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Controlling Powdery Mildew; Use of Arbuscular Mycorrhizal Fungi as Biocontrol Agent instead of Chemical Fungicides

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ABSTRACT

Plant protection based solely on modern fungicides could lead to genetic changes in neurons of animal and humans that contribute to cases of autism and Alzheimer disease, unless the bio control agents is applied or replaced. Most researches is focused on the strongest chemical fungicides, dangerous to human health, for effective plant disease control and we believe that non-chemical control methods such as biological agents like AMF are of great importance. The aim of study was to investigate whether the soil inoculation with *Glomus* isolates of arbuscular mycorrhizal fungi (AMF) affected control of powdery mildew disease of apple MM_{111} and its survival and growth instead of use of chemical fungicides such as Flint and Stroby. Twenty apple seedlings were randomly arranged to 4 treatments, each with 5 replicates (T1, control = no AMF, no fungicide, T2= Flint fungicide, T3= Stroby fungicide and T4 = only AMF mixture) and were monitored throughout 9 weeks. All seedlings were exposed to powdery mildew on week 6 and only T2 and T3 plants treated by fungicides after developing mildew colonies on the leaves. Results showed that, seedling length in plants cultivated in AMF-inoculated-soil was significantly higher than other treatments especially in weeks 1-4 and weeks 6-9. Leaf growth rate of all plants during the experimental growth period non-significantly increased between treatments with the exception of first week that did show a significant increase in leaf growth rate of group 4 plants, even after exposure to disease. T4 samples showed a high average of leaves numbers ($P<0.05$) in compared to other groups followed by T3 samples during the experimental growth period. The data from this study confirmed the response of seedling and leaf growth rates of apple seedlings to mycorrhizal colonization. It was concluded that plants cultivated in soil inoculated to AMF throughout 6 weeks had higher resistance and growth rates against *Podosphaera leucotricha* fungi as an agent of powdery mildew disease in apple seedling and it can be considered as an applicable strategy in biocontrol measures against pathogens when most researches is focused on chemical fungicides.

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INTRODUCTION

Podosphaera leucofricha is an obligate, parasite that overwinters on apple as mycelium in dormant buds infected during the previous growing season [1]. The "primary infection phase" of the disease is initiated by conidia produced on overwintering mycelium at bud break, which infect young leaves, flowers and shoots. Newly formed conidia from these sources are inoculum for the "secondary infection phase", which is the infection of healthy leaves during the growing season [2]. The reduction of primary inoculum and the protection of leaves, fruit and buds from secondary infections are two areas of concern for effective disease control measures. Timely application of fungicides is widely used to prevent new infections and to reduce the number of spores produced on new lesions.

Powdery mildew (PM), caused by *P. leucofricha* (Ell. & Ev.) Salm., is an important disease of apple in the interior of British Columbia. Disease severity and need for control measures are related to host susceptibility and to the intended market for the cultivar [3]. The pathogen may cause death of vegetative shoots or flower buds, and russetting of fruit [4]. The grower's primary concern with mildew is the russet symptoms that markedly reduce fruit quality [4]. Infected young trees of susceptible cultivars may be seriously damaged or become poorly shaped because of retarded vegetative growth or loss of terminal buds. In British Columbia the very susceptible apple cultivars, such as Mclntosh and Golden Delicious, are treated regularly with fungicides for control of fruit russet. The fungicides most commonly used for powdery mildew. Several new cultivars have been recently introduced, which also will require regular fungicide treatments [5]

The most promising new fungicides for control of powdery mildew are the broad-spectrum, sterol-inhibiting compounds [6]. With the exception of the morpholines, all sterol inhibitors have a common site of action within the biosynthesis pathway and are grouped together as demethylation inhibitors or DMIs [7]. Stroby and Flint are often called stroby fungicides and are very effective for controlling Black Spot (scab), mildew, and black rot. They provide adequate control of rust diseases when applied ahead of rains, but they have very little post-infection activity against rust diseases. For apple scab and mildew, they can provide roughly 48 hr of post-infection activity, but they are not effective for arresting apple scab after lesions are visible on foliage.

All stroby-containing fungicides carry labels stating that combined usage for any product in this group is limited to four applications per year. Thus, one can apply a maximum of four sprays per year that contain Stroby, Flint, or Pristine, otherwise controlling disease, not guarantee. For example, if Flint is applied three times to control mildew, then Pristine can be used only one time during summer. Based on using methods and rates of sprays of mentioned fungicide for control powdery mildew, it is detected that the additional time is required for a good controlling plants against types of pathogens that markedly reduce fruit quality, while there is several biological solutions for improving resistance of plants via boosting mineral nutrition against different pathogens or the attacker lifestyle [8]. For example, colonization of the original soil by AMF can boost resistance/tolerance of plant such as apple seedling against powdery mildew in an uninterrupted manner without spending additional costs to fungicides, sprays times and labor costs.

Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource. Arbuscular mycorrhizal (AM) associations have been shown to reduce damage caused by soil-borne plant pathogens. This prophylactic ability of AM fungi could be exploited in cooperation with other rhizospheric microbial antagonists to improve plant growth and health. The most important roles and benefits [9-11] of the Arbuscular Mycorrhiza (AM) are: 1- Increasing plant nutrition by exploring and deploying soil volumes; 2- Increase plant nutrition by obtaining a form of nutrition that is not available to the plant; 3- Increasing the tolerance of plants against dirt pathogens such as phytophthora, fusarium and pityum; 4- Improve water and plant relationships, plant hormonal changes; 5- Increased plant crop, increased food supply and reproduction; 6- Mycorrhiza can cause changes in the form of growth and root and vascular tissues; 7- The network of mycorrhiza fungi and plant roots provide a reasonable level of nutritional for a population of soil bacteria that increases host growth; 8. Hyphae of mycorrhiza arbuscular fungi contributes to the soil structure, their role in the physical aggregation of the soil is questionable, but sticky secretions such as Glomalin may be important; 9. Mycorrhizal fungi affect carbon storage in soil due to their impact on the quantity and quality of organic matter; 10- Arbuscular mycorrhiza increases plant resistance to environmental stresses such as dryness, cold and root pathogens; 11. Mineral absorption of soil, especially low-mobility elements such as phosphorus, zinc and copper, is considered to be the main function of mycorrhiza.

Common benefits for the plant are improved plant nutrition and/or increased capability to cope with adverse conditions. In the case of arbuscular mycorrhizal (AM) associations, the symbioses alter plant physiology, leading to a better mineral nutrition and to increased resistance/tolerance to biotic and abiotic stresses and or pathogens. Enhanced resistance/tolerance to soil-borne pathogens has been widely reported in mycorrhizal plants [12]. Although it is clear that the symbiosis may also impact plant interactions with aboveground attackers, the outcome of those interactions is less clear and seems to depend largely on the attacker lifestyle [8]. This finding points to a differential regulation of plant defense signaling pathways.

Although Flint and Stroby was registered for the control of powdery mildew on apples and grapes in Iran, it is unclear whether soil inoculated by AMF are comparable to these DMI fungicides, which are used to control powdery mildew on apples in Iran. Hence, this study compares the activities of arbuscular mycorrhizal fungi in compared to DMI fungicides on seedling and leaf growth rates of apple seedlings infected to powdery mildew disease under controlled conditions in the greenhouse.

MATERIAL AND METHODS

The study was conducted during the 2011/04 season in Iran on Maling merton (MM_{111}) apple seedlings which cultivated in soil with and without AMF, infected to powdery mildew (*Podosphaera leucotricha*) and treated by fungicides or AMF-inoculated soil. The fungicides used in these experiments [Flint and stroby, Kersoxim-methyl & Trifloxy strobin (% 50) WG, are a pre-mix products containing the *strobilurin trifloxystrobin*; registered in pome and stone fruits] were commercial formulations provided by the manufacturers.

MM_{111} apple seedlings were planted through tissue culture to free from any contamination by microorganisms in institute of tissue culture, Pishtaz Bldg., Karaj/Safadasht, Iran then all seedlings replaced in 10-cm dia. pots in a soil mixture containing equal volumes of loam, sand and vermiculite, perlite and coco-pit. Selected seedlings for trial were transferred to larger pots (35-cm dia.) containing 50% sterile sand and 50% AMF-inoculated soil. Prior to starting the experiment inoculation concentration of AMF were cleaned from soil of all new pots with the exception of those selected as maycorrhizal treatment (5 pots).

The symbiosis of the roots of apple seedlings of control group and treatment 4 (plants inoculated with Mycorrhiza) has been studied. Forty-two days after inoculation of apple seedlings with inoculum containing mycorrhizal fungi, apple rootstocks were sampled to determine if they have been able to coexist. The investigation of the seedlings showed that there was no fungal organ in the root of the control plants that had not received any inoculum, whereas the plants that were planted in the soil containing the mycorrhizal inoculum were created fungal structures (Figure 1). The rate of mycorrhizal symbiosis in these roots was moderate (less than 30%).

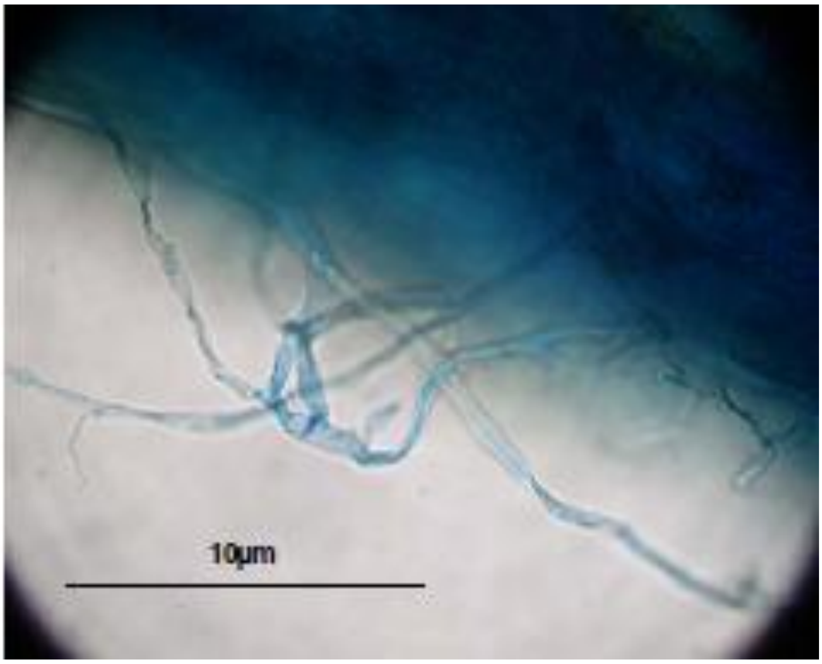

Figure 1. Mycorrhiza spp. in the roots of inoculated seedlings with Mycorrhiza

Therefore, twenty apple seedlings were selected and randomly arranged to four groups and five replicates including Treatment 1 as control group= without AMF mixture and fungicide; T2= Flint fungicide in 6th week; T3= Stroby fungicide in 6th week; and T4 = AMF mixture, which were monitored throughout 9 week. All groups' seedlings were exposed to Powdery Mildew on 6th week. Only T4 plants were cultivated in soil inoculated to AMF while only T2 and T3 plants treated by fungicides after developing mildew colonies on the leaves. The active ingredient (a.i.) dosages applied for the DMI materials were those recommended by the manufacturer. The experimental pots were placed in the greenhouse (22°C day, 18°C night, 77- 84% RH) for germination and subsequent growth for approximately 9 weeks so that plants protected against pesticides for disease or insect up to 6th week.

The inoculum source was infected apple shoots from an eight year old Jonagold tree in Research Station orchard in the *Iranian Research Institute of Plant Protection*. The fungus was identified as *Podosphaera leucotricha* (Ell. & Ev.) Salm. on the basis of symptom development and a comparison of the morphological characters of the conidia and fruiting bodies with those described for *P. leucotricha* by Ogawa and English [7]. The infected shoots were placed in a 1°C cold storage room for approximately 4 hrs while the fungicide suspensions were being prepared. Maling merton (MM_{111}) seedlings were sprayed to runoff using a hand operated mister. The leaves were allowed to dry for 30-min before inoculation with *P. leucotricha* conidia. Each treatment consisted of 5 seedlings (replicates). A conidial suspension was prepared by brushing conidia from diseased shoots into sterile water containing 20 pl/mL of Triton X 100 according to used method of Dekker [13]. The concentration was adjusted to 8.0 x 1011 conidi d.m.l. with a haemacytometer. Within 15-min of preparation the suspension was sprayed on the leaves. Seedlings length and leaves numbers of experimental apple seedlings were monitored and measured in day 1 and weekly during a 9-wks trial (Figure 2).

All data from the trial were analyzed by ANOVA using the GLM procedure of SAS software [14], which was appropriate for a randomized complete block design. When significances were detected ($P < 0.05$), values were compared post-hoc using the Duncan test. The results are expressed as averages and their Standard Error (SE).

Figure 2. Seedlings length in control and mycorrhizal plants after 4 weeks of Mycorrhiza inoculation

RESULTS AND DISCUSSION

Results of seedling length and leaves number of apple seedlings of treatments are shown in Tables 1 and 2, respectively. Seedling length in apple seedlings cultivated in AMF-inoculated-soil was higher than other treatments so that significant differences were observed in weeks 1-4 and weeks 6-9. Leaf growth rate of all experimental plants in weeks of plant growth non-significantly increased between treatments with the exception of first week that did showed a significant increase in leaf growth rate of group 4 plants (grown in AMF mixtures) compared to other treatments, even after infecting to disease. The data confirmed the response of seedling and leaf growth rates of apple seedlings to mycorrhizal colonization.

In the present study, results related to seedling length in different treatments indicated that apple seedlings cultivated in AMF-inoculated-soil did showed a higher continuous growth than control groups or those treated with fungicides. Hence, it seems mycorrhizal soil resulted in boosting seedling growth in apple seedling (MM_{111}). Our findings are agreement with results of Hause and Fester [15], Xu and Madden [16] and Stevens et al. [17]. Fortuna et al. [18] reported that soil contain arbuscular mycorrhizal fungi (AMF) via an beneficial interactions between plant and AMF improved plant nutrition and/or increased capability to cope with adverse conditions [19]. In the case of arbuscular mycorrhizal (AM) associations, the symbioses alter plant physiology, leading to a better mineral nutrition and to increased seedling and leaf growth rates and resistance/tolerance to biotic and abiotic stresses and or pathogens [20, 21].

Comparison of average of seedlings length revealed that in spray fungicides to T2 and T3 plants in 6^{th} week, had no deterrent effect on seedlings in the subsequent weeks (seventh, eighth, ninth). Researchers reported that Flint and Stroby fungicides that were used against *Podosphaera leucotricha*, had no effect on the seedlings length or leave numbers [22, 23]. Average of leave numbers in different treatment significantly (P<

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0.05) increased between treatments in first week that did show highest leaf effects in group 4 plants (fertilized by AMF mixtures) compared to other treatments, even after infected to disease.

These results could be due to boosting nutritional minerals of plants planted in soil inoculated by AMF which finally lead to a fast growth and leaf effects compared to those grown in AMF-deficient-soils [20, 24-26].

Table 1. Comparing the average of seedlings length during the experimental growth period

Date \ Treatment	T1	T2	T3	T4	Standard errors	Significant level
Day 1	17.40	17.00	18.60	19.20	0.815	NS
Week 1	21.60^{b}	22.40^{b}	24.20^{b}	33.00^{a}	2.866	*
Week 2	24.00^{b}	23.60^{b}	28.80^{ab}	38.20^{a}	3.789	*
Week 3	26.20^{b}	25.40^{b}	32.40^{ab}	42.60^{a}	4.793	*
Week 4	29.40^{ab}	27.80^{b}	35.00^{ab}	46.40^{a}	5.499	NS
Weed 5	35.00	32.20	38.80	54.40	6.912	NS
Week 6	42.20^{ab}	37.60^{b}	43.20^{ab}	66.60^{a}	8.202	*
Week 7	46.40^{ab}	39.80^{b}	48.40^{ab}	71.20^{a}	8.571	*
Week 8	48.60^{ab}	42.20^{b}	54.60^{ab}	74.20^{a}	8.587	*
Week 9	52.20^{ab}	43.80^{b}	60.20^{ab}	78.60^{a}	8.467	*

^{a,b} Values in the same row and variable with no common superscript differ significantly; Values are means of 5 observations per treatment and their standard errors. Treatment 1 (T1) = control (non-AMF mixture, non-fungicide); T2 = non-AMF mixture + fungicide Flint in 6th week; T3 = non-AMF mixture + fungicide Stroby in 6th week; T4 = AMF mixture; NS= p>0.05; *= p<0.05; **= p<0.01.

Table 2. Comparing the average of leaves numbers during the experimental growth period

Date \ Treatment	T1	T2	T3	T4	Standard errors	Significant level
Day 1	13.20	11.20	14.00	13.80	1.185	NS
Week 1	15.00^{b}	14.80^{b}	18.20^{ab}	23.00^{a}	2.143	*
Week 2	16.60	15.60	20.80	24.00	2.704	NS
Week 3	17.20	16.60	22.00	25.00	3.033	NS
Week 4	19.60	18.60	22.60	27.60	3.136	NS
Weed 5	21.60	21.60	24.60	31.40	3.991	NS
Week 6	26.60	24.00	26.80	35.40	4.622	NS
Week 7	28.40	25.80	30.60	38.20	4.693	NS
Week 8	30.80	28.00	34.20	39.00	5.137	NS
Week 9	32.80	29.60	38.00	43.60	5.146	NS

^{a,b} Values in the same row and variable with no common superscript differ significantly; Values are means of 5 observations per treatment and their standard errors. Treatment 1 (T1) = control (non-AMF mixture, non-fungicide); T2 = non-AMF mixture + fungicide Flint in 6th week; T3 = non-AMF mixture + fungicide Stroby in 6th week; T4 = AMF mixture; NS= p>0.05; *= p<0.05; **= p<0.01

CONCLUSION

The data confirmed the response of seedling and leaf growth rates of apple seedlings to mycorrhizal colonization. From the results of this study, it was concluded that plants cultivated in soil inoculated to AMF throughout 6 weeks had higher resistance and growth rates against *Podosphaera leucotricha* fungi as an agent of Powdery Mildew disease in apple seedling and it can be considered as an applicable strategy in bio control measures against pathogens.

Nowadays, many works has been performed for improving plant growth and crop production. Most researches is focused on the strongest chemical fungicides, dangerous to human health, for effective plant disease control and we believe that non-chemical control methods such as biological agents like AMF are of great importance. Meanwhile, our work suggested that the combined use of both arbuscular mycorrhizal fungi and most safely fungicides is an effective strategy to management of diseases such as powdery mildew; so that, using AMF-fertilized-soil at the foot of the trees in early spring and then use fungicides only once (instead of three phases) in order to decline additional sprays times and labor costs is recommended.

DECLARATIONS

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Authors' Contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

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