FUNGAL FERMENTATION PRODUCTS FOR CONTROL OF ROOT-KNOT NEMATODES

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Introduction

Nematodes are an increasing problem in all of Georgia's cotton production areas. Results from a recent survey of cotton fields in Georgia showed that 69% of the sampled fields had root-knot nematodes (Kemerait, R., 2005). In 2004, according to Georgia Cooperative Extension Service estimates, plant-parasitic nematodes caused $40 million in crop losses on cotton, and incurred 82% of the cost of pesticides used for disease control (Pearce, M. A., et. al., 2004). Although average damage levels due to nematodes may be in the 10 % range, these losses are not evenly distributed, and growers with problem fields are experiencing much higher levels of crop loss. Our research on cotton in Georgia has indicated that cotton yield losses due to nematodes may be as high as 60-70% in fields infested with root-knot or reniform (Noe, 1994, 1998), Total crop failures are possible with extreme pest pressures. Populations of these parasitic nematodes may increase 200-300% per year under cotton

A critical need exists for the development of new nematode management options for cotton production. Commercially-acceptable cotton cultivars that are resistant to nematodes are not yet available, and breeding of new resistant cultivars is proceeding slowly. Chemical control of nematodes on cotton relies mainly on Temic (aldicarb), and Telone (1-3 dichloropropene). The use of traditional chemical pesticides for control of nematodes is both expensive and hazardous. The primary emphasis of this project is the development of novel nematicidal compounds derived from microbial culture filtrates. These nematicides are more targeted against nematodes and are less hazardous to the environment than traditional pest-control chemicals. Our hypothesis is that the effective use of new biologically-based nematicides can significantly reduce production costs and enhance consumer acceptance of the resulting cotton products, both for fiber and feed.

Materials and Methods

Soil samples were collected from locations in Georgia with differing soil types and habitats. Soilborne fungi were isolated from these samples by dilution-plating and use of selective growth media. Several thousand isolates of fungi were recovered, from which approximately 150 isolates were selected for further evaluation as producers of nematicidal compounds. For evaluation, each fungus was placed in flasks containing nutrient agar and fermented with aeration on platform shakers for 10 days. As an in-vitro assay, liquid cultures were micro-filtered (0.22 μm) and pipetted into sterile microwell plates with freshly-hatched Southern rootknot nematode (*M. incognita*) juveniles. Sterile water was used as a control treatment. Nematode survival rates were

determined at 2, 4, 24, and 48 hours after suspension, with 6 replications per isolate. Liquid fungal-culture filtrates also were applied to a sterile soil mix in 6" greenhouse pots. Control treatments of sterile water, and a filtrate of the nutrient agar used for fermentation were also applied. Southern rootknot nematode (*M. incognita*) eggs were added to the pots, and cotton cv. DPL5415 RR was planted in each pot to serve as a susceptible host. Each treatment was applied to 6 replications. Plants were grown on greenhouse benches for 45 days. Plant roots were then removed from the pots and washed, and the nematode eggs were collected and counted. Total numbers of nematode eggs were compared using ANOVA followed by mean separation (LSD) for each fungal-isolate treatment and the controls. After mass screening of the fungal collection, isolates were selected and further evaluated for biocidal production using different evaluation protocols. The methods used were similar to the greenhouse screening, but with different soil types, culture media, and fermentation protocols.

Results and Discussion

After preliminary selection from the several thousand fungal isolates that were recovered from soil samples, approximately 150 fungi were further evaluated for production of nematicidal compounds. Of the 150 that were screened in the laboratory tests, 3 isolates showed enough root-knot nematode control when applied to soil planted with cotton to warrant their selection for phase 3 evaluation and testing. This final phase of testing included repeated greenhouse screens, evaluation of rates and application methods for nematode control, and a determination of the efficacy of heat-killed and dried fermentation residues for nematode control in soil.

Two fungal isolates were tested in three different soil types for efficacy in killing root-knot nematodes on cotton. One soil each from north and south Georgia, and a greenhouse mix were placed in 6" pots and treatments consisting of two candidate culture filtrates, a control fungal culture (no effect on nematodes), raw culture media, and water were applied. There was no significant interaction among the soils and treatments ($P < 0.05$), so results from all three soils were combined, giving a total of 18 replications per treatment. Both of the candidate culture filtrates reduced nematode egg numbers per gram of root weight ($P < 0.05$) across the three soil types, with the nematicidal treatments reducing the number of eggs by an average of 60% compared to the control treatments.

We continue to observe variability in nematode control results from the soil-treatment evaluations, and development of a final product is slowed by the need to evaluate various protocols for stabilizing the nematicidal activity of selected fungal isolates. In one series of experiments, fungal culture filtrates were dried and applied as a powder at varying rates to soil in greenhouse pots, with 6 replications per treatment. Two of the fungal isolates tested reduced root-knot nematode egg numbers by an average of 65% when applied in a powdered formulation ($P < 0.05$). In another experiment, culture filtrates of selected fungal isolates were pH-adjusted to determine the possibility of enhancing the nematicidal formulations by changing the acidity of the solutions. In two trials with 6 replications each, pH adjustment did not significantly enhance the nematicidal activity of any of the culture filtrates tested ($P < 0.05$). Other experimental approaches for evaluation of application rates, methods, and timing of treatments are planned to further the development of an effective and reliable nematicidal preparation.

Acknowledgments

This work was supported in part by the Georgia Cotton Commission and Cotton Incorporated.

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