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Interaction between fungi mycorrhiza and Treflan herbicide on root colonization of clover (*Trifolium repens*)

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Abstract

There is little information on the issues such as interaction between mycorrhiza fungi and herbicide application and their effects on plant growth. Therefore, factorial experiment was arranged in completely randomized design with three replications in Shahrood University of Technology in 2013. Experimental factors were combination of two factors: (1) non mycorrhiza (control), *Glommus mosseaeand Glommus intraradices* as mycorrhiza inoculums. (2) Herbicide treatments included four levels of Treflan (0, 1000, 1500 and 2000 g a.i. ha^{-1}). The results showed that soil inoculated with mycorrhiza, increased dry weight shoot and root, fresh weight shoot and root, root and shoot height and colonization[%] of *Trifolium repens*in the low herbicide concentrations (1000 g a.i. ha^{-1}) as compared to non-inoculated treatments significantly. Based on our results, mycorrhiza fungi can alleviate crop stress due to low doses of Treflan injury through increasing of plant growth.

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Introduction

However, increasing societal concern, [27, 29] over the effects of herbicides on human health and the environment has led to more regulation and suggestions for alternative control tactics [5, 40, 47]. Biological weed control is one of the alternatives [45], but vesicular-arbuscular mycorrhiza (VAM) fungi have not been previously considered as bio control agents. We are now beginning to realize, however, that responses by one plant to selective stresses [21, 25, 36], are communicated to other plants, and that VAM fungi are involved in this process because their mycelia form a living bridge between root systems [16, 26]. Mycorrhiza associations formed by *Glomeromycotan* fungi are known as arbuscular mycorrhizas or vesicular-arbuscular mycorrhizas, and abbreviated as VAM [3]. VAMs are considered as endo-mycorrhiza as they produce arbuscules, hyphae and vesicles within the root cortex cell [39]. VAM fungi form symbiotic associations with the roots of a wide variety of plant species, including many agricultural crop species [22]. In this symbiosis, the host plant provides the fungus with soluble carbon sources and the fungus facilitates the host plant with increased access to water and nutrients from soil [11]. This increased access of VAM fungi to nutrients is happened through their extra radical hypae that extend outside the host root up to several centimeters in the soil, allowing the fungi to absorb soil nutrients otherwise unavailable to the host plant [13]. However, the relative abundance of VAM fungi within roots mostly depends on soil conditions that directly or indirectly affect the rapidity of fungal spore germination and plant root colonization such as growth and infectivity of both internal and external hyphae of the fungi [42]. Although VAM fungi are present in soils of all textures from sandy to those with high clay content and at a wide range of soil pH [4], but many soil factors such as conventional tillage, soil compaction, high fertilizer and pesticides application shave a negative impact on VAM functions [11].

The symbiotic relationship between mycorrhiza and host plant can be seriously hampered by the neglectful interference of human activities such as over application of pesticides mainly herbicides in modern intensive agricultural systems [46]. Herbicides, despite of their control on weeds, have the potential to affect beneficial non-targeted soil microbes including VAM fungi [17]. However, several authors have reported different effects of herbicides on VAM symbiosis, which ranges from no adverse effects to slightly or highly toxic effects [6, 10, 31, 33, 35, 38, 46]. However, their results showed that the types of herbicide and its rate of application are an important factor in mediating the toxic effects on mycorrhizae [18, 21]. Direct effects of herbicides on root colonization and sporulation by VAM fungi have been evaluated [21, 36, 46] and were variable and often species- and dosage-dependent [9]. The beneficial effects of VAM were found to disappear when herbicides were applied at higher doses [35]. Herbicides, when applied as foliar spray or via root system, showed the deleterious effects on the VAM-host interaction. Recent studies have shown that application of foliar-applied herbicides might affect the VA mycorrhiza symbiosis through affecting the photosynthesis of the host plant [19]. On the other hand, the VAM-mediation uptake of soil-applied herbicides into herbicide-sensitive host plant may lead to added injury or stress that ultimately affects the growth and development of host plant [30]. However, many herbicides are not directly fungi-toxic but affect these obligately biotrophic organisms indirectly by altering host-plant functions [9, 12, 41]. The resulting inhibition of the endophyte may have further effects on host-plant physiology, but these effects on the individual host are little-known, and even less well-known for plant associations. Thus, the evaluation of the effects of herbicide application on VAM-plant association deserves increased attention. It is also important to identify herbicides that have toxic effects on the efficacy of VAM-plant symbiosis so that alternatives may be considered. Mycorrhiza colonization, however, was reduced in field plots through applications of the fungicide benomyl as a soil drenches [32]. Therefore, the present study was conducted to determine the effects of widely-used herbicide on colonization of VAM fungi in roots of *Trifolium repens*plants in response to herbicides application.

Materials and methods

Pot experiment were conducted to determine whether the arbuscular mycorrhiza (AM) fungi modifies the effects of herbicide on clover (*Trifolium repens*) in 201 3respectively.The trials were carried out at the Research Farm of Shahrood Universityof Technology, situated about 5 km west of Shahrood in the city of Bastam (36° 25'E, 54° 58'N, 1349 ma.s.l.). The mean annual temperature is 14.4°C and the mean annual rainfall is 156.5 mm, most regularly falling in winter and early spring, with low rainfalls during summer and autumn. Factorial experiments were conducted in completely randomized design with three replications. Experiment was conducted on6 weeks grown clover in 2013. Treatments were factorial combination of two factors: (1) non mycorrhiza (control), *Glommus mosseae* and *Glommus intraradices* as mycorrhiza inoculums. (2) Four levels of the Treflan herbicide (0, 1000, 1500 and 2000 g a.i. ha^{-1}). The soil used in these experiments was collected from depth of 0– 20 cm. Soil was then air-dried, sieved to 2 mm and homogenized. A 1:1 (v.v) mixture of sand (1–2 mm) and farm soil was used as the growth medium in order to enhance the permeability of the soil.Some chemical and physical properties of the soil are given in Table 1. Plastic pots of [30 cm diam. and 40cm depth] were used in this trial with a central hole, containing 3 Kg unsterilized soil.

Table 1. Some physicochemical characteristics of the soil used in these experiments.

Mechanical composition [%]						
Sand	**Clay**	**Loam**	**Available P (mg kg^{-1} soil)**	**Organic matter (%)**	**Ec (dS m^{-1})**	**pH**
30%	45%	23%	5.43	0.41	0.7	7.67

For mycorrhiza treatments, 100 g inoculums were thoroughly mixed with 250 g soil. Seeds of clover [*Trifolium repens*] were sown in the pots maintained at near field with a capacity of [60–70% WHC] in order to provide normal environmental conditions. Seedlings were thinned to five plants per pot in the three leaf stages. At harvest, 42 days after seedlings of clover, shoot height and shoot dry weight were obtained respectively. Clover shoots and roots were dried for 48 at 70° C and weighed. All root samples were carefully washed under tap water to remove adhered soil particles. Root systems of the plants were thoroughly rinsed with distilled water, wiped with tissue paper, and weighed immediately. A portion of sub-sample fresh roots was taken from each treatment for the determination of the proportion of root length colonized by the AM fungus.

Determination of root colonization

The proportion of total clover root length colonized by the AM fungus was determined by cutting a random sub-sample of 1.0 g of fresh roots into 0.5- to 1.0-cm-long segments. Mycorrhiza root segments were cleared in 10% KOH for 10 min at 90° C in a water bath, rinsed in water, and then stained with 0.1% Trypan Blue for 3–5 min. at 90° C in a water bath. The grid –line intersect method [15] was used to determine total root length and the percentage length of root colonized by mycorrhiza fungi.

Statistics

Statistical analysis of data was performed with Statistical software of SAS. Significant differences between means refer to the probability level of 0.05 by LSD test.

Results and discussion

Shoot height

The herbicide decreased Shoot height of inoculated and non-inoculated clover, when applied at all rates of application. However, the height of AM infected plants, were higher when the herbicide was applied at the concentration of 1000 than at the concentration of 1500 and 2000 g a.i.ha^{-1}. No differences between both doses of herbicide were found in the non AM infected plant, however, the height of non-inoculated plants was decreased by the herbicide at all rates of application. Clover height was increased significantly under mycorrhiza colonization [table 3]. The AM-mediated uptake of herbicides may lead to added injury or stress when an herbicide sensitive crop comes into contact with residues present in the soil [18]. But, other researchers suggested that mycorrhiza colonization significantly increased the concentration of P and other nutrition's in both shoot and root of clover plant at all levels of soil salinity [23]. Miransari *et al.* [28] also reported that even under compact conditions, the increased P uptake, had an important role to alleviate the salt stress due to AM inoculation. The Enhanced N and P uptake results in an enhanced shoot and root growth, respectively. It seems that AM infected plants can alleviate plant growth and height by Fe uptake. Enhanced nutrients uptake is due, in part, to the hyphal extension into the soil and subsequent transfer of them back to the root. Recent researches have established that AM fungi are able to absorb organic compounds, macro and micro nutrients from the soil and transfer them to plants [14].

Seed germination

Results showed that pre-emergence application of Treflan and inoculation with mycorrhiza did not affect seed germination and emergence clover [table 2]. Other studies have shown that inhibitors of photosynthesis at photosystem-II herbicides such as simazine and trefolan have been ineffective or have little impact on the germination process [1].

Plant root fresh and dry and shoot fresh weight

Herbicide had a significant effect on clover root fresh and dry and shoot fresh weight [$p<0.01$]. Root fresh and dry and shoot fresh weight was decreased by increasing the herbicide concentration. root fresh and dry and shoot fresh weightof clover plant was not affected by low level [1000 g a. i.ha^{-1}] of herbicides, but increasing herbicide concentrations to 1500 and 2500 g a.i.ha^{-1} significantly decreased root fresh and dry and shoot fresh weight of both mycorrhiza and non-mycorrhiza clover plants than control, thoughroot fresh and dry and shoot fresh weight of mycorrhiza plant was significantly greater than that of non-mycorrhiza plant in low herbicide rate [table 3]. This is in agreement with the results reported by Garcia-Romeria *et al.* [12]. They reported that shoot dry weights of AM fungi infected pea plants, were higher when the cyanazine was applied at the rate of 0.05 mgml $^{-1}$ than applied at the rate of 0.1 ml per pot.

But no differences were found between root fresh and dry and shoot fresh weights in both herbicide rates in the non AM fungi infected plants. However, the dry matter of non- inoculated plants was decreased by the herbicide at all rates of herbicide application. But in both media VA mycorrhizas alleviate the effect of herbicide on plant growth, when applied at intermediate 1000 g a.i. ha-1 levels. Similar results have been found with other photosynthetic inhibitor herbicides [35]. Based on our results the beneficial effect of AM fungi disappears, when the trflon was applied at the agronomical rate of [2000 g a.i. ha^{-1} and greater], in all tested treatments. However, our study suggests that under standard agricultural practices herbicide residues in soil, accumulated from repeated applications, may eliminate the better tolerance of AM plants to herbicide.

Mycorrhiza colonization

Results showed that herbicide application reduced the percentage of root colonization of clover significantly by mycorrhiza as compared to control [fig.1].In greenhouse studies, in which simazine and paraquat were added to the soil at the recommended amounts, citrus root colonization was decreased significantly by *Glomus etunicatum* [31]. Herbicides have been made only to control weeds and are not designed for AM fungi. Many studies have also shown that herbicides will not have negative effects on AM fungi. Busse *et al.* [7], reported that triclorpyr, imazapyr and sulfometuron methyl herbicides did not alter the capability of mycorrhiza fungi to infect roots, even at concentrations detrimental to seedling growth. Pesticides have also been reported to stimulate colonization of plant roots by AM fungi. This may be due to a reduction or elimination of competing microorganisms or to a pesticide mediated change in plant metabolism resulting in increased production of materials stimulatory to AM fungi in the rhizosphere [37]. The growth inhibition that cyanazine produces in plants is mainly attributed to the blockage of photosynthesis [20]. The inhibition of photosynthesis by herbicides has been already demonstrated when applied as foliar spray as well as via the root [24]. The carbon requirements of AM fungi must be supplied by the host photosynthate [2]. Hence, any factor which modifies the photosynthetic product, might affect VA mycorrhiza development available for distribution [7], so we can expect that trayazin group herbicides can decrease mycorrhiza fungi pollution through inhibition of photosynthesis in photosystem II.

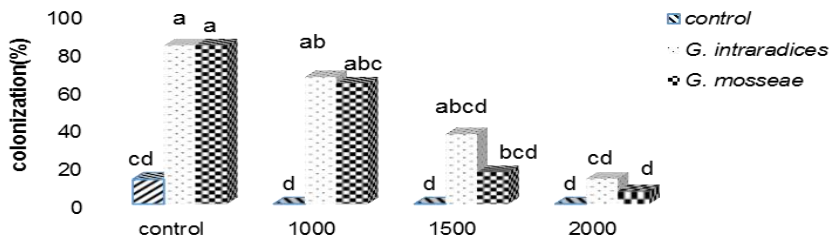

Figure 1- Mean comparison interaction of mycorrhizal fungi and Treflan herbicide (g a. i. ha^{-1}.) on clover colonization (%).

Shoot dry weight

The results of the analysis variance showed that the Shoot dry weight was significantly affected by all treatments [P≤0.01] in this experiment [Table 2].Mean comparison table showed that the highest and lowestShoot dry weightwere obtained by a treatment of *G.intraradices* + non Treflanherbicide [control] and non mycorrhiza [control] + 2000 [ga.i.ha^{-1}.] Treflan herbicide, respectively [fig.2].However, the dry matter of non- inoculated plants was decreased by the herbicide at all rates of herbicide application. But in both media VA mycorrhizas alleviate the effect of herbicide on plant growth, when applied at intermediate 1000 g a.i. ha^{-1} levels.This is in agreement with the results reported by Garcia- Romeria *et al.* [12]. They reported that shoot dry weights of AM fungi infected pea plants, were higher when the cyanazine was applied at the rate of 0.05 mgml^{-1} than applied at the rate of 0.1 ml per pot.

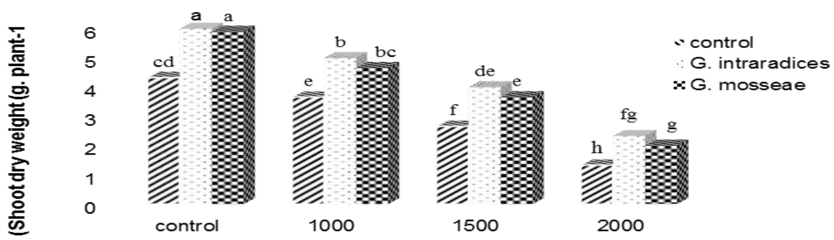

Figure 1- Mean comparison interaction of mycorrhiza fungi and Treflan herbicide (g a. i. ha^{-1}.) on clover shoot dry weight.

Shoot height

The results of the analysis variance showed that the Shoot height weight was significantly affected by all treatments [P≤0.01] in this experiment [Table 2]. Mean comparison table showed that the highest and lowest Shoot height weights were obtained by a treatment of *G.intraradices* + non Treflan herbicide [control] and non mycorrhiza [control] + 2000 [ga.i.ha-1.] Treflan herbicide, respectively [fig.3].

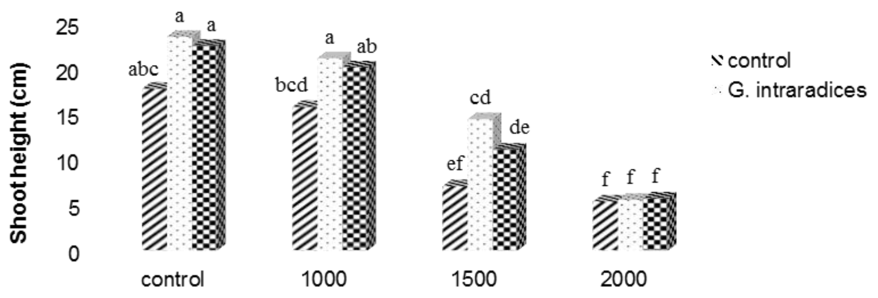

Figure 3- Mean comparison interaction of mycorrhizal fungi and Treflan herbicide (g a. i. ha-1.) on clover shoot height (cm).

Table 2. Analysis of variance for effects treatments of mycorrhiza fungi and Treflan herbicide on some characteristic growth and colonization [%] in clover plant.

Resource changes	df	Germination	dry weight Shoot	dry weight root	fresh weight Shoot	fresh weight root	Shoot height	Root height	Colonization
Repetition	2	17.6	2.86	0.77	6.02	7.86	0.19	9.19	11.11
Mycorrhiza	2	46.52 Ns	6.02**	7.19**	32.02**	26.77**	23.11**	61.01**	7536.25**
Treflan	3	117.73 Ns	20.69**	31.95**	125.36**	111.43**	84.85**	473.42**	5269.44**
Treflan×mycorrhiza	6	1 Ns	0.13**	0.45Ns	0.91 Ns	1.29 Ns	2.18 Ns	8.88**	913.88**
Cv%	-	2.75	12.46	13.63	12.54	12.03	15.21	9.38	9

**: Significant at = 1%, ns: Not significant

Table 3. Means comparison of the main effects of studied treatments on some clover properties.

Treatments	dry weight root	fresh weight Shoot	fresh weight root	Shoot height
Mycorrhiza				
control	5.1 a	7.8 a	8.91a	6.16a
G. *intraradices*	6.6 a	10.83a	11.58a	8.38a
G. *mosseae*	6a	9.5a	9.08a	6.83a
LSD	2.7	4.79	4.3	4.07
Treflan				
control	5.01a	7.33 a	7.73a	6.7a
1000	4.88a	6.55a	6a	5.33a
1500	3.66a	5.72 a	5.5a	4a
2000	2.22a	3.33a	3a	3.66a
LSD	5.52	10.95	10.32	9

Means of data followed by the same letter in each column, are not significantly different using the LSD test at 5% level. Means in theparenthesis are standard errors.

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