Influence of ultraviolet light protectants on the persistence of the entomopathogenic fungus *Beauveria bassiana*

S. Hemalatha*, K. Ramaraju and S. Jeyarani

ABSTRACT

Influence of nine UV protectants on the persistence of the entomopathogenic fungus *B. bassiana* (Bb 111) was evaluated in the laboratory. Among the UV protectants screened with the local *B. bassiana* (Bb 111) isolate, skimmed milk, yeast extract, starch, molasses and tinopal were found to be promising and offered good conidial protection with more than 70 per cent germination even after 60 minutes of UV exposure. Other UV protectants *viz*., folic acid (66.19%) and sucrose (61.91%) offered moderate protection of *B. bassiana* (Bb 111) conidia. Skimmed milk at 2 and 5 per cent concentration consistently recorded higher germination of 85.71 and 89.29 per cent respectively at 60 minutes of exposure. Upon 90 minutes of UV exposure, in *B. bassiana* (Bb 111) alone, the spore viability was drastically reduced to 27.38 per cent, whereas skimmed milk and starch at 5 per cent offered 71.43 and 53.85 per cent conidial germination respectively.

Keywords: *B. bassiana*, UV protectants, spore germination

MS History: 04.03.2017 (Received)-30.11.2017 (Revised)- 08.12.2017 (Accepted)

Citation: S. Hemalatha, K. Ramaraju and S. Jeyarani 2017. Influence of Ultraviolet light protectants on the persistence of the entomopathogenic fungus, *Beauveria bassiana*. *Journal of Biopesticides,* **10**(2): 130-134.

INTRODUCTION

The entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill has shown considerable potential for the management of insect pests (Feng *et al.,* 1994). *Beauveria bassiana* conidia are hyaline and rapidly killed by sunlight (Daoust and Pereira, 1986; Inglis *et al.,* 1993). It has been observed that conidia of *B. bassiana* are relatively susceptible to sunlight which causes impact on establishment of inoculums required to cause beauveriosis in insects, and decrease the efficacy of conidia applied on foliage. The ultraviolet radiation-B (UV-B; 280-320nm) component of sunlight is detrimental to all microorganisms (Tevini, 1993). A number of UV protectants and adjuvents have been used to protect and enhance the persistence of entomopathogenic viruses (Ignoffo and Batzer, 1971; Shapiro *et al.,* 1983; Martingoni and Iwai, 1985; Shapiro, 1989, 1992). Several report revealed *Bacillus thuringiensis* Berliner (Morris, 1983; Cohen *et al.,* 1991), the entomopathogenic fungus *Metarhizium flavoviride* Gams & Rozsypal (Moore *et al.,* 1993), and the nematode *Steinernema carpocapsae* (Weiser) (Nickle and Shapiro, 1992) when exposed to artificial UV-B radiation are showing lesser efficacy. In the present study, investigation was undertaken to identify the UV protectants that can increase the persistence and viability of *B. bassiana* spores.

MATERIALS AND METHODS

Photostability of the native fungal pathogen, *B. bassiana* (Bb 111) was evaluated by using SUNTEST CPS$^+$ solar simulator.

PREPARATION OF INOCULUM

B. bassiana (Bb 111) spores were suspended in different UV protectants *viz.,* ascorbic acid, folic acid, molasses, yeast extract, starch, sucrose, skimmed milk powder, zinc oxide and tinopal, at the concentrations of one per cent each. One mL of *B. bassiana* (Bb 111) spore suspension (10^6 spores mL^{-1}) containing different UV protectant were placed in sterilized embryo cups and exposed to UV radiation in Sun Test CPS$^+$ solar simulator for 0, 30 and 60 minutes respectively. After the

UV exposure, the spore germination percentage was assessed.

Effect of UV protectants on spore germination

Ten μL of an aqueous spore suspension in 0.05 per cent Tween 80 containing 1 x 10^6 spores ml^{-1} was spread on a cavity slide coated with thin film of SMA+Y medium. The inoculated slides were kept in Petri dishes lined with blotting paper moistened with sterile water. Petri dishes were sealed and placed in incubators set at four test temperatures. Three replicates were maintained for each isolate. The slides were observed under a compound microscope after 24 hrs of inoculation and the spores were considered as germinated when the germination peg was at least twice the diameter of the spore. Percentage germination was then determined by counting 100 spores for each plate at 40 X magnification. Based on the above experiment, UV protectants showed the higher germination of *B. bassiana* spores were selected at three different concentrations (1, 2 and 5 per cent). The isolate *B. bassiana* (Bb 111) (10^6 spores mL^{-1}) mixed with the UV protectant at three different concentrations as mentioned above were exposed to UV radiation for 0, 30, 60, 90, 120, 150 and 180 minutes respectively in a solar simulator and the germination per cent was assessed.

RESULTS

Results on screening of nine different UV protectants with *B. bassiana* (Bb 111) for conidial viability revealed a varying response to UV radiations. Addition of UV protectants in control as standard check as without exposure to UV radiation showed no effect on the viability of *B. bassiana* conidia. At 30 minutes of UV exposure, *B. bassiana* (Bb 111) added with skimmed milk, sucrose and yeast extract recorded the highest conidial germination and were found to be on par with each other. Observations after 60 minutes of UV exposure on conidial germination showed drastic reduction up to 48.57 per cent in case of *B. bassiana* (Bb 111) without UV protectant. Among the UV protectants

screened, skimmed milk, yeast extract starch. Molasses and tinopal were found to be promising and offered good conidial protection with more than 70 per cent germination even after 60 minutes of UV exposure. Other UV protectants *viz.*, folic acid and sucrose offered moderate protection of *B. bassiana* (Bb 111) conidia. Ascorbic acid and zinc oxide were found to show no effect as UV protectant as observed by the germination percentage up to 52.38 and 50.95 per cent respectively on a par with control (Table 1).

Table 1. Screening of UV protectants on the survival of *B. bassiana* (Bb 111) spores exposed in solar simulator

Treatments	Germination of spores (%)		
	0 minute	30 minutes	60 minutes
Bb + Ascorbic acid	78.57 (68.39)	59.05 $(57.16)^{f}$	52.38 $(53.24)^{d}$
Bb + Folic acid	92.38 (76.47)	86.67 $(73.08)^{abc}$	66.19 $(61.28)^{bc}$
Bb + Molasses	96.19 (78.78)	80.95 $(69.76)^{d}$	71.91 $(64.56)^{ab}$
Bb + Yeast Extract	93.81 (77.33)	87.62 $(73.64)^{ab}$	74.76 $(66.19)^{a}$
Bb + Starch	94.76 (77.91)	85.24 $(72.25)^{bc}$	73.33 $(65.37)^{a}$
Bb + Sucrose	92.86 (76.76)	87.62 $(73.64)^{ab}$	61.91 $(58.81)^{c}$
Bb+ Skimmed milk	95.24 (77.34)	89.52 $(74.79)^{a}$	76.19 $(67.02)^{a}$
Bb + Zinc oxide	89.05 (74.49)	83.33 $(71.14)^{cd}$	50.95 $(52.37)^{d}$
Bb + Tinopal	90.47 (75.33)	85.71 $(72.57)^{abc}$	70.47 $(63.74)^{ab}$
Bb alone	90.95 (75.62)	68.09 $(62.37)^{e}$	48.57 $(50.95)^{d}$

**B. bassiana* @10^6 spores mL^{-1}; UV protectants @ 1 per cent; Figures in parentheses are arc sin transformed values; In a column means followed by a common letter (s) are not significantly different at P = 0.05 by LSD

Based on the preliminary screening, three UV protectants *viz.,* skimmed milk, starch and yeast extract were selected for further studies and tested at three concentrations (1, 2 and 5 %) over a period of 0 to 180 minutes of UV exposure in a solar simulator. Observations on the conidial germination showed significant variation with the UV exposure periods. The pattern of conidial growth was significantly reduced in *B. bassiana* (Bb 111) without UV protectant after 60 minutes of exposure, whereas an effective UV protectant was observed in terms of higher conidial germination after 60 minutes of UV-exposure at different concentration. A concentration dependent protection was also observed, when different concentrations of effective UV protectants were added with *B. bassiana* conidia. Skimmed milk at 2 and 5 per cent concentration consistently recorded higher germination of 85.71 and 89.29 per cent respectively at 60 minutes of UV-exposure. However, after 90 minutes of UV-exposure, in *B. bassiana* alone, the spore viability was drastically reduced to 27.38 per cent, whereas skimmed milk and starch at 5 per cent offered 71.43 and 53.85 per cent conidial germination respectively. Skimmed milk at 5 per cent concentration showed good protection and exhibited the highest conidial germination of 52.38, 21.43 and 15.47 per cent at 120, 150 and 180 minutes respectively. This was followed by yeast extract which recorded 38.64, 27.27 and 7.95 per cent conidial germination at 120, 150 and 180 minutes respectively (Table 2). At lower concentration of two per cent, skimmed milk offered higher conidial germination as against control. Evaluations on screening of UV protectants revealed skimmed milk as maximum effective UV protectant followed by yeast extract with moderate level of UV protection. Starch offered the least protection on prolonged exposure.

DISCUSSION

UV protestant has the ability to reflect or absorb the energy of UV rays. In case of absorption, energy gained is dissipated as heat or molecular changes, eliminating or reducing the cellular damage caused by sunlight exposure. Effective protectants should be able to form a thin pellicle around conidia, protecting them from UV for certain period of time. In the present investigation, addition of UV protectants to unexposed *B. bassiana* (Bb 111) conidia had no influence on the germination. Upon 60 minutes of exposure to UV radiation, all the UV protectants increased the conidial viability, whereas a strong negative growth impact was observed on conidial germination in the control, *B. bassiana* alone.

Among the compounds screened as UV protectants which can enhance *Beauveria* spore growth were skimmed milk, starch, yeast extract, molasses and tinopal with more than 70 per cent conidial germination at 60 minutes of exposure. Similar results were also observed by Santos *et al.* (2011) where one per cent starch or skimmed milk provided a strong degree of photoprotection over the shorter irradiation period, resulting in a germination rate almost 30 per cent higher than in control.

A concentration dependent protection was observed when different concentrations of promising UV protectants were exposed to longer irradiation period up to 180 minutes. As the period of exposure increased, higher conidial germination was noticed in *B. bassiana* (Bb 111) + skimmed milk compared to other treatments. Skimmed milk at 2 and 5 per cent provided the most effective protection in all the exposure periods. This result is in accordance with Edgington *et al.* (2000). Skimmed milk powder has several effects on the efficacy of *B. bassiana* spores such as preserving of viability (Stephan and Zimmermann, 1998; Abadias *et al.*, 2001). The effectiveness of the skimmed milk may be attributed to the protein content which absorbs UV-B radiation and acts as a protection barrier over the surface of the spores (Jaques, 1970).

Table 2. Influence of selected UV protectants on the survival of *B. bassiana* (Bb 111) spores exposed in solar simulator

Treatment	Dose (%)	Germination per cent in different time interval (minutes)						
		0	30	60	90	120	150	180
Bb+ Skimmed milk	1.0	94.05	86.90	77.38	53.57 (47.05)	41.67 (40.21)	11.91 (20.18)	4.76 (12.48)
	2.0	96.43 (79.09)	92.86 (74.50)	85.71 (67.79)	63.09 (52.59)	44.05 (41.58)	14.28 (22.20)	9.52 (17.97)
	5.0	96.88 (79.81)	94.05 (75.88)	89.29 (70.89)	71.43 (57.69)	52.38 (46.36)	21.43 (27.58)	15.47 (23.16)
Bb + Starch	1.0	96.15 (78.68)	83.65 (66.15)	67.31 (55.12)	47.62 (43.64)	14.28 (22.20)	5.95 (14.12)	0.00 (0.00)
	2.0	97.83 (81.51)	90.38 (71.93)	71.15 (57.51)	52.38 (46.36)	23.81 (29.21)	7.14 (15.49)	0.00 (0.00)
	5.0	97.12 (80.23)	94.23 (76.10)	73.08 (58.74)	53.85 (47.20)	27.38 (31.55)	9.52 (17.97)	3.57 (10.89)
Bb + Yeast extract	1.0	94.32 (76.21)	84.09 (66.49)	62.50 (52.24)	42.05 (40.42)	26.14 (30.75)	11.36 (19.69)	0.00 (0.00)
	2.0	95.45 (77.68)	92.05 (73.61)	76.14 (60.76)	52.27 (46.30)	35.23 (36.44)	23.86 (29.24)	5.68 (13.78)
	5.0	97.73 (81.32)	95.45 (77.68)	81.82 (64.76)	53.41 (46.95)	38.64 (38.45)	27.27 (31.48)	7.95 (16.38)
Bb alone	-	92.86 (74.49)	80.95 (64.12)	52.38 (46.36)	27.38 (31.55)	9.52 (17.97)	0.00 (0.00)	0.00 (0.00)

**B. bassiana* @10^6 spores mL^{-1}; Figures in parentheses are arc sin transformed values

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the Department of Biotechnology (DBT), New Delhi for the fund provided.

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