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Research Article

Biopriming of *Momordica charantia* Seeds with *Enterobacter* to Improve Nutritional and Biochemical Attributes

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The increasing world population needs a standard balanced diet to address malnutrition problems. For this purpose, seed priming is one of the best techniques, which helps to increase the production of functional and nutritional food crops. Different techniques have been used for seed priming, but biological priming is the most frequently used because biocontrol agents offer a friendly environment for the growth of food crops. In this study, *Momordica charantia* L. seeds were subjected to a strain of *Enterobacter* sp. FD17 as a biocontrol agent at different time exposures (i.e., 24 h, 48 h, and 72 h). Leaf growth, flavonoids, chlorophyll content, amino acids, soluble sugars, protein, and total soluble phenolics were studied in the vegetative stage. The yield of nutritive components was evaluated from fruit, peel, and pulp of *M. charantia*. Biopriming was revealed to improve the final emergence rate, mean emergence time, seedling vigor, emergence index, and vigor indices I and II. Among the growth parameters, the root (0.45 ± 0.045 g) and shoot fresh weight (1.23 ± 0.05 g), leaf area (15.52 ± 1.5 cm), shoot length (30.33 ± 0.58 cm), number of flowers (6 ± 1.0), fruit weight (96.33 ± 1.15 g), and germination percentage (56.67 ± 11.55%) were also improved. Among biochemical analyses, biopriming improved chlorophyll a (6.33 ± 0.58 mg/g) and b (8.58 ± 2.5 mg/g), total soluble sugar (33.13 ± 2.24%), and total chlorophyll content (9.0 ± 1.5 mg/g). The nutritional analysis showed that free amino acids (1.43 ± 0.02 mg/g), total soluble sugar (42.53 ± 1.65%), ash (20.53 ± 2.57%), and catalase (347.47 ± 34.76 U/g) were increased in fruit, while crude fiber (3.62 ± 0.1%) and peroxidase (5.61 ± 0.34 U/g) in peel and protein and metabolizable energy in peel and fruit were increased. Among the water, acetone, and methanol extracts, the maximum antibacterial activity was shown by methanol extracts of leaves against Gram-negative and Gram-positive bacterial species (i.e., *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively) with inhibitory diameters of 3 mm. Biopriming also improved the phenolic contents in the leaves and fruits of *M. charantia*. Biopriming treatment was also revealed to be directly correlated with antiglycation activity. Therefore, biopriming treatment on seeds could be used to manipulate plant cell metabolism with a substantial improvement in phenolic content, antibacterial activity, and growth of *M. charantia*.

1. Introduction

Our agriculture faces issues related to climate change and reductions in production, biodiversity, and resources. Nutraceuticals and functional foods are a cheap solution to this problem. Specific defense mechanisms in plants are strongly dependent on the state of seed priming. Priming has been divided into three different states, including a priming phase, a postchallenge primed state, and a transgenerational primed state [1]. In developing countries like Pakistan, herbal medicines have become more important in solving the problems of the health-seeking populations of these countries. Seed priming is a technique that is applied to seeds before sowing. In this technique, the seeds are moderately

hydrated to a point where metabolic processes related to pregermination begin but without actual germination. The seeds are dried almost completely to handle normally. Among the different priming techniques, biopriming gives an extra advantage to manage biotic stress, and therefore, this priming method has gained more attention [2]. In seed biopriming, beneficial microbes are utilized to improve the physiological performance of seeds and enhance their ability to withstand stress, and therefore it is an environmentally friendly and advantageous technique of seed priming [3].

A wide range of bacteria (e.g., *Azotobacter*, *Bacillus*, *Burkholderia*, *Arthrobacter*, *Klebsiella*, *Azospirillum*, *Agrobacterium*, *Rhizobium*, *Streptomyces*, phosphate-solubilizing bacteria, *Pseudomonas fluorescens*, and *Enterobacter*) and/or fungi (e.g., vesicular-arbuscular mycorrhiza, *Trichoderma viride*, and *Trichoderma harzianum*) have been found to be promising biopriming agents, whether these agents are used as biopesticides or biofertilizers [4].

Momordica charantia is usually used as a vegetable and is also an important medicinal plant economically. The common name of *M. charantia* is bitter gourd [5]. *M. charantia* is beneficial in the physiological and metabolic processes of the human body. *M. charantia* juice increases body stamina and prevents chronic fatigue. Various parts of *M. charantia* are used for the treatment of different diseases, including anemia, blood disorders, diabetes, cancer, diarrhea, cholera, and bronchitis [6]. Bitter gourd has biologically active plant chemicals such as proteins, steroids, alkaloids, triterpenes, flavonoids, saponins, and other acids that exhibit antibacterial, antifungal, antiviral, antifertility, anticarcinogenic, antidiabetic, and hypoglycemic properties [7].

Bitter gourd can reduce blood glucose in diabetic patients. Bitter gourd decreases the amount of glucose in the blood by inhibiting the enzyme α-glucosidase, which causes the breakdown of oligosaccharides to monosaccharides [8]. Bitter gourd affects glucose transport channels, which decrease glucose transport into the blood. This impact is important for the treatment of both types of diabetes (i.e., type I and type II). Bitter gourd contains a chemical known as charantin that is used to reduce blood glucose. A regular high sugar concentration has been observed in type I and type II diabetes, which could increase the risks of inflammation, blindness, oxidation in the whole body, kidney diseases, and heart attack [9]. The aim of this research work was to compare the growth parameters (i.e., mean germination time and percent germination, determination of vigor indices, shoot and root lengths, and total fresh and dry weights), biochemical changes (i.e., photosynthetic pigment, total soluble sugar and free amino acids, and antibacterial activity), nonenzymatic antioxidant (i.e., total phenolic contents and total flavonoid contents), and antiglycation activity induced in *M. charantia* by a biological priming method (i.e., treated with *Enterobacter* sp. strain FD17 for different time exposures of 24 h, 48 h, and 72 h).

2. Materials and Methods

2.1. Sample Collection.
The seeds of *M. charantia* of a local variety were provided by AARI (Ayub Agriculture Research Institute), Faisalabad, Pakistan. Taxonomical identification of *M. charantia* seeds was carried out at the Department of Botany of Government College University, Faisalabad. The pot experiment was conducted with three replicates in a completely randomized manner in June under natural conditions of the environment to study growth and biochemical and antibacterial activity. A field experiment with a complete randomized block design was conducted in March with five replicates under natural environmental conditions to study the nutritional and nutraceutical potential.

2.2. Selection and Level Optimization

2.2.1. Biological Treatment.
Before biopriming, *M. charantia* seeds were thoroughly cleaned and then surface sterilized using mercuric chloride solution (1%) for three minutes. A set of samples with three replications and each sample comprising 40 seeds were used. The seeds were thoroughly washed with distilled water before subjecting to biopriming. *Enterobacter* (FD17) was applied to bitter gourd seeds. An inoculum of the selected strain (FD17) was prepared in a 200 ml Erlenmeyer flask containing 10% TSA broth (Trypton soya agar). The flask was inoculated with the selected bacterial strain and incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 100 rpm at optimized temperature levels, i.e., 24 h, 48 h, and 72 h at $28 \pm 1°C$. An absorbance of 0.5 was achieved, measured with an optical density meter (Biolog1 Model-21907; Biology Inc.) at wavelength 535 nm, by dilution to maintain a uniform cell density (10^7–10^8 CFU/ml) prior to seed inoculation. Finally, the suspension of bacterial strain was injected into sterilized peat (100 ml/kg, seed to peat ratio: 1:1 w/w). For inoculation, seed dressing was carried out on inoculated peat. In case of control, the seeds were coated with the same slurry but autoclaved without inoculum. Seeds inoculated/uninoculated were seeded in pots having soil (5 kg) [10].

2.3. Growth Parameters

2.3.1. Mean Germination Time and Percent Germination.
The incubation proportion was measured at the end of the seventh day of incubation by the process designated by Soad et al. [11].

$$Gp = \frac{Ng}{Np} \times 100, \qquad (1)$$

where "Ng" is the last number of seeds emerged and "Np" is the total number of seeds seeded.

Similarly, the mean growth time (MGT) in days was calculated as follows:

$$MGT = \frac{\sum (Dn)}{\sum n}, \qquad (2)$$

where "n" is the number of seeds germinated on day "D" and "D" is the number of days counted from the beginning of the germination test.

2.3.2. Determination of Vigor Index and Number of Leaves.
Following equations were used to determine seedling vigor [12].

$$\text{Vigor index I} = \text{germination percentage} \times \text{seedling length},$$
$$\text{Vigor index II} = \text{germination percentage} \times \text{seedling dry weight.} \qquad (3)$$

The leaves of all the plants in the field were counted manually in every row and the mean was taken. Roots and shoots were used to determine length and dry weight of seedlings.

2.3.3. The Length of the Shoot and Primary Root (cm).
The length of the shoot and the primary root from the ground to the ligule of the upper leaf of the plant in each pot was measured using a tape and the average length of each plant/shoot was determined. For the length of the primary root, the plants were taken from the land at the time of harvest. A scale was used to record the root length and the average was used.

2.3.4. Total Fresh and Dry Weight (g).
The total fresh weight was measured by adding weight of the root and shoots of every plant, while the total dry weight of the plant was measured based on the dry weights of the root and shoot.

2.4. Biochemical Parameters

2.4.1. Photosynthetic Pigments.
The method described by Arnon [13] was used to determine photosynthetic pigments. Total 0.5 g of fresh *M. charantia* leaves were ground using pestle and mortar in acetone solution (80%). After grounding, the solution was filtered and distilled water was used to make the final volume up to 10 ml of the filtrate. A spectrophotometer was used to note the absorbance at 480, 645, and 663 nm.

2.4.2. Total Soluble Sugar and Free Amino Acids.
Total soluble sugars were measured using the method defined by Van Handel [14] and the method of Hamilton et al. [15] was used to determine total free amino acids.

2.4.3. Antibacterial Testing.
Three extractions (i.e., water, acetone, and methanol) of fresh leaves of *M. charantia* were prepared. One hundred milligrams of leaf sample were used for each extraction. Broth Micro-Dilution Method [16] was employed to measure the antibacterial activity. Briefly, to prepare microdilution trays, a 2–6 fold dilution of the sample extract was used volumetrically. Each subsequent dilution step was performed using a new pipette and the extract was dispensed into the microdilution tray. Cultures of selected bacterial species (i.e., *P. aeruginosa* and *S. aureus*) were grown in their proper growth medium for inoculum preparation, and suspension (0.01 ml) was carefully transferred to broth. The growth of each bacterial species was maintained at 5×105 CFU/ml. Standardization of the inoculum was performed using the growth method and used for inoculation of each well of the microdilution tray. A volume not exceeding 10% was delivered in the well. Incubation was carried out in an ambient air incubator after a colony count of inoculum suspension for 16–20 hours at $35 \pm 2°C$. Finally, the lowest concentration (which completely inhibited the microbial growth) of leaf extract was determined in the microdilution wells and expressed as minimal inhibitory concentration (MIC).

2.5. Nonenzymatic Antioxidant

2.5.1. Total Phenolic Content (TPC).
The Folin–Ciocalteu reagent method [17] was used to measure total phenolic contents. To confirm oxidation of the Folin–Ciocalteu reagent (1 ml), dilutions were prepared and 7.5% sodium carbonate (2 mL; w/v) was used for neutralization. A final volume of 7 ml was maintained by adding distilled water. A spectrophotometer with a 1 cm cell was used to measure the absorbance at 765 nm of the resulting blue color after two hours of incubation at room temperature in the dark. To calibrate the curve, a standard (i.e., gallic acid) was used.

2.5.2. Total Flavonoid Content.
The colorimetric assay [18] was employed to determine total flavonoid content with minor modifications. Briefly, one milliliter of dilute sample was taken in a volumetric flask containing distilled water (4 ml) followed by the immediate addition of 5% $NaNO_2$ (0.6 ml), 10% $AlCl_3$ (0.5 ml) after 5 min, and 1 m NaOH (2 ml) after 1 min. The reaction flask was subsequently diluted by adding distilled water (2.4 ml) immediately and mixed. The absorbance at 510 nm was observed for the pink solution. To calibrate the curve, quercetin (μg/g) was taken as a standard. The total flavonoid content of the samples was measured with the help of the following linear equation ($y = 0.0019x + 0.6157$) based on the calibration curve.

2.6. Enzymatic Antioxidant

2.6.1. Catalase Activity.
The method of Aebi [19] was employed to check catalase activity with minor modifications. Briefly, in 1.5 ml of 1 m phosphate buffer (pH 7.0), half grams of leaves, peel, and fruit of the plant were ground in a prechilled mortar to homogenize. After centrifugation (15,000 rpm, 15 min at 40°C), catalase activity of the supernatant was checked. In a cuvette, phosphate buffer and H_2O_2 (3.0 ml) were taken and 40 μl of enzyme extract was added rapidly and thoroughly mixed. The time taken to decrease the absorbance by 0.05 units was measured at 240 nm on a spectrophotometer (Genesys 10-S, USA). The enzyme quantity required to decrease the absorbance at 240 nm by 0.05 units was taken as one enzyme unit.

2.6.2. Peroxidase Activity.
The method of Sadasivam and Manickam [20] was employed to determine peroxidase (POD) activity using 20 mm guaiacol and hydrogen peroxide as a substrate. Briefly, total 0.5 g of plant material was ground and extracted in a prechilled mortar by adding 3 ml of 0.1 m phosphate buffer (pH 7.0). After centrifugation (18,000 rpm, 15 min at 5°C), the supernatant was used as an enzyme source within 2–4 hours and stored on ice until POD analysis. Then the buffer solution (3 ml), guaiacol solution (0.05 ml), enzyme extract (0.1 ml), and hydrogen peroxide (0.03 ml) were pipetted into a cuvette. The mixture was shaken well, and the absorbance was recorded in a spectrophotometer. The time required for the mixture to increase absorbance by 0.1 (Δt) at 430 nm was recorded and used in the following calculations:

$$\text{the enzyme specific activity units} \left(g^{-1} \text{ f. wt.} \right) = \left[\frac{500}{\Delta t} \right] \times \left[\frac{1}{1000} \right] \times \left[\frac{TV}{VU} \right] \times \left[\frac{1}{f. wt.} \right], \tag{4}$$

where Δt = change in time (min), TV = total volume of extract (ml), UV = volume used (ml), and f. wt. = weight of fresh leaf tissues (g).

2.7. Antiglycation Activity

2.7.1. Sample Extraction and AGE Assay.
Methanol (50%) was added to one gram of the sample. The solution was centrifuged at 1500 rpm for ten minutes. To check the antiglycation activity, the supernatant was employed. A characteristic absorbance was used to check advanced glycation end products (AGEs) as given by Matsuda et al. [21]. Briefly, bovine serum albumin (BSA) (150 μl in 1 ml of Na$_3$PO$_4$ buffer at pH 7.2), d-glucose (150 μl), and sample (150 μl) were added and incubated for seven days at room temperature. Absorbance at 440 nm was calculated with a spectrophotometer. D-glucose was not added in the control and was used as a blank. The readings were taken in duplicates.

2.8. Percentage Inhibition (IC_{50}%).
The IC_{50} value is used to show the amount of a substance that is required to inhibit (i.e., *in vitro*) a specific biological component or process by 50%. The following equation was used to calculate the % inhibition:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100. \tag{5}$$

2.9. Statistical Analysis.
Complete randomized experiments were used in all analyses. Analysis of variance was applied to measure the significant differences in various factors. The least significant difference test was used to compare means to the final significant difference. The computer software CoStat (CoHort 2003 v.6.2) was used to check the overall interaction of all factors for significance.

3. Results and Discussion

3.1. Effect of FD17 Bacterial Strain Treatment on Germination

3.1.1. Final Emergence Rate.
The *Momordica charantia* seeds were treated with the bacterial agent *Enterobacter* sp. The FD 17 strain was used for this purpose. The seeds were dipped in bacterial culture at time exposures of 24 h, 48 h, and 72 h (Figure 1).

In Figure 1, it was found that *M. charantia* seeds treated at 72 hours with the FD17 bacterial strain have a high emergence rate of 70% compared to 24 and 48 hours. It was revealed that the control group treated with water had a high final emergence rate of 22% compared to the control group without water treatment, which had a final emergence rate of 18%. Therefore, compared to untreated samples, the percentage of the final emergence rate in seeds treated with FD17 bacterial strain was found to be significant ($p < 0.05$).

3.1.2. Mean Emergence Time.
The *M. charantia* seeds treated at 72 hours with FD17 bacterial strain have the highest mean emergence time compared to 24 and 48 hours (Figure 2). Furthermore, it was also revealed that the control group treated with water had a higher mean emergence rate compared to the control group without water treatment.
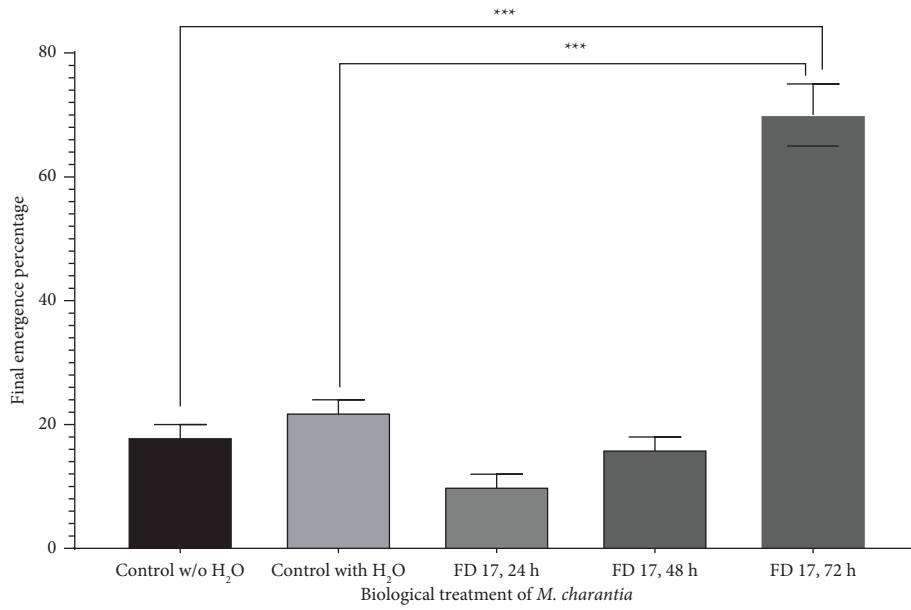
FIGURE 1: Percentage of final emergence of *Momordica charantia* seeds after biopriming with the microbial agent *Enterobacter* sp. strain FD17 for time exposures of 24, 48, and 72 hours. Values are given as mean (±SD). $p < 0.05$ is the significant difference in treatments.
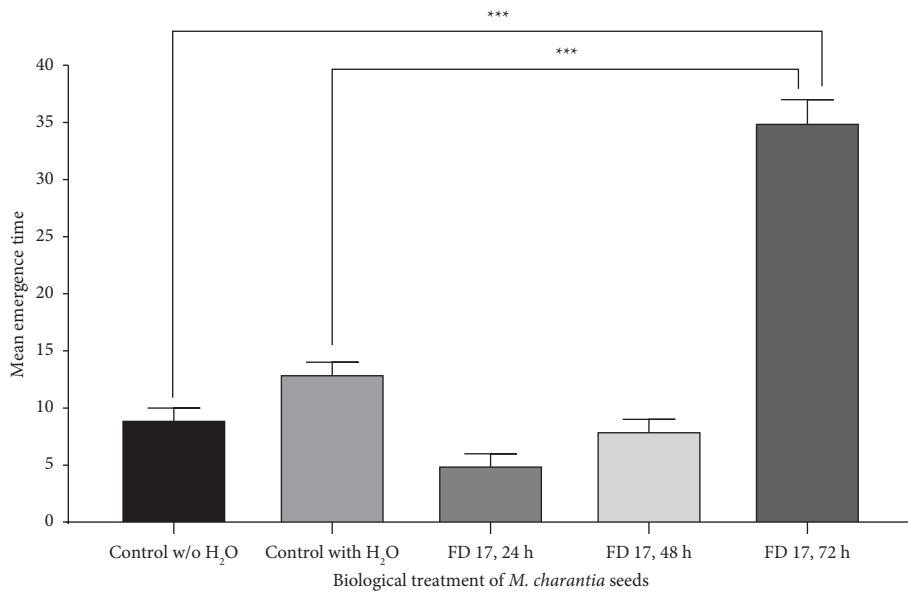

FIGURE 2: Emergence time of *M. charantia* seeds treated with the bacterial agent *Enterobacter* sp. strain FD17 for time exposures of 24, 48, and 72 hours. Values are given as mean (±SD). $p < 0.05$ is the significant difference in treatments.

Therefore, the mean emergence time was significant ($p < 0.05$) in *M. charantia* seeds treated with bacterial strain FD17 compared to untreated samples.

3.1.3. Seedling Vigor. It was found that *M. charantia* seeds treated at 72 hours with the FD17 bacterial strain were found to have the highest seedling vigor compared to 24 and 48 hours (Figure 3). Furthermore, it was explored that the control group (treated with water) had higher seedling vigor

compared to the control group without water treatment. Therefore, compared to untreated samples, the seedling vigor of *M. charantia* seeds treated with the bacterial strain FD17 was found to be significant ($p < 0.05$).

3.1.4. Emergence Index. Figure 4 shows that *M. charantia* seeds treated with bacterial strain for 72 h had the highest emergence index compared to treatments of 24 h and 48 h and the control groups with and without water treatment.
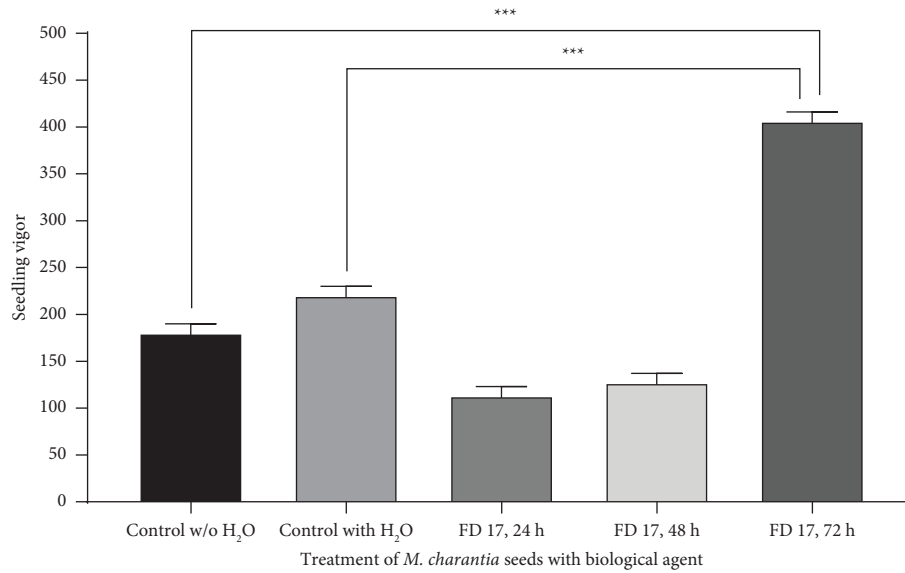
Figure 3: Seedling vigor of *M. charantia* seeds treated with microbial agent *Enterobacter* sp. strain FD17 for time exposures of 24, 48, and 72 hours. Values are given as mean (±SD). $p < 0.05$ is the significant difference in treatments.
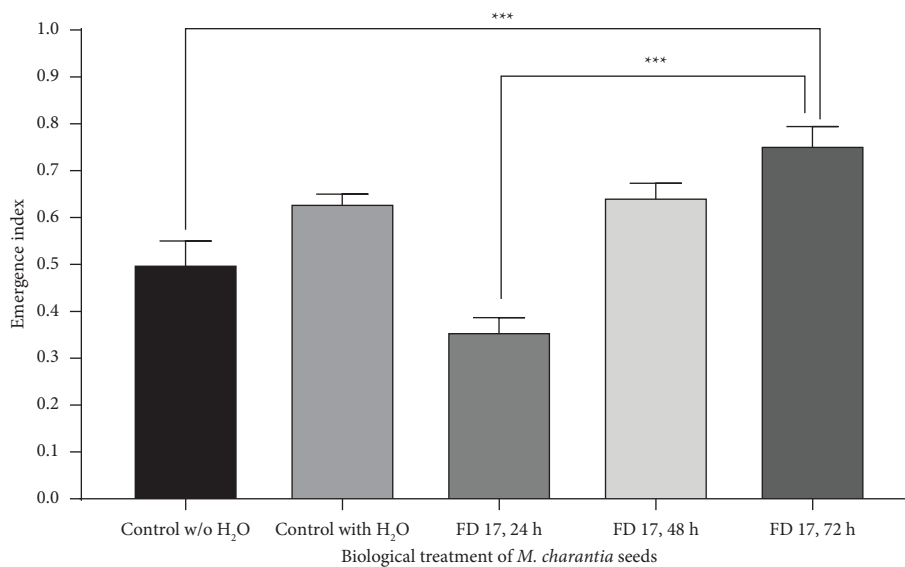

Figure 4: Emergence index of *M. charantia* seeds treated with *Enterobacter* sp. strain FD17 for time exposures of 24, 48, and 72 hours. Values are given as mean (±SD). $p < 0.05$ is the significant difference in treatments.

Therefore, compared to untreated samples, the emergence index in the seeds of *M. charantia* treated with the bacterial strain FD17 was found to be significant ($p < 0.05$).

3.1.5. Vigor Indices I and II. *M. charantia* seeds treated with *Enterobacter* sp. for 72 hours showed the highest values for vigor indices I and II compared to *M. charantia* seeds treated for 24 and 48 hours and control groups with and without water treatment (Figures 5(a) and 5(b)). Therefore, compared to untreated samples, the vigor indices I and II of *M. charantia* seeds treated with the bacterial strain FD17 were found to be significant ($p < 0.05$).

3.2. Growth Attribute. The plant phenotype after biopriming with its improved germination and growth is shown in Figure S1. The growth parameters of the *M. charantia* seeds treated with the bacterial strain FD17 are given in Table 1. The fresh weight of the root was found to be 0.45 ± 0.045 g, while the weight of the root of the control group was 0.37 ± 0.02 g. Similarly, the fresh weights of the shoots from the treated and controlled seeds were found to be 1.23 ± 0.05 g and 0.84 ± 0.08 g, respectively. The leaf area of the treated seeds was found to be 15.52 ± 1.50 cm and that of the control group was 11.37 ± 0.55 cm. The length of the shoot and the length of the root of the treated seeds were
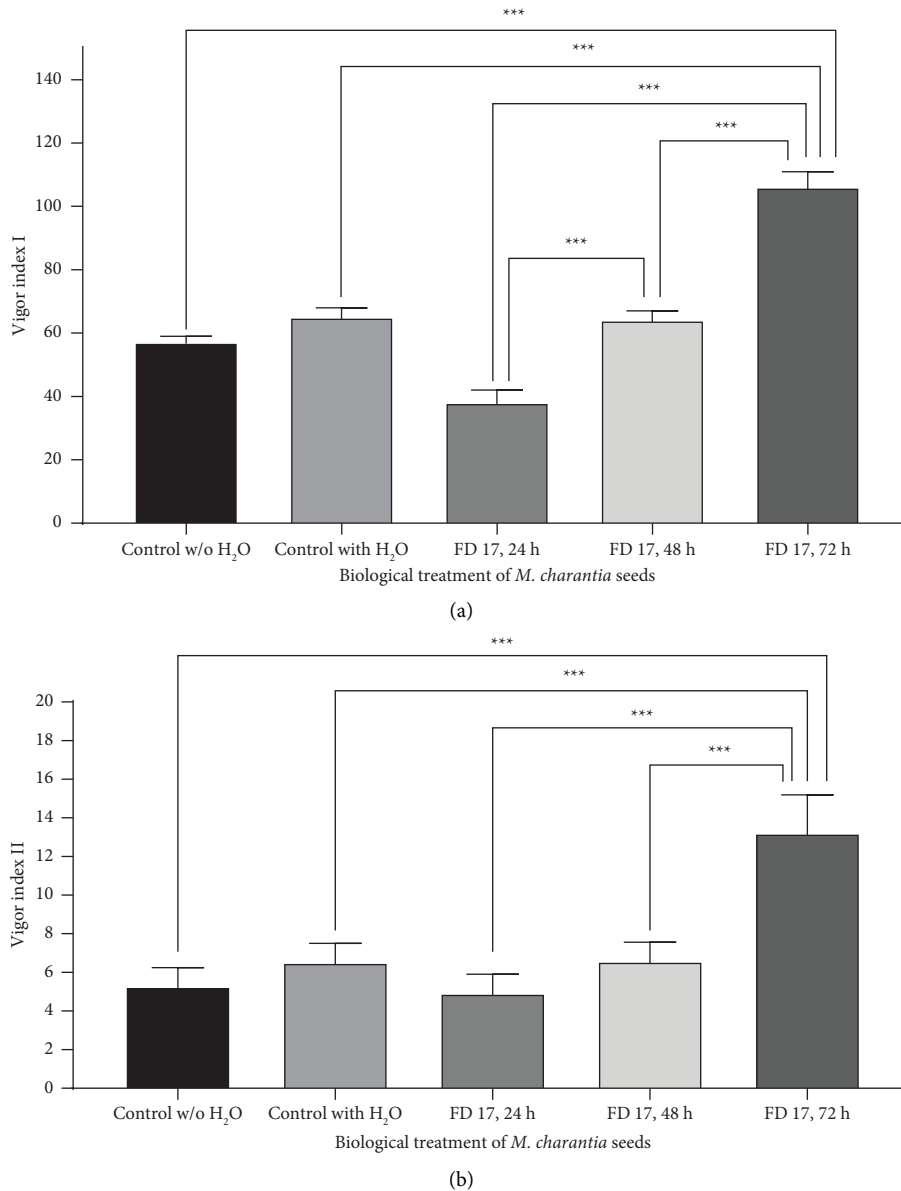
(a)

(b)

FIGURE 5: Vigor indices of *M. charantia* seeds treated with *Enterobacter* sp. strain FD17 for time exposures of 24, 48, and 72 hours. (a) Vigor index I. (b) Vigor index II. Values are taken as mean (±SD). $p < 0.05$ is the significant difference in treatments.

found to be 30.33 ± 0.58 cm and 2.97 ± 0.06 cm, respectively, while in the case of the untreated seeds, the length of the root and the length of the shoot were 4.33 ± 0.29 cm and 21.83 ± 2.75 cm, respectively. The weight of the fruits of the treated seeds was 96.33 ± 1.15 g and that of the control group was 94.67 ± 5.03 g. The percentage of germination of treated seeds was found to be 56.67 ± 11.55 and that of the control group was 26.67 ± 5.77. From these growth attributes, it was explored that there is a significant difference between all growth parameters compared to the control group. The

length of the root and shoot, the weight of the root and shoot, the leaf area, and the germination parameters were found to be higher in the treated samples compared to the seeds of the untreated sample.

3.3. Protein and Total Soluble Sugar. The statistical analysis of total soluble sugar revealed that there was a nonsignificant ($p < 0.05$) effect ($p < 0.05$) of bacterial treatment, while according to the analysis of variance data ($p < 0.05$), the

TABLE 1: Growth parameters of *M. charantia* seeds treated with bacterial strain FD17.

Growth parameters	Control *M. charantia*	Treated *M. charantia*
Root fresh weight (g)	0.37[a] ± 0.02	0.45[a] ± 0.045
Shoot fresh weight (g)	0.84[b] ± 0.08	1.23[a] ± 0.05
Leaf fresh weight (g)	0.20[a] ± 0.045	0.16[a] ± 0.02
Leaf area (cm)	11.37[b] ± 0.55	15.52[a] ± 1.50
Shoot length (cm)	21.83[b] ± 2.75	30.33[a] ± 0.58
Root length (cm)	4.33[a] ± 0.29	2.97[b] ± 0.06
No. of flowers	1.33[b] ± 0.58	6.0[a] ± 1.0
Fruit weight (g)	94.67[a] ± 5.03	96.33[a] ± 1.15
Germination (%)	26.67[b] ± 5.77	56.67[a] ± 11.55

The results are presented as means of 3 replicates. Values are given as mean (±SD). "a" and "b" show treatments with a mean significant difference ($p < 0.05$).

protein in the leaves showed a significant difference. Compared to control plants, the protein level was found to be lower in plants that were treated with the FD17 microbial strain (Table 2).

3.4. Phenolic Content and Free Amino Acids.
Phenolic content and free amino acids decreased significantly in plants treated with the bacterial strain FD17 according to statistical analyses ($p < 0.05$). The phenolic content and free amino acids were found to be higher in the control group (Table 2).

3.5. Chlorophyll Content and Flavonoids.
According to analysis of variance data ($p < 0.05$), both chlorophyll a and chlorophyll b revealed a significant increase after bacterial treatment. Compared to the control group, the plants treated with FD17 bacterial strain showed a positive effect on chlorophyll a and chlorophyll b levels, while the total chlorophyll result was nonsignificant ($p < 0.05$) for the treated plants (Table 2). Bacterial treatment was explored to reduce flavonoids in *M. charantia* plants after treatment with *Enterobacter* strain FD17 (Table 2).

3.6. The Nutritive Analysis of Peels and Fruits

3.6.1. Free Amino Acids.
Statistical analysis of free amino acids in fruit and peel revealed nonsignificant results ($p < 0.05$) in *M. charantia* plants after seeding with *Enterobacter* strain FD17 (Table 3). The free amino acids decreased slightly in the peel of the treated plants, while these increased slightly in the fruit of the treated plants compared to the control group.

3.6.2. Crude Fiber.
The crude fiber in the peel indicated a significant effect of microbial treatment according to the variance of the data ($p < 0.05$). The treated plants with the bacterial strain FD17 exhibited lower crude fiber in the fruit compared to control plants and therefore expressed nonsignificant ($p < 0.05$) results in the treated plants (Table 3).

3.6.3. Protein.
Statistical analysis of protein in peel and fruit had shown significant results ($p < 0.05$) in treated plants, and protein content was found to increase in both peel and *M. charantia fruit* after seed priming with *Enterobacter* stain FD17 (Table 3).

3.6.4. Total Soluble Sugar.
The results of statistical analysis according to the variance of data ($p < 0.05$) of total soluble sugar in *the* fruit and peel of *M. charantia* demonstrated significant effects of biopriming. For fruit, treated plants showed better results compared to control plants as the percentage of total soluble sugar increased significantly, while it decreased in the peel of treated plants (Table 3).

Similarly, the percentage of oil and ash content showed nonsignificant results in both the peel and the fruit of *M. charantia* after treatment with the *Enterobacter* strain FD17. Bacterial treatment showed a significant increase in the concentration of peroxidase and catalase enzymes in the fruit of treated plants. Metabolizable showed a significant increase in both the peel and the fruit of the treated plants (Table 3).

3.7. Antiglycation.
Antiglycation activity was found to increase significantly in *M. charantia* fruit after seed preparation with *Enterobacter* strain FD17 (Table 4).

3.8. Antibacterial Activity of M. charantia Leaf Extracts.
The antibacterial activity of various leaf extracts (i.e., water, acetone, and methanol) of *M. charantia* was explored against *S. aureus* and *P. aeruginosa* bacteria (Figure 6). In the case of Gram-negative bacteria, the maximum activity was shown by methanol extract followed by acetone and water (Figure 6(a)). The microbial treatment enhanced the antibacterial activity in all extractions. However, in the case of Gram-positive *Staphylococcus aureus* bacteria, the highest activity was displayed by methanol followed by water and acetone. Treatment with the FD17 bacteria strain had reduced antibacterial activity in the extraction of methanol and acetone compared to the control (Figure 6(b)).

TABLE 2: Biochemical parameters of the leaves of *M. charantia* seeds bioprimed with the bacterial strain FD17.

Biochemical analysis	Control	Bioprimed with FD17
Phenolic content (mg/g)	$10.211^a \pm 0.01$	$5.56^b \pm 0.03$
Total soluble sugar (%)	$28.431^a \pm 3.11$	$33.13^a \pm 2.24$
Protein (mg/g)	$27.40^a \pm 1.162$	$5.68^b \pm 0.07$
Free amino acid (mg/g)	$4.071^a \pm 0.06$	$2.0^b \pm 0.1$
Total chlorophyll content (mg/g)	$7.16^a \pm 1.03$	$9.0^a \pm 1.5$
Chlorophyll-a (mg/g)	$2.850^b \pm 0.0$	$6.33^a \pm 0.58$
Chlorophyll-b (mg/g)	$3.780^a \pm 0.66$	$8.58^a \pm 2.50$
Flavonoid (mg/g)	$6.86^b \pm 0.01$	$1.03^a \pm 0.05$
Catalase (U/g)	$542.4^a \pm 17.86$	$459.8^a \pm 70.78$
Peroxidase (U/g)	$5.681^a \pm 0.01$	$2.68^b \pm 0.64$
Anthocyanins (mg/g)	$0.66^a \pm 0.002$	$0.67^a \pm 0.01$
Carotenoids (µg/g)	$5.031^a \pm 0.06$	$3.17^b \pm 0.29$

The results are shown as means of 3 replicates. Values are given as mean (±SD). "a" and "b" show the treatments with a significant difference ($p < 0.05$).

TABLE 3: Proximate and nutritive analyses of the peel and fruit of *M. charantia* after seed priming with *Enterobacter* strain FD17.

Proximate analysis	Control *M. charantia*		Treated *M. charantia*	
	Peel	Fruit	Peel	Fruit
Free amino acids (mg/g)	$11.03^b \pm 1.05$	$0.44^a \pm 0.05$	$9.57^a \pm 0.31$	$1.43^a \pm 0.02$
Crude fiber (%)	$3.4^a \pm 0.44$	$3.08^a \pm 0.04$	$3.62^b \pm 0.10$	$2.81^a \pm 0.02$
Protein (mg/g)	$26.78^b \pm 0.68$	$26.62^b \pm 1.57$	$28.63^a \pm 0.15$	$76.20^a \pm 3.56$
Total soluble sugar (%)	$31.16^a \pm 0.07$	$29.0^b \pm 3.46$	$30.82^b \pm 0.12$	$42.53^a \pm 1.65$
Ash (%)	$15.82^a \pm 0.75$	$14.93^a \pm 0.46$	$15.38^a \pm 0.75$	$20.53^a \pm 2.57$
Peroxidase (U/g)	$2.43^a \pm 0.57$	$2.24^b \pm 0.41$	$5.61^a \pm 0.34$	$3.52^a \pm 0.53$
Catalase (U/g)	$374.3^a \pm 17$	$322.9^a \pm 48.52$	$290.13^a \pm 4.69$	$347.47^b \pm 34.76$
Metabolizable energy (kcal/100 g)	$17.04^b \pm 0.76$	$17.1^b \pm 0.79$	$28.94^a \pm 0.86$	$28.87^a \pm 0.81$
Oil (%)	$0.97^a \pm 0.01$	$0.958^a \pm 0.01$	$0.96^a \pm 0.01$	$0.959^a \pm 0.01$

The results are shown as means of 3 replicates. Values are given as mean (±SD). "a" and "b" show the treatments with a significant difference ($p < 0.05$).

TABLE 4: Antiglycation activity of *M. charantia* fruit after seed priming with *Enterobacter* strain FD17.

Activity	Control *M. charantia*	Treated *M. charantia*
Antiglycation	$0.45^a \pm 1.49$	$0.90^b \pm 0.052$

The results are shown as means of 3 replicates. Values are given as mean (±SD). 'a' and 'b' show the treatments with a significant difference ($p < 0.05$).
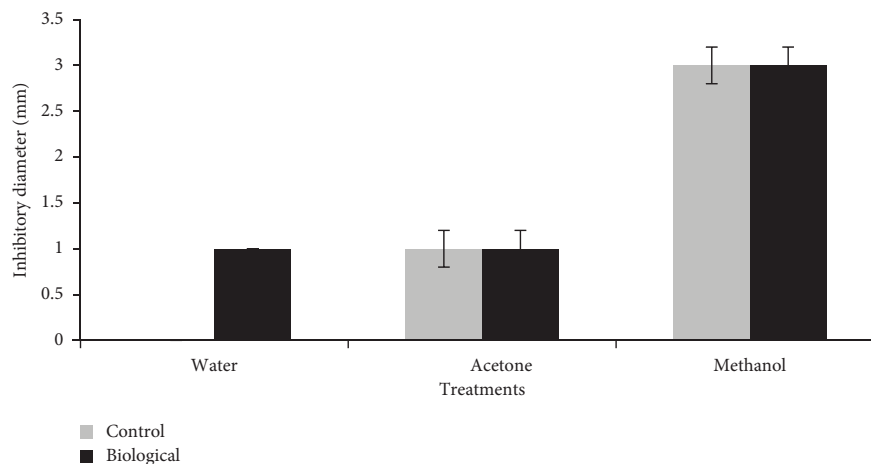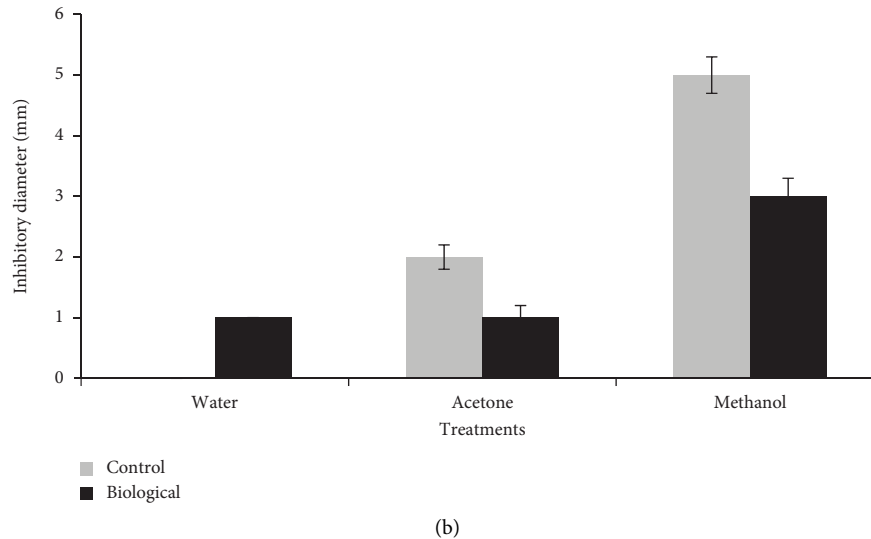

(a)

FIGURE 6: Continued.

(b)

FIGURE 6: Antibacterial activity of leaf extracts (water, acetone, and methanol) of *M. charantia* after seed priming with *Enterobacter* FD17. (a) Antibacterial activity against *P. aeruginosa*. (b) Antibacterial activity against *S. aureus*.

3.9. Phenolic Profile. The freeze-dried samples of *M. charantia* (i.e., leaves and fruits) indicated the presence of several phenolic acids. According to the analysis of variance, the quantitative variation was found to be significant in microbial FD17 treated and untreated plants. In *M. charantia* leaves, quercetin, benzoic acid, and sinapic acid levels improved significantly after treatment with FD17, while chlorogenic acid, syringic acid, *M*-coumaric acid, and cinnamic acid decreased in leaves of treated plants (Table 5, Figure S2). In the case of the *M. charantia* fruit, there was a significant increase in chlorogenic acid and *M*-coumaric acid, while a decrease was observed for quercetin and sinapic acid (Table 5, Figure S3).

3.10. Correlation of Antibacterial Activity vs Primary and Secondary Metabolites. The antibacterial activity of *M. charantia* leaf extracts against a Gram-negative *P. aeruginosa* after treatment with *Enterobacter* strain FD17 showed a direct correlation with catalase enzyme and secondary metabolites such as vanillic acid and coumaric acid, while showing an indirect correlation with ferulic acid and a nonsignificant correlation with peroxidase, total chlorophyll contents, chlorophyll a, chlorophyll b, soluble sugar, protein, amino acids, anthocyanin, flavonoids, carotenoids, quercetin, sinapic acid, syringic acid, cinnamic acid, chlorogenic acid, and benzoic acid (Table 6).

Similarly, Gram-positive *S. aureus* presented a positive correlation with biochemical parameters such as proteins and free amino acids and showed a negative correlation with chlorophyll a, total chlorophyll contents, and soluble sugar. *S. aureus* expressed a direct correlation with cinnamic acid, *M*-coumaric acid, vanillic acid, chlorogenic acid, and syringic acid. On the other hand, it was indirectly correlated with quercetin, sinapic acid, benzoic acid, and ferulic acid (Table 6).

3.11. Correlation of the Phenolic Profile. The phenolic profile of the *M. charantia* fruit after seed priming with *Enterobacter* strain FD17 showed a direct correlation with ferulic acid and coumaric acid to metabolizable, peroxidase, and protein, while it revealed an indirect correlation with catalase and soluble sugar. Benzoic acid in fruit had a positive correlation with metabolizing energy, peroxidase, and protein, whereas there was a negative correlation with catalase and soluble sugar. Sinapic acid, cinnamic acid, and quercetin showed an indirect correlation to metabolizable, peroxidase, and protein, while a direct correlation to catalase and soluble sugar was obtained, and a nonsignificant correlation with ash, crude fiber, oil, and free amino acids was observed. The phenolic components also had a positive correlation with catalase and soluble sugar and negatively correlated with the metabolism of energy, peroxidase, and protein (Table 7). The metabolizable energy is physiologically useful that is obtained when carbohydrates, fats, and proteins are catabolized.

3.12. Antiglycation Level Correlation. The level of antiglycation increased after microbial treatment of *M. charantia* seeds. A positive correlation with catalase (-0.98^*), protein (-0.97^{**}), and the phenolic profile, i.e., coumaric acid (0.94^{**}), sinapic acid (-0.94^{**}), quercetin (-0.94^{**}), and chlorogenic acid (0.94^{**}), was observed in fruit. Antiglycation exhibited a negative correlation with peroxidase, ash, soluble sugar, crude fiber, and free amino acids in the *M. charantia* fruit.

4. Discussion

To improve the speed and uniformity of seed germination, seed priming has been used as a strategic presowing technique [22, 23]. Upon seed priming, various biochemical

TABLE 5: Phenolic acid profile of *M. charantia* leaves and fruit after treatment with *Enterobacter* strain FD17 by HPLC.

Sr. no.	Phenolic acids	Retention time (min)	Concentration (ppm)			
			Control leaf	FD17 treated leaf	Control fruit	FD17 treated fruit
1	Quercetin	2.82	3.77	6.46	2.96	0.86
2	Benzoic acid	14.65	1.64	40.81	ND	ND
3	Chlorogenic acid	15.87	5.19	ND	ND	6.08
4	Syringic acid	17.01	3.31	ND	ND	ND
5	M-coumaric acid	19.73	5.81	ND	ND	2.22
6	Cinnamic acid	24.71	16.25	ND	11.58	ND
7	Sinapic acid	26.19	ND	6.63	14.78	4.17

ND: not detected.

processes of seeds are stimulated which play a vital role in the breakdown of dormancy of the reserved seed food and its mobilization. During germination, seed priming also improves enzymatic activity, resulting in an early emergence of the embryonic part [24] with better synchrony [25]. In response to presown treatments, altered germination characteristics are supposed to be correlated with improved metabolic activities, which eventually lead towards enhanced plant growth [26]. For example, in a study, the magnetic field was applied for the manipulation of plant cell metabolism, resulting in an improvement in antimicrobial activity, plant growth, and phenolic contents of *M. charantia* [5]. Similarly, in another study, for the development of food crops with improved pharmaceutical and nutritional values, $ZnSO_4$ was used for the seed priming of *M. charantia* [9]. Previously, different types of chemicals, plant growth regulators, and vitamins have been investigated to get better agronomic yield [27]. Despite their successful findings, these chemicals were not found to be very effective in their regular application in developing countries (e.g., Pakistan) [9].

Enterobacter sp. strain FD17 had positive reports for growth and improvement in yields of some crops (e.g., maize) and the current work also proved similarity to the findings of Naveed et al. [10], where the fresh weight of the root (21%), the fresh weight of the leaf (112%), the fresh weight of the shoot (48%), and the length of the shoot (38%) increased after seed priming with this species. The response of *M. charantia* to FD17 differed from the previous work of our own research group [28]. Another variety of *M. charantia* was compared for its phytochemicals in two growth stages. In that study, in the seedling stage, there was no effect of treatment on total chlorophyll content, but later in the flowering stage, control plants remained with similar content, whereas the leaves of treated plants showed a significant increase. The present findings can be partially agreed on since the data presented here are for leaves in the vegetative stage. Furthermore, the difference in outcomes could be due to the varietal difference or due to the difference in the FD17 inoculum protocol followed in this study. In addition to bacterial strains, researchers have also employed fungal strains as biopriming agents. For example, in a study, Afrouz et al. [29] used *Trichoderma harzianum* as a biopriming agent and investigated the tolerance of seedlings from two genotypes of maize to cold stress. The emergence of the seedlings and the physiological parameters were revealed to be enhanced as a result of pretreatments with *T. harzianum*.

Enterobacter spp. has been confirmed to be nonhemolytic by blood hemolysis test and therefore has been revealed safe for animals and humans [30]. There are many bacterial species (e.g., *Enterobacter cloacae*, *Enterobacter amnigenus*, *Bacillus anthracis*, *Bacillus cereus*, and *Klebsiella pneumoniae*) that have been well studied for their association with plant growth-promoting abilities, and all these bacterial species have been considered as opportunistic pathogens for humans [31]. However, based on genetic differences in their virulence-associated genes possessed by some of their strains, pathogenicity and virulence factors of a bacterial species have been found to vary between different strains of similar species [32].

Primary metabolites act as feedback to metabolic pathways as metabolic precursors, which include photosynthetic pigments [33], enzymes [34], and soluble sugars [35]. In the event of better accumulation of primary metabolites, there would be activation of enzymes and consequently improved secondary metabolites. The leaves of primed *M. charantia* plants showed an accumulation of soluble sugars better than those of nonprimed plants. Formerly, Shahzad et al. [28] had observed an enhancement in catalase activity in response to FD17 priming in a local race of *M. charantia*. In contrast to their findings, in the current study, the Black King variety of *M. charantia* showed a decrease in catalase activity in plants treated with FD17, pointing out that the allosteric response of catalase to exogenous applications differs from variety to variety for the same species. However, a minor difference in seed treatment method and timing could also be the reason behind this difference in the FD17 priming effect on catalase activity.

Kumar et al. [36] pointed out the importance of plant-derived secondary metabolites as a potential antimicrobial remedy. Different phenolics have been studied for their antimicrobial activities [37, 38] against pathogenic bacterial species [39]. A similar trend was shown in the current study. With very few exceptions, sinapic acid, vanillic acid, p-coumaric acid, and ferulic acid showed highly significant correlation with the antibacterial activity of the leaf extract of *M. charantia* against *S. aureus* and *P. aeruginosa*. The different extractions used for the antibacterial analysis differed

TABLE 6: Correlation matrix of antimicrobial activity with enzymes, primary, and secondary metabolites of *M. charantia* leaves.

Bacteria	Enzymes		Primary metabolites						Secondary metabolites	
	Peroxidase	Catalase	Chl-tot	Chl-a	Chl-b	Proteins	Soluble sugar	Amino acids	Carotenoid	Anthocyanin
P. aeruginosa	ns	0.7***	ns	ns	ns	ns	ns	ns	ns	ns
S. aureus	ns	ns	−0.9**	−0.9**	ns	0.8***	−0.7***	0.7***	ns	ns

Bacteria	Secondary metabolites									
	Flavonoid	Quercetin	Vanillic acid	Sinapic acid	M-coumaric acid	Cinnamic acid	Syringic acid	Benzoic acid	Chlorogenic acid	Ferulic acid
P. aeruginosa	ns	ns	1***	ns	1***	ns	ns	ns	ns	−1***
S. aureus	ns	−1***	1***	−1***	1***	1***	1***	−1***	1***	−1***

*, **, and *** indicate significant correlation; ns: nonsignificance at $p < 0.05$.

TABLE 7: Correlations between fruit phenolics and nutritional characteristics of *M. charantia* after seed bioprimed with FD17 strain of *Enterobacter*.

Acids	Metabolizable energy	Peroxidase	Catalase	Soluble sugar	Protein	Ash	Oil	Crude fiber	Amino acid
Ferulic acid	0.9***	0.97**	−0.97**	−0.9*	0.9**	Ns	Ns	Ns	Ns
Coumaric acid	0.9***	0.97**	−0.97**	−0.9*	0.9**	Ns	Ns	Ns	Ns
Benzoic acid	0.9***	0.97**	−0.97**	−0.9*	0.9**	Ns	Ns	Ns	Ns
Sinapic acid	−0.9***	−0.97**	0.97**	0.9*	−0.9**	Ns	Ns	Ns	Ns
Cinnamic acid	−0.9***	−0.97**	0.97**	0.9*	−0.9**	Ns	Ns	Ns	Ns
Quercetin	−0.9***	−0.97**	0.97**	0.9*	−0.9**	Ns	Ns	Ns	Ns
Phenolic acid	−0.9***	−0.97**	0.97**	0.9*	−0.9**	Ns	Ns	Ns	Ns

*, **, and *** indicate significant correlation; Ns indicates nonsignificance at $p < 0.05$.

in their MIC against both pathogens, where the best was exhibited by methanol followed by acetone and water. The antibacterial activity against both species differed for their correlation with secondary metabolites. The differences in the constituents of the cell membrane of Gram-positive and Gram-negative bacterial species could be the reason for the increased sensitivity of Gram-positive bacterial species towards different extractions [40]. However, for the primary metabolites, there was no significant correlation. At later stages, the response of the blackberry variety might be linked with some better outcomes that could be explored in future studies. Although the possibility of this attribute being varietal specific could not be completely ignored, as in previous studies, a different metabolic response of *Trachyspermum ammi* was observed after similar seed treatment [41].

Glycation can cause damage to proteins and induction of oxidative stress. In the body, the excessive accumulation of advanced glycation end products (AGEs) has been found to be associated with various adverse health conditions, including metabolic disorders such as diabetes mellitus [42] and even sometimes tumor [43]. Antiglycation has been proposed as a tactic that slows human aging and lowers inflammasome activity [44]. Phenols are involved in the human antiglycation process through antioxidant ability and protein interactions. Furthermore, they can trap or block receptors for advanced glycation end products [45, 46]. In the current study, it was revealed that antiglycation activity could be enhanced by FD17 seed treatment. The plants primed with FD17 for their antiglycation capacity were directly correlated with the amounts of coumaric acid and chlorogenic acid. The results related to these treatments confirmed the previous findings of Aljohi et al. [47]. In the future, the secondary metabolites reported in this study should be tested *in vivo* in different animal models to explore and confirm their medicinal properties.

5. Conclusions

The biological seed priming technique has been preferred because the bioagents used in this technique provide a friendly environment for the growth of nutritional food crops and vegetables. In the current study, after treatment of *Momordica charantia* seeds with *Enterobacter* strain FD17, the percentage of germination, growth, total amino acids in the peel, chlorophyll content, and phenolic content improved. FD17 treatment could be used for the manipulation of plant cell metabolism with increased growth and antibacterial activity. Treatment also showed a direct correlation with antiglycation activity, and therefore it can be concluded that the treatment of *Enterobacter* strain FD17 could be followed by pharmacists to overproduce metabolites of interest in *M. charantia* with better antiglycation activity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Supplementary Materials

Figure S1: the plant phenotype after biopriming with *Enterobacter* spp. showing improved germination and growth. Figure S2: HPLC chromatograms of phenolic profile of *M. charantia* leaves—(a) after seed priming with *Enterobacter* strain FD17; (b) control. Figure S3: HPLC chromatograms of phenolic profile of *M. charantia* fruit—(a) after seed priming with *Enterobacter* strain FD17; (b) control. (*Supplementary Materials*)

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