Journal of Biological Control, 31(1): 50-55, 2017, DOI: 10.18311/jbc/2017/15556

Research Article

Evaluation of carrier materials for formulating entomopathogenic fungus *Lecanicillium Lecanii* (Zimmermann) Zare and Gams

P. R. NITHYA* and O. P. REJI RANI

Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Trivandrum, Kerala

**Corresponding author E-mail:* menitz91@gmail.com

ABSTRACT: Carrier material plays a pivotal role in maintaining the viability and virulence of an entomopathogenic fungus (EPF). Evaluation studies using sunflower oil, groundnut oil and talc as the basic carrier materials and their enrichment with chitin and chitosan, was conducted. The ideal proportion of carrier material and technical ingredient and viability were assessed based on number of colony forming units (cfu) over a period of storage of 3 months. Chitin enriched ground nut oil (GNO + Chitin) and chitin enriched sunflower (SFO + chitin) were observed as the best carriers for formulating the fungus. Ideal proportion of carrier and technical ingredient in oil formulations was found to be 65:35 per cent. The number of cfu observed with chitin enriched GNO and SFO by the end of three months of storage was 2.27 x 10^6 ml^{-1} and 2.20 x 10^6 ml^{-1} under room temperature respectively. Enrichment of oil formulations (GNO and SFO) with chitin could thus sustain viability as well as spore count of *L. lecanii* till the end of experimental period (three months).

KEY WORDS: Groundnut oil, Carrier, entomopathogenic fungus, *Lecanicillium lecanii,* sunflower oil

(Article chronicle: Received: 02.02.2017; Revised: 24.03.2017; Accepted: 28.03.2017)

INTRODUCTION

In the present era of organic farming, biocontrol agents like entomopathogens play a key role in eco-friendly pest management tactics. *Lecanicillium lecanii* is a promising fungal bio agent primarily infesting sucking pests *viz.* aphids, scales, mealy bugs and whiteflies which has gained great scope for insect pest management (Hall and Papierok, 1982; Cuthbertson and Walters, 2005; Diaz *et al.,* 2009; Park and Kim, 2010; Ujjan and Shahzad, 2012*).*

The popular trend of formulating bio pesticides is mainly in solid carriers like talc, peat, lignite, clay, etc. However, these solid formulations suffer from major setbacks like shorter shelf life, high contamination and low field performance (Hedge, 2002). The carrier should be carefully chosen in such a way to enhance the inherent potential of the formulated organism. Liquid formulations offer longer shelf life, with high purity, carrier-free activity, easiness in handling and application, convenience in storage and transport, better quality parameters and enhanced export potential (Pindi and Satyanarayana, 2012).

Formulating entomopathogens in oil was found to increase their efficiency (Prior *et al.,* 1988). The carrier material and other constituents used in a bioformulation should be non-inhibitory to the infective propagule *i.e.,* conidia, inert on the target crop plant, at the same time should maintain viability during storage. Groundnut oil was proved as a better carrier for *Verticillium lecanii* compared to sunflower oil, as germination in the former was found to be excellent (Verhaar*et al.,* 1999). Lomer and Lomer (2001) observed comparatively higher germination of *Metarhizium anisopliae* (Metschnikoff) Sorokinand *Beauveria bassiana* (Balsamo) Vuilleminin in diesel: sunflower oil mixture (7:3) than diesel: groundnut oil mixture (7:3). Sunflower oil formulation of *Nomurea rileyi* Farlow Samson (Vimaladevi*et al*., 2002) and oil combination of coconut oil and soya bean oil in the ratio 50:50 with *M. anisopliae* (Batta *et al*., 2003) were reported to improve viability at storage. Similar works were carried out using talc as carrier also. Talc formulations of *L. lecanii* prepared by mixing Molasses Yeast Broth (MYB) plus two percent polyethylene glycol (PEG) and sterilized talc in the ratio 50:50 maintained higher viability than prepared from Potato dextrose Broth (PDB). Banu (2013) prepared dry formulations by mixing *L. lecanii* multiplied in PDB and Sabouraud Dextrose Broth (SDB) with yeast extract with talc in the ratio 1:2.

Bottleneck in microbial formulation technology is lesser shelf life which can be addressed with addition of suitable additives to bioformulations for improving their

shelf life. Presence of chitin in formulation enhanced viability of *M. anisopliae* (St. Leger *et al.,* 1986). Addition of chitin in wheat bran was reported to induce conidia production in *B. bassaina* formulations (Gerding-gonzalez *et al.,* 2007). Sriram and coworkers (2010) reported that two or five per cent chitin helped in maintaining the number of colony forming units (10^6 cfu g^{-1}) in the formulation upto six months of storage. They also reported that addition of colloidal chitin at 0.2 per cent in production medium enhanced the shelf-life by additional two months. Abdel-Kader *et al.* (2012) reported that carriers, sawdust + talc + chitosan and sawdust + chitosan maintained viability of *Trichoderma harzianum* in a dry formulation over a period of three and five months, respectively. Present study evaluated the basic carrier materials, sunflower oil (SFO), groundnut oil (GNO) and talc and their enrichment with chitin and chitosan for formulating a better formulation of *L. lecanii* having better efficiency and improved shelf life.

MATERIALS AND METHODS

Preparation of Spore Concentrate

Spores were harvested from SDB following the procedure of Kim, *et al.* (2007) with some modifications. The 14 days old culture was filtered through sterilized Whatman No.1 filter paper to remove mycelia. The filtrate was centrifuged at 12000 rpm for 25 min in Hermile labortechnic Z323K centrifuge to obtain spore pellet. The pellet was repeatedly washed with sterile distilled water and resuspended in 10 ml sterile distilled water and this concentrated spore suspension was utilized for preparation of formulations.

Preparation of Colloidal Chitin

Crude chitin (40 g) was slowly added into 250 ml of cold 0.25 N HCI with vigorous stirring and kept overnight at 4 º C in a refrigerator. The mixture was filtered through glass wool into 2 L ice cold water with rapid stirring using a magnetic stirrer. The gelatinous white material formed beneath was separated by filtration through a Whatman No. 1 filter paper. The chitin pellet was washed repeatedly with tap water until the pH became neutral (Roberts and Selitrennikoff, 1988).

Preparation of formulations

Formulations were prepared by mixing *L. lecanii* spore suspension (having 8.12 x 10^8 spores ml^{-1}) with sterilized carriers viz. sunflower oil, groundnut oil talc at proportions mentioned in Table 1. Colloidal chitin 0.1 per cent was as additive in enriched oil formulations while crude chitin 0.5 per cent and crude chitosan 5 per cent were added to talc formulations. 30 ml / 30 g of each formulations were prepared. Liquid formulations were stored in glass vials with bakelite lid and dry formulations were stored in polypropylene covers under room temperature (28±3˚C, 85±5%). Spores suspended in sterile distilled water alone served as control and all the treatments were replicated thrice. Spores suspended in sterile distilled water alone served as control and all the treatments were replicated thrice. Spore count and cfu were estimated at fortnightly interval for a period of three month and the data was subjected to Analysis of Variance.

Table 1. List of carriers, additives and proportions of spore suspension used for preparing the formulations

Type of formulation	Carrier + additive	Proportion (Carrier : Spore suspension)
Basic	Sunflower oil (100 %)	50 :50
		60 :40
		65:35
	Groundnut oil (100 %)	50 :50
		60 :40
		65:35
	Talc	50 :50
		60 :40
		65:35
Enriched	Chitin enriched sunflower oil	50 :50
		60 :40
		65:35
	Chitin enriched groundnut oil	50 :50
		60 :40
		65:35
	Chitin enriched talc	50 :50
		60 :40
		65:35
	Chitosan enriched talc	50 :50
		60 :40
		65:35
Sterile distilled water (99.90 %) + colloidal chitin (0.1 %)		
Sterile distilled water (100 %) (Check)		

Viability Test

Colony forming units (cfu) per gram /millilitre is considered as viability of spores (Derakhshan et al. 2008). One gram /millilitre of formulations sterile water for making serial dilution. Prepared serial dilutions were plated at one microliter per plate on SDA medium as per spread plate method. The cfu counts were recorded on seventh day after plating. Cfu were estimated at fortnightly interval for a period of three month and the data was subjected to Analysis of Variance.

cfu = Number of colonies x Dilution factor
Weight/ Volume of sample (g/l)

Estimation of Spore Count

One gram/ one millilitre sample each of the formulations were suspended separately in 10 ml of sterile water at

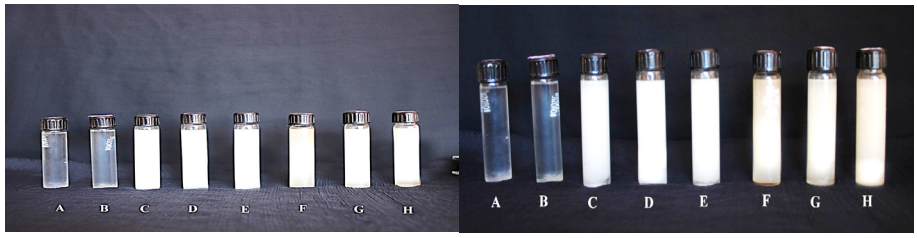

Plate 1. *Lecanicillium lecanii* formulations in groundnut oil (GNO)/ sunflower oil (SFO). A. Distilled water, B. Distilled water + chitin, C. GNO / SFO + Spore concentrate (SS) 50:50, D. GNO / SFO + SS 60:40, E. GNO / SFO + SS 65:35, F. (GNO / SFO + chitin) + SS 50:50, G. (GNO / SFO + chitin) + SS 60:40, H. (GNO / SFO + chitin) + SS 65:35.

forthnightly intervals. The spore count was assessed after making necessary dilutions using Neubauer's haemocytometer. This was repeated 90 days of storage and the data was subjected to Analysis of Variance.

Spores ml^{-1} = (n) x 10^4, where 'n' is the average number of spores in the four one millimetre corner squares of haemocytometer.

RESULTS AND DISCUSSION

Effect of carrier materials on conidial viability

The conidial viability of *Lecanicillium lecanii* in the formulations was evaluated at 15 days interval using cfu counts observed for three months (Table 2). The results proved the superiority of two enriched oil carriers, chitin enriched ground nut oil (GNO + chitin) and the chitin enriched sunflower oil (SFO + chitin) in terms of viability of *L. lecanii*. After 90 DAS, the enriched formulations, GNO + chitin 65:35, SFO + chitin 65:35, GNO + chitin 60:40 and GNO + chitin 50:50 were found to be superior, with a cfu of 2.27 x 10^6 ml^{-1}, 2.20 x 10^6 ml^{-1}, 2.16 x 10^6 ml^{-1} and 2.10 x 10^6 ml^{-1}, respectively. Enriched formulation, SFO + chitin 60:40 was on par with the basic formulation, GNO 65:35 (1.66 x 10^6 ml^{-1}). Among the basic oil formulations, both GNO and SFO were better in maintaining viability of spores when compared to talc based basic formulation. The decline in number of viable colonies ranged from 2.67 x10^6 ml^{-1} at 15 DAS to 1.29 x 10^6 ml^{-1} at 90 DAS in basic oil formulations and 1.30 x10^6 ml^{-1} at 15 DAS to 6.00 x 10^4 ml^{-1} at 90 DAS in basic talc formulations. However, chitin enriched talc formulation was superior to basic talc formulation (2.13 x10^6 ml^{-1} @ 15 DAS to 6.00 10^4 ml^{-1} @ 90 DAS).

Table 2. Effect of carrier materials on the viability of *Lecanicillium lecanii* formulations stored at room temperature

Sl no.	Carrier : Spore concentrate	*Mean number of cfu (x 10^6 spores ml^{-1})					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	SFO + SC (50 : 50)	2.67 (1.63)	2.43 (1.56)	2.23 (1.49)	2.03 (1.43)	1.56 (1.25)	1.33 (1.15)
2	SFO + SC (60 : 40)	2.77 (1.66)	2.43 (1.56)	2.23 (1.49)	1.97 (1.40)	1.63 (1.28)	1.29 (1.14)
3	SFO + SC (65 : 35)	2.90 (1.70)	2.46 (1.57)	2.30 (1.52)	2.09 (1.45)	1.70 (1.30)	1.49 (1.22)
4	(SFO + CC 0.1 %) + SC (50 : 50)	2.97 (1.72)	2.63 (1.62)	2.60 (1.61)	2.13 (1.46)	1.86 (1.37)	1.60 (1.26)
5	(SFO + CC 0.1 %) + SC (60 : 40)	3.13 (1.77)	2.90 (1.70)	2.63 (1.62)	2.27 (1.51)	2.13 (1.46)	1.66 (1.29)
6	(SFO + CC 0.1 %) + SC (65 : 35)	3.07 (1.75)	3.10 (1.76)	2.7 (1.64)	2.50 (1.58)	2.36 (1.54)	2.20 (1.48)
7	GNO + SC (50 : 50)	2.83 (1.68)	2.53 (1.59)	2.3 (1.52)	2.13 (1.46)	1.66 (1.29)	1.36 (1.17)
8	GNO + SC (60 : 40)	2.90 (1.70)	2.50 (1.58)	2.37 (1.54)	2.37 (1.54)	1.73 (1.32)	1.53 (1.24)
9	GNO + SC (65 : 35)	2.93 (1.71)	2.57 (1.60)	2.4 (1.55)	2.46 (1.57)	1.73 (1.32)	1.66 (1.29)
10	(GNO + CC 0.1 %) + SC (50 : 50)	3.13 (1.77)	3.00 (1.73)	2.67 (1.63)	2.53 (1.59)	2.20 (1.48)	2.16 (1.47)
11	(GNO + CC 0.1 %) + SC (60 : 40)	3.30 (1.82)	3.20 (1.79)	2.90 (1.70)	2.53 (1.59)	2.23 (1.49)	2.10 (1.45)
12	(GNO + CC 0.1 %) + SC (65 : 35)	3.40 (1.84)	3.23 (1.80)	3.16 (1.78)	2.59 (1.61)	2.46 (1.57)	2.27 (1.51)
13	Talc + SC (50 : 50)	1.30 (1.14)	1.30 (1.14)	1.13 (1.06)	0.79 (0.89)	0.33 (0.58)	0.06 (0.24)
14	Talc + SC (65 : 35)	1.40 (1.18)	1.37 (1.17)	1.10 (1.05)	0.93 (0.96)	0.50 (0.71)	0.06 (0.24)
15	Talc + SC (80 : 20)	1.50 (1.22)	1.30 (1.14)	1.30 (1.14)	1.09 (1.05)	0.90 (0.95)	0.33 (0.58)
16	(Talc + C 5 %) + SC (50 : 50)	2.13 (1.46)	1.73 (1.32)	1.46 (1.21)	1.06 (1.03)	0.63 (0.79)	0.06 (0.24)
17	(Talc + C 5 %) + SC (65 : 35)	2.57 (1.60)	2.37 (1.54)	2.23 (1.49)	1.83 (1.35)	1.19 (1.09)	0.66 (0.81)
18	(Talc + C 5 %) + SC (80 : 20)	2.53 (1.59)	2.30 (1.52)	2.20 (1.48)	1.93 (1.39)	1.30 (1.14)	0.85 (0.92)
19	(Talc + CS 0.5 %) + SC (50 : 50)	2.43 (1.56)	1.90 (1.38)	1.72 (1.31)	1.11 (1.05)	0.73 (0.85)	0.07 (0.26)
20	(Talc + CS 0.5 %) + SC (65 : 35)	2.50 (1.58)	2.26 (1.50)	2.13 (1.46)	1.69 (1.30)	1.13 (1.06)	0.63 (0.79)
21	(Talc + CS 0.5 %) + SC (80 : 20)	2.63 (1.62)	2.40 (1.55)	2.20 (1.48)	1.83 (1.35)	1.33 (1.15)	0.90 (0.95)
22	SC+ CC	1.93 (1.39)	1.87 (1.37)	1.57 (1.25)	1.40 (1.18)	1.26 (1.12)	1.16 (1.08)
23	SC	1.73 (1.32)	1.50 (1.22)	1.23 (1.11)	0.99 (1.00)	0.82 (0.91)	0.63 (0.79)
	CD (0.05)	0.064	0.072	0.107	0.134	0.128	0.378

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are square root transformed values

Table 3. Effect of carrier materials on the spore count of *Lecanicillium lecanii* formulations stored at room temperature

Sl no.	Carrier : Spore Concentrate	*Mean spore count (x 10^8 spores ml^{-1})					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	SFO + SC (50 : 50)	3.36(1.83)	2.61(1.62)	2.05(1.43)	1.51(1.23)	1.05(1.03)	0.76(0.87)
2	SFO + SC (60 : 40)	3.63(1.91)	2.57(1.60)	2.24(1.50)	1.59(1.26)	1.06(1.03)	0.76(0.87)
3	SFO + SC (65 : 35)	3.79(1.95)	2.66(1.63)	2.29(1.51)	1.60(1.27)	1.22(1.10)	0.83(0.91)
4	(SFO + CC 0.1 %) + SC (50 : 50)	4.15(2.04)	3.84(1.96)	2.87(1.69)	2.35(1.53)	2.03(1.42)	1.38(1.18)
5	(SFO + CC 0.1 %) + SC (60 : 40)	4.56(2.14)	4.13(2.03)	3.26(1.81)	2.58(1.61)	2.11(1.45)	1.51(1.23)
6	(SFO + CC 0.1 %) + SC (65 : 35)	4.53(2.13)	4.19(2.05)	3.78(1.95)	2.82(1.68)	2.24(1.50)	1.65(1.29)
7	GNO + SC (50 : 50)	3.77(1.94)	3.01(1.74)	2.12(1.46)	1.79(1.34)	1.36(1.17)	0.72(0.85)
8	GNO + SC (60 : 40)	3.94(1.99)	3.36(1.83)	2.84(1.68)	1.94(1.39)	1.41(1.19)	0.72(0.85)
9	GNO + SC (65 : 35)	4.19(2.05)	3.56(1.89)	2.76(1.66)	2.23(1.49)	1.45(1.20)	0.86(0.93)
10	(GNO + CC 0.1 %) + SC (50 : 50)	5.23(2.29)	4.67(2.16)	3.52(1.88)	2.74(1.66)	2.19(1.48)	1.54(1.24)
11	(GNO + CC 0.1 %) + SC (60 : 40)	5.33(2.31)	4.56(2.14)	3.70(1.92)	2.68(1.64)	2.29(1.51)	1.60(1.27)
12	(GNO + CC 0.1 %) + SC (65 : 35)	5.21(2.28)	4.65(2.16)	3.94(1.98)	2.82(1.68)	2.32(1.52)	1.67(1.29)
13	Talc + SC (50 : 50)	1.71(1.31)	1.11(1.05)	1.04(1.02)	0.54(0.74)	0.23(0.48)	0.02(0.13)
14	Talc + SC (65 : 35)	2.25(1.50)	1.67(1.29)	1.14(1.07)	0.72(0.85)	0.28(0.53)	0.05(0.22)
15	Talc + SC (80 : 20)	2.28(1.51)	1.91(1.38)	1.18(1.09)	0.82(0.91)	0.31(0.55)	0.06(0.24)
16	(Talc + C 5 %) + SC (50 : 50)	2.07(1.44)	1.76(1.33)	1.19(1.09)	0.73(0.85)	0.43(0.65)	0.07(0.27)
17	(Talc + C 5 %) + SC (65 : 35)	3.12(1.77)	2.02(1.42)	1.53(1.24)	1.15(1.07)	0.74(0.86)	0.39(0.63)
18	(Talc + C 5 %) + SC (80 : 20)	3.08(1.75)	2.54(1.59)	1.81(1.34)	1.36(1.17)	0.90(0.95)	0.60(0.77)
19	(Talc + CS 0.5 %) + SC (50 : 50)	2.27(1.51)	1.51(1.23)	1.27(1.13)	0.84(0.92)	0.52(0.72)	0.08(0.29)
20	(Talc + CS 0.5 %) + SC (65 : 35)	2.89(1.70)	2.31(1.52)	1.98(1.41)	1.21(1.10)	0.79(0.89)	0.56(0.75)
21	(Talc + CS 0.5 %) + SC (80 : 20)	2.86(1.69)	2.55(1.60)	1.82(1.35)	1.41(1.19)	0.83(0.91)	0.53(0.73)
22	SC+ CC	2.44(1.56)	2.35(1.53)	1.95(1.40)	1.57(1.25)	1.00(1.00)	0.69(0.83)
23	SC	2.32(1.52)	1.82(1.35)	1.29(1.14)	1.08(1.04)	0.66(0.81)	0.33(0.57)
	CD (0.05)	0.057	0.119	0.090	0.100	0.115	0.124

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are square root transformed values

Effect of Carriers on Spore Count

The spore count of *L. lecanii* formulations stored under room temperature using different carriers showed considerable variations under storage (Table 3). The overall observations based on spore count revealed that the chitin enriched formulations with GNO and SFO as carriers in proportions 65:35, 60:40 and 50:50 were the best. Though the spore count observed was 10^8 ml^{-1} even after three months of storage, there was a slight decline in the number at the end of three months. The spore count of enriched oil formulations ranged from 5.33 x 10^8 spores ml^{-1} @ 15 DAS (for GNO + Chitin 60:40) to 1.38 x 10^8 spores ml^{-1} @ 90 DAS (for SFO + chitin 50:50). The spore count in chitin and chitosan enriched talc formulations were equally good as basic formulations of GNO and SFO. The decline in number of spores ranged from 2.86 x 10^8 spores ml^{-1} @ 15 DAS to 3.9 x 10^7 spores ml^{-1} @ 90 DAS in enriched talc formulations and 3.36 x 10^8 spores ml^{-1} @ 15 DAS to 7.20 x 10^7 spores ml^{-1} @ 90 DAS in basic oil formulations. However, chitin enriched talc formulation was superior to basic talc formulation (1.17 x 10^8 spores ml^{-1} @ 15 DAS to 2.00 x 10^6 spores ml^{-1} @ 90 DAS).

Of the basic and enriched carrier materials evaluated for their ability to maintain conidial viability and spore count, chitin enriched ground nut oil (GNO + Chitin) and chitin enriched sunflower (SFO + chitin) were observed as the best carriers. Ideal proportion of carrier: technical ingredient in oil formulations was found to be 65:35. The corresponding spore count was 1.67 x 10^8 spores ml^{-1} and 1.65 x 10^8 spores m^{-1}, respectively. The basic as well as chitin enriched oil formulations were found to be superior in terms of viability than the chitin and chitosan enriched talc formulations. The enriched talc formulations were better than basic talc formulations in maintaining viability.

The experiment demonstrated that ideal proportion of carrier : technical for oil based liquid formulations was 65:35 and for talc based dry formulation it was 80:20. The present finding points out to the possibility of formulating an entomopathogen with lesser proportion of technical ingredient (80:20) as against the proportion (50:50) suggested by Burges and Jones (1998). But, there is a dearth of knowledge regarding standardization of the proportion in which carrier is to be mixed with technical ingredient while formulating a mycoinsecticide. However, perusal of literature revealed that various workers have tested the efficacy of mycoinsecticide formulations prepared in oil *i.e.,* formulation of *N. rileyi* F. in the ratio 50:50 based on sunflower oil (Vimaladevi *et al*., 2002); 50:50 oil combination of coconut oil and soya bean oil mixed with spore suspension of *M. anisopliae* in distilled water (Batta, 2003). Dry

formulations of *V. lecanii* based on talc prepared by earlier workers were in the ratio 50:50 (Derakhshan *et al.,* 2008) and 65:35 (Banu, 2013). Conversely, in the present work 50:50 formulations based on talc were inferior to others.

The results proved the superiority of two carriers, one using enriched ground nut oil (GNO + chitin) and the other using enriched sunflower oil (SFO + chitin) in terms of viability of *L. lecanii*. Verhaar *et al*. (1999) reported an excellent germination of *L. lecanii* spores in arachid (ground nut) oil compared to sunflower oil. While studying the viability of *M. anisopliae* in various formulations Alves *et al.* (2002) found that ground nut oil was superior for retention of viability than mineral oils, where the conidial viability was more than 90 per cent even after 40 weeks of storage. They also observed that the number of viable spores declined over storage time. High viability of *L. lecanii* spores in SFO formulation compared to talc based formulations was earlier reported by Banu and Gopalakrishnan (2012). The increased viability observed with arachid oil and sunflower oil may be due to the protective action of these on the mucilaginous outer envelope of *L. lecanii* spores which is usually lost while drying them for preparation of formulations.

Conidia are infective propagules in EPF ultimately leading to mortality of susceptible insects. The virulence of entomopathogens is generally assessed in terms of mortality of susceptible host insect. In this context apart from spore count, estimation of cfu is more relevant for assessing the effectiveness of formulations over the period of storage. The data on spore count revealed that the chitin enriched formulations with GNO and SFO were superior with respect to spore count. Presence of chitin in the formulation may enhance virulence, as chitin induces production of chitinases, which are important cuticle degrading enzymes (St. Leger *et al.,* 1986b; Mohanty and Prakash, 2004; Fang *et al*., 2005). The viability observed before and after three months of storage in this study was 10^6 cfu/ml and spore count was in the range of 10^8 spores ml^{-1}. The effective dose of *L. lecanii* was reported to be 10^7 spores ml^{-1} (Nirmala *et al.,* 2006; Wenzel and Filho 2011).

The chitin enriched oil formulations thus could maintain the viability and spore count in an effective range even after three months of storage. Chitin addition improved the bioformulations by suppressing the common contaminant like *Penicillium* (Knudsen *et al.,* 1990) attributing to the fact that saprophytic organisms are unable to utilize chitin as carbon source. Pavlyushin *et al.* (2005) observed better retention of viability of *T. viride* in chitin and chitosan based formulations, even after a longer period of storage. Addition of chitin two or three per cent to wheat bran induced higher conidia production in alginate pelleted formulations of *B. bassiana* (Gerding-gonzalez *et al.,* 2007). Chitin addition (two or five per cent) in talc formulations helped in maintaining high cfus in talc based formulations of *T. harzianum* and also enhanced shelf life by additional two months (Sriram *et al.,* 2010). However, enrichment of vegetable oil with chitin to improve storage properties of EPF was not seen attempted earlier.

In short, enrichment of oil formulations (GNO and SFO) with chitin could sustain viability as well as spore count of *L. lecanii* till the end of experimental period (three months).

Conidia are infective propagules in EPF ultimately leading to mortality of susceptible insects. The virulence of entomopathogens is generally assessed in terms of mortality to susceptible host insect. In this context apart from spore count, estimation of cfu is more relevant for assessing the effectiveness of formulations over the period of storage. Presence of chitin in the formulation may enhance virulence, as chitin induces production of chitinases, which are important cuticle degrading enzymes. Enrichment of oil formulations (GNO and SFO) with chitin could sustain viability as well as spore count of *L. lecanii* till the end of experimental period (three months).

ACKNOWLEDGEMENTS

The authors are thankful to Kerala Agricultural University, Kerala, for providing facilities to carry out this study.

RFERENCES

Abdel-Kader MM, El-Mougy NS, Aly MDE, Lashin SM. 2012. Long activity of stored formulated bio-agents against some soil-borne plant pathogenic fungi causing root rot of some vegetables. *J Appl Sci Res*. **8**(4): 1882–1892.

Alves RT, Bateman RP, Gunn J, Prior C, Leather SR. 2002. Effects of Different Formulations on Viability and Medium-Term Storage of *Metarhizium anisopliae* Conidia. *Neotrop Entomol.* **31**(1): 91–99.

Banu JG, Gopalakrishnan N. 2012. Development of formulations of a native entomopathogenic fungus, *Lecanicillium lecanii* and testing virulence against mealybug, *Paracoccus marginatus* infesting cotton. *Indian J Plant Prot*. **40**(3): 182–186.

Banu JG. 2013. Effect of different storage conditions on spore viability of *Lecanicillium lecanii* formulations and infectivity to mealybug, *Paracoccus marginatus*. *Int J Plant Prot*. **6**(2): 334–337.

Batta YA. 2003. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes). *Crop Prot*. **22**: 415–422.

Batta YA, Rahman M, Powis K, Baker G, Schmidt O. 2011. Formulation and application of the entomopathogenic fungus: *Zoophthora radicans* (Brefeld) Batko (Zygomycetes: Entomophthorales). *J Appl Microbiol*. **110**: 831–839.

Cuthbertson AGS, Walters KFA, Northing P. 2005. The susceptibility of immature stages of *Bemisia tabaci* (G.) to the entomopathogenic fungus *Lecanicillum muscarium* (*V. lecanii* (Zimm.) on tomato and verbena foliage. *Mycopathologia* **159**: 23–29.

Derakhshan A, Rabindra RJ, Ramanujam B. 2008. Effect of storage of formulations on viability of *Verticillium lecanii*(Zimmermann) Viegas and its virulence to *Brevicorne brassicae*(L). *J Biol Sci.* **8**: 498–501.

Diaz BM, Oggerin M, Lastra CCL, Rubio V, Fereres A. 2009. Characterization and virulence of *Lecanicillium lecanii* against different aphid species. *BioControl* **54**(6): 825–835.

Gerding-Gonzalez M, France A, Sepulveda ME, Campos J. 2007. Use of chitin to improve a *Beauveria bassiana* alginate-pellet formulation. *Biocontrol Sci Technol*. **17**(1): 105–110.

Hall RA, Papierok B. 1982. Fungi as biocontrol agents of arthropods of agricultural and medical importance. *Parasitology* **84**: 205–240.

Hegde SV. 2002. Liquid biofertilizers in Indian agriculture. *Biofertilizer News Lett.* **12**: 17–22.

Kim JJ, Goettel MS, Gillespie DR. 2007. Potential of *Lecanicillium* species for dual control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea. Biol Control* **40**: 327–332.

Knudsen GR, Johnson JB, Eschen DJ. 1990. Alginate pellet formulation of a *Beauveria bassiana* (Fungi: Hyphomycetes) isolate pathogenic to cereal aphids. *J Econ Entomol*. **83**: 2225–2228.

Lomer CH, Lomer CJ. 2001. Collection of Insect Pathogens. *Lubilosa Tech Bull.* **2**: 24p.

Nirmala R, Ramanujam B, Rabindra RJ, Rao NS. 2006. Effect of entomofungal pathogens on mortality of three aphid species. *J Biol Control* **20**: 89–94.

Park H, Kim K. 2010. Selection of *Lecanicillium* strains with high virulence against developmental stages of *Bemisia tabaci*. *Mycobiology* **38**: 210–214.

Pavlyushin VA, Tyuterev SL, Novikova II, Popova EV, Bykova GA, Boikova IV, Khatskevich LK. 2005. New preparations for combined protection of plants against diseases of various etiology. *Russ Agric Sci*. **12**: 7–12.

Pindi PK, Satyanarayana SDV. 2012. Liquid Microbial Consortium: A Potential Tool for Sustainable Soil Health. *J Biofertil Biopestici*. [e-journal] **3**(4). Available from: *http://www.omicsonline.org/liquid-microbial-consortium-a-potential-tool-for-sustainable-soil-health.pdf.* ISSN 2155-6202.

Prior C, Jollands P, Patourel GL. 1988. Pathogenicity test of *Beauveria bassiana* (Balsamo) against oil palm bagworm (*Metisaplana* Wlk). *Elaeis* **5**(2): 92–101.

Roberts WK, Selitrennikoff CP. 1988. Plant and bacterial chitinases antifungal activity. *J Gen Microbiol*. **134**: 169–176.

Sriram S, Palanna KB, Ramanujam B. 2010. Effect of chitin on the shelf- life of *Trichoderma harzianum* in talc formulation. *Indian J Agric Sci.* **80**(10): 80–82.

Ujjan AA, Shahzad S. 2012. Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis erysimi*) on canola (*Brassica napus* L). *Pak J Bot.* **44**(6): 2081–2086.

Verhaar MA, Hijwegen T, Zadoks JC. 1999. Improvement of the efficacy of *Verticillium lecanii* used in biocontrol of *Sphaerotheca fuliginea* by addition of oil formulations. *BioControl* **44**: 73–87.

Vimaladevi PS, Prasad YG, Chowdary A. 2002. Effect of drying and formulation of conidia on virulence of the entomofungal pathogen, *Nomuraea rileyi* (Farlow) Samson. *J Biol Contr.* **16**(1): 43–48.

Wenzel IM, Filho BA. 2011. Effect of pesticides on the pathogenicity of conidia of *Lecanicillium lecanii* on *Tetranychus urticae*. *Arq Inst Biol*. **78**(2): 261–266.