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## Enhanced growth of cabbage and red beet by *Trichoderma viride*

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### ABSTRACT

The main agricultural importance of *Trichoderma* species was not so far away mainly suppression of plant diseases. Novel investigations emphasize their potential to stimulate plant growth independently of suppression of any plant disease. However, *Trichoderma* spp. and even biotypes of same species vary between each other in its effectiveness both in the control of plant pathogenic fungi and in the promotion of plant growth and increasing yield. The subject of this study was to evaluate growth promoting effect of two indigenous *T. viride* strains STP16 and STP8 on cabbage and red beet. Increment of fresh and dry weight accomplished by STP16 was statistically significant so, it could be concluded that growth enhancement of 27% at red beet and 29% at cabbage is significant.

**Key words:** cabbage, plant growth, red beet, *Trichoderma*

### IZVLEČEK

#### POSPEŠENA RAST ZELJA IN RDEČE PESE Z DODATKOM GLIVE *Trichoderma viride*

Agronomski pomen gliv iz rodu *Trichoderma* je bil do nedavnega v zatiranju rastlinskih bolezni. Novejše raziskave poudarjajo njihov potencial vzpodbujanja rasti rastlin neodvisno od zaviranja katerekoli bolezni. Pospeševanje rasti in povečevanje pridelka kot nadzor patogenih gliv pa se pri vrstah iz rodu *Trichoderma* razlikuje med biotipi iste vrste v učinkovitosti. Predmet te raziskave je bil ovrednotiti učinek na povečanje rasti zelja in rdeče pese dveh samoniklih sojev glive *T. viride*, STP16 in STP8. Povečanje sveže in suhe teže obeh vrtnin je bilo ob prisotnosti seva STP16 statistično značilno, 27 % pri rdeči pesi in 29 % pri zelju.

**Ključne besede:** zelje, rdeča pesa, rast, *Trichoderma*

## 1 INTRODUCTION

The members of the genus *Trichoderma* are rhizosphere competent fungi which taking part in the decomposition of plant debris in the soil. But, since the early 1930s when Weidling (1934) reported that *T. lignorum* produce and excretes a "lethal principle" in the surrounding, the scientists become involved in investigation of antifungal ability of various *Trichoderma* species although *T. harzianum* arisen as the most prominent species of the genus. Along with revelation of diverse antifungal mechanisms of *Trichoderma* the ability to promote plant growth, to increase plant height, leaf area and dry weight were perceived. Firstly was this ability treated as side effect of suppression of plant pathogenic fungi (Baker, 1988; Chang at

al., 1986; Inbar at al., 1994; Ousley at al., 1994). Other possible explanations of this phenomenon include: control of minor pathogens leading to stronger root growth and nutrient uptake (Ousley at al., 1993), secretion of plant growth regulatory factors such as phytohormones (Windham at al., 1986; Chang at al., 1986; Baker, 1988) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley at al., 1994a). Furthermore, positive influence of *Trichoderma* spp. to a faster germination and increases in percentage of emergency were perceived also (Celar and Valič, 2005; Koch, 2001; Gupta and Sharma, 1995). Recently, it is speculated that *Trichoderma*

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positive effect on plant growth is independent ability and equally remarkable and significant as its antifungal ability because growth enhancement has been observed in the absence of any detectable disease and in sterile soil (Celar and Valič, 2005; Altomare et al., 1999). Therefore, today is considered that the direct effects of these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems.

Metabolic products of various *Trichoderma* strains are not identical and they have selective character to different plant species and even a variety (Celar and Valič, 2005; Gupta and Sharma, 1995). There are numerous papers about positive influence on plant growth of *T. harzianum* and *T. viride* in lettuce, cucumbers and bell peppers (Bal and Altinatas, 2006; Bal and Altinatas, 2008; Poldma et al., 2000; Yedidia et al., 2001) and *T. virens*, *T. tomentosum* and *T. longipile* in cabbage (Rabeendran et al., 2000). In progress are investigations of effectiveness differences between species and biotypes of same species in the plant growth promotion. Seems that may be due to better interaction of some *Trichoderma* species or some biotypes with certain plant species because root exudates may induce or inhibit their mycelial growth (Bal and Altinatas, 2008).

Nowadays are *Trichoderma* species considered as opportunistic plant symbionts because they colonise root surface and even penetrate into the epidermis (outer layer of root tissue) and a few cell layers below this level establishing pseudo-mycorrhizal relationship with plant host (Harman, 2006). This intimate relationship between *Trichoderma* sp. and the host root cells is what induces localized and systemic resistance plant responses to pathogen attack. For the fungus, abundant healthy roots are environment where it grows and proliferates best because plant derived sucrose which is an important resource provided to the *Trichoderma* sp. cells. Furthermore, roots are resort of plant pathogenic fungi and nematodes, the target for *Trichoderma* as micoparasite and nematofag. The plants also benefit from this relationship through increased root and shoot growth and increased macro- and micronutrient uptake. Therefore, *Trichoderma* sp. may be benefit as growth promotant (biofertiliser) as well as

pathogen control agent (mycofungicide) and their application may lower the production costs and environmental impact.

The *Trichoderma* potential as biocontrol agent is utilized through the commercial production of *Trichoderma* – based biofungicides, which account for about 60% of the biofungicides market. The spectra of *Trichoderma* – based products signed as biofertilizer in Croatia has tendency to expand due to the easier registrations. The availability and diffusion of *Trichoderma* sp. based biofertilizers is more widespread than commonly known. Mostly permitted for use in organic farming in Europe are: RootShield, Plant Box and Bio Trek (northern Europe), Binap (Switzerland, Sweden, UK), Bio fungus (Belgium), Supersivit (Czech Republic), Trichodex (Italy), Trifender (Hungary) and Trianum (Avantagro, Spain) (Topolovec-Pintarić et al., 2011; Robson et al., 2007). Today in Croatia only *Trichoderma* based biofertilizer is Trifender (Bioved, Hungary) and is legal to sell from 2009 (distributed by ZKI Sljeme) while, only biofungicide is Trichodex (Makhteshim, Israel) registered as mycofungicide for uses against grey mould in vineyards.

Since 2007 we have conducted investigation of antagonistic ability of indigenous *T. viride* isolates against some of important plant pathogenic fungi like *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Botrytis cinerea*. As two indigenous *T. viride* strains, STP16 and STP8 achieved good antagonistic activity it seemed appropriate to also investigate whether, would have a direct effect on plant growth when no disease pressure was present. The mode of effective dispersal of fungal inocula became an issue.

The development of formulation and delivery systems for antagonistic microorganisms is of great importance in the field of biological control. After rearing beneficial organism in laboratory their reintroduction in rhizosphere can be difficult even if it is soil borne organism such as *T. viride*. Rhizosphere is environment full with spectrum of microbes with whom reintroduced *T. viride* must compete, trying to colonize available sites along the plant roots. Therefore, it need to be applied in low-cost formulation with highly densities inocula and engineer to maintain fungal propagules viable

during the transport, storage and application. To accomplish mentioned goals wet or dry fungal biomass needs to be immobilized within a matrix forming material, such as gelatinized polysaccharide or an oil emulsion. Matrix is serving as carrier of fungal inoculums. Most of the examples on different types of fungal inocula for biological control include peat, granular vermiculite or clay mixtures, grains and alginate

pellets. Encapsulation in formulation of alginate pellets has been found to be successful for the delivery of *Trichoderma sp.* (Walker and Connick, 1983; Fravel et al., 1985; Lewis and Papavizas, 1985; Knudsen and Bin, 1990; Nipoti et al., 1990; Leštan and Lamar, 1996; Mafia et al., 2003). In this work we chose to encapsulate fungal inocula into form of alginate pellets.

## 2 MATERIALS AND METHODS

Two indigenous *Trichoderma viride* Pers. strains were used: STP16 originated from parasited sclerotia of *Athelia rolfsii* while STP8 originated from sclerotia of *Sclerotinia sclerotiorum*. Cultures were maintained at 25 °C on potato dextrose agar (PDA, Biolife, Italy) slants.

The alginate pellets were prepared as described previously (Topolovec-Pintarić et al., 2011). The culture of isolates were grown on Petri dishes 10 cm in diameter containing 20 ml of PDA and incubated in humid chamber at 25 °C for 7 days until conidiation occurred. After incubation the substrate altogether with hyphal biomass and conidia from two Petri dishes were upraised with spatula and transferred into glass with 50 ml sterile DI water. These were mixed by common blender at low speed for 3-5 min in order to make a suspension. The final concentration to be used contained  $4 \times 10^6$  spore ml<sup>-1</sup>. The suspension was mixed with 100 g l<sup>-1</sup> talcum (Kemig, Croatia) and 10 g l<sup>-1</sup> sodium alginate (Fluka, Switzerland). The formed matrix was then placed in a separator funnel modified in order to allow suspension to dripping into a 0.1 M suspension of calcium gluconate (Kemig, Croatia) under stirring on magnetic agitator. Drops of alginate matrix dripped into calcium gluconate suspension transformed to gelatinized spherules or pellets. Pellets were removed from suspension within 10 min, rinsed with distilled water and allow to dry on waxed paper under a sterile vertical flow for 12 -24 h.

Two sets of experiments (white cabbage (Ditmar) and red beet (Crimson Globe)) were carried out in the plastic tunnel according to randomised complete block design in 5 replications (4 plants

per replication). The seedlings were planted in raised beds 250 x 100 cm wide and 250 mm deep edged with scaffold planks and fulfil with sterilized potting compost Potgrond P (Klasmann-Deilmann GmbH, Germany). Pellets of *T. viride* strains STP16 and STP8 were added manually during planting into the root zone of seedlings. The equal number of cabbage and red beet plants was grown without addition of pellets as control. The planting was conducted at the beginning of May. After that first application the pellets were added manually four times more at two weeks intervals in the root zone. Control beds were omitted. The 4 g of pellets were added per plant in each application. Plant protection practices were not applied during the growing period. Mature plants were pullout from the beds 48 days after planting. The roots of plants treated with pellets were rinsed with tap sterile distillate water. In such manner obtained suspension from each test plant were poured onto PDA in the Petri dish. After incubation in humid chamber at 25 °C for five days examination was conducted for *T. viride* presence. All plants were weighed, each cabbage head and red beet root, and after the fresh weight (*FW*) was recorded they were dried to constant weight, dry weight (*DW*). An analysis of variance ( $p=0.05$ ) and t-test were performed on *FW* and *DW* data.

Evaluation of growth promotion by *T. viride* was interpreted as Index of growth and calculated as follows:  $I = 100 (T-C) C^{-1}$  where *I* or index growth is percent of growth promotion with respect to control, *C* is plant growth represented with *FW* and *DW* at control and *T* is plant growth represented with *FW* and *DW* in the treatment.

### 3 RESULTS AND DISCUSSION

Two indigenous *T. viride* strains STP16 and STP8 were evaluated for growth promotion effects in order to confirm the hypothesis that biotypes of same species differ in their abilities to inducing plant growth. The most effective strains will colonize roots and provide benefits for at least the life of annual crops (Harman, 2006). Furthermore, even if the *T. viride* is present only on roots the enhancement of growth; both on the root and on the foliage can be asses (Harman, 2000; Harman, 2004a; Harman, 2004b; Harman, 2004c). Therefore, the trial was set to investigate whether strains would have a direct effect on plant growth when no disease pressure was present, although they showed good antagonisms against soil-borne pathogens in previous investigation. In order to estimate the hypothesis that presence of *T. viride* on root can enhance the foliage we choose cabbage as represent of leafy green vegetables and red beet as root vegetable.

In conducted trial the indigenous *T. viride* isolates STP16 and STP8 enhanced plant growth in only one trial vegetation season. Influence of strains STP16 and STP8 on red beet growth was estimated by weighing the root (table 1). Fresh weight was increased by both isolates, STP16 ( $FW = 725$  g) and STP8 ( $FW = 607.5$  g). There was no statistically significant difference between isolate's influences although STP16 significantly increased fresh weight in comparison to control ( $FW = 569$  g) while STP8 did not. Dry weight was greater at STP16 treatment ( $DW = 13.1$  g) and statistically significant in comparison to STP8 treatment ( $DW = 12.2$  g) and control ( $DW = 11.6$  g). Growth-promotion index (*I*) showed that STP16 treatment increased root fresh weight for 27.42% while STP8 treatment for only 6.44%. Index calculated for dry weight showed that STP16 increased dry weight for 12.93% and STP8 for 5.17%.

**Table 1:** Effect of *Trichoderma viride* strains on red beet yield.

Treatment	Fresh weight (g)	SEM	Dry weight (g)	SEM	$I_{FW}$ (%)	$I_{DW}$ (%)
STP16	725 <sup>a</sup>	150	13.1 <sup>a</sup>	0.218	27.42	12.93
STP8	607.5 <sup>ab</sup>	133	12.2 <sup>b</sup>	0.213	6.44	5.17
Control	569 <sup>b</sup>	202	11.6 <sup>b</sup>	0.277	–	–

<sup>a, b</sup> Mean in each column, with same letters are not significantly different at  $p < 0.05$ .

*SD* = standard deviation;  $I_{FW}$  = index of growth promotion for fresh weight;  $I_{DW}$  = index of growth promotion for dry weight.

Influence of strains STP16 and STP8 on cabbage growth was estimated by weighing the heads (table 2). Fresh weight was greater at STP16 treatment ( $FW = 1666.5$  g) than at STP8 treatment ( $FW = 1372.5$  g) but not statistically significant. In comparison to control ( $FW = 1291$  g) STP16 and STP8 significantly increased fresh weight. Dry weight was slightly but statistically significant increased at STP16 treatment ( $DW = 8.2$  g) against STP8 treatment ( $DW = 7.2$  g) which was statistically equal to control ( $DW = 6.3$  g). Growth-promotion index showed that STP16 treatment promote fresh weight for 29% while STP8 treatment only for 6.3% and dry weight for 30.16% while STP8 for 14.29%.

Calculation of growth-promoting index allowed comparison of strain's abilities in relation to control. Taking into account that increment of fresh and dry weight accomplished by STP16 was

statistically significant it could be concluded that growth enhancement of 27.42% at red beet and 29% at cabbage is significant. Here represented result of *T. viride* influence on cabbage growth differs from previous reports of Rabeendran at al. (2000). They reported that *T. longipile* and *T. tomentosum* promote cabbage growth of seedlings old 28 days but the effect was not maintained through to the 42 days assessment. We consider the discrepancy between here presented results and Rabeendran observations (Rabeendran at al., 2000). as confirmation of speculation that different *Trichoderma* species acting differently on various host plants. Unfortunately, we were unable to compare growth promotion effect of *T. viride* on red beet owing to lack of similar investigation.

Observations here described, different ability of two new *T. viride* autochthonous isolates in enhancing growth of different plant species,

supporting hypothesis that growth promotion of *Trichoderma* is not species dependent as well as that biotypes of same species differ in their abilities for inducing plant growth.

To accomplish introduction of *T. viride* into the rhizosphere, the fungal culture was encapsulated into the alginate pellets. Pellets allowed effective dispersal of fungal inocula into the soil and enable fungal transfer onto the roots of plants. Colonisation of the roots was confirmed with isolation of strains from root of every plant originated from beds amended with pellets. The alginate matrix used for the formation of pellets remained in the soil after the harvesting. With the

time the alginate matrix slowly decayed, possibly because the substrate was sterile, without microbiological activity and only the regular watering enables the decomposition of the pellets. Some of the remaining pellets were taken to the laboratory where the isolates STP16 and STP8 were regenerated from the pellets to the PDA medium. Therefore, the alginate pellets can be considered as good formulation for encapsulation and preservation of *T. viride* inocula. Furthermore, alginate pellets shown to be formulation easy for application. Obtained results warrant further investigation toward field application and perhaps commercialization of the product for the market.

**Table 2:** Effect of *Trichoderma viride* strains on cabbage yield.

Treatment	Fresh weight (g)	SEM	Dry weight (g)	SEM	$I_{FW}$ (%)	$I_{DW}$ (%)
STP16	1666.5 <sup>a</sup>	409	8.2 <sup>a</sup>	0.184	29	30.16
STP8	1372.5 <sup>a</sup>	484	7.2 <sup>b</sup>	0.242	6.3	14.29
CONTROL	1291 <sup>b</sup>	595	6.3 <sup>b</sup>	0.293	–	–

<sup>a, b</sup> Mean in each column, with same letters are not significantly different at  $p < 0.05$ .

$SD$  = standard deviation:  $I_{FW}$  = index of growth promotion for fresh weight;  $I_{DW}$  = index of growth promotion for dry weight.

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