Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 2, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE BENEFITS OF USING USEFUL MICROORGANISMS IN DROUGHT MITIGATION AND INCREASING SOIL FERTILITY - AN OVERVIEW

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Abstract

In recent decades, drought has represented a major setback for the world's agricultural economy, causing substantial reductions in global agricultural production that threaten the amount of food and food security. Thus, are necessary new alternatives to improve the sustainability of agricultural yield, to cope with various stress factors, including drought and high temperatures. Beneficial microorganisms (fungi, rhizobacteria) play an important role in mitigating the stress caused by drought by modulating the enzymatic and non-enzymatic antioxidant systems of plants and by producing phytohormones, osmolytes which represent the primary mechanisms through which they mitigate the effects of water stress, improving plant growth parameters and soil characteristics. In this study, we presented a review of studies found in the literature regarding the currently known implications of beneficial microorganisms for drought tolerance, including their mechanisms of action and also implication of them in increase of soil fertility.

***Key words**: water stress, yield, crops, nutrients, plants.*

INTRODUCTION

The increasing demand for food to sustain a growing global population necessitates the maximization of agricultural production, even under extreme conditions, which have become more frequent due to global climate change, while simultaneously conserving non-renewable resources along with the environment (Raza et al., 2019).
In nature, plants confront numerous stress factors (drought, extreme temperatures, heavy metals, salinity, limited availability of soil nutrients) individually or in combination, factors which can have a negative impact on plant growth and development, productivity, and soil ecosystem (Bera et al., 2022; Teshome et al., 2020).
In recent decades, drought (water deficit) has represented a major setback for the global agricultural economy, causing losses worth hundreds of millions of dollars each year and substantial reductions in global agricultural production (Zhang et al., 2022). Additionally, drought results in the loss of diversity in rhizospheric microbial flora, soil fertility, and nutrient availability, with negative consequences on the functional traits (morphological, physiological, and biochemical) of the plant (Ali et al., 2022).
Therefore, it is essential to find and develop alternative methods to mitigation drought, improve soil fertility, and retain water in the soil, methods which can contribute to enhancing plant performance under extreme environmental conditions.
Lately, researchers focused on various approaches and methods of enhancement to increase drought resistance in different crops (Qaim, 2020). However, this requires time and is a long-term approach. For instance, employing biotechnological methods to develop drought-resistant and tolerant varieties in cereals and vegetables has made significant contributions to improving drought resistance traits in both model and crop plants. Nevertheless, their accessibility to farmers has been limited due to high costs, complexity,

ethical considerations, and concerns regarding toxicity.

An innovative technique increasingly studied and implemented in sustainable agricultural systems, is the use of beneficial microorganisms (rhizobacteria, symbiotic bacteria, mycorrhizal and non-mycorrhizal fungi) that promote and enhance plants' tolerance mechanisms to adverse environmental conditions (Tripathi et al., 2021; Poudel et al., 2021; Hanaka et al., 2021; Poveda, 2020; Guler et al., 2016; Mastouri et al., 2012; 2010; Bae et al., 2009), stimulating plant growth, development, and resistance to abiotic factors. This approach is novel and efficient for enhancing plant tolerance to climate change and can play a significant role in the development of sustainable agricultural systems (Rajesh et al., 2016).

Among these groups of microorganisms, *Trichoderma* spp. is a rhizospheric fungus of significant agricultural and environmental importance, capable of conferring numerous beneficial effects through secondary metabolites (xylanases, peptaibols, epipoly-thiodioxopiperazines, terpenes, pyrones, siderophores, etc.) in promote plant growth and nutrition (Harman et al., 2004), induction systemic resistance to abiotic and biotic factors (Kashyap et al., 2017).

Species of *Trichoderma* spp. are fungi found in soil ecosystems and rhizosphere (Harman et al., 2004). They are avirulent and form symbiotic relationships with plants, colonizing their roots and leading to significant alterations in metabolism by modifying hormone content, soluble sugars, phenolic compounds, and amino acids, photosynthetic intensity, transpiration rate, and water content (Brotman et al., 2013).

Based on these arguments, the present study evaluated current knowledge regarding the use of various *Trichoderma* species to mitigate the negative effects of drought on the nutrition, physiological, and morphological parameters of different crop and horticultural plants, as well as soil fertility improvement.

THE USE OF BENEFICIAL MICROBIAL AGENTS TO ALLEVIATE THE EFFECTS OF DROUGHT

Recently, researchers have focused their activity on identifying, using, and applying beneficial microbial agents to improve the sustainability of agricultural production, increase crop nutrition efficiency and productivity, and new evidence suggests that these agents help plants cope with a variety of stress factors, including drought, high temperatures, salinity (Rubin et al., 2017; Colla et al., 2015).

Beneficial microorganisms play a crucial role in mitigating drought stress by modulating plants enzymatic and non-enzymatic antioxidant systems and by producing compounds such as phytohormones (indole 3-acetic acid, cytokinins, gibberellins, ethylene, ABA, salicylic acid, jasmonic acid), osmolytes, which represent the primary mechanisms through which they alleviate the effects of water stress and improve plant growth characteristics under drought conditions (Ullah et al., 2019). During drought stress, plants generate excessive amounts of reactive oxygen species (ROS), which cause oxidative damage to cells, such as membrane injury, increased lipid peroxidation, protein degradation, and ultimately cell death. Plants, on the other hand, defend themselves by activating antioxidant systems (both enzymatic and non-enzymatic) to protect against damage and maintain normal cellular function under drought stress.

In general, water deficiency reduces nutrient absorption through roots and transport from roots to stems. However, the use of certain microorganisms can aid in nutrient uptake from the soil even under water restrictions (Khoshmanzar et al., 2020). Mastouri et al. (2010) speculated that since the interaction between plant and *Trichoderma* mostly occurs in the rhizosphere, such a mechanism is likely linked to an increase in water absorption efficiency due to enhanced root capabilities, thus increased water uptake. Nevertheless, it has been demonstrated that *Trichoderma* spp. enhances plant drought tolerance even from the germination phase.

Inoculation of sugar cane plants with *Trichoderma asperellum* showed higher absorption of nitrogen and sulfur in both unstressed and drought-stressed plants (Scudeletti et al., 2021). Increased absorption of nitrogen and sulfur can occur directly through the production of hydrolases (proteases and chitinases), which are responsible for degrading rhizospheric proteins and chitin (Khoshmanzar et al., 2020), as well as indirectly assisting in the provision of simple compounds for degradation by heterotrophic nitrogen-fixing microorganisms. Multiple pieces of evidence have shown that *Trichoderma* spp. species are directly involved in nitrogen metabolism, mediated by the activation of nitrate reductase in plants (Sherameti et al., 2005).

Trichoderma spp. can enhance plant drought tolerance by activating the antioxidant system against dehydration-induced damage (Guler et al., 2016; Brotman et al., 2013; Mastouri et al., 2012), solubilizing minerals (including solubilization through acidification, redox, chelation, and hydrolysis), and delaying drought-induced changes by promoting stomatal opening, increasing leaf chlorophyll content, and enhancing photosynthesis.

Another valuable and interesting characteristic of plant root colonization by *Trichoderma* spp. strains is the improvement of root development (Contreras-Cornejo et al., 2009), proliferation of secondary roots, accompanied by alterations in root architecture and increased pH, providing better water and nutrient absorption and increase in seedling fresh weight and leaves surface (Pelagio-Flores et al., 2017).

It has been reported that crop productivity in the field increased by up to 30% after the addition of strains of *Trichoderma hamatum* or *Trichoderma koningii*. Numerous reports have indicated that *Trichoderma* stimulates plant growth in radishes, cucumbers, peppers, lettuce, and tomatoes (Contreras-Cornejo et al., 2016; Brotman et al., 2013; Bae et al., 2009).

Recently, the positive effects of using *Trichoderma* strains to mitigate drought stress effects on tomatoes has been described in published studies (Rawal et al., 2022; Cornejo-Ríos et al., 2021; Mona et al., 2017).

Furthermore, plant colonization by *Trichoderma* has stimulated the antioxidant mechanism (Mastouri et al., 2012), thereby reducing plant sensitivity to stress and/or delaying stress-induced changes in gene expression. Increased tolerance to abiotic stress in several plant species after inoculation with *Trichoderma,* has also been associated with increased osmolyte production (Zhang et al., 2019; Mona et al., 2017).

Scudeletti et al. (2021) results have shown that inoculating sugar cane plants with *Trichoderma asperellum* improves not only agronomic and nutritional parameters but also physiological metabolism and plant production under drought conditions. Additionally, numerous other studies have supported that *Trichoderma* spp. can increase tomato production and quality (Khoshmanzar et al., 2020), wheat (Shukla et al., 2015), rice (Shukla et al., 2012) under drought conditions.

Trichoderma spp. species are widely recognized as producers of phytohormones (e.g., auxin) and hormone-like compounds called harzianolide and svolenine, substances that, together with auxin, aid in cell wall extension (De Sousa et al., 2020). These compounds not only enhance stem weight but also, promote root and lateral root development (De Sousa et al., 2020), which are involved in water and nutrient absorption.

On the other hand, it has been found that IAA-producing bacteria (*Bacillus*) mediate drought tolerance through various mechanisms, such as increasing water permeability, water absorption, ROS scavenging, improving root architecture, as well as inducing a large number of stress-related genes (Etesami and Maheshwari, 2018).

Corn plants inoculated with beneficial microorganisms under drought conditions showed greater plant biomass, soluble substances, sugars, and higher leaf water content and osmotic potential compared to uninoculated corn plants (Sandhya et al., 2010). A subsequent study also demonstrated similar findings in corn plants after inoculation with drought-tolerant bacterial strains such as *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Paenibacillus favisporus* during drought stress (Sandhya et al., 2011). For example, cucumber plants inoculated with microbial strains of *B. subtilis* SM21, *B. cereus* AR156, and *Serratia* sp. XY21 increased leaf proline content three to four times, protecting them from dehydration during drought (Wang et al., 2012).

Thus, the application of microbial consortia significantly improves plant drought tolerance by modulating their antioxidant system. The use of microbial consortia is a long-term solution, a cost-effective strategy, and environmentally beneficial as it boosts plant immunity, assimilates nutrients, and helps crops tolerate abiotic stress factors.

Specialized literature describes several successful research studies on the use of biopreparations based on *Trichoderma* spp. formulations for managing abiotic stress. Both solid and liquid formulations are utilized to produce active and viable inoculants using conidia of *Trichoderma* strains, which are more tolerant to unfavorable environmental conditions during the application of the formulated product in the field (Aamir et al., 2020).

Applying these formulations in the early stages of crop growth provides maximum benefits regarding root development and nutrient absorption. However, plant response to treatment with *Trichoderma* spp. has varied depending on the crop, variety, application method (i.e., seeds, roots, and soil), inoculum concentration, formulation type, soil and environmental conditions, etc. (López-Bucio et al., 2015).

THE PROTECTIVE MECHANISMS USED BY MICROORGANISMS UNDER DROUGHT CONDITIONS

Beneficial microorganisms with specific characteristics in agricultural ecosystems can increase drought resistance through various mechanisms that improve water circulation in the soil and its absorption by the plant (Chepsergon et al., 2014).

Vilela et al. (2017), demonstrate that in drought-stressed plants, reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and hydroxyl radicals can increase to toxic concentrations, which can significantly affect cell membranes and DNA, deactivate antioxidant enzymes, and lead to lipid peroxidation, resulting in morphological, physiological, and metabolic changes in plants.

Some researchers, highlighted the existence of multiple pathways in plants that transform toxic levels of ROS into a less toxic form. Thus, *Trichoderma* strains stimulate the activation of these pathways by enhancing the expression of genes encoding the component enzymes, increasing the capacity for ROS removal (Mastouri et al., 2010). For example, if metabolic pathways in chloroplasts are improved, it is expected that photosynthetic efficiency will increase by reducing damage caused by superoxide anion and other reactive species involved in photosynthesis.

Trichoderma spp. colonizes the roots and remains limited to the cortex and outer layers of the host plant root epidermis, modulating gene expression both in roots and stems. Roots inoculated with *Trichoderma* have shown increased levels of antioxidant enzymes, primarily superoxide dismutase (SOD), as well as elevated levels of peroxidase (POD), glutathione reductase, glutathione-S-transferase (GST), and other detoxifying enzymes in leaves. SOD represents the first line of cellular defense against abiotic stress generated by ROS, being the primary scavenger of superoxide radicals, which converts the toxic superoxide (O_2^-) into hydrogen peroxide and oxygen through a process called dismutation reaction.

Arora et al. (2002) explained that the enzymes catalase and peroxidase are capable of converting toxic H_2O_2 into water and oxygen under water stress conditions. However, increased SOD activity alone cannot protect plants from the toxic effects of free oxygen radicals, hence CAT and POD are necessary to eliminate the toxicity of H_2O_2.

Gusain et al. (2014), observed increased drought tolerance in rice plants due to the application of *Trichoderma harzianum* T35 and highlighted that *T. harzianum* stimulated the activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), thus preventing oxidative damage in rice by rapidly removing reactive oxygen species (ROS). Mastouri et al., (2012), observed increased tolerance of tomatoes to water stress induced by the application of *T. harzianum* T22, which was attributed to its ability to eliminate harmful reactive oxygen species, accompanied by an increase in antioxidant enzyme activity.

It has been reported that crop productivity in the field increased by 30% after the addition of *T. hamatum* or *T. koningii* due to their production of cytokinin-like molecules (e.g., zeatin), indole- 3- acetic acid a precursor of auxin, and gibberellin GA3, or related molecules. The production and controlled application of these compounds could be useful for enhancing the biological fertility of crops (Tucci et al., 2011).

In a previous experiment conducted by Björkman Bjorkman (1998), showed that maize seeds subjected to oxidative stress had significantly reduced vigor; however, subsequent treatment with *Trichoderma -T22* restored vigor. *Trichoderma* spp. helps plants withstand stress caused by drought and high temperatures by activating the antioxidant system and enhancing defense signaling pathways in roots and stems, thereby strengthening plant growth (López-Bucio et al., 2015; Contreras-Cornejo et al., 2009). Additionally, plants colonized by *Trichoderma* secrete various compounds (such as auxins, gibberellins, enzymes, antioxidants, soluble substances, and compounds like phytoalexins and phenols) that provide tolerance to abiotic stress and enhance root system branching capacity (López-Bucio et al., 2015; Brotman et al., 2013).

Trichoderma strains applied to plants under water stress, improve the content of antioxidant pigments and proline (Mona et al., 2017; Mastouri et al., 2010), with positive effects also observed on seed germination and seedling vigor, inducing physiological protection against oxidative damage.

Numerous studies have demonstrated that *Trichoderma* spp. can enhance plant drought tolerance by stimulating root development, promoting secondary root proliferation, increasing root density, and altering root system architecture, while also activating antioxidant protection against dehydration-induced damage (Guler et al., 2016; Brotman et al., 2013; Mastouri et al., 2012; Contreras-Cornejo et al., 2009).

THE USE OF BENEFICIAL MICROBIAL AGENTS AS BIOFERTILIZERS

Although significant results have been achieved through the use of drought-resistant and heat-tolerant varieties (Fullana-Pericas et al., 2019), the use of biofertilizers has garnered increased attention due to their capacity to sustainably increase crop production and quality when applied both to the soil and to vegetation (Itelima et al., 2018).

A proper management of beneficial microorganisms in agriculture to allow for increased assimilation of minerals from the soil without production losses will lead to a more sustainable production system. Optimal growth and development of plants require nutrients in the soil to be available and in balanced quantities (Chen, 2006). Soil fertility can be restored by applying the concept of integrated soil fertility management based on managing soil nutrients through the conservation of natural resources and increased input efficiency.

Biofertilizers (microbial inoculants) are important components of integrated soil management, playing a key role in regulating soil nutrient activity through enzymatic activity followed by nutrient dynamics in the rhizosphere. This helps maintain soil structure and fertility, making it an environmentally friendly measure with low costs and a renewable source of nutrients for plants, successfully replacing chemical fertilizers in sustainable agriculture systems (Mahanty et al., 2017).

Biofertilizers are products containing living or latent cells of various beneficial microorganisms (bacteria or fungi) that, when applied to seeds, plants, soil, or foliage, colonize the rhizosphere, soil, or interior of the plant. They stimulate plant growth and development by transforming nitrogen and phosphorus nutritional elements into assimilable forms through biological processes such as phosphate solubilization (Verma and Pandey, 2022; Simarmata et al., 2015; Colla et al., 2015; Chen, 2006) and the production of bioactive compounds capable of inducing various plant responses leading to the development of induced systemic tolerance conditions, making the plant more resistant to adverse environmental conditions (Figure 1).

The use of biofertilizers for plants containing beneficial microorganisms (*Trichoderma* spp., *Bacillus* spp., and rhizobacteria) increases the accessibility and efficiency of nutrient absorption, maintains soil moisture and soil health, enhances crop production, and can be considered a sustainable and environmentally friendly approach for production stability through minimal input usage (Colla et al., 2015).

The specialized literature provides numerous pieces of evidence that the application of biofertilizers offers numerous benefits to the soil-plant system, including the improvement and stabilization of soil physical functions and properties, enhancing the soil's capacity to sequester carbon, and long-term productivity enhancement (Figure 1). Numerous researchers have experimented with the beneficial effects of applying *Trichoderma* in soil fertilization for various vegetable and cereal varieties. *Trichoderma* and its secondary metabolites enhance the absorption and efficiency of macronutrients, such as nitrogen (Fiorentino et al., 2018) or phosphorus (Garcia-Lopez et al., 2018), and oligo/micronutrients, such as iron, zinc, copper, and manganese (De Santiago et al., 2011).

Trichoderma spp. plays a vital role in the soil nutrient cycle through their mobilization and absorption. Numerous studies have indicated that *T. harzianum* can solubilize a range of nutrients for plants (Khan et al., 2016; Saravanakumar et al., 2013; Altomare et al., 1999). The colonization of cucumber roots by *T. asperellum* increases the availability of P and Fe through a significant increase in plant biomass (Yedidia et al., 2001). Seed treatment with *Trichoderma* spp. can reduce nitrogen requirements by 30–50% in various crops (Shoresh and Harman 2008).

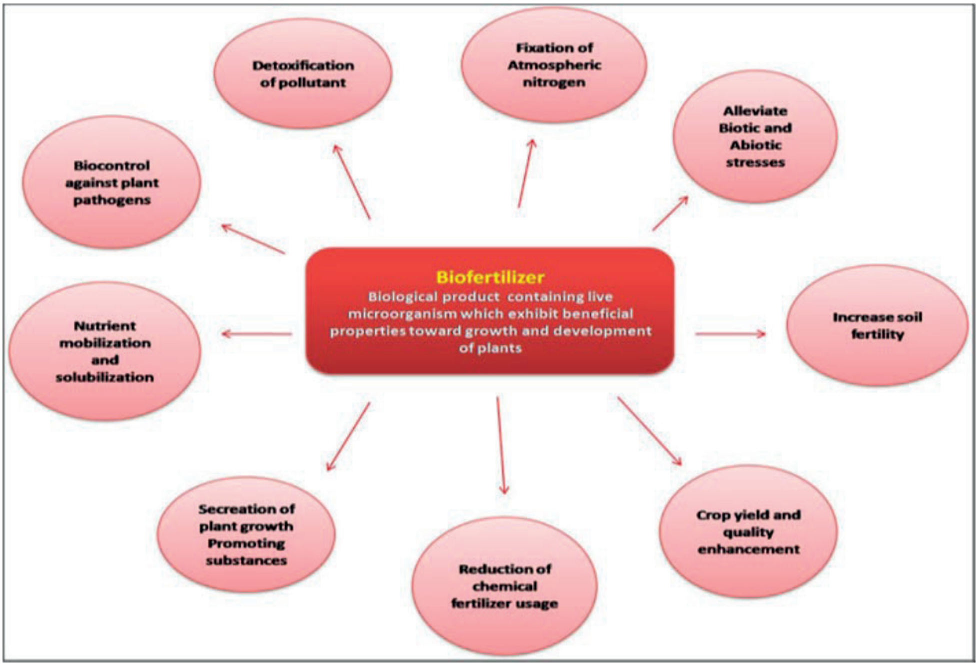

Figure 1. A summary on mechanism of biofertilizer and its importance in agriculture (according to Verma and Pandey, 2022)

The application of the *T. harzianum* (Th 37) formulation (20 kg ha^{-1}) in sugar cane increased the availability of primary nutrients N, P, and K by 27, 65, and 44%, respectively (Singh et al., 2010). The role of *Trichoderma* spp. in solubilizing tricalcium phosphate and other phosphates has been well investigated, with results indicating increased phosphorus availability for plants (Saravanakumar et al., 2013). The application of *T. harzianum* in combination with other beneficial microbial agents has led to higher contents of N, P_2O_5, K_2O, Fe, and Mg in chickpea leaves (Mohammadi et al., 2010). Yadav et al. (2009), demonstrated that *T. viride* has great potential to restore soil fertility and stimulate the growth of sugar cane plants.

Biofertilizers enriched with *Trichoderma* spp. have intensified plant growth in tomatoes, assimilatory pigments in leaves, and mineral content (P, K, Ca, Mg, Cu, Fe, Mn, and Zn) in tomato roots, increasing production by 12.9% compared to recommended NPK doses. Taken together, these reports suggest that *Trichoderma* spp. enriched bioproducts can reduce the application of chemical fertilizers and, therefore, can be considered an important practice in sustainable agriculture.

Trichoderma spp. has attracted special attention because it mineralizes organic nutrients by producing large quantities of extracellular enzymes that allow them to use plant residues as nutritional material (Contreras-Cornejo et al., 2009). This may be due to their abundance in soil under diverse climatic conditions, their ability to degrade a variety of organic substrates in soil, metabolic versatility, and competitive saprophytic capacity. Thus, *Trichoderma* spp. represents one of the viable options of modern crop cultivation technologies, where considerable emphasis is placed on environmental impact. The low cost and ease of obtaining *Trichoderma*-based biofertilizers are of particular importance in producing stress-resistant and high-quality agricultural crops in the current context of global climate fluctuations and population explosion.

CONCLUSIONS

Fungal strains from the genus *Trichoderma* induce drought tolerance, and their abundance increases under water-limiting conditions.

Reduction of oxidative stress by increasing the activity of antioxidant enzymes and attenuating reactive oxygen species in plants under water stress has been reported as one of the mechanisms of action of the *Trichoderma* fungus. Another mechanism includes the production of phytohormone analog metabolites that enhance plant growth and development in the presence of water stress. Increased water absorption efficiency due to modifications in root density, length, and number of secondary roots has also been proposed as another mechanism used by this fungus.

Microbial biofertilizers stimulate plant growth and nutrition by increasing the availability or absorption of nutrients, through the action of phytohormones, or by decomposing organic residues.Microbial biofertilizers can replace or reduce the use of chemical fertilizers and, therefore, can be considered an important practice in sustainable agriculture, preventing environmental pollution.

ACKNOWLEDGEMENTS

This work was performed within the ADER 6.3.1./2023-2026 research project funded by the Ministry of Agriculture and Rural Development.

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