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Research Note

Influence of storage conditions on viability and infectivity of talc based WP formulation of *Beauveria bassiana* against rice hispa, *Dicladispa armigera* (Olivier)

PURNIMA DAS*, L. K. HAZARIKA[1], D. BORA[2], K. C. PUZARI[3] and SURAJIT KALITA[4]

Department of Entomology

[1] Retd. Dean, Faculty of Agriculture

[2] Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat 785 013, Assam, India

[3] Department of Life Sciences, Dibrugarh University, Dibrugarh

[4] Krishi Vigyan Kendra, Bongaigaon, Assam Agricultural University (AAU), Jorhat 785 013, Assam

** Corresponding author E-mail:* purnima.dashazarika@gmail.com

ABSTRACT: Shelf life of talc based wettable powder formulation of *Beauveria bassiana* (Bals.) Vuill. was evaluated at room temperature (24±1°C), refrigerated condition (4°C) and in deep freeze condition (–4°C to –6°C). At room temperature, viability of conidia lasted up to 180 days with 20.22×10^7conidia/gm, having 48% pathogenicity, whereas under refrigeration the same lasted for 210 days having the same conidial density but with 69.45% pathogenicity; under deep refrigeration, though viability lasted for 300 days with same conidial load, but substantially reduced pathogenicity to 20%.

KEY WORDS: *Beauveria bassiana*, shelf life, wettable powder, formulation, pathogenicity

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Continuous research on *Beauveria bassiana* (Bals.) Vuill. for last two decades in this laboratory has established that a strain isolated from rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae) is a proven mycoinsecticide and it has been recommended for managing rice pests including hispa in this part of India (Hazarika and Puzari 1990, 1994, Das *et al*., 2012). However, commercialization of this fungus as an alternative to synthetic insecticides demands the development of a formulation, which facilitates the fungus to survive under storage as well as in the field for a considerable length of time and to be applied with ease, with existing spraying machines so as to enhance efficacy. It is seen that short shelf life of a formulation acts as a major impediment for commercialization of mycoinsecticides (Lane *et al*., 1991) and during storage; most of the formulations lose virulence because of high temperature and decrease of nutrients. Besides, for an effective formulation choice of a carrier material is also important so does the type of formulation. In a review, Faria and Wraight (2007) reported that 20.5% of formulated fungal products are wettable powders (WP) because of their ease of application as suspension in water. The talc-based formulation are persistent (Navon and Aschor, 2000), effective and cheaper for pest and diseases management in different crops (Rajacommare *et al*., 2002; Saravanakumar *et al*., 2007; Rajendra *et al*., 2007; Kavino *et al*., 2007) and talc is a carrier for inducing sporulation (Das *et al*. 2006). Therefore, keeping in view the above, the present study was undertaken to formulate the mass-produced *B. bassiana* spores into a sprayable talc-based WP and to conduct a bioassay-guided evaluation against *Dicladispa armigera*.

Mass production of inoculum in submerged culture

Beauveria bassiana was cultured on PDA medium in Petri dishes and incubated at 25±1°C for 14 days. From there a 2 mm disc of culture was transferred to 1000 ml Borosil conical flask containing sterilized 500 ml potato broth enriched with 1 per cent dextrose and 1 percent peptone under laminar flow hood and incubated again at the same temperature. After 15 days of incubation the culture was blended in an electric mixture for 10-15 minutes to get homogenous slurry. The slurry was then filtered through a muslin cloth and then through a Whatman No. 1 filters paper under aseptic conditions to remove hyphae or clumps so as to obtain a conidial suspension. Conidial

concentration per ml was ascertained as per method described below.

Blending of inoculum into carrier

Five hundred grams of talc powder (100 mesh) were taken in a metal tray which was then sterilized by heating to 50°C for 3 hours, pH was adjusted at 7. After cooling, the talc powder was mixed with the homogenous fungal slurry in 1:1 ratio and dried under laminar flow hood for 72 hours under aseptic condition. After drying to approximately 8-10% moisture, the product [wettable powder (WP)] was packed in sterilized polypropylene bags, sealed and stored at room temperature (22–27°C), refrigerated (4°C) and deep-freeze (–4°C to –6°C) conditions; each condition was considered as a treatment. For initial record, conidial load was determined, for which ten g of the WP was mixed in 35 ml double-distilled water using a rotary blender-cum-mixer for 10 min and the mixture was then filtered once through a muslin cloth and also through a Whatman No. 1 filter paper to remove hyphae or clumps so as to obtain a uniform suspension composed of conidia. The suspension was further shaken in a vortex mixer for 15 sec. For each treatment 50 samples were taken for conidial counts using an improved Neubauer hemocytometer (Hazarika and Puzaria 1995) and means were expressed as number/gm. One ml of each suspension was smeared over a clean slide and it was incubated for six hours inside a BOD incubator maintained at 25±1°C and 95±2% RH. Conidial germination test was performed as per method of (Franscisco *et al.*, 2006) and rate of germination was expressed in percentage.

Shelf life of WP formulation

At an interval of 30 days, samples were drawn from each treatment to bioassay against the target insect and to determine corresponding conidial density and conidial germination in order to find out how long the WP formulation thus prepared could remain effective in the shelf. To estimate conidial density, ten g of the WP was mixed in 35 ml double-distilled water using a rotary mixture for 10 min and the mixture was passed through a muslin cloth and then filtered through a Whatman No. 1 filter paper to remove hyphae or clumps so as to obtain a suspension composed of conidia. The suspension was further shaken in a vortex mixer for 15 sec. Conidia were counted from 50 samples for each treatment using an improved Neubauer hemocytometer (Hazarika and Puzari 1995) and means were expressed as number/gm. One ml of each suspension was smeared over a clean slide and it was incubated for six hours inside a BOD incubator maintained at 25±1°C and 95±2% RH. Conidial germination test was performed as per method of Franscisco *et al.* (2006) and rate of germination was expressed in percentage. Thus each treatment was replicated six times.

Pathogenecity

A culture of *D. armigera* was maintained in a net house throughout the year as per method of Deka (1992) by using potted rice plants (variety Culture-1). For pathogenecity test, 20-laboratory-reared 2-day old adult of *D. armigera* starved for 6 hours were released on the 20-day-old seedlings of rice grown in pot and caged with paired lantern chimney atop to another (Deka and Hazarika 1996). Twenty ml of inoculum was mixed with Tween 80 at 0.023 ml/l and the emulsion was sprayed over the leaf surface of seedlings (Phukan *et al*., 2008). Six replications were kept for each storage condition with CRD. Mortality of adults was recorded on 7th day, which were corrected (Abbott, 1925). The process *in vitro* was repeated to 360 days at an interval of 30 days.

Table 1, 2 and 3 shows the pathogenecity, conidial density/g, and percent of conidia dried of *B. bassiana* under three conditions. At room temperature, though conidial population was maintained at a strength of 20.22×10^7 conidia/g to 180 days, but it differed significantly between 90 and 180 days, whereas under refrigeration, conidial density remained significantly unchanged for 180 days (Table 2). When samples were drawn on 210th day, the conidial density was reduced to 22.00×10^7 conidia/g and it was significantly different from those sampled either on 90th or 180th day (Table 2). Under deep refrigeration (-4±1°C), density did significantly differ from each other till 270th day, which, however, was significantly reduced on 300th day (Table 3).

At room temperature, concomitant to conidial load pathogenecity of propagules was reduced to 48% on 180th day, from that of 70% on 150th day of storage (Table 1), reduction, ranged between 70 and 86%. Similarly, under refrigeration, viability and infectivity were also affected significantly over age of the culture. At refrigeration, the infectivity of the viable propagules was reduced to 69.45% on 210 days of storage from that of 87% on 30 days, and beyond 210 days the viability and infectivity of the spores were totally lost. Under deep freeze, the viability of the propagules lasted up to 10 months showing 20% infection from that of 87% on 30 days, and beyond which, no viability and infectivity were observed. In all these storage conditions with increase in age, the strength, viability of the spores and infectivity of the culture declined significantly, and beyond 180 days of storage, infectivity of the viable spores was lost at room temperature;

Table 1: Shelf life and virulence of *Beauveria bassiana* talc formulation under room temperature

Age of Culture (Days)	Room Temperature (22-27°C)		
	* Mortality (%)	**(%) Conidia Density (x107)	** Conidial Dried
30	85.97±1.00	35.32±1.20	-
60	86.10±0.56	36.26±0.22	0.10±0.29
90	85.23±1.11	35.20±0.52	0.30±0.11
120	70.00±0.65	25.00±0.20	20.11±0.71
150	70.17±1.09	23.00±1.00	25.39±0.82
180	48.00±0.45	20.22±1.45	36.62±0.81
210	–	–	100±1.00
240	–	–	–
270	–	–	–
300	–	–	–
330	–	–	–
360	–	–	–
(P = 0.05)	8.11	4.30	5.11

* Mean of six replications
** Mean of 50 samples

Table 2: Shelf life and virulence of *Beauveria bassiana* of talc formulation under refrigerated condition

Age of Culture (Days)	Room Temperature (22-27°C)		
	* Mortality (%)	**(%) Conidia Density (x107)	** Conidial Dried
30	87.00±1.29	35.67±1.10	1.45±0.67
60	83.02±1.03	35.50±0.20	2.12±0.57
90	85.00±1.12	34.30±0.90	2.22±0.63
120	86.25±0.64	34.29±1.20	2.29±0.45
150	73.00±1.01	34.50±0.22	2.28±1.15
180	70.29±0.69	34.20±0.62	30.80±1.40
210	69.45±1.06	22.00±0.90	30.00±1.00
240	–	–	100.00±0.25
270	–	–	–
300	–	–	–
330	–	–	–
360	–	–	–
(P = 0.05)	7.25	8.20	9.60

* Mean of six replications
** Mean of 50 samples

Table 3: Shelf and virulence of *Beauveria bassiana* of talc formulation under deep freeze

Age of Culture (Days)	Room Temperature (22-27°C)		
	* Mortality (%)	**(%) Conidia Density (x107)	** Conidial Dried
30	87.00±0.88	35.41±1.40	–
60	87.87±1.21	39.00±1.30	1.40±0.30
90	88.00±1.00	38.98±0.80	0.73±0.22
120	87.00±1.25	38.90±1.33	1.50±0.30
150	86.00±1.00	38.98±1.27	0.70±0.09
180	77.10±1.12	39.40±1.40	0.25±0.40
210	70.65±1.11	39.20±1.01	0.70±0.21
240	72.75±1.00	39.18±1.00	1.00±0.30
270	20.00±0.62	39.00±1.02	1.40±0.30
300	–	20.21±2.12	44.30±0.80
330	–	–	100±1.11
360	–	–	–
(P = 0.05)	5.00	9.10	4.11

* Mean of six replications
** Mean of 50 samples

however, the same was observed on 210 and 270 days in refrigerated and deepfreeze conditions. Talc powder was the best carrier to retain maximum number of viable propagules up to 180 days of storage, as was earlier reported in case of *Trichoderma longibrachiatum* (Gade *et al.*, 2008; Yashwant *et al.*, 2010, Sahid *et al*., 2011). Storage temperature influenced markedly shelf life of *B. bassiana* WP which is true for any other formulations of entomopathogenic fungi. The germination rate of WP was near 100% at room temperature on 90th day observation which was however; observe by Simkova (2009) at 4°C for 90 days. On the contrary, this kind of result was observed on 150th and 210th day of observation on when WP was stored at 4 ± 1° C and -4 ± 10°C, respectively. The highest percentage of germinating conidia (98.67% after 90 days of storage) was observed at -20°C (Hong *et al*., 1997). It supports our findings that decrease in temperature increases the longevity of conidia of *B. bassiana* (Stathers *et al*., 1993; Morley-Davies *et al*., 1995; Hong *et al*., 1997). Moreover, fungal spores are relatively delicate and their viability diminishes with time depending on environmental conditions, which in this case the storage relative humidity, temperature (Moore *et al.*, 2000), age of the culture, endogenous reserves (Bidochka *et al*., 1991; Lane *et al*., 1991) pH (Hallswarth and Magan1996) and light (Sandhu *et al*., 1993). Some fungi such as

B. bassiana, Lecanicillium lecanii, M. anisopliae and *Paeciliomyces fumosoroseus* are also endowed with good nutritional reserves. Some spores require exogenous sources of carbon, oxygen and water or nitrogen for germination, while others do not require exogenous sources of nutrients but they utilize their reserved materials such as carbohydrates, lipids or proteins (Moore-Landecker 1972). Hence conidial viability was observed up to 270 days. According to (Morley-Davies *et al.*, 1995) prefered storage temperature was found to be 0°C and 20°C, however this may not be true in all cases. The type of formulation ultimately selected depends upon the biology of the insect and physical properties of the soil, ecosystem characteristics, habits and habitats of the target pest. Nevertheless, currently registered mycoinsecticides, for example *Isaria fumosorosea* SPF198 produced in soybeans or ice can survive 6-12 months at room temperature (Kim *et al*., 2010).

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