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RESEARCH ARTICLE

Seed biopriming with soil microorganisms antagonize allelopathic effect of weeds residues on pearl millet germination

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Abstract

Aim This study aims to evaluate the impact of seed primimg with soil microorganism on the germination and metabolism of pearl millet seeds when exposed to the allelopathic effects of some specific weed extracts.

Methods Pearl millet seeds were categorized into five distinct groups. Four of these groups were subjected to priming with different soil microorganisms: *Bacillus velezensis*, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Trichoderma viride*. The remaining, fifth group underwent hydropriming. Subsequently, these groups were subjected to germination in the presence of weed extracts, a process that extended over five days. Following germination, various factors were assessed, including germination percentage, radicle and plumule length, and seed vigor. Furthermore, the study encompassed the analysis of biochemical parameters such carbohydrate and phytate hydrolysis, oxidative stress markers, antioxidant enzyme activity, and secondary metabolite.

Results The findings of the study revealed that biopriming of pearl millet seeds with soil microorganisms led to a significant enhancement in germination, even when exposed to different weed extract treatments. This improvement was chiefly manifested through heightened levels of antioxidant enzymes, which mitigate the oxidative stress induced by the weed treatments. Moreover, the biopriming process improved the hydrolysis in germinated seeds, resulting in energy savings and a reduction in carbon utilization for secondary metabolism through the shikimic acid pathway and the phenylpropanol pathway. This facilitated the production of defense molecules like phenols and flavonoids.

Conclusion Seed priming with soil microorganism ultimately bolsters the seeds' tolerance against allelochemicals originating from weed residue treatments.

Keywords *Bacillus velezensis* · Hydrolysis enzymes · *Pseudomonas fluorescens* · Secondary metabolism · Seed priming · *Serratia marcescens* · *Trichoderma Viride*

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Introduction

In the modern era, the global climate change phenomenon presents a substantial risk to food crop production. Researchers are actively searching for stable crops that can withstand climate change conditions while retaining their high nutritional value (Vambe et al. 2023). Among these promising crops, pearl millet stands out due to its nutritional richness and inherent ability to thrive in challenging environmental conditions, including low rainfall, poor soil fertility, high temperatures, and drought. These attributes make it a viable candidate to potentially replace staple crops like corn, wheat, and rice (Singhal et al. 2022; Pandey and Bolia 2023).

Millet crops flourish during hot and humid rainy seasons, which unfortunately create an ideal environment for the rapid spread of weeds. These invasive plants cause loss in the crop yield. Weeds often compete with pearl millet for essential resources such as water, nutrients, and sunlight. The allelopathic chemicals released by weeds can further intensify this competition, affecting the ability of pearl millet to access the resources it needs for growth (Samota et al. 2022; Chinyo et al. 2023). Allelopathic compounds released by weeds may interfere with the nutrient uptake mechanisms of pearl millet. This can lead to nutrient deficiencies in the soil, negatively impacting the growth and development of the crop (Qureshi et al. 2024). Allelopathic substances released by weeds can have direct toxic effects on pearl millet. These chemicals may hinder seed germination, root development, and overall plant growth, leading to reduced crop yields (Choudhary et al. 2023). Weed allelopathy can also influence the composition and activity of soil microbial communities. Changes in the soil microbiome may impact nutrient cycling, soil structure, and overall soil health, affecting the growth of pearl millet (Scavo et al. 2019; Blaise et al. 2021; Kotra et al. 2023). Millets face unique challenges when it comes to managing weeds during their early growth stages as they are characterized by their wider row spacing and slower initial growth phase. Therefore, it becomes crucial to implement effective weed control measures early in the growth cycle to maximize crop yields (Ajeesh et al., 2022; Gangaiah and Yadav 2024; Yonli et al. 2024).

Weeds pose a significant threat to agricultural production, resulting in decreased product quality and market value. Weeds engage in fierce competition with crops for essential resources such as water, nutrients, light, and space, ultimately leading to reduced crop yields (Padu et al. 2023). Historically, farmers have relied on chemical herbicides to manage weed growth, but unfortunately, these chemicals have negative effects on soil quality, plants (Kakhki et al. 2022a). Naseri et al. (2018) reported that using herbicide increased the root rot incidence in bean plants. Also, chemical herbicides are toxic to humans causing various health problems (Yates et al. 2024). Additionally, the widespread use of chemical herbicides has led to weed resistance. Hence, there is a growing need to develop alternative strategies like mechanical (hand weed controlling, planting at appropriate date and other agroecological properties (Kakhki et al. 2022b; Naseri and Kakhki 2022; Naseri 2023), choosing resistance cultivars (Naseri and Safaee 2023), and biological methods for effective weed control to ensure sustainable agriculture (Raza et al. 2021).

Biological control methods for weeds have gained significant attention as safe and effective alternatives. Bioherbicides encompass the use of plant extracts or microorganisms such as fungi or bacteria (Verma et al. 2020). Microbial bioherbicides contain microorganisms like bacteria (Fang et al. 2022), fungi (Kakhaki et al. 2017; Todero et al. 2020; Tan et al. 2024), viruses (Charudattan 2024) or protozoa (Marini et al. 2021) as their active components. These bioherbicides have the potential to manage a wide range of weeds, with each active component exhibiting relatively specific activity against its target weeds (Jampílek and Kráľová 2022). The use of soil microorganisms as bioherbicides relies on their ability to detoxify allelochemicals secreted in the soil by weeds. Moreover, these microorganisms can be host-specific, promoting crop plant growth while inhibiting weed growth (Bo et al. 2020; Ramesh and Abinaya 2022; Roberts et al. 2022; Jelena et al. 2023; Charudattan 2024; Tan et al. 2024).

Addressing the issue of weed residue is another crucial aspect to minimize its adverse effects on crop plant growth. Various weed control methods leave behind weed residue in the soil, which can transport allelochemicals to crop plants even in the absence of actively growing weeds (Rogers 2020). Khatri et al. (2023) reported the harmful impact of *Ageratina adenophora* leaf residues on the production of two rabi crops. Additionally, Padu et al. (2023) discovered that the germination of seeds and nutrient uptake in various crops significantly decreased when exposed

to extracts from weed species such as *Poa annua*, *Erigeron annuus*, and *Stellaria media*. Hence, it is equally important to find safe strategies that enhance crop plants' tolerance to allelochemicals from weed residues (Xiao et al. 2020).

From the perspective of weed residue phytotoxicity, this study aims to assess the impact of priming pearl millet seeds with specific soil microorganisms to alleviate the allelopathic effects of certain weed extracts. The investigation focuses on the effects of seed priming with soil microorganism on pearl millet germination and metabolic status in the presence of weed extracts.

Materials and methods

Preparation of soil microorganisms

We used *Bacillus velezensis* (ATCC 6051), *Pseudomonas fluorescens* (ATCC 13,525), *Serratia marcescens* (ATCC 13,880), and *Trichoderma viride* (ATCC 28,020) as these soil microorganisms are commonly used as biocontrol agents against different plant diseases. We acquired these strains from the American Type Culture Collection in USA. These microorganisms were cultured following the manufacturer's guidelines to create a microbial suspension at a concentration of 10^8 colony-forming units per milliliter (CFU/mL). This microbial suspension served as the biopriming solution.

Preparation of weed residue extracts

We used the most common weeds that can invade the pearl millets cultivated lands. Weed species, including *Brachiaria ramose*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Digitaria marginata*, *Paspalum paspaloides*, *Panicum dichotomiflorum*, and *Sorghum halepense*, were collected the weeds in vegetative stage from various farms in Qilwah, Al-Baha region, Saudi Arabia. The whole plant was (leaves, stems and root system) were thoroughly cleaned, air-dried, and subsequently ground into a fine powder. An extract was prepared for each weed species by mixing 20 g of the weed powder with 100 mL of distilled water, followed by overnight incubation at room temperature with periodic shaking. After incubation, the mixture was filtered through filter paper (Whatman n°1), and the resulting filtrate was stored in dark bottle in the refrigerator at 1 °C for use as the weed extract.

Germination of pearl millet seeds

Pearl millet seeds were obtained from Millborn Seeds. The seeds were subjected to surface sterilization using 70% sodium hypochlorite for 15 min, followed by multiple rinses with sterile distilled water. The seeds were then divided into five groups. The first group underwent hydropriming, while the remaining four groups were bioprimed using the prepared microbial suspension at a concentration of 10^8 CFU/mL of *B. velezensis*, *P. fluorescens*, *S. marcescens*, and *T. viride*. Ten seeds of each group were placed in 10 cm-diameter Petri dishes containing a piece of cotton saturated with either one of the prepared weed extracts (20 mL) or water (20 mL) as a control. The experiment included forty experimental groups (Supplementary Table S1). The forty groups were incubated in dark at 30 °C and 40% humidity for a week. After incubation, the germination percentage, radicle, and plumule length were measured, and the seed vigor index was calculated using the formula:

$$\text{Seed vigor} = \text{germination percent} \times \text{seedling length}$$

Following this, the entire seedlings were rapidly frozen using liquid nitrogen and stored at -18 °C for subsequent biochemical analysis.

We repeated the experiment three times successively and we collected the samples from each time and stored − 18 °C for subsequent biochemical analysis.

Activity of hydrolysis enzymes during germination

The hydrolysis of stored organic compounds is a critical biochemical process during germination, as it provides the essential simple compounds required for the embryo's energy during germination (Batool et al. 2022).

Organic Phosphate Hydrolysis: Phytase activity was determined using the K-Phytase kit 09/21 (Megazyme, Wicklow, Ireland). One unit of the enzyme was defined as the enzyme that liberates 1 µmol of phosphate per minute. Phytate content was determined using the K-PHYT Phytic Acid (Phytate)/Total Phosphorus Kit 05/19 (Megazyme, Wicklow, Ireland).

Starch Hydrolysis: Amylase activity was assessed according to the protocol provided by K-CERA 02/22 (Megazyme, Wicklow, Ireland). One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar per minute. Total

soluble sugar was determined using the phenol-sulfuric acid method (Nielsen 2017).

Oxidative stress markers and antioxidant enzyme activities

Oxidative stress markers Hydrogen peroxide (H_2O_2) content was determined using the method described by Alexieva et al. (2001), and lipid peroxidation was assessed by measuring malonyl dialdehyde (MDA) levels as per Li (2000).

Antioxidant enzymes Crude enzyme extracts were obtained from the germinated seedlings by centrifuging the homogenate of 5 g of germinated seeds in cold phosphate buffer (pH 6.5) containing 1 mM EDTANa2 (Saroop et al. 2002). The activity of antioxidant enzymes was measured in this crude extract. Superoxide dismutase (SOD) activity was determined based on the nitro blue tetrazolium (NBT) reduction method, following the procedure of Beyer (1987). Polyphenol oxidase (PPO) and peroxidase activities were determined by the oxidation of pyrogallol, following the method of Kar and Mishra (1976).

Components of secondary metabolism

Shikimic acid content The shikimic acid content was determined in germinated seedlings according to Zelya et al. (2011).

Phenylalanine ammonia-lyase The activity of phenylalanine ammonia-lyase was assessed using the method outlined by Rösler et al. (1997), which involves measuring the formation of cinnamic acid.

Phenols and flavonoids Total phenols and total flavonoids were extracted from sprouting seedlings, following the methodology outlined by Campbell and Ellis (1992). The quantification of phenolic content was conducted using the Folin-Ciocalteu assay, while the assessment of flavonoid content was carried out using the $AlCl_3$ assay, as described by Pallab et al. (2013).

Statistical analysis

The data from the three experiments were inputted into SPSS (version 14), where the mean and standard deviation were computed for each treatment based on three replicates. The results were presented as mean ± standard deviation (SD). To assess the significance of the differences between treatment groups and the control, paired T-tests were employed. A significance level of $p < 0.01$ was chosen to determine statistical significance.

Results

Effect of soil microorganisms on pearl millet germination under weed extract treatment

The results indicate that priming with soil microorganisms led to improved germination parameters under weed treatment conditions (Table 1; Fig. 1). Weed treatment significantly reduced the germination percentage of hydroprimed seeds. The lowest germination percentage (46.667%) was observed in hydroprimed seeds affected by *B. ramose* or *P. dichotomiflorum*. In contrast, under weed treatments, seeds primed with soil microorganisms exhibited a non-significant decrease in germination percentage compared to the control group. Notably, S. marcescens and T. viride demonstrated the best germination percentage under weed treatment conditions. Under the treatment of *Cynodon dactylon*, *Dactyloctenium aegyptium*, and *Digitaria marginata*, the germination percentage was 93.33% in the *S. marcescens*-primed seeds and 96.667% in *T. viride*-primed seeds (Table 1).

Another important parameter in healthy germination is seed vigor, which depends on the seedling length. Soil microorganisms priming significantly improved the radicle and plumule length (Fig. 1) and thus the seed vigor (Table 1) under both normal conditions and weed treatment. Under normal conditions, the highest seed vigor index was 1043.3 in the case of *T. viride*-primed seeds, while it was 683.1 in the control seeds. Also, under weed treatment, *T. viride*-primed seeds showed the highest vigor index, 957.0, under the *Cynodon dactylonI* treatment, while the lowest seed vigor index was recorded, 132.2, in the hydroprimed seeds under *Panicum dichotomiflorum* treatment (Table 1).

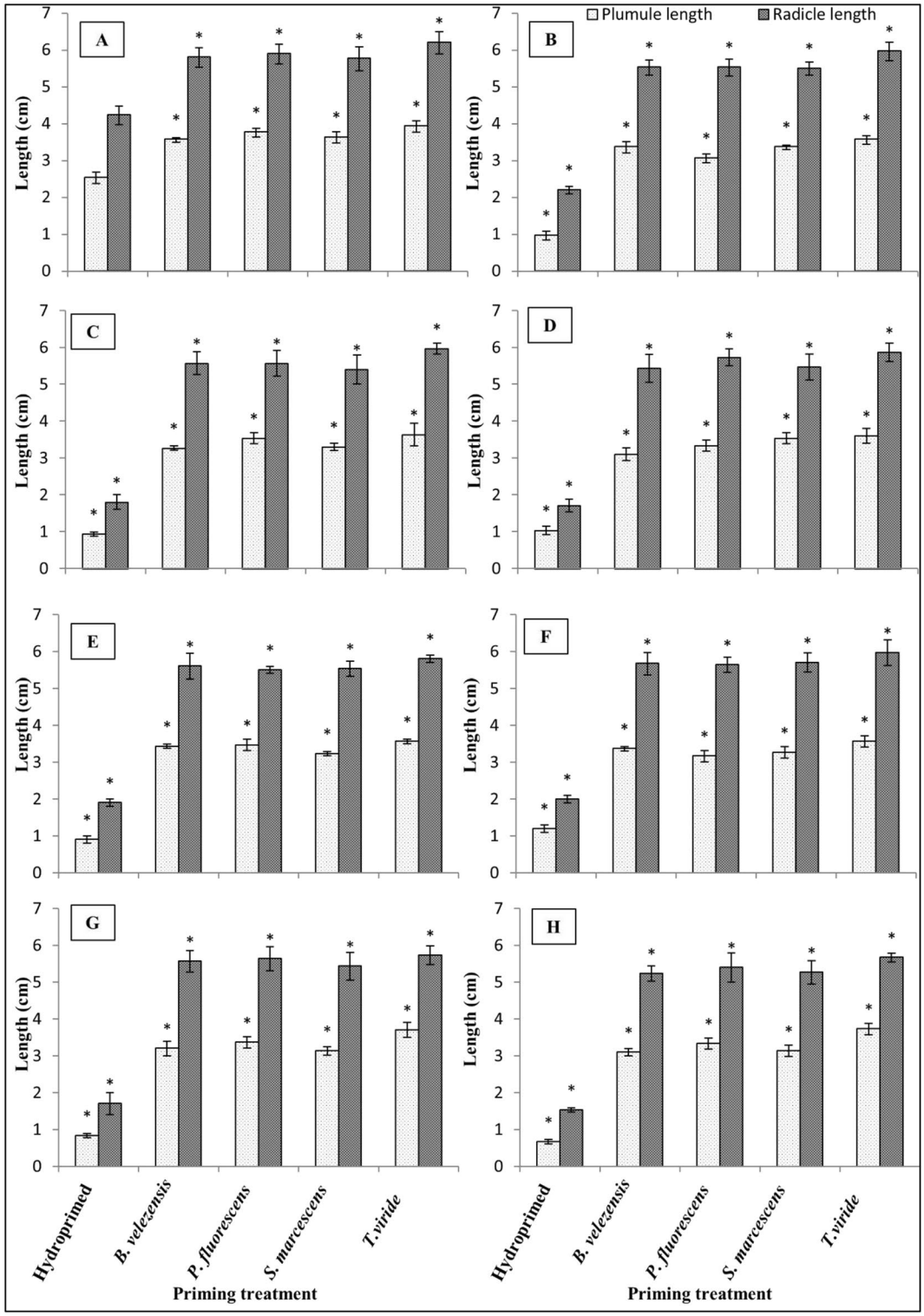
Effect of soil microorganisms on hydrolysis enzymes in germinated pearl millet under weed extract treatment

The phytase enzyme catalyzes the hydrolysis of organic phosphate stored in seeds, and thus, organic

Table 1 Effect of biopriming with different soil microorganisms on the germination percent and vigor index of pearl millet under different weeds extract treatment

Priming	Hydroprimed		*Bacillus velezensis*		*Pseudomonas fluorescens*		*Serratia marcescens*		*Trichoderma viride*	
Weed treatment	Germination (%)	Vigor Index	Germination (%)	Vigor Index	Germination (%)	Vigor Index	Germination (%)	Vigor Index	Germination (%)	Vigor Index
Non-treated (water)	96.7	683.1	100.0	966.7*	100.0	996.7*	100.0	970.0*	100.0	1043.3*
Brachiaria ramose	46.7*	161.8*	86.7	797.3*	86.7	771.3*	83.3	763.9*	93.3	917.8*
Cynodon dactylon	53.3*	161.8*	83.3	761.1*	86.7	814.7*	93.3	840.0*	96.7	957.0*
Dactyloctenium aegyptium	66.7*	202.2*	86.7	765.6*	86.7	811.8*	93.3	868.0*	96.7	944.1*
Digitaria marginata	53.3*	165.3*	83.3	777.8*	86.7	803.1*	93.3	846.2*	96.7	934.4*
Paspalum paspaloides	63.3*	221.7*	86.7	808.9*	86.7	788.7*	86.7	803.1*	93.3	917.8*
Panicum dichotomiflorum	46.7*	132.2*	86.7	785.8*	86.7	806.0*	86.7	768.4*	86.7	843.6*
Sorghum halepense	53.3*	133.3*	83.3	719.4*	83.3	752.8*	86.7	754.0*	86.7	840.7*

Data are means of three replicates. Data followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$


Plumule length
Radicle length
A
B
C
D
E
F
G
H
Length (cm)
Hydroprimed
B. velezensis
P. fluorescens
S. marcescens
T. viride
Priming treatment

◂**Fig. 1** Effect of priming with soil microorganisms on plumule and radicle length under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment,**F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

phosphate hydrolysis depends on phytase activity. Under weed treatment, organic phosphate hydrolysis significantly decreased in the hydroprimed germinated seeds, as phytase activity notably decreased, leading to a significant accumulation of phytate in the hydroprimed seeds germinated under weed treatment. The lowest phytase activity recorded was 0.50 U/g fresh weight in hydroprimed seeds treated with *Panicum dichotomiflorum* and *Sorghum halepense*. On the other hand, priming with soil microorganisms significantly improved organic phosphate hydrolysis by increasing phytase activity, resulting in decreased phytate content under both normal and weed treatment conditions. The phytase activity reached approximately 2.99 U/g fresh weight in *T. viride*-primed seeds under normal conditions, and this activity decreased to about 2.70 U/g fresh weight under weed treatments (Figs. 2 and 3).

Table 2 illustrates the changes in amylase activity and soluble sugar content in germinated pearl millet seeds subjected to weed treatment. Amylase is the enzyme that catalyzes the conversion of storage starch to soluble carbohydrates, thus governing carbohydrate hydrolysis in germinated seeds. Under weed treatment, hydroprimed germinated seeds exhibited a significant decrease in carbohydrate hydrolysis, characterized by a significant reduction in both amylase activity and soluble sugar content.

The amylase activity in hydroprimed seeds germinated under normal conditions was 2.40 U/g fresh weight, and this activity dropped to 0.74 U/g fresh weight in hydroprimed seeds under *Paspalum paspaloides* treatment. In contrast, germinated seeds primed with soil microorganisms displayed notably higher amylase activity than the control group, both under normal conditions and weed treatment. Amylase activity increased from 2.40 U/g fresh weight in hydroprimed seeds under normal conditions (control) to 4.05 U/g fresh weight in *T. viride*-primed germinated seeds under normal conditions. Under weed conditions, soil microorganisms priming protected amylase activity, ranging from 3.33 to 3.88 U/g fresh weight, which is significantly higher than that of the control. Interestingly, despite the significant increase in amylase activity in soil microorganism-primed seeds compared to the control, there was a non-significant difference observed in soluble sugar content between the control group and the soil microorganism-primed seeds under weed treatment (Table 2).

Effect of soil microorganisms on oxidative stress markers and antioxidant enzyme activities in germinated pearl millet under weed extract treatment

Hydrogen peroxide is one of the reactive oxygen species that can induce peroxidation in various biological macromolecules such as lipids, proteins, and nucleic acids. H_2O_2 and malonyl dialdehyde (MDA), a product of lipid peroxidation, are recognized as markers of oxidative stress. These oxidative stress markers showed a significant increase in hydroprimed germinated seeds under weed treatment. The H_2O_2 content escalated in hydroprimed seeds from 1.80 µg/g fresh weight under normal conditions (control) to over 4.00 µg/g fresh weight under weed treatments. Similarly, the MDA content in hydroprimed germinated seeds increased from 0.48 µM/g fresh weight under normal conditions to more than 3.80 µg/g fresh weight under weed treatment. On the other hand, soil microorganism priming demonstrated a significant reduction in the content of oxidative stress markers under normal conditions. Although the oxidative stress markers increased under weed treatment in soil microorganism-primed germinated seeds, their content was not significantly different from that of the control. Under weeds treatment H_2O_2 content in soil microorganism-primed seeds ranged from 1.62 to 1.76 µg/g fresh weight, which was not significantly different from that of the control (1.80 µg/g fresh weight). Similarly, under weed treatments, MDA content in soil microorganism-primed seeds ranged from 0.35 to 0.44 µM/g fresh weight, which was not significantly different from that of the control (0.48 µM/g fresh weight) (Table 3).

Plants mitigate the oxidative stress through the activity of the antioxidant enzymes. Results in Figs. 4, 5 and 6 show the changes in superoxide

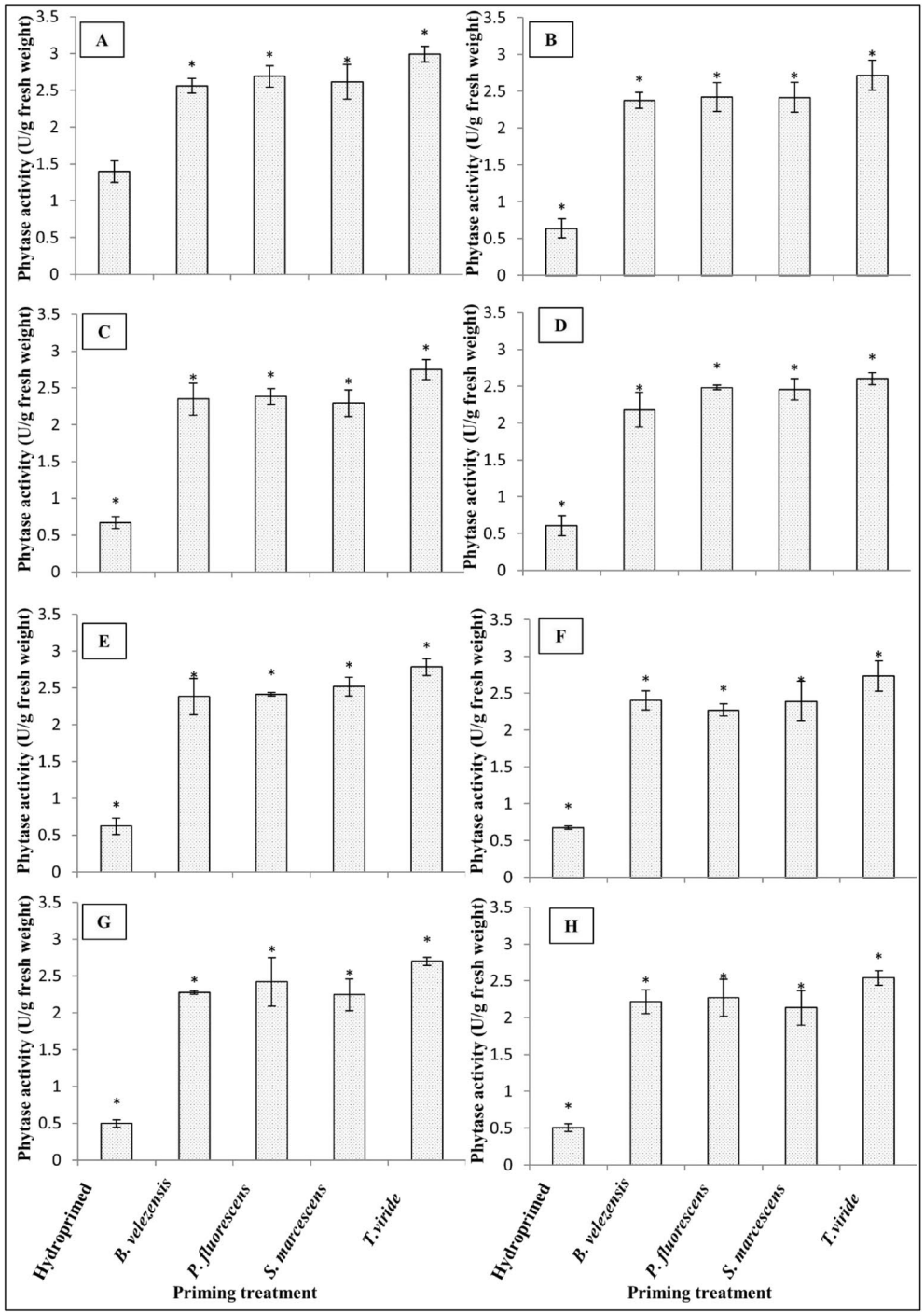


Fig. 2 Effect of priming with soil microorganisms on phytase activity in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

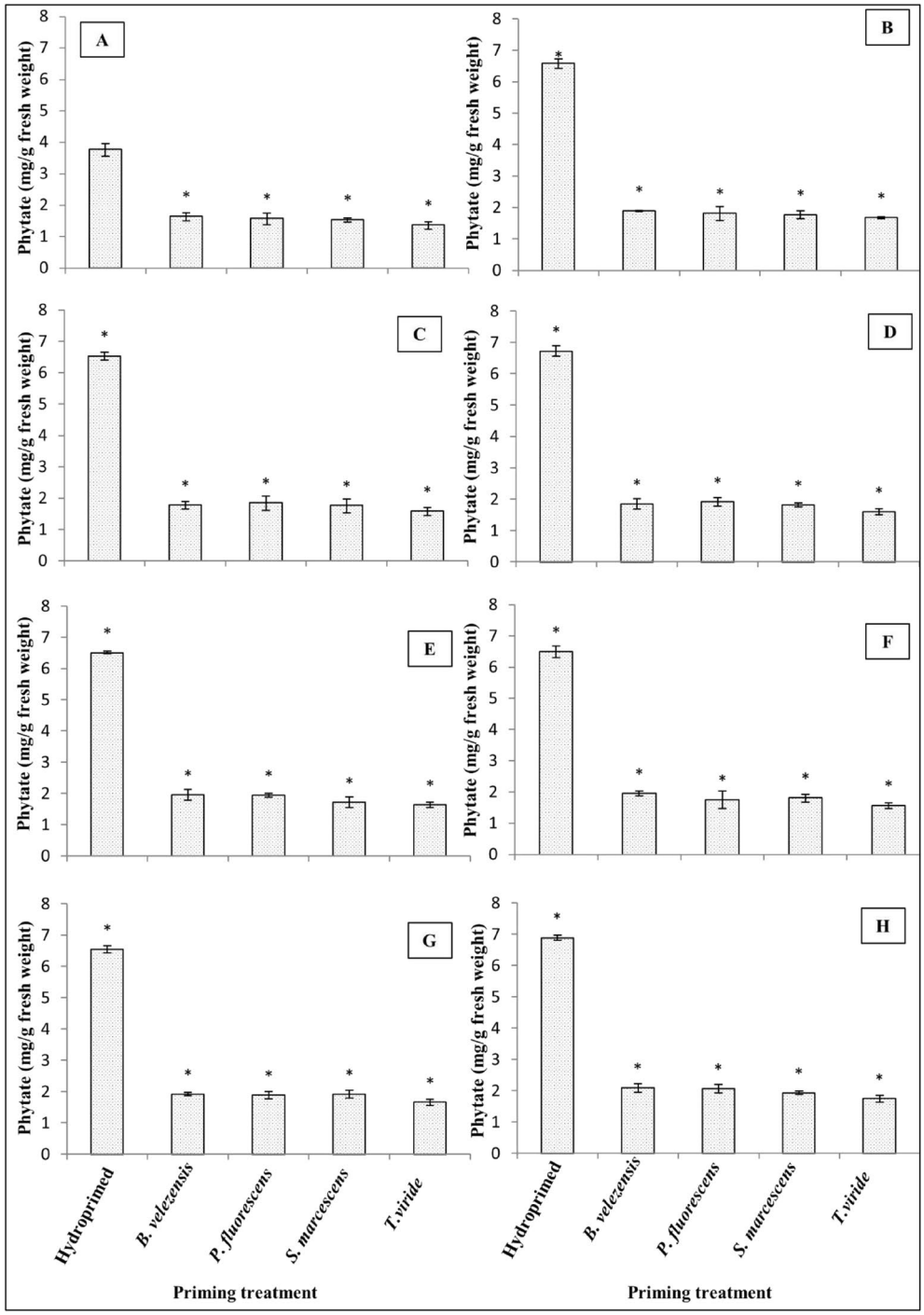


Fig. 3 Effect of priming with soil microorganisms on phytate content in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D**: seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

Table 2 Effect of biopriming with different soil microorganisms on the amylase activity and soluble carbohydrate content in pearl millet seeds germinated under different weeds extract treatment

Priming	Hydroprimed		*Bacillus velezensis*		*Pseudomonas fluorescens*		*Serratia marcescens*		*Trichoderma viride*	
Weed treatment	Amylase activity	Soluble carbohydrates	Amylase activity	Soluble carbohydrates	Amylase activity	Soluble carbohydrates	Amylase activity	Soluble carbohydrates	Amylase activity	Soluble carbohydrates
Non-treated (water)	2.40 ± 0.163	70.08 ± 2.224	3.80 ± 0.166*	77.93 ± 2.233*	3.88 ± 0.100*	80.67 ± 1.000*	3.79 ± 0.140*	79.19 ± 1.620*	4.05 ± 0.150*	82.20 ± 2.464*
Brachiaria ramose	1.06 ± 0.117*	55.81 ± 1.155*	3.56 ± 0.078*	69.07 ± 0.805	3.49 ± 0.070*	72.18 ± 1.442	3.59 ± 0.121*	69.34 ± 2.407	3.88 ± 0.185*	73.67 ± 0.950
Cynodon dactylon	0.93 ± 0.090*	55.39 ± 1.500*	3.54 ± 0.120*	71.37 ± 2.100	3.52 ± 0.184*	70.81 ± 0.956	3.58 ± 0.257*	69.74 ± 1.055	3.72 ± 0.168*	72.71 ± 1.002
Dactyloctenium aegyptium	0.93 ± 0.133*	55.47 ± 1.309*	3.57 ± 0.166*	69.87 ± 2.472	3.41 ± 0.150*	71.34 ± 2.184	3.52 ± 0.162*	69.30 ± 0.501	3.86 ± 0.121*	73.15 ± 2.205
Digitaria marginata	0.91 ± 0.150*	56.03 ± 0.998*	3.47 ± 0.194*	68.70 ± 2.321	3.66 ± 0.115*	71.24 ± 1.471	3.48 ± 0.076*	68.51 ± 0.502	3.86 ± 0.093*	73.80 ± 1.906
Paspalum paspaloides	0.74 ± 0.189*	56.24 ± 1.301*	3.46 ± 0.228*	70.29 ± 2.255	3.66 ± 0.115*	69.93 ± 0.678	3.63 ± 0.122*	69.03 ± 1.021	3.79 ± 0.213*	72.18 ± 1.230
Panicum dichotomiflorum	0.97 ± 0.135*	53.23 ± 0.588*	3.44 ± 0.297*	67.96 ± 1.578	3.66 ± 0.125*	70.77 ± 2.057	3.37 ± 0.161*	69.77 ± 1.100	3.72 ± 0.072*	72.74 ± 1.008
Sorghum halepense	0.88 ± 0.124*	53.86 ± 1.070*	3.33 ± 0.220*	69.53 ± 1.051	3.35 ± 0.174*	69.93 ± 1.021	3.34 ± 0.116*	67.28 ± 1.719	3.64 ± 0.155*	70.77 ± 1.124

Data are means of three replicates. Data followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

Table 3 Effect of biopriming with different soil microorganisms on oxidative stress markers (H_2O_2 content (μM g^{-1} fresh weight), Malonyl di aldehyde (MDA) (μg g^{-1} fresh weight)) content in pearl millet seeds germinated under different weeds extract treatment

Priming	Hydroprimed		*Bacillus velezensis*		*Pseudomonas fluorescens*		*Serratia marcescens*		*Trichoderma viride*	
Weed Treatment	H_2O_2 (μg g^{-1} fresh weight)	MDA(μM g^{-1} fresh weight)	H_2O_2 (μg g^{-1} fresh weight)	MDA(μM g^{-1} fresh weight)	H_2O_2 (μg g^{-1} fresh weight)	MDA(μM g^{-1} fresh weight)	H_2O_2 (μg g^{-1} fresh weight)	MDA(μM g^{-1} fresh weight)	H_2O_2 (μg g^{-1} fresh weight)	MDA(μM g^{-1} fresh weight)
Non-treated (water)	1.80 ± 0.136	0.48 ± 0.056	0.79 ± 0.038*	0.24 ± 0.035*	0.82 ± 0.053*	0.24 ± 0.021*	0.81 ± 0.032*	0.28 ± 0.024*	0.79 ± 0.012*	0.28 ± 0.035*
Brachiaria ramose	7.35 ± 0.767*	3.84 ± 0.396*	1.64 ± 0.111	0.35 ± 0.079	1.56 ± 0.186	0.36 ± 0.064	1.63 ± 0.136	0.39 ± 0.044	1.67 ± 0.180	0.42 ± 0.058
Cynodon dactylon	7.17 ± 0.670*	3.89 ± 0.267*	1.49 ± 0.061	0.36 ± 0.055	1.65 ± 0.162	0.37 ± 0.046	1.76 ± 0.082	0.41 ± 0.074	1.67 ± 0.150	0.42 ± 0.021
Dactyloctenium aegyptium	7.30 ± 0.766*	4.13 ± 0.556*	1.67 ± 0.103	0.36 ± 0.046	1.60 ± 0.066	0.39 ± 0.039	1.63 ± 0.075	0.41 ± 0.031	1.71 ± 0.072	0.43 ± 0.060
Digitaria marginata	7.29 ± 0.889*	4.11 ± 0.183*	1.64 ± 0.137	0.35 ± 0.042	1.64 ± 0.099	0.39 ± 0.038	1.67 ± 0.097	0.42 ± 0.026	1.62 ± 0.218	0.43 ± 0.051
Paspalum paspaloides	7.52 ± 0.494*	3.92 ± 0.807*	1.60 ± 0.110	0.36 ± 0.045	1.63 ± 0.187	0.42 ± 0.019	1.69 ± 0.155	0.41 ± 0.066	1.67 ± 0.143	0.42 ± 0.023
Panicum dichotomiflorum	7.61 ± 0.754*	3.99 ± 0.343*	1.61 ± 0.146	0.39 ± 0.052	1.69 ± 0.152	0.39 ± 0.012	1.64 ± 0.152	0.39 ± 0.049	1.55 ± 0.203	0.42 ± 0.036
Sorghum halepense	7.48 ± 0.683*	4.40 ± 0.175*	1.66 ± 0.145	0.39 ± 0.051	1.68 ± 0.144	0.39 ± 0.055	1.64 ± 0.142	0.40 ± 0.036	1.66 ± 0.218	0.44 ± 0.034

Data are means of three replicates. Data followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

dismutase (SOD), peroxidase (POX), and polyphenol oxidase (PPO) activities, respectively, in the hydroprimed and soil microorganisms primed germinated seeds under the different treatments of weeds extract. The priming with soil microorganisms significantly increased the antioxidant enzymes under weed treatments, while the hydroprimed seeds showed non-significant changes in the antioxidant enzymes under weed treatment except for the superoxide dismutase, which showed a significant increase under weed treatments (Fig. 4).

Change in secondary metabolism components

Under various stress conditions, plant cells induce secondary metabolic pathways to produce compounds that aid in stress resistance. Shikimic acid and phenylalanine ammonia-lyase (PAL) are considered pivotal in various secondary metabolic pathways. Figures 7 and 8 present the changes in shikimic acid content and PAL activity in the different studied groups, respectively. In the case of shikimic acid, soil microorganisms priming significantly reduced the content to 3.03 μg/g fresh weight in the control, dropping below unity under normal conditions or weed treatment. Conversely, hydroprimed germinated seeds showed a significant increase in shikimic acid content under weed treatment (Fig. 7). Phenylalanine ammonia-lyase, acting as the gateway to secondary metabolism, exhibited significantly higher activity in seeds primed with soil microorganisms under weed treatments, while its activity remained unchanged in hydroprimed seeds under weed treatments (Fig. 8).

Phenols and flavonoids represent the primary secondary metabolites. Under weed treatment, soil microorganisms significantly increased total phenols and total flavonoids. Phenol content rose from 15.42 mg/g fresh weight in the control to over 42.00 mg/g fresh weight in soil microorganisms-primed germinated seeds under weed treatment. Flavonoid content increased from 48.90 μg/g fresh weight in the control to over 115.00 μg/g fresh weight in soil microorganisms-primed germinated seeds under weed treatment. Conversely, hydroprimed germinated seeds under weed treatment showed non-significant changes in total phenols and total flavonoids compared to the control (Table 4).

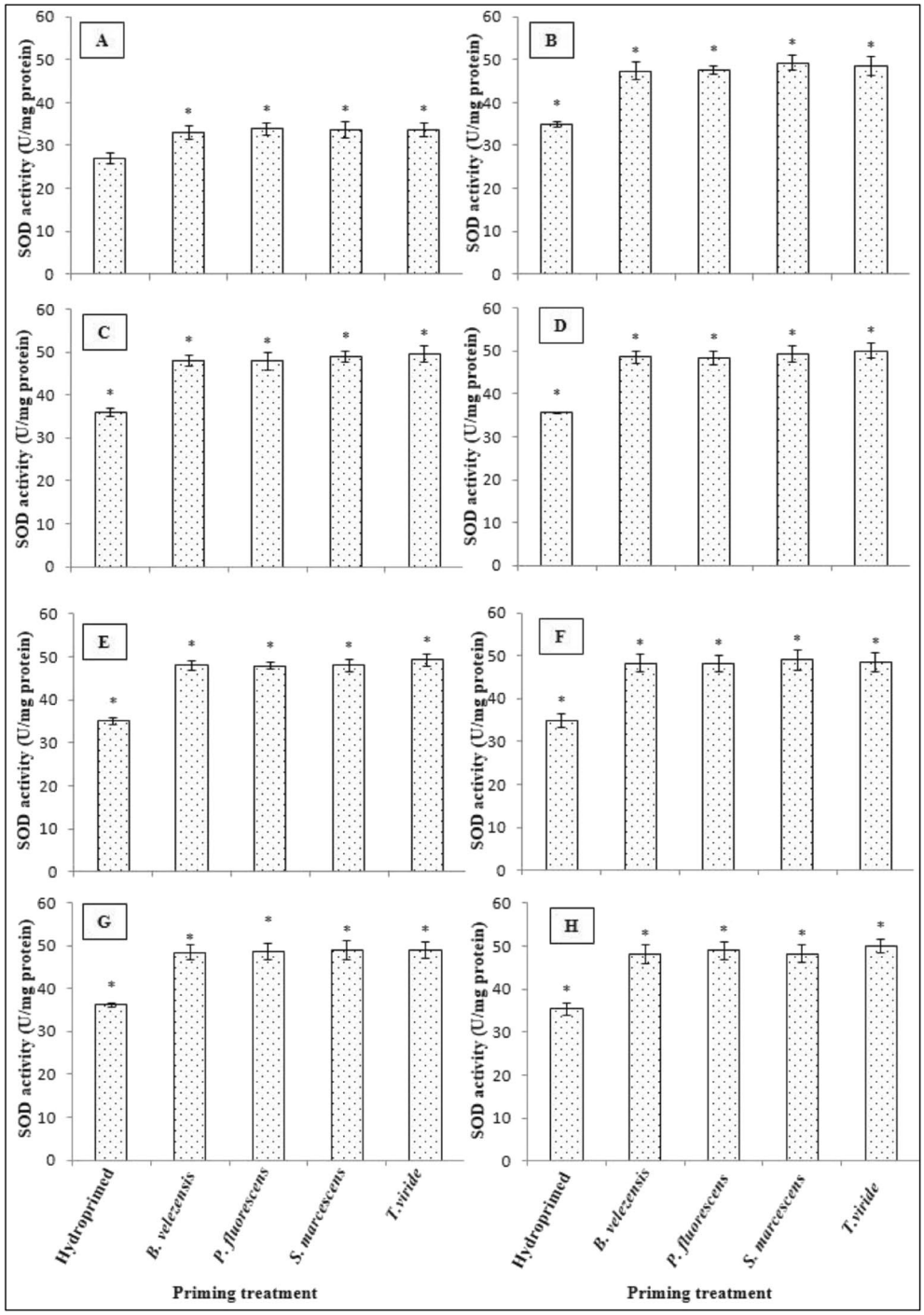


Fig. 4 Effect of priming with soil microorganisms on super oxide dismutase (SOD) in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C**: seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment,**E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

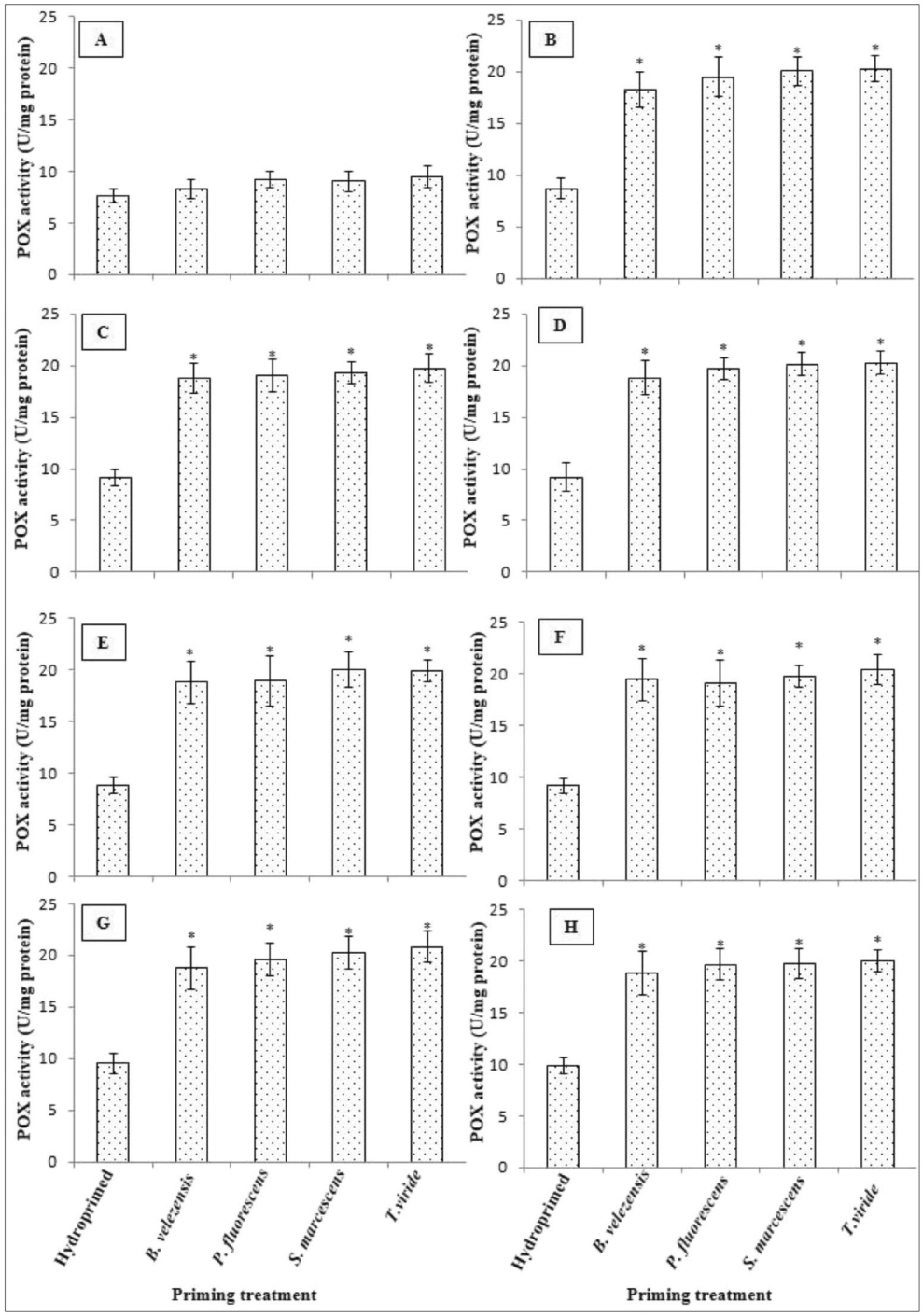


Fig. 5 Effect of priming with soil microorganisms on peroxidasse (POX) in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

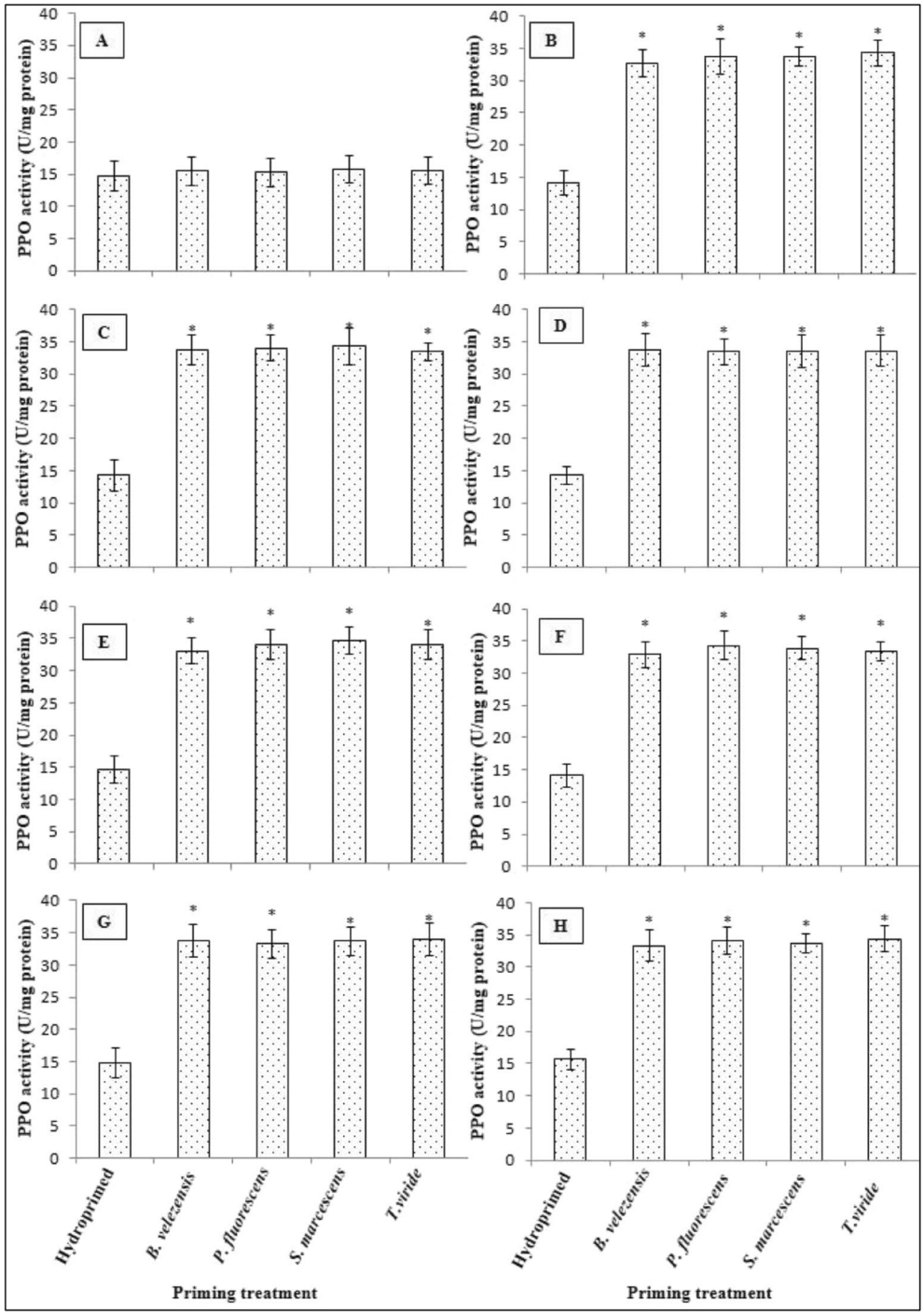


Fig. 6 Effect of priming with soil microorganisms on polyphenol oxidase (PPO) in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

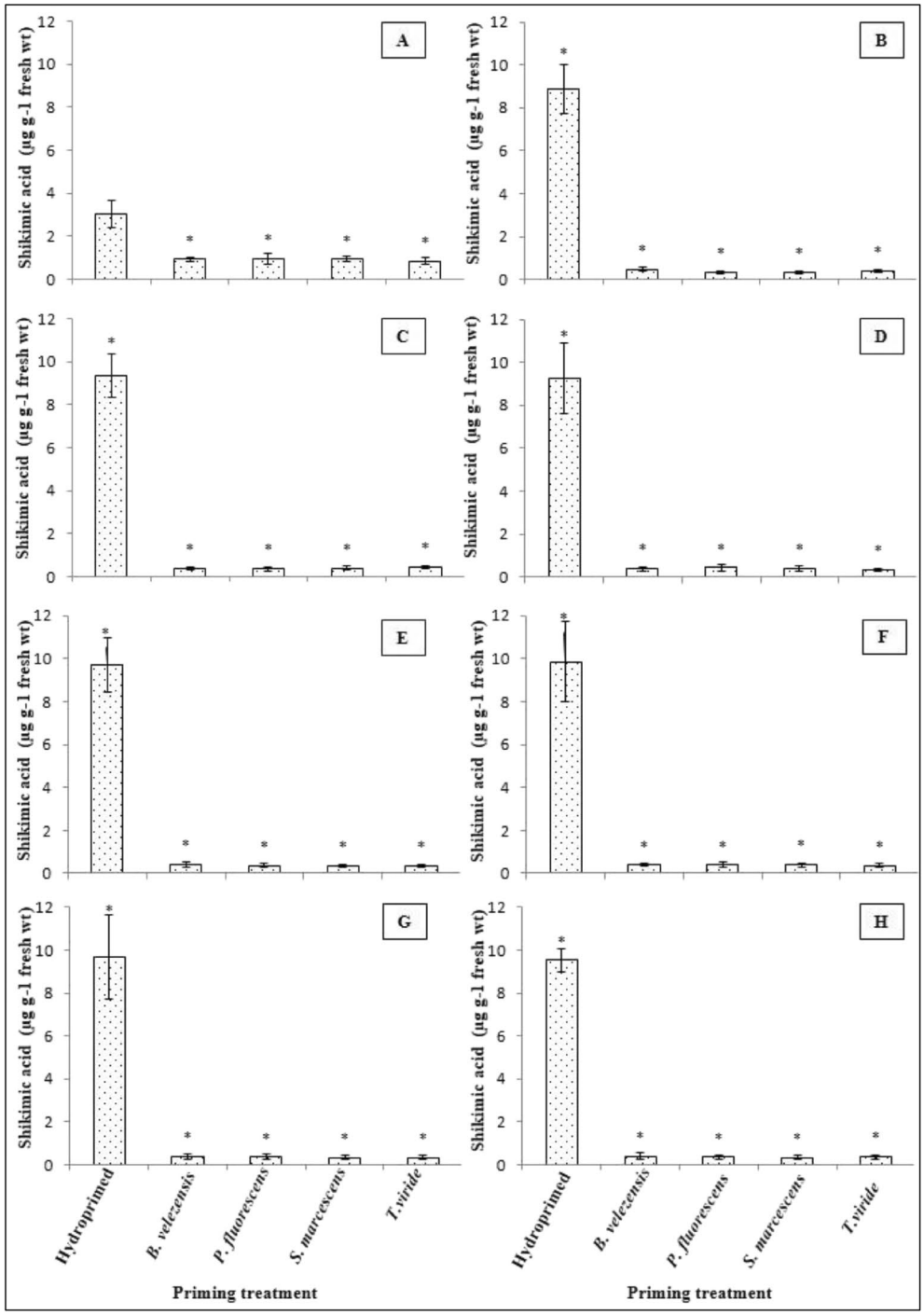


Fig. 7 Effect of priming with soil microorganisms on shikimic acid in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment, **E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

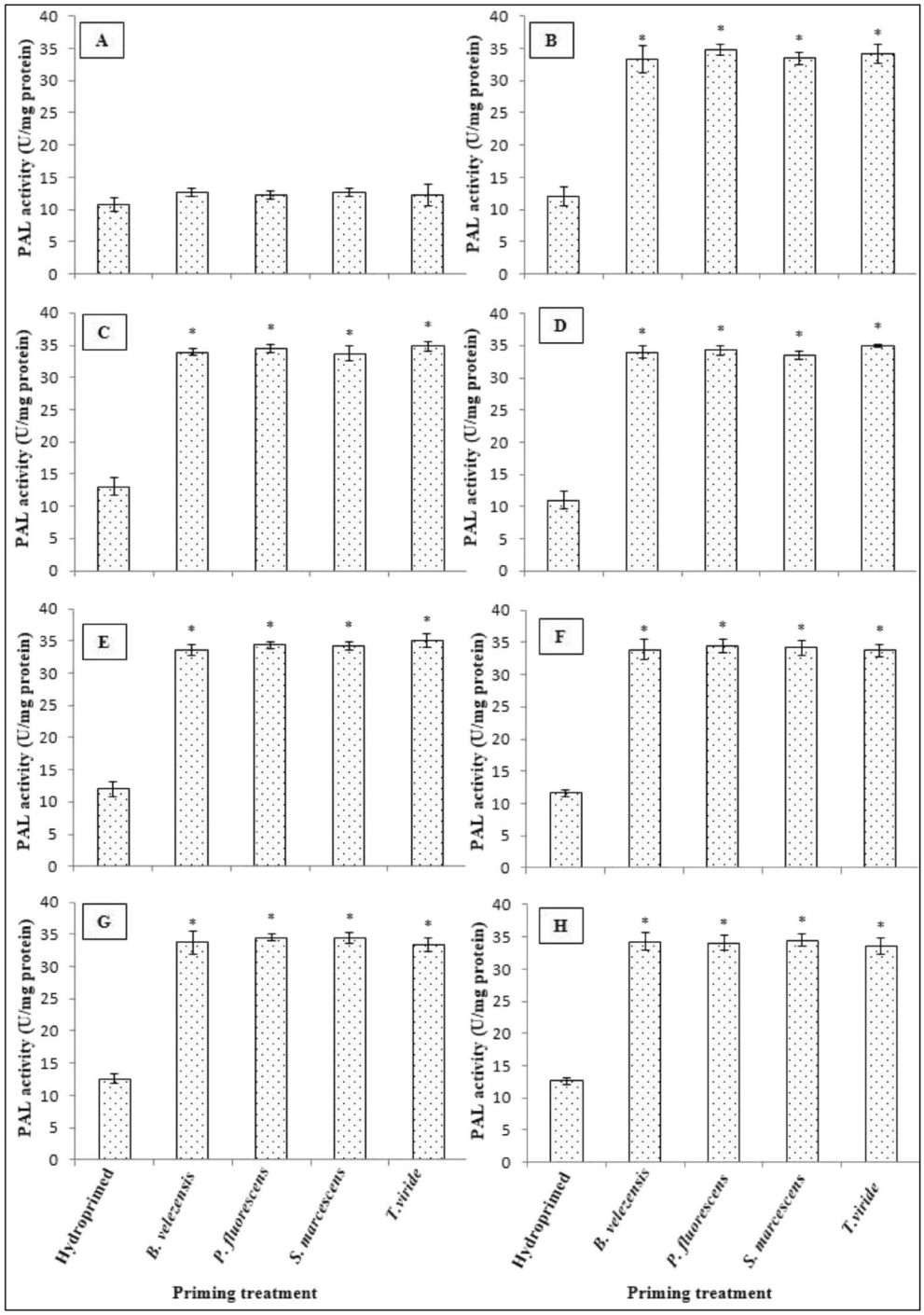


Fig. 8 Effect of priming with soil microorganisms on phenyl alanine ammonia lyase (PAL) in seeds germinated under under different weeds treatments. **A** seeds germinated on normal conditions (water), **B** seeds germinated under *Brachiaria ramose* treatment, **C** seeds germinated under *Cynodon dactylon* treatment, **D** seeds germinated under *Dactyloctenium aegyptium* treatment,**E** seeds germinated under *Digitaria marginata* treatment, **F** seeds germinated under *Paspalum paspaloides* treatment, **G** seeds germinated under *Panicum dichotomiflorum* treatment, **H** seeds germinated under *Sorghum halepense* treatment. Data are means of three replicates. Columns followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

Table 4 Effect of biopriming with different soil microorganisms on secondary metabolites (Total phenols content (TPC) (mg g^{-1} fresh weight), Total flavonoids (TFC) (µg g^{-1} fresh weight)) content in pearl millet seeds germinated under different weeds extract treatment

Priming	Hydroprimed		Bacillus velezensis		Pseudomonas fluorescens		Serratia marcescens		Trichoderma viride	
Weed Treatment	TPC (mg g^{-1} fresh weight)	TFC (µg g^{-1} fresh weight)	TPC (mg g^{-1} fresh weight)	TFC (µg g^{-1} fresh weight)	TPC (mg g^{-1} fresh weight)	TFC (µg g^{-1} fresh weight)	TPC (mg g^{-1} fresh weight)	TFC (µg g^{-1} fresh weight)	TPC (mg g^{-1} fresh weight)	TFC (µg g^{-1} fresh weight)
Non-treated (water)	15.42 ± 2.157	48.90 ± 2.789	17.83 ± 1.320	51.29 ± 2.005	16.76 ± 1.266	52.20 ± 3.768	17.34 ± 1.139	52.25 ± 3.611	17.83 ± 1.289	53.00 ± 3.606
Brachiaria ramose	16.56 ± 0.807	50.89 ± 3.432	42.90 ± 1.714*	115.36 ± 4.638*	43.66 ± 1.259*	118.96 ± 4.382*	44.83 ± 1.988*	119.69 ± 1.211*	44.18 ± 1.399*	119.04 ± 3.485*
Cynodon dactylon	17.00 ± 1.402	51.63 ± 4.521	43.90 ± 1.348*	118.39 ± 2.639*	44.32 ± 2.066*	119.64 ± 3.073*	44.51 ± 1.076*	118.16 ± 4.583*	44.94 ± 1.114*	120.69 ± 3.569*
Dactyloctenium aegyptium	16.30 ± 1.175	52.10 ± 3.518	43.02 ± 1.311*	117.32 ± 3.490*	43.61 ± 2.193*	121.09 ± 2.809*	43.81 ± 1.333*	120.06 ± 4.410*	45.53 ± 1.340*	119.09 ± 4.891*
Digitaria marginata	17.08 ± 1.214	53.78 ± 5.961	44.05 ± 1.241*	119.73 ± 2.988*	44.39 ± 1.840*	119.09 ± 3.553*	44.16 ± 1.029*	120.49 ± 3.701*	45.41 ± 1.370*	119.79 ± 4.127*
Paspalum paspaloides	16.21 ± 1.095	52.84 ± 4.031	42.76 ± 1.361*	119.20 ± 3.771*	44.03 ± 1.848*	118.56 ± 4.361*	44.43 ± 1.853*	118.76 ± 4.084*	44.69 ± 1.440*	119.09 ± 3.005*
Panicum dichotomiflorum	16.66 ± 1.467	52.23 ± 3.412	43.35 ± 1.827*	119.75 ± 3.095*	42.91 ± 1.303*	119.53 ± 4.405*	42.77 ± 1.380*	120.23 ± 3.857*	45.22 ± 0.692*	119.93 ± 3.794*
Sorghum halepense	17.35 ± 1.490	52.55 ± 4.203	44.12 ± 1.503*	119.38 ± 1.004*	43.62 ± 1.623*	118.30 ± 4.954*	44.16 ± 1.442*	120.08 ± 3.808*	44.69 ± 1.261*	119.55 ± 4.883*

Data are means of three replicates. Data followed by asterisk are significantly different from the control according to paired T test, $P < 0.01$

Discussion

Soil microorganisms are very effective in improving plant growth from germination to fruiting, besides their positive role in improving plant tolerance against different stress conditions (Ramandi and Seifi 2023). Seed biopriming with rhizosphere plant growth-promoting microorganisms is a well-reported technique for introducing soil microorganisms to improve plant germination and growth under different stress conditions (Mitra et al. 2021). Our results showed that seed priming with soil microorganisms improved pearl millet germination under weed extract treatments that significantly reduced the germination percent, plumule length, radicle length, and seed vigor in the hydroprimed seeds. The reduction of germination through the allelopathic effect of the weeds can be explained through the inhibition of nutrient uptake through the reduction in gibberellin content, which negatively affects the hydrolysis enzyme activity, which supplies the embryo with simple nutrients needed for energy production for healthy germination (Choudhary et al. 2023). Zareen et al. (2022) reported the allopathic effect of weeds on the germination of chickpeas and wheat due to the role of the allelochemicals in the availability of nutrients. Similarly, Padu et al. (2023) reported the inhibitory effect of the weeds on the germination of *Hordeum vulgare*, *Brassica juncea*, *Triticum aestivum*, and *Eleusine coracana* due to the effect of the allelochemicals. *Casuarina equisetifolia*-L leaf aquatic extract negatively affected the seed germination and growth of wheat, maize, mustard, and lentils (Ahmed et al. 2019). This allelopathic effect of *C. equisetifolia* is related to 2,4-di-tert-butylphenol (2,4-DTBP), which is the major allelochemical of the *C. equisetifolia* litter exudates (Xu et al. 2022).

The present work shows that priming with soil microorganisms improved the germination under the weeds treatments, this in the same line with what is reported that the soil microorganisms can mitigate the effect of the weeds; thus, they are a candidate for safe bioherbicide production (Neto et al. 2021). Similarly, de Matos et al. (2019) reported that soil microorganisms improved the interaction between maize and weeds, and maize growth increased with the improvement in soil microorganism activity. Also, Raza et al. (2021) reported the effectiveness of the rhizobacteria in biocontrolling the weeds in wheat agriculture. Soil microorganisms can induce germination under weed treatments by detoxifying allelochemicals. This process aids seeds in germinating without being adversely affected by the toxic impact of the weeds. Alternatively, they can stimulate the production of growth hormones such as gibberellins, which activate hydrolytic enzymes, promoting a healthier germination process (Makhaye et al. 2021; Xiao et al. 2020).

The hydrolysis process during seed germination is the key to all metabolic pathways in the germinated seeds (Nautiyal et al. 2023). Carbohydrates and phytate are the main storage materials in cereal seeds such as rice, wheat, and pearl millet (Samtiya et al. 2020; Hassan et al. 2021). The hydrolysis of these storage materials is the source of energy for various metabolic activities during germination. Additionally, they serve as a reservoir of building blocks for other essential components required for seedling growth and resistance against adverse conditions (Batool et al. 2022). The different stress conditions mainly affect seed germination by disturbing the hydrolysis processes (El Moukhtari et al. 2023). The weed residue in the soil is one of the environmental stresses that can inhibit the germination of the crop seeds by deactivating the hydrolysis process (de Souza Coelho et al. 2022). Similarly, the weed extract in the present study significantly reduced the carbohydrate and phytate hydrolysis by affecting the amylase and phytase activity. This inhibition is related to the toxic allelochemicals in the weed extract (Zareen et al. 2022). Seed priming can mitigate the adverse effects of different environmental stresses on seed germination (Rasheed et al. 2022). Priming the pearl millet seeds with soil microorganisms significantly improved the phytate and carbohydrate hydrolysis under weed treatments. Improving carbohydrate and organic phosphate hydrolysis and their immobilization during germination helps the seeds germinate under stress conditions (Wang et al. 2022). Similarly, Ghannad et al. (2022) reported that the activation of hydrolytic enzymes improved the germination of *Dracocephalum kotschyi* dormant seeds. *Trichoderma* biopriming increased the germination of soya beans through the activation of hydrolysis enzymes and nutrient mobilization (Paul and Rakshit 2023). Soil microorganisms priming increased the hydrolysis enzymes activity is related to the enhancement of its catalytic properties, and increasing its affinity to the substrate makes it more resistant to the differences in the surrounding conditions under stress (Al-Hazmi

and Naguib 2022; Al Hijab et al. 2024). In addition, priming with soil microorganisms induces gibberellin synthesis, which is the main phytohormone responsible for the activation of the hydrolysis enzymes during seed germination (Singh et al. 2018; Makhaye et al. 2021).

Production of reactive oxygen species (ROS) under stress conditions is a well-documented metabolic reaction that induces oxidative stress if there is not sufficient antioxidant machinery to antagonize the harmful effect of these excess reactive oxygen species. The excess of these ROS attacks the large molecules of the cells, such as lipids, proteins, and nucleic acids, causing their denaturation and deactivation. Lipid peroxidation is one of the most common markers of oxidative stress (Kumar et al. 2021). Weed treatments significantly increased the oxidative stress markers, hydrogen peroxide, and lipid peroxidation, in the hydroprimed seeds, while in the seeds primed with soil microorganisms, there was no significant change in the oxidative stress markers. Šoln et al. (2022) reported that allelochemicals from weeds cause a burst of ROS in the plant cells, which results in oxidative stress and then cell death. The seed biopriming with soil microorganisms mitigates the toxic effect of the excess ROS and prevents oxidative stress by improving the antioxidant enzyme activity, which detoxifies the excess ROS (Mitra et al. 2021). Pérez-García et al. (2023) reported the ability of the seed biopriming with soil microorganisms to up-regulate the antioxidant enzymes in seedlings, which ceases the negative effect of the ROS. Similarly, the present results showed that the pearl millet priming with soil microorganisms significantly increased the activity of the antioxidant enzymes that save the oxidative stress marker content, similar to the control, which means the mitigation of the oxidative stress induced by the weed treatments. Also, Haroon et al. (2022) reported the efficiency of soil microorganism priming in mitigating oxidative stress in wheat seeds germinated under stress. The positive role of the soil microorganisms in activation the antioxidant enzymes under stress is well reported as soil microorganisms help in induction defense genes which activates the antioxidant enzymes (Pérez-García et al. 2023). Beside soil microorganisms priming improve the kinetics parameters of the antioxidant enzymes (Al Hijab et al. 2024).

Secondary metabolism is a specialized metabolic pathway that produces specialized compounds that help plants adapt to different environmental conditions (Rajčević et al. 2023). These secondary metabolites have a significant role in the defense mechanism and tolerance against different stress conditions, improving plant growth and yield. The secondary metabolites include many different compounds found in small amounts under normal conditions, but their content increases according to the plant's needs under different stress conditions. Thus, the induction of secondary metabolism under different stress conditions is a common strategy in tolerant plant species (Chakraborty 2022). Shikimic acid is an important precursor for many secondary metabolite syntheses (Jha and Mohamed 2022). Its accumulation in plant tissues is an indicator of impairment in the secondary metabolism pathways (Guo et al. 2021). The pearl millet seeds primed with soil microorganisms showed a significant decrease in the shikimic acid content under weed treatments, while the hydroprimed seeds showed accumulation in the shikimic acid content under weed treatments. The accumulation of the shikimic acids is an indicator of the sensitivity to stress conditions, as the tolerant species can consume the shikimic acid to synthesize the secondary metabolites used in the defense mechanism (Malalgoda et al. 2020; Sweellum and Naguib 2023). Another important component in the secondary metabolism pathways is Phenylalanine ammonia-lyase (PAL). PAL is a key enzyme for the gate of secondary metabolism in plants. PAL shares in the phenylpropanoids synthesis such as phenols and flavonoids. The activity of PAL is very important for plant growth under stress (Barros and Dixon 2020). The soil microorganism priming significantly increased the PAL in pearl millet germinated seeds under weed treatments, while for the other hydroprimed seeds, the increase was non-significant compared to the control. Similarly, Qin et al. (2022) reported that the stress-tolerant plants have higher PAL activity than the sensitive ones. The increase in PAL expression and activity increased the seed vigor index and germination under different stress conditions (Zhang et al. 2022). Plant growth-promoting microorganisms share in the induction and activation of phenylalanine ammonia-lyase to increase the plant's tolerance to different external stimuli (Sarraf et al. 2023). The activity of PAL is a major point in the formation of phenols and flavonoids, which participate in the plant's tolerance against different stresses (Tak et al. 2023). Liu et al. (2023) reported that the high PAL transcription and activity in *Euryale ferox* resulted in high polyphenol and flavonoid content. Similarly,

the present results show that the significant increase in PAL activity is accompanied by a significant increase in phenols and flavonoid content in pearl millet seeds primed with the soil microorganisms germinated under weed treatments. This is in line with reports by Lourenzi et al. (2022) that soil plant growth-promoting microorganisms can induce the synthesis of secondary metabolites such as phenols and flavonoids in plants under the allelopathic effect of different weeds. Also, Pérez-García et al. (2023) reported that rhizobacteria priming can improve the seed vigor index and germination under stress conditions through the activation of the synthesis of bioactive compounds such as phenols and flavonoids. Phenols and flavonoids are widespread secondary metabolites that have an important role in plant tolerance against different stresses. They act as non-enzymatic antioxidants, which mitigate oxidative stress (Kaur et al. 2023; Mishra et al. 2023). Shikimate and Phenylalanine ammonia-lyase play a pivotal role in phenol and other secondary metabolite biosynthesis, bridging primary metabolism with secondary metabolism (Tak et al. 2023). This bridging is very clear in the present study, as in the pearl millet seeds primed with soil microorganisms; however, the amylase activity increased significantly and the soluble sugar content remained with a non-significant change than that of the control. This is in line with Wei et al. (2023), who reported that starch metabolism and phenylpropanoid biosynthesis are involved in defense responses to stress.

Seed biopriming with soil microorganisms can mitigate the toxic effects of weed on pearl millet growth. This provide insights into sustainable agricultural practices, reducing reliance on synthetic chemicals, promoting beneficial microbial communities, integrating biological control, aligning with agroecological principles, and addressing food security challenges in specific agroecosystems. This knowledge contributes to a more holistic and environmentally friendly approach to weed management, supporting the long-term sustainability of agricultural systems.

Conclusion

Biopriming pearl millet seeds with specific soil microorganisms significantly enhanced germination under various weed extract treatments by optimizing seed metabolism, increasing antioxidant enzymes, and improving the hydrolysis process. This approach not only alleviated oxidative stress induced by weeds but also conserved energy for primary metabolism, enabling resources to be redirected to secondary metabolism for the formation of defense molecules. This knowledge supports a holistic and environmentally friendly weed management approach, contributing to the long-term sustainability of agricultural systems.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary file.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests We declare that we have no competing interests.

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