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Biosolubilization of Different Nutrients by *Trichoderma* spp. and their Mechanisms Involved: A Review

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Abstract
Trichoderma is an effective biocontrol agent. It uses different mechanisms to manage and control the spread of a disease by a pathogen. This property of Trichoderma has been long known. Recently, there have been investigations into the mineral solubilising ability of the bioagent and it has been shown to solubilise minerals like phosphorus, iron, copper, manganese, zinc etc. which are essential for plant growth and development. As it is known, these elements are abundant in the soil but are mainly present in forms that cannot be taken up by the plants. Hence activities to increase the availability of these minerals are essential for the uptake by the plants, which is possible through the activity of soil microorganisms and plant roots in the rhizosphere. Here, we have made an attempt to summarise the mechanisms, that Trichoderma spp. use in order to solubilize these minerals and hence increase their bioavailability.

Keywords: Biosolubilization, *Trichoderma* spp., Mineral nutrients, Mechanisms of solubilisation

Introduction
Trichoderma is a very effective biological control agent for the management of plant diseases, especially the soil borne diseases. It is free living and is a common fungus in the soil and the root ecosystem. It is highly interactive in these environments and also in the foliar environment. Trichoderma uses different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretions for reducing the growth and survival of plant pathogens (Kumar et al., 2017). The antagonistic fungus, Trichoderma inhibitis the pathogen growth by coiling around its hyphae and producing volatile substances and cell wall degradation enzymes like glucanases, chitinases, proteases etc. (Al Naemi et al., 2016). Examples of such interactions are *T. harzianum* acting against *Fusarium oxyporum*, *F. roseum*, *F. solani*, *Phytophthora colocaciae* and *Sclerotium rolfsii* (KüçüK and Kivanç., 2004). The biocontrol ability of different Trichoderma spp. has been effectively exploited and applied in agriculture, as a management method against various plant diseases. There are different formulations of *Trichoderma* like Ecofit (T. viridae), Plant biocontrol agent -1 (*T. harzianum*), Ecoderma (*T. viridae*+ *T. harzianum*) etc. available in the market for the same (Ghazanfar et al., 2018).
Apart from this property of the fungus, direct effects on plants such as increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage rate of seed germination and stimulation of plant defences against biotic and abiotic damage has been shown by some rhizosphere competent strains of Trichoderma (Shoresh et al., 2010). Some of these properties of the fungus have been studied, and the mechanisms involved behind them, have been deciphered. One of such properties of Trichoderma apart from the biocontrol activity, that has attracted attention is the mineral solubilising activity of the bioagent.
The circulation of nutrients in soil are affected, because many nutrients are found in sparingly soluble or insoluble state in the soil (Halifu et al., 2019). Sixteen nutrients are known to be essential for plant growth and development.

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Among the nutrients except Carbon, hydrogen and oxygen the remaining nutrients like nitrogen, phosphorus, potassium, manganese, zinc, copper, iron etc. are absorbed from soil solution as inorganic ions or oxides by growing plant roots (Fageria and Baligar, 2005). Many of these nutrients are fixed in the soil and arenot available for plant growth. In order to convert these unavailable forms of nutrients into available forms, the activity of soil microflora and microfauna is essential where they use different mechanisms to increase the bioavailability of the nutrients, which may include production of organic acids, enzymes, chelating agents, siderophore production etc. (Rashid et al., 2016). Different strains of the biocontrol agent Trichoderma also have the ability to solubilise these minerals and thus make them available to the plants. The mechanisms involved behind the solubilisation of different minerals by various strains of *Trichoderma* have been studied. This review paper deals with the ability of such *Trichoderma* strains to solubilise certain minerals and the mechanisms, the biocontrol agent uses for the process.

Trichoderma spp. as a mineral solubilizer

Though large amount of fertilizers are used in modern agriculture, nutrient deficiencies pose significant agronomic problems (de Santiago et al., 2013). For example, most of the Phosphorus (P) in soil is fixed in the form of insoluble phoshphates, hence this element becomes a major growth-limiting nutrient, despite the large quantities of P found in soil (Fernández et al., 2007). Elements like Iron (Fe), Manganese (Mn), Copper (Cu) and Zinc (Zn), which are involved in a number of physiological processes in the plants are also not always active in the soil (Li et al., 2015). The deficiencies of Fe, Mn, Cu and Zn have a great impact on the yield and quality of agricultural produce (Li et al., 2015). Thus, methods that increase the bioavailability and uptake of these important nutrients are necessary and of particular scientific interest. The availability of micronutrients at the plant root surface is mediated to a large extent by the biological activities of microorganisms like that of *Trichoderma* spp. (Marschner H, 1995).

The different research works conducted to investigate the increase in mineral availability due to the activity of *Trichoderma* spp. till now, show that the different strains of species like *T. harzianum*, *T. viridae* and *T. koningiopsis*, are able to solubilize minerals namely: Phosphorus and Potassium among the macronutrients and Zinc, Manganese, Copper, Iron etc. among the micronutrients. The process of solubilization by the microorganism is done through different mechanisms, depending upon the source of the mineral and the ability of the strain to solubilize them.

Soubilization of Phosphorus:

In most natural soils, Phosphorus (P) is fixed as insoluble iron and aluminum phosphates in acidic soils with pH lower than 5.0 or as calcium phosphates in alkaline soils with pH above 7.0 (Altomare et al., 1999). These unavailable forms of phosphorus like calcium phosphate is made available to plants by the activity of the soil and rhizosphere microorganisms through a mechanism that is thought to involve the release of organic acids which dissolves these insoluble forms (Cunningham and Kuiack., 1992; Goldstein, 1995). Different Trichoderma strains have also shown to produce organic acids for the process of Phosphorus solubilisation, though it is not the only mechanism found behind the process.
Eg: The first report of *Trichoderma koningiopsis* from India, is the strain NBRI-PR5 which has been bio prospected for different PGPR attributes including Phoshphorus solubilization. The process of Phosphorus solubilization by the strain is mediated by the production of organic acids. The strain is shown to produce acids like acetic acid, malic acid etc. (Tandon et al., 2020). Due to the production of these organic acids, by the strain, there’s a lowering of pH of the surroundings, where the Trichoderma application is done. This enhances the activity of acid phosphatase in the soil, thus providing another mechanism by which the inorganic source of phosphorus i.e. Tricalcium phosphate could besolubilised (Tandon et al., 2020).
In strains like *T. harzianum* SQR T037, a different mechanism for the solubilisation of organic source of phosphorus is seen. Phytate is an organic source of phosphorus found in soil and accounts for almost 70% of the soil phosphorus. Phytase is an enzyme that is responsible for the solubilisation of phytate and is shown to be produced by SQR T037 (Li et al., 2015). Hence, this strain mediates phosphorus availability through the action of phytase in

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soil. It competes with the plant roots for phytate and when the mycelium degrades with age, phosphorus is made available to plants (Li et al., 2015).

Some other strains like *T. harzianum* T-22, THB 10, and *T. viridae* TV97 have also shown to solubilise inorganic phosphorus in soil (Altomare et al., 1999 and Rudresh et al., 2005). The studies conducted on these strains, clearly indicate that mere production of organic acid, need not be the only mechanism behind the solubilisation of the element. Rather, it is a process involving a combination of different mechanisms, ranging from organic acid production to enzyme secretion.

Solubilization of the micro elements namely Manganese (Mn), Zinc (Zn), Copper (Cu) and Iron (Fe)

Manganese deficiency is visible in many soils. Some soils may contain upto 3000ppm of manganese, yet most of the element is unavailable for plant use. The manganese present in rocks and minerals are only available to the plants, when these sources undergo weathering. Manganes present in available forms in soils occur as exchangeable manganese, manganese oxides and in association with organic matter.(Schulte and Kelling., 1999). The *T. harzianum* strain T-22solubilises Mn by reducing Mn+4 to Mn+2 through the production of reducing metabolites (Altomare et al., 1999). Whereas, in case of *T. harzianum* strain T1, solubilisation of Mn+4, takes place either by reduction or chelation activity (Küçük et al., 2008).In contrast to these mechanisms involved, the strains like SQR T037 are not at all able to solubilise MnO2(Li et al., 2015).

Iron is the fourth most abundant element found on earth though it is largely present in forms that can't be taken up by the plants. Nearly 1-5% of soil consists of iron, mostly in the unavailable form. Plants uptake iron in the form of Fe2+ions. (Schulte., 1999a).The divalent form of copper is mostly found in the soils. But most of the copper in soil is unavailable to the plants because a major portion of the element is found in the crystal lattices of primary and secondary minerals and also bound to the organic matter in soil (Mengel et al., 2001). Plants are able to uptake copper available in Cu2+ form. The role of microorganisms in the uptake of these elements is essential. Eg: The plants and microorganisms release organic molecules and metabolites, that are able to form complexes with Fe(III) or can reduce this element. The iron present in minerals is solubilised by the siderophores released by the microbes and the plant exudates likephytosiderophores, organic acids and flavonoids(Colombo et al., 2013). As it is seen these elements have to be either reduced or incorporated into complexes that can be taken up by the plants and it is shown that some of the Trichodermal strains are able to facilitate the mechanism.

The mechanisms involved behind the solubilisation of copper and iron by different strains of *Trichoderma* is almost similar. The mechanisms may involve reducing Cu(II) and Fe(III) into absorbable forms by the plants or involves the production of chelating molecules or siderophores that may form a complex with the mineral ions in soils and these complexes are in turn taken up by the plants. Eg; The strain T-22 produces diffusible metabolites capable of reducing Fe(III) and Cu(II) (Altomare et al., 1999). The diffusible metabolites are found to be different for both the elements and are heat stable and non-proteinaceous(Küçük et al., 2008). Similar to the production of reducing metabolites, synthesis of chelating substances appears to be a constitutive trait of T-22. Compounds in T-22 culture filtrates are able to chelate iron (Altomare et al.,1999). Therefore, chelation accounts at least partially for solubilization of this nutrient.

The strain SQR T037 is shown to solubilise Fe2O3and CuO. The solubilisation of Fe2O3involves multiple mechanisms like chelation, reduction of Fe(III) and acidification. Ferric reductase activity is also seen in the culture filtrates of the strain. The strain is also shown to produce siderophores(Li et al., 2015). A combination of these different mechanisms is the possible reason behind the solubilisation of iron and copper by the strain.

A major portion of zinc found in soil is in unavailable form. Soils contain upto 2-25 ppm of exchangeable and organic zinc. But, a large portion of the element is present as iron and manganese oxides and other non-available forms (Schulte., 1999b). Different strains of Trichoderma have exhibited their ability to solubilise metallic zinc, through a no. of mechanisms like:

a. In the strain T-22, although the reducing activity found in culture filtrates accounts, at least partially, for solubilization of metal oxides, solubilization of metallic zinc occurs by a different process. T-22 proved to have the ability in culture to accelerate the oxidative dissolution of metallic zinc, releasing Zn2+. The effect

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of the fungus includes the release of complexing ligands which sequester $Zn2+$, thereby increasing the dissolution of metallic zinc in the culture medium (Altomare et al., 1999).

b. In The strain SQR T037, the mechanism involved was a different one. It solubilised zinc through the production of organic acid by the strain. Four organic acids are found in the culture filtrates of SQR T037, namely: Lactic acid, Citric acid, tartaric acid, and succinic acid (Li et al., 2015)
c. Another strain that is shown to solubilise zinc is the *T. harzianum* strain T1. T1 is proved to have the ability in culture to accelerate the oxidative dissolution of metallic zinc to $Zn+2$(Küçük et al., 2008).

Conclusion

Minerals are very essential for plant growth and development. Deficiencies of these minerals, in the plants leads to the development of various symptoms, which are a result of hinderance to the essential functions in plants, posed due to the lack of one or a few of the essential minerals. As seen in majority of the cases, most of the minerals, are naturally present in the soil, but only a very few portion of these minerals are available for plant uptake and nutrition. These minerals that are present, but not available to the plants have to be converted into forms, which can be utilized by the plants. This process is facilitated by the activity of soil microorganisms which alter the rhizospheric soil environment and in turn lead to solubilisation of some of the minerals.

A number of microorganisms have been identified for their ability to solubilise different elements in soil. Microbes like *Azospirillumbraziliense, Bacillus megaterium, Pseudomonas putida, Aspergillusniger, Penicillium* spp. etc. are known to solubilisedifferent sources of phosphorus(Corbett et al., 2017). For solubilisation of elements like potassium, bacteria such as *Bacillus mucilaginosus, B. edaphicus, Acidothiobacillusferooxidans, B. circulans* etc. have been identified (Etesami et al., 2017). Many more microorganisms are being tested for their abilities to solubilise fixed elements from soil. The exploitation of this activity of the microbes, provides us with an eco-friendly method of providing essential minerals to the plants.

Trichoderma, in this aspect can prove to be very beneficial. The biocontrol activity of this microorganism has been known very well for a certain period of time now. Its ability to solubilise a few minerals essential for plant growth is an area of study which is gaining interest. It has been shown to solubilise minerals like phosphorus, iron, copper, manganese, zinc etc. The ability of the biocontrol agent has been seen to vary with strain. It can't be said that all the species of *Trichoderma*, can solubilise a particular set of minerals equally. Even within the species, the property of solubilisation is shown to vary among the strains. Another factor that has shown to affect solubilisation of minerals, is the type of source of mineral that is involved. Also, it can't be said that all the *Trichoderma* strains, use the same mechanism for solubilisation of a particular element. For example: In the above cases, solubilisation of iron in some strains like T-22, may involve the production of reducing metabolites, at the same time in case of SQR T037, iron solubilisation involved a number of mechanism ranging from chelation to acidification of the media. For the solubilisation of phosphorus, the main mechanism that is found is the production of organic acids in most of the strains. The organic acids are known to chelate the cationic counterpart of phosphorus ions and and release inorganic phosphorus into the medium. But the mere production of organic acid cannot be said as the only mechanism behind Phosphorus solubilisation. This comes from the proof that the *Trichoderma viridae* strain TV97, produces a higher amount of organic acid than the *T. harzianum* strain THB10, but the latter proves to be an efficient Phosphorus solubiliser than the former (Rudresh et al., 2005).

Hence, given from the above situation we can say that solubilisation of mineral by *Trichoderma* spp. appears to be strain specific. From the above example we can also see that, amongst the various species of *Trichoderma, T. harzianum* proves to be a potential species for the solubilisation process. Various research works are also being carried out to see the potential of the Trichoderma spp. to solubilise other major minerals like potassium. Once, this ability of the fungus is exploited to the full extent, it may prove to be a potent biocontrol agent cum biofertilizer.

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