Liquid Fermentation Can Mitigate Mycotoxin Production in *Myrothecium verrucaria*, a Mycoherbicide for Kudzu and Hemp Sesbania Control

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INTRODUCTION

Kudzu (*Pueraria lobata*) is an exotic invasive weed in the southeastern U.S., and an over-wintering host for Asian soybean rust (*Phakopsora pachyrhizi*). Kudzu now occurs from Florida to New York, westward to central Oklahoma and Texas, with heaviest infestations in Alabama, Georgia, and Mississippi (Miller, 1985).

Hemp sesbania (*Sesbania exaltata*) is one of the most important agronomic weeds in row crops and rice in the mid-southern U.S. and ranks as one of the most troublesome weeds in Arkansas, Louisiana, and Mississippi rice fields reducing seed quality and crop yield.

Fungal spores of *Myrothecium verrucaria* IMI 361690 (MV), exhibit excellent biocontrol potential for several weed species, including hemp sesbania and kudzu when formulated with Silwet L-77 surfactant (SW). Although MV can effectively control several weeds, mycotoxin production (e.g. trichothecene verrucarins) has hindered commercial interest. Our objectives were to examine the possibility of reducing or eliminating trichothecenes by cultural manipulation. More specifically, we sought to develop methodology to rapidly produce mycelial inoculum in liquid fermentation, as this is the desired method to mass-produce microbial bioherbicides.

MATERIALS AND METHODS

Inoculum production Mycelial inocula of MV (Figure 1). was produced in shake flasks and laboratory fermenters in soyflour-cornmeal liquid medium (SCM) and Richards V-8 solution (RV8) at 125 rpm and 28ºC for 48 h. The mycelium was then collected and assayed for trichothecenes via HPLC. A greenhouse bioassay was conducted by spraying kudzu and hemp sesbania seedlings with MV mycelium formulated with 0.2% SW.

Field tests Kudzu test plots were established near Eden, MS, and treated weekly for a period of 6 weeks, beginning on 9/12/06. Kudzu plants were ~0.90 - 1.0 m tall at the first treatment. Hemp sesbania plants were established in rice plots in Stoneville, MS, and treated on 7/20/06 when plants were ~1.0 - 1.5 m tall. In all tests, raw fermentation product in 0.2% SW was sprayed at a rate of ~300 L/ha using a CO_2 backpack sprayer. Kudzu and hemp sesbania were rated weekly for weed control.

RESULTS AND DISCUSSION

Inoculum production MV mycelium grew rapidly in both media, producing thick vegetative growth after 24 h (Fig. 1A-C). CFU counts of raw product averaged ~ 2 x 106/ml. Greenhouse bioassays revealed that the raw fermentation product was highly efficacious in infecting and killing both kudzu and hemp sesbania (Figure 2). HPLC analysis revealed that trichothecene levels were either eliminated or significantly reduced (Fig. 3). Because there appeared to be no differences in growth or bioassay effects, MV grown in SCM was selected for use in field trials.

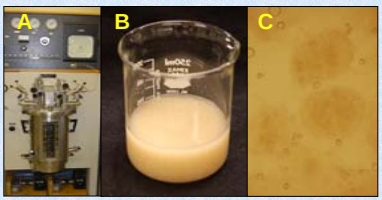

Figure 1. MV mycelium produced in liquid fermentation; A = fermenter, B = mycelial mass, C = microscopic assessment of MV mycelium.

Field tests Kudzu and hemp sesbania were severely damaged after 24 h. Over 90% of each species was controlled within 48 h. (Fig. 4 & 5). Regrowth of kudzu required weekly applications for continual control until the first killing frost occurred in mid-October (Fig. 6). However, a single application of MV mycelium provided season-long control of hemp sesbania (Fig. 7).

Figure 2. Biocontrol of kudzu (A) and hemp sesbania (B) with MV mycelial formulation.

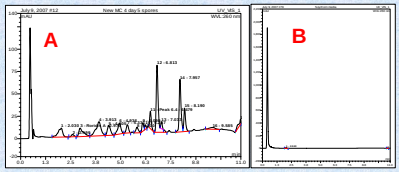

Figure 3. HPLC measurement of trichothecenes in MV spores (A) and mycelium (B).

Figure 4 . Kudzu controlled by MV mycelial formulation 48 HAT.

Figure 5. Hemp sesbania controlled in rice by MV mycelial formulation 48 HAT.

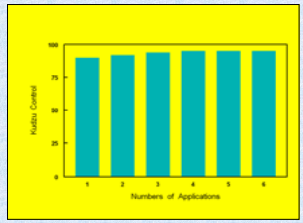

Figure 6. Six weekly re-applications of MV controlled kudzu until the first killing frost occurred.

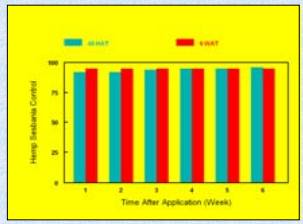

Figure 7. Hemp sesbania in rice controlled by MV mycelial formulation. No regrowth occurred 6 WAT.

SUMMARY AND CONCLUSIONS

- MV mycelium can be rapidly produced on inexpensive agricultural products.
- MV mycelium controls kudzu with repeated applications and hemp sesbania with a single application.
- Trichothecene production in MV mycelium is greatly reduced or eliminated.
- MV mycelium provides a safe, highly efficacious bioherbicidal product.
- These findings may improve probability of EPA registration and commercial development.

ACKNOWLEDGEMENTS

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