Investigation of the mechanism of biological control of the spot blotch pathogen *Bipolaris sorokiniana* by *Trichoderma harzianum* T-22

It is projected that food production must increase by 70% to feed an additional 2.3 billion people by 2050; without an increase in farm productivity an additional 1.6 billion hectares of arable land is needed. One solution to increase the productivity of agricultural plants is to reduce crop losses due to plant pathogens. Biological control is one method of sustainably minimising these losses.

Work conducted at Newcastle University by a previous BSPP-funded student identified that the commercially-available biocontrol fungus *Trichoderma harzianum* T-22 inhibits spore germination and development of *Bipolaris sorokiniana*, which causes spot blotch of wheat and barley, and that T-22 spores exude compounds that inhibit germination of *B. sorokiniana* spores. The aim of my project was to investigate the inhibitory secondary metabolites produced by T-22 spores and the whole fungus.

My work involved extracting metabolites from culture filtrate from T-22 grown in malt extract broth for 14 d and from spores. Spore suspensions were prepared from cultures grown on potato dextrose agar (PDA), diluted 10-fold and 100-fold and left for 72 h to allow spores to exude compounds. Also, 1 g of Trianum-P, a commercially available formulation (from Koppert B.V.) containing T-22 spores, was added to sterile distilled water in the recommended ratio of 1:5 or in a ratio of 1:10 and left for 24 h to allow compounds to be exuded. All suspensions were centrifuged and the supernatants were kept. Metabolites were extracted using ethyl acetate. The ethyl acetate phase was removed and evaporated, and the residues were suspended in 500 µl of ethyl acetate.

The effects of the extracted compounds on fungal growth were measured. 10 µl of each of the extracts was pipetted onto a *B. sorokiniana* fungal plug at the centre of a one-fifth concentration PDA plate and the plate was left for 48 h. A post hoc Dunnett test indicated that there were significant differences between the effects of all the treatments and the control ($p < 0.005$). Extract from 10-fold diluted T-22 spore suspension had the greatest effect, reducing colony diameter by approximately 50% compared to the control, followed by extracts from the commercial formulation diluted 1:5 or 1:10 and from T-22 culture filtrate. Extract from 100-fold diluted T-22 spore suspension had the least effect (approximately 25% reduction in colony diameter).

An additional experiment investigated the effect of compounds from Trianum-P on the lengths of *B. sorokiniana* germ tubes. Trianum-P was suspended in either sterile water or ethyl acetate in volumes of 5 ml and 10 ml and released compounds were extracted as described above. 5 µl of concentrated Trianum-P extract was added to 45 µl of concentrated *B. sorokiniana* spore suspension and the mixture pipetted onto a PDA-coated microscope slide. The inoculated microscope slides were incubated at 28 ^{o}C for 4 h. Germ tube lengths of 20 spores in each treatment were measured. Compounds extracted in ethyl acetate significantly inhibited hyphal growth compared to the control, whereas compounds extracted in water did not, as indicated by a post hoc Dunnett test. The extract that caused the largest inhibition was from Trianum-P spores suspended in 10 ml of ethyl acetate, which reduced hyphal length by 51%.

A lot more work and research is needed in this area, but I feel these results provide evidence that secondary metabolites produced by *Trichoderma harzianum* may be able to contribute to reducing the severity of spot blotch disease of cereal crops.

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