

Role of mycorrhiza and bacillus in solubilizing P from its sources and their impact on P content and dry mass of tomato grown in sandy soil

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Abstract

The aim was to investigate the effectiveness of mycorrhiza and bacillus in extracting P from its sources. Tomato plants were cultivated in sandy soils at three different sources of P (rock phosphate 27% P₂O₅, cattle bone 21% P₂O₅ and superphosphate 12.5% P₂O₅) until fruit maturity. All sources of P were added at rate 80 Kg P₂O₅ feddan⁻¹. On the other hand, root of tomato seedling, were inoculated by biofertilizers in rate Cont. = 0, BM= 5 ml, AM=5 g and (BM=2.5 ml+ AM=2.5 g), finally main and subplot were replicated three time. The result showed that P concentration and P uptake in fruits were obviously raised in inoculated plants by biofertilizer under CB and SP sources. Under RP source, AM only resulted in markedly increased P concentration and P uptake in tomato fruits. Inoculation cattle bone (CB) and superphosphate (SP) with bacillus or mycorrhiza led to improve dry mass of different plant organs particularly generative organ (fruit). Inoculation rock phosphate (RP) with mycorrhiza only increased dry mass of various plant organs particularly fruits.

In conclusion, the highest P concentration and uptake in tomato fruits as well as, the highest dry mass of tomato plants was obtained when P sources particularly (CB) were inoculated by only mycorrhiza (AM).

Key words: Cattle bone (CB), Superphosphate (SP), rock phosphate (RP), arbuscular mycorrhiza (AM), bacillus megaterium (BM)

1. Introduction

In the agricultural sector, phosphorus (P) is a crucial macronutrient needed to promote plant growth and development [1]. The application of phosphorus increases plant development, this improves soil fertility, preserves soil structure, lowers the risk of soil erosion, and stabilizes contaminated sites [2]. Natural rock phosphate (RP) stocks, the primary source of conventional P fertilizers, are projected to be 18 billion tones in size and may run out within the next 50 to 100 years given present economic and technological trends [3]. Alternatives that are less expensive and safe for the environment are being researched. Utilizing organic waste as a source of nutrients for plants, such as cattle bone (CB) [4-5], is one of the options [6-7]. Cattle bones are by-products of the meat industry that are obtained during the processing of raw animal resources, and they include head and skeleton bones from the abattoir [3]. However, these insoluble P fertilizers are suitable to fertilize sandy soil due to sandy soils are recognized for having a low capacity to retain nutrients [8]. thus, P leaching can happen in sandy soils and when P fertilizer is applied repeatedly in areas where soil P sorption is low [9].

Bacillus megaterium is known as a vital phosphate-solubilizing microorganisms, which are a promising alternative to produce fertilizers by biotechnological routes of RP solubilization [10]. Whereas, mycorrhizal plants are known to be more effective at absorbing nutrients from the soil, particularly phosphorus, which is why they are advantageous to tomato plants [11]. Mycorrhizal fungi can increase P bioavailability *in vitro* by reducing soil pH, resolving Pi, activating produced phosphatases, and increasing soil contact area through AM hyphae [12-13].

Tomato plant (*Solanum lycopersicum*) is regarded as the second most important vegetable crop after the potato [14]. Tomato is a crucial plant for human nutrition and one of the most commercially significant vegetables in the world [15]. According to Abu-Alrub *et al.* [16], the high production of tomatoes in intensive crops is also a result of the extensive nitrogen and phosphate fertilizer applications. In this case, solubilizing P from its sources has become an imperative that can be done by inoculating tomato roots with bacillus and mycorrhiza due to, the coinoculation between mycorrhiza and bacillus megaterium can have synergistic effects on plant growth, nutrient absorption [17-18]. So far, all studies confirm the role of bacillus and mycorrhiza in solubilizing P from its sources and increasing tomato yield, however, during this study, our hypotheses are: (I) Inoculating plants with mycorrhiza and bacillus improve phosphorus acquisition from its source. (II) Mycorrhiza and bacillus are microbial specific and dependent on P source.

2. Material and Methods

Field experiment was executed on sandy soil under drip irrigation system in a private farm. Seeds of tomato plants (*Solanum Lycopersicon cv. 010*) were germinated in the arboretum on 20 November, 2022, one months old seedlings were transplanted into the field. Field experiment was arranged in split plot design where, the main plot (first factor) was P source with a three source {rock phosphate (RP $P_2O_5 = 27\%$), cattle bone (CB $P_2O_5 = 20\%$) and conventional superphosphate (SP $P_2O_5 = 12.5\%$)}. The ground application of P sources was 80 Kg P_2O_5 feddan-1 in subplot (second factor) four inoculant-fertilizer treatments {without bio (Cont.), *Bacillus megaterium* (BM= 5 ml) and arbuscular mycorrhiza (AM=5 g) and combination both bacillus megaterium and arbuscular mycorrhiza (BM 2.5 ml +AM 2.5 g). All treatments were fertilized by recommended dose of nitrogen (N) and potassium (K); tomato fruit were harvested at two time before final harvest. Finally, plants were harvested 90 days after start of treatments, stem, leaves, and fruit parts were separated and dried at 70 °C for determination of dry mass and P was determined in fruit using atomic absorption -standard procedures outlined by Cotteine [19]. ***Data was statically analyzed in split plot design using R program***

3. Results

3.1. Concentration and uptake of P in plant:

Our expectation was that tomato plant accumulates P in their source organs (leaves) and particularly the main sink organ (fruits) because plants were harvested during maturity stage. Therefore, we determined P in leaves and fruits only.

3.1.1. Phosphorus in tomato leaves

Inoculant-fertilizer had no significant effect on P concentration in leaves. Meanwhile, P concentration in leaves were influenced significantly by kind of P sources. Where, RP and SP resulted in increased P concentration in leaves compared to CB. Concerning the interaction between phosphorus sources and inoculant-fertilizers, we find that the reason for the superiority of both rock phosphate (RP) and regular phosphate (SP) is due to the superiority of bacillus (BM) in RP and mycorrhiza (AM) in SP by increasing P concentration (1.8 mg g⁻¹ DM) in tomato leaves. The P concentration was in range 0.8 to 1.8 mg g⁻¹ DM. Leaves of plants grown

in uninoculated sources contained about 1 mg g⁻¹ DM (Fig. 1A). Uptake of P in tomato leaves was not significantly influenced by P sources, meanwhile; inoculant-fertilizer had significant effect on P uptake in leaves (Fig.2 A). Inoculation plant root by bacillus (BM) resulted in improved P acquisition in leaves, however, other treatments (AM or BM+AM) had no effect on P uptake in fruits (Fig. 2 A). This finding attributed to superior of BM when it was inoculated in rock phosphate (RP), where only P uptake of its leaves reached more than twice of control plant leaves, followed by the content of the leaves of growing plants on superphosphate (SP) and inoculated with mycorrhiza (AM). The highest values more than 60 mg plant⁻¹ leaves, while, most P uptake in leaves in range 20 to 25 mg plant⁻¹ leaves (Fig.2 A).

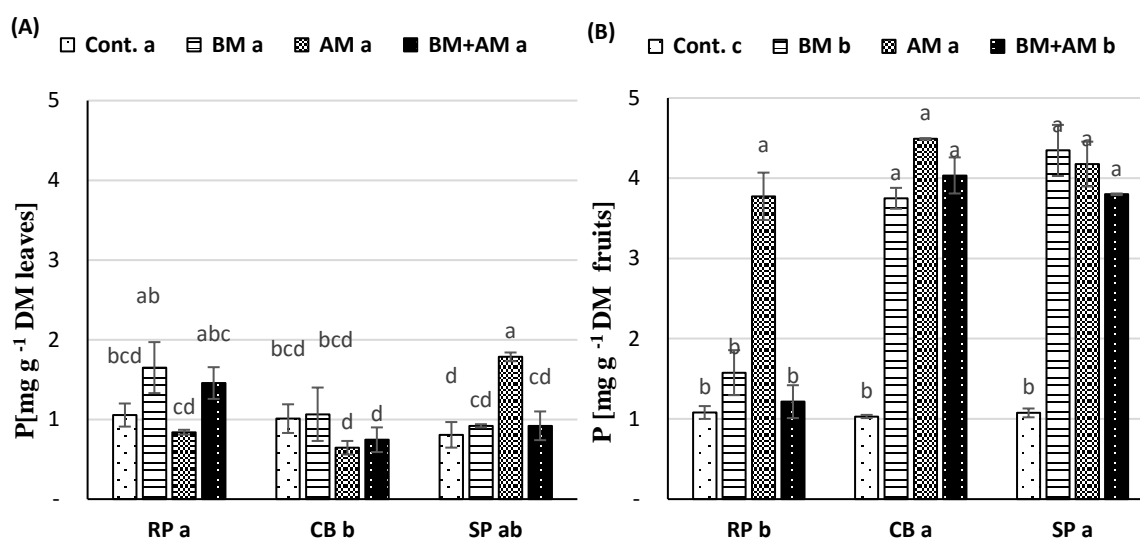


Fig. 1 Impact of P sources and inoculant-fertilizer and their combination on P concentration in leaves (A) and fruits (B) of tomato plant. Different letters refer to least significant differences among mean whether in main plot, subplot, or interaction among both. Vertical lines represent the standard errors of means (n=3)

3.1.2. Phosphorus in tomato fruit

Inoculant-fertilizer and P sources had significant impact on P uptake and P concentration in tomato fruits (Fig. 1 B, Fig. 2 B.). where, P concentration in tomato fruits was the highest (4 mg g⁻¹ DM fruits), when the plant was inoculated with AM only. The uninoculated plants had a very low concentration in their fruits (no more than one milligram per gram dry mass of fruits). While the fruits of plants injected with BM or mixture (BM+AM) ranged from 3 to 3.6 mg g⁻¹ DM fruits (Fig. 1B). The uptake of P in fruits gradually increased from 0.11, 0.52, 0.69 and 0.86 g plant fruits⁻¹ for the treatments Cont., (BM+AM), BM and AM respectively (Fig.2B). This increment in P in inoculated plants by AM reached 8 times as compared to P in control plants. These findings reveal that inoculant-fertilizers have crucial role in solubilizing P from various sources. In comparison to RP, CB and SP treatments were associated with higher concentration and uptake of P in tomato fruit. This result attributed to inoculated plant by BM or BM+AM were not able to acquire P from RP, in contrast, P concentration and uptake in fruits increased when these plants were injected by inoculant-fertilizer in other P sources (CB and SP).

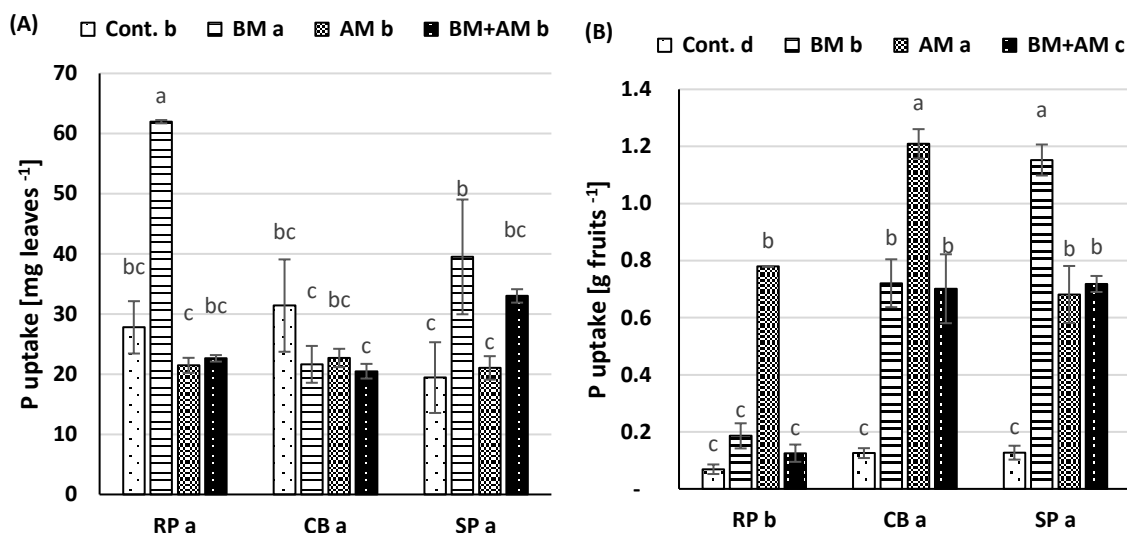


Fig. 2 Impact of P sources and inoculant-fertilizer and their combination on P uptake in leaves (A) and fruits (B) of tomato plant. Different letters refer to least significant differences among mean whether in main plot, subplot, or interaction among both. Vertical lines represent the standard errors of means (n=3)

Table 1. Effect P sources and Inoculant-fertilizer on dry mass of different plant organs. Different letters within a column indicate significant (Tukey-Kramer's test, $P < 0.05$) differences of organ mass among treatments

Treatment		Dry mass of different plant organs			
		Stem	Leaves	Fruits	Total
P sources	Inoculant-fertilizer	(g plant ⁻¹)			
RP	Cont.	11 ^f	20 ^{cd}	69 ^g	98 ^g
RP	BM	13 ^f	33 ^{abcd}	155 ^{cdef}	176 ^{ef}
RP	AM	25 ^{bcd}	35 ^{abc}	208 ^{bc}	268 ^b
RP	BM+AM	14 ^{ef}	17 ^d	130 ^{defg}	133 ^{fg}
CB	Cont.	12 ^f	26 ^{bcd}	97 ^{fg}	137 ^{fg}
CB	BM	21 ^{de}	29 ^{abcd}	182 ^{cd}	225 ^{cd}
CB	AM	34 ^a	40 ^{ab}	269 ^a	363 ^a
CB	BM+AM	28 ^{abc}	38 ^{abc}	174 ^{cd}	260 ^{bc}
SP	Cont.	25 ^{bcd}	28 ^{abcd}	105 ^{efg}	152 ^f
SP	BM	22 ^{cd}	46 ^a	256 ^{ab}	255 ^{bc}
SP	AM	27 ^{abcd}	29 ^{abcd}	162 ^{cde}	209 ^{de}
SP	BM+AM	31 ^{ab}	35 ^{abc}	213 ^{bc}	268 ^b

3.2. Dry mass in aerial plant organs

Sources of P and inoculant-fertilizers had significant effect on dry biomass of stems, leaves, fruits and total dry biomass (Table 1). In comparison to rock phosphate (RP), conventional superphosphate (SP) and cattle bone (CB) were associated with higher dry mass of stem, fruit and total plant, whereas inoculant-fertilizer treatments resulted in increased dry mass of stem, fruits and total plants compared to control treatment while dry mass of leaves was no significantly affected by investigated treatments (Table 1). Most leaves in maturity stage are susceptible to senescent and fall down so, the effect of P source and inoculant-fertilizer did

not appear on leaves, but appears on stems and fruits. Regarding the interaction effect between P sources and biofertilizer, plants inoculated and fertilized with superphosphate (SP) and especially cattle bones (CB), their dry mass in different organs was higher than that of their counterparts fertilized with rock phosphate (RP) regardless of the type of inoculant. Moreover, mycorrhiza-inoculated plants have the highest dry mass of various plant organs particularly under the cattle bone (CB) source.

4. Discussion

Main aims of this study were to assess (i) if inoculation by inoculant-fertilizers improve external phosphorus acquisition from its sources and is dependent on the type of P source? (ii) inoculant-fertilizer efficiency is specific depending on AM, BM, and their combination (AM+BM).

4.1. Phosphorus in plant (concentration and uptake):

For healthy growth and development, tomato plants need phosphorus, and a phosphorus deficiency can significantly lower crop yield. Precipitated P-containing minerals, which are described as minerals that contain P as a structural element are one type of inorganic P form [20]. In our investigation, three different P sources RP, CB, and SP were treated with inoculant-fertilizer (AM, BM, and both BM+AM). Due to P was determined in leaves and fruit at maturity stage, P concentration in fruit reached to 3-times P concentration in leaves, thus, most of P in leaves was remobilized to generative organs (fruits) Consequently, P concentration and uptake in fruit must be discussed.

The results demonstrated that AM, BM, or their combination (BM+AM) considerably improved tomato plants' nutritional status with regard to P in fruits. Unexpectedly, the concentration or uptake of phosphorus in fruits may be lower when inoculated with a mixture of mycorrhiza (AM) and bacillus (BM) under RP source (Fig. 1, B and Fig.2 B). while under CB and SP, BM, AM and their combinations had the same effect on P concentration and uptake in tomato fruit (Fig. 1, B and Fig.2B). Thus, combining AM and BM did not produce results that were distinguishable from individual inoculation with one.

Crop plants and arbuscular mycorrhizal (AM) fungus collaborate in a symbiotic relationship to aid in the nutrition of phosphorus [21]. However, AM are unable to extract P on their own from sources of P that are less accessible, like rock phosphates [13]. Contrary to what we obtained, AM inoculation is the only one that led to an increase in the P concentration and P content in tomato fruits (Fig. 1, B and Fig 2, B). Also, phosphates can be solubilized and released from organic and inorganic P compounds in nature by bacillus. Bacillus can improve plant growth and nutrient uptake when BM and AM in coinoculation [13]. In contrast, according to another studies, the specific combination of bacillus and mycorrhiza did not demonstrate an improvement in the extraction of P from P sources [21]. Then that is what occurred during our study, mixing BM and AM did not add anything valuable in terms of phosphorus concentration or growth and yield of tomato plants. Mycorrhiza and bacillus are both known to encourage plant growth and nutrient uptake. Also, bacillus can stimulate plant growth either through enhancement in acquisition of nutrients or through stimulation of other plant growth-promoting rhizobacteria [21].

Why the combination between mycorrhiza and bacillus reduces P acquisition from rock phosphate?

According to our results, coinoculation with BM and AM did not improve P concentration in fruits of inoculated plants under RP source compared to under CB or SP sources. This finding attributed to CB and SP sources might be contains available P compared to RP source because previous studies illustrated that the combination between mycorrhiza and bacillus may lead to synergistic effects only benefit plants when additional P was also supplied [22]. Consequently,

results showed that P concentrations available in the soil regulate P mobilization and immobilization to determine the bacterial P contribution to plants [23]. In general, when the available P level is low in soils, AM and BM compete for the P, and this competition is not stimulated by the fungi. With additional P supply, BM improved AM hyphal growth, and the BM activities were stimulated by the fungi [22-13]. Up to now, the interactions and synergistic effects between mycorrhiza and bacillus in the context of P extraction may require further study.

4.2. Biomass accumulated in different tomato organs

The effects of mycorrhizal and bacillus inoculation on tomato plant development and yield have been the subject of numerous studies. P-solubilizing bacteria and mycorrhizal fungi can be used to dramatically improve tomato plant growth and yield [24]. The obtained results from (Table 1) emphasise that dry mass of the various plant organs is directly proportional to their phosphorus content. As the inoculation of mycorrhiza and bacillus of the cattle bone (CB) and superphosphate (SP) led to an increase in dry mass for all plant organs, while the vaccination of rock phosphate provided positive results only when it was inoculated with mycorrhiza (AM) only. Whereas, the best results were obtained from inoculated plants with mycorrhiza (AM) which were fertilized by cattle bone (CB). Tomato plants can produce much more biomass when inoculated with mycorrhizal fungus [25-26]. Also, bacillus species can directly stimulate plant growth through enhancement in nutrient acquisition or through stimulation of plant growth [21], the findings imply that mycorrhiza and bacillus inoculation of tomato plants may enhance growth and yield.

This is what we have achieved by fertilizing with unavailable P sources and inoculating them with mycorrhiza and bacillus to ensure the availability of an appropriate amount of phosphorus throughout the growing season and avoid washing it from the soil profile in what is known as slow-release of fertilizers which were added to sandy soils which is characterized by low cation exchange capacity (CEC)

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