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Ability of *Trichoderma harzianum* from Semi Arid Soils to Enhance Antioxidant Defense of Maize Seedlings under Water Stress

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors managed the analyses of the study and literature searches. Also, the authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Determine the effect of different inoculum concentrations of *T. harzianum* from semi - arid soils on the activity of antioxidant enzymes of maize seedlings under water stress.
Methods and Results: This study employed a three-factor factorial (3×4×4) design, arranged in a completely randomized design (CRD) with three replications. Three maize varieties (H614, H629 and H6210) were treated with four concentrations of *T. harzianum* (0, $1x10^5$, $1x10^7$and $1x10^{10}$ spore/ml and thereafter grown under four osmotic potential regimes (0, -0.3, -0.6 and -0.9 MPa). Results from the study showed that *T. harzianum* had a significant effect on Superoxide dismutase (SOD) and catalase (CAT) activity of maize seedlings and did not enhance either maize seed germination or seedling growth. The activity of SOD and CAT was significantly enhanced by *T. harzianum* in all the three varieties of maize. Optimum SOD and CAT activity were recorded in seeds treated with 10^7 spores/ml of *T. harzianum*. Under normal growth conditions (0MPa), SOD and CAT activities were not enhanced by *T. harzianum*. However, under severe water stress (-0.9MPa), maximum activity of the enzymes was registered in all the three varieties of maize.

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Conclusion: Maize seedling colonization by *T. harzianum* enhanced systems of antioxidative enzymes. Maximum activity of these enzymes was recorded under severe water stress (0.9MPa) mainly in seedlings treated with 10^7 spores/ml of *T. harzianum*. Consequently *T. harzianum* from semi-arid soils may be employed to improve maize plants' tolerance to water stress.
Significance and Impact: With rapid increase in human population, coupled with global climate change, there is need to devise a cheap and safe option to increase the production of food crops. The ability of *T. harzianum* in promoting plant growth precisely maize under stress is of importance.

Keywords: T. harzianum; maize; water stress; SOD and CAT.

1. INTRODUCTION

Water stress or drought stress is an inevitable and recurring feature in global agriculture. It is one of the most devastating environmental stresses. Water stress limits growth and productivity of main crop species, reducing yields to less than half [1]. Also it has been reported that, about one-third of the world's potentially arable land suffers from water shortage [2]. Maize (*Zea mays* L.) also known as queen of cereals is an important cereal crop grown all over the world [3] and is central to developing nations' agriculture and food security. Most cereals, maize being one of them are drought-sensitive. Significant yield losses can occur in even a mild water stress during reproductive phase [4]. Water stress brings about physiological, biochemical and molecular changes in plants which oversees growth and productivity. One such biochemical mechanism includes antioxidant enzymatic system (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) etc.), which protect plant cells against the detrimental effects of reactive oxygen species (ROS) generated under variety of environmental stresses [5].

[6] showed that *Trichoderma spp*. are cosmopolitan fungi found in agricultural, forest, desert soils. They also colonize roots of various plants found in different ecosystems including maize. They have been defined as plant symbiont opportunistic avirulent organisms, able to colonize plant roots and to produce compounds that stimulate growth and plant defense mechanisms under suboptimal conditions. *Trichoderma spp*. are the most common research tools as microbial inoculants which have been mostly used as biocontrol agents. However, in the recent years, they have become popular as plant growth promoters [7]. For *Trichoderma* to effectively augment plant development, it must be able to establish in the spermosphere of germinating seeds, distribute on the emerging radicle and colonize the developing root [8]. Research shows that colonization of host roots with *Trichoderma* strains enhances entire tolerance to biotic and abiotic stresses [9,10]. Such kind of augmented tolerance to biotic and abiotic stress is believed to be due to enhanced root growth and the nutritional status of plants [11].

An increase in damaging levels of reactive oxygen species (ROS) is a common feature in plants in the presence of abiotic stresses [12]. Even though, the ability of *Trichoderma spp.* mainly T22– to alleviate varied types of stress is suggested to be mediated by enhanced redox buffering capacity of the colonized plants. For example, under water deficit, lipid peroxide content of colonized tomato seedlings was lower than in the control seedlings [13]. Lipid peroxidation is commonly associated with oxidative damage [14] when the level of ROS exceeds the capacity of the antioxidant defense system [12,15]. While changes in ROS level may act as a signal to activate a host of defense mechanisms, continued production of high levels of ROS under ongoing stress causes damage to plants [12].

Superoxide dismutase is the main scavenger of superoxide radicals, which converts the toxic superoxide (O_2^-) to hydrogen peroxide and oxygen, through a process called dismutation reaction: $2O_2^- + 2H+ \longrightarrow H_2O_2 + O_2$. The enzyme embodies the first line of cell defence against ROS generated abiotic stresses like drought in plants, therefore, preventing the tissue damage due to oxidative stress. CAT and POD enzymes are able to convert toxic H_2O_2 to water and oxygen. under water stress only elevated SOD activity cannot protect the plants from toxic effect of oxygen free radical hence CAT and POD is needed to remove toxicity of H_2O_2 [16]. On the other hand, *Trichoderma* strains have been reported to enhance the activity of these pathways, through improved expression of genes encoding the component enzymes [13]. For example, if these pathways are enhanced in the chloroplasts, then it is expected that the

photosynthetic efficiency will increase by reducing damage by the superoxide anion and other reactive species involved in photosynthesis [17]. *Trichoderma spp.* augments protection against ROS perhaps by increasing ROS scavenging capacities. Proteomics of roots inoculated with *Trichoderma* showed an increase in levels of anti-oxidative enzymes mainly Superoxide dismutase (SOD) as well as increased levels of peroxidase, glutathione-reductase and Glutathione-S-transferase (GST), and other detoxifying enzymes in leaves [18]. In a recent experiment performed by [19], *T. harzianium* (T-35) benefited rice plants by increasing their tolerance to severe drought stress through the reduction of oxidative stress by enhancing the production of SOD, CAT and POD anti-oxidative enzymes. Furthermore, studies have shown that microbes in harsh habitats are adapted to their environments and they have the ability to transfer this ability to their host plants grown in such habitats like arid and semi arid area [20]. Therefore, the main goal of this study is to investigate the ability of *T. harzianum* isolated from semi- arid soils to enhance the antioxidant defense in maize seedlings.

2. MATERIALS AND METHODS

2.1 Soil Sample Collection

Soil samples were collected from the semi- arid rangeland of Marigat area, Baringo County Kenya. The area is located between latitude 00^{o} 26-00^{o}32'N and longitude 36^{o} 00'36^{o} 09' E. The climate is semi-arid with an average altitude of 900 M above the sea level. A total of 60 g of soil samples were randomly collected from the rhizosphere of grass plants and bare soil in 10 cm depth using a sterile soil auger. The samples were then transferred into sterile polyethylene bags and transported to the Microbiology laboratory, at the University of Eldoret, Kenya within 24 hours of collection. These samples were used for isolation of *T. harzianum*.

2.2 Isolation of *T. harzianum*

[21] method for *T. harzianum* isolation was adopted with slight modifications. Ten grams of the soil sample made up to 1000 ml using sterile distilled water in a sterile conical flask. The soil suspension was left for one hour at room temperature to release conidia and hyphae adhering to soil particles. Serial dilutions up to 10^{-3} were prepared from the suspension and 1 ml aliquots were then spread-plated onto Potato Dextrose agar (PDA) medium supplemented with 50 mg/l of streptomycin antibiotic to inhibit bacterial growth. The plates were then incubated at 28℃ and 35℃ for seven days. Under 35℃, growth inhibition of all species of *Trichoderma* has been reported except for *T. harzianum* [22]. Distinct colonies of *T. harzianum* were picked based on their on their morphological characteristics as described by [23]. To obtain pure cultures of *T. harzianum,* streaking was done on fresh PDA medium twice. Microscopic examination and measurements of conidiophores and conidia were made from slide preparations stained with lactophenol-cotton blue and observed under a light microscope under ×400. Pure cultures of T. *harzianum* were then taken to Kenya Agricultural and livestock Research Organization (KALRO) Njoro, Kenya for confirmation.

2.3 Inoculum Production of *Trichoderma spp*

The study adopted [24] method for production of *T. harzianum* inoculum. However, slight modification was made to suit the present study. The pure cultures obtained above were sub-cultured aseptically in eight 90 mm diameter Petri plates each containing 15 ml of a freshly autoclaved PDA media. Incubation of the eight plates was done at 28℃ for ten days. On the tenth day, spore suspensions from the fungus inoculum were prepared by flooding the surface of the agar slant with 10 ml sterile distilled water and the culture surface gently scraped to extricate the spores. The spore suspensions derived from the eight Petri plates were transferred separately to 500 ml flasks containing 400 ml sterile distilled water. Flasks were then shaken for 2 minutes to ensure that the spores are appropriately mixed. Four concentrations of the fungal spore (0, $1x10^{5}$, $1x10^{7}$and $1x10^{10}$ spore/mL) were determined using a haemocytometer under a light microscope at ×400 magnifications. The control was made up of autoclaved spores of *T. harzianum*. The autoclaving process was done at 121℃ for 15 minutes [25].

2.4 Water Stress Induction

Polyethylene glycol 6000 (PEG) at different concentrations was prepared to establish different levels of osmotic potential. Approximately 0, 143.18, 213.64 and 267.97 g of PEG were dissolved in 1000 ml distilled water to

generate four osmotic stress levels (0, -0.3, -0.6 and -0.9 MPa, respectively).The control was made up of only distilled water with no PEG.

2.5 Research Design

The study employed a three- factor factorial design (3×4×4) replicated three times. Maize seeds belonging to (H614, H629 and H6210) varieties with no cracks or any visible deformations were used in this study. Surface sterilization was done for 5 minutes with 1% sodium hypochlorite solution, followed by rinsing with distilled water three times and finally air dried. Wet seed treatment method was adopted, where seed coating was done by applying 2% of starch (adhesive) on the maize seeds. Subsequently, maize seeds were dipped in seed coating suspension of 0, 1×10^5, $1x10^7$ and 1×10^{10} spores/mL *Trichoderma harzianum* for 2 minutes. The seeds were finally germinated on petri dishes lined with whatman filter paper satured with distilled water under 0, -0.3, -0.6 and -0.9 MPa for ten days.

2.6 Enzyme Extraction from Plant Samples

Extraction of (SOD and CAT) enzymes from plant samples was done according to [26]. Both water stressed and control maize seedlings were evaluated for antioxidative enzymes' activity after 10 days of germination. Fresh weight of 0.5 g leaf sample was taken and then placed in a freezer at -10℃ for 24 hrs. The frozen leaf sample was then finely ground by pestle in a frozen motor to prevent the loss of enzymes' activities. The frozen powder was added to 10 mL of phosphate buffer (pH 7.5). The homogenate was centrifuged at 15000 × *g* for 10 min at 25℃ and supernatant was used as enzyme source for catalase (CAT) and superoxide dismutase (SOD).

2.7 Assay of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined according to Kong et al. [27]. A 3 ml sample of the reaction mixture was made up 0.1 ml of 1.5 M Na_2CO_3, 0.2 ml of 200 mM methionine, 0.1 ml of 3 mM EDTA, 0.1 ml of 2.25 mM *p*-nitroblue tetrazolium chloride (NBT), 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1 ml of distilled water and 0.05 ml of enzyme samples. A tube containing reaction mixture without the enzyme extract was used as control. The reaction was started by adding 0.1 ml 60 µM riboflavin and placing the tubes below a light source for 15 minutes. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm. An illuminated blank without protein gave the maximum reduction of NBT, and therefore, the maximum absorbance at 560 nm. Superoxide dismutase activity was presented as absorbance of blank minus absorbance of sample, giving the total inhibition, calculated per microgram protein. The activity of SOD was expressed as U mg $^{-1}$ protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

2.8 Assay of Catalase (CAT) Activity

Determination of CAT activity was done according to Lum et al. (2014). A total of 3 ml of the assay mixture (0.5 ml of 0.2 M phosphate buffer (pH 7.5), 0.3 ml of H_2O_2, 0.1 ml of the reaction mixture and 2.1 ml of distilled water was prepared. Change in optical density was measured at 240 nm at 0 min and 3 min on UV- spectrophotometer. The molar extinction coefficient of H_2O_2 at 240 nm was taken as 36 μmol^{-1} cm^{-1} and the results were expressed as µmol H_2O_2 min^{-1} g^{-1} protein [26].

2.9 Statistical Analysis

The experiment for the activity of SOD and CAT enzymes was carried out using (4×3×3) factorial design with three replicates. The mean values (±SE) of SOD and CAT enzymes activity of the three replicates were calculated. The mean values were then analyzed by a three-way analysis of variance (ANOVA) using Statgraphics programme to determine the activity of antioxidant enzymes (SOD and CAT). The means were separated using Tukey's test.

3. RESULTS

3.1 Isolation of *T. harzianum*

At 28℃ and 35℃, *T. harzianum* grew uniformly and formed white mycelia within five days.

After ten days of growth, the fungus displayed green conidia, at both 28 and 35℃. The conidia production was dense at the center and towards the margins. It was also observed that, conidia production by the fungus was not different at 28 and 35℃ as shown in Plate 1.

Table 1 showed that concentration of *T. harzianum* and osmotic potential affected SOD and CAT activities significantly ($p<0.05$).

Concentration of *T. harzianum* by osmotic potential and maize variety by osmotic potential interactions were also significant ($p<0.05$) for SOD and CAT activities. However, maize variety, interactions for maize variety by *T. harzianum* concentration and maize variety by *T. harzianum* concentration by osmotic potential had no significant ($p>0.05$) effect on SOD and CAT activities. At low osmotic potential, increase in concentration of the fungus increased CAT and SOD activity until 10^7 spores/ml of the fungus. Further increase in concentration (10^{10} spores/ml of *T. harzianum*) led to a stabilization of the activity.

Results showed that SOD activity increased significantly ($p<0.05$) with decrease in osmotic potential in both treated and untreated maize seedling across the three varieties of maize (Table 2). Also SOD activity increased with increase in concentration of *T. harzianum* upto 10^7 spores/ml of the fungus before stabilizing with 10^{10} spore/ml of the fungus. Maize varieties did not differ significantly in SOD activity at the same osmotic potentials with the same spore concentration of *T. harzianum*. Under normal growth condition (0 MPa), SOD activity increased significantly ($p<0.05$) from 15.0 U g^{-1} protein in control to 15.2 U g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Further increase (15.5 U g^{-1} protein) in SOD activity was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum*. Stabilization of SOD activity (15.5 U g^{-1} protein) was recorded in control seedlings treated with 10^{10} spores/l of the fungus for the three varieties of maize as shown in Table 2.

Under severe water stress (-0.9 MPa), SOD activity increased significantly ($p<0.05$) from 194 U g^{-1} protein in control to 337 U g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Maximum SOD activity (893 U g^{-1} protein) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum* with stabilization of SOD activity (892U g^{-1} protein) recorded in seedlings treated with 10^{10} spores/ml of the fungus across the three varieties of maize (Table 2).

Seedlings treated with 10^7 and 10^{10} spores/ml concentrations of *T. harzianum* were not significantly different ($p>0.05$) in SOD activity in all the three varieties of maize. However, they were significantly different ($p<0.05$) from seeds treated with 10^5 spores/ml of *T. harzianum* and control (Table 2). Furthermore, seedlings treated with 10^5 spores/ml of *T. harzianum* showed significant ($p<0.05$) SOD activity from control irrespective of maize variety.

Table 1. Effects of main factors and their interactions on SOD and CAT activities

Source of variation	SOD activity			CAT activity		
	F-ratio	P-value	Effect	F-ratio	P- value	Effect
Concentration of *T. harzianum* (CT)	1.4E+07	<0.05	**	13440.00	<0.05	**
Osmotic potential (OP)	5.4E+07	<0.05	**	33327.53	<0.05	**
Maize variety (V)	1049.00	>0.05	NS	10.01	>0.05	NS
CT×OP	6071030.00	<0.05	**	3354.41	<0.05	**
CT×V	38.10	>0.05	NS	0.97	>0.05	NS
OP×V	799.74	<0.05	**	5.42	<0.05	**
CT×OP×V	178.68	>0.05	NS	2.38	>0.05	NS

***Significant at p < 0.05. NS denotes not significant at p<0.05*

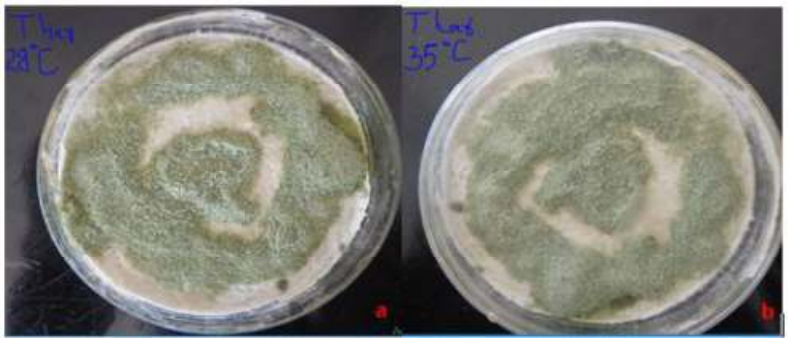

Plate 1. Conidia 10 day old *T. harzianum* on PDA at (a) 28 and (b) 35℃

Table 2. Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the SOD activity (U g^{-1} protein) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	SOD activity (U g^{-1} protein)		
		Maize variety		
		H614	H629	H6210
0	0	15.054±0.06[a]	15.079±0.08[a]	15.023±0.08[a]
	-0.3	47.338±0.01[d]	47.369±0.07[d]	47.319±0.06[d]
	-0.6	150.440±0.04[g]	178.478±0.05[g]	150.378±0.02[g]
	-0.9	194.379±0.06[h]	194.378±0.04[h]	194.311±0.04[h]
10^5	0	15.212±0.03[b]	15.219±0.09[b]	15.209±0.01[b]
	-0.3	97.579±0.02[e]	97.583±0.10[e]	97.573±0.06[e]
	-0.6	194.112±0.03[h]	194.117±-0.07[h]	194.102±0.07[h]
	-0.9	337.777±0.01[k]	337.781±0.09[k]	337.729±0.07[k]
10^7	0	15.560±0.07[c]	15.572±0.06[c]	15.557±0.10[c]
	-0.3	126.801±0.01[f]	126.825±0.02[f]	126.791±0.04[f]
	-0.6	336.769±0.05[j]	336.772±0.07[j]	336.760±0.09[j]
	-0.9	893.564±0.06[l]	893.570±0.1[l]	893.555±0.07[l]
10^{10}	0	15.520±0.01[c]	15.527±0.05[c]	15.518±0.06[c]
	-0.3	126.616±0.03[f]	126.631±0.06[f]	126.609±0.07[f]
	-0.6	336.358±0.09[i]	336.367±0.07[i]	336.351±0.01[i]
	-0.9	892.912±0.08[l]	892.926±0.05[l]	892.905±0.08[l]
F-ratio		177.68	178.59	178.66
P value		<0.05	<0.05	<0.05
Effect		**	**	**

Means followed by the same letter within the same column are not significantly different at P < 0.05.
*** denotes significant at p<0.05*

Similarly, CAT activity increased significantly ($p<0.05$) with decrease in osmotic potential in both treated and untreated maize seedling across the three varieties of maize (Table 2). CAT activity increased with increase in concentration of *T. harzianum* upto 10^7 spores/ml of the fungus before stabilizing with 10^{10} spore/ml of the fungus. Maize varieties did not differ significantly in CAT activity at the same osmotic potentials with the same spore concentration of *T. harzianum*.

CAT activity increased significantly ($p<0.05$) from 0.01µmol H_2O_2 min^{-1} g^{-1} protein in control to 0.06 µmol H_2O_2 min^{-1} g^{-1} protein in control seedlings treated with 10^5 spores/ml of *T. harzianum*. Seedlings treated with 10^7 spores/ml of *T. harzianum* recorded highest CAT activity (0.09 µmol H_2O_2 min^{-1} g^{-1} protein), while stabilization of CAT activity (0.09 µmol H_2O_2 min^{-1} g^{-1} protein) was recorded in seedlings treated with 10^{10} spores/ml of the fungus at 0 MPa a cross the three varieties of maize as shown in Table 3.

At -0.9 MPa, CAT activity increased significantly ($p<0.05$) from 1.0 µmol H_2O_2 min^{-1} g^{-1} protein in control to 1.3 µmol H_2O_2 min^{-1} g^{-1} protein in seedlings treated with 10^5 spores/ml of *T. harzianum*. Maximum CAT activity (4.0 µmol H_2O_2 min^{-1} g^{-1} protein) was recorded in seedlings treated with 10^7 spores/ml of *T. harzianum*. Stabilization of CAT activity (4.0 µmol H_2O_2 min^{-1} g^{-1} protein) was recorded in control seedlings treated with 10^{10} spores/ml of the fungus for the three varieties of maize as shown in Table 3.

4. DISCUSSION

In this study, we isolated *T. harzianum* from semi- arid soils. There was no doubt that the isolated fungus was *T. harzianum* since growth at 35℃ was recorded. [22] found that the capability of *T. harzianum* to grow at 35℃ was useful in distinguishing it from other *Trichoderma* species.

Findings from the present study clearly showed that *T. harzianum* played a key role in enhancing maize seed germination and early seedling growth under water stress. Seeds treated with *T. harzianum* showed significant difference in germination from control at water stress. Seeds respond to *T. harzianum* very early in germination, even before the radicle protrudes [6]. Also, *Trichoderma spp.* have been shown to augment seed germination by enhancing phase

III imbibition (cell elongation, followed by radicle protrusion). The present results are in agreement with those of [13]. The authors found that tomato seeds that were treated with *T. harzianum* (T22) showed higher seed germination percentage than untreated tomato seeds.

Table 3. Effects of four concentrations of *T. harzianum* (0, 10^5, 10^7 and 10^{10} spores/ml) on the CAT activity (µmol H_2O_2 min^{-1} g^{-1} protein) of three varieties of maize (H614, H629 and H6210) at four osmotic potential levels (0, -0.3, -0.6 and -0.9 MPa)

Conc. of *T. harzianum* (spore/ml)	Osmotic potential (MPa)	CAT activity (µmol H_2O_2 min^{-1} g^{-1} protein)		
		Maize variety		
		H614	H629	H6210
0	0	0.016±0.009[a]	0.015±0.011[a]	0.017±0.007[a]
	-0.3	0.076±0.013[bc]	0.076±0.016[bc]	0.081±0.009[c]
	-0.6	0.874±0.011[f]	0.873±0.003[f]	0.883±0.010[f]
	-0.9	1.071±0.008[g]	1.070±0.009[g]	1.075±0.014[g]
10^5	0	0.066±0.007[b]	0.075±0.017[b]	0.079±0.016[b]
	-0.3	0.117±0.008[c]	0.117±0.020[c]	0.119±0.011[c]
	-0.6	1.147±0.008[h]	1.174±0.016[h]	1.150±0.019[h]
	-0.9	1.333±0.009[i]	1.332±0.008[i]	1.335±0.007[i]
10^7	0	0.097±0.012[c]	0.095±0.009[c]	0.097±0.008[c]
	-0.3	0.504±0.013[d]	0.500±0.015[d]	0.509±0.017[d]
	-0.6	3.623±0.010[j]	3.619±0.011[j]	3.626±0.017[j]
	-0.9	4.083±0.009[k]	4.079±0.007[k]	4.085±0.011[k]
10^{10}	0	0.094±0.011[c]	0.097±0.008[c]	0.094±0.018[c]
	-0.3	0.502±0.012[d]	0.497±0.014[d]	0.502±0.015[d]
	-0.6	3.637±0.011[j]	3.623±0.017[j]	3.638±0.017[j]
	-0.9	4.082±0.014[k]	4.078±0.010[k]	4.080±0.009[k]
F-ratio		2.38	2.31	2.41
P value		<0.05	<0.05	<0.05
Effect		**	**	**

Means followed by the same letter within the same column are not significantly different at P < 0.05.
*** denotes significant at p<0.05*

The study revealed that SOD and CAT activities were recorded even in untreated seedlings under severe water stress (-0.9 MPa). Plants develop a variety of mechanisms to acclimatize themselves to forever changing environments. These mechanisms are facilitated through multiple signal transduction pathways acting in a global signal network [28]. Previously, [29] had reported an increase in SOD activity that was correlated to induced resistance of plants to drought stress. Furthermore, SOD enzyme embodies the first line of cell defence against reactive oxygen species (ROS) generated by abiotic stresses like drought in plants, therefore, preventing tissue damage due to oxidative stress [30]. The enzyme converts superoxide radicals to hydrogen peroxide. *Trichoderma spp.* induces systemic changes in gene expression through a complex signal transduction network with methyl jasmonate (MeJA) playing the pivotal role [18]. MeJA induces expression of genes encoding antioxidant enzymes. MeJA may play a signaling role in the expression of genes encoding antioxidant enzymes as well. Similar findings were reported by [10] when proteomics of shoots inoculated with *Trichoderma* showed an increase in levels of anti-oxidative enzymes mainly Superoxide dismutase as well as increased levels of peroxidase, glutathione-reductase and Glutathione-S-transferase (GST), and other detoxifying enzymes in leaves.

In the present study, *T. harzianum* increased SOD and CAT activities significantly in all the three varieties of maize under water stress as compared to control plants. These results are in agreement with those of [31] where a transient increase in intracellular ROS was detected 5 to 10 min after treating soybean cell culture with culture filtrate of *T. atroviride.* Furthermore, [32] also reported that *T. harzianum* enhanced the activity of antioxidant enzymes in tomato plant subjected to water stress.

SOD converts superoxide radicals to hydrogen peroxide, while CAT enzyme converts hydrogen

peroxide to water and oxygen [30]. Increased activity of SOD alone cannot protect plants from toxic effect of oxygen free radicals and therefore, other enzymes like CAT and POD are required to get rid of hydrogen peroxide toxicity [16].

5. CONCLUSION

This study presents evidence that maize seedling colonization by *T. harzianum* enhances systems of antioxidative enzymes. Maximum activity of these enzymes was recorded under severe water stress (-0.9 MPa) mainly in seedlings treated with 10^7 spores/ml of *T. harzianum*. This consequently indicates that, one of the mechanisms that *T. harzianum* especially those isolated from semi-arid soils employ in improving plant tolerance to water stress is through the reduction of oxidative stress via increased. SOD and CAT activities. Treatment of seed or plants that could simultaneously confer resistance to abiotic stresses would be of importance to agricultural plant production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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