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RESEARCH ARTICLE

# Biopriming using microbial consortia and biostimulants as a technique to increase seed quality of *Solanum melongena L.* by modifications in morphological and metabolic constituents

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## Abstract

Brinjal (*Solanum melongena* L.) is a widely cultivated vegetable in tropical and subtropical regions. High-quality seeds are crucial for successful crop production, necessitating rapid and uniform field emergence. In the new wave of the Green Revolution, organic wastes were replaced by agrochemicals. This study was conducted from January to March 2024 to standardize biopriming techniques for brinjal using various organic inputs and microbial cultures to improve seed quality. Brinjal seeds cv. PLR 2 were treated with organic inputs (Panchagavya, Egg fermented extract, Fish fermented extract and effective microorganism solution) and liquid microbial cultures (*Azospirillum* and Pink Pigmented Facultative Methylotroph). The bioprimed seeds were assessed for their physiological attributes and anatomical and biochemical changes. Compared with the control, biopriming with 2% Fish fermented extract significantly improved the germination rate (19.4%), germination energy (40.9%), root length (44.4%), shoot length (24.4%) and seedling vigour index (68.1%). This increase was attributed to the nutritional and bioactive compounds in the fish by-products, which promoted root development and nutrient absorption. The biochemical parameters revealed increased α-amylase activity, protein and amino acid content in the bioprimed seeds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed beneficial root volatile compounds in the roots of treated seeds. An evaluation of the plant growth-promoting activities of the biopriming agents revealed the superiority of fish-fermented extract over other bioagents. Therefore, this method promotes sustainable agriculture and improved crop productivity, providing a scientific basis for biopriming as a viable alternative to conventional seed treatments.

## Keywords

Amino Acids**;** biostimulants; biopriming; protein content; root volatile compounds; sterols

## Introduction

Seed is the fundamental and most crucial input for sustainable agriculture, as the effectiveness of all other inputs largely depends on the quality of the

seeds. High-quality seeds alone are estimated to contribute directly to about 15-20% of total crop production, and this can increase to as much as 45% with the efficient management of other agricultural inputs. The seed industry in India has made remarkable advancements, particularly over the past 30 years. The future of agricultural production will significantly rely on the development of improved crop varieties and hybrids supported by efficient and cost-effective seed production technologies.

High-quality seeds are essential for successful stand establishment, as they must germinate rapidly and uniformly to produce vigorous seedlings with minimal sensitivity to environmental factors (1). Rapid and uniform field emergence is particularly critical in transplanted vegetables, such as tomatoes, brinjal and chilli. Therefore, ensuring the availability of high-quality seeds to farmers at an affordable price and at the correct time is vital for enhancing and maintaining agricultural productivity. However, achieving high seed quality and maintaining a high germination rate can be challenging under field conditions, especially under varying environmental conditions.

Advanced seed quality enhancement techniques, such as seed priming, have been developed to address these challenges. Seed priming is a pre-sowing treatment that improves seed physiological conditions favourable for germination. This process involves controlled seed hydration (imbibition), allowing seeds to undergo the initial reversible germination phase without radical protrusion through the seed coat (2). Seed priming has enhanced synchronized germination and emergence, improved seedling performance, and offered better protection against soil- and seed-borne pathogens. Among the various priming methods, biopriming - a technique involving bio-stimulants such as beneficial microorganisms or natural substances - is gaining popularity (3). Bio-stimulants are particularly advantageous for solanaceous vegetable crops, such as brinjal, as they increase nutrient uptake potential crops by supporting the development of robust and efficient root systems and thus lead to enhanced overall plant health, making them more productive under field conditions (4).

However, chemical-based seed priming has several drawbacks, including high costs and potential toxicity to beneficial soil microbes, plants and ecosystems (5). In contrast, seed biopriming is an eco-friendly alternative that positively affects vegetable seeds by enhancing physiological seed quality parameters and promoting plant growth (6). Previous studies have shown that bio-primed seeds in crops such as finger millet (7), tomato (8) and chilli (9) result in improved seed germination, seedling vigour and plant growth compared to nonprimed seeds.

Brinjal (*Solanum melongena* L.) is one of the most widely cultivated and popular vegetables, particularly in Northeast China and tropical and subtropical areas worldwide. It is primarily grown during the kharif and summer seasons under irrigated conditions. China is the world's leading producer of brinjal, followed by India, where the cultivated area, production and productivity of brinjal are approximately 6.747 lakh hectares, 12,764.93 metric tons and 18.9 tons per hectare, respectively (2022-2023) (10). The increasing demand for brinjal is attributed to its nutritional and medicinal properties. However, one of the significant challenges in brinjal cultivation is its lower germination rate.

Given this context, the present study aims to standardize biopriming techniques to enhance seed germination and seedling vigour in brinjal. This study addresses a specific research gap by exploring the effectiveness of biopriming on brinjal seeds, conducting anatomical and biochemical analyses and identifying root volatile compounds in bioprimed seeds compared to nonprimed seeds. Brinjal is a suitable model for this research due to its agricultural importance and the need to improve its germination rates and overall crop performance. This research will contribute to sustainable farming practices and provide valuable insights into improving brinjal cultivation through biopriming.

## Materials and Methods

### Seed materials and Bio agents

Brinjal seeds cv. PLR 2 were collected from the Vegetable Research Station, Palur, Tamil Nadu, India. A laboratory experiment was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore, during 2023-24. The organic inputs such as Panchagavya, Egg fermented extract, Fish fermented extract and EM (Effective microorganisms) solution were collected from the Nammazhvar Organic Farming Research Centre, TNAU, Coimbatore. Similarly, liquid microbial cultures such as *Azospirillum* and PPFM were collected from the Department of Agricultural Microbiology, TNAU, Coimbatore.

### Experimental Design

In this experiment, the seeds were subjected to biopriming with organic inputs and liquid microbial culture at different concentrations for 8 hrs, followed by shade drying for 12 hrs to dry back to the original moisture content (11). The treatment details are as follows: $T_0$-Control (Nonprimed seeds), $T_1$- Seed biopriming with 3% Panchagavya, $T_2$- Seed biopriming with 2 % Egg fermented extract, $T_3$- Seed biopriming with 2 % Fish fermented extract, $T_4$- Seed biopriming with 5 % EM solution (Effective microorganisms), $T_5$- Seed biopriming with *Azospirillum* liquid culture at a 1:50 dilution, $T_6$- Seed biopriming with PPFM liquid culture at a 1:100 dilution.

### Assessment of seed physiological quality parameters

A standard germination test was conducted via the roll towel method following the procedure outlined in ISTA (12). In this experiment, four replicates of 100 seeds each were used. The seeds were sown in moistened germination paper and kept in a germinator at 25 ± 2°C and an RH of 95 ± 2%. Evaluations were conducted to record the germination percentage after 14 days of sowing, expressed in percentage.

Germination (%) = Number of normal seedlings/ Total number of seeds sown x 100 (Eqn. 1)

Germination energy (GE) was calculated per the guidelines by Maguire *et al.* (13).

The observations' mean root length and mean shoot length were calculated in cm to calculate seedling growth. Furthermore, the seedling vigour index was computed and the mean values were reported in whole numbers (14).

Vigour index I = Germination (%) x Total seedling length (cm)

(Eqn. 2)

Vigour index II = germination (%) x Dry matter production (g per 10 seedlings) (Eqn. 3)

### Anatomical changes in primed seeds

Three replicates of five seeds each were taken from each treatment and soaked overnight. The next day, the seed coats were carefully removed to expose the embryo. Afterwards, they were subjected to microscopic examination using the Euromex Microscope Hollands IMAGE 1.0 to analyze the anatomy of the seeds. The present study utilized a 17.5x magnification lens and the results obtained varied with different crops. The average length of cotyledons/embryos (mm) and the length of radicle (mm) were estimated using Euromex software with calibration corresponding to the magnification of the images.

### Biochemical analysis during seed germination

Biochemical assays such as a-amylase activity, protein content, and total free amino acids were carried out by Paul et al. (15), Ali-Khan and Youngs (16) and Misra et al. (17), respectively.

### Identification of root volatile compounds through GC-MS analysis

### Extraction and analysis of volatile compounds

Brinjal seeds were bioprimed with 2% Fish fermented extract (Best treatment) and untreated seeds were maintained for germination in a germination room at 25 ± 2°C and 90 ± 3% relative humidity for 14 days. Root samples collected from 14-day-old seedlings were finely ground with a mortar and pestle. The resulting liquid was passed through a column (20 mm in diameter) containing 100 ml of XAD-4 resin, followed by elution with 50 ml of methanol. The eluate was then condensed using a rotary evaporator (Model IRA@ RV 10) at 40°C. The resulting solution with a final volume of 25 ml was subsequently refrigerated at -20°C until further use.

The samples were analyzed using GC-MS (18) (Model: GC Agilent - 7890A, MS Agilent - 5975C MSD). A One µL aliquot of the reaction mixture was injected directly into the gas chromatograph. The following conditions were applied: initial temperature was set at 80$^0$C for one minute, then increased up to 250$^0$C at a rate of 8$^0$C per minute and further increased to 300$^0$C at 12$^0$C per minute and held for 5 minutes. The total GC run time was 30 minutes, with the injector temperature maintained at 240$^0$C.

### Statistical design

Analysis of variance was carried out, and comparisons were performed using Duncan's Multiple Range Test (DMRT). The mean difference was significant at P-values < 0.05. The data represent the means from three replicates with standard errors. Different letters within each column indicate significant differences between treatments, as determined by Duncan's multiple range test at P < 0.05. Graphs were generated using Microsoft Excel (2019) and GraphPad Prism version 5.8. The data was further analyzed using R-software's principal component analysis (PCA).

## Results

### Efficacy of Fish Fermented extract on seedling growth parameters

The results revealed that compared with nonprimed seeds, standardized seeds primed with organic inputs and liquid microbial cultures showed significant increases (P <0.05) in seedling attributes such as germination percentage, germination energy, root and shoot length (cm), vigour index I and vigour index II. Among the treatments, priming with 2 % Fish fermented extract for 8 hrs ($T_3$) showed an increase in the germination percentage (19.4%) followed by $T_6$ (16.4%) compared with the control (Fig 1). Furthermore, the speed of germination (40.9%), root length (44.4%), shoot length (24.4%) and seedling vigour index (68.1%) were higher in 2 % Fish fermented extract for 8 hrs ($T_3$) followed by PPFM liquid culture at a 1:100 dilution for 8 hrs ($T_6$) than in control. A highly statistically significant difference was observed among the treatments regarding the germination rate and vigour of the seedlings compared with those of the untreated seeds. Generally, priming treatments increase the germination rate (%) and seedling vigour compared to the control (without priming). The improved seedling parameters were significantly improved (P <0.05) because of the effect of biopriming with fish-fermented acid (Table 1). However, increasing the concentration of biopriming agents affected seed germination and other seed quality parameters.

### Impact of anatomical structure changes in primed and nonprimed Seeds

The present study subjected brinjal PLR 2 seeds to different biopriming agents for 8 hrs. The results demonstrated a statistically significant increase (P <0.05) in the embryonic parameters, *viz.,* average length of the radicle and cotyledon/embryo for the PLR 2 varieties of Brinjal seeds subjected to different treatments compared with the nonprimed seeds. Among the various concentrations of biopriming agents used, $T_3$, followed by $T_6$, had the maximum embryo and radicle length. The extent to which an increase in embryo size was observed is discussed below. The corresponding lengths of embryo and radicle length were recorded in ($T_3$) 2 % Fish fermented extract for 8 hrs (15.22 mm and 6.96 mm) (Fig 2) followed by ($T_6$) PPFM liquid culture @1:100 dilution for 8 hrs (14.20 mm and 6.84mm) respectively.

In contrast, the minimum length was recorded (10.49 mm and 4.94 mm) in untreated seeds (Table 2). The enhanced anatomical potential of the embryo may have led to the significant improvement in germination and seedling growth observed with biopriming with 2 % fish-fermented extract for 8 hrs. However, increasing the concentrations of

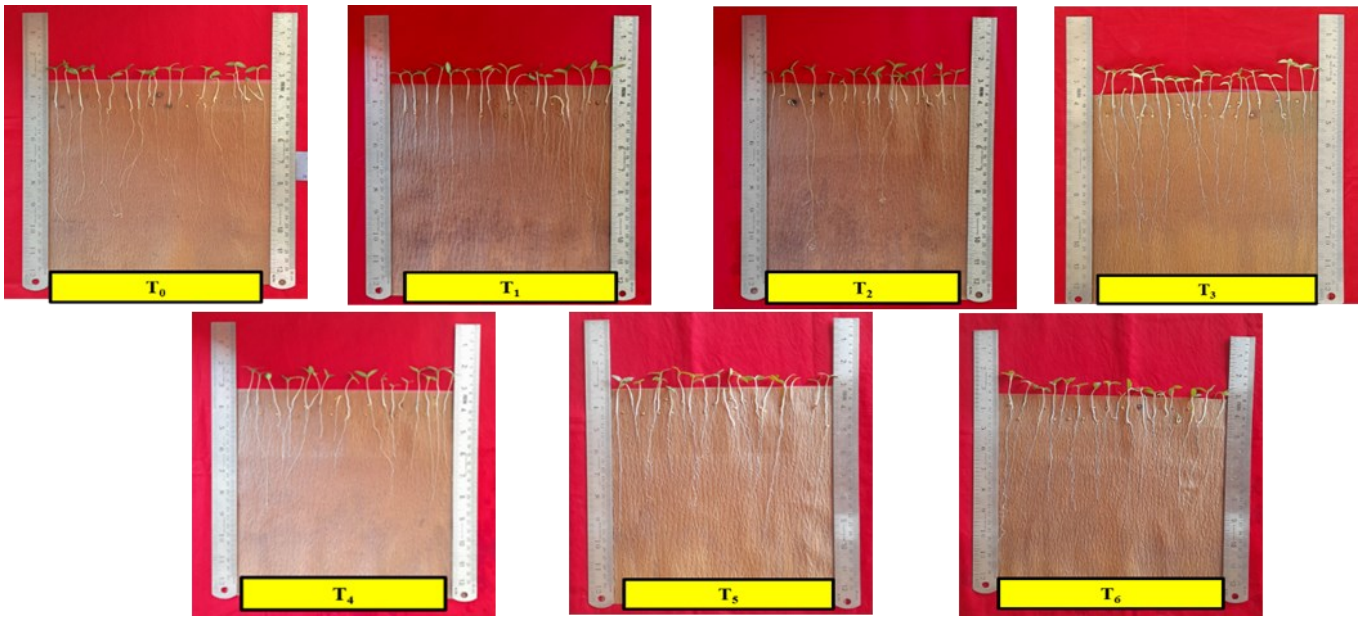


**Figure 1.** Effect of seed biopriming on germination and seedling vigour of brinjal cv. PLR 2

Treatments are as follows: $T_0$-Control (Non primed seeds); $T_1$- Seed biopriming with 3% Panchagavya; $T_2$- Seed biopriming with 2 % Egg fermented extract; $T_3$- Seed biopriming with 2 % Fish fermented extract; $T_4$- Seed biopriming with 5 % EM solution (Effective microorganisms); $T_5$- Seed biopriming with *Azospirillum* liquid culture at a 1:50 dilution; $T_6$- Seed biopriming with PPFM liquid culture at a 1:100 dilution.

**Table 1.** Effect of seed biopriming on physiological parameters of brinjal cv. PLR 2

| Treatments | Germination energy | Germination (%) | Root length (cm) | Shoot length (cm) | Vigour index I | Vigour index II |
|---|---|---|---|---|---|---|
| $T_0$ | 12.7± 0.20 $^{e}$ | 67(54.9) ± 1.8 $^{d}$ | 8.1 ± 0.05 $^{e}$ | 4.5 ± 0.04 $^{f}$ | 844 ± 19.50 $^{e}$ | 1.41 ± 0.02 $^{f}$ |
| $T_1$ | 15.9± 0.26 $^{cd}$ | 76(60.7) ± 1.7 $^{bc}$ | 9.9 ± 0.02 $^{cd}$ | 4.7 ± 0.10 $^{ef}$ | 1110 ± 27.15 $^{cd}$ | 2.13 ± 0.05 $^{e}$ |
| $T_2$ | 16.3± 0.14 $^{c}$ | 75(60.0) ± 1.9 $^{bc}$ | 10.2 ± 0.24 $^{c}$ | 4.9 ± 0.07 $^{cd}$ | 1133 ± 13.56 $^{c}$ | 2.33 ± 0.05 $^{d}$ |
| $T_3$ | 17.9± 0.20 $^{a}$ | 80(63.7) ± 2.4 $^{a}$ | 11.7 ±0.26 $^{a}$ | 5.6 ± 0.05 $^{a}$ | 1419 ± 1.48 $^{a}$ | 3.44 ± 0.06 $^{a}$ |
| $T_4$ | 15.7± 0.01 $^{d}$ | 74(59.3) ± 1.7 $^{c}$ | 9.6 ± 0.02 $^{d}$ | 4.7 ± 0.03 $^{de}$ | 1058 ± 20.92 $^{d}$ | 2.07 ± 0.04 $^{e}$ |
| $T_5$ | 16± 0.19 $^{cd}$ | 77(61.3) ± 1.5 $^{abc}$ | 10.8 ± 0.07 $^{b}$ | 5.0 ± 0.05 $^{bc}$ | 1217 ± 26.60 $^{b}$ | 2.70 ± 0.06 $^{c}$ |
| $T_6$ | 16.8± 0.19 $^{b}$ | 78(62.0) ± 1.8 $^{ab}$ | 11.0 ± 0.10 $^{bc}$ | 5.1 ± 0.07 $^{b}$ | 1256 ± 9.80 $^{b}$ | 3.04 ± 0.03 $^{b}$ |

Treatments are as follows: $T_0$-Control (Non primed seeds); $T_1$- Seed biopriming with 3% Panchagavya; $T_2$- Seed biopriming with 2 % Egg fermented extract; $T_3$- Seed biopriming with 2 % Fish fermented extract; $T_4$- Seed biopriming with 5 % EM solution (Effective microorganisms); $T_5$- Seed biopriming with *Azospirillum* liquid culture at a 1:50 dilution; $T_6$- Seed biopriming with PPFM liquid culture at a 1:100 dilution.

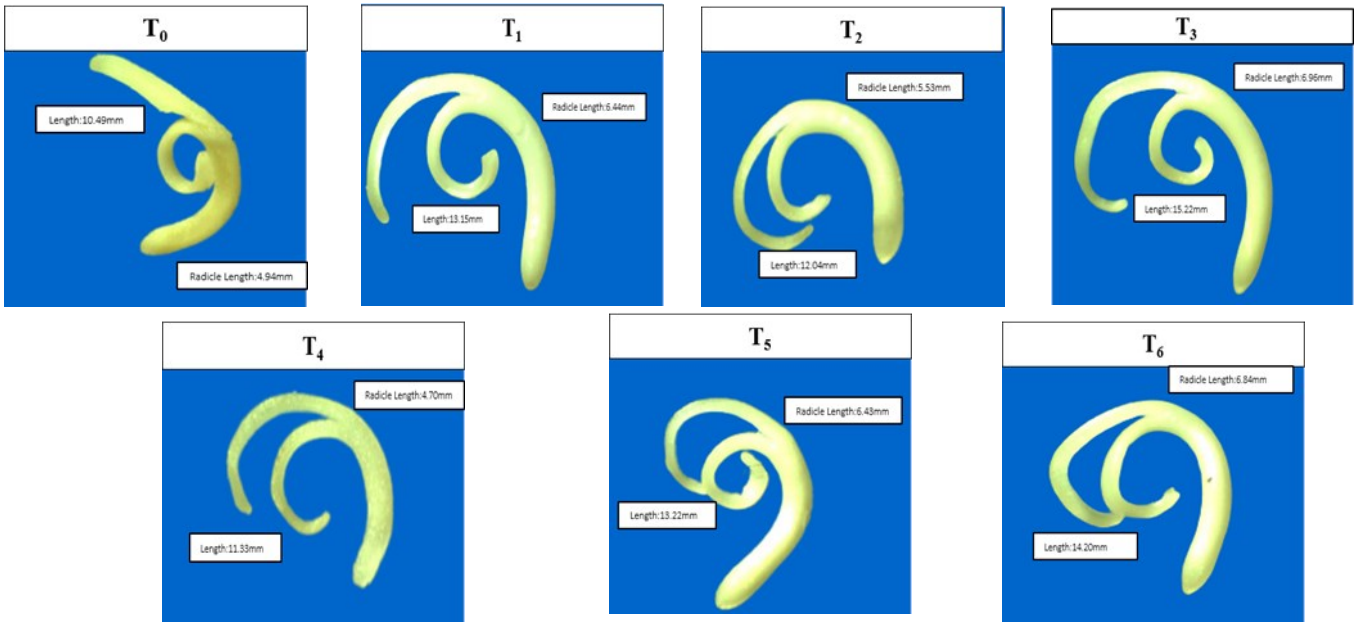


**Figure 2.** Microscopic analysis (Euromex) of embryo showing different internal morphology (length of radicle and cotyledon)

Treatments are as follows: $T_0$-Control (Non primed seeds); $T_1$- Seed biopriming with 3% Panchagavya; $T_2$- Seed biopriming with 2 % Egg fermented extract; $T_3$- Seed biopriming with 2 % Fish fermented extract; $T_4$- Seed biopriming with 5 % EM solution (Effective microorganisms); $T_5$- Seed biopriming with *Azospirillum* liquid culture at a 1:50 dilution; $T_6$- Seed biopriming with PPFM liquid culture at a 1:100 dilution.

**Table 2.** Effect of biopriming on seed anatomical parameters of brinjal cv. PLR 2

| Treatments | Average length of embryo/ cotyledon (mm) | Average length of radicle (mm) |
|---|---|---|
| $T_0$ | 10.49 ± 0.131$^{e}$ | 4.94 ± 0.061$^{d}$ |
| $T_1$ | 13.15 ± 0.253$^{c}$ | 6.44 ± 0.131$^{b}$ |
| $T_2$ | 12.04 ± 0.238$^{d}$ | 5.53 ± 0.023$^{c}$ |
| $T_3$ | 15.22 ± 0.158$^{a}$ | 6.96 ± 0.076$^{a}$ |
| $T_4$ | 11.53 ± 0.126$^{d}$ | 4.94 ± 0.121$^{d}$ |
| $T_5$ | 13.22 ± 0.275$^{c}$ | 6.43 ± 0.117$^{b}$ |
| $T_6$ | 14.20 ± 0.288$^{b}$ | 6.84 ± 0.071$^{a}$ |

Treatments are as follows: $T_0$-Control (Non primed seeds); $T_1$- Seed biopriming with 3% Panchagavya; $T_2$- Seed biopriming with 2 % Egg fermented extract; $T_3$- Seed biopriming with 2 % Fish fermented extract; $T_4$- Seed biopriming with 5 % EM solution (Effective microorganisms); $T_5$- Seed biopriming with *Azospirillum* liquid culture at a 1:50 dilution; $T_6$- Seed biopriming with PPFM liquid culture at a 1:100 dilution.

biostimulants and liquid biofertilizers affected the length of the embryo/cotyledon and radicle.

## Effects of biopriming on biochemical changes during germination

The a-amylase activity (mg maltose min$^{-1}$), protein content (%) and total free amino acids (µg g$^{-1}$) of brinjal seeds were strongly influenced by seed biopriming with the addition of fish waste among the different treatments and nonprimed seeds. Among the treatments, α-amylase activity was more significant in bioprimed seeds, with 2 % Fish fermented acid for 8 hrs ($T_3$) during seed germination recorded (0.498 mg maltose min$^{-1}$) followed by ($T_6$) PPFM liquid culture at a 1:100 dilution for 8 hrs recorded (0.451 mg maltose min$^{-1}$) than in nonprimed seeds which have 0.235 mg maltose min$^{-1}$(Fig. 3A). The results showed an increase in α-amylase activity in the bioprimed seeds, along with a corresponding increase in the germination percentage.

The presence of the fish waste amendments increased the protein content of $T_3$(15.40%), followed by $T_2$(15.17%) and the lowest protein content was recorded in nonprimed seeds (14.07%) (Fig.3B). The total free amino acid content was higher in $T_3$(0.116µg g$^{-1}$) followed by $T_6$ has (0.101µg g$^{-1}$) in the primed seeds when compared to the nonprimed seeds (0.042µg g$^{-1}$) (Fig. 3C). The germination percentage and α-amylase activity were significantly positively correlated (Fig. 3D).

## GC-MS analyses of root volatile compounds in bioprimed seeds

Gas Chromatographic - Mass Spectrometry analysis of root volatile compounds collected from 14-day-old seedlings revealed consistent differences in the composition of volatile blends released by the Fish fermented extract compared with those released by the untreated control.

The eighteen compounds identified from the roots of non-treated seedlings (Supplementary Table 1A) included 2 Pyrrolidinemethanol, ethanol, 2-phenoxy, benzoic acid, 4-ethoxy-, ethyl ester, 1,3-benzenedicarboxylic acid, bis (2-ethylhexyl) ester, diphenyl sulfone, campesterol, stigmasterol and gamma-sitosterol. The identified compounds have antimicrobial, antiviral, antibacterial, antifungal and anticancer activities. The primary chemical constituents were benzoic acid, 4-ethoxy-, ethyl ester with a peak area of 27.76 % with a retention time of 9.83 and 2 Pyrrolidinemethanol, 1-methyl- with a peak area of 14.83% and a retention time of 5.77 (Fig 4).

In contrast, 21 chemical constituents have been identified from the roots of brinjal seeds bioprimed with fish-fermented acid (Supplementary Table 1B), including decane, 2-pyrrolidine methanol, rhodopin, acetamide, hexanedioic acid, 1,2-benzene dicarboxylic acid, bis(2-methyl propyl) and mono (2-ethylhexyl) ester, campesterol, stigmasterol and gamma-Sitosterol. The identified compounds have antimicrobial, antiviral, antibacterial, antifungal, antioxidant and insecticidal activities. The primary chemical constituents were 2-pyrrolidine methanol, 1-methyl- with a peak area of 33.10 % with a retention time of 5.76 and stigmasterol with a peak area of 9.70% and a retention time of 21.55 (Fig. 5). Additionally, the heatmap analysis results showed significant differences in metabolite expression patterns between the two groups (Fig. 6).

## Principal component analysis

The relationship between measured seedling growth parameters was demonstrated through principal component analysis (PCA) (Fig. 7). It is an effective statistical tool for reducing multiple correlated variables into a smaller set of principal components.

The PC 1 axis explains 94.35% of the total variance in the data. The PC 2 axis explains 4.39% of the total variance in the data. PC 1 and PC 2 explain a significant portion (98.74%) of the total variance. Shoot length, vigour index II, free amino acids, vigour index I and root length had strong positive loadings on PC 1 and PC 2, indicating that they contributed significantly to the variation explained by these components. Moreover, the germination percentage, germination energy, α-amylase content and protein content (%) had weaker loadings on PC 1 but still contributed to the variation.

When $T_0$ is positioned on the positive side of PC 2, this treatment is distinct from the other treatment. Its unique position suggests it has characteristics significantly different from the other treatments and is mainly influenced by the variables contributing to PC 2. $T_1$, $T_2$ and $T_4$ are clustered in the negative quadrants of PC 1 and PC 2. Their proximity suggests that they have similar characteristics. $T_3$ is positioned positively along PC 1, indicating that variables with high positive loadings on PC 1 characterize it. $T_5$ and $T_6$ are positioned closer to the origin, suggesting that these treatments have more average values for the variables measured and do not exhibit extreme characteristics in either direction.

# Discussion

Seed priming using biostimulants derived from natural fish

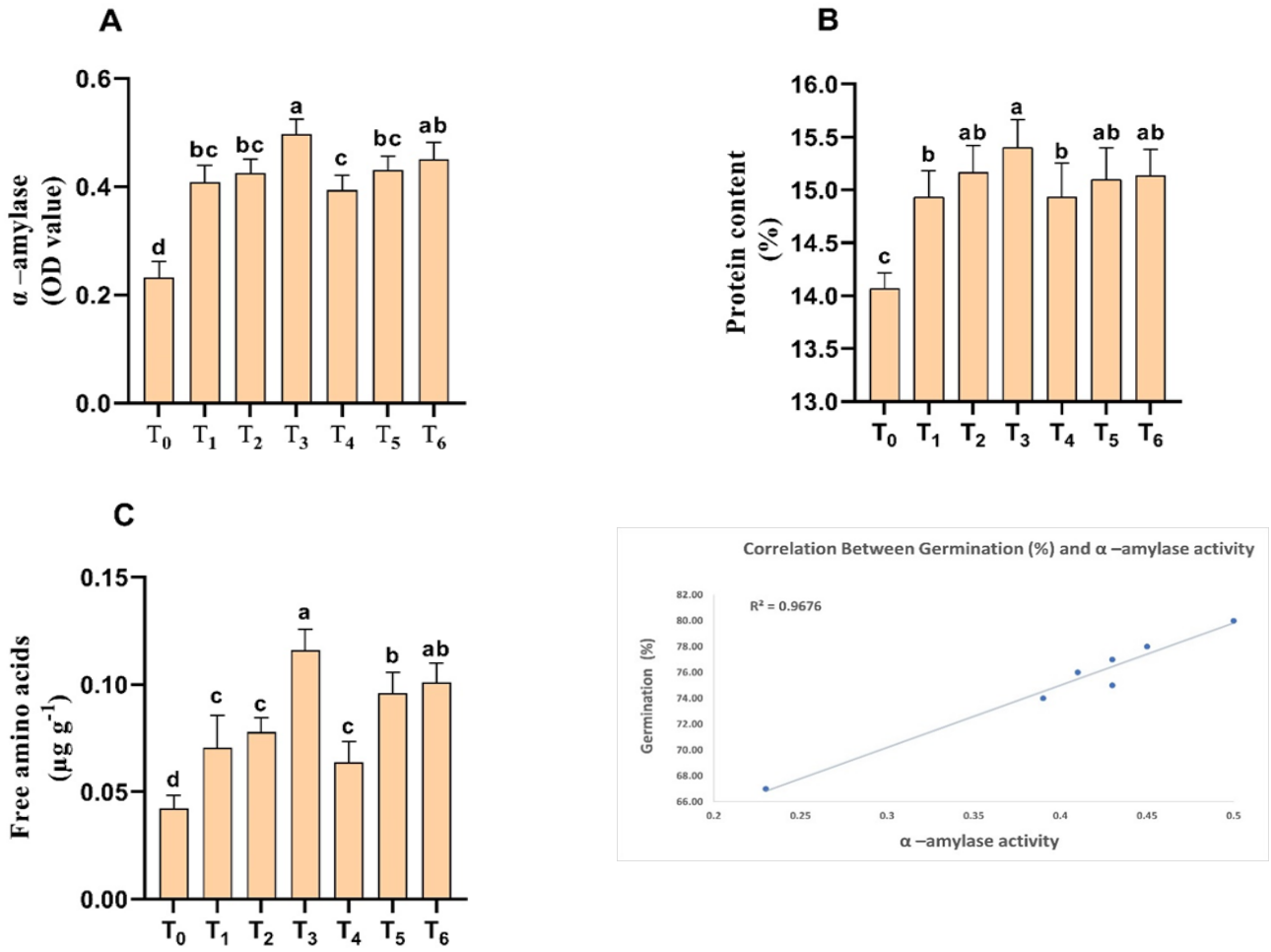


**Figure 3.** The effect of seed biopriming on (A) α-amylase activity (B) Protein content (C) Total free amino acids (D) Correlation analysis between germination

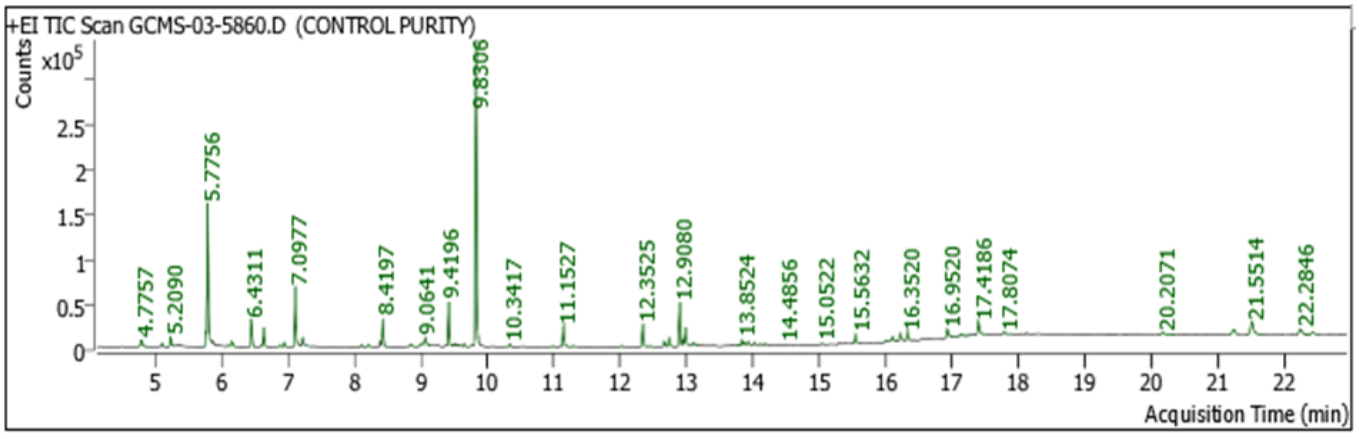


**Figure 4.** GC-MS chromatogram of methanolic extracts from 14 days brinjal seedlings of untreated seeds.

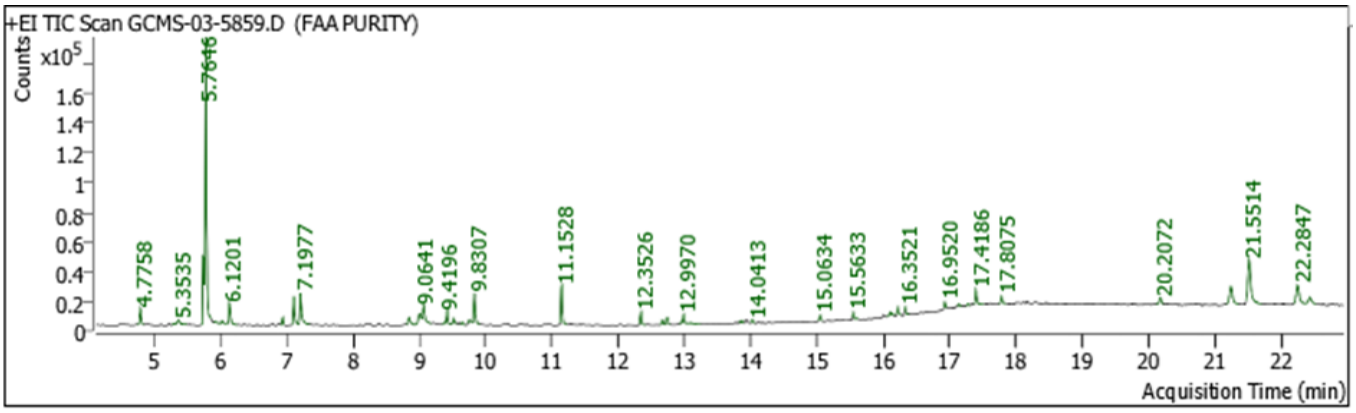


**Figure 5.** GC-MS chromatogram of methanolic extracts from 14 days brinjal seedlings of bioprimed seeds with fish fermented acid

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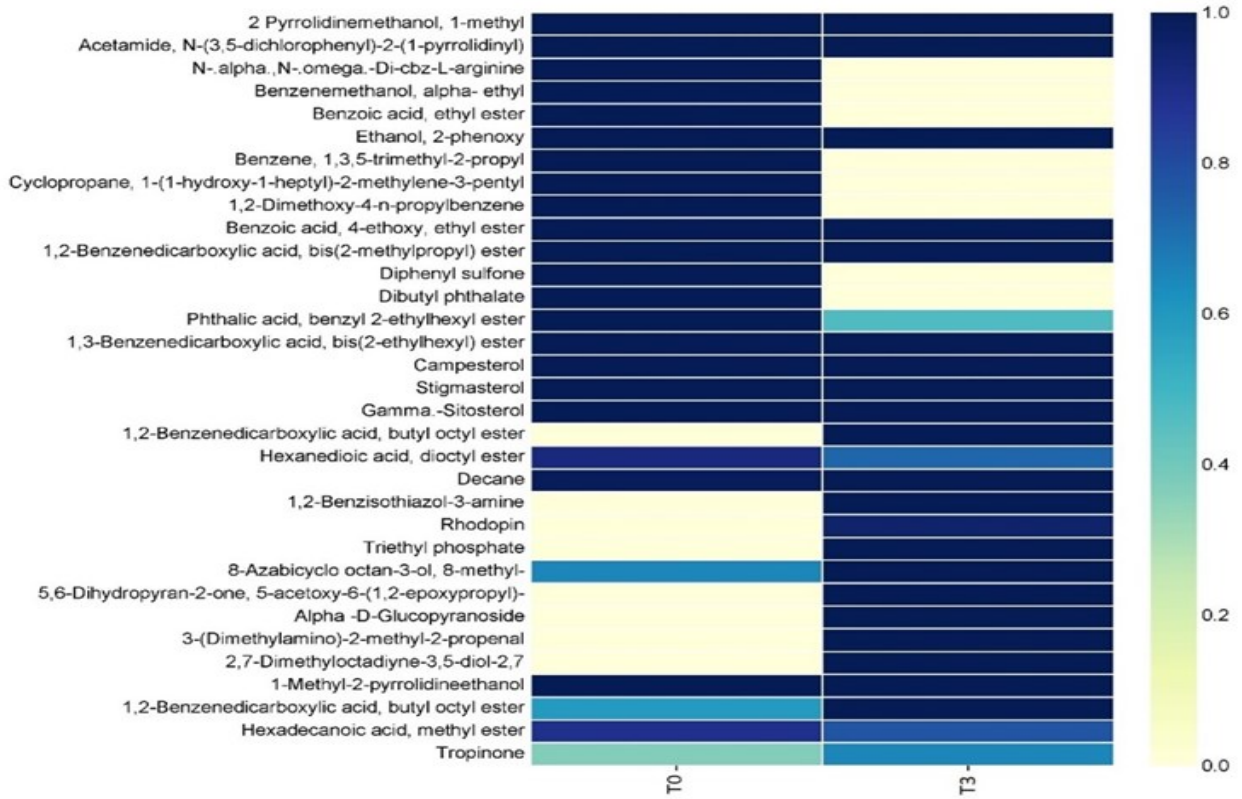


**Figure 6.** Heatmap visualization - The colour gradient from yellow to blue represents the range of metabolite expression levels, with yellow indicating lower levels and blue indicating higher levels. Treatments are $T_0$ - Control (Non-primed seeds); $T_3$ - Seed biopriming with 2 % Fish fermented extract.

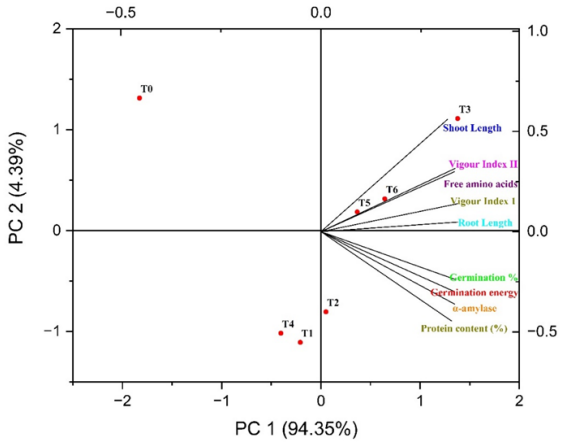


**Figure 7.** The principal component analysis shows treatments' impact on variables such as seed quality and biochemical parameters of brinjal cv.PLR 2.

products represents one of the most innovative and effective methods to enhance seed germination, promote early radicle protrusion and improve seedling establishment under abiotic stress conditions (19). Using animal-derived protein hydrolysates in agriculture, such as fish by-products, enhances plant growth and crop development while potentially reducing the use of chemical fertilizers (20). Fish by-products contain significant amounts of proteins, fat, amino acids and peptides following protease hydrolysis. Animal-derived protein hydrolysates contain large amounts of amino acids (rich in proline and glycine, which are essential for the central metabolism in developing seeds) and peptides (which play a role as signal molecules in plant physiology, regulating defensive mechanisms in response to stress, influencing the plant growth and development) (21) and contain small amounts carbohydrates, phytohormones, mineral elements and phenols. Peptides also promote the synthesis of plant growth hormones, such as gibberellins, cytokinins and auxins. These hormones play crucial roles in cell division, elongation and overall plant growth, leading to improved germination rates and healthier seedlings. These by-products are excellent for use as biostimulants in organic agriculture, similar to other animal protein hydrolysates (22).

Fish waste also contains macro and micronutrients, which increase root growth activity and the expression of plant growth regulators (23). Amino acids are a vital source of nitrogen for plant growth. When these nutrients are applied to seeds, they are readily available for uptake, increasing their metabolic processes, which can enhance germination and early seedling vigour. Interestingly, the current study demonstrated that, compared to nonprimed seeds, brinjal seeds priming with 2 % fish-fermented acid increased the seed quality parameters.

However, some studies have shown that fish protein hydrolysates may positively affect plant growth and seedling vigour. Seed priming with fish protein hydrolysates (FPH) at 2.5 ml/l and 5.0 ml/l shows enhancement of seed vigour of soybean, tomato and corn seeds (24). Similarly, these results are in parallel with (25), who reported that the seed rehydration treatment with FPH (2ml $1^{-1}$ concentration) increased the average plant height and weight and enhanced seedling vigour in peas. Previous reports have suggested that using fish waste increases the fresh and dry weights of shoots, roots and bulbs in onions and similar results were recorded with the growth enhancements in okra (26).

Furthermore, Madende *et al.* (27) reported that fish

waste is rich in amino acids that can enhance plant growth, protect plants from ecological stress factors and perform an essential function in metabolic signalling by regulating nitrogen uptake in the roots (28). Protein hydrolysates enhance plant growth by improving nutrient uptake and metabolism. This improvement is due to increased soil microbial activity, as well as in root length, density and the number of lateral roots (29).

According to Liatile *et al.* (30), Xcell Boost (fish protein hydrolysate) is a highly beneficial biostimulant for increasing spinach resilience to drought stress. This may also be helpful for other crops cultivated in semiarid and arid regions, resulting in increased plant growth attributes and lettuce yield (31). As previously discussed, many fish-fermented products have been studied, revealing positive effects on various crop plants. Altogether, these products are also applied to supply nitrogen (N), sometimes in combination with phosphorus (P). In some cases are enriched as compost and less often as a complete fertilizer that fulfills a crop's nutritional requirements.

In the present study, bio-primed seeds show an increase in the average length of the embryo compared to the control. These findings are consistent with those of earlier studies (32), which revealed that enhancing the anatomical potential of okra embryos led to significant improvements in seed germination and seedling growth, particularly when subjected to humid priming for 4 hrs. The accelerated speed of germination and improved germination observed in pre-hydrated seeds were likely due to the enlargement of the embryo (33). Vijayalakshmi *et al.* (34) also suggested that enhanced seed germination and speed of emergence with an adequate supply of soluble carbohydrates to the developing embryo were facilitated by an increase in α-amylase activity. However, few reports have documented the anatomical changes associated with seed growth attribute enhancement. Notably, the embryo's size increases even in seeds without radicle protrusions. This suggested that significant growth occurred in the embryonic plant before the cell division phase, likely due to cell elongation and increased embryo size.

PHs are known to exhibit hormone-like activities, particularly auxin, gibberellin and their hydrolytic enzymes, which collectively stimulate seed germination and promote seedling growth. The presence of amino acids and peptides in Fish Protein Hydrolysates (FPH) can activate critical enzymes involved in the breakdown of macromolecules that facilitate the development and growth of the embryo, leading to earlier and improved seedling emergence. One of the essential enzymes is α-amylase, which breaks down stored starches into simpler sugars that the seedling uses as an energy source. It also promotes the activity of other enzymes involved in protein synthesis, which are necessary for the growth and development of seedlings.

The present investigations revealed that these biochemical changes increase the germination rate and seedling vigour by increasing protein and amino acid contents in bioprimed seeds. Several studies have investigated the increase in protein content of bioprimed brinjal seeds treated with fish waste may be attributed to enhanced nitrate uptake and biological nitrogen fixation. This process involves an enzyme complex associated with L-arginine, which is crucial for protein biosynthesis (35). Similarly, in strawberries (36), fish waste combined with dual inoculation of arbuscular mycorrhizal fungi and *Trichoderma viride* led to a slight increase in protein and amino acid. These results followed Shahsavani *et al.* (37), who revealed an increase in the seed protein percentage of Vigna sinensis due to fish waste and *Pseudomonas* bacteria and their interactions compared to the control.

Root exudates aid in the initial colonization of rhizospheric bacteria and serve as a significant nutrient source for microorganisms in the rhizosphere. The stimulation of microorganisms in the rhizosphere appears to be due to organic compounds released by the roots, which increases the plant's dry weight up to 20% (38). Based on GCMS analysis results, VOCs released from the roots, such as hexadecenoic acid, are known to have antibacterial, antifungal activity, antioxidant, nematicide and 5-alpha reductase inhibitor (39). Rhodopin is an identified carotene known for its natural antioxidant properties (40). Amino acids such as 8-Azabicyclo octan-3-ol are tropane alkaloids, similar to other significant categories of plant secondary metabolites predominantly found in the Solanaceous family. These plants' roots produce tropane alkaloids, which are then transported to the leaves and reproductive organs (41). Benzoic acid, 4-ethoxy- and ethyl ester have antibacterial properties against test pathogens (42) and are antioxidants, anti-inflammatory and free radical scavengers (43). Diphenyl sulfone was also found to possess antibacterial activity (44).

Some other groups of root volatile compounds, such as phytosterols (esters), are released by roots. Phytosterols are commonly present in seeds, grains, beans, nuts (45) and cereals. These compounds, similar to those in vegetable oils, include sitosterol, campesterol, stigmasterol, etc. (46) in corn and cereal seeds also found (47). Stigmasterol has antibacterial resistance *in Channa punctate* against *Vibrio harveyi* infection (48) and Bassuany *et al.* (49) concluded that presoaking flax seeds in stigmasterol could improve salt tolerance, leading to enhanced growth and photosynthetic pigments. γ-sitosterol is a vital plant sterol reported to exhibit anticancer and anti-inflammatory activity (50). Bioprimed seeds produce more root volatile compounds, are resistant to pathogens and promote seedling growth.

## Conclusion

The study demonstrated that biopriming with fish-fermented extract significantly enhances seed growth attributes in brinjal. The results showed that seeds soaked in 2% fish-fermented extract for 8 hours improved germination rate, root length, shoot length and overall seedling vigour index compared to nonprimed seeds. The biochemical analysis also revealed increased α-amylase activity, protein content and other vital parameters in bioprimed seeds. These findings suggest that fish-fermented extract can be an efficient and environmentally friendly alternative to chemical fertilizers, promoting

agricultural sustainability and contributing to higher crop quality and yield. Future research should focus on the long -term effects of biopriming and explore its applicability to other crops to validate these promising results further.

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## Authors' contributions

CH carried out the experiments and prepared the original draft of the writing. VV Conceptualization. SK and EP supervised the work and drafted and reviewed the manuscript. MK participated in the sequence alignment and editing. KP visualization. All authors have read and agreed to the published version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

### Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this work, the author(s) did not use any generative AI or AI-assisted technologies. The content is entirely the result of the author(s)' original work, and they take full responsibility for the publication's content.

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