

Testing of efficacy of arbuscular mycorrhizal product Symbivit® on three species of balcony plants

Experiments conducted in the Research Institute of Ornamental Gardening by Dr. Sramek and Ing. Dubsky, Pruhonice, Czech Republic



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Summary:

Effects of the inoculation of rooting substrate with arbuscular mycorrhizal fungi, with *Trichoderma harzianum*, and with both microorganisms and the effect of application of fungicide Previcur were tested in an experiment with three species of balcony plants (*Verbena*, *Torenia*, *Diascia*) propagated from cuttings. Mycorrhizal inoculation positively affected fresh weight, dry weight and total shoot length of *Verbena* plants and dry weight and total shoot length of *Torenia* plants. Mycorrhiza improved leaf colour of all three species, the greatest differences of this parameter between inoculated and uninoculated plants were found particularly before transplantation, whereas they almost diminished at the end of the experiment. *Trichoderma harzianum* had no effect on any growth parameter or leaf colour. Plants in the treatments inoculated with both micro-organisms showed similar positive growth response as in the treatments inoculated with mycorrhizal fungi.

Purpose/Aims:

The aim of this work was to evaluate the effect of inoculation of rooting substrate with arbuscular mycorrhizal fungi and with *Trichoderma harzianum* or with both micro-organisms together on the growth of three balcony plants *Verbena*, *Torenia* and *Diascia* propagated from cuttings.

Procedure/Methods:

Herbaceous cuttings of three balcony plant species were rooted in substrate containing 4 volume parts of peat-perlite (3:1) substrate and 1 volume part of Vermiculite with or without inoculum (Table 1). The experiment involved four treatments: control uninoculated plants (C), plants inoculated with the AMF (M), plants inoculated with *Trichoderma harzianum* (T) and plants inoculated with both AMF and *Trichoderma* (MT). The AMF inoculum contained spores, mycelium and colonized root fragments of three pure AMF cultures: *Glomus fistulosum*, *G. mosseae* and *G. intraradices*. All cultures have been maintained separately on maize grown in Vermiculite-sand mixture (5:1) for 4 months in the greenhouse. One volume part of the AMF inoculum was mixed with 9 volume parts of Vermiculite for better homogenisation and this mixture was then added to peat-perlite substrate. The dose of inoculum was 0.02 L per 1 L of substrate. Inoculum of *Trichoderma harzianum* was applied as a product "Supresivit" (Fytovita s.r.o., Praha, Czech Republic) in concentration 0.002 g per one litre of substrate.

Table 1: Experimental design and treatments of the experiment with inoculation of three species of balcony plants vegetatively propagated from cuttings and inoculated with arbuscular mycorrhizal fungi and *Trichoderma harzianum*.

Plants	<i>Verbena</i> Temari hybrid 'Pink' <i>Diascia elegans</i> <i>Torenia</i> hybrid 'Summer Wave'
Variants	C – control M - arbuscular mycorrhizal fungi T - <i>Trichoderma harzianum</i> MT - arbuscular mycorrhizal fungi + <i>Trichoderma harzianum</i>
No of replications	5
No of plants in each replicate	<i>Verbena</i> 20, <i>Torenia</i> 10, <i>Diascia</i> 10
Rooting substrate	peat-Perlite substrate + Vermiculite (with or without mycorrhizal or <i>Trichoderma</i> inoculum), volume ratio 4:1
Pinching	<i>Verbena</i> and <i>Torenia</i> above the second leaf couple
Substrate	mixture of peat, composted bark and clay (volume ratio 2:2:1) with 3 g/l of limestone, 2.5 g/l of NPK fertilizer
Fertilization	3× 0.2 % nutrient solution of Kristalon Blue (20/6/19)

Trays with unrooted cuttings were placed to the greenhouse with day/night temperature 20/18 °C. They were covered by PE foil for 10 days and by porous foil for the next 7 days. After one-month cultivation without fertilizer application the rooted cuttings were transplanted into the growing substrate. The length of the longest lateral shoot of *Verbena* plants was estimated on April 20, 1998 before transplanting. Fresh weight, dry weight, total shoot length (*Verbena*, *Torenia*), leaf area, and number of inflorescences (only for *Verbena*) were estimated at the end of the experiment when plants reached marketable size. *Diascia* plants were evaluated only visually according to colour of their leaves and size classes of the plants. The root samples from 10 randomly selected plants per treatment were stained with Trypan blue according to Phillips, Hayman (1970) using lactoglycerol instead lactophenol and mycorrhizal colonization was determined by grid-line intersect method (Giovannetti, Mosse 1980).

Results/Discussion:

Mycorrhizal inoculation of rooting substrate positively affected growth and quality (leaf coloration) of all three tested plant species. Visual differences were apparent mainly before transplanting. Leaves of plants with in both mycorrhized treatments (M, MT) were healthy green in comparison with chlorotic leaves of plant without mycorrhiza (C, T). Before transplanting significant effect of mycorrhizal inoculation on the growth of lateral shoots was found at *Verbena* plants (Table 2). Relatively low level of available nutrients in the rooting substrate (Table 3) without additional fertilisation facilitated favourable conditions for appropriate function of AMF and our findings were consistent with the results of other authors (Johnson et al., 1982; Chang, 1994). After transplanting into the growing substrate with higher available nutrient level (Table 3) and after application of fertiliser solution the differences in leaf colour became less apparent, but growth trends were the same as in the rooting substrate. Detailed evaluation at the end of the experiment confirmed positive influence of the AMF on the plant growth. Mycorrhiza significantly increased fresh weight, dry weight, total shoot length and number of inflorescences of *Verbena* plants and dry weight and total shoot length of *Torenia* plants (Tables 2, 4, 5). Application of *Trichoderma harzianum* into rooting substrate did not affect plant growth nor leaf colour in comparison with control plants (Tables 2, 4, 5). Nevertheless, *T. harzianum* showed compatibility with AMF inoculation and no inhibitory effects on mycorrhizal growth response of mycorrhized plants occurred. Moreover, there was no inhibitory effect of *T. harzianum* on the development of mycorrhizal colonization by contrast to previously reported reduction of extraradical mycelium of *G. intraradices* (Green et al., 1999). Nevertheless, no inhibition of P transport via hyphae was found in that study, therefore the authors suggested that *T. harzianum* exploited only dead mycelium of the AMF. By contrast Calvet et al., (1993) found the increase of AMF colonization after preinoculation of substrate with *Trichoderma aureoviride*.

Table 2. Effect of inoculation with arbuscular mycorrhizal fungi and *Trichoderma harzianum* on the growth of *Verbena* - nondestructive analysis of plants uninoculated (C) or inoculated with arbuscular mycorrhizal fungi (M), *Trichoderma harzianum* (T) or combination of both inoculants (MT). ¹ANOVA results, means followed by the same letter are not significantly different within one factor and parameter according to Duncan's Multiple Range test, p<0.05.

Factor			Lateral shoot (cm)	Total shoot length (cm)
Mycor.x Trich.	00	(C)	2.9 c	83.0 b
	10	(M)	5.5 a	98.2 a
	01	(T)	2.5 c	78.1 b
	11	(MT)	5.6 a	95.3 a

Table 3. Chemical properties of substrates used in the experiment.

Substrates	pH	EC mS/cm	N-NH ₄ mg/100g	A-NO ₃ mg/100g	P ₂ O ₅ mg/100g	K ₂ O mg/100g	CaO mg/100g
Rooting substrate	5.47	0.48	5	14	2	30	620
Growing substrate	5.18	1.62	4	44	53	52	970

Table 4: Effect of inoculation with arbuscular mycorrhizal fungi and *Trichoderma harzianum* on the growth of *Verbena* - destructive analysis of plants uninoculated (C) or inoculated with arbuscular mycorrhizal fungi (M), *Trichoderma harzianum* (T) or combination of both inoculants (MT) ¹ANOVA results, means followed by the same letter are not significantly different within one factor and parameter according to Duncan's Multiple Range test, p<0.05.

Factor			Fresh weight(g)	Total shoot length (cm)	No of inflorescences	Mycorrhizal Colonization (%)
Mycor.x Trich.	00	(C)	8.8 b	84 b	0.05 b	0 b
	10	(M)	11.3 a	106 a	1.00 a	25 a
	01	(T)	8.7 b	84 b	0.10 b	0 b
	11	(MT)	10.3 ab	111 a	0.60 ab	21 a

Table 5. Effect of inoculation with arbuscular mycorrhizal fungi and *Trichoderma harzianum* on the growth of *Torenia* - destructive analysis of plants uninoculated (C) or inoculated with arbuscular mycorrhizal fungi (M), *Trichoderma harzianum* (T) or combination of both inoculants (MT). ¹ANOVA results, means followed by the same letter are not significantly different within one factor and parameter according to Duncan's Multiple Range test, p<0.05.

Factor			Shoot dry weight (g)	Total shoot length (cm)	Mycorrhizal Colonization (%)
Mycor.x Trich.	00	(C)	1.09 b	101 b	0 b
	10	(M)	1.31 ab	134 a	20 a
	01	(T)	1.17 b	104 b	0 b
	11	(MT)	1.44 a	120 ab	19 a

In general no negative effects of AMF inoculation has been found in any treatments what favours practical applications and confirms results of most practical tests with AMF inoculations in horticulture (Nemec, 1987). Low specificity of AMF to host plant seems to be of a great advantage, however, chemical properties, especially nutrients concentrations, of cultivation substrate can influence development and efficiency of introduced symbionts. That was indicated also by higher response of plants to AMF inoculation in early stages of cultivation in less fertile substrate. Particular care should be paid to quality and purity of inocula at its preparation since AMF cannot be grown axenically and therefore there is a danger of inoculum contaminants e.g. root pathogens transfer from open pot cultures commonly used for inoculum production to inoculated substrate. As suggested by Chang (1994) the combination of crop - AMF species should be also tested for particular cultivation system before large scale application.

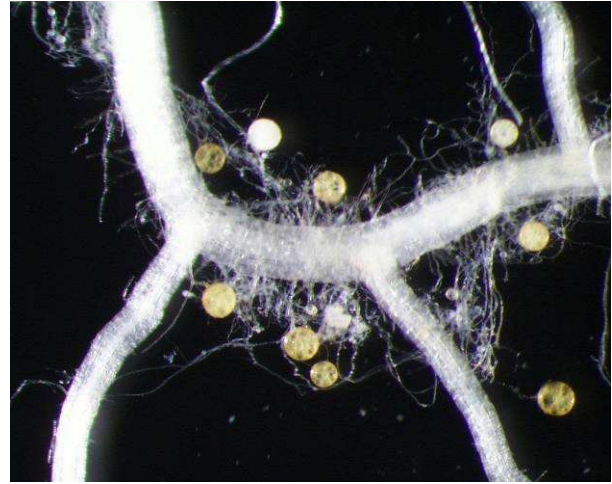
The inoculation with AMF itself or in combination with *Trichoderma harzianum* can be a feasible biotechnology used in horticulture for better exploitation of nutrients from substrates and for improvement of growth of horticultural crops.

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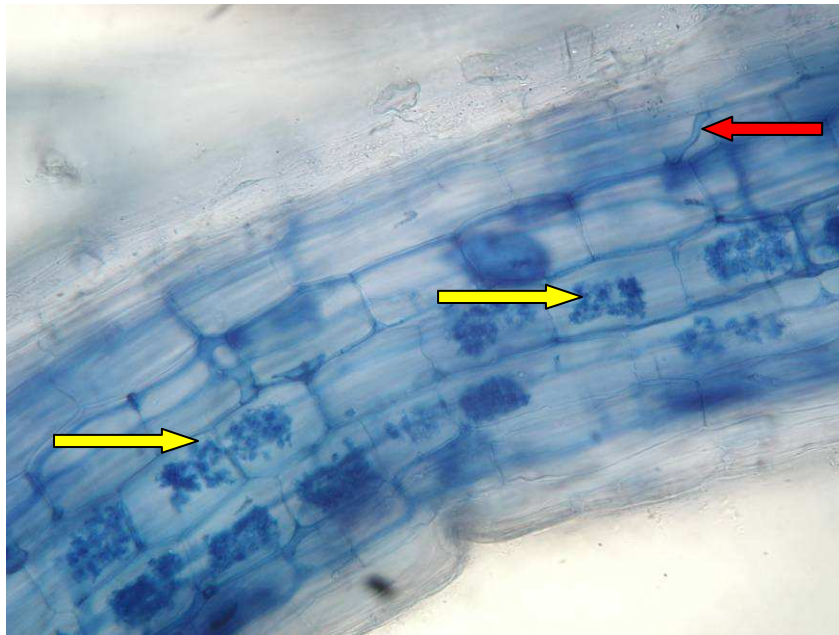
Inoculated (bottom) plants of *Verbena* showed highly significant increase in flowering compared to uninoculated plants (top) of the same age.



Root colonized by endomycorrhizal fungus. Circle objects are spores, entangled in extraradical mycelium.



Uninoculated, i.e. control (left) and inoculated (right) plants of *Verbena*, *Torenia* and *Diascia* - already at early stages of propagation there was a visible effect of mycorrhizal inoculation



Mycorrhizal root with visible arbuscules (yellow arrows) and running hyphae (red arrow).