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Influence of conidial age on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* against Third Instar Larvae of *Spodoptera litura*

S Dayakar
Department of Entomology, Agricultural College, Rajamahendravaram, Andhra Pradesh.

ABSTRACT

Bio assays were conducted with *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin against third instar larvae of *Spodoptera litura*. Conidial concentrations of 10^4 to 10^9 and 5 X10^7 conidia ml^{-1} were prepared from the cultures having 10, 15, 20, 25 and 35 days age to assess the LC_{50} and LT_{50-}, respectively. The probit analysis of data showed an increase in LC_{50} and LT_{50} values with the increase in age of the conidia. Conidia cultured on Sabouraud's Dextrose Agar (SDA) medium at 25^0C at the age of 10 days showed LC_{50-} value of 12.50 and 17.52 X 10^5 conidia ml^{-1} with *B. Bassiana* and *M. anisopliae*, respectively. There was a sharp increase in LC_{50} values with 20, 25 and 35 day old cultures. The LT_{50} value of a 10 day old culture of *B. bassiana* and *M. anisopliae* were 113.09 and 119.31h, respectively at 5X10^7 conidia ml^{-1} as compared to 153.79 and 155.85 h, respectively with 35 day old cultures. From the present studies, it can be advocated that there was a reduction in mortality of host larvae to fungal infection beyond 35 days of age and the use of 10 or 15 day old cultures of fungi is advantageous.

Key words: *Beauveria bassiana, Metarhizium anisopliae and Spodoptera litura*

The recognition of deleterious effects of pesticides has prompted the development of alternate methods and one of the feasible alternatives to chemicals is microbial control (*Dayakar and Kanaujia, 2010*). Insect pathogens such as fungi, bacteria, virus, nematodes and protozoans serve as potential microbial agents. There are more than 700 species of fungi mostly Deuteromycetes and Entomophthorales from about 90 genera that are pathogenic to insects (*Charnley, 1989*). Despite the large number of entomogenous fungi reported from insects, only about 20 species have been investigated so far in insect management. Beauveria bassiana (Balsamo) Vuillemin and Metarhizium anisopliae (Metchnikoff) Sorokin were used on larger scale (*Moore and Prior, 1996*) for the management of insect pests.

Large scale use of fungi as microbial pesticides requires mass production of virulent strain. Repeated sub-culturing for the mass production of entomopathogenic fungi on culture media attenuates the virulence. Attenuation and enhancement of virulence of entomopathogenic fungi in general was observed by repeated sub culturing followed by passaging through insect host (Dayakar and Kanaujia, 2004). The increased virulence of B. bassiana and M. anisopliae when cultured on media containing host larval extract against S. litura was well documented (Dayakar and Subbarao, 2011). It is evident from the above findings that the virulence depends on environmental factors. Age of the conidia is one important factor that influences the pathogenecity. Survival of spores of B. bassiana and also their infectivity to H. zea greatly declined when the fungus was stored in culture tubes alone or with silica gel crystals in continuous shade for prolonged periods (Bell, 1975). The relation between age of certain entomopathogenic fungal cultures in the light of their infectivity to the host insects was already identified by Le Grande and Cliquet (2013). Further, Ignoffo et al. (1985) observed that conidia of *N. rileyi* could lose about 50 per cent of their original infectivity in a period of two weeks when kept at an ambient temperature of 25°C. The fungal cultures grown on carrot agar medium of the ages 10 and 15 days were significantly more virulent against H. armigera and S. litura than the 35-day old cultures (Prasad, 1989). He also noticed that LT_{50} increases gradually with the increase in the age of the cultures. In view of the above, the bio assays were, therefore, undertaken to determine the

effect of age on the pathogenecity of *B. bassiana* and *M. anisopliae* against *S. litura*.

MATERIAL AND METHODS

Different fungal isolates, MUCL-38502 of *B. bassiana* and MUCL-8237 of *M. anisopliae* originally obtained from Belgian Co-Ordinated Collection of micro-organisms (BCCM™) were used in the present investigations against tobacco caterpillar, *Spodoptera litura* Fabricius. Sabouraud Dextrose Agar (SDA) medium was prepared and transferred aseptically into sterilised petridishes @ 20 ml per plate and were inoculated with conidia of *B. bassiana* and *M. anisopliae* in a laminar airflow. The culture plates were maintained at 25±2°C and 95±5% RH in an incubator.

Conidial suspensions containing 5 x 10^7 conidia ml^{-1} of *B. bassiana* and *M. anisopliae* were prepared from cultures of different ages *viz.,* 10, 15, 20, 25 and 35 days grown on SDA medium was sprayed on third instar larvae of *S. litura* to study the effect of age of the fungus culture on virulence. The standard procedures laid down by *Rombach et al. (1986)* was followed for the preparation of conidial concentrations and that for the viability test (*Gillespie, 1986*).

The bio-assays were carried out with third instar larvae of *S.litura*. Twenty third instar larvae were taken in Petri plate (90 by 15 mm) lined with sterilized filter paper were sprayed under Potter's tower at 40±2 lbs $inch^{-1}$ with 2 ml of each concentrations. Conidial suspensions from respective ages diluted to the concentrations from 10^4 to 10^9 conidia ml^{-1} were used to assess the LC_{50} and that for LT_{50} a conidial suspension containing 5x10^7 conidia ml^{-1} were used. Control larvae were sprayed only with 0.02 per cent Tween 80® solution. The mortality due to mycosis was recorded at regular intervals and the cumulative mortality data on eighth day was used for probit analysis (*Finney, 1964*).

RESULTS AND DISCUSSION

Bio efficacy of entomogenous fungi: The results of bioassay indicated that the pathogenecity of conidia decreased with the increase in age. The dose mortality and time mortality response of both the fungi increased with the increase in age of the conidia.

Mean lethal concentration (LC_{50}): The results of the bioassay tests with different aged conidial concentrations of *B. bassiana* and *M. anisopliae* against third instar larvae of *S. litura* showed an increase in LC_{50} and LT_{50} values with the increase in the age of the conidia (Table 1 and 2). *B. bassiana* and *M. anisopliae* fungus cultures of 10 days old showed the LC_{50} values of 12.50 and 17.52 x 10^5 conidia ml^{-1}, respectively and that for 15 days was 15.76 and 20.28 x 10^5 conidia ml^{-1}, respectively. There was a sharp increase in LC_{50} with 20, 25 and 35 day-old cultures over 10 and 15 day-old cultures (Table 1). The potency decreased with the increase in the age of the fungal culture. Maximum LC_{50} was recorded with both the fungi with conidial concentrations prepared from 35 day old conidia. There was 5.4, 11.6 and 27.3 fold increase in LC_{50} over 10 day old conidial culture with *B. bassiana* and 4.7,11.1, and 23.3 fold increase in LC_{50} over 10 day old conidial culture with *M. anisopliae* when treated with 20, 25 and 35 day old conidial concentrations, respectively (Table 3).

The time of harvest of the conidia from the fungus culture is critical for the performance of the fungal pathogen. Conidiogenesis in *B. bassiana* starts after six days on agar medium (*Domsch et al., 1980*). Sporulation of *B. bassiana* generally took about nine days on SDA agar and it can readily be judged since the aerial hyphae collapse into a powdery mass (*Smith and Grula, 1981*). In the present study, conidia of *B. bassiana* cultures grown on SDA medium at 25°C of the ages 10 and 15 days caused significantly higher mortalities than that of 35 day old cultures (Table 1) which was evidenced by lower LC_{50}- values. It was emphasized that entomopathogenic fungal cultures grown on carrot agar medium of the ages 10 and 15 days were significantly more virulent against *Heliothis armigera* and *S. litura* than the 35 day old cultures (Prasad, 1989). In another study with *Dactylaria higginsii* against *Cyperus rotundus* (*Kadir et al., 2011*) it was concluded that the infection rate of conidia harvested from 15 day-old cultures was faster compared to the infection rate of conidia harvested from other culture ages against purple nut sedge.

The χ^2 test showed homogeneity of the test population which indicates the good fit of the observed and expected response (Table 2). This ultimately accounted for the precision of the regression. A good fit of the regression also is a reflection of the precision of the technique and procedure adopted. The slope functions were very low in all the bio assays (<1.0) indicating that the dose dependent responses were

not well pronounced. Slopes of the dose response lines for entomogenous fungi generally have been less steep than those for other entomopathogens as established (Ignoffo *et al*., 1981). The shallow dose mortality response seems to be typical for fungus insect interactions (Rombach and Gillespie, 1988). In the present study, the slope function for *B. bassiana* with 10 day old conidia was maximum (0.222) indicating more virulence than 35 day old conidia (0.1847). The slope functions of *M. anisopliae* were 0.2353 and 0.1855 for conidia obtained from 10 day old culture and 35 day old culture, respectively. The value of the slope produced by regression analysis indicates level of uniformity of the response of the insects to the fungus applied, the greater the value of the slope the greater the uniformity of the response. The slope functions recorded in the present study confirms the above reports.

Mean Lethal Time (LT_{50}): The results of the probit analysis of time mortality data from bio assays indicated that the LT_{50} values (at $5X10^7$conidia ml^{-1}) ranged from 113.09 to 153.79 hours with *B. bassiana* and 119.31 to 155.85 hours with *M. anisopliae* (Table 2). Among the two fungi tested, *B. bassiana* showed more virulence than *M. anisopliae*. The time taken for killing 50% population increased with the increase in age of the conidia. The increase in LT_{50} with *B. bassiana* was 1.18, 1.27 and 1.36 folds with 20, 25 and 35 day old cultures, respectively, in comparison to 10 day old culture (Table 3). A positive relationship between increase in the age of the conidia and LT_{50} estimate was observed with the fungi indicating the lower virulence of older conidia than the 10 day old conidia.

The LT_{50} values (Table 2) increased gradually as the fungal age advanced. The infectivity of *B. bassiana* to *H. zea* greatly declined when the fungus was stored in culture tubes for prolonged periods (*Bell, 1975*). Conidia of *N. rileyi* could lose about 50 per cent of their original infectivity in a period of two weeks when kept at an ambient temperature of 25°C *(Ignoffo et al., 1985)*.

Present findings indicated that there was a reduction in mortality of host larvae due to fungal infection beyond 35 days of age which may be due to the decrease in viability of the conidia. Similar results were also reported with *B. bassiana* conidia (*Ignoffo et al., 1981*). The loss in virulence may be attributed to the decrease in vigour of the germinating conidia. Since the most important function of chitinase, lipase and protease is to aid in the penetration of the insect cuticle, virulence is more likely to be correlated with enzyme production by germinating conidia than the mycelium (*Robert, 1981*). From the present studies, it was advocated that the use of 10 or 15 day old cultures of fungi is advantageous over use of aged conidia. In the era of WTO, the use of insecticides is given last preference and the use of potential alternatives like microbials are essential. To incorporate the microbials in bio-intensified pest management strategies mass multiplication of fungi is necessary and hence the present studies have bearing in effective use of entomopathogenic fungi in insect pest management.

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Table 1. Dosage-mortality responses of third instar *S. litura to *B. bassiana* and *M. anisopliae* conidia of different ages.**

Fungus	Age	χ^2	Regression equation	LC_{50} (conidia ml^{-1}) ($x10^5$)	Fiducial limits (95%) ($x\ 10^5$)
B. bassiana	10	0.05	Y = 4.7565 + 0.2220x	12.50	3.0464 - 51.2659
	15	0.09	Y = 4.7664 + 0.1951x	15.76	3.2422 - 76.6310
	20	0.13	Y = 4.6310 + 0.2016x	67.64	14.5626 - 314.1737
	25	0.45	Y = 4.6214 + 0.1749x	146.12	22.9617- 929.8553
	35	0.09	Y = 4.5320 + 0.1847x	341.61	50.9448 - 2290.7197
M. anisopliae	10	0.06	y= 4.7074 + 0.2353x	17.52	4.6940 – 65.3525
	15	0.12	y= 4.7050 + 0.1908x	20.28	4.0891 – 100.6265
	20	0.26	y= 4.6696 + 0.1718x	83.73	13.6731 – 512.6943
	25	0.06	y= 4.5518 + 0.1955x	196.01	35.7264 – 1075.3850
	35	0.14	y= 4.5157 + 0.1855x	408.71	59.1077 – 2826.0996

* @ 360 per bioassay

Table 2. Time mortality responses of third instar *S. litura to different aged conidia of *B.bassiana* and *M. anisopliae*.**

Fungus	Age	χ^2	Regression equation	LT_{50} (h)#	Fiducial limits (95%) ($x\ 10^5$)
B. bassiana	10	0.94	y = 3.3350 + 2.4755x	113.09	99.4056 – 128.6400
	15	2.42	y = 3.1384 + 2.6863x	118.36	105.4104 – 132.8328
	20	0.48	y = 3.2320 + 2.3692x	133.80	117.4344 – 152.4432
	25	1.47	y = 3.4381 + 2.0138x	143.16	121.8912 – 168. 1320
	35	0.90	Y = 3.2659 + 2.1496x	153.79	130.5528 – 181.1688
M. anisopliae	10	0.86	y = 3.0917 + 2.7400x	119.31	106.5288 – 133.6128
	15	0.69	y = 3.3360 + 2.2857x	124.48	108.9696 – 142.1976
	20	0.63	y = 3.4834 + 2.0111x	136.24	116.7240 – 159.0168
	25	0.67	y = 3.2016 + 2.2888x	146.53	126.7244 – 169.4376
	35	0.43	y = 3.2278 + 2.1811x	155.85	132.2280 – 183.6936

#at $5x10^7$ conidia ml^{-1} *@ 360 per bioassay

Table3. Influence of conidial age on the larvae to *B. bassiana and M. anisopliae*

Days	LC_{50}		LT_{50}		Fold increase over 10 days (10 day old culture=1)			
					LC_{50}		LT_{50}	
	B. bassiana	*M. anisopliae*	*B. bassiana*	*M. anisopliae*	*B. bassiana*	*M. anisopliae*	*B. bassiana*	*M. anisopliae*
10	12.50	17.52	113.09	119.31	-	-	-	-
15	15.76	20.28	118.36	124.48	1.26	1.15	1.05	1.04
20	67.64	83.73	133.80	136.24	5.41	4.78	1.18	1.14
25	146.12	196.01	143.16	146.53	11.69	11.18	1.27	1.23
35	341.61	408.71	153.79	155.85	27.32	23.33	1.36	1.31

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