fertilizer and Bioenzyme1** are the most effective treatments, consistently resulting in the greatest plant growth across all weeks.
- **Bioenzyme2** shows some improvement over Control but is less effective than Fertilizer and Bioenzyme1.
- **Control** consistently shows the least growth.
- In all treatment show steady increase in length over the weeks. F and B1 tretment consistently lead to greater growth than control and B2. In week 2 F and B1 show significantly higher length

compare to control and B2. In week 5 F and B1 growth is almost equal. This interpret that the use of Bioenzyme gives equal results as Fertilizer gives.F and B1 shows more growth as compare to B2 and control.

This shows that Bioenzyme can be replaced by chemical fertilizers (because in week 5 both B1 and F has almost equal growth).

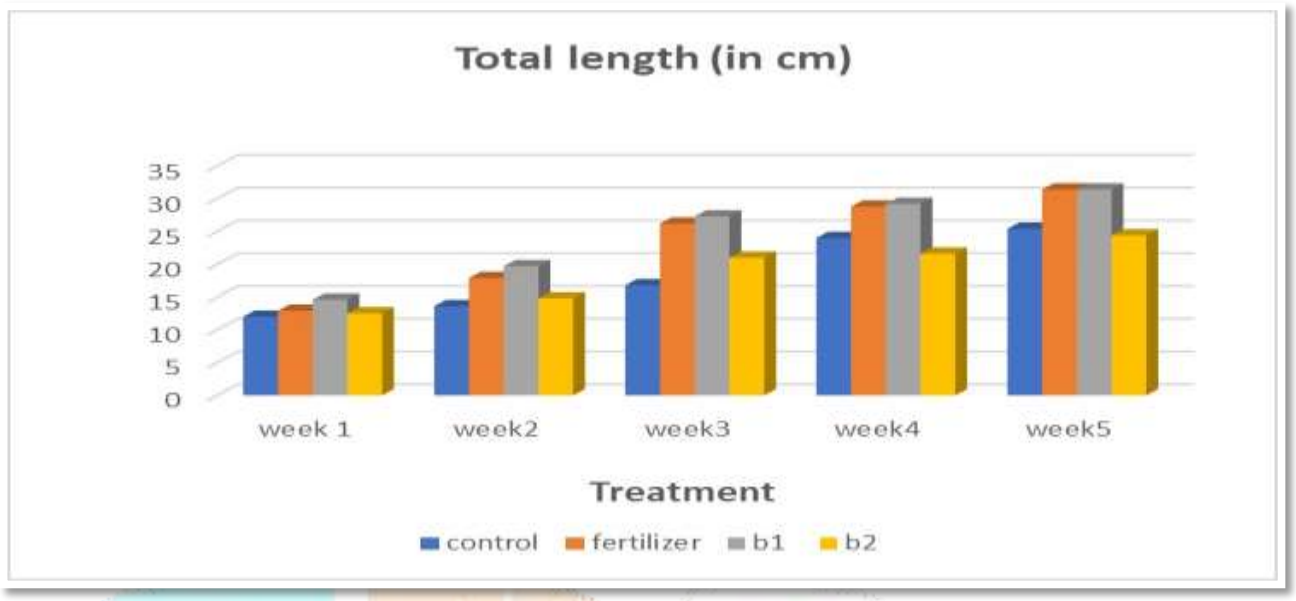

fig: Effect of different treatment on Biochemical parameters of Pea (Pisum sativam)

fig: Different treatment in field

CONCLUSION:

The present study successfully demonstrated the production of bioenzyme using organic waste materials through a natural fermentation process involving fruit peels (such as citrus or papaya), jaggery (as a carbohydrate source), and water. The fermentation period of approximately 60 days yielded a nutrient-rich, acidic liquid rich in beneficial microorganisms, enzymes, and plant-growth-promoting .When applied to Pisum sativum (pea plants), the bioenzyme showed significant positive effects on various growth parameters compared to untreated controls or those treated with chemical fertilizers. Key findings include:

- **Enhanced Germination Rate**: Seeds treated with bioenzyme exhibited a higher and faster germination rate, indicating improved seed vigor and metabolic activity.

- **Improved Plant Growth**: There was a notable increase in plant height, number of leaves, and stem thickness. The enzymatic and microbial action of the bioenzyme likely improved nutrient uptake, stimulated root development, and enhanced overall plant metabolism.

- **Soil Health Improvement:** Application of bioenzyme enhanced the soil's microbial diversity and organic content. This contributed to better soil structure, water retention, and long-term fertility.

- **Increased Yield Parameters**: The number and weight of pods produced per plant were higher in the bioenzyme-treated group, reflecting its role in reproductive success and yield optimization.

- **Eco-friendly Alternative:** Bioenzyme serves as a sustainable and environmentally friendly substitute for chemical fertilizers and pesticides. It reduces environmental pollution and promotes circular waste management by reusing kitchen waste.

The use of naturally fermented bioenzyme offers a sustainable, low-cost, and eco-friendly approach to improving the growth and productivity of Pisum sativum. It promotes healthier plants, improves soil quality, and reduces dependence on synthetic agrochemicals. Given its effectiveness and accessibility, bioenzyme has great potential for adoption in organic farming and small-scale agriculture.

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