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Full Length Research Paper

Determining the effects of *Trichoderma harzianum* and some mycorrhizal fungi on plant growth and against *Rhizoctania solani* Kühn in *Lilium* under *in vivo* conditions

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This study was conducted to determine the effects of microbial fertilizer containing *Trichoderma harzianum* Kuen 1585 (Tr), biological preparation (BRDI) (containing *Glomus aggregatum, G. clarim, G. deserticola, G. intraradices, G. monosporus, G. mosseae, Gigaspora margarita* and *Paraglomus brasilianum*), and *Glomus* sp. collected from agricultural areas in Aydin province on plant growth in *Lilium* and *Rhizoctonia solani* Kühn. (Rs) bulb and root rots under *in vivo* conditions. Study was conducted in 2 stages: at stage 1, effects of Tr, BRDI and *Glomus* sp. on growth of lily plants were observed and four treatments, Tr (20 g/kg), BRDI (40 cc/L), *Glomus* sp. (10 spore/g) and control, took place in this trial. At stage 2; effects of Tr, BRDI and *Glomus* sp. on Rs were observed and Rs (2%), Rs + Tr, Rs + BRDI, Rs + *Glomus* sp. and control treatments were used in the study. Treatments at stage 1 had significant effects on flower stalk length, length of flower bud and flower head diameter. Flowers with the longest stalk (62.03 cm) were obtained from control and this was followed by flowers harvested on lily plants treated *Glomus* sp. Flowers obtained from the bulbs treated *Glomus* sp., had the highest flower head diameter (15.27 cm). Significantly positive effects were observed on root growth of bulbs obtained from Tr and *Glomus* sp. treatments. *Glomus* sp. gave the best result, and disease severity which is 4.73 in Rs was reduced to 1.20 with *Glomus* sp. Similar results were also obtained in terms of fresh root weight.

Key words: *Lilium*, biocontrol, plant growth, *Trichoderma* sp., *Rhizoctonia* spp.

INTRODUCTION

Ornamental geophytes, also called “flower bulbs” contribute significantly to the global ornamental industry, and are utilized for commercial bulb and flower production (Kamenetsky and Miller, 2010). This is a very large and diverse group of species which includes *Lilium*s. As one of the commercial cut flower species that can be produced as an alternative to carnation and rose which have the highest production rates among commercial cut flowers produced in Turkey, *Lilium* is a species belonging to the *Liliaceae* family. *Liliumlongiflorum* and *L. candidum* are important species as commercial cut flowers. In addition, there are groups called “Asiatic Hybrids”, “Oriental Hybrids” and “LA Hybrids” obtained as a result of cross breeding of *L. longiflorum* with Asiatic Hybrids, and there is a wide range of variety diversity among these groups.*Lilium* is a plant species of perennial geophytes. On lily plants, a flower stalk grows up to 45 to 150 cm depending on the species and varieties, and this flower stalk consists of 1 to 12 flower buds (Uzun, 1984; Korkut, 2004). Geophytes exhibit great diversity in morphology, growth, and developmental biology and physiological responses to environmental factors (Kamenetsky, 2009; Benschop et al., 2010). Morphologically, geophytes are composed of basal plate, apical meristem and scales. Many factors

Abbreviation: Tr, *Trichoderma harzianum;* **BRDI,** biological preparation; **Rs,** *Rhizoctonia solani.*

influence the growth of lilies such as soil type, temperature, lightning, watering, the nutrient element contents of bulbs, nutrient level of growing medium, age of the bulb and soilborne diseases. In terms of bulb structure, geophytes are classified in two groups, geophytes having bulbs with tunica and bulbs without tunica (Zencirkıran, 2002). *Lilium* is one of the geophytes having a bulb without tunica (Wilkins, 1980: Hartmann et al., 1997). It is sensitive against diseases, because it does not have a dry scale structure which functions as a protection against external factors, their bulbs have higher moisture content when compared with other geophytes, and they have thicker and succulent scales; thus, it is easier for pathogens to go through the scales. The sensitivity of lilies to soil born diseaes depends on the variety and growing conditions.It is possible to specify fungal pathogens, which are important in *Lilium* production, as *Rhizoctonia solani, Phytophthora* ssp., *Fusarium* spp., *Verticillium* spp., *Pythium* spp., *Botrytis* sp. (Miller, 1998; Chase, 2005; Gümrükcü and Gölükcü, 2005). Among plant production activities, control of soil borne pathogens is one of the significant factors increasing the cost. However, some methods such as starting production in sterilized environments, using resistant plant species/ varieties and solarisation, are used in control of these pathogens as well as chemical methods. But, from time to time satisfactory results are not achieved in plant production by using these methods.

Furthermore, sustainable farming systems strive to minimise the use of synthetic pesticides and to optimise the use of alternative management strategies to control soil-borne pathogens (Harrier and Watson, 2004). For this purpose, biological control methods became prominent in recent years.Mycorrhizal fungi are present almost everywhere and approximately 90% of higher plant species are normally with mycorrhizal, such that some plants cannot grow normally without a proper mycorrhizal partner (Kendrick, 1992; Marschner, 1995). Mycorrhizal have a role on plants in water uptake and intake of some nutrient elements such as P, Fe, Mn, K, N (Hayman, 1982; Smith et al., 1992; Demir, 1998; Davies et al., 2000). Arbuscular mycorrhizae (AM) have been shown to be an important tool in the biological control of soil-borne pathogens including *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Verticillium* and *Thielaviopsis* as well as various nematodes not only in sustainable but also in organic agriculture (Harrier and Watson, 2004). Therefore, recently more studies are conducted on use of mycorrhizal fungi in organic agriculture. However, agricultural factors such as pesticides, fertilizers and soil tillage have an effect on mycorrhizal population in soil. Hence, it has been highlighted that in organic agriculture, agricultural activities should be performed by taking mycorrhizal fungi into consideration (Gosling et al., 2006). Another important point that should be emphasized is specifying and using entophyte species. Thus, in greenhouse studies performed in an attempt to determine mycorrhizal fungi improving growth of corn plant, it was reported that isolates taken from different habitats have different adaptation abilities and lead to different outcomes, therefore it was emphasized that it is important to select entophytes with high adaptation abilities by taking vegetation, soil type into consideration (Kühn et al., 1987). It is well known that mycorrhizal fungi have positive effects on plant growth. *Glomus intraradices* and *G. etunicatum* which were inoculated to strawberry plants showed increased crown width, leaf area, and dry root weight (Grabowski et al., 1999). Additionally, it was observed that mycorrhizal fungi prolonged the vase life of cut flowers (Besmer and Koide, 1999), and in another study conducted with *Chrysanthemum morifolium*, it was revealed that the number of flowers, fresh and dry stem weights were increased (Johnson et al., 1982). Furthermore, *Glomus* sp. isolates significantly reduced the percentage of infection caused by *Fusarium oxysporum* f. sp. *lycopersicum* and *Fusarium oxysporum* f. sp. *vasinfectum*, respectively in tomato and pepper (Al-Momany and Al-Raddad, 1998) and that caused by *R. solani* in potato (Yao et al., 2002). Similarly, *G. etunicatum* and *G. monosporum* reduced *Phytophthora fragariae* colonization in strawberry *in vitro* conditions (Norman and Hooker, 2000).Many studies have been performed on fungal biological control agents, particularly on *Trichoderma* spp. The principal mechanisms for control have been assumed to be those primarily acting upon the pathogens and included mycoparasitism, antibiosis and competition for resources and space. Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are also very important (Howell, 2003; Harman, 2006). Studies revealed that *Trichoderma* spp. also stimulates systemic immunity (Hanson, 2000; Hoitink et al., 2006). Similarly, it was determined that *Trichoderma* spp. increased the number of flowers and buds, fresh and dry weight of shoots in flower production (Ousley et al., 1994).This research was aimed at determining the effects of microbial fertilizer containing *Trichoderma harzianum* Kuen 1585 (Tr), bio-preparation (BRDI) containing *Glomus aggregatum*, *G. clarim*, *G. deserticola*, *G. intraradices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita* and *Paraglomus brasilianum,* and *Glomus* sp. collected from corn fields in Aydin province, on plant growth of lilies and against *Rhizoctonia solani* Kühn. (Rs) that has a negative effect on growth of *Lilium* plants and causing decay in bulbs. *In-vivo* conditions and its corresponding results were also analyzed.

MATERIALS AND METHODS

This study was conducted in 2007-2008 in Adnan Menderes University. During the vegetation period, plants were grown in a special climatic controlled room set for 24°C, 60% humidity and

14/10 h light/ dark photoperiod was maintained. In the experiment, *Lilium* bulbs with 10 to 12 cm circumference, and Longiflorum and Asiatic hybrids (LA hybrids) cv, "Ceb Dazzle" were used as plant material. *Lilium* bulbs used in the study were pre-cooled by keeping at 4°C for 8 weeks and they were imported from Holland. Bulbs were transported in boxes filled with moist peat, and between receipt and planting time the bulbs were packed in moist peat and stored at 4 to 5°C in a refrigerator.

In this study, microbial fertilizer with *T. harzianum* Kuen 1585 (Tr) content (Sim Derma, Simbiyotic Company), bio-organics root dip inoculant (BRDI) a commercial preparation (containing *Glomus aggregatum*, *G. clarim*, *G. deserticola*, *G. intraradices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita*, *Paraglomus brasilianum*) and *Glomus* sp. isolated from province of Aydin (Yıldız, 2010) were used in order to determine the effects on plant growth and against soil borne *Rhizoctonia solani* (Rs) (AG4). Studies were conducted in 2 stages; at the 1st stage: Tr, BRDI and *Glomus* sp. were applied on *Lilium* bulbs prior to planting and their effects on plant growth were observed. At the 2nd stage; the effects of treatments of Tr, BRDI and *Glomus* sp. on *Lilium* bulbs grown in *R. solani* Kühn. inoculated soil was observed.

In trial, medium used for planting of *Lilium* bulbs were prepared with soil: peat: sand (1:1:1 by volume) and it was mixed homogenously. This mixture was also sterilized twice in an autoclave once in every two days for 90 min under 121°C before filling pots. The lily bulbs used in the 1st and 2nd stages of trial, were planted in plastic cylindrical pots with 1.2 L volume, 9.5 and 14.0 cm diameter at the bottom and top respectively, and 12.5 cm depth, on the 9th of November 2007. In each pot, one bulb was planted and at the end of vegetation period only one cut flower stalk was harvested from each pot. Three repetitions were used in the study and in total 15 bulbs were used in each treatment – 5 bulbs for each repetition. In all treatments, bulbs were planted in 5 cm depth. The bulbs planted in pots were irrigated with water without nutrient elements until the sprout emergence on bulbs were completed, after completion of sprout emergence nutrient elements were applied to growing substrate via irrigation. They were irrigated with nutrient solution 2 to 3 times a week. As nutrition, "Hoagland" nutrient solution (Hoagland and Amon, 1950) was used.

Effects of Tr, BRDI and *Glomus* sp. on plant growth

Surface of *Lilium* bulbs to be applied Tr, BRDI and *Glomus* sp. were disinfected in 1% sodium hypochlorite and rinsed in sterilized water prior to treatments. Then for Tr treatment, the bulbs were covered with *T. harzianum* preparation such that there was 20 g/kg bulb. In BRDI treatment, *Lilium* bulbs were inoculated with mycorrhizal fungi by dipping into 40 cc/L slurry. In *Glomus* sp. treatment 30 g root inoculums colonizing spore, mycelium and mycorrhizae were added in each pot where bulbs were planted and it was mixed into the substrate mixture. Bulbs used in the control were moistened only with sterilized pure water and upon moisturizing; they were planted in pots containing sterilized mixture prepared as aforementioned. At this stage of the experiment, 4 different treatments; Tr, BRDI, *Glomus* sp. and control were used.

In the trial, observations and measurements were made on bulbs and emergence rate of shoots, emergence time of shoots, rate of flower bud formation and time of flower bud formation were determined. In addition, plant growth was observed by measuring length of *Lilium* plants every week until harvest time. Furthermore, cut flower stalks on *Lilium* plants grown at Stage 1 of trial, were harvested in the date of the first flower on that stalk was opened and measurements related with quality criteria were taken on flower stalk. To this extent; flower stalk length (cm), raceme length (cm), flower stalk diameter (mm), number of nodes, number of leaves, leaf length (cm), number of flower bud, flower bud length (cm), diameter of flower head (cm), vase life (day), fresh flower stalk weight (g) and dry flower stalk weight (g) were measured. Additionally, after harvesting flower stalks, bulbs were left to grow in the pots until the end of vegetation period. At the end of vegetation period, when the aerial parts of lily plants dried, bulbs were deemed to be in dormant period and they were lifted up, then the values related to the root growth performance of bulbs such as root fresh and dry weights were obtained.

Effects of Tr, BRDI and *Glomus* sp. on *R. solani*

At this stage of the study, 2% *R. solani* inoculum was added and mixed in sterilized pot substrate mixture prepared as aforementioned (Sneh et al., 1998). In the control, the pots were filled with only sterilized mixture. After that Tr, BRDI and *Glomus* sp. as explained at Stage 1 of this study, were applied to *Lilium* bulbs and they were planted into the pots. Lily bulbs used in control treatment were planted in pots upon irrigating only with water. This stage of study consisted of 5 different treatments; control, Rs, Rs + Tr, Rs + BRDI and Rs + *Glomus* sp.

During the trial, length of *Lilium* plants were measured once on the same day of each week until harvesting time, thus changes in length of plants according to the treatments were determined. Flower stalks were harvested when the first flower on flower stalk opened. As in the 1st Stage of trial, in the 2nd Stage bulbs were left to grow in the pots until the end of vegetation period after harvesting flower stalks. At the end of vegetation period when the aerial parts of lily plants were dried, bulbs were lifted up and the values of root fresh weight (g) and root dry weight (g) of lifted bulbs were obtained in order to determine the root growth regime of bulbs. Moreover, bulbs were rated in accordance with the following stated 0 - 8 scale in order to determine the severity of diseases caused by Rs (Schneider, 1998).

Disease assessment

For all treatments in both stages of trial, disease severity per bulb was rated on a scale from 0 to 8 (Schneider, 1998) with;

0: 0% of the bulb surface area infected, no distinct *R. solani* symptoms visible;
1: up to 5% of the bulb surface covered with small *R. solani* lesions;
2: up to 12.5% of the bulb surface covered with lesions;
3: up to 25% of the bulb surface covered with lesions;
4: up to 50% of the bulb surface covered with lesions, and/or one bulb scale disrupted;
5: >50% of the bulb surface infected and/or at least two bulb scales disrupted;
6: no daughter bulbs developed, mother bulb apparently healthy;
7: bulb almost completely decayed;
8: bulb completely decayed, plant dead.

Production of *R. solani* inoculum

With the purpose of producing *R. solani* inoculums, inoculums produced in soil-cornmeal culture was used (135 g sand, 15 g cornmeal and 20 ml potato juice) (200 g potatoes/ 1 L pure water), and sterilized in small size autoclave bags once in two days under 121°C for 60 min (Turhan and Turhan, 1989). Three discs which included 4 mm mycelium taken from border of colonies grown for one week in Potato Dextrose Agar (PDA) were left in bags containing sand culture and bags were kept in incubators for 21 days under 24°C for development of inoculum. In order to achieve

Table 1. Abbrevitions used in text.

Trait symbol	Trait description	Trait symbol	Trait description
FSL	Flower Stalk Length	FSFW	Flower Stalk Fresh Weight
RL	Raceme Length	FSDW	Flower Stalk Dry Weight
DFS	Diameter of Flower Stalk	RFW	Root Fresh Weigth
NN	Number of Nodes	RDW	Root Dry Weigth
NL	Number of Leaves	RCR	Root Colonization Rate
LL	Leaf Length	ERS	Emergence Rate of Shoot
NFB	Number of Flower Bud	ETS	Emergence Time of Shoot
FBL	Flower Bud Length	FBFR	Flower Bud Formation Rate
DFH	Diameter of Flower Head	FBFT	Flower Bud Formation Time
VL	Vase life		

Table 2. The effects of treatments on ERS, ETS, FBFR,FBFT and on root growth of *Lilium* bulbs at stage 1.

Treatment	ERS (%)	ETS (day)	FBFR (%)	FBFT (day)	RFW (g)	RDW (g)	RCR (%)
Control	93.33	11.67	80.00	43.43	7.64	0.904	-
Tr	93.33	10.43	86.67	47.67	11.63	1.127	-
BRDI	100.00	7.67	86.67	33.70	7.93	0.678	39
Glomus sp.	93.33	11.70	73.33	41.70	10.59	1.015	65
LSD 5%	ns	ns	ns	ns	ns	ns	

ns, Non-significant ; *significant at P≤ 0.05; **significant at P≤ 0.01.

once in two days without opening. Inoculums produced were mixed homogenous growth of inoculums, bags were shaken and mixedin pots with sterilized mixture in a ratio of 2% (Sneh et al., 1998).

Calculation of root colonization rate

Root colonization rate (RCR) was calculated for *Lilium* plants produced at each stage. In order to calculate the percentage of AM fungi colonization, Grid-Line Intersect Method was used (Giovenetti and Mosse, 1980). Then, 0.5 g pieces were taken from colored roots and 1 to 1.5 cm pieces were cut and observed under microscope. By using the following formula, percentage of root colonization was calculated;

$$\text{AM Colonization (\%)} = \frac{\text{Number of roots colonized with AM}}{\text{Total number of roots}} \times 100$$

Table 1 shows the abbreviations used in text to define the morphological characters which were measured and to define traits used in the trial.

Statistical analysis

The trial was conducted in the completely randomized block design with three replicates. Each group of data obtained from the study conducted in the two stages was subjected to analysis of variance (ANOVA) using Duncan statistical analysis program. The mean values were compared using the least significant difference (LSD) test by JMP (SAS, 1996) in order to study whether these variations are of statistical significance. All analyses of significance were made at the $P<0.05$ level of significance.

RESULTS

Effects of Tr, BRDI and *Glomus* sp. on plant growth

At stage 1 of the study, observations related to plant growth were made on *Lilium* bulbs starting from planting in order to determine effects of Tr, BRDI, *and Glomus* sp on plant growth. To this extent, rate and time of emergence of shoots, and rate and time of flower bud formation were determined. Statistical analysis was performed on these values and as a result of variance analysis, it was observed that there were no variations between treatments (Table 2). Emergence rates of lily bulbs varied between 93.3 and 100 %. As shown in Table 2 from the study, mycorrhizal root colonization rate was determined for BRDI and *Glomus* sp. applied bulbs; RCR of *Glomus* sp. was higher than of BRDI. The values of mycorrhizal RCR were 39% for BRDI and 65% for *Glomus* sp. However, it was seen that there were no correlation between plant growth and quality, and mycorrhizal colonization rate. All lily bulbs planted upon applying BRDI at this stage were emerged and ERS of bulbs were 100%. Similarly, the formation rate of flower buds following the vegetation period of bulbs did not have significant variation between treatments, and in accordance with the treatments, flower buds formed in 73.33 to 86.67% of bulbs planted (Table 2).

Values related to root growth performances of bulbs grown in treatments at this stage were also shown in Table 2. Highest RFW (11.63 g) was obtained in Tr, and

Table 3. The change of plant length of lilies according to the treatments at stage 1.

Treatment	Plant length (cm)								
	27.11 2007	04.12 2007	11.12. 2007	18.12. 2007	25.12. 2007	01.01. 2008	08.01. 2008	15.01. 2008	22.01. 2008
Control	6.37	10.78	19.21	28.29	35.03	40.93	42.15	43.34	45.12
Tr	6.33	10.49	17.18	24.52	30.98	36.98	39.08	40.79	42.18
BRDI	9.07	14.11	22.76	30.61	37.50	39.63	41.17	41.70	42.07
Glomus sp.	5.98	9.97	17.62	25.58	32.46	36.85	39.62	40.87	42.16

*Date.

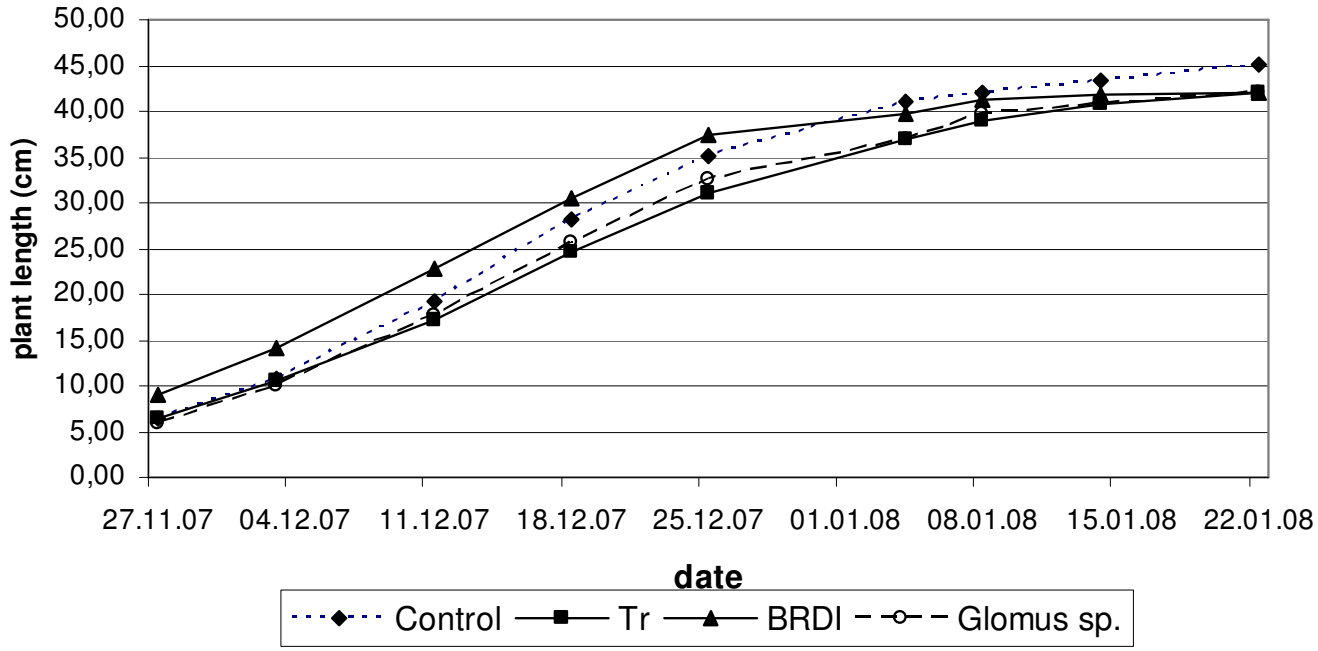

Figure 1. The change of plant length of lily bulbs grown in treatments at stage 1.

analysis of variance of the complete data set related RFW indicated insignificant differences among treatments. Similar results were obtained as RDW in this stage of experiment. When RDW was considered, Tr treatment provided higher values but there were no significant differences between the treatments. In addition, time of shoot emergency and flower bud formation of bulbs were analyzed for each treatment in the study. As a result of assessment, however, it was seen that there were numerical time variations between treatments, there were not any significant statistical variation in ETS and FBFT according to the treatments. In bulbs grown in treatments, ETS values varied between 7.67 and 11.7 days. An analysis on the FBFT values obtained in treatments indicated that the latest flower bud formation was in Tr treated bulbs with 47.67 days.

Effects of treatments in trial on length of plants were assessed by measuring length of shoots starting from the first emergence until harvest time (Table 3). As shown in Figure 1, BRDI treated bulbs had better growth of shoots during the initial growth period following planting when compared with other treatments. At stage 1; variance analysis were performed on values related with quality criteria of flower stalks harvested from treatments and it was observed that treatments have statistically significant effects on FSL ($p = 0.01$), FBL and DFH ($p = 0.05$) quality criteria. Values obtained from treatments related with FSL which is an important quality criteria ranged between 52.68 and 62.03 cm, flowers with longest stalk were produced on bulbs grown in the control. Respectively, *Glomus* sp. (54.82 cm), Tr (54.06 cm) and BRDI (52.68 cm) treatments followed this. When compared with the control, treatments in stage 1 did not provide any positive effects on plant growth; FBL values in BRDI and *Glomus* sp. treatments, DFH values in *Glomus* sp. were higher than other treatments. Accordingly, DFH value in *Glomus* sp. was the highest with 15.27 cm; this was followed by flowers harvested from control with 14.85 cm. In respect

Table 4. The average values of investigated characteristics related to quality of flower stalks.

Treatment	FSL (cm)	RL (cm)	DFS (mm)	NN	NL	LL (cm)	NFB	FBL (cm)	DFH (cm)	VL (day)	FSFW (g)	FSDW (g)
Control	62.03[a]	13.05	5.806	55.1	57.3	9.40	2.033	10.01[b]	14.85[ab]	8.5	42.34	4.27
Tr	54.06[ab]	13.20	5.812	48.5	50.0	8.93	1.933	9.99[b]	14.20[b]	8.3	36.89	3.98
BRDI	52.68[b]	13.91	5.966	48.3	49.6	8.57	1.933	10.56[a]	14.52[b]	7.2	37.90	3.68
Glomus sp.	54.82[ab]	13.38	6.065	48.1	50.7	8.30	2.167	10.36[ab]	15.27[a]	8.7	38.61	3.82
LSD %1	1.859**	ns	ns	ns	ns	ns	ns	3.355*	1.895*	ns	ns	ns

ns, Non-significant ; *significant at P≤ 0.05; **significant at P≤ 0.01.

of FBL values, BRDI and *Glomus* sp. treatments shared top two ranks respectively with 10.56 and 10.36 cm. Length of racemes (RL) on flower stalk where there are nodes of flower buds did not show any variation of statistical significance according to the treatments. RL on stalks ranged between 13.05 and 13.91 cm between treatments (Table 4).

It was observed that the treatments at stage 1 did not have statistically significant effect on DFS which is an important quality criteria in terms of sturdiness of flower stalks, between the treatments. However, flowers harvested on *Lilium* bulbs treated with *Glomus* sp. had the highest stalk diameter (6.065 mm), this treatment was followed by BRDI (5.966 mm). As shown on Table 4, DFS value obtained in control which was at the end of the list, was 5.806 cm. Leaf numbers on flower stalks were counted and the number of leaves were compared between treatments, and it was determined that control had the highest number of leaves and was followed by *Glomus* sp., Tr and BRDI respectively. Moreover, lengths of leaves on flower stalks did not vary according to the treatments and length of leaves ranged between 8.3 and 9.4 cm. In this part of the trial, depending on Tr, BRDI and *Glomus* sp. applied on *Lilium* bulbs, highest number of flower buds (2.167 pcs) were obtained on flower stalks harvested from *Glomus* sp. applied bulbs. However, treatments did not influence the NFBs on flower raceme, and no statistical effects of Tr, BRDI and *Glomus* sp. were observed on flower stalk fresh weight (FSFW) and flower stalk dry weight (FSDW), which were other quality parameters considered in this study, although, both parameters were the highest in flower stalks harvested from the control (Table 4).

Effects of Tr, BRDI and *Glomus* sp. on *R. solani*

When severity of disease caused by *R. solani* on *Lilium* bulbs and effects of treatments against *R. solani* were assessed, bulbs grown in pots where only *R. solani* was inoculated had the highest level of disease severity, however, it was classified in the same group as Rs + BRDI treatment. This was followed by Rs + Tr and Rs + *Glomus* sp. (Table 5).

In Stage 2 of this study, when fresh and dry root weights were analyzed, it was found that fresh and dry root weights showed significant variations at p = 0.05 level between treatments. RFW was the highest in Rs + *Glomus* sp. with 9.002 g. This was followed by control with 7.638 g, while Rs had the lowest value with 4.939 g. Moreover, RDW was highest in control with 0.904 g and Rs treatment had the lowest value with 0.496 g (Table 6).

Plant lengths measured every week starting from the planting of *Lilium* bulbs are given in Table 7. As shown Figure 2, in combined treatments of Rs and Tr, BRDI and *Glomus* sp., plant lengths were lower when compared with the control. Furthermore, as shown in Table 7, plant lengths of bulbs grown in Rs + Tr, Rs + BRDI, Rs + *Glomus* sp. treatments were 35.71, 39.00 and 42.31 cm, respectively. Result also indicated that the length of *Lilium* plants produced by inoculating only Rs were the shortest with mean value of 33.19 cm.

DISCUSSION

One of the disease-causing pathogen in ornamental geophytes plants, which also includes *Lilium* is *R. solani* (Miller, 1998; Chase, 2005). *R. solani* Kühn. is an important soil borne pathogen which has wide range of hosts (Sneh et al., 1998) and also it causes disease problems in geophytes which can be propagated by using bulbs such as *Tulipa* sp. (Schineider, 1998, Schineider et al., 1999, 2001). Thus, *R. solani* used in this study lead to high disease severity on *Lilium* bulbs (Table 5). Last decades biologic agents against the control of diseases are becoming more common. Most common biologic fungal agents are *Trichoderma* spp. (Harman, 2006) and mycorrhizal relationship, which is the oldest symbiotic life form in nature (Smith and Read, 2008). Therefore, biologic agents were used against pathogens.

When effects of Tr, BRDI and *Glomus* sp. were assessed, Tr, BRDI treatments had relatively positive effects on Rs, and it was observed that the lowest disease severity caused by Rs was in *Glomus* sp. treatment. Plants inoculated with Rs isolated from strawberry had disease severity of 67% and this value was reduced to 31% in plants which were treated with Rs

Table 5. Disease severity caused by *R. solani* on *Lilium* bulbs.

Level	Mean
Control	0.00[d]
Rs	4.73[a]
Rs+Tr	3.47[b]
Rs+BRDI	3.93[ab]
Rs+*Glomus* sp	1.20[c]
LSD 5%	1.99

ns, Non-significant; *significant at P≤ 0.05; **significant at P≤ 0.01.

Table 6. The effect of *R.solani* on root growth of *Lilium* bulbs at stage 2.

Treatment	RFW (g)	RDW (g)	RCR (%)
Control	7.648[ab]	0.904[a]	-
Rs	4.939[b]	0.496[b]	-
Rs+Tr	5.849[b]	0.547[b]	-
Rs+BRDI	5.470[b]	0.511[b]	25
Rs+*Glomus* sp.	9.002[a]	0.782[ab]	64
LSD %1	1.812*	1.812*	

ns, Non-significant ; *significant at P≤ 0.05; **significant at P≤ 0.01

Table 7. The change of plant length of lilies according to the traits at stage 2.

Treatments	Plant length (cm)								
	27.11 2007*	04.12 2007	11.12. 2007	18.12. 2007	25.12. 2007	01.01. 2008	08.01. 2008	15.01. 2008	22.01. 2008
Control	6.37	10.78	19.21	28.29	35.03	40.93	42.15	43.34	45.12
Rs	4.77	8.49	13.64	18.91	25.03	30.64	31.68	32.43	33.19
Rs+Tr	6.48	9.01	14.56	22.33	29.14	33.54	34.72	35.16	35.71
Rs+BRDI	6.37	10.45	19.18	26.81	34.03	37.92	38.63	38.92	39.00
Rs+*Glomus* sp.	5.36	8.30	15.53	23.31	29.90	36.69	38.58	40.92	42.31

*Date.

and BioOrganics (Bayözen and Yıldız, 2009). Plants with AM formation provide considerable protection against several soil borne fungal pathogens (Dehne, 1982), and this reduces the infection percentage of pathogen in plants (Al-Momany and Al-Raddad, 1998; Demir, 1998; Norman and Hooker, 2000). To this extent, in this study, disease severity was 4.73 in treatment where only Rs was inoculated, and severity of disease caused by Rs was 1.20 on bulbs treated with *Glomus* sp. and planted in pots filled mixture inoculated Rs (Rs + *Glomus* sp.) (Table 5). Plants inoculated with *Glomus* sp. have better root growth performance when compared with plants inoculated with only pathogen (Zheng et al., 2004). In this study, RFW values of lily plants grown by Rs + *Glomus* sp. were higher than the values obtained on lily plants inoculated with only Rs. Thus, in terms of RCR, plants inoculated with mycorrhizae, *Glomus* sp. (64%) had better colonization when compared with BRDI (25%) (Table 6).

It is reported that *Trichoderma* spp. stimulates plant growth in different mechanisms (Harman, 2006). However, isolate used in our study did not present any significant effect. Moreover, it was observed that *Trichoderma* spp. and the other treatments in the 1st stage of the trial did not have any significant effects on emergence of shoots on bulbs and flower bud formation (ERS, ETS, FBFR and FBFT). Emergence rate of bulbs ranged between 93.3 and 100%. Assuredly, it was thought that variation in emergence rate of shoots in treatments were caused by the amount of reserved mineral elements in the bulbs and physiological structure of bulbs. In this stage of the study, mycorrhizal

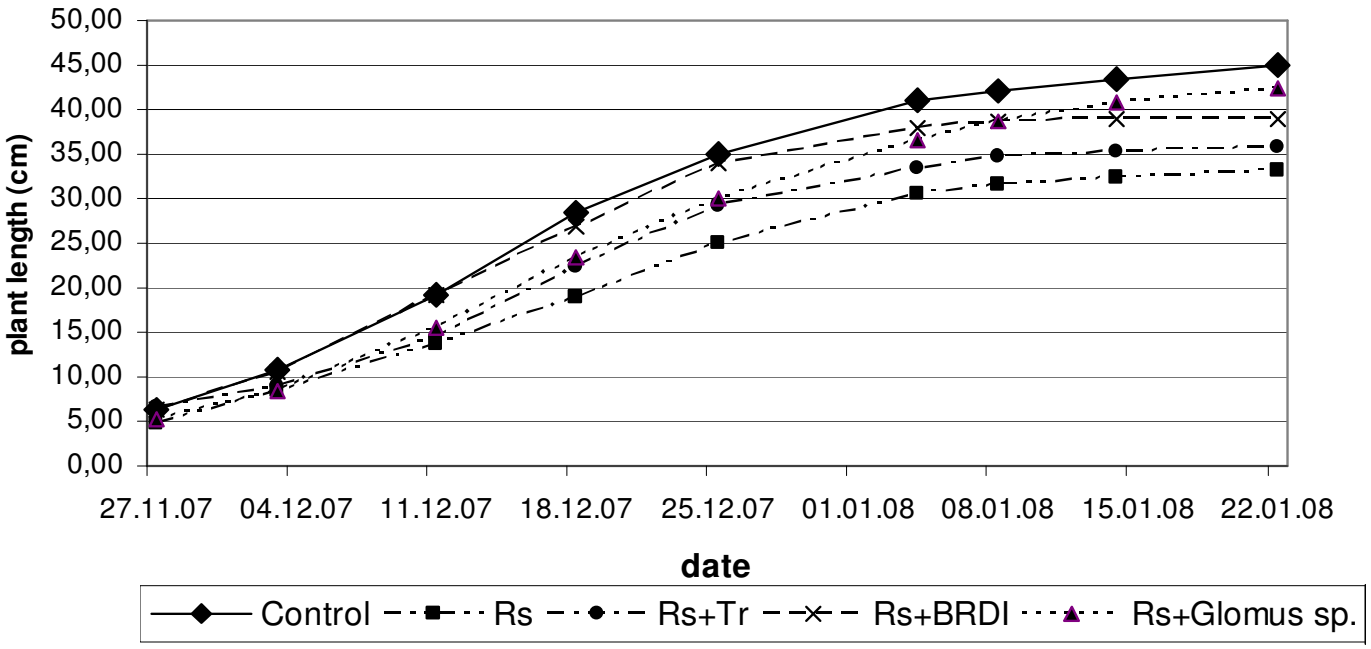

Figure 2. The change of plant length of lily bulbs grown in treatments at stage 2.

colonization rates in roots of BRDI and *Glomus* sp. applied on bulbs were measured and mycorrhizal colonization rates were 39 and 65%, respectively (Table 2). However, there was no parallelism between plant growth and quality criteria, and mycorrhizal colonization rate. As it is known, it is important to use local species in mycorrhizae treatments (Kühn et al., 1987; Gosling et al., 2006). However, plant species may also show different reactions in regards to mycorrhizal colonization. Thus, in their study, Scagel and Schreiner (2006) used 'Majestic Red' and 'Pot of Gold' calla lily cultivars, and they reported that reactions asserted against mycorrhizal colonization varied in different plant varieties. It was reported that Majestic Red variety used in study blossomed later than Pot of Gold variety.

When AM fungi have a symbiotic relationship with host plants, they stimulate water intake and some nutrient mineral intake of the plant (Hayman, 1982; Demir, 1998; Kim et al., 2002). However, in this study, generally, control plants and Tr, BRDI and *Glomus* sp. applied plants did not have any significant variation in terms of growth performance. It was concluded that this situation is caused by the fact that lilies obtain nutrient elements which were required at initial growth periods from nutrients stored in the bulb. In addition, the effects of treatments on plant growth were analyzed and it was observed that there was a significant variation among criteria such as FSL, FBL and DFH. *Glomus* sp. treatment led to a significant variation in DFH and provided the best results. Moreover, when it was analyzed in terms of FBL, *Glomus* sp. and BRDI had the highest values with 10.56 and 10.36 cm, respectively.

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