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RESEARCH ARTICLE

Effect of different storage conditions on spore viability of *Lecanicillium lecanii* formulations and infectivity to mealybug, *Paracoccus marginatus*

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ABSTRACT

Talc based formulation of *Lecanicillium lecanii*, a native entomopathogenic fungus isolated from mealy bug was developed at Central Institute for Cotton Research, Regional Station, Coimbatore. *L. lecanii* spores produced in sorghum grains, sabouraud dextrose broth with Yeast extract (SDYB) and potato dextrose broth (PDB) were formulated in talc and stored at room temperature (27±2°C) and refrigerator (9±2°C). Viability and virulence of spores was monitored at monthly intervals for six months. Among different formulations tested, *L. lecanii* multiplied on SDY broth and formulated in talc supported maximum viability and virulence. Among two storage temperatures tested, formulation stored at 9±2°C supported maximum viability and virulence. In general, spore viability was reduced with increase in storage duration and temperature.

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INTRODUCTION

Lecanicillium lecanii is a widely worked potential entomopathogenic fungus that is highly effective against wide range of insects hosts belonging to Homoptera, Orthoptera , Coleoptera and Lepidoptera (Goettel *et al.*, 2008). A native isolate of *L.lecanii* was found to be highly effective against mealybug, *Paracoccus marginatus* and *Phenococcus solenopsis* infecting cotton in India (Banu *et al.*,2009 and 2010). Formulation of entomopathogens is a critical step towards their implementation as biocontrol agents. Formulation must be devised in combination of ingredients to ensure that the active component effectively reaches the target pests. The proper formulation of a fungal biocontrol agent, therefore, requires an understanding of the life cycle of the pathogen, and effects of environmental factors on its biology, combined with knowledge of the target hosts biology (Feng *et al.*, 1994). The development of formulations inexpensive, which minimizes the susceptibility of fungi to environmental stress, and is compatible with suitable application technologies that may enhance the efficacy of entomopathogenic fungi as biocontrol agents. Formulation plays an important role in delivering the fungal entomopathogens to the target environment. Formulation development must also consider ecological and environmental factors to maximize the biocontrol efficacy (Jackson *et al.*, 2010). The talc based formulation containing beneficial microbes was found to be effective and cheaper for management of insect pests (Saranya and Ushakumari, 2011; Shahid *et al.*, 2011). Formulation and storage temperature plays an important role in viability and virulence of spores (Banu and Gopal Krishnan *et al.*, 2012). Keeping these facts in view, a laboratory experiment was carried out to identify the suitable medium and optimum temperature for storage based on spore viability for *L. lecanii* and virulence against *P. marginatus* infecting cotton in India.

MATERIAL AND METHODS

Fungus :

Lecanicillium lecanii used in this study was isolated

from naturally infected mealybug, *Phenococcus solenopsis* (Tinsley) (Hemiptera: Psuedococcidae) from Coimbatore district, Tamil Nadu, India during 2009.The fungal isolate was cultured on sabouraud dextrose agar with Yeast extract (SDAY) medium for 7-10 days at 25°C.

Preparation of formulations :

The media tried were sorghum grains, potato dextrose broth (PDB) and sabouraud dextrose broth with yeast extract (SDYB). *L.lecanii* was multiplied in SDYB and one ml spore suspension was inoculated and left for complete sporulation at 27 ± 1°C. After completion of sporulation, sorghum grains were air dried for 12 – 18 hours and powdered using warring blender under aseptic conditions. Talc powder (Grade 5) was heated in hot air oven at 70°C for three days using metal pans. Two hundred and fifty grams of powdered sorghum grain media (spore laden) was thoroughly mixed with 750g of talc powder using pestle and mortar. *L.lecanii* multiplied in potato dextrose broth and sabouraud dextrose broth with yeast extract was mixed with talc at 1:2 ratio (500 ml: 1 kg). To the mixture, 5 g of carboxy methyl cellulose was added as sticker and dried in shade for 72 h, powdered and stored in polypropylene bags at two temperatures (9 and 27 ± 2°C) (Jeyarajan *et al*., 1994).

Viability test :

The viability of conidia was tested at 30 days interval upto six months. The test was conducted by spraying 1 x 10^8 conidia/ml of formulation on SDAY medium on glass slides. Viability was estimated after 15 hours. Conidia with germ tubes equal to or greater than the width were considered to have germinated. For each treatment, three separate fields were observed for germination.

Virulence of formulations against mealybug, *P. marginatus*:

Virulence of two formulations stored at room temperature at (27±2°C) and refrigerator (9±2°C) was evaluated for six months at 30 days interval. mealybug, *P. marginatus* culture maintained in laboratory was used for the study. To test the virulence initial spore load was adjusted to 1 x 10^7 conidia/ ml and sprayed onto mealybug adults by using hand atomizer. Insect mortality was recorded at 12 hours interval upto 10 days. Dead insects were transferred to moist filter paper to observe the growth of test fungus.

RESULTS AND DISCUSSION

The fungal viability was affected by the formulation, storage temperature and duration of storage. Among three media tested, talc based formulation of SDYB maintained the maximum fungal viability followed by PDB. Irrespective of the medium tested, formulations stored at 9 ± 2°C recorded significantly more spore viability than at 27 ± 2°C. Viability over time decreased and the difference in viability among six storage duration were significant. Spore viability rapidly declined with increase in storage time at all the temperature regimes (9 and 27 ± 2°C) in sorghum grains, SDYB and PDB (Table 1). Generally the spore viability in grain based formulations declined more rapidly than in broth based formulations. This could be due to more uniform and higher moisture levels in liquid based formulations than in grain based formulations.

Virulence studies revealed that media, temperature and storage duration had significant effect on mealybug mortality. Among three media tested SDYB caused highest mortality of mealybug followed by PDB. Formulations stored at 9 ± 2°C caused significantly high mortality of mealybug. Virulence was also found to decrease over time (Table 2).

Storage temperature is the most important abiotic factor that affects the shelf-life of biological formulations by maintaining them in a state of low metabolic activity (Sandoval-Coronado *et al*., 2001). Stathers *et al*. (1993) found that long term conidial viability is maintained at low storage temperature.

Table1 : Effect of formulation, storage temperature and storage period on spore viability of *Lecanicillium lecanii*

Formulation	Storage temperature (°C)	Spore viability (%) at different days of storage						Mean
		30	60	90	120	150	180	
Sorghum+Talc	27 ± 2	82.00 (64.92)	79.33 (63.00)	62.67 (52.34)	46.00 (42.70)	40.00 (39.23)	36.00 (36.87)	65.90 (55.28)
	9 ± 2	94.00 (76.13)	92.00 (73.65)	74.33 (59.56)	65.67 (54.13)	62.33 (52.14)	56.33 (48.64)	
SDY broth+Talc	27 ± 2	96.33 (79.14)	90.67 (72.29)	88.67 (70.38)	74.00 (59.36)	66.00 (54.34)	59.67 (50.58)	81.22 (65.81)
	9 ± 2	98.00 (82.05)	94.33 (76.24)	84.00 (66.45)	79.67 (63.21)	73.33 (58.93)	70.00 (56.80)	
Potato dextrose	27 ± 2	97.33 (80.74)	80.33 (63.69)	70.33 (57.00)	62.67 (52.34)	59.67 (50.58)	53.67 (47.10)	72.56 (59.79)
broth +Talc	9 ± 2	97.00 (80.28)	86.67 (68.60)	76.33 (60.90)	64.33 (53.33)	60.33 (50.97)	62.00 (51.95)	
Mean		94.11 (72.21)	87.22 (69.58)	76.06 (61.11)	65.39 (54.18)	60.28 (51.03)	56.28 (48.66)	
Temperature	27 ± 2	69.19 (57.59)	–	–	–	–	–	–
	9 ± 2	77.26 (63.0)	–	–	–	–	–	–
CD (P=0.05)	Formulation = (0.74)		Temperature =(0.60)		Storage duration =(1.04)			

*Figures in the parantheses are arc sine transformed values

Table 2 : Effect of formulation, storage temperature and storage period on mortality of *P.marginatus* by *Lecanicillium lecanii*

Formulation	Storage temperature (°C)	Mortality (%) at different days of storage						Mean
		30	60	90	120	150	180	
Sorghum+Talc	27 ± 2	78.67 (62.50)	68.67 (55.97)	62.00 (51.95)	34.00 (35.66)	33.67 (35.47)	32.00 (34.45)	57.94 (50.05)
	9 ± 2	90.00 (71.62)	78.00 (62.04)	71.67 (57.84)	50.00 (45.00)	50.67 (45.38)	46.00 (42.70)	
SDY broth+Talc	27 ± 2	92.33 (73.94)	77.33 (61.58)	71.67 (57.84)	56.33 (48.64)	54.00 (47.30)	49.00 (44.43)	69.14 (57.17)
	9 ± 2	92.67 (74.34)	68.67 (55.97)	62.00 (51.95)	47.00 (43.28)	46.33 (42.90)	45.33 (42.32)	
Potato dextrose	27 ± 2	92.67 (74.34)	68.67 (55.97)	62.00 (51.95)	47.00 (43.28)	46.33 (42.90)	45.33 (42.32)	62.06 (52.90)
broth+Talc	9 ± 2	94.00 (76.13)	74.00 (59.37)	66.33 (54.54)	49.67 (44.81)	50.00 (45.00)	48.67 (44.24)	
Mean		90.28 (72.40)	74.44 (59.72)	68.00 (55.61)	49.94 (45.00)	49.33 (44.60)	46.28 (42.83)	
Temperature	27 ± 2	59.54 (51.14)						
	9 ± 2	66.56 (55.57)						
CD (P=0.05)	Formulation = (0.59)	Temperature = (0.48)		Storage duration = (0.84)				

*Figures in the parantheses are arc sine transformed values

Conidial viability declined due to high temperature and high moisture contents (Hedgecock *et al.*, 1995).

In the present study, viability of spores was found to be decreased over time and formulations stored at 9 ± 2°C recorded maximum viability and virulence. This agrees with the findings of Chen *et al*. (2008) who reported that the temperature was the most critical factor influencing the conidial storage stability. They revealed that both conidial germination and infection of host decreased with storage temperature ranging from 15 to 35°C. Storage temperature was found to affect the viability and shelf-life of entomopathogenic fungal formulations. Derakhshan *et al.* (2008) also reported that the viability of *V.lecanii* stored in refrigerated condition was significantly higher than at room temperature. In addition to being viable, the fungal propogules must also possess the ability to infect and kill the insect host under the environmental conditions where the entomopathogen is to be used. The shelf-life of fungal entomopathogen can be affected by nutritional and environmental conditions present during production and drying (Jackson *et al.*, 2010). Further studies are in progress to evaluate the efficacy of these two formulations under field conditions.

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