

MM 3.3: Commercialisation of bioagent mass-production technologies in intensive cotton districts

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Screening of antagonistic organisms against cotton pathogen, *Verticillium dahliae*

Twenty-five isolates of *Trichoderma* belonging to *T. viride* (7 isolates), *T. harzianum* (10 isolates), *T. virens* (3 isolates) *T. pseudokoningii* (2 isolates) and an isolate each of *T. koningii*, *T. hamatum*, *T. deliquescens*, were tested against *V. dahliae* by Dual culture test. The percent inhibition of the pathogen and over growth of antagonists on the pathogen is presented in Table.1. The percent inhibition of *V. dahliae* with different isolates of antagonists ranged from 67.8 to 75.2 and over growth of antagonists on *V. dahliae* ranged from 2.5mm to 29.2mm. Based on % inhibition of *V. dahliae* and extent of overgrowth of antagonist on *V. dahliae*, PDBCTV-3, PDBCTV-11, PDBCTV-13, PDBCTH-1, PDBCTH-2, PDBCTH-4, PDBCTH-6, PDBCTH-7, PDBCTH-8, PDBCTH-V2 and PDBCTVS-9 were identified as promising against *V. dahliae*, showing good inhibition (71.2-75.2%) and good overgrowth (22.5-29.2 mm) on *V. dahliae*.

Effect of promising isolates of *Trichoderma* sp. on seed germination and seedling growth of cotton.

Twelve promising isolates of *Trichoderma* sp. belonging to *T. viride* (PDBCTV-11, TV-13, TV-97 and TV-CICR), *T. harzianum* (PDBCTH-4, TH-10, TH-V2, CPCRI-TH-KS, CPCRI-TH-KD, IISR-GTH7, IISR-TH-P26) and *T. virens* (PDBCTVs-9) were tested for their effect on seed germination and seedling growth of cotton by paper towel method. Hundred seeds were treated with the spore suspension of each isolate at the spore dose of 2×10^6 spores and dried and planted on paper towel. The percentage seed germination, the root length and shoot length were recorded and based on these observations, vigor index was worked out. All twelve isolates of *Trichoderma* tested showed higher seed germination (92.5-100%) and vigor index (2090.0-2660.0) compared to the untreated seeds which showed 75.0% seed germination with vigor index of 1455.0. Among the twelve isolates of *Trichoderma* tested, *T. harzianum* (CPCRI-TH-KD) showed highest seedling vigor index of 2660.0 followed by *T. harzianum* (IISR-TH-P26) showing vigor index of 2457.0.

Shelf-life of talc formulations of *B. bassiana* and *M. anisopliae* prepared from the fermentor biomass of different production media *B. bassiana* was grown in three different production media viz., Jaggery soya broth (JSB), Corn meal broth (CMB) and Jaggery Soya yeast broth (JSYB) in a fermentor (10 liter capacity) for 7 days and talc formulations were prepared from the fermentor biomass obtained from these three media by mixing with sterilized talc at 1:1 ratio and then drying to 8% moisture. Similarly talc formulations of *M. anisopliae* were prepared from fermentor biomass (7days) obtained from, Potato dextrose broth (PDB), Sabouroud dextrose yeast broth (SDYB), Jaggery Soya yeast broth (JSYB) and Jaggery soya broth (JSB). After drying, the samples were packed in polypropylene bags and stored at room temperature (17-36°C) and refrigerated temperature (6-8°C) for shelf life studies. The samples were drawn at monthly intervals and the cfu counts were estimated on PDA plates.

The cfu counts in talc formulations of *B. bassiana* prepared from corn meal broth (CMB) remained as per the CIB standard (minimum of 1.0×10^8 cfu/g) for a period of seven months (5.4×10^8 cfu/g), when stored at room temperature and for ten months at refrigerated temperature (2.0×10^8 cfu/g). The shelf life of the talc formulations of *B. bassiana* prepared from JSB and JSYB media remained for six months when stored at room temperature (1.3×10^8 and 1.1×10^8 cfu/g respectively) and nine months at refrigerated temperature (1.4×10^8 and 2.4×10^8 cfu/g respectively). Thus these studies indicate that the talc formulations of *B. bassiana* prepared from CMB had longer shelf at room and refrigerated temperature compared to other media.

Among the four production media tested for their effect on the shelf life of talc formulations of *M. anisopliae*, the talc formulations prepared from Jaggery soya yeast broth (JSYB) showed longest shelf life of ten months at room temperature (2.3×10^8 cfu/g) and twelve months at refrigerated temperature (1.8×10^8 cfu/g). The shelf life of talc formulations prepared from JSB, SDYB and PDB media had shelf life of 7, 6 and 5 months respectively at room temperature and 10, 10 and 8 months respectively at refrigerated temperature. Thus these studies indicate that the talc formulations of *M. anisopliae* prepared from JSYB had longer shelf at room and refrigerated temperatures compared to other media.

Effect of addition of Polyethylene Glycol (Osmoticant) to the production media on shelf life of *B. bassiana*, *M. anisopliae* and *N. rileyi*

***B. bassiana*, *M. anisopliae*:** Polyethylene glycol (PEG, 200), a known osmoticant was added at 2% concentration into the production medium of corn meal agar (CMB) for *B. bassiana* and Jaggery Soya yeast broth (JSYB) for *M. anisopliae* in a fermentor (10 liter capacity) and talc formulations were prepared from the fermentor biomass obtained after 7 days from these media by mixing with sterilized talc at 1:1 ratio and then drying to 8% moisture. The cfu counts recoded in the formulations prepared from the PEG amended production media were significantly higher than the formulations prepared from un-amended media in all the samples stored at room temperature. However, addition 2% PEG to the production medium did not extend the shelf life of the talc formulations of *B. bassiana* and *M. anisopliae* beyond the shelf life of the formulations prepared from the un-amended media.

The shelf life of talc formulations of *B. bassiana* remained 7 months in PEG- amended as well as un-amended media and that of *M. anisopliae* remained 10 months in PEG-amended as well as un-amended media
***N. rileyi*:** In case of *N. rileyi*, PEG (200) was added at 1, 2, 3% concentrations to the production medium of Sabouroud maltose yeast broth (SMYB) in stationary culture. Talc formulations were prepared from these cultures and dried to 8% moisture and stored at room temperature for shelf studies. The cfu counts of *N. rileyi* recorded in fresh talc formulation and samples stored for 2, 3, 4 and 5 months are presented in table 7. Addition of PEG at 1-3% concentrations to the SMYB did not influence the cfu counts of *N. rileyi* in talc formulation during storage at room temperature. Shelf life remained of *N. rileyi* in talc formulations same (4 months) in PEG-amended and un-amended media.