RESEARCH ARTICLE

Biofilm biofertilizer enhances bioactive compounds including antioxidant activity in hybrid rice grains

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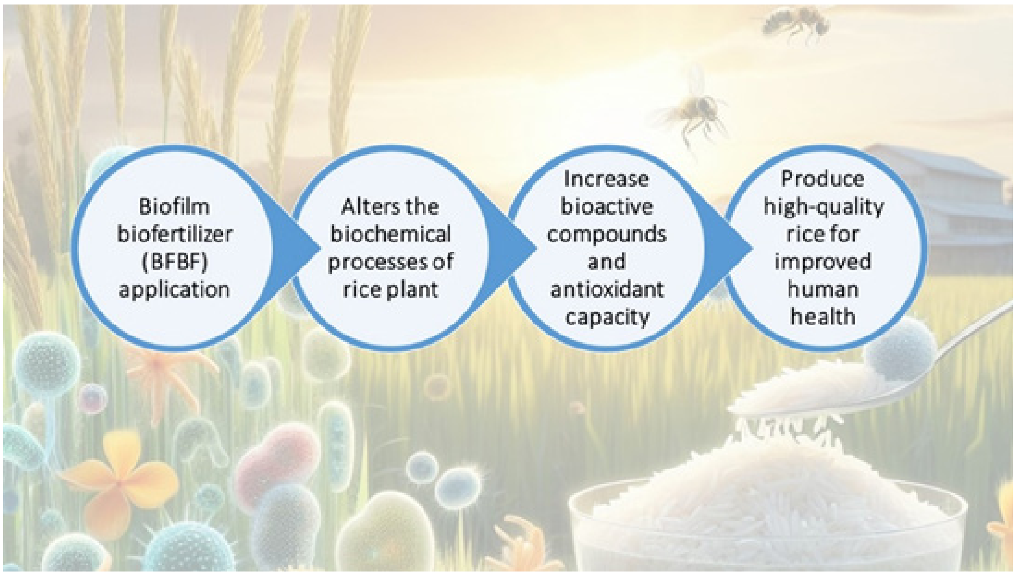

Highlights

- Effect of biofilm biofertilizer (BFBF) on antioxidants and bioactive compounds in rice grains was analysed.
- BFBF significantly increased total phenolic, flavonoids, antioxidant capacity, and Gamma amino butyric acid in most rice varieties.
- BFBF alters the biochemical processes of the rice plant
- BFBF shows its potential to produce high-quality rice for improved human health

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Biofilm biofertilizer enhances bioactive compounds including antioxidant activity in hybrid rice grains

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Abstract: Excessive use of chemical fertilizers (CF) in rice cultivation has adversely affected soil and crop productivity and human and environmental health. This has led to the introduction of bio-organic inputs like biofilm biofertilizers (BFBF), which have been shown to increase crop yields and soil quality with reduced CF use. However, the effect of the BFBF on rice grain quality has been understudied. The present study investigated the effect of BFBF on bioactive compounds in rice grains, including antioxidants, which are essential in protecting against diseases related to oxidative stress. The study compared two practices i.e. CF practice and BFBF practice, using six hybrid rice varieties. Harvested grain samples were analyzed for total phenolics (TPC), flavonoids (TFC), and Gamma amino butyric acid (GABA) contents, and antioxidant capacity (TAC). Results showed that the BFBF significantly ($p < 0.05$) increased TPC, TFC, and GABA in most rice varieties. The TAC was also significantly ($p < 0.05$) higher (30-200%) in the rice grains produced by the BFBF practice. In conclusion, the BFBF alters the biochemical processes of the rice plant leading to improved medicinal properties showing its potential to produce high-quality rice, which is essential to improve human health.

Keywords: Antioxidants; Biofilm biofertilizers; Grain quality; Rice paddy

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food in Asia, and it is a crucial source of vitamins, minerals, fibers, bioactive compounds, and antioxidants (Monks et al., 2013). An antioxidant is a substance that considerably delays or prevents oxidation of an oxidizable substrate when present in low concentrations compared to those of the oxidizable substrate (Halliwell, 1990). Antioxidants influence the cellular redox status of animal and human plasma, which could offer protection against chronic diseases related to oxidative stress (Liu et al., 2018). Oxidative stress is defined as disequilibrium between oxidants and antioxidants, which arises from a failure of endogenous antioxidants to slow or stop the assembly of reactive oxygen species, which are free radicals produced in response to injury or irritation. If the generation of free radicals exceeds the scavenging capacity of cells, the surplus free radicals can react with biological macromolecules, and cause oxidative damage. Moreover, this oxidative damage plays a crucial role in the molecular mechanisms behind many diseases, like cancer and diabetes (Halliwell, 1990). Due to these consequences, the importance of studying the antioxidant capacity of foods in the Asian context has increased and the attention has recently shifted to using antioxidant-rich extracts from rice, which may employ health-promoting effects in many ways (Anggraini et al., 2015; Inagaki et al., 2013).

In rice cultivation, chemical fertilizers (CF) have been used extensively around the world to increase crop yield. However, long-term usage of excessive CF incurs harmful effects causing a reduction in soil and crop productivity (Zhang et al., 2010; Rahman & Zhang, 2018). It results in many adverse consequences while reducing the sustainability of conventional agroecosystems (Singh et al., 2018; Meepegamage et al., 2021). As a remedy, the application of organic inputs (e.g. biofertilizers) is gaining popularity among farmers, because they are considered sustainable options to improve soil quality (Meena et al., 2015; Meepegamage et al., 2021). Among biofertilizers, Biofilm biofertilizer (BFBF) is an effective bio-product introduced to agriculture a decade ago (Seneviratne et al., 2008). Biofilms consist of communities of bacterial, fungal, algal, or other microbial cells that produce extracellular polymeric substances (EPS) to offer structure and protection to the communities (Kokare et al., 2009). The biofilms can be developed *in-vitro* using beneficial microbes to be used as BFBF (Seneviratne et al., 2008). The BFBF increases crop yield and improves soil quality while recovering soil livability that has been damaged by conventional agricultural practices (Seneviratne et al., 2011; Seneviratne et al., 2008). It has also been reported that the BFBF reduces the application of CF up to ca. 50% while increasing grain yield and soil carbon sequestration by ca. 20-30% and ca. 30%, respectively (Premarathna et al., 2021; Rathnathilaka et al., 2022; Jayasekara et al., 2022; Premarathna et al., 2023). Currently, the BFBF practice has been adopted in over 16% of total rice cultivation (i.e. ca. 0.75 M ha) in Sri Lanka, showing its potential to address current farming challenges (Ekanayake et al., 2023). However, there is a lack of information on the effect of BFBFs on the bioactive compounds like antioxidants of rice grains, which is very important as explained above.

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This study reports the effect of BFBF on antioxidants and some other bioactive compounds of rice grains.

MATERIALS AND METHODS

Experimental sites

The experiment was conducted in Ampara district (7.2912°N), a major rice-growing area in Sri Lanka. The research site had a typical subtropical climate with a mean annual rainfall of 1,000 mm during the study period of 2019. Initial soil properties were not significantly different due to high variability. Therefore, those properties, soil pH (6.48), moisture (55.7%), porosity (35.3%), particle density (2.13 g cm^{-3}), bulk density (1.63 g cm^{-3}), labile carbon (0.06%), microbial biomass carbon (110 mg kg^{-1}), organic carbon (0.75%), total nitrogen (0.08%), total phosphorus (0.2%), total potassium (0.3%) were pooled and the means were calculated.

Treatments and sample collection

In nine locations (i.e. L1 – L9) in Ampara district, farmers maintained two consecutive, uniform paddy field plots, each ca. 0.4 ha for two treatments, which are now being practiced in the districts, viz. (a) CF practice (340 kg NPK/ha, Department of Agriculture (DOA) recommendation), and (b) BFBF practice (225 kg NPK/ha + 2.5 L BFBF/ha [the BFBF is a patented commercial product (Sri Lanka patent no. 15958, 2013), and hence exact composition cannot be revealed due to Intellectual Property Rights reasons]. The plots were treated separately without mixing the treatments and the rice crop was cultivated according to cultural practices recommended by the DOA, Sri Lanka. The study was carried out in the Dry season of 2019 in 18 farmer fields at nine locations in Ampara district. The rice varieties used were At 373, At 362, At 311, Bg 358, Bg 350, and Bg 300, all of which are 3.5-month-old varieties. At the crop maturity, five paddy grain samples were collected from random positions of each plot to evaluate the antioxidants in rice grains.

Sample preparation

The collected paddy grains samples were de-husked, powdered and sieved through a 0.25 mm mesh. The powdered sub-sample (0.1 g) was mixed with 5 ml of 80% methanol and vortexed for 15 min. It was kept in a water bath at 60 °C for 40 minutes while vortexing at ten-minute intervals. After centrifugation at 4,000 rpm for 5 min, the supernatant was decanted into a 15 ml centrifuge tube and the remaining was re-extracted with 5 ml of 80% methanol. Supernatants were pooled and stored at -20 °C before chemical analyses.

Quantification of total phenolics

The total phenolic contents (TPC) of rice grains were determined using the Folin-Ciocalteu method with modifications (Yafang et al., 2011). Modifications in brief, samples or standards containing 0.5 ml solution of gallic acid were added to 15 ml centrifuge tubes and 4 ml of distilled water was added into each tube. Then, Folin-Ciocalteu phenol reagent (0.5 ml) was added and mixed thoroughly. After 3 min, 1 ml of saturated sodium carbonate was added and incubated for 2 hours at 30 °C. Then absorbance was read at 760 nm using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). TPC was expressed in mg of gallic acid equivalents (GAE) per 100 g dry weight (DW) as standard.

Quantification of total flavonoids

The total flavonoid contents (TFC) of rice grain samples were determined according to the $AlCl_3$ colorimetric method described by Mammen and Daniel (2012) with minor modifications. Modifications in short, a sample of 1 ml containing 10 mg of solution of each plant extract was added to 0.5 ml of distilled water. Then, 0.075 ml of 5% sodium nitrite solution was added to the mixture followed by incubation for 6 minutes after which 0.15 ml of 10% $AlCl_3$ was added, shaken, and left to stand for 6 minutes at room temperature (27 - 30 °C). After that, 0.5 ml of 1 mol L^{-1} sodium hydroxide was added and the mixture was diluted using 0.275 ml of distilled water, mixed, and left for 15 minutes. Then, absorbance was read at 500 nm using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). TFC was expressed in Trolox equivalents (TE) per 100 g dry weight (DW) as standard.

Quantification of total antioxidant capacity

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation-based assay was performed. The stock solution of ABTS was prepared according to Re et al. (1999), by reacting 7 mol L^{-1} ABTS stock solution with 2.45 mol L^{-1} potassium persulfate. This stock solution was kept in the dark at room temperature (27 - 30 °C) for 12-16 hours before use. The working solution of ABTS was prepared by diluting 10 ml of stock solution using 800 ml of ethanol (50:50, v/v) to obtain an absorbance of 0.75 - 0.80 at 734 nm (Serpen et al., 2012).

Quantification of gamma amino butyric acid content

The extraction procedure of Komatsuzaki et al. (2007) involved mixing 3 g of hybrid rice powder with 30 mL of 70% ethanol, followed by centrifugation and repeated extraction was used. The supernatant was evaporated and dissolved in water. An aliquot of the extract was treated with borate buffer, phenol reagent, and sodium hypochlorite, then heated and cooled. The optical density was measured at 630 nm. Standard solutions of GABA were prepared for calibration. The extract was analyzed using high-performance liquid chromatography (HPLC).

Statistical analysis

Data analysis was done using R software. Comparisons of treatments in all locations were done using two-sample *t*-test after confirmation of the normal distribution of data using a normality test. Linear regression analysis was conducted to determine the relationships between bioactive compounds and antioxidant activity.

RESULTS AND DISCUSSION

Effect of BFBF on TPC and TFC of rice grains

The TPC of all six rice varieties was significantly higher ($p < 0.05$) in the BFBF practice, except the Bg-300 when compared to the CF practice (Figure 1). This finding is in agreement with Ochoa-Velasco et al. (2016) who reported that the application of biofertilizer coupled with 75% of the nitrogen dose significantly increased the synthesis of phenol contents compared with the full nitrogen dose or no fertilizer treatments. Further, Al-shakankery et al. (2014) reported that there was a significant increase in the polyphenol content of maize grains, which were treated with biofertilizer when compared to the control maize plants. Phenolic compounds are secondary metabolites that have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants (Larson, 1988). Polyphenols play a vital part in the protection of plants against UV radiation, pathogens, and herbivores, and help maintain structural integrity for the cell wall (Klepacka & Fornal, 2006; Inglett et al., 2011).

The TFC was also significantly higher ($p < 0.05$) in the BFBF practice than that of the CF practice, except L3 and L7, and ranged between 892 -1678 mg/kg, and 698 - 1143 mg/kg in the BFBF practice and CF practice, respectively (Figure 2).

Effect of biofilm biofertilizer on antioxidant capacity of rice grains

The antioxidant activity in rice grains significantly increased in the BFBF practice over the CF practice except for one location ($p < 0.05$, Figure 3). Antioxidants are compounds that inhibit the initiation or propagation of oxidative chain reactions (Halliwell, 1990; Velioglu et al., 1998). Generally, the presence of antioxidants is important in the preservative effects, as many plant spices and herbs suggest (Hirasa & Takemasa, 1998).

Moreover, the antioxidant activity is linearly and positively related to the TPC and TFC (Figures 4 & 5). It has been reported that the TPC and TFC contribute to antioxidant activity in plants (Muflihah et al., 2021).

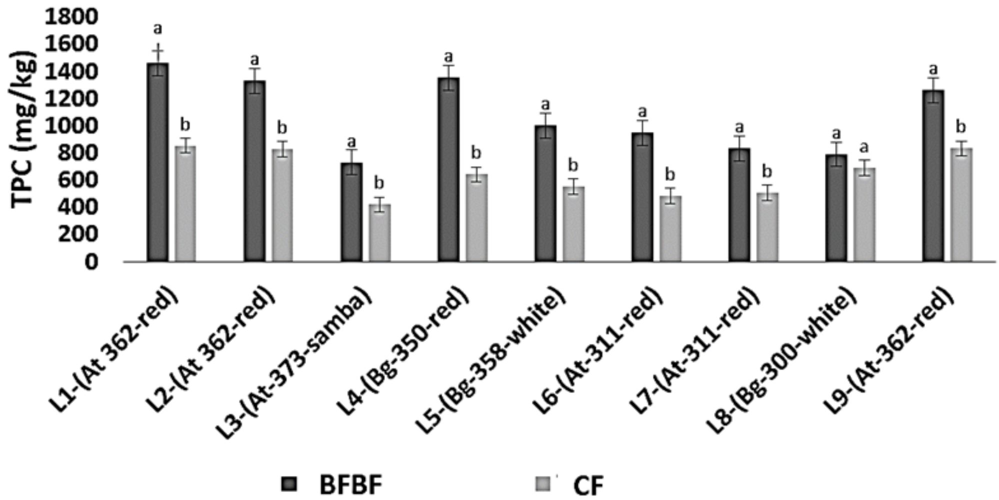

Figure 1: Total Phenolic Content (TPC) in different rice varieties produced by the biofilm biofertilizer (BFBF) and chemical fertilizer (CF) practices at different locations in the Dry season of 2019. L1 – L9: nine locations. Rice varieties are mentioned within the parentheses. Pooled standard deviation of each practice is shown in the error bars.

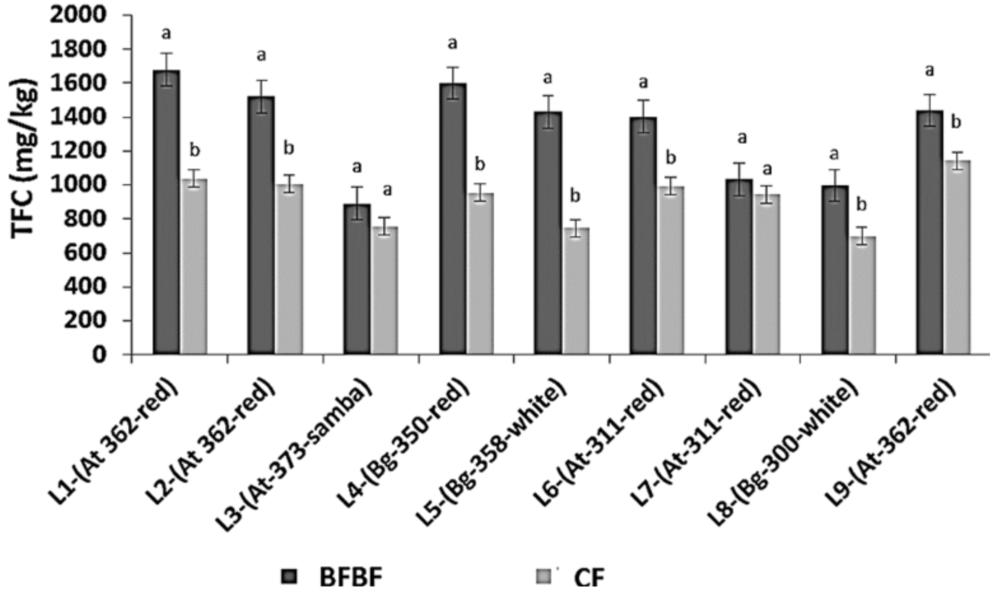

Figure 2: Total Flavonoid Content (TFC) in different rice varieties produced by the biofilm biofertilizer (BFBF) and chemical fertilizer (CF) practices at different locations in the Dry season of 2019. L1 – L9: nine locations. Rice varieties are mentioned within the parentheses. Pooled standard deviation of each practice is shown in the error bars.

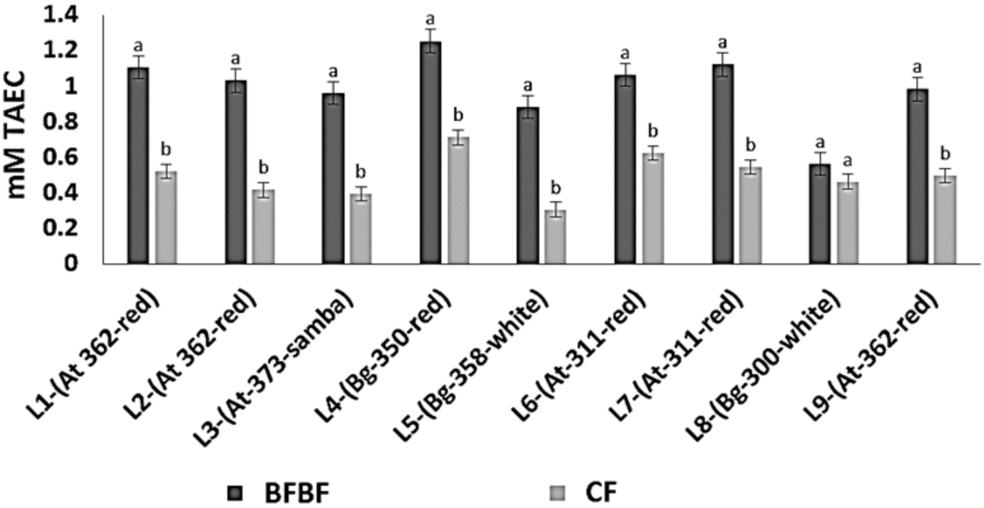

Figure 3: Total antioxidant capacity in rice grains of different rice varieties produced by the biofilm biofertilizer (BFBF) and chemical fertilizer (CF) practices at different locations in Dry season 2019. L1 – L9: nine locations. Rice varieties are mentioned within the parentheses. Pooled standard deviation of each practice is shown in the error bars.

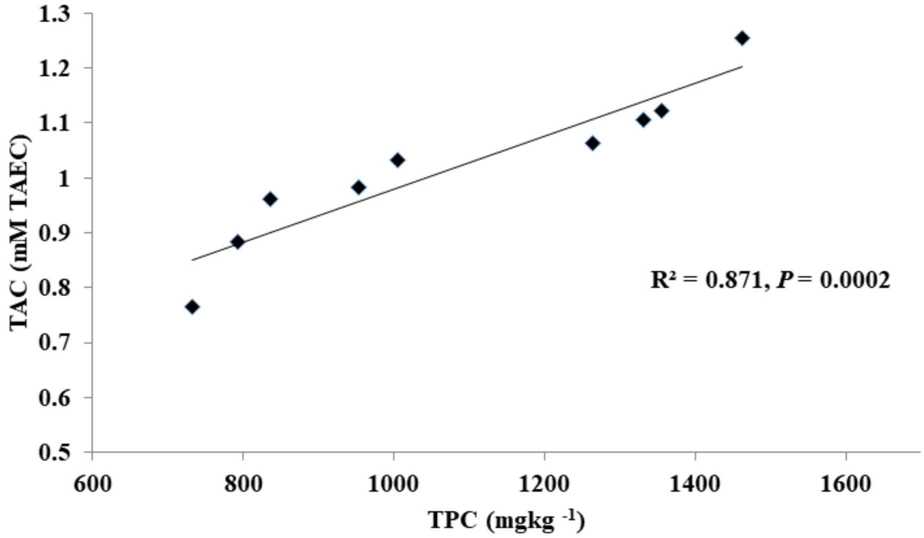

Figure 4: Relationship between total phenolic content (TPC) and total antioxidant capacity (TAC) in rice grains produced by the biofilm biofertilizer and chemical fertilizer practices at different locations in Dry season 2019.

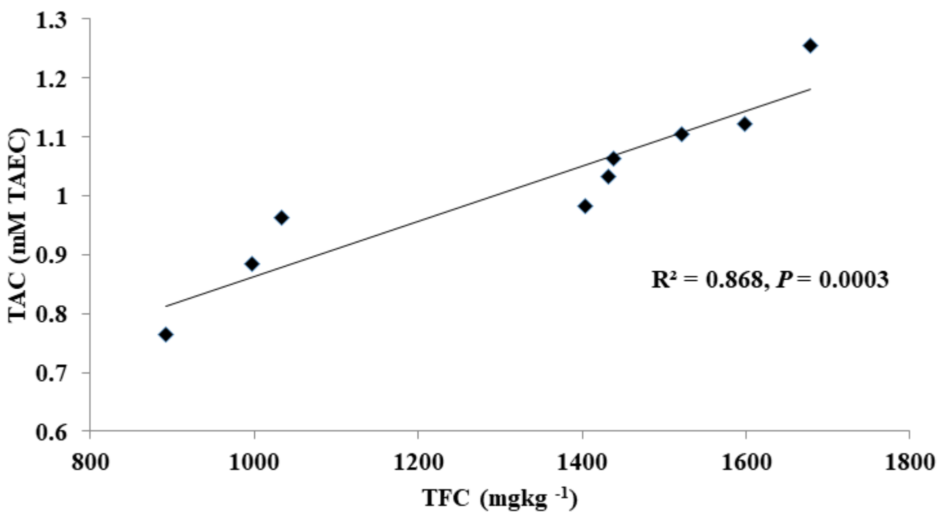

Figure 5: Relationship between total flavonoid content (TFC) and total antioxidant capacity (TAC) in rice grains produced by the biofilm biofertilizer and chemical fertilizer practices at different locations in Dry season 2019.

The TAC increased due to an increase in antioxidants and bioactive compounds. Rice produced from the BFBF practice would be healthier due to its higher contents of antioxidants and bioactive compounds than the rice produced from the CF practice (Kaur et al., 2017). It was observed that the gamma amino butyric acid (GABA) content was significantly higher in the rice produced from the BFBF practice in five locations out of the nine (Figure 6). The increase in GABA content could be ascribed to the activation of glutamate decarboxylase that catalyzes the decarboxylation of L-glutamic acid to carbon dioxide and GABA, which causes the decrease of glutamic acid (Charoenthaikij et al., 2010; Cornejo et al., 2015). GABA is a non-protein amino acid, which is an essential inhibitory neurotransmitter in the mammalian central nervous system (Kittibunchakul et al., 2021), and it has a role in controlling stress and anxiety, altering cognitive and brain processes, boosting mood, and encouraging sleep in mammals (Rashmi et al., 2018), ameliorating blood flow in the brain, inhibiting cancer-cell proliferation (Okada, et al., 2000; Oh & Oh, 2004; Karladee & Suriyong, 2012), preventing diabetic conditions, modulating blood cholesterol levels (Ngo & Vo, 2019), and antihypertensive activity (Akama et al., 2020). In addition, the GABA content was significantly correlated with the TPC and TFC contents (Figures 7 & 8). Generally, GABA increases phenolic and flavonoid content by promoting phenylalanine ammonia-lyase (PAL) activity (Liu et al., 2024; Wang et al., 2021).

CONCLUSION

The present study clearly showed that the application of BFBF significantly enhanced the total phenolic content, total flavonoid content, antioxidant capacity, and GABA content in rice grains compared to CF, suggesting its potential for producing healthier rice with improved nutritional and functional properties. Further studies should compare the medicinal properties of the BFBF-applied hybrid rice and traditional rice, while rapidly implementing the BFBF practice in large-scale rice cultivations.

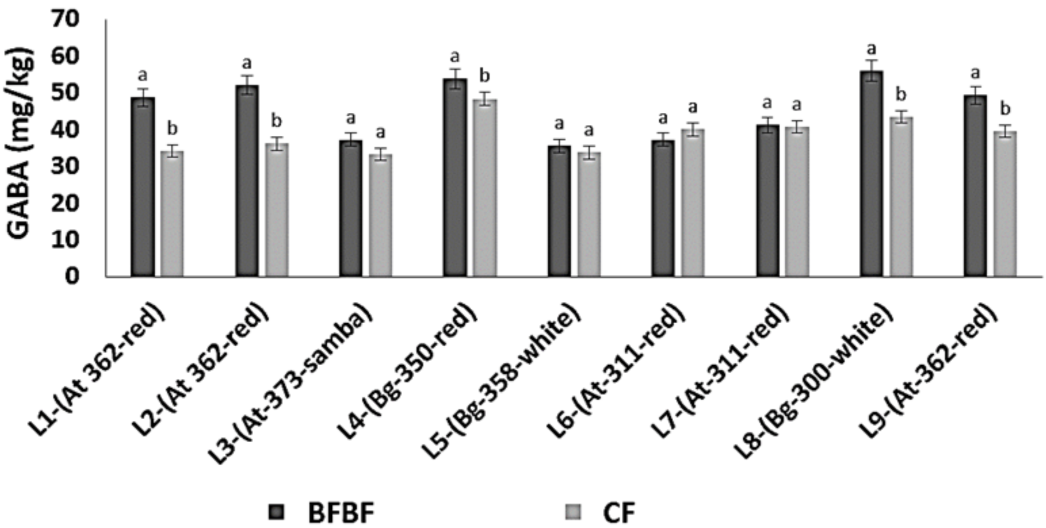

Figure 6: Gamma amino butyric acid (GABA) content in rice grains produced by the biofilm biofertilizer and chemical fertilizer practices at different locations in the Dry season of 2019.

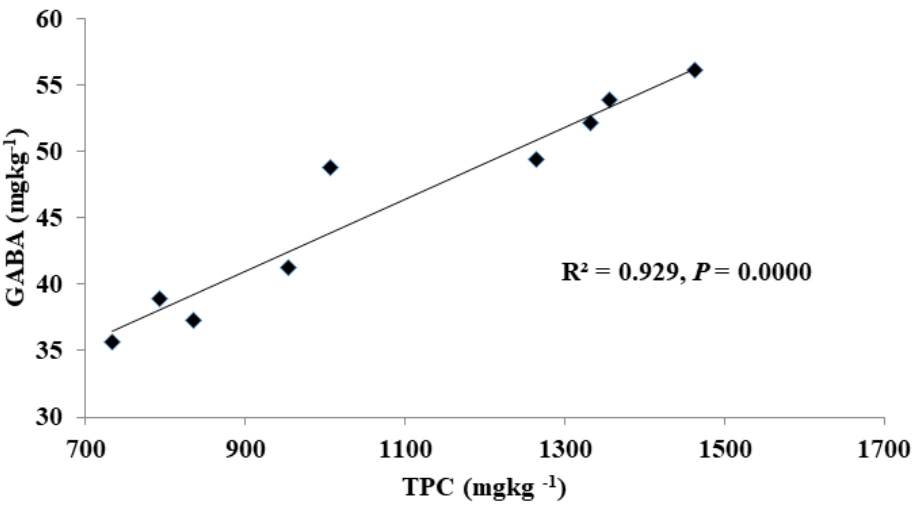

Figure 7: Relationship between total phenolic content (TPC) and gamma amino butyric acid (GABA) in rice grains produced by the biofilm biofertilizer and chemical fertilizer practices at different locations in Dry season 2019.

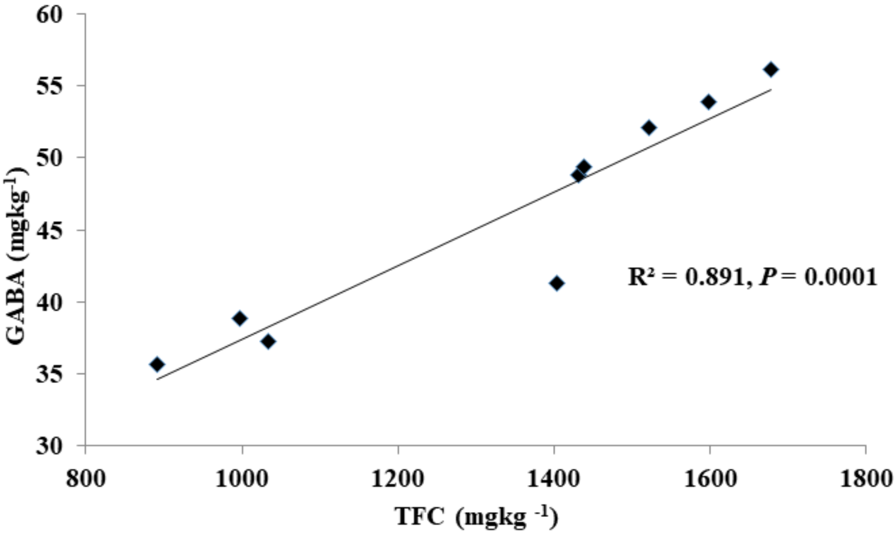

Figure 8: Relationship between total flavonoid content (TFC) and gamma amino butyric acid (GABA) in rice grains produced by the biofilm biofertilizer and chemical fertilizer practices at different locations in Dry season 2019.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest.

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