FIELD TESTING OF ENTOMOPATHOGENIC OR POTENTIALLY PLANT GROWTH PROMOTING FUNGAL STRAINS FOR THE CONTROL OF CABBAGE ROOT FLY (*Delia radicum* L.) AND THEIR RHIZOPLANE COMPETENCE

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ABSTRACT

The aim of this research was to assess the ability of 6 entomopathogenic or potentially plant growth promoting fungal species to protect cauliflower plants against cabbage root fly (CRF) and to test their rhizoplane competence in a field experiment. The following fungal species were tested: *Trichoderma atroviride* (1 strain), *T. koningiopsis* (1), *T. gamsii* (1), *Beauveria bassiana* (1), *Metarhizium anisopliae* (2) and *Clonostachys solani* (1). The fungi were isolated from various substrata in Slovenia. A commercial product based on *B. bassiana* (Naturalis) was used as a positive control. The field experiment mimicked semi-normal agronomic practice in cauliflower production. Amount of 2.2 x 107 were applied to individual 4 wk-old cauliflower plantlets as a drench 8 hours before they were transplanted to the field. All fungal isolates were infective to one or more CRF life stages (eggs, larvae, pupae or imago), as assessed in previous laboratory bioassays. *Clonostachys solani* and the product Naturalis increased the average plant weight at harvest. *Trichoderma atroviride* and *M. anisopliae* (1154) treated plants had the lowest mortality. The lowest number of pupae and live larvae were counted in the Naturalis treatment followed by both *M. anisopliae* treatments. The highest number of pupae and living larvae were counted in the control treatment. *Clonostachys solani* and all *Trichoderma* species were reisolated from the rhizoplane 85 days after application. The results indicate that rhizoplane competence of the tested fungal species varies considerably, potentially due to different ecological preferences of the fungal species.

Keywords: biological control, brassicas, biopesticides, Diptera, pest

IZVLEČEK

POLJSKI PRESKUS VARSTVA CVETAČE PRED KAPUSOVO MUHO (*Delia radicum* L.) Z ENTOMOPATOGENIMI ALI POTENCIALNO RAST SPODBUJAJOČIMI SEVI GLIV IN DOLOČANJE NJIHOVE RIZOSFERNE KOMPETENCE

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Namena raziskave sta bila a) oceniti varstvo cvetače pred kapusovo muho (KM) in b) oceniti prilagojenost 6 vrst entomopatogenih ali potencialno rast spodbujajočih gliv na rizosfero v okviru poljskega poskusa. Preskušane so bile naslednje vrste gliv: *Trichoderma atroviride* (1 izolat), *T. koningiopsis* (1), *T. gamsii* (1), *Beauveria bassiana* (1), *Metharhizium anisopliae* (2) in *Clonostachys solani* (1). Glive smo izolirali iz različnih substratov v Sloveniji. Za pozitivno kontrolo smo uporabili komercialni izdelek, ki temelji na *B. bassiana* (Naturalis). Poljski poskus je bil oskrbovan v skladu z ustaljeno agronomsko prakso iz katere je bila izvzeto varstvo rastlin s fitofarmacevtskimi sredstvi. 4 tedne stare sadike cvetače smo zalili z 2,2 x 10^7 konidijev 8 ur pred presajanjem na polje. Vsi izolati gliv so bili kužni za eno ali več razvojnih faz KM (jajčeca, ličinke, bube ali odrasli osebki), kar smo potrdili v predhodnih laboratorijskih poskusih. Pri tretiranjih s *C. solani* in pripravkom Naturalis je bila povprečna masa rastlin ob spravilu večja. Pri tretiranjih s *T atroviride* in *M. anisopliae* (1154) je propadlo manj rastlin kot pri ostalih tretiranjih. Najmanj bub in živih ličink smo našteli pri postopkih Naturalis in obeh izolatih *M. anisopliae*. Največje število bub in živih ličink smo našteli pri kontroli. *C. solani* in vse vrste *Trichoderma* sp. smo uspeli ponovno izolirati s površine korenin po končanem poskusu. Ti rezultati kažejo, da se prilagojenost testiranih vrst gliv na rizosfero precej razlikuje, verjetno zaradi različnih ekoloških potreb posameznih vrst.

Ključne besede: biotično varstvo, bioinsekticidi, Diptera, kapusnice, škodljivec

1 INTRODUCTION

Brassicaceous plants are attacked by a wide range of pest insects (Klingen *et al*., 2002). Specifically the cabbage root fly species *Delia radicum* and *Delia floralis* present major threats for many brassica crops in Europe (Vanninen *et al*., 1999). No sustainable control strategies are currently available; however, several studies have indicated that larvae of *Delia* sp. can be biologically controlled using entomopathogenic fungi (Vanninen *et al*., 1999; Klingen *et al*., 2002; Bruck *et al*., 2005).

Entomopathogenic fungi kill dipterous insects after they are ingested or through infection via external contact (Thomas and Read, 2007; Toledo *et al*., 2007). Both mechanisms imply that an effective biological control agent should live in juxtaposition of either the crop or the insect pest. The goal of the presented study was to evaluate if inoculated entomopathogenic fungi were able to protect cauliflower roots under field conditions and to determine their plant compatibility.

The experiments were performed with fungi already identified as entomopathogens and with soil fungi. We hypothesized that different ecological preferences of the various isolates will have an effect in their ability to attack *D. radicum* and consequently provide various kinds of protection to the plants, qualitatively and quantitatively. We also hypothesized that various isolates differ in their root colonization ability and that they may have a different effect on plant vigor and growth. The aims of the research were to assess whether (i) the 7 entomopathogenic or potentially plant growth promoting fungi (EPF) can protect cauliflower plants against cabbage root fly (CRF) in a field experiment, (ii) the EPF are rhizoplane competent or able to persist on or in roots, and (iii) the fungal isolates have an effect on plant growth and survival.

2 MATERIALS AND METHODS

2.1 Fungal cultures tested

The tested fungal isolates were *Metarhizium anisopliae* (isolate 1154 and 1868), *Beauveria bassiana* (1174), *Clonostachys solani* (1828)*, Trichoderma atroviride* (1873)*, T. koningiopsis* (1874) and *T. gamsii* (1876). The product 'Naturalis' based on the entomopathogenic fungus

Beauveria bassiana, at a recommended concentration of 0.1 %, was used as a positive control.

2.2 Protection against cabbage root fly

The field experiment mimicked semi-normal agronomic practices in cauliflower production, with phytopharmaceutical protection excluded from the usual practice. An amount of 2.2 x 10^7 conidia were applied to individual, 4-wk-old cauliflower plantlets as a drench 8 hours before they were transplanted to the field.

2.3 Rhizoplane competence and endophytism

The fungal isolates were further tested for their rhizoplane competence and ability to survive as endophytes in cauliflower (*Brassica oleracea* var. *botrytis*). Rhizoplane competence, which describes the ability of a fungus to become infested on the immediate root surface, was assessed by placing root pieces, 3-times washed with sterile water, on semi selective media (Strasser and THSM media). The ability of the fungi to survive as endophytes in root tissue was assessed similarly but with an additional washing step with 70 % ethanol for XX seconds. Before they were placed on agar media, root pieces were air-dried under sterile conditions.

2.4 Biostimulating effects

Plant weight and mortality was assessed at experiment endpoint to provide us with the information on possible biostimulating effects of the fungal inoculums.

3 RESULTS AND DISCUSSION

3.1 Protection against cabbage root fly

All EPF treatments decreased the number of CRF pupae and living larvae. The effect was most pronounced when *Metarhizium anisopliae* (1154) was applied, followed by the 'Naturalis' positive control and *Metarhizium anisopliae* (1868), however, this reduction was not statistically significant (Figure 1).

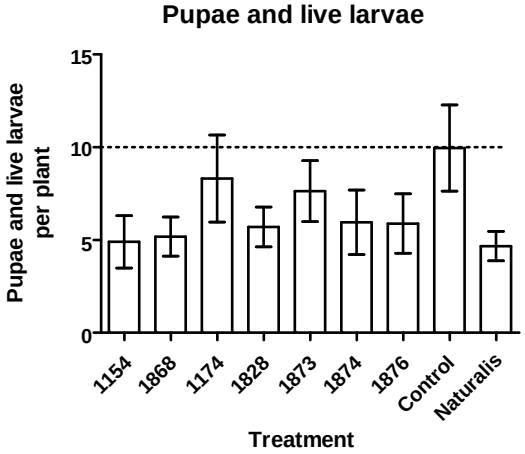

Figure 1: Protection of cauliflower roots indirectly assessed by counting living CRF larvae and pupae in individual root systems. The isolates tested were: *M. anisopliae* (1154 and 1868), *B. bassiana* (1174), *C. solani* (1828), *T. atroviride* (1873), *T. konigiopsis* (1874) and *T. gamsii* (1876). *Naturalis* - commercial product based on *B. bassiana*.

3.2 Rhizoplane competence and endophytic colonization

Clonostachys solani (1828), *Trichoderma atroviride* (1873), *T. konigiopsis* (1874) and *T. gamsii* (1876) were successfully isolated from the rhizoplane. This indicates that our isolates could potentially survive on the rhizoplane throughout the duration of the field experiment. Endophytic colonization was not observed.
Only some fungal isolates were successfully reisolated from the rhizoplane. This is probably because of different ecological preferences of the tested fungi (Harman *et al*., 2004; Pava-Ripoll *et al*., 2011).

3.3 Biostimulating effects

Some treatments decreased (1154, 1174, 1828, 1873, 1874, Naturalis), while others (1868, 1876) increased plant mortality. Some treatments decreased (1154, 1873, 1876), while others (1868, 1828, 1874, Naturalis) increased plant weight (Figure 2). However, these effects were not statistically significant.

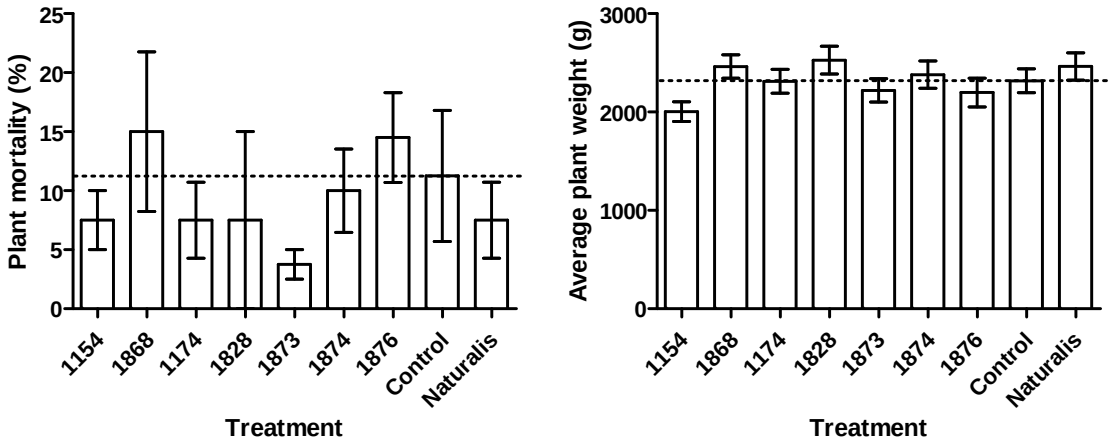

Figure 2: The effect of fungal isolates on plant mortality (left) and fresh biomass production (right). The isolates tested were: *M. anisopliae* (1154 and 1868), *B. bassiana* (1174), *C. solani* (1828), *T. atroviride* (1873), *T. konigiopsis* (1874) and *T. gamsii* (1876).

4 CONCLUSIONS

The added isolates offered some level of protection to the cauliflower roots as the number of pupae and live larvae was lower as in the control treatments. *Clonostachys solani, Trichoderma atroviride, T. konigiopsis* and *T. gamsii* were successfully isolated from the rhizoplane of cauliflower roots more then 60 days after inoculation and planting in the field. None of the fungi was isolated as an endophyte. Some treatments (1154, 1174, 1873 and Naturalis) decreased plant mortality. Treatments 1868, 1828, 1874 and Naturalis increased plant weight. All of the parameters observed were not significantly different from the control treatment. This is probably due to high control group variation as well as the low number of replicates (4). The experiment will be repeated. Hopefully, by incorporating this new data, we will be able to calculate significant difference between treatments.
The reduction of the pupae in the root systems as well as the successful isolation of our input fungal species from cauliflower rhizoplane suggests that the tested fungal species could provide a fungal-based biological control strategy against *Delia radicum*.

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