Floriculture and Ornamental Biotechnology ©2012 Global Science Books

Effect of Microbiological Products on Bulblet Development of *Lilium* spp. in Scale Culture

Antra Balode*

Faculty of Agriculture, Latvia University of Agriculture, Liela 2, LV 3001, Latvia

Corresponding author: * **antra.balode@llu.lv**

ABSTRACT

The aim of the present study was to investigate the effects of two locally produced microbiological products *Trihodermins* B-J and *Vitmins* on scale promotion and bulblet development in *Lilium*. Lily scales were given three treatments: untreated controls; *Vitmins* solution (10 ml L^{-1}) and *Vitmins* solution (10 ml L^{-1}) + *Trihodermins* B-J (dry powder form, at 10 g kg^{-1} in the substrate). After treatment, scales were placed in a polyethylene bag in peat moss. Bulbs of three cultivars from different groups were used: Asiatic hybrid 'Gardenja', *Longiflorum* × Asiatic (LA) hybrid 'Sonora' and Trumpet hybrid 'Elegija'. The scales were incubated in a plastic greenhouse at 15°C for 12 weeks; at 5°C for 10 weeks; and 18°C for 4 weeks. The effectiveness of microbiological products was evaluated by the number of bulblets per scale, the diameter of bulblets, shoot height, root length, and the percentages of dead plants. Significant difference ($P < 0.05$) between shoots and roots among the three cultivars and variants were found. The mean values obtained for shoots by treatment with *Vitmins* + *Trihodermins* B-J were: 'Gardenja' - 138.5 ± 9.8 mm, 'Sonora' – 102.0 ± 4.7 mm and 'Elegija' – 7.0 ± 3.0 mm. The coefficient of variation for number of bulblets per scale was recorded in the range from 19.7 to 39.2%. Treatment with *Vitmins* + *Trihodermins* B-J resulted in a significantly higher number of bulblets per scale and greater shoot height ($P < 0.05$ vs. control).

Keywords: *Fusarium oxysporum* f.sp. *lilii*, disease rating, peat moss, propagation, *Trihodermins* B-J, Vitmins
Abbreviations: CFU, colony forming units

INTRODUCTION

Bulbs of the genus *Lilium* can be propagated by regeneration of scale bulblets on detached scales (Bonnier *et al*. 1996). When propagated by scales, they can be damaged (broken off at the basal plate), and subjected to infection.

Often a considerable percentage of the new bulblets fail because of contamination by soil organisms. The soil borne fungus *Fusarium oxysporum* f.sp. *lilii* Imle is the most serious pathogens of lily (Straathof *et al.* 1993) and can survive in the soil at least tree years without host plant (Baayen *et al*. 1998). The disease can be controlled by disinfecting bulbs before planting in combination with soil fumigation (Straathof *et al.* 1993). Bulblets are dipped into a fungicide to protect against soil borne diseases, such as *Fusarium* spp. and *Cylindrocarpon* spp. (Grassoti 1997; McRae 1998).

Measures against soil borne diseases may not always result in sufficient control, or may result in a high input of pesticides (Boer *et al.* 2005). To reduce environmental pollution created by application of chemicals, microbiological products and plant growth regulators are being trialed. The use of biological agents to induce plant resistance mechanism against pathogens represents an ecologically friendly alternative to fungicides that must be repeatedly used for control of plant diseases (Elad 2000). Microorganisms that antagonize fungal pathogens can be used to control root diseases that are otherwise difficult to treat conventional means (Broekaert 1994).

Several reports have demonstrated the successful use of biological control against *Fusarium* spp. in greenhouse environments (Reid *et al*. 2002; Rose *et al.* 2004; Sage 2005). The fungus *Trichoderma* spp. has had a positive effect in biological control. *Trichoderma* spp. produces metabolites that demonstrate antibiotic and mycoparasitic activity against a wide range of phytopathogens (molds, root rots, black scab, grey rot, white rot, powdery mildew, wilt and fruit rot) (El Neshawy *et al.* 1999; Clarkson *et al.* 2004). *Trichoderma* spp. compete with pathogens for nutrients; acts as parasite in the cells of pathogen fungus; produces antibiotic compounds (glioxine, viridine, trichodermine), thus inhibiting the pathogen (Shternshis 2005).

Trichoderma spp. are widely distributed all over the world, and fungi belonging to this genus, are easily isolated from soil (Vanacci and Gullino 2000; Mako and Alimova 2006). The mechanism of action of *Trichoderma harzianum* Rifai, which controls foliar pathogens, has also been attributed to induce defense against pathogens (Elad 2000). *Trichoderma* spp. has also demonstrated positive effects on biological control of vegetable diseases (Inbar *et al.* 1994). *Trichoderma viride* Pers. can also inhibit the development of cucumber, tomato and pepper seedlings as a "minor pathogen" (Menzies 1993). The species *Trichoderma harzianum* has also been described as a biocontrol agent to control several fungi such as *Sclerotinia minor* and *S. sclerotiorum* (Sanchi *et al*. 2005). Most of biological control research has concentrated on *Gliocladium* and *Trichoderma*, mycoparastitic *Pythium*, non-pathogenic *Fusarium*, binucleate *Rhizoctonia*, as well as antagonistic bacteria belonging to the genus *Bacillus*, fluorescent *Pseudomonas* and *Streptomyces* (Vanacci and Gullino 2000). Application of various microorganisms (*Pseudomonas* spp., *Azotobacter* spp.) stimulated seed germination and rooting of plant cuttings (Lielpetere 2009).

The use of biological agents to induce plant defense mechanism against pathogens represents an ecologically friendly alternative to fungicides, which must be repeatedly used for control of plant disease (Elad 2000).

MATERIALS AND METHODS

Biological control agents

The microorganisms were obtained from the Microbial Strain Collection of Latvia. In the trial were used *Trihodermins* B-J (further - *Trihodermins*) and *Vitmins*, both selected trade names, produced by the Latvian company Bioefekts (SME, Latvia

Received: 17 December, 2010. Accepted: 20 December, 2012.

Short Communication

www.bioefekts.lv).

Trihodermins is a microbiological product for plant protection and contains cells of *Trichoderma harzianum* 8-21 and *Trichoderma viride* 1-5. *Trihodermins* dry powder form (10^7–10^9 CFU/g of peat moss) was used.

Vitmins regulate plant growth because it contains beneficial metabolism products – auxins, cytokines, amino acids and gibberellin – released by various microorganisms (*Pseudomonas* spp., *Azotobacter* spp.). Application of *Vitmins* stimulate seed germination and rooting of plant cuttings. *Vitmins* also promotes plant ability to absorb mineral nutrients (Lielpetere 2009). In this trial *Vitmins* solution form (10 ml L^{-1}) was used.

Plant material

In September, 2009, bulbs from the *Lilium* collection of the Latvia University of Agriculture in Jelgava were harvested and used for scale bulblet induction.

Three cultivars from three groups: Asiatic hybrid 'Gardenja', the *Longiflorum* × Asiatic (LA) hybrid 'Sonora' and the Trumpet hybrid 'Elegija' were used as plant material. All cultivars are registered in The International Lily Register (Leslie 1982).

1. Treatments

For each cultivar, scales from 10 bulbs were detached for propagation. Scales were treated in three ways: 1) Water, as control; 2) *Vitmins* solution (10 ml L^{-1}) and 3) *Vitmins* solution plus *Trihodermins* dry powder form (10 g kg^{-1}), added to substrate in a polyethylene bag. The exposure time for the liquid treatments was 15 minutes. There were 4 replicates of 10 scales each. After liquid treatment, scales were placed in moist peat moss in a polyethylene bag and stored in a plastic greenhouse at 15°C for 12 weeks; at 5°C for 10 weeks; and 18°C for 4 weeks to regenerate scale bulblets.

2. Evaluation

After incubation, scales with bulblets were removed from polyethylene bags and evaluated. Measurements were made of the number of bulblets per scale, bulblet diameter (mm), shoot height (mm) and root length (mm). Disease incidence was determined by counting the number of healthy and dead plants during the harvesting.

No pesticides were used in the trial. The obtained data were analyzed by dispersion analysis and means separation were determined using Fisher's least significant difference (FLSD) test ($P < 0.05$).

RESULTS AND DISCUSSION

For cultivar 'Gardenja' the two biological treatments yielded longer shoots than the untreated controls and there were significant difference ($F_{fact} > F_{0.05}$). For 'Sonora' *Vitmins* + *Trihodermins* treated scales produced significantly higher shoots ($F_{fact} > F_{0.05}$) compared to the *Vitmins* and control treatment. 'Elegija' shoots were very short (0.7 mm), and there were no differences between the three treatments (**Fig. 1A**). For 'Gardenja' and 'Sonora', the effect of biological treatments on the root length were significant ($F_{fact} > F_{0.05}$) compared to the control. 'Elegija' showed no significant differences between treatments (**Fig. 1B**).

In all cases the control treatment yielded significantly smaller bulblets than the *Vitmins* and *Vitmins* + *Trihodermins* treated scales (represents significance level of $P < 0.05$) (**Table 1**). The diameter of bulblets was characterized with great phenotypic variability.

The reason bulblet size varied is: 1) cultivar (some cultivars have larger bulblets than others) and 2) treatment. Presumably the environment did not vary a lot within the experiment, as the physical size of the experiment was very very small.

The resistance to *Fusarium* spp. was tested. Trial results indicate resistance differences between the various cultivars and treatments (**Table 2**).

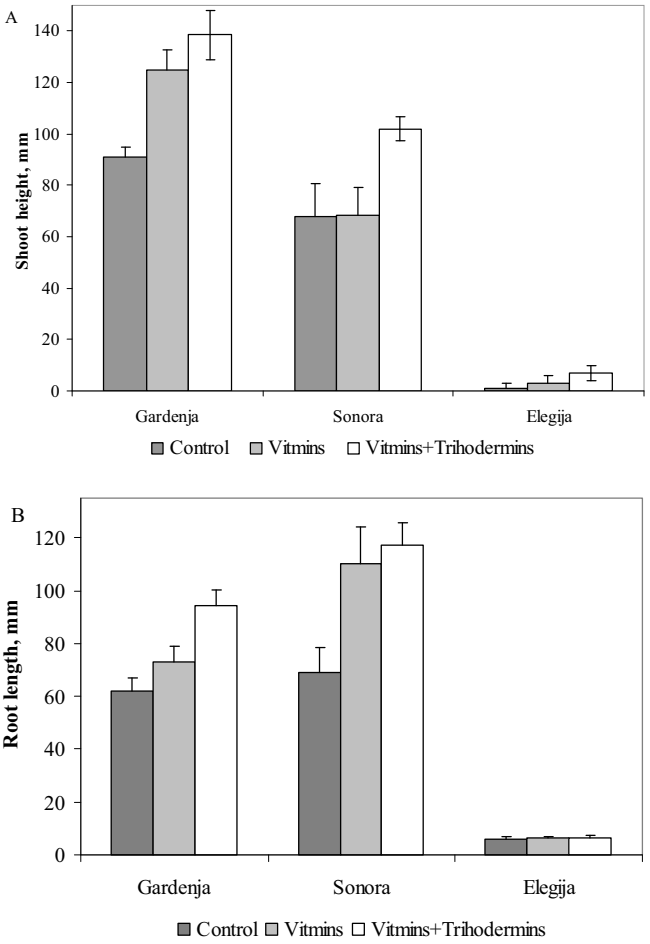

Fig. 1 The effect of different plant growth regulators on the shoot height (**A**) and root length (**B**) after 26 weeks of incubation. Values represent mean ± standard error (SE).

Table 1 The effect of different plant growth regulators on the diameter of bulblets (in mm) regenerating from scales (n = 40).

Treatment	Gardenja	Elegija	Sonora
Control	8.8	12.6	10.0
Vitmins 10 ml L^{-1}	10.4	14.4	10.9
Vitmins 10 ml L^{-1} + *Trihodermins* 10 g kg^{-1}	11.7*	14.7*	11.5*

* Significant differences ($P < 0.05$) from the control.

Table 2 Number of bulblets tested for *Fusarium* resistance, percentage of plants that died during propagation after 26 weeks of incubation.

Cultivar	No. of bulblets	Control	*Vitmins* 10 ml L^{-1}	*Vitmins* 10 ml L^{-1} + *Trihodermins* 10 g kg^{-1}
Gardenja	92	20.0	7.5*	6.3*
Elegija	133	5.1	2.5*	1.3*
Sonora	94	17.2	13.3*	12.5*

* Significant differences ($P < 0.05$) from the control.

All cultivars had a significant difference ($F_{fact} > F_{0.05}$) between the average indices of variants (treated and untreated). In these plants, significant increase in resistance was detected ($F_{fact} > F_{0.05}$) – treatment with *Vitmins* + *Trihodermins* had enhanced resistance to the *Fusarium* spp.

It was found, spraying with fungal suspension *Trichoderma viride* and *T. harzianum*-based products, significantly decreased the severity of diseases (Sternshis 2005). Our results concur with the literature findings that *T. harzianum* and other species are the antagonists against soil borne diseases (Vanacci and Gullino 2000).

The product *Vitmins* contains microorganisms *Pseudomonas* spp. that increase growth of leaves and decrease the severity of diseases. The results confirm that metabolites

(antibiotics and hydrolytic enzymes) of *Trichoderma* spp. and *Pseudomonas* spp. are very important in the decrease of disease severity against *Fusarium oxysporum* (Lielpetere 2009).

De Boer *et al*. (2005) reported that antagonistic *Pseudomonas* spp. strains producing the antibiotic 2,4-diacetylphloroglucinol or producing biosurfactants showed *Pythium* spp. root rot control of different bulb crops in pot experiments under controlled conditions. It was found that strains of *Pseudomonas* isolated from soil have proved to be capable of lysing hyphae of *Fusarium* species (Orlikowski and Skrzypczak 1992). The species *Pseudomonas putida* has also been described as a biocontrol agent to control several fungi such as *Botrytis elliptica* (Liu *et al*. 2008).

CONCLUSIONS

To assist propagation by scales and to stimulate growth of bulblets and avoid infection, microbiological products have been used - bulb scales treated with *Vitmins* solution 10 ml L^{-1} + *Trihodermins* 10 g kg^{-1}, substrate used - peat moss. The scales of bulbs treated with *Trihodermins* showed not only a reduction in disease incidence, but also produced larger bulblets.

REFERENCES

Baayen RP, Forch MG, Waalwijk C, Bonants PJM, Loffler HJM, Roebroeck EJA (1998) Pathogenic, genetic and molecular characterisation of *Fusarium oxysporum* f.sp. *lilii. European Journal of Plant Pathology* **104**, 887-894

De Boer M, Van den Ende E, Van Os G, Bijman V, De Weerd R (2005) Integrated Management of soil borne diseases in flower bulb production. *Acta Horticulturae* **673**, 73-78

Bonnier FJM, Jansen RC, Van Tuyl JM (1996) Long term lily scale bulblet storage: Effects of temperature and storage in polyethylene bags. *Annals of Applied Biology* **129**, 161-169

Broekaert WF (1994) Antifungal proteins and their application in the molecular breeding of disease-resistant plants. *Acta Horticulturae* **355**, 209-211

Clarkson JP, Mead A, Payne T, Whipps JM, Warwick HRI (2004) Effect of environmental factors of *Sclerotium cepivorum* isolate on sclerotial degradation and biological control of white rot by *Trichoderma. Plant Pathology* **53**, 353-362

Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection* **19**, 709-714

El Neshawy S, Osman N, Okasha KH (1999) Biological control of neck rot and black mould of onion. *Egyptian Journal of Agricultural Research* **77**, 125-137

Inbar J, Abramsky M, Cohen D, Chet I (1994) Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *Plant Pathology* **100**, 337-346

Grassoti A (1997) The effects of scale incubation temperature treatments on number and growth of regenerated bulblets, to reduce production time of commercial lily bulbs. *Acta Horticulturae* **430**, 221-226

Guzhov JL (1978) Ways of use regularity of modicative variability of quantitative traits in plant breeding. Scientific news of the Academy of Sciences (*Izvestija*). *Biology* **3**, 418-429 (in Russian) **Гужов ЮЛ** (1978) Пути использования в селекции растений закономерностей модификационной изменчивости количественных признаков

Leslie AC (1982) *The International Lily Register*, The Royal Horticultural Society, London, (supplements 10, 19), 377 pp

Lielpetere A (2009) *Biological Plant Protection Products and Microbiological Preparation*, Riga, SIA Bioefekts, 132 pp (in Latvian) **Lielpētere A** (2009) Bioloģiskie augu aizsardzības līdzekļi un mikrobioloģiskie preparāti

Liu YH, Huang CJ, Chen CY (2008) Evidence of induced systemic resistance against *Botrytis elliptica* in lily. *Phytopathology* **98**, 830-836

Mako GA, Alimova FK (2006) The growth kinetic of *Trichoderma harzianum* G-(432) in greenhouse soil of Tataristan (Russia). *Biotechnology* **5 (3)**, 280-283

McRae EA (1998) *Lilies: A Guide for Growers and Collectors*, Timber Press, Portland, Oregon, 391 pp

Menzies JG (1993) A strain of *Trichoderma viridum* pathogenic to germinating seedlings of cucumber, pepper and tomato. *Plant Pathology* **42**, 784-794

Orlikowski LB, Skrzypczak Cz (1992) Biological control of pathogens on ornamental bulb plants. *Acta Horticulturae* **325**, 763-768

Reid TC, Hausbeck MK, Kizilkaya K (2002) Use of fungicides and biological control in the suppression of *Fusarium* crown and root rot of asparagus under greenhouse and growth chamber conditions. *Plant Diseases* **86**, 493-498

Rose S, Yip R, Punja ZK (2004) Biological control of *Fusarium* and *Pythium* root rots on greenhouse cucumbers grown in rockwool. *Acta Horticulturae* **635**, 73-78

Sage DO (2005) Propagation and protection of flower bulbs: Current approaches and future prospects, with special reference to *Narcissus*. *Acta Horticulturae* **673**, 323-334

Sternshis MV (2005) Bioproducts for plant protection in Siberia: Application and enhancement of activity. *Journal of Agricultural Technology* **1**, 1-18

Straathof ThP, Jansen J, Löffler HJM (1993) Determination of resistance to *Fusarium oxysporum* in *Lilium. Phytopathology* **83**, 568-572

Vanacci G, Gullino ML (2000) Use of biocontrol agents against soil-borne pathogens: Results and limitations. *Acta Horticulturae* **532**, 79-87