# Effects of arbuscular mycorrhizal fungi against apple Powdery Mildew disease

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**Abstract:**The study was conducted to examine effects of mixture of arbuscular mycorrhizal fungi (AMF) and two most prevalent fungicides on Powdery Mildew disease of apple seedlings (Maling merton, $MM_{111}$). Twenty seedlings were subjected to completely randomized design (CRD) in the following treatments (5 replicates): control (non-AMF mixture, non-fungicide, T1), non-AMF mixture + fungicide Flint in 6th week (T2), non-AMF mixture + fungicide Stroby in 6th week (T3) and AMF mixture (T4), which were monitored for a period of 9 week. Seedlings were exposed to powdery mildew on 6th week and only T3 and T4 plants sprayed one time by fungicides after developing mildew colonies on the leaves. Mildew colonies counted on the all positions of apple seedling leaves (-4 to 4) after a 25-d-treating period that was started from week 6. Results indicated that the most mildew colonies were related to control plants, while lowest colonies numbers were observed in plants treated by Flint (T2), and followed by those inoculated by AMF without using any fungicide (T4). It was concluded that soil inoculation by mixture of AMF had effects similar to Flint and better than Stroby fungicide on decreasing powdery mildew colonies in apple seedling and thus, the combined use of both AMF and fungicides can be considered as a protective strategy against powdery mildew of apple. [Yousefi, Z., Riahi, H., Khabbaz-Jolfaei H., Zanganeh S. **Effects of arbuscular mycorrhizal fungi against apple Powdery Mildew disease.** Life Science Journal. 2011;8(4):108-112] (ISSN:1097-8135). http://www.lifesciencesite.com.

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## 1. Introduction

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. 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 (Whipps, 2004).

Powdery mildew (PM), caused by *Podosphaera leucotricha* (Ell. & Ev.) Salm., caused by *P. leucotricha* is an important disease of apple in Iran, Islamic Republic of. Disease severity and need for control measures are related to host susceptibility and to the intended market for the cultivar (Yoder and Hickey, 1983). In Iran the very susceptible apple cultivars, such as Maling merton ($MM_{111}$) and Golden Delicious, are treated regularly with fungicides for control of fruit russet. The fungicides most commonly used for powdery mildew. 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.

The most promising new fungicides for control powdery mildew are the broad-spectrum, sterol-inhibiting compounds (Ogawa and English 1991). Stroby and Flint are often called stroby fungicides and are 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 is not guarantee. For example, if Flint is applied three times to control mildew, then Stroby can be used only one time during summer.

Based on using mentioned fungicides (methods and sprays rates) 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 aboveground attackers (Pozo and Azcón-Aguilar, 2007). 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, repetitious sprays times as well as labor costs.

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 mildew of apple plants under controlled conditions in the greenhouse.

## 2. 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.

The fungicides used in these experiments [Flint and stroby, Kersoxim-methyl and 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).

Apple seedlings on $MM_{111}$ rootstocks had approximately 17±2 cm length with 3-month age. Twenty seedlings were subjected to completely randomized design (CRD) in the following treatments (5 replicates): control (non-AMF mixture, non-fungicide, T1), non-AMF mixture + fungicide Flint in 6$^{th}$ week (T2), non-AMF mixture + fungicide Stroby in 6$^{th}$ week (T3) and AMF mixture (T4), which were monitored throughout 9 week. Seedlings were exposed to powdery mildew on 6$^{th}$ wk. and only T3 and T4 plants sprayed one time by fungicides after developing mildew colonies on the leaves. Mildew colonies counted on the all positions of apple seedling leaves (-4 to 4) after a 25-d-treating period that started from week 6.

The active ingredient (a.i.) dosages of fungicides 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 6$^{th}$ 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* 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 (1991). 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 (1982). 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. The seedlings were inoculated using the method Dekker (1982) developed to evaluate powdery mildew on $MM_{111}$ leaves.

Mildew development was estimated by counting colonies on leaves day 25 after infection to mildew pathogen. Each small white spot at least 3-mm in diameter was counted as a colony. Mildew colonies were counted on both surfaces of nine leaves at positions -4 to +4, where leaf 0 was the youngest leaf behind the shoot apex at the time of inoculation and -4 was the next unrolled leaf and youngest leaf at the shoot tip when the colonies were counted (Jeger et al. 1986). One plant was removed from each treatment because it had on average fifteen times more colonies than the mean, and therefore had been apparently infected with powdery mildew before the start of the experimental period of resistance.

### 2.1. Statistical analyses

All data from the trial were analyzed by ANOVA using the GLM procedure of SAS software (SAS Institute, 1998), which was appropriated 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).

## 3. Results

Results of colonies numbers on leaves in different positions are shown in Table 1. The controlling disease by Flint was significantly boosted in plants of group 2 (Table 1), and values followed by plants cultivated in AMF-fertilized-soil after infected to *Podosphaera leucotricha* (P<0.01) (Figure 1). Results indicated that the most mildew colonies were related to control plants, while lowest colonies numbers were observed in plants treated by Flint (T2), and followed by those inoculated by AMF without using any fungicide (T4).

No significant differences in mildew colonies numbers were observed between treatments in the leaves of positions 4, 3 and 2, while mildew colonies of the leaves of positions 1 to -4 were significantly (P<0.01) decreased by Flint, followed by mixture of AMF. Mildew colonies in the leaves of plants treated by Stroby not significantly decreased in position -4.

**Table 1.** Comparing averages of colonies numbers of leaves in positions (-4 to +4)

| Treatment / Traits | Treatment 1 | Treatment 1 | Treatment 1 | Treatment 1 | Standard errors | Significant level |
|---|---|---|---|---|---|---|
| **Leave -4** | $1.20^{ab}$ | $0.00^{b}$ | $2.00^{a}$ | $0.60^{ab}$ | 0.316 | ** |
| **Leave -3** | $3.80^{a}$ | $0.00^{b}$ | $2.20^{a}$ | $0.20^{b}$ | 0.282 | ** |
| **Leave -2** | $3.60^{a}$ | $0.00^{b}$ | $2.40^{ab}$ | $0.80^{a}$ | 0.339 | ** |
| **Leave -1** | $2.40^{a}$ | $0.00^{b}$ | $2.10^{a}$ | $0.80^{a}$ | 0.367 | ** |
| **Leave 0** | $3.60^{a}$ | $0.20^{a}$ | $0.60^{ab}$ | $0.20^{b}$ | 0.353 | ** |
| **Leave +1** | $2.00^{a}$ | $0.00^{b}$ | $0.40^{ab}$ | $0.80^{a}$ | 0.316 | ** |
| **Leave +2** | $0.60^{b}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ | 0.200 | ns |
| **Leave +3** | $0.80^{a}$ | $0.00^{b}$ | $0.40^{ab}$ | $0.00^{b}$ | 0.223 | ns |
| **Leave +4** | $0.80^{ab}$ | $0.00^{b}$ | $0.40^{a}$ | $0.00^{b}$ | 0.223 | 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.

## 4. Discussion

Comparing average of colonies numbers in leaves of apple seedlings between treatments revealed a significant different (P < 0.01); so that, average of highest colonies numbers was related to T1 and T3 (control and Stroby-treated groups, respectively) and lowest value was related to plants treated by Flint in 6th week (T2) and those planted in AMF-inoculated-soil (T4). Data from current study confirmed that groups fertilized by mycorrhizal inoculum had lower colonies similar to those treated by Flint. Therefore, this result revealed that the soil contain arbuscular mycorrhizal fungi has a continual effect on prevent further contamination of the older leaves.

Results from the current study are agreement with researchers (by fungicides: Wilcox et al., 1992; Sholberg and Haag, 1994; and by AMF-inoculated-soil: Azcón-Aguilar and Barea, 1996; Pozo et al., 2008).

Daft and Nicolson (2011) by study of influence of inoculum concentration of mycorrhiza on growth and infection in tomato found that enrichment of soil using inoculum concentration of arbuscular mycorrhizal fungi were significantly affected and improved plant growth and crop production. To date the most studies were done related to effects of soil inoculation by AMF on final level of root diseases and rot (Fortuna et al., 1996; Xu and Madden; 2002; Fortin et al., 2002; Turk et al., 2006; Redecker and Raab, 2006; Wehner et al., 2009; Mitre et al., 2010) while there is little reference and relatively centralized researches which examined the secondary effect of mycorrhizal fungi-inoculated-soils in plants on containment of pollution of leaves like powdery mildew (Azcón-Aguilar and Barea, 1996; Whipps, 2004; Pozo et al., 2008). Nowadays the chemical fungicide were widely used by farmers and producers of agricultural products against powdery mildew disease; hence, most researches is focused on the strongest and most safely fungicides for effective

disease control and often non-chemical methods such as biological control using AMF are of secondary importance (Valiuškaitė et al., 2009).

Fortuna et al. (1996) 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 (Wehner et al., 2009).

## 5. Conclusion

Results indicated that soil inoculation via AMF (T2) for apple seedlings ($MM_{111}$) was effective on decreasing percentage of infection similar to those treated by fungicide Flint. It was concluded that plants cultivated in soil inoculated to AMF throughout 6 weeks had higher resistance against *Podosphaera leucotricha* fungi as an agent of powdery mildew disease in apple seedling and it can be considered as a protective strategy in fruiting plants for reduce the negative effects of infectious fungi. From the presented study it is suggested that the combined use of both arbuscular mycorrhizal fungi and fungicides is an effective strategy to management of diseases.

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