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Effects of Arbuscular Mycorrhizal Fungi on Mildew Infection Percentage in Apple Seedling

Zohreh Yousefi

Young Researchers Club, Central Tehran Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

In this study effects of soil fertilization via inoculation with a mixture of arbuscular mycorrhizal fungi (AMF) (isolates *Glomus*) and fungicide (Flint and Stroby) on controlling Powdery Mildew disease in apple seedling (Maling merton, MM_{111}) was evaluated. Apple seedlings were arranged via CRD in 4 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 throughout 9 week. Seedlings were exposed to Powdery Mildew on week 6 and T3 and T4 plants groups treated by fungicides after developing mildew colonies on the leaves. Results indicated that soil inoculation via AMF (T2) for apple seedlings (MM_{111}) was more effective on decreasing percentage of infection than those treated by fungicide; however, the controlling disease by Flint was significantly boosted ($P<0.01$). It was concluded that plants cultivated in soil inoculated to AMF throughout 6 weeks had higher resistance against 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.
KEY WORDS: Arbuscular mycorrhizal fungi, fungicide, Powdery Mildew, apple seedling.

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. Enhanced resistance/tolerance to soil-borne pathogens has been widely reported in mycorrhizal plants [1]. Although it is clear that the symbiosis may also impact plant interactions with, the outcome of those interactions is less clear and seems to depend largely on the attacker lifestyle [2]. 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 angatonists to improve plant growth and health [3]. Powdery mildew (PM), caused by *Podosphaera leucofricha* (Ell. & Ev.) Salm., is an important disease of apple in Iran. Disease severity and need for control measures are related to host susceptibility and to the intended market for the cultivar (Yoder and Hickey 1983).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 [5]. 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 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 most promising new fungicides for control powdery mildew are the broad-spectrum, sterol-inhibiting compounds [6]. Strobilurin fungicides include Sovran, Flint, Stroby and the pyraclostrobin component found in Pristine. 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 hrs of post-infection activity, but they are not effective for arresting apple scab after lesions are visible on foliage.

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 [2]. 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.

***Corresponding Author:** Zohreh Yousefi (Ph.D.). Young Researchers Club, Central Tehran Branch, Islamic Azad University, Tehran, Iran. Email: m.yousefi65@gmail.com

MATERIALS 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 6th week (T2), non-AMF mixture + fungicide Stroby in 6th week (T3) and AMF mixture (T4), which were monitored throughout 9 week. Seedlings were exposed to powdery mildew on 6th 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 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* 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 [6]. 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 [7] 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.

All data from the trial were analyzed by ANOVA using the GLM procedure of SAS software [8], 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

RESULTS

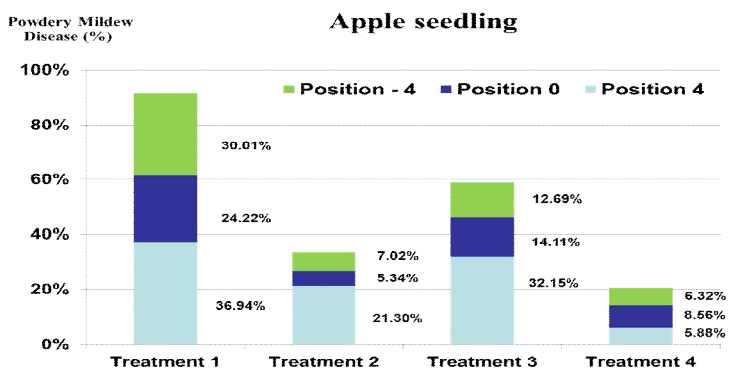

Figure 1. Percentages related to powdery mildew disease in apple seedlings of different groups (in three positions and or in total). The presented graph is calculated and designed based on data presented in table 1. Mildew severity infection percentages was calculated for average of conidia number based on a criterion that criteria for most infected leaves in position 4 = 2295 conidia, position 0 = 1589 conidia and position -4 = 2016 conidia and totally (4, 0 and -4) = 5900 conidia which was considered as 100% infection. Hence, the percentage of the infected leaves on each seedling in different groups was calculated.

Results of Mildew severity infection percentages on leaves in different positions are shown in Figure 1. The controlling disease by Flint was significantly boosted in plants of group 2 (Table 1), while plants cultivated in AMF-fertilized-soil

showed lowest infection in leaves of three positions (4, 0 and -4) after infected to *Podosphaera leucofricha* compared than those planted in non-AMF mixture, exposed to disease and treated by fungicides ($P<0.01$).

Comparison of leaves positions between treatments did showed higher infection in the older leaves (position 4) in compared with newest leaves (position -4) in control (T1) and fungicide-treated (T2 and T3) treatments. Thus, those planted in by soil-fertilized-AMF from head had higher conidia numbers in leaves of position zero in compared with -4 leaves.

DISCUSSION

Results indicated that soil inoculation via AMF (T2) for apple seedlings (MM_{111}) was more effective on decreasing percentage of infection than those treated by fungicide; however, the controlling disease by Flint was significantly boosted ($P<0.01$).

Results from the current study are agreement with researchers [by fungicides: 9, 10; and by AMF-inoculated-soil: 11, 12). Daft and Nicolson [13] 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 [14, 15, 16, 17, 18] 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 [1, 11, 12]. 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 [19]. Fortuna et al. [14] 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 [17].

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 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.

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