Suppression of *Verticillium dahliae* by *Brassica juncea* seed meal amendment

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Introduction

Biofumigation is worldwide discussed as a potential control method to *Verticillium dahliae*. The method is defined as incorporation of brassicaceous green manures containing glucosinolates (GSLs). These compounds are hydrolyzed by the enzyme myrosinase to isothiocyanates (ITCs), which have a toxicological effect to microsclerotia. Recent studies have shown that the GSL concentration of the biomass of Brassicaceae and the release efficiency of ITCs is too low to eliminate microsclerotia in natural soils. GSLs are found in all parts of the plant, but the highest concentrations are found in the seed or seed meal that remains after oil extraction.
Therefore *Brassica* seed meal amendments as an alternative biofumigation method can be more effective, because higher ITC concentrations are generated in soil. Also the use of seed meals allows more rigorous control by increasing the application rates.
In previous studies the best effect to microsclerotia of *V. dahliae* was achieved by *Brassica juncea* seed meal amendments containing Sinigrin as a single GSL, which is hydrolyzed to 2-propenyl-ITC. The objective of a present study was to evaluate the biofumigation potential of seed meals of different *B. juncea* cultivars using a bioassay under standardized optimal conditions.

Material & methods

Seeds of five cultivars of *B. juncea* were obtained from P. H. Petersen Saatzucht, Lundsgaard. Defatted seed meals were produced by removing the oil using a mill and stored at 4 °C. To assess the biofumigation potential of the seed meals they were mixed with a rate of 0.4 % (vol./vol.) in a sterile quartz sand filled in glass flasks and artificially infested with 200 microsclerotia/g sand (Fig. 2). Autoclaved seed meal with a deactivated glucosinolate-myrosinase-system served as a control. Water was added to adjust an optimum moisture level of 60% of maximum water capacity. Flasks were sealed immediately and incubated at 20 °C for 48 h. The sand of each flask was air dried and analyzed for viable microsclerotia using a wet sieving detection method (Neubauer and Heitmann 2011). GSL concentration of the meals was analyzed by HPLC. Additionally *B. juncea* seed meal was tested with different doses in six different natural infested soils using the same bioassay. On the basis of dose-efficacy curves the LD_{90} values were calculated.

Fig. 1: Brassica juncea: flowering plants (left); seeds (middle;) defatted seedmeal (right)

Fig. 2: Bioassay: assessment of seedmeal

Results and Discussion

The Sinigrin contents of tested *B. juncea* seed meals ranged from 91.4 to 108.1 µmol g^{-1} (Table 1). All seed meals showed in the bioassay an efficacy of 100 % to *V. dahliae* when applied in a dose with 0.4 % (Fig. 3). The efficiencies of the seed meal amendments can be compared with the maximum amounts of 2-propenyl ITC release calculated on the measured Sinigrin concentrations. Considering the LD_{90} value of 2-propenyl ITC (89.0 nmol/g sand), determined in a similar test system, the impact of seed meals can be explained widely as an ITC-related biofumigation effect.

Additionally seed meal of cultivar 'Energy' was tested with different application rates in six different natural infested soils and the artificially infested sterile quartz sand using the bioassay (Fig. 4). On the basis of dose-efficacy curves the LD_{90} values were calculated (Tab. 2). In natural infested soils the ITC effect was reduced considering that 2-propenyl-ITC was prone to rapid microbial degradation and sorption to organic matter. According to soil characteristics the calculated doses to achieve an effect of 90 % varied between 0.5-2.2% (vol./vol.). Assuming an incorporation depth of 10 cm and a soil bulk density of 1,35 g cm^{-3} application rates of 2.5-11.0 tons per hectare would be necessary in practice.

Cultivar	Sinigrin concentration µmol / g
Terrafit	99.0
Energy	91.4
101	108.1
102	107.1
103	103.1

Table 1: Sinigrin concentration of seed meals of different *Brassica juncea* cultivars.

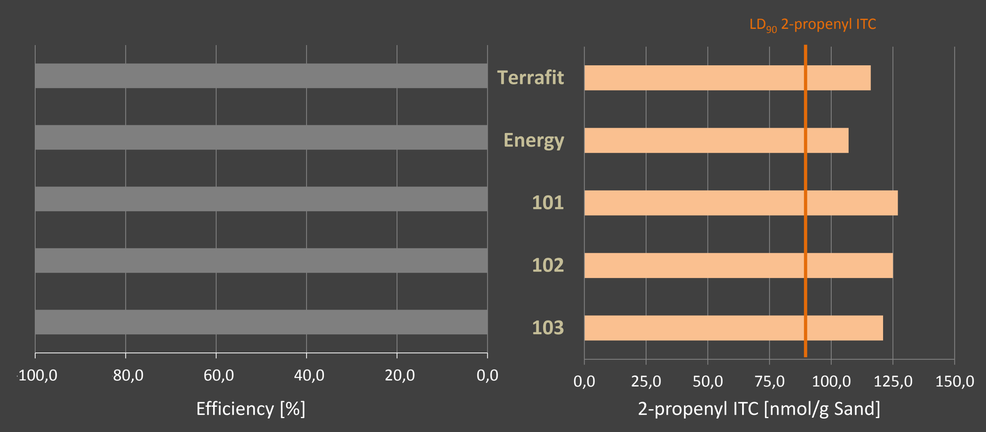

Fig. 3: Efficiency of *B. juncea* seed meal amendments with 0.4 % (vol./vol.) to microsclerotia of *V. dahliae* (left) and total ITC-liberating of applied biomass on basis of GSL concentration (right).

Soil/Site	Soil type	Infestation level microsclerotia/g soil	LD_{90} % (vol./vol.)
control	sterile quartz sand	129,9	0,1
A	silty sand	9.9	0.6
B	silty sand	72.0	0.5
C	silty sand	40,1	0,7
D	silty sand	23.1	0,5
E	sand	47.6	2,2
F	sand	42.9	1,3

Table 2: Effect of seed meal `Energy´ on *V. dahliae*: LD_{90} values determined in sterile quartz sand and different natural infested soils on basis of dose-efficacy curves.

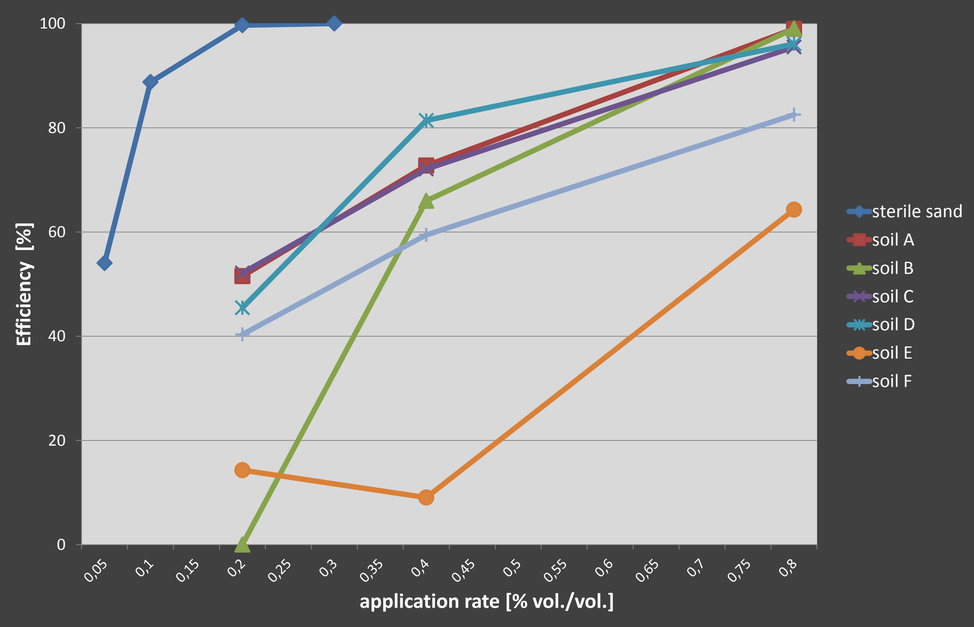

Fig. 4: Efficiency of *B. juncea `Energy´* seed meal amendments to microsclerotia of *V. dahliae* with different doses in six different natural infested soils and artificially infested sterile quartz sand.

References: NEUBAUER C., HEITMANN B. , 2011: Quantitative detection of *Verticillium dahliae* in soil as a basis for selection of planting sites in horticulture. Journal für Kulturpflanzen, 63 (1). S. 1-8, 2011

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