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Soil incorporation of brassica materials and seed treatment with *Trichoderma harzianum*: Effects on melon growth and soil microbial activity

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ABSTRACT

Brassica nigra defatted seed meal (DSM) and liquid formulation were added to three different soils in a pot experiment to investigate its effect on the growth of melon, on soil microbial size and on six enzyme activities. DSM was added as a pre-sowing treatment; the liquid formulation, based on *B. carinata* oil and *B. nigra* DSM, was then repeatedly applied as fertilizer with allelopathic activity. Brassica materials were also evaluated in combination with the melon seed treatment with an isolate of *Trichoderma harzianum*. This beneficial fungus was previously selected for tolerance to toxic compounds released by brassica DSM. At the flowering stage, brassica treatments determined a doubling in shoot dry weight in all soils; this was paralleled by an increase of both soil microbial biomass (+46%) and enzyme activities, up to +90%. The combination of the applications of brassica materials and seed treatment with *T. harzianum* also increased root length by 20%. These effects were most probably due to enhanced microbial activity and enhanced biogeochemical cycles of nutrients caused by the treatments with brassica materials and the stimulation of root activity by *T. harzianum*.

1. Introduction

The soil incorporation of brassica chopped tissues or defatted seed meal (DSM) represents a sustainable technique for soil disinfestation, also known as biofumigation, which has gained considerable attention in the last decades (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Lazzeri et al., 2013). *Brassicaceae* cells typically contain the glucosinolate–myrosinase defensive system, able to release, by enzymatic hydrolysis, a number of biologically active compounds including mainly isothiocyanates and, at lower concentrations, nitriles, epithionitriles and thiocyanates (Agerbirk and Olsen, 2012). Allelopathic effects are reported towards several soil pests and pathogens, like fungi (Larkin and Griffin, 2007), nematodes (Lazzeri et al., 2009) and wireworms (Furlan et al., 2010) following the use of brassica plants or DSM-based products (Lazzeri et al., 2008, 2010). More recently, a liquid formulation has also been defined, based on a water emulsion of *B. carinata* oil containing small amounts of *B. carinata* DSM, making it possible to utilize these materials also during cultivation by drip irrigation (De Nicola et al., 2013).

The soil incorporation of brassica derived materials also provides a significant amount of organic matter (OM) and nutrients. OM is a fundamental benefit for soil fertility, especially in warm cultivation areas viz. in the Mediterranean one, where soil OM decline is causing increasing risks of desertification (Jones and Montanarella, 2003). Indeed, OM has positive effects on physical, chemical and biological soil properties. Some authors also highlighted the potential of brassica DSM as an organic source of N in agricultural production systems due to their N content (5–6%) and C:N ratio (8:1) (Gale et al., 2006). Fertilization with brassica DSM increased N uptake and improved yields and quality of several crops like wheat, barley, sugar beets and carrots (Kucke, 1993 Snyder et al., 2009).

Notably, the actual release of biologically active glucosinolate-derived products may have an impact not only on soil-borne pathogens but also on the beneficial soil microflora. So far, the effects on microbial size, structure or function of non-target soil

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Table 1
B. nigra defatted seed meal characterization for its main components (% of DW).

Moisture	4.1 ± 0.7
Residual oil	7.7 ± 0.2
Nitrogen	6.6 ± 0.1
Phosphorous	1.1 ± 0.1
Carbon	47.2 ± 0.3
Ashes	7.1 ± 0.4
Total glucosinolates(μmoles gr^{-1} DW)	133.6 ± 0.6

DW: dry weight. Values are reported as the mean of 4 replications ± standard deviation

biota have rarely been investigated. Alterations of communities of both pathogenic and saprophytic soil micro-organisms were observed after *B. napus* seed meal application to orchard soils (Cohen et al., 2005). More recently, Pane et al. (2012) found that *B. carinata* DSM mitigated negative effects of solarization on microbial size and activity, with a positive effect on lettuce yield. Thus, brassica DSM can be useful both in controlling soil-borne pathogens and in improving soil properties and plant yield.

Biofumigation is not the only available non-chemical method to control soil-borne pathogens. *Trichoderma* (*phylum Ascomycota*, ord. *Hypocreales*, fam. *Hypocreaceae*) is a soil free-living filamentous fungus which has been considered for decades as a biocontrol agent acting against soil-borne pathogens by direct and indirect mechanisms. More recently, similarly to brassica plants and DSM, many other functions have been revealed, leading to consideration of *Trichoderma* as an avirulent, opportunistic plant symbiont, able to enhance plant growth and productivity (Harman et al., 2004, 2011). Plant root colonization followed by root cortex penetration represents the first step in establishing communication with the plant by the release of small proteins, peptides and volatiles as signalling molecules. Plant systemic responses are induced and variations in plant gene expression occur. The main advantages conferred by *Trichoderma* to the plant are: increased plant growth, especially of roots, systemic resistance to plant biotic and abiotic stresses and improved nitrogen use efficiency (Shoresh et al., 2010; Harman and Mastouri, 2010). Instead of massive soil application, seed inoculation with *Trichoderma* represents an effective tool to vehicle selected *Trichoderma* strains in the rhizosphere, in direct contact with the developing seedlings (Lifshitz et al., 1986; Mastouri et al., 2010).

Sustainable agricultural systems should rely first of all on reduced chemical inputs, maintaining crop yield and productivity at high levels. The adoption of integrated eco-friendly techniques and products can also exploit the synergy that often derives from their combined use. In this view, the combined use of vegetal amendments such as brassica DSM and beneficial microorganisms such as *Trichoderma* could represent a sustainable way to fulfill this requirement. A previous work indicated the existence of a synergy between *B. carinata* DSM amending and massive *T. harzianum* application to soil in controlling damping-off caused by *Pythium ultimum* on sugarbeet (Galletti et al., 2008).

The aim of this work was to evaluate the effect of the soil incorporation of brassica based materials and the seed treatment with a selected *T. harzianum* isolate, on the growth of melon (*Cucumis melo* L.) and on the microbial size and enzymatic activity of the soil.

2. Materials and methods

2.1. Brassica seed meal based products

2.1.1. B. nigra *DSM characterization*

DSM of *B. nigra* (L.) W.D.J. Koch was obtained by an endless screw press (Agrium Italia S.p.A., Livorno, Italy) and the main components are reported in Table 1. Moisture content was determined by oven-drying DSM at 105 °C for 12 h. Nitrogen and phosphorous content were determined by the Kjeldhal method and by the spectrophotometric vanado-molybdate method (Cottenie et al., 1982), respectively. Residual oil was determined by the standard Soxhlet extraction method, using hexane as solvent, and glucosinolate content was determined following the ISO 9167-1 (1992), with some minor modifications as reported by Lazzeri et al. (2011). Glucosinolates were represented for more than 90% by 2-propenyl glucosinolate (trivial name, sinigrin) and by 4-hydroxy glucobrassicin. The high sinigrin content gives the meal a strong biofumigant potential, due to the release of the corresponding allyl-isothiocyanate by enzymatic hydrolysis, via myrosinase.

2.1.2. Liquid formulation

This formulation consisted of a water emulsion based on 2.6% (w/v) *B. carinata* oil, containing 4.4 % (w/v) of *B. nigra* DSM, ground at 0.05 mm, with the addition of 6.9% (w/v) animal protein hydrolysate, containing 9% N. The formulation was prepared according to the procedure described by De Nicola et al. (2013) and based on a patent (Lazzeri et al., 2008). Differently from the De Nicola et al. (2013) procedure, *B. nigra* DSM was used instead of *B. carinata* DSM and the emulsion was not filtered before use.

2.2. T. harzianum *isolate and seed treatment*

The wild-type *T. harzianum* B41 isolate, from the CRA-CIN collection, was chosen for this experiment among those isolates that proved to be tolerant to *B. carinata* biologically active compounds in a previous screening (Galletti et al., 2008). *T. harzianum* B41 was isolated from *B. nigra* rhizosphere and was able to grow *in vitro* in direct contact with *B. carinata* DSM after an initial moderate lag phase. It displayed also features of antagonism against phytopathogenic fungi, like the ability of growing over *Fusarium oxysporum*, *Sclerotium rolfsii* and *Pythium ultimum* colonies (Galletti et al., 2008).

Commercial melon seeds of the F1 hybrid 'Summer dream' (Four – Blumen SRL, Piacenza, Italy), not chemically treated, were treated by a preparation of *T. harzianum* B41. The fungus was cultured in Potato Dextrose Broth for 7 days at 30 °C; the liquid culture was then vortexed, obtaining a preparation containing mycelium fragments and spores (5×10^8 spores ml^{-1} at the haemocytometer count). Seeds were wet with this preparation under sterile flow, at the dose of 0.75 ml g^{-1} of seed. The excess liquid was removed and the treated seeds were left to dry under sterile flow, and were then stored at 4 °C until sowing. The seed germinability was assessed on filter paper before and after the treatment to verify the absence of phytotoxic effects, according to ISTA (1979).

2.3. Soil characteristics

Soil samples from three different farms specialized in melon cultivation, located in Cabras (A) and Arborea (B and C), Oristano, Sardinia, Italy, were collected and used in the greenhouse experiment. The main physico-chemical properties of these 3 soils are reported in Table 2. Texture was determined by pipette method; pH

Table 2
Physico-chemical characteristics of the three Sardinian soils utilized in the experiment.

Soil	A	B	C
Sand (g kg^{-1})	193	441	350
Silt (g kg^{-1})	250	249	159
Clay (g kg^{-1})	557	310	492
CaCO3 (g kg^{-1})	207	345	265
pH	8.3	8.1	7.8
Total N (g kg^{-1})	1.5	1.4	1.3