

# Effect of Some Bacteria and Iron Chelators on Potato Colonization by Arbuscular Mycorrhiza Fungi Inoculated by Rhizoctonia

Ali Baradar<sup>1\*</sup>, Roohallah Saberi-Riseh<sup>1</sup>, Ebrahim Sedaghati<sup>1</sup> and Abdolreza Akhgar<sup>2</sup>

<sup>1</sup>Department of Plant Protection, College of Agriculture, Vali-e-Asr University of Rafsanjan, Iran; eng.baradar@gmail.com, roohia2002@yahoo.com, sedaghati@vru.ac.ir

<sup>2</sup>Department of Soil Science, College of Agriculture, Vali-e-Asr University of Rafsanjan, Iran; arakhgar@yahoo.com

## Abstract

**Background/Objectives:** Arbuscular mycorrhiza is a symbiosis between plants and glomeromycota fungi. This relationship gives several important benefits to the plants including increasement tolerance against abiotic and biotic stresses. **Methods/Statistical Analysis:** In this study, the effects of Plant Growth Promoting Rhizobacterium (PGPR) and chemical chelating factors (EDTA, EDDHA) in the presence of Rhizoctonia solani AG3 were studied on mycorrhizal colonization on potato. To investigate the mycorrhizal colonization percentage, root staining was performed. The existence of hyphae, vesicle and arbuscule estimate as Arbuscular mycorrhiza colonization. **Findings:** The results revealed that interaction of chelating factor of iron (EDTA and EDDHA) mycorrhizal colonization and bacterial strains had positive effects on root colonization and in consequence leads to increase fresh and dry weight, other growth factors and chlorophyll. **Application/Improvements:** It is concluded that combinations of chelating agents (EDTA and EDDHA) may increase the growth and resistance to soil borne pathogens.

**Keywords:** Arbuscular Mycorrhiza, Chelators, Colonization, Potato, Rhizoctonia

## 1. Introduction

Arbuscular mycorrhiza is a obligate symbionts-and surviving of this fungi depend on a host. Colonization of plant roots can be induced by an AMF hypha. The specificity of interaction of the arbuscula rmycorrhizal with its host is very low<sup>1</sup>. Tolerant for soil pathogens was induced by AM fungi. Also results showed that disease symptoms of several fungi such as a *Gaeumannomyces*, *Fusarium*, *Chalara* (*Thielaviopsis*), *Rhizoctonia*, *Sclerotium* and *Verticillium*, oophyceous micro-organisms and nematodes reduced in the interaction of AM. It has been proposed that excreted materials from roots are important factors in the initial phase of infection in the soil pathogens. In this case, AM fungi controls pathogen population by reducing the available plant roots material<sup>2</sup>. AM fungi may also affect pathogens through

the infection sites. Results showed that arbuscules reduced the penetration of root cells by soils pathogens<sup>2</sup>. Arbuscular mycorrhizal fungi have great impact on surroundings microorganisms' community and changed the roots microflora. Excreted compounds of roots alternatively modified to other nutrition materials for other microbes by AM<sup>3</sup>. The rhizosphere of plants is colonised by a large number of microbes. Some of them are pathogens and some others such as nitrogen-fixing bacteria are beneficial for growth promoting. The effects of Mycorrhizal on rhizosphere bacteria depend to taxonomic positions<sup>3</sup>. The microbial community in the rhizosphere also influences germination, spore production and hyphal growth of AM fungi. Bacteria that are beneficial to mycorrhizal fungi are often called the as mycorrhiza (or mycorrhization) helper bacteria (MHB), or mycorrhization promoting rhizobacteria<sup>2,4</sup>. Plant growth

\*Author for correspondence

promoting rhizobacteria have stimulatory and inhibitory effects on mycorrhizal growth<sup>5</sup>. Surface sterilization of spores, often reduced germination frequency of the spores. Some bacterial strains also induced AMF spore germination rates<sup>3</sup>. However, the reduced germination may be due to use of sterilization materials that causes damage on mycorrhizal spores<sup>6</sup>. Rhizobacterial influences AMF colonization and growth of its hyphae. In certain soils, some AM fungi that fail to produce external communication materials. This is due to a high frequency of mycorrhizal inhibitory bacteria, or lack of beneficial bacteria. The effects of biocontrol antagonists on mycorrhizal development have been investigated in several studies. The overall results from them are that these organisms do not seem to be antagonise the AM fungi<sup>2,3</sup>. Colonization of potato by *R. Solani* and AM fungi was not found to be competitive manners; however, during the inoculation of plant roots, accumulation of phytoalexins increases the tolerance of plants<sup>7</sup>.

Studies have also found that most Iranian soils are alkaline in nature, because there are a lot of minerals in them, such as limestone, etc., and therefore it is necessary use the type of special micro element that don't sediments and also easily absorbed by the plant. So, to fix these problems often chelating EDTA and EDDHA are used. According, the aim of this study is evaluates colonization of potato roots by *Glomus mossea* and *G. intraradices* in different applications of these species. Also, their effect is studied in association with bacterial strains of *Pseudomonas fluorescens* and chemical chelating factors of iron on Marfona cultivar in the presence of crown rot and stems canker pathogens of potato.

## 2. Material and Methods

### 2.1 Fungal and Bacterial Isolates and Potato Cultivar

Isolate of *Rhizoctonia solani* AG3, *Glomus intraradices* and *G. mossea* were obtained from Mycology and biological control Collections, Department of Plant Protection, Vali-e-Asr University of Rafsanjan. Barley seeds were used for preparing the pathogen inoculum. 500cm<sup>3</sup> barley seeds and 300 ml water poured into 1-liter flask and sterilized two times at 121<sup>o</sup>C, 1.5 atmosphere pressure for 1 hour with one day interval<sup>8</sup>. Five segments of 5mm (7 days old) colony of fungi in PDA transferred into the flasks and stored at 24<sup>o</sup>C for 20 days. Due to the high sensitivity of Marfona cultivar to *Rhizoctonia solani*, tuber of this cultivar was applied in this study.

### 2.2 Chemical Chelating Agents

Two grams of Fe-EDTA and Fe-EDDHA chelating agents were dissolved in irrigation water and added to pots each containing 3 kg of soil.

### 2.3 Greenhouse Experiments

Pots containing 3 kg sterile soil from potato fields with appropriate EC (8 ds/m) and pH (0.5) were used to do the tests in this study. 50 g of Mycorrhizal propagules containing soil and root mixed with substrate and one tuber was planted. Bacterial strains and chemical chelating factors were added in the first irrigation phase of this treatment.

### 2.4 Staining the Roots and Determination of Root Colonization

To investigate the mycorrhizal colonization percentage, root staining was performed according to the method of Philips and Hyman<sup>9</sup>. Root colonization percentage was determined randomly based on Bearman and Lyndrmn<sup>10</sup> in 30 pieces of 1 cm stained roots segment. Each piece of root evaluated under microscope. The existence of hyphae, vesicle and arbuscule estimate as AM colonization. The estimated percentage of root length colonization was calculated by comparing to the total root length.

## 3. Results and Discussion

### 3.1 Mycorrhizal Colonization of *Glomus Mossea* and *Glomus Intraradices*

The results of potato root colonization by these mycorrhizal indicate that *G. mossea* and *G. intraradices* root colonisation rate were significantly different and about 66/33% and 22/16% respectively (Table 1). However, in the treatment contain *R. solani* as plant pathogens AM colonization rate were reduced but the reduction rate was not significant.

### 3.2 The Effect of *P. Fluorescens* on AM Colonization

In the compared to the control, bacterial treatments had different effect on mycorrhizal colonization according to fungal species and bacterial strains at the presence of *R. solani* AG3. Our results show that, in comparison with the control, AM colonization significantly increases in interactive treatment of *G. mossea* with *P. fluorescens* F<sub>140</sub> and *G. intraradices* with *P. fluorescens* T<sub>17-4</sub> in the presence

**Table 1.** Average percentage of mycorrhizal colonization roots in the presence and lack of pathogenic

Control	In presence of <i>R.solani</i> AG3	Mycorrhizal species
66.33	60.16	<i>Glomus mosseae</i>
22.16	18.33	<i>Glomus intraradices</i>

of *R. solani* AG3. However, maximum mycorrhizal colonization was observed in interactive treatments of *P. fluorescens* F<sub>140</sub> and *P. fluorescens* T<sub>17-4</sub> with *G. mosseae* in absence of fungal pathogen (Table 2). After all, in mycorrhizal-bacterial interactions, *G. intraradices* more efficient than *G. mosseae* and it is due to higher production of internal organs in this species. It has been reported that bacterial species having stimulating effects on AMF colonization are called mycorrhiza helper bacteria<sup>11</sup> and these promotion cause by stimulation spore germination and mycelial development<sup>12,13,14</sup>.

### 3.3 The Effect of Chemical Chelating Agents on AM Colonization

Both iron chemical chelating factors, EDTA and EDDHA, increase AM colonization. Though colonization of both mycorrhizal species decreased in interaction with these chelating factors, compared to the control. The presence of *R. solani* in pots containing mycorrhizal fungi with chemical chelating factors of iron had negative effects on performance on AM colonization (Table 3). It is noteworthy that *G. intraradices* has high colonization

**Table 2.** Percentage of mycorrhizal colonization in interactions with bacterial strains

Control	In presence of <i>R. solani</i> AG3	Bacterial strains – Mycorrhizal species
59.67	24.67	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>G. mosseae</i>
26.16	47.5	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>G. intraradices</i>
59.5	36.5	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>G. mosseae</i>
4.5	11.5	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>G. intraradices</i>
34.33	48.5	<i>P. fluorescens</i> F <sub>140</sub> + <i>G. mosseae</i>
27.16	18.33	<i>P. fluorescens</i> F <sub>140</sub> + <i>G. intraradices</i>

**Table 3.** Percentage of mycorrhizal colonization in interactions with chemical chelatings

Control	In presence of <i>R. solani</i> AG3	Mycorrhizal species - chemical chelatings
83	75	<i>Glomusmosseae</i> + EDTA
80	71.67	<i>Glomusmosseae</i> + EDDHA
70	34.67	<i>Glomusintraradices</i> +EDTA
35	21	<i>Glomusintraradices</i> + EDDHA

only in the presence of EDTA, while *G. mosseae* showed high colonization effect in the presence of both chelating factors. Nevertheless, addition of fungal pathogen to all treatments led to decrease this effect. However, it can be concluded that chemical chelating factors of iron increase colonization.

### 3.4 The Effect of Chemical Chelating Agents and Bacterial Strains on AM Colonization

The results revealed that interaction of chemical chelating factor of iron (EDTA and EDDHA) mycorrhizal colonization and bacterial strains had positive effects on colonization and in consequence leads to increase fresh and dry weight, other growth factors and chlorophyll (Table 4). This may arise from uptake of metallic elements and increase in thaloid size. It has been thought that beside the plant nutrient uptake the competition for space and nutrients, changes

**Table 4.** Percentage of mycorrhizal colonization in association with bacterial strains and chemical chelating agents

Control	In presence of <i>R. solani</i> AG3	Bacterial strains - Mycorrhizal species - Chemical chelatings
78	8.5	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>Gm</i> +EDTA
70	62	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>Gm</i> +EDDHA
61	30	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>Gi</i> +EDTA
65	45	<i>P. fluorescens</i> VUPf <sub>5</sub> + <i>Gi</i> +EDDHA
7.5	40	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>Gm</i> +EDTA
58	58	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>Gm</i> +EDDHA
43	34	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>Gi</i> +EDTA
27	36.5	<i>P. fluorescens</i> T <sub>17-4</sub> + <i>Gi</i> +EDDHA
75	18	<i>P. fluorescens</i> F <sub>140</sub> + <i>Gm</i> +EDTA
16.41	2.5	<i>P. fluorescens</i> F <sub>140</sub> + <i>Gm</i> +EDDHA
30.25	52.5	<i>P. fluorescens</i> F <sub>140</sub> + <i>Gi</i> +EDTA
33	23	<i>P. fluorescens</i> F <sub>140</sub> + <i>Gi</i> +EDDHA

in root system, mycorrhizosphere effect and the activation of plant defense mechanisms are responsible for disease inhibition by AMF<sup>11,15</sup>. The same researchers acknowledged that the competition for iron (siderophore production) was used for the inhibition of *R. solani*. So, it has been reported that dual application of AMF and bacterium inhibited the pathogen more efficiently<sup>6</sup> and caused decrease plant deaths<sup>11</sup>, than single applications. Moreover, it has been stated that dual applications of both AMF and rhizobacteria (PGPR) are becoming efficient by inhibiting parasitic growth of any pathogen on the plant root<sup>6</sup>. Their mutual establishment also improves plant rooting and enhances plant growth and nutrition<sup>11</sup>. AMF and rhizobacteria, as the most important symbionts of rhizosphere, have shown stimulating<sup>11-15</sup> or inhibiting<sup>16</sup> effects on each other or on the growth of plants and pathogens. This was also confirmed in our study. Linderman<sup>15</sup> reported that some antagonistic interactions might occur among the microorganisms inhibiting the same pathogen. It is not clear how AMF root colonization may be affected by soil-borne pathogens. Some reports stated that AMF root colonization was not affected by pathogens such as *Fusarium* spp. and some confirmed that AMF was negatively affected by the pathogens in different pathosystems and the root colonization was reduced<sup>17</sup>. Recent studies have demonstrated that underlying mechanisms of these differential interactions are still unclear<sup>18</sup>. However, it has been hypothesized that these effects may be related to the species and varieties of bacteria and the conditions in the rhizosphere<sup>19,20</sup>. The present study demonstrates that the two groups of rhizosphere organisms, AMF and rhizobacteria, can coexist without exhibiting adverse effects on each other. Moreover, it is concluded that suitable combinations of these biocontrol agents may increase the plant growth and resistance to pathogens. In future studies, therefore more detailed investigations of the relationships in various pathosystems and of the interactions between the microorganisms and the host plant are needed for further developing the biocontrol of the related diseases.

## 4. References

- Harrison MJ. The arbuscular mycorrhizal symbiosis. Plant-Microbe Interactions. US: Springer; 1997. p. 1-12.
- Azcon-Aguilar C, Barea JM. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. Mycorrhiza. 1996 Feb; 6(6):457-64.
- Linderman RG, Paulitz TC, Hornby D. Mycorrhizal-rhizobacterial interactions. Biological Control of Soil-Borne Plant Pathogens. CAB International; 1990. p. 261-83.
- Garbaye J. Tansley review no. 76 helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist. 1994 Oct; 128(2):197-210.
- Ravnkov S, Jacobsen I. Effects of *Pseudomonas fluorescens* DF57 on growth and P uptake of two arbuscular mycorrhizal fungi in symbiosis with cucumber. Mycorrhiza. 1999 Apr; 8(6):329-34.
- Budi SW, Blal B, Gianinazzi S. Surface-sterilization of *Glomus mosseae* sporocarps for studying endomycorrhization in vitro. Mycorrhiza; 1999 Aug; 9(2):65-8.
- Yao M, Tweddell R, Desilets H. Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micro-propagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. Mycorrhiza. 2002 Oct; 12(5):235-42.
- Sneh B, Burpee L, Ogoshi A. Identification of rhizoctonia species. APS Press; 1991.
- Philips JM, Hyman DS. Improved procedures clearing root and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Mycological Research. 1970; 55:158-61.
- Bearmann B, Linderman R. Quantifying vesicular-arbuscular mycorrhizae: a proposed method towards standardization. New Phytologist. 1981 Jan; 87(1):63-7.
- Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, Bonfante P, Azcon-Aguilar C. Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. Applied and Environmental Microbiology. 1998 Jun; 64(6):2304-7.
- Meyer JR, Linderman RG. Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. Soil Biology and Biochemistry. 1986; 18(2):185-90.
- Edwards SG, Young JPW, Fitter AH. Interactions between *Pseudomonas fluorescens* biocontrol agents and *Glomus mosseae*, an arbuscular mycorrhizal fungus, within the rhizosphere. FEMS Microbiology Letters. 1998 Sep; 166(2):297-303.
- Gupta SS. Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiology Ecology. 2003 Aug; 45(3):219-27.
- Linderman RG. Effects of mycorrhizas on plant tolerance to diseases. Arbuscular Mycorrhizas: Physiology and Function. Netherlands: Springer; 2000. p. 345-65.
- Soderberg KH, Olsson PA, Baath E. Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal

- colonisation. *FEMS Microbiology Ecology*. 2002 Jun; 40(3):223–31.
17. Zambolim L, Schenck NC. Reduction of the effects of pathogenic, root-infecting fungi on soybean by the mycorrhizal fungus, *Glomus mosseae*. *Phytopathology*. 1983; 73(10): 1402–5.
  18. Johansson JF, Paul LR, Finlay RD. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology*. 2004 Apr; 48(1):1–13.
  19. Siddiqui IA, Shaukat SS. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant-growth-promoting rhizobacteria *Pseudomonas aeruginosa* (IE-6S+) and *P. fluorescens* (CHA0). *Journal of Phytopathology*. 2002; 150(8–9):500–6.
  20. Anjaiah V, Cornelis P, Koedam N. Effect of genotype and root colonization in biological control of fusarium wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. *Canadian Journal of Microbiology*. 2003 Feb; 49(2):85–91.