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In vitro screening of the effect of three glucosinolate derived nitriles on soil-borne fungi

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Introduction

Materials and methods

Glucosinolates are allelochemicals present in all plants of the order Capparales that are hydrolysed endogenous enzymes by (myrosinases) forming a variety of compounds with biological activity.1 'Biofumigation' is the term used to describe the effect of these compounds on soil-borne pathogens and it normally has been attributed to isothiocyanates. At acidic pH and in the presence of redox co-factors such as glutathione, glucosinolate hydrolysis yields also nitriles, which are more hydrophilic and stable than isothiocyanates.1

Three nitriles (allyl-, benzyl- and phenethyl cyanide) were purchased from Sigma-Aldrich (St. Louis, MO). Each nitrile was diluted in an emulsion of hydrolysed rapeseed oil² and afterwards added to cooled (50-60°C) PDA medium (0.5 % v:v). The compounds were initially tested at 1 mM and four-five additional concentrations of each nitrile were further tested in order to determine LD₅₀. Aphanomyces pisi, Gaeumannomyces euteiches var. graminis var. tritici, Pseudocercosporella herpotricoides var. herpotricoides and Verticillium dahliae were the fungi chosen due to their economic importance. The Petri dishes were inoculated with the fungi and incubated at 22 °C for a varying number of days depending on rate of fungi growth. Five replicates per treatment were prepared. Control Petri dishes consisted of the PDA medium with emulsion (0.5% v:v).

Results

Allyl nitrile was the least effective of the three nitriles tested although the four fungi were very differently affected. Aphanomyces and Gaeumannomyces were activated in their growth even at 20 mM of allyl nitrile, whereas Pseudocercosporella and Verticillium were only weakly inhibited (less than 10%) at 8 mM and 35 mM respectively.

LD₅₀ values for benzyl nitrile ranged between 1.5-2.5 mM for all fungi, except for Gaeumannomyces with LD₅₀ values of about 0.5 mM (Figure 1-left). Phenethyl nitrile was even more effective than benzyl nitrile, with LD₅₀ values lower than 1.5 mM except for Verticillum with LD₅₀ values of almost 2.5 mM (Figure 1-right).



Conclusions

Allyl nitrile did not inhibit significantly the growth of any of the fungi tested, in contrast to the high toxicity of allyl isothiocyanate (Sarwar et al., 1998). The fungi showed different resistance against benzyl and phenethyl nitriles. In the case of the cereal pathogen Gaeumannomyces, the LD₅₀ value for phenethyl nitrile was below 1.5 mM, which is up to 50 times higher than the LD₅₀ value reported for phenethyl isothiocyanate (Smith and Kirkegaard, 2002). Despite their lower toxicity compared to isothiocyanates, nitriles are generally more soluble in water, which may allow them to persist longer in soils. Nitriles are produced at low pH and in the presence of thiol groups and ferrous ions, which can be quite common in soils, therefore, the role of nitriles in biofumigation should also be considered .

References

- 1. Sørensen, H. 1990. In: Rapeseed/Canola: Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold Publisher.
- 2. Sørensen, J. C. 2001. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
- 3. Sarwar, M., Kirkegaard, J. A., Wong, P. T. W., Desmarchelier, J. M. 1998. Plant and Soil. 201, 103-112.
- Smith, B. J., Kirkegaard, J. A. 2002. Plant Pathology. 51, 585-593