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Efficiency of Elite Fungicide for Control of Pistachio Gummosis

Mohammad Moradi[*]*, Amir Hossein Mohammadi, Masoumeh Haghdel*

Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran

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Abstract

Several species of *Phytophthora* cause crown and root rot diseases of herbaceous and woody plants. Crown and root rot of pistachio trees cause significant damages in infected orchards. The effect of foliar application with Elite (fosetyl-Al) in 2 and 2.5 g/l was evaluated in greenhouse experiments. The frequency of mortality, fresh and dry weight of roots and shoots, height, intensity of crown root colonization using CAMA-PARP medium was determined. Under greenhouse experiments, foliar application with Elite increased height, fresh and dry weight of shoots and root either in inoculation with and without *Phytophthora drechsleri.* The effects of Elite were more pronounced in roots, which increased the fresh and dry weight of root 1.3 and 2.5 times compared to those not sprayed with Elite, respectively. On the other hand, the application of Elite before or on the day of inoculation significantly reduced the frequency of mortality, which ranged from 35 to 90% ($P \leq 0.01$). Crown and root colonization of pistachio seedling was affected by both the concentration of Elite and reduced the frequency of crown and root colonization of seedling. When fungicide and pathogen were applied at the same time, the frequency of colonization reduced to 18% and 36% for 2 and 2.5 g/l, respectively, and 43% and 60% when seedlings were treated with fungicide before *P. drechsleri* inoculations. The highest effect was seen in foliar application of Elite seven days before inoculation in 2.5 g/l. Further investigations have been conducted to understand the effect of Elite in infected trees as well as modeling of Elite application via soil drench, foliar application or trunk injection.

Keywords: Aluminium Fosetyl, Crown, Elite, Foliar spray, *Phytophthora*, Root rot.

Introduction

Crown and root rot (CRR) of fertile and non-fertile pistachio trees is one of the most destructive soil-borne diseases throughout Iranian orchards. The disease can be caused by multiple species of *Phytophthora* spp. (Banihashemi and Moradi 2004; Mirabolfathy *et al.,* 2001; Fani *et al.,* 2005; Mostowfizadeh-Ghalamfarsa *et al.,* 2008). *Phytophthora pistaciae* and *P. drechsleri* are the most widely distributed species found in the orchards (Mirabolfathy *et al.,* 2001; Banihashemi and Moradi 2004). In severe cases, nearly all trees may be affected, which can cause the total loss of orchard and reduce the number of trees by up to 80% over a 5-10 year time span (Moradi 2015 a & b), which drastically affects pistachio production in infected orchards. The yearly damage caused by the disease has been estimated to range between 2% to 11% (Moradi 2015a and b).

Different approaches have been recommended to reduce disease incidence and severity, including resistant rootstocks and application of cultural, chemical and biological methods (Moradi 2015a & b). Control of CRR is very difficult, because the disease agents are soil-borne and *Phytophthora* species are polycyclic in the orchards. The applications of fungicides, such as Captan, Mancozeb, Fosetyl-Al and Bordeaux mixture, have failed to manage CRR under field conditions.

However, most assays under laboratory and greenhouse conditions were affective in inhibiting the

[*]**Corresponding author**: Email: moradi@pri.ir

growth of pathogen or protecting seedlings from infection (Mirabolfathy *et al*., 1990; Abousaeidi *et al.*, 1990). Preliminary studies have shown that the application of Elite in infected orchards reduced the severity of disease and mortality of fertile trees.

Many studies have shown that application of fosetyl-Al via foliar, trunk injection and soil drench controlled many plant diseases caused by *Phytophthora* spp. (Fernandez-Escobar *et al.*, 1999; Shearer *et al.*, 2004; Jung *et al.*, 2009; Fenn and Coffey, 1984; Hoover and Bates, 2012; Matheron and Mircetich, 1985; El-Hamalawi *et al.*, 1995; Silva *et al*., 2016). In tree species, the effects of foliar spray of fosetyl-Al were evaluated in comparison to the control *P. cactorum* (Leber and Cohn) Sohroet on apple (Orlikowski *et al.*, 1986). *P. cinnamomi* Rands on avocado (Coffey *et al.*,1984; Darvas *et al.*, 1984) and walnut (Matheron and Mircetich, 1985), *P. citrophthora* (Smith & Smith) Leonian on walnut (Matheron and Mircetich, 1985) and citrus (Farih *et al.*, 1981).

Several investigations have shown a complex mode of action of phosphite, acting directly on the pathogen and indirectly by stimulating host-defense responses by means of activation of phytoalexin production. (Fenn and Coffey, 1984; Coffey and Bower, 1984; Guest and Grant, 1991; Gisi, 2002; McDonal *et al.*, 2001).

There is scarcity information on the use of fosetyl-Al to prevent CRR by *Phytophthora* spp. in pistachio orchards. Therefore, the current study evaluated the efficacy of fosetyl-Al in prevention of CRR on pistachio trees under greenhouse conditions.

Materials and Methods

Fungicides

In all experiments, Elite (Fosetyl-Al, aluminium tris-o-ethyl-phosphonate, 80%WDG, Shangdong Dacheng, China) were used.

Plants

The experiments were carried out Pistachio Research Institute in Rafsanjan during 2013-2014.

The seeds (cv. Musiabadi) were sterilized for five minutes in 0.5% (v/v) solution of NaOCl, then drained the solution and washed three times with sterilized distill water. Afterwards, the seeds were soaked for 24 hours in sterilized distill water and sterilized in Benomyl (50%WP) (2 g/L w/v). The seeds were transferred in a mixture of sand and perlite (1:1 v/v) for germination for 3 to 5 days. The germinated seeds were planted 1-2 cm deep in 5 L pots (10 seeds per pot) filled with a mixture of sterilized sand and clay (2:1) in a deep of 1-2 cm. After seedling emergence, plants were thinned to four seedlings per pot and grown under greenhouse conditions with a 12-hour light photoperiod at 27 ± 2ºC.

Inoculum preparation and plant inoculation

In all experiments, a virulent strains of *P. drechsleri* originally isolated from infected pistachio trees in the Rafsanjan region were obtained from the Iranian Plant Protection Institute (IPPI) culture collection and maintained on corn meal agar (CMA) (Himedia, Pvt. Ltd., India). Inoculum of *P. drechsleri* was prepared on wheat seed. Wheat seeds (100g) were soaked for 24 hours in tap water after surface sterilization. After removing the excess water, seeds were autoclaved three times at 1.5 atm pressure and 121˚C for 20 minutes for three consecutive days. The seeds were inoculated with five day old of colonies of *P. drechsleri* and incubated for three weeks at 27˚C in the dark. The flasks were gently shaken every three days. Six-month-olds seedlings (cv. Mosiabadi) were inoculated with ten grams of colonized wheat seeds and covered with sterile sand.

Immediately after the inoculation, the pots were flooded. After 12 hours, the excess water was drain out of the pots and kept under a 12-hour photoperiod. To assess the efficacy of Elite, seedlings were uprooted forty five days after inoculations. Seedling mortality was evaluated via visual assessments and by culturing small pieces of root and crown on CMA+PARP medium (Masago *et al.*, 1977) with some modifications (Pimaricin 10 mg/L, Ampicillin 250 mg/L, Ri-

fampicin 10 mg/L, Terrachlor (PCNB) 75% WP 100 mg/L in 1000 ml corn meal agar). Plant height, fresh and dry weight of shoots and roots were measured or each seedling and replication.

The experiment was carried out in a complete randomized design, where plants were sprayed with 0 (control), 2 or 2.5 g Elite/L (Company recommended) and inoculated with *P. drechsleri* at one of three time periods from seven days prior, at the same time, to seven days after, spray application.

Results

Seedlings mortality

As shown in Fig. 1, a high mortality rate was seen in sample that was inoculated with *P. drechsleri* alone and those sprayed with Elite fungicide seven days after inoculation. Hence, spraying Elite seventh day after the inoculation had no effects on seedling mortality. Spraying Elite at the same time or seven days before *P. drechsleri* inoculation significantly reduced seedling mortality compared with the control (no spraying, Fig. 2). The lowest mortality rates were observed in seedlings sprayed with Elite before *P. drechsleri* inoculation. No significant differences were observed between doses 2 and 2.5 g/l of Elite to reduce disease mortality. The reduction rate of mortality for doses of 2 and 2.5 g/l Elite were 85% and 90%, respectively. No seedling mortality was observed in no-inoculation control and seedling received only Elite.

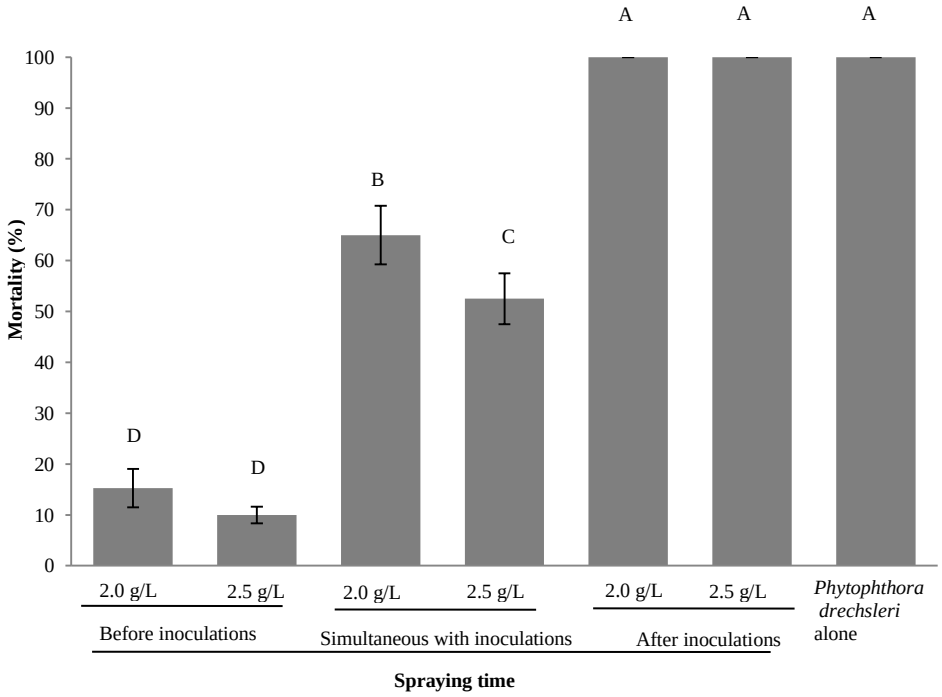

Fig. 1. Effects of Elite spraying on the rate of mortality in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range tests at P=0.01

Colonization of seedling

Crown and root colonization of pistachio seedling was affected by both concentration of Elite spraying at concentration of 2 and 2.5 g/l (Fig. 3). The highest rates of colonization was seen in seedlings inoculated either with *P. drechsleri* alone or application of Elite seven days of pathogen inoculations which reached to 100%. Spraying Elite with both concentrations simultaneously or seven days before pathogen inoculations

significantly reduced the frequency of crown and root colonization of seedlings. When fungicide and pathogen were applied at the same time, the frequency of colonization was reduced18% and 36% for 2 and 2.5 g/l, respectively, and 43% and 60% when seedlings were treated with fungicide before *P. drechsleri* inoculations.

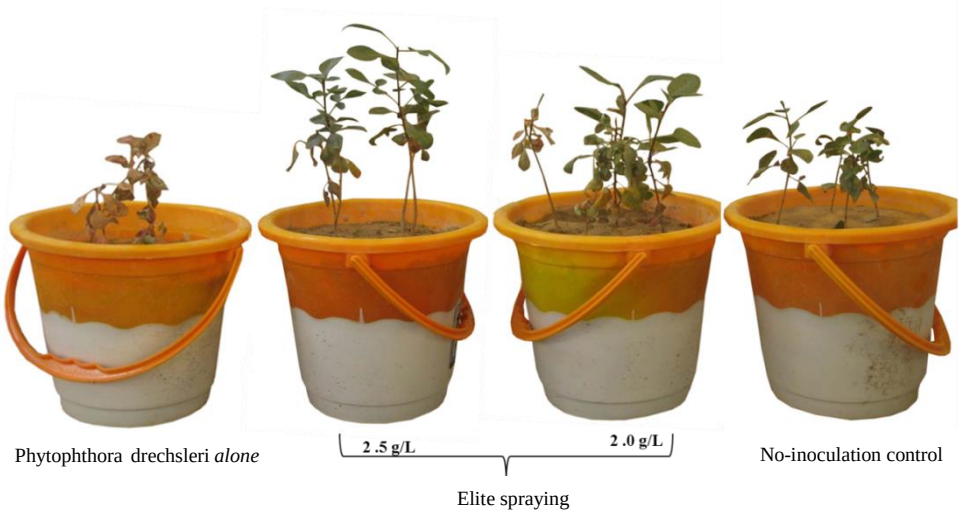

Fig. 2. Effects of Elite spraying in 2 and 2.5 g/l on the mortality of pistachio seedling inoculated with *Phytophthora drechsleri* in greenhouse experiments

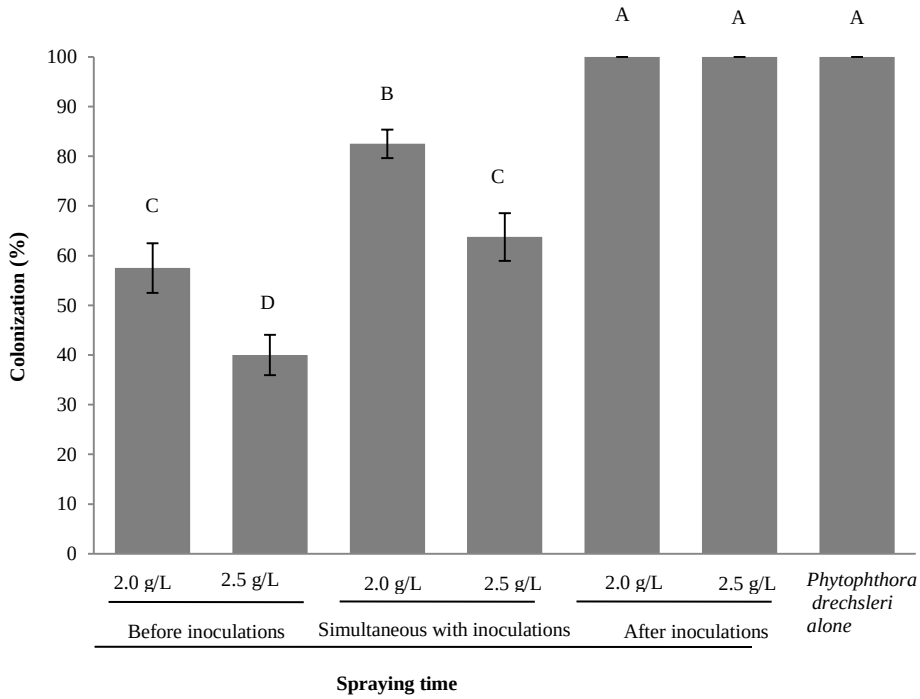

Fig. 3. Effects of Elite spraying on disease severity in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drec hsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range test. at P=0.01

Seedling height

Plant height was not significantly affected by Elite application in both concentrations either before or in simultaneous inoculations with *P. drechsleri.* Seedling inoculated with *P. drechsleri* alone or 7 days before fungicide application had significantly lower height compared to other treatments (Fig. 4).

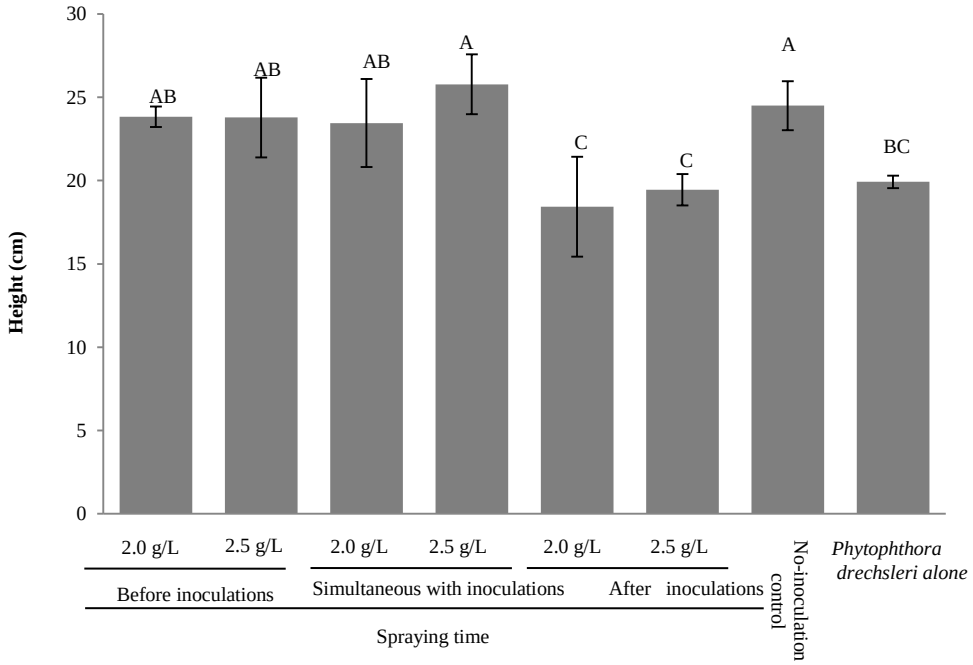

Fig. 4. Effects of Elite spraying on plant height in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse

Elite spraying either before or at the same time of inoculation with *P. drechsleri* had significant effects on fresh and dry weight of root in concentration of 2.5 g/l compared to the other treatments (Figs. 5 and 6). This could indicate the level of fungicide to inhibit fungal colonization as well as positive effects of Elite on root development. Spraying seedling with Elite alone increased fresh and dry weight of seedling 1.3 to 2.5 times higher than no-spraying control seedling, with highest effect in 2.5 g/l of Elite (Fig. 7).

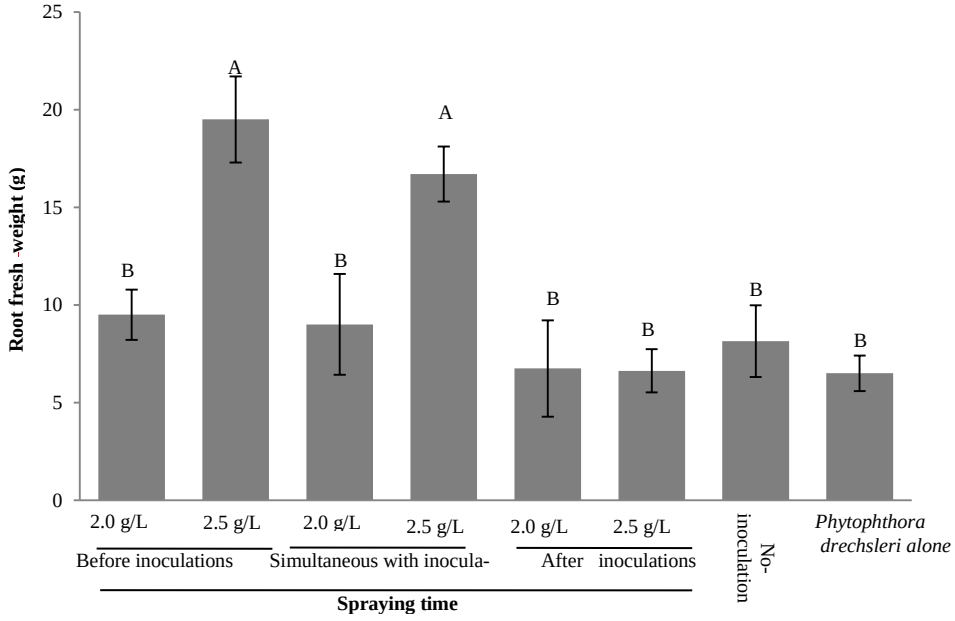

Fig. 5. Effects of Elite spraying on fresh weight of root in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range tests. at P=0.01

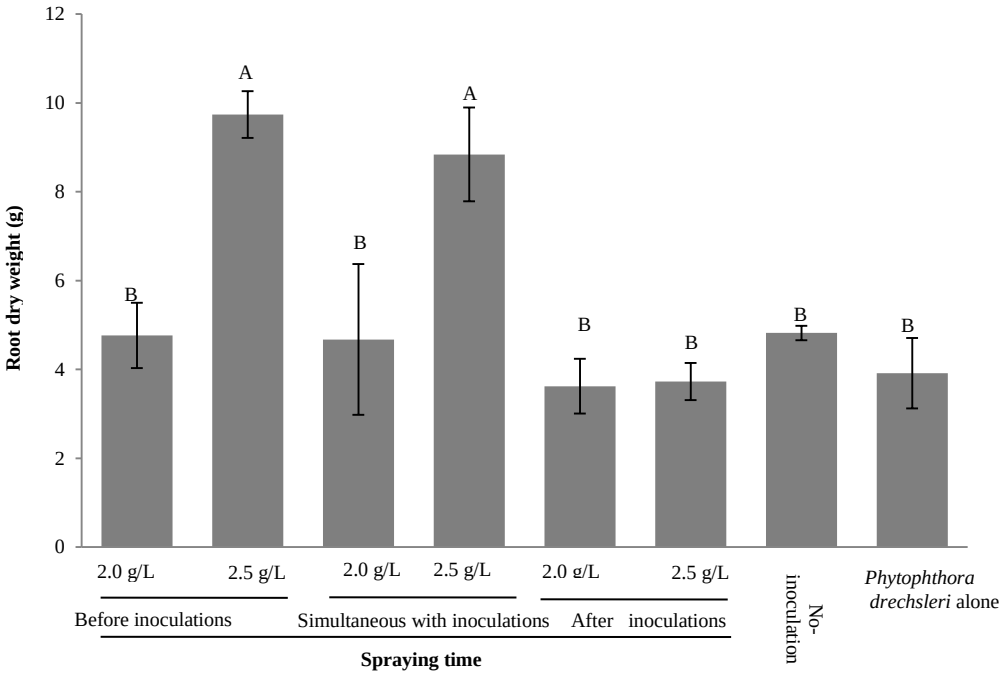

Fig. 6. Effects of Elite spraying on dry weight of roots in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range test. at P=0.0

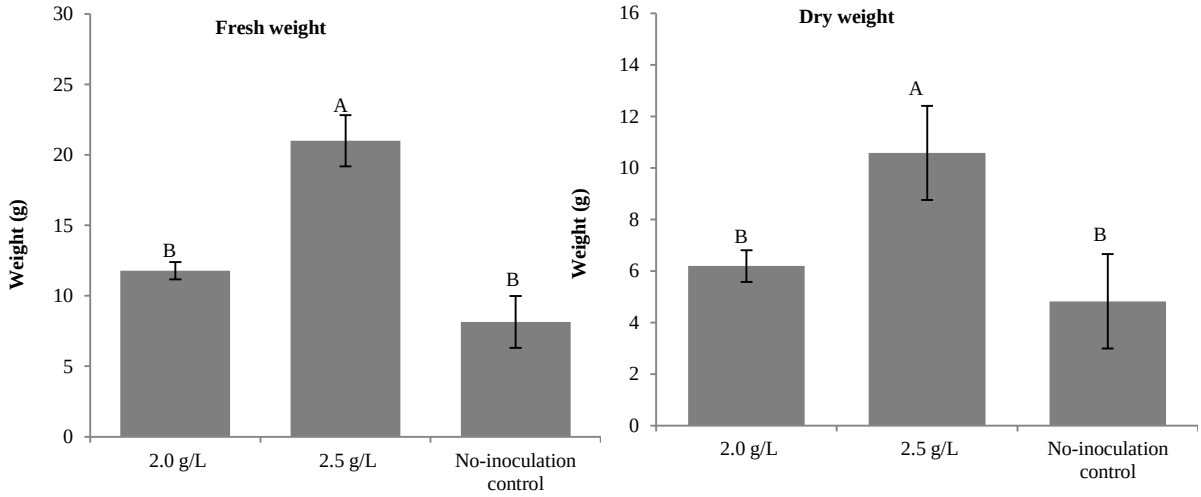

Fig. 7. Effects of Elite spraying on fresh and dry weight of root in pistachio cultivar (cv. Mosiabadi) in greenhouse experiments Columns with the same letter are not significantly different according to Duncan's multiple range tests. at P=0.01

Fresh and dry shoot of seedling

Similar to other parameters, seedling sprayed with Elite had higher fresh and dry weight of shoot compared to the control seedlings that were not sprayed (Figs. 8 and 9). The highest rates of fresh and dry weight of shoot were observed in seedling sprayed with 2.5 g/l compared to other treatments. The lowest shoot weight was in the seedling inoculated with *P. drechsleri* alone or seven days before fungicide application. Spraying pistachio seedlings with Elite alone did not have a significant effect in increasing shoot weight compared to control seedlings (Fig. 7).

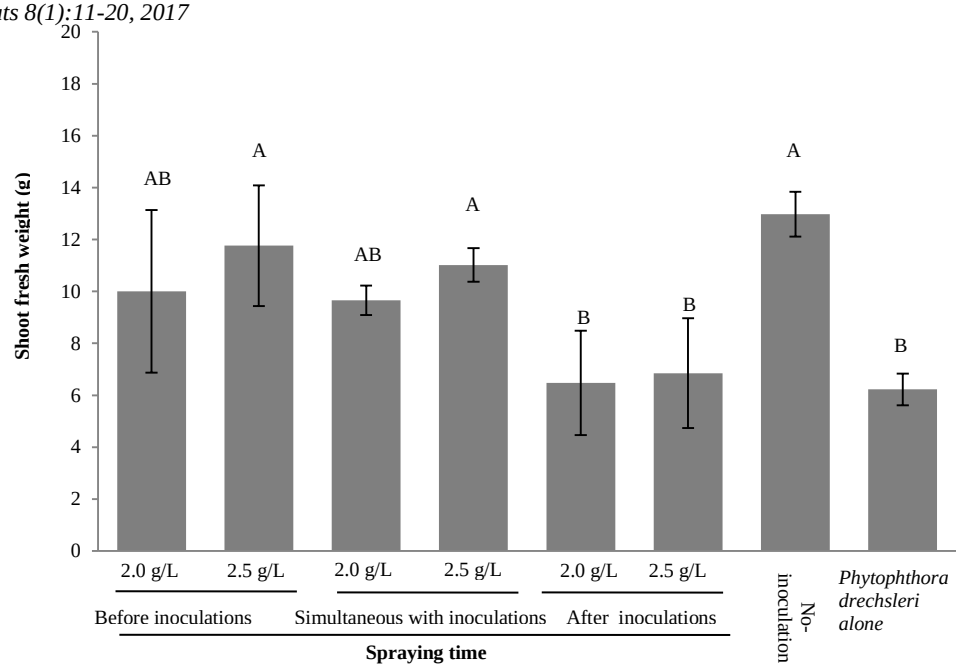

Fig. 8. Effects of Elite spraying on fresh weight of shoots in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range test. at P=0.01

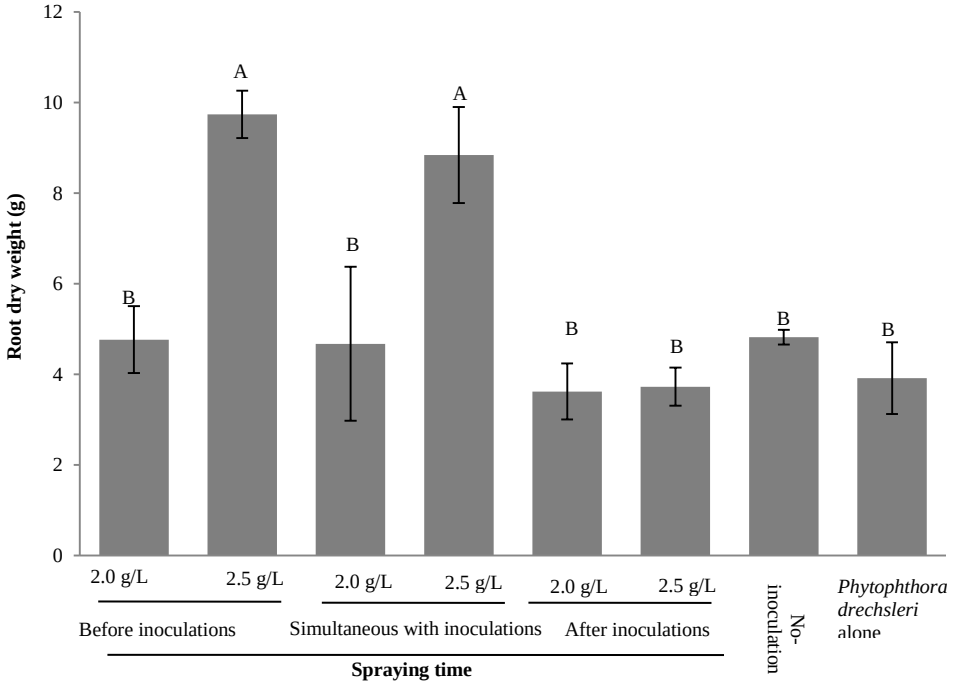

Fig. 9. Effects of Elite spraying on dry weight of shoots in pistachio cultivar (cv. Mosiabadi) inoculated with *Phytophthora drechsleri* in greenhouse experiments. Columns with the same letter are not significantly different according to Duncan's multiple range test. at P=0.01

Discussion

Crown and root rot (CRR) of pistachio trees is one of the most destructive soil-borne diseases throughout of Iranian orchards. There is little information on the different approaches to prevent CRR cited by *Phytophthora* spp. in pistachio orchards. In addition, non-compliance prevention methods by growers and tradetional cultural practices have led to devastating disease epidemics in infected areas. On the other hand, traditional fungicides, such as Copper-based compounds or Lime, are not able to control the disease due to very low permeability of the bark. Many studies have shown that fosetyl-Al is able to control CRR in several plants species (Aberton *et al.,* 1999; Fernandez-Escobar *et al.,* 1999; Hardy *et al.,* 2001).

Overall, the application of Elite before *P. drechsleri* caused a significant reduction in seedling

mortality and disease severity compared to other treatments. The fungicide reduced the growth of pathogens by moving through the vascular system and induced defense mechanisms, although more experiments on this are necessary. Preliminary experiments have shown the presence of Elite in crown and root tissue three days after foliar spraying (data not published). The direct effects of phosphonate / fosetyl-Al and activation of defense mechanisms against *Phytophthora* species in many plant species has been documented (Crane and Shearer, 2014; Daniel and Guest, 2006, Eshraghi *et al.,* 2011; Fenn and Coffey, 1984; Grant *et al.,* 1990; Guest and Grant, 1991; Lim *et al.*, 2013; Mcgrath 2004; Shearer and Fairman, 2007; Silva *et al.,* 2016; Smillie *et al.*, 1989).

In tobacco plants, spraying with fosetyl-Al caused rapid increments in concentrations of phytoalexins, lignins and ethylenes as well as destructive activity of phenylalanine on pathogen compared to plants without foliar application, indicating the activation of plant defense mechanisms (Nemestothy and Guest, 1990). It should be noted that within the plant tissue, fosetyl-Al is converted to phosphite (Cohen and Coffey, 1986; Lim *et al*., 2013; Mcgrath 2004; Smillie *et al*., 1989).

Studies on the mode of action of phosphite ion in *Eucalyptus* have shown that low concentrations of phosphite in roots induced defense mechanisms. In high concentrations, defense mechanisms remained unchanged (Jackson *et al* 2000).

Spraying inoculated and non-inoculated pistachio seedlings with Elite had a positive trend of increasing different growth estimated parameters, which was more pronounced in fresh and dry weight of roots. In most cases, the concentration 2.5g/l of Elite either in seedling inoculated and non-inoculated with *P. drechsleri* had a significant effect on estimated parameters such as mortality and disease severity. Bielenin and Jones (1988) showed a significantly increase in root dry weight of Cherry after spraying fosetyl-Al to control crown and root rot of Cherry, while no effects was observed in bud growth.

Based on the results, the concentration of 2.5 g/l of Elite can be assessed in infected orchards to manage the CRR caused by *Phytophthora* spp. Further investigations should be conducted to understand the effect of Elite in infected trees as well as modeling of its application via soil drench, foliar application or trunk injection.

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