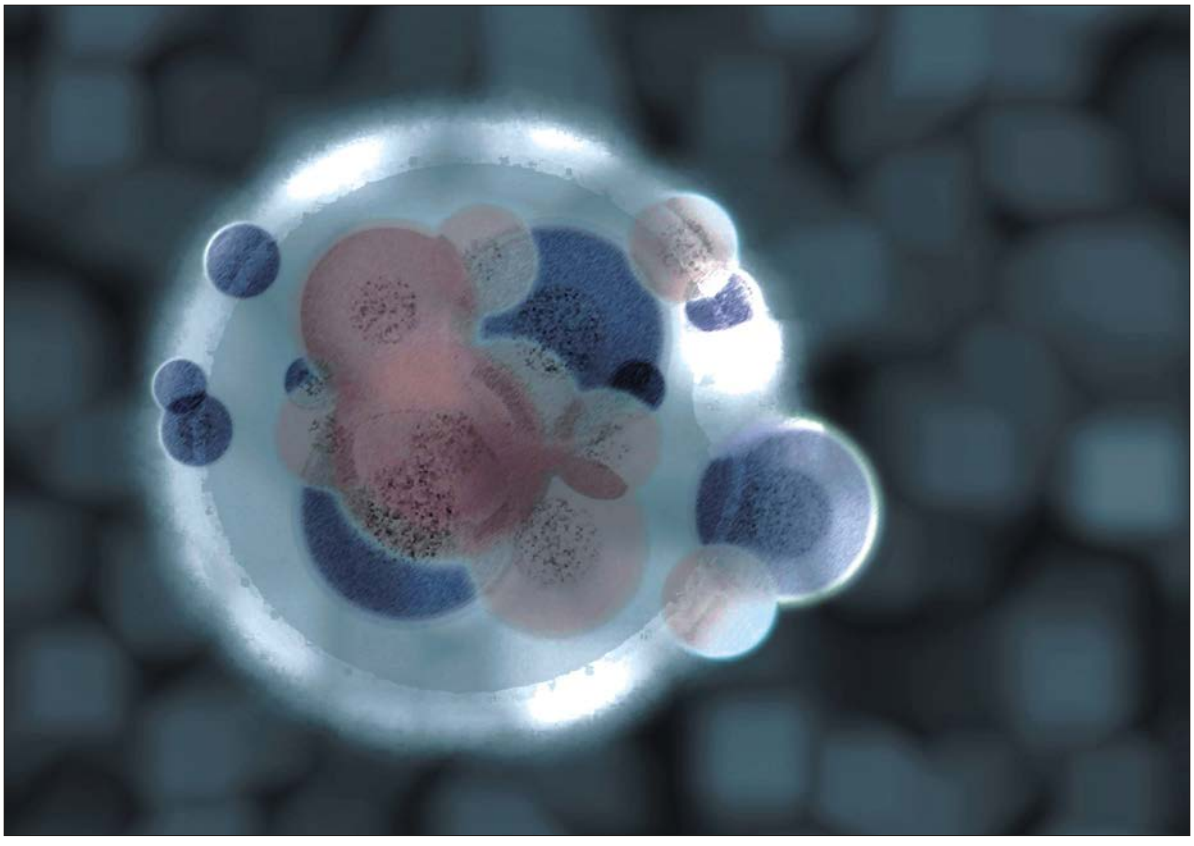

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Effect of Storage Conditions of Formulations on Viability of *Verticillium lecanii* (Zimmerman) Viegas and its Virulence to *Brevicoryne brassicae* (L.)

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Abstract: Studies were conducted on the effect of culture media, storage temperature and moisture content on viability and virulence of *Verticillium lecanii* to *Brevicoryne brassicae*. Among nine media evaluated, Molasses Yeast Broth (MYB) plus 2% Polyethylene Glycol (PEG) maintained the fungus viability higher than other media followed by MYB plus 1% PEG and rice powder. Viability in refrigerator temperature was significantly higher than in room temperature. Among three moisture levels tested, viability at 5 and 10% were on par and were significantly higher than at 15%. Viability over time decreased and the differences in viability among the three storage times were significant. Storage time and media had significant effect on aphid mortality. Mortality decreased over storage time but the rate of decrease in aphid mortality was less than the rate of decrease in the fungal viability.

Key words: *Verticillium lecanii*, moisture, temperature, storage, virulence, *Brevicoryne brassicae*

INTRODUCTION

Verticillium lecanii is an entomopathogenic fungus primarily of aphids and scales. Many isolates of this fungus demonstrate high pathogenicity to several species of aphids such as *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas), *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) (Askary *et al*., 1998; Derakhshan *et al*., 2007; Kim *et al*., 2007).

Large-scale utilization of entomopathogenic fungi for the control economically important crop pests calls for standardization of commercially viable mass production and formulation techniques. The maintenance of conidial viability in formulations during storage is crucial for obtaining effective insect control (Consolo *et al*., 2003). Formulation must be compatible with the agent, enhance its performance and ideally, must maintain an adequate shelf-life of the agent in order to be successful. The type of medium and inoculum has been found to greatly influence the stability and pathogenicity of the bioherbicidal propagules (Elzein *et al*., 2004). Polyethylene Glycol (PEG) has been shown to have varied effects on biomass characteristic in addition to its influence on the shelf-life and field performance different fungal species (Kumar *et al*., 2005). Apart from media, temperature and moisture content are also the major factors which influence conidial longevity (Hong *et al*., 1997).

In a previous study (Derakhshan *et al*., 2007), we tested 25 isolates of entomopathogenic fungi against cabbage aphid, *B. brassicae*, in which Vl-7 isolate of *V. lecanii* was found to be the most virulent isolate. In this study, we have investigated the factors influence on viability and virulence of *V. lecanii* formulations during storage.

MATERIALS AND METHODS

The experiments were conducted at the Project Directorate of Biological Control (PDBC), Bangalore, India during 2005-2006. Isolate Vl-7 of *V. lecanii* originally isolated from *Bemisia tabaci*, was obtained from PDBC.

The shelf life of the fungus in talc formulations prepared using fungal biomass produced in Potato Dextrose Broth (PDB) (200 g potato, 20 g dextrose and 1000 mL distilled water), Molasses Yeast Broth (MYB) (30 g sugarcane molasses, 5 g yeast extract and 1000 mL distilled water), MYB supplemented by 1 and 2% Polyethylene Glycol (PEG) and cereal grain powder

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formulations prepared from rice, ragi, sorghum, corn and wheat at three different moisture levels (5, 10 and 15%) and two temperature levels viz., room (18-32°C) and refrigerator (4-7°C) conditions, was assessed at different intervals, i.e., 0, 60, 120 and 180 days.

Preparation of formulations: The fungus was cultured in PDB and MYB (0, 1, 2% PEG) media for 14 days and then mixed with sterilized talc powder in 1:1 ratio. In case of grain powders, the fungus was grown on grains followed the method described by Nirmala *et al.* (2006). After 14 days of growth, the grains were powdered and the formulations were dried to 5, 10 and 15% moisture content and then packed into polyethylene bags and were stored at room and refrigerator conditions.

Viability tests: To determine the fungus colony forming units per gram (cfu g^{-1}), one gram of formulations was suspended in 10 mL of 0.05% sterilized Tween-80 solution for making serial dilution. Prepared serial dilutions were plated at one milliliter per plate on PDA medium as per dilution plating method. The plates were gently rotated for uniform spreading of spore suspension and incubated at 25±2°C, 90±3% RH and 12:12 photoperiod. Each treatment had three replications. The CFU counts were recorded on 7th day after plating.

Pathogenicity tests: Pathogenicity of the fungus in different formulations stored at room conditions at 10% initial moisture content was assessed against *B. brassicae* using bioassay technique. For bioassay, healthy cabbage leaves were rinsed initially with distilled water for 10 min and the leaf surface further was sterilized in 0.25% sodium hypochlorite solution for 3 min, then the leaves were again rinsed three times with sterile distilled water and air-dried in a Laminar Flow Chamber (LFC). Working in the sterile LFC, the detached leaves were placed individually over sterilized 1% agar in Petri plates. Inoculation of aphids with different formulations were carried out by immersing the young adult apterous aphids (10-11 days old) in suspensions (1×10^7 cfu mL^{-1}) in a Buchner funnel for 5-10 sec and then the aphids were transferred on the sterilized filter paper. Control aphids were treated with 0.05% sterilized Tween-80 only. Twenty inoculated aphids/replication were transferred to the leaf discs by the help of a brush. Then the Petri dishes were incubated at 25±2°C and 80±3% humidity. Each treatment was replicated three times.

Statistical analysis: Analysis of variance (ANOVA) was used to analyze percentage mortality data after arcsine transformation to normalize the data. Percentage mortality (at 7th day post-treatment) was also adjusted for natural mortality in controls using Abbott (1925) formula before analysis. All data were analyzed using three-way analysis of variance for a completely randomized design. Means were compared using Duncan's multiple range test ($p = 0.05$).

RESULTS AND DISCUSSION

The fungal viability was affected by culture media, storage temperature and moisture content of the formulations ($p<0.01$). Among the nine media evaluated, talc powder formulation of MYB plus 2% PEG maintained the fungus viability higher than other media followed by MYB plus 1% PEG and rice powder formulation. The results indicate the importance of adding PEG for spore longevity during storage. Among the grain powders, rice powder formulation maintained the fungus viability better than other grains followed by ragi. Talc formulation of PDB was the poorest to maintain the fungus viability. Viability in refrigerator temperature was significantly higher than in room temperature. Among the three moisture levels tested, viability at 5 and 10% were on par and were significantly higher than at 15%. Viability over time decreased and the differences in viability among the three storage times were significant (Table 1, 2).

These findings are in accordance with those of Moore *et al.* (1996) who reported the conidia moisture contents need to be reduced to around 5% for optimal storage. Daoust and Roberts (1983) also mentioned that the survival of *M. anisopliae* conidia in storage is best at either high or low humidities (90 or 10%), with intermediate levels being detrimental. Stathers *et al.* (1993) found that long-term conidial viability is maintained better at low storage temperatures. Conidial viability declined due to high temperatures and high moisture contents (Hedgecock *et al.*, 1995).

Bioassay results revealed that storage time and media had significant effect on aphid mortality ($p<0.01$). Mortality decreased over time storage but the rate of decreasing in aphid mortality is less than the rate of decreasing in the fungus viability (Table 3). This confirms reports that factors other than germination influence on the virulence of entomopathogenic fungi (Inglis *et al.*, 1997; Fargues *et al.*, 1997; Dimbi *et al.*, 2004). Among 9 media tested, MYB plus 2% PEG and MYB plus 1% PEG caused highest aphid mortality followed by rice powder. There were no significant differences among MYB, ragi, sorghum and PDB as well as wheat and corn powders. After six months storage, highest and lowest reductions in aphid mortality were seen in PDB and MYB+ 2% PEG (Table 3). The results are in agreement with Kleespies and

Table 1: Effect of storage and media on % viability of *V. lecanii* in refrigerator condition

Initial moisture content	Media	Days after storage 60	120	180
5	Talc powder (PDB)	79.29^{hi}	59.06^{ef}	35.78^{ef}
	Talc powder (MYB)	85.42^{c}	63.70^{d}	38.93^{def}
	Talc powder (MYB+1%PEG)	88.10^{ab}	69.53^{b}	50.39^{abc}
	Talc powder (MYB+2%PEG)	89.95^{a}	72.74^{a}	57.59^{a}
	Grain powder (Ragi))	82.73^{d}	62.96^{d}	42.83^{ede}
	Grain powder (Rice)	85.07^{c}	63.80^{d}	43.19^{cde}
	Grain powder (Sorghum)	79.80^{efg}	58.86^{efg}	34.97^{ef}
	Grain powder (Wheat)	80.95^{def}	57.83^{fg}	36.47^{ef}
	Grain powder(Corn)	81.36^{def}	58.57^{fg}	37.38^{ef}
10	Talc powder (PDB)	79.23^{ghi}	58.21^{fg}	36.60^{ef}
	Talc powder (MYB)	85.09^{c}	63.41^{d}	38.37^{def}
	Talc powder (MYB+1%PEG)	88.11^{ab}	69.11^{b}	54.35^{a}
	Talc powder (MYB+2%PEG)	89.95^{a}	73.19^{a}	57.08^{a}
	Grain powder (Ragi))	82.62^{d}	63.35^{d}	42.35^{cde}
	Grain powder (Rice)	84.81^{c}	63.47^{d}	42.60^{cde}
	Grain powder (Sorghum)	79.55^{fgh}	58.09^{fg}	33.33^{f}
	Grain powder (Wheat)	80.84^{def}	56.92^{gh}	35.96^{ef}
	Grain powder(Corn)	81.10^{def}	57.94^{fg}	36.84^{ef}
15	Talc powder (PDB)	77.60^{ij}	53.43^{i}	32.31^{e}
	Talc powder (MYB)	80.81^{def}	60.60^{e}	35.73^{ef}
	Talc powder (MYB+1%PEG)	86.65^{bc}	66.71^{c}	52.04^{ab}
	Talc powder (MYB+2%PEG)	89.16^{a}	71.74^{a}	55.49^{a}
	Grain powder (Ragi))	81.64^{de}	60.78^{e}	39.45^{bcd}
	Grain powder (Rice)	81.79^{e}	60.81^{e}	40.60^{def}
	Grain powder (Sorghum)	78.30^{hij}	55.49^{hi}	30.64^{fg}
	Grain powder (Wheat)	81.14^{edf}	53.69^{hi}	33.49^{f}
	Grain powder(Corn)	76.34^{j}	54.79^{hi}	32.78^{f}

Means followed by the similar superscripts letter(s) in the columns are not significantly different at 5% by Duncan's multiple range test (DMRT)

Table 2: Effect of storage and media on % viability of *V. lecanii* in room condition

Initial moisture content	Media	Days after storage 60	120	180
5	Talc powder (PDB)	75.03^{k}	52.18^{kl}	28.37^{l}
	Talc powder (MYB)	82.90^{cd}	60.60^{ef}	35.01^{fgh}
	Talc powder (MYB+1%PEG)	86.16^{b}	65.15^{c}	45.71^{c}
	Talc powder (MYB+2%PEG)	89.36^{a}	72.16^{a}	56.12^{a}
	Grain powder (Ragi))	82.03^{cde}	62.33^{d}	42.17^{d}
	Grain powder (Rice)	83.88^{c}	62.13^{de}	42.17^{d}
	Grain powder (Sorghum)	78.87^{hij}	55.59^{hi}	31.56^{jk}
	Grain powder (Wheat)	82.75^{cd}	54.90^{hi}	34.31^{hi}
	Grain powder(Corn)	79.89^{efg}	56.18^{gh}	36.03^{fg}
10	Talc powder (PDB)	76.03^{jk}	50.82^{l}	27.59^{l}
	Talc powder (MYB)	82.68^{cd}	60.57^{ef}	34.59^{gh}
	Talc powder (MYB+1%PEG)	83.55^{c}	64.92^{c}	45.45^{c}
	Talc powder (MYB+2%PEG)	89.16^{a}	71.93^{a}	56.16^{a}
	Grain powder (Ragi))	82.03^{cde}	62.19^{de}	42.08^{d}
	Grain powder (Rice)	83.92^{c}	61.79^{de}	41.87^{d}
	Grain powder (Sorghum)	79.08^{fgh}	54.72^{hi}	61.83^{jk}
	Grain powder (Wheat)	82.26^{cd}	54.13^{ij}	32.88^{ij}
	Grain powder(Corn)	79.59^{fgh}	54.10^{ij}	34.86^{fgh}
15	Talc powder (PDB)	71.56^{l}	57.35^{g}	21.86^{m}
	Talc powder (MYB)	81.21^{def}	59.04^{f}	31.67^{jk}
	Talc powder (MYB+1%PEG)	82.51^{cd}	63.91^{c}	44.62^{c}
	Talc powder (MYB+2%PEG)	87.83^{ab}	70.21^{b}	52.51^{b}
	Grain powder (Ragi))	79.94^{efg}	59.19^{f}	36.35^{f}
	Grain powder (Rice)	81.85^{cde}	60.06^{f}	39.35^{e}
	Grain powder (Sorghum)	77.62^{hij}	52.79^{ijk}	30.70^{k}
	Grain powder (Wheat)	77.04^{ijk}	52.61^{jk}	30.30^{k}
	Grain powder(Corn)	78.46^{hij}	52.81^{jk}	30.89^{k}

Means followed by the similar superscripts letter(s) in the columns are not significantly different at 5% by DMRT

Table 3: Influence of storage and media on infectivity of *V. lecanii* to *B. brassicae* (% aphid mortality)

Media	Days after storage 60	120	180
Talc powder (PDB)	61.54^{bc}	51.85^{d}	37.04^{f}
Talc powder (MYB)	67.31^{c}	55.56^{bc}	38.89^{e}
Talc powder (MYB+1%PEG)	72.14^{ab}	62.96^{a}	46.30^{b}
Talc powder (MYB+2%PEG)	72.38^{ab}	62.11^{a}	54.15^{a}
Grain powder (Ragi))	67.31^{abc}	55.56^{bc}	42.59^{d}
Grain powder (Rice)	73.08^{a}	57.41^{b}	44.44^{c}
Grain powder (Sorghum)	69.23^{ab}	53.70^{c}	38.89^{e}
Grain powder (Wheat)	67.31^{abc}	48.15^{e}	35.19^{g}
Grain powder(Corn)	63.46^{abc}	48.15^{e}	35.19^{g}

Means followed by the similar superscripts letter(s) in the columns are not significantly different at 5% by DMRT

Zimmermann (1998) who observed increased viability of blastospores produced in 5% PEG amended media compared to un-amended medium. Hallsworth and Magan (1994) reported that reduction in water activity by PEG can lead to the accumulation of trehalose in spores that were more pathogenic than those produced on control media.

One fundamental objective with microbial pesticides is long-term storage with no loss of product viability and virulence. The results presented here showed that media has significant effect on fungal viability during storage as well as its virulence. Conidia produced in MYB supplemented by 2% PEG 200 maintained viability and virulence of the fungus higher than other media at both room and refrigerator conditions. This indicates that selected media for mass production not only must supported higher conidial yield but also be able to keep viability and virulence of the fungus in desirable condition.

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