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Bioefficacy and shelf life of conidial and chlamydospore formulations of *Trichoderma harzianum* Rifai

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ABSTRACT: **The conidia and chlamydospores of *Trichoderma harzianum* produced by solid state and liquid fermentation, respectively, were formulated as powder and tested for their comparative bioefficacy and shelf life. Both the propagules did not differ significantly in reducing root rot incidence in chickpea caused by *Rhizoctonia solani*. Conidial formulation retained optimum amount of viable propagules (>10^6 cfu/g) even after 180 days of storage at room temperature but in chlamydospore formulation, viable propagules reduced to less than 10^6 by 150 days.**

KEY WORDS: Bioefficacy, chlamydospores, conidia, formulation, shelf life, *Trichoderma harzianum*

Fungi in the genus *Trichoderma* are promising bioprotectants against many plant pathogens. Biomass of these fungi used for biological control should contain right kind of propagules. *Trichoderma* species produce three kinds of propagules, which are hyphae, chlamydospores and conidia (Papavizas, 1985). Biomass of *Trichoderma* produced for biological control must retain high level of viability after drying. Since hyphae typically do not withstand drying, hyphal biomass is not useful. Conidia are produced more abundantly than chlamydospores in solid state fermentation. Conidia are difficult to be produced in submerged conditions (Papavizas *et al.*, 1984; Harman *et al.*, 1991). However, a suitable medium was developed by Harman *et al.* (1991) which supported production of large quantities of conidia of *Trichoderma harzianum* Rifai in submerged shake-flask cultures but the biomass was not formulated and tested for shelf life. Adequate information is not available on comparative bioefficacy and shelf life of conidial and chlamydospore propagules of *Trichoderma*. In the present study, conidial and chlamydospore propagules of *T. harzianum* were produced, formulated and tested for bioefficacy and shelf life.

MATERIALS AND METHODS

Bioagent and Growth conditions

Trichoderma harzianum isolate PDBCTH-10 is an effective bioagent against *Rhizoctonia solani* of chickpea (Prasad and Rangeshwaran, 2000a). Molasses-soy medium was used for mass production of *T. harzianum* (Prasad and Rangeshwaran, 2000b). Starter culture of the bioagent was prepared by inoculating 2 ml spore suspension from a slant culture to 100 ml molasses-soy medium in 250 ml Erlenmeyer flasks. The flasks

were kept on shaker for 3 consecutive days and the culture broth was used as starter culture to initiate fermentation in molasses-soy medium using 2 L laboratory fermenter (VirTis, Virtis Inc. USA). The fermenter was run for 7 days at 500 rpm, 32°C temperature and 2 LPM aeration. The 7-day-old culture broth from this fermenter served as chlamydospore inoculum (also contained bits of mycelium). For obtaining conidial inoculum, 3-day old culture broth from shaker culture was spread on potato dextrose agar plates and was incubated at 25°C for four days. Biomass from these plates served as conidial inoculum (which also contained mycelial fragments).

Preparation of conidial and chlamydospore formulation

Conidial formulation was prepared by blending conidia along with medium (PDA) in sterile water and mixed with kaolin powder in 1:2 ratio (Prasad and Rangeshwaran, 2000C). Chlamydospore formulation was prepared by harvesting fermenter biomass (FB) on 7th day and mixed with talc powder in 1:2 ratio. Both formulations were air-dried and moisture level (determined by using moisture analyzer) in two formulations was maintained at 11percent. Gum acacia powder (0.5%) was mixed to act as sticker for seed treatment. For maintaining uniform number of propagules in both formulations, initially, the product was diluted with sterilized talc powder.

Bioefficacy of *Trichoderma harzianum* propagules

A pot culture experiment was conducted in a completely randomized block design under greenhouse conditions to test the bioefficacy of *Trichoderma harzianum* propagules. *Rhizoctonia solani* (not identified to anastomosis group), which incites root rot in chickpea, was selected as test pathogen. The pathogen was grown on wheat bran for 15 days and used @ 3g/kg soil. The conidial and chlamydospore formulations were applied @ 10g/kg chickpea seed (cv. Annegiri). Fungicide (captan) was applied @ 2.5g/kg seed. Pathogen alone treatment served as check. Four replications were maintained for each treatment. Seedling emergence was recorded after 10 days of sowing. Incidence of root rot was recorded at 30 days after sowing. The root rot incidence was calculated by using number of plants infected divided by total number of plants in each pot and multiplied by hundred. The experiment was repeated twice and pooled data were analyzed for statistical significance.

Shelf life of conidial and chlamydospore propagules

Formulations of bioagent were stored at room temperature (25±2°C) in polythene bags. One-gram samples were drawn at 30 day interval for 180 days and colony forming units (cfu) were estimated by dilution plate method using *Trichoderma* selective medium (Elad and Chet, 1983). Three replications were maintained for each treatment (formulation). The data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

Bioefficacy of *Trichoderma harzianum* propagules- Greenhouse studies

The bioefficacy of conidial and chlamydospore formulations of *T. harzianum* was tested against *R. solani* incited root rot of chickpea. Seedling emergence was 92.5 percent in conidial and chlamydospore treatments (Table 1). In pathogen control, the seedling emergence was only 75 percent. The root rot incidence in conidial and chlamydospore treatments was 17.5 and 20 percent, respectively. Though chlamydospore treatment resulted in less root rot incidence, it was not significantly different from conidial treatment. Earlier studies (Papavizas and Lewis, 1989; Lewis *et al.*, 1990) showed that chlamydospore preparations of *Trichoderma* and *Gliocladium* were more effective than conidial preparations in controlling diseases caused by *Sclerotium rolfsii* and *R. solani*. Our observations did not indicate much difference in efficacy of conidial or chlamydospore propagules in reducing *R. solani* incited root rot of chickpea. This could be due to the reason that the formulations were used immediately after preparation and also that the conidial preparation by solid state fermentation showed better survivability.

Table 1. Effect of *T. harzianum* propagules on root rot of chickpea incited by *R. solani*

Bioagent propagules	Seedling emergence (%)	Root rot incidence (%)*
Conidia	92.5	17.5 (26.6)
Chlamydo spores	92.5	20.0 (26.1)
Pathogen control	75.0	67.5 (59.0)
C D (P= 0.05)	(N.S)	(12.7)

* Figures in parentheses are angular transformed values.

Shelf life of conidial and chlamydospore propagules

The viable propagule counts of conidial and chlamydospore formulations taken at 0 day and at 30 days of storage at room temperature are not significantly different. The population levels in both formulations are above 10^8cfu/g (Fig. 1). However, significant differences in the number of viable propagules between conidial and chlamydopsore formulations (log 7.65 for conidia and log 7.47 for chlamydospore) were observed at 60-180 days. The population levels in both formulations were significantly different during the storage period of 60 to 180 days. The number of viable propagules in chlamydospore formulation declined to less than 10^6 cfu/g by 150 days. The minimum recommended population of fungal bioagents in any formulation for seed treatment is more than 10^6 cfu/g (Jeyarajan and Angappan, 1998). The conidial formulation retained more than the recommended levels even at 180 days after storage. Papavizas *et al.* (1984) obtained better shelf life using conidia of *T. viride* from 6-day-old agar culture and 15-day-old fermenter broth. They observed that at 25°C conidial inoculum retained 0.5×10^7 viable propagules but FB retained no viable propagules after 5 months of storage. In our study, conidia produced on solid substrate and chlamydospores produced in liquid fermentation were used for shelf life studies. The propagules of *Trichoderma* produced in liquid fermentation will have thinner cell walls and may have lesser desiccation tolerance and shelf life (Papavizas *et al.*, 1984). Hence, chlamydospores produced in liquid fermentation could have lost their viability faster than conidia. We have used 7-day-old fermenter biomass (chlamydospores and mycelium) for shelf life studies. Papavizas *et al.* (1984) used an incubation period of 15 days to get optimal level of fully mature chlamydospores under liquid fermentation. Though we had used relatively immature chlamydospores (7-day-old), shelf life obtained was similar to 15-day-old chlamydospores. It was commonly felt that 15 day production period is too long for commercial production. Jin *et al.* (1996) reported the production of optimum quantity of biomass mostly containing conidia and mycelia of *T. harzianum* by 3 days of growth in a laboratory scale fermenter. They succeeded in increasing the desiccation tolerance

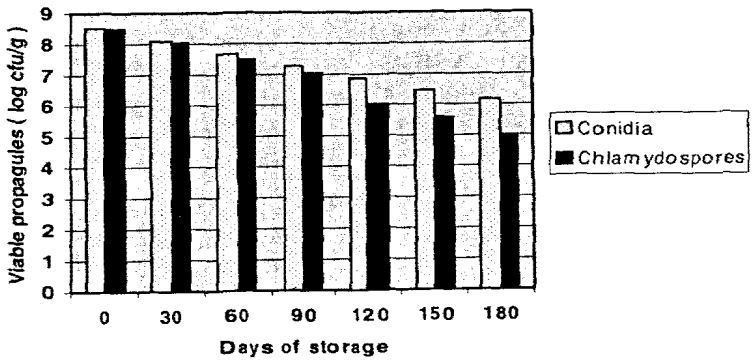

Fig. 1 Shelf life of conidia and chlamydospores of *T. harzianum* after storage. Values in bars for each storage period differ significantly except at 0 and 30 days from each other at P=0.05.

of conidia by lowering the water potential of growth medium and growing at a higher temperature (32°C). However, they did not formulate the biomass and assess the shelf life. Similarly, the previous studies reported production of chlamydospores (Papavizas *et al.*, 1984) and conidia (Harman *et al.*, 1991) by liquid fermentation, without further formulation and testing for shelf life. The conidia produced on solid substrate are known to have thicker cell walls compared to those produced in liquid medium (Munoz *et al.*, 1995). In this investigation, we produced the conidia of *T. harzianum* on solid medium, formulated and evaluated its shelf life in order to understand the effect of its production process (solid state fermentation) on the shelf life.

Conidia produced by solid state fermentation in our experiments survived longer than chlamydopsores and exhibited almost equal bioefficacy as compared to chlamydopsores produced under submerged fermentation. But longer incubation period is required for commercial production of conidia using solid substrate. Also, chances of contamination are more and developing a formulation from such solid state products for seed treatment is very difficult. So research efforts needs to be diverted to identify a suitable submerged production system, which allows production of maximum conidia with enhanced shelf life.

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