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ORIGINAL RESEARCH ARTICLE

Effect of Abiotic Stress Conditions on Growth and Sporulation in Mycopesticidal Isolates of *Beauveria* species

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ABSTRACT

Thirty isolates of *Beauveria* species were evaluated for *in vitro* growth and sporulation under abiotic stress conditions viz., different temperatures, organophosphorous pesticides and fungicides at recommended dosages. Optimal temperature for the fungal isolates proved to be 30°C however both the parameters could be recorded at 7 to 33°C. Among the organophosphorous pesticides and fungicides tested, the fungal isolates were more susceptible to Dichlorovos and Bavistin.

Key words: Abiotic stress, temperature, pesticides, fungicides, *in vitro* compatibility, growth and spore output.

INTRODUCTION

Desirable trait of a potential microbial control agent is its ability to attain high levels of disease in the target populations, able to grow, sporulate on dead insect and furnish inoculum for further infections. Entomopathogenic fungi defer from other entomopathogens in terms of their mode of parasitism as well as survival in nature which are strongly influenced by abiotic factors. This unique feature of microclimate dependency is of major importance with respect to the formation, germination and survival of various types of infective spores or conidia that attach to host cuticle, germinate and subsequently penetrate it (Carruthers and Hural 1990).

Approaches to deploy these fungi in biocontrol programs rely on the ability of fungi to withstand environmental conditions in order to persist and establish infection in the host population. The effectiveness of these entomopathogenic fungi in field is determined by a variety of factors influencing its pathogenicity which include temperature, relative humidity, light, air, nutrient availability and host physiological status (Milner and Soper 1981). Magan (1997) expressed the opinion that development of fungal biocontrol agents in field environments is critically dependent on the fluctuations in relative humidity and temperature. Soil is a natural reservoir for moribund insects that die due to entomopathogenic fungal infection (Keller and Zimmerman 1989). Soil temperature, moisture, pH, content of organic matter and conductivity (Lingg and Donaldson 1981) have been found to affect the survival and long term efficacy of these fungi. The foremost abiotic stress factor that has to be surpassed by the fungal pathogen is the temperature sufferance and prior knowledge regarding thermal tolerance of biocontrol agent is very essential before targeting it at field level, wherein fluctuating climatic conditions exist in different environments. An effort to understand the tolerance to different temperatures has been attempted by some investigators in *B. bassiana* (Fargues *et al*, 1997; Ekesi *et al*, 1999; Lacey *et al*, 2001).

In view of its wide host range, *B. bassiana* is being dispensed in alliance with chemical/synthetic compounds in several IPM programs. A major limitation for the use of entomopathogenic fungi is their susceptibility to chemical pesticides which checks their survival in the fields where pesticide residues prevail. Many investigators have reported the effects of chemical pesticides on the growth of entomopathogenic fungi. *bassiana* (Padmaja *et al*, 2002, Tedders 1981, Fuher 1980, Machowicz *et al*, 1980). Failures in the performance of entomopathogenic fungi in biocontrol programs is mainly due to the fungicidal activity of chemical fungicides used in crop protection against plant pathogenic fungi. In

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view of the above factors, it is necessary to scrutinize the effect of abiotic stress factors on the entomopathogenic fungus under *in vitro* conditions prior to their use at the field level.

MATERIALS AND METHODS

1. FUNGAL CULTURES

Twenty four endemic isolates of *Beauveria bassiana* were isolated from diseased larvae from various agricultural fields of Andhra Pradesh, India. Six isolates which includes, one *B. Brongniartii* species were procured from USDA - ARS, ITCC and EMBRAPA. (Padmini and Padmaja 2010). Pure cultures were established and maintained in SDAY (Sabouraud's dextrose agar yeast) medium.

2. PREPARATION OF SPORE SUSPENSIONS

Spores were harvested from the surface of 15 day old *Beauveria* cultures grown at 25ºC on agar slants of SDAY medium by scraping and suspending in 5 ml of sterile 0.02% Tween 80 solution. The suspension was then vortexed to decimate spore clumps in order to obtain homogenous mixtures which were then adjusted to $2x10^8$ /ml concentration using haemocytometer.

3. GROWTH AND SPORULATION OF *BEAUVERIA* ISOLATES AT DIFFERENT TEMPERATURES

Autoclaved SDAY medium was poured into pre sterilised petri plates which were then allowed to solidify. A well of 2 mm diameter was made with a pre sterilized cork borer at the centre of solidified medium in petri plates and 50 µl of spore suspension was inoculated with micro pipette. Three replicates were maintained for each individual temperature as well as fungal isolate. The petri plates were incubated at lower temperatures in range of 6ºC, 7ºC, 8ºC, 10ºC, 14ºC, 18ºC and a high temperature range of 25ºC, 30ºC and 33ºC.

4. COMPATIBILITY OF *BEAUVERIA* ISOLATES WITH DIMETHOATE AND DICHLORVOS (ORGANOPHOSPHOROUS PESTICIDES)

Dimethoate(0,0-Dimethyl S-(N-methyl carbamoyl methyl) phosphorodithioate) and dichlorvos (2, 2-Dichlorovinyl dimethyl phosphate) were obtained from the local pesticide dealer and required quantities (2 ml/L dimethoate and 1.3 ml/L dichlorvos) of the pesticides were added to the autoclaved SDAY medium at 45ºC prior to plating in pre sterilised petri plates so as to get 1x concentration, i.e., recommended dosage. SDAY medium devoid of pesticides were used as controls.

5. COMPATIBILITY OF *BEAUVERIA* ISOLATES WITH COPPER OXYCHLORIDE AND BAVISTIN (COPPER AND BENZIMIDAZOL FUNGICIDES)

Copper oxychloride (Dicopper chloride trihydroxide) and bavistin (Methyl N (1H-benzomidazol-2yl) carbamate) were obtained from the local fungicide dealer and required amounts (1.5 g/L copper oxychloride and 1 g/L bavistin) were amended to autoclaved SDAY medium at 45ºC so as to give 1x concentration i.e., field recommended dose and medium thus prepared was plated in pre sterilized petri plates. SDAY medium devoid of fungicides were used as controls.

6. INCOULATION OF FUNGAL SPORE SUSPENSION AND DATA COLLECTION

Wells of 2 mm diameter were made using a sterile cork borer in the centre of solidified medium in petri plates and 50µl of $2x10^8$/ml spore suspension was inoculated into each well with help of micro pipette. Three replicates were maintained for each isolate, treatment as well as controls and incubated at room temperature for a period of 10 days. Data pertaining to radial growth and spore output for all parameters were recorded on particular days of incubation with a graduated scale (mm) for the former and flooding petri plates with 5 ml of 0.02% Tween 80 for the later and determining concentration with the help of haemocytometer.

7. DATA ANALYSIS

Statistical evaluation of the data for all experiments was performed using STATISTICA version 6.0. The data pertaining to radial growth and spore output were subjected to factorial analysis of ANOVA and growth of each isolate at different parameters was compared.

RESULTS

GROWTH AND SPORULATION OF *BEAUVERIA* ISOLATES AT DIFFERENT TEMPERATURES

Lower temperatures (7ºC to 20ºC):

As the temperature increased from 7ºC to 20ºC rate of growth also increased in the isolates but sporulation did not follow a consistent pattern. At 7ºC, only 13 isolates showed radial growth and maximum attained was 5 mm in B15, B18 and B27. In B33, there was complete inhibition of sporulation. At 8ºC except B8, B13, B14 and B29 remaining isolates showed growth while B20 and

B30 did not show sporulation in spite of considerable radial growth. Maximum growth was observed in B19, B32 (7mm) and lowest in B31 and B39 with 4mm on 7th day of incubation. Highest spore output was observed in B40 with $2x10^9$/ml and lowest in B16 with $1x10^4$/ml. At 10ºC, maximum growth of 8mm in B18 and spore output of $5.4x10^8$ /ml was recorded. At 14ºC maximum growth observed was in B18 on the 7th day of incubation (**Table 1**) and minimum of 7mm was observed in B31, B40 and B28. Highest spore output was observed in B32 with $6.8x10^8$/ml and lowest was observed in B7 with $1.6x10^5$/ml. At 18ºC, all the thirty isolates displayed considerable growth and sporulation at this temperature. Maximum radial growth of 10 mm was observed in B18 and B32 whereas highest spore output of $5.2x10^8$/ml and lowest of $1.1x10^6$/ml in B8 was observed. At 20ºC all the isolates of *Beauveria* showed profuse vegetative growth and also sporulation. Highest growth of 17 mm was observed in B32 and B40 and the lowest of 12 mm in B27. While maximum spore output was observed in B13 with $1.18x10^9$/ml though radial growth was 13.6 mm on 7th day and minimum was observed in B20 with $1.6x10^5$/ml.

Higher temperatures (25ºC to 33ºC):

At 25ºC, vegetative growth and sporulation were observed in all the isolates which displayed maximum growth of 13 mm by B8 and B26 on 7th day. Minimum growth was recorded in B30 followed by B20 and B33 with 5 and 5.5 mm in the later two. Regarding sporulation, highest of $8.75x10^8$/ml was recorded in B13 and lowest of $1.2x10^6$/ml was recorded in B35. At 30ºC also, all the isolates grew well showing s maximum of 22 mm in B8 on 7th day of incubation. On the other hand, minimum growth as well as spore output was observed in B16. Whereas, maximum spore output of $1.37x10^9$/ml in B6 was recorded though radial growth was not maximum compared to rest of the tested isolates. At 33ºC, 10 isolates out of 30 responded in terms of growth as well as sporulation. There was no measurable growth in isolates till 10th day so the data pertaining to radial growth and sporulation were measured on 15th day of incubation. Highest radial growth of 9.6 followed by 7.2 mm was observed in B7 and B19 while lowest of 4mm in B32 was observed (**Table 2**). Regarding spore output, maximum of $4.1x10^8$/ml in B7 and minimum of $3.9x10^5$/ml in B30 was recorded.

COMPATIBILITY OF *BEAUVERIA* ISOLATES WITH DIMETHOATE AND DICHLORVOS (ORGANOPHOSPHOROUS PESTICIDES)

Dimethoate:

In vitro radial growth and spore output response of *Beauveria* isolates towards this pesticide was variable for all the 30 isolates in dimethoate amended medium. Moreover, radial growth, among the isolates was in the range of 30% to 140% where as spore output ranged from 0.8 to 150% showing stimulation in both parameters. In some of the isolates though there was considerable vegetative growth, mycelium did not show sporulation and out of 30 isolates, 23 displayed >60 % radial growth in the presence of dimethoate. Highest radial growth (140%) as well as sporulation (150%) were recorded by B33 (**Table 3**) while lowest radial growth was shown by B6 (30%) and spore output by B28 (0.8%).

Dichlorvos:

At 1x concentration, it proved to be deleterious to some of the *Beauveria* isolates showing 100% inhibition in both *in vitro* parameters however, permitting vegetative growth in only 9 out of 30 isolates. Radial growth among the isolates ranged from 36% to 95% while spore output ranged from 0.93% to 100%. However in few isolates even though where there was significant growth, spore output could not be observed (B18, B22, B38, and B41). Conversely, isolates B12, B15, B24, B25, B27, B29, B33 and B35 recorded >60% radial growth with highest in B33 (115%) followed by B29 (95%) control (**Table 3**). On the other hand, highest spore output was recorded by B35 (100% control). B33 isolate exhibited stimulation in terms of both *in vitro* parameters towards organophosphorous pesticides studied.

COMPATIBILITY OF B*EAUVERIA* ISOLATES WITH COPPER OXYCHLORIDE AND BAVISTIN (COPPER AND BENZIMIDAZOL FUNGICIDES)

Copper oxychloride:

At 1x concentration, copper oxychloride permitted vegetative growth of all *Beauveria* isolates but significant reduction in conidial production was observed in almost all the isolates. Spore output was completely inhibited in six isolates while radial growth ranged from 53.3% (B40) to 105% (B32). According to the procedure followed by Filho *et al*, (2001), the isolates were categorized into different categories depending on the response to fungicide. The isolates showing 0-30% radial growth in the presence of fungicide were said to be very toxic, 31-45% was toxic, 46-60% were moderately toxic and >60% were

compatible. Except two isolates i.e., B6 and B40, all the *Beauveria* isolates were compatible showing considerable radial growth above 60% (**Table 4**).

Bavistin:

At 1x concentration, bavistin inhibited growth of all 30 isolates even beyond 15 days of incubation. So, the concentration was dropped down to 0.5x concentration i.e., half the recommended dosage for further experimentation. Bavistin at 0.5x concentration permitted growth only in 11 out of 30 isolates in the range of 34.6% (B20) to 69.2% (B12) while spore output was in the range of 1% to 82.5% (**Table 4**). As the fungitoxicity was much prominent, only those isolates showing >50% radial growth were considered to be compatible. Moreover bavistin at 0.5x dosage significantly affected the macroscopic picture of the colonies of *Beauveria* resulting in poor sporulating mycelial colonies with distorted appearance. Isolates which did not show any trace of vegetative growth beyond 15 days of inoculation were transferred to control medium and resurgence of vegetative growth was observed. Isolates B7, B22, B26 and B37 regained normal growth while rest of the isolates did not revive from the toxic affect. For statistical analysis of data, only the isolates which showed vegetative growth were considered.

The data pertaining to radial growth and spore output were subjected to factorial analysis of ANOVA and growth of each isolate at different parameters were compared (P=<0.05). Prior to analysis, the *in vitro* radial growth and sporulation data was log transformed. The factorial ANOVA showed highly significant differences (P = 0.00) among the *Beauveria* isolates for both parameters were calculated. For temperature, values of F=140.9 for radial growth and F=17.45 for spore output were scored. While the value of F=5761 for radial growth and F=23946 for spore output for dimethoate were recorded. On the other hand, for dichlorvos F=319773 for radial growth and F= 2.639481 for spore output were recorded. For copper oxychloride F=15.08 for radial growth and F=25973 for spore output. On the other hand, for bavistin F=10554 for radial growth and F= 3621 for spore output were observed.

Table 1: Radial growth and spore output of *Beauveria* isolates at lower temperatures

	7°C		8°C		10°C		14°C		18°C		20°C	
Isolate number	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10thday**	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10th day**
B6	4.00 ± 0.0	$3.0x10^4$	6.00 ± 0.0	$3.5x10^5$	6.00 ± 0.00	$2.0x10^6$	7.5 ± 0.00	$3.1x10^6$	10.3 ± 0.03	$1.2x10^7$	1.33 ± 0.03	$4.25x10^8$
B7	4.00 ± 0.0	$1.0x10^4$	6.00 ± 0.0	$8.0x10^4$	6.60 ± 0.03	$3.6x10^5$	7.5 ± 0.05	$4.2x10^5$	10.0 ± 0.05	$5.0x10^6$	1.40 ± 0.00	$2.30x10^8$
B8	-	-	5.00 ± 0.0	0	7.00 ± 0.00	$3.1x10^4$	8.5 ± 0.05	$1.6x10^5$	11.0 ± 0.05	$1.1x10^6$	1.50 ± 0.00	$5.25x10^7$
B12	4.00 ± 0.0	$1.0x10^4$	5.00 ± 0.0	$5.0x10^4$	6.00 ± 0.00	$4.5x10^5$	7.5 ± 0.05	$6.5x10^6$	11.0 ± 0.00	$7.5x10^6$	1.36 ± 0.05	$7.00x10^7$
B13	-	-	-	-	6.00 ± 0.00	$2.2x10^5$	7.5 ± 0.00	$4.3x10^7$	10.6 ± 0.03	$8.5x10^7$	1.36 ± 0.03	$1.18x10^9$
B14	-	-	-	-	6.00 ± 0.00	$1.2x10^5$	9.0 ± 0.00	$4.9x10^5$	10.6 ± 0.03	$7.5x10^6$	1.60 ± 0.05	$1.85x10^8$
B15	5.00 ± 0.0	$3.0x10^4$	5.00 ± 0.0	$4.0x10^4$	6.00 ± 0.00	$3.7x10^5$	7.5 ± 0.05	$7.1x10^6$	11.0 ± 0.00	$2.9x10^7$	1.36 ± 0.03	$2.87x10^8$
B16	4.00 ± 0.0	$1.0x10^4$	6.00 ± 0.0	$1.0x10^4$	6.30 ± 0.03	$1.5x10^5$	7.5 ± 0.00	$5.7x10^5$	10.3 ± 0.03	$1.7x10^6$	1.33 ± 0.03	$4.25x10^7$
B18	5.00 ± 0.0	$4.0x10^4$	6.00 ± 0.0	$6.0x10^4$	8.00 ± 0.00	$3.3x10^8$	10.0 ± 0.0	$2.6x10^6$	11.6 ± 0.03	$1.0x10^8$	1.50 ± 0.00	$4.75x10^8$
B19	4.00 ± 0.0	$1.6x10^5$	7.00 ± 0.0	$2.8x10^5$	7.50 ± 0.05	$3.5x10^5$	8.0 ± 0.00	$1.5x10^7$	10.6 ± 0.03	$1.8x10^8$	1.40 ± 0.00	$3.93x10^8$
B20	-	-	5.00 ± 0.0	0	6.00 ± 0.00	$2.6x10^4$	8.0 ± 0.00	$1.0x10^6$	10.0 ± 0.00	$2.0x10^6$	1.30 ± 0.00	$1.60x10^5$
B22	-	-	5.00 ± 0.0	$2.0x10^8$	6.60 ± 0.03	$2.7x10^5$	8.0 ± 0.00	$3.2x10^6$	11.6 ± 0.08	$1.7x10^8$	1.60 ± 0.00	$6.75x10^7$
B23	-	-	4.50 ± 0.0	$1.3x10^5$	6.00 ± 0.00	$7.0x10^4$	10.0 ± 0.0	$2.0x10^7$	12.0 ± 0.00	$1.5x10^7$	1.60 ± 0.00	$1.35x10^8$
B24	4.00 ± 0.0	$1.0x10^8$	6.00 ± 0.0	$3.0x10^4$	7.00 ± 0.00	$5.4x10^8$	6.5 ± 0.00	$2.1x10^5$	11.3 ± 0.03	$2.2x10^7$	1.40 ± 0.00	$1.77x10^8$
B25	-	-	6.00 ± 0.0	$7.0x10^4$	7.00 ± 0.00	$4.1x10^5$	8.0 ± 0.00	$8.1x10^6$	11.6 ± 0.03	$9.5x10^7$	1.40 ± 0.00	$2.90x10^8$
B26	4.00 ± 0.0	$1.1x10^4$	6.00 ± 0.0	$2.0x10^4$	7.00 ± 0.00	$3.3x10^5$	8.5 ± 0.05	$6.2x10^6$	11.3 ± 0.03	$5.1x10^7$	1.60 ± 0.00	$9.00x10^7$
B27	5.00 ± 0.0	$4.0x10^4$	6.00 ± 0.0	$3.0x10^4$	6.00 ± 0.00	$1.9x10^5$	8.0 ± 0.00	$2.0x10^7$	10.3 ± 0.03	$3.0x10^7$	1.20 ± 0.00	$1.50x10^8$
B28	-	-	5.00 ± 0.0	$8.0x10^4$	6.50 ± 0.05	$1.8x10^5$	7.0 ± 0.00	$1.9x10^7$	9.00 ± 0.00	$1.0x10^7$	1.40 ± 0.00	$4.37x10^8$
B29	-	-	-	-	6.00 ± 0.00	$8.0x10^4$	9.0 ± 0.00	$4.9x10^6$	10.3 ± 0.00	$2.0x10^7$	1.30 ± 0.00	$6.75x10^7$
B30	-	-	5.00 ± 0.0	$7.0x10^4$	6.00 ± 0.00	$8.0x10^4$	7.5 ± 0.05	$5.3x10^6$	10.0 ± 0.00	$5.0x10^6$	1.50 ± 0.00	$1.10x10^9$
B31	-	-	4.00 ± 0.0	$3.0x10^4$	6.00 ± 0.00	$4.0x10^4$	7.0 ± 0.00	$7.0x10^7$	10.0 ± 0.00	$1.7x10^7$	1.36 ± 0.03	$5.50x10^7$
B32	-	-	7.00 ± 0.0	$1.0x10^5$	6.50 ± 0.05	$1.5x10^7$	8.0 ± 0.00	$6.8x10^8$	12.3 ± 0.03	$5.2x10^8$	1.70 ± 0.00	$2.12x10^8$
B33	4.00 ± 0.0	-	6.00 ± 0.0	0	7.50 ± 0.01	$4.0x10^4$	8.5 ± 0.05	$3.1x10^6$	11.0 ± 0.00	$1.7x10^7$	1.40 ± 0.00	$1.72x10^8$
B35	-	-	5.50 ± 0.0	$3.0x10^4$	7.00 ± 0.00	$2.9x10^5$	8.0 ± 0.00	$5.0x10^6$	19.6 ± 0.03	$4.0x10^7$	1.36 ± 0.03	$8.75x10^7$
B37	-	-	6.00 ± 0.0	$2.0x10^4$	6.60 ± 0.03	$1.9x10^5$	8.5 ± 0.05	$2.9x10^6$	10.3 ± 0.03	$1.0x10^7$	1.40 ± 0.00	$5.85x10^7$
B38	-	-	5.00 ± 0.0	$8.0x10^8$	6.50 ± 0.05	$2.6x10^5$	8.0 ± 0.00	$3.1x10^5$	11.0 ± 0.00	$2.5x10^6$	1.50 ± 0.00	$6.00x10^8$
B39	-	-	4.00 ± 0.0	$1.0x10^4$	5.00 ± 0.00	$9.0x10^7$	8.0 ± 0.00	$2.1x10^6$	11.0 ± 0.00	$9.0x10^7$	1.60 ± 0.00	$7.80x10^8$
B40	4.00 ± 0.0	$2.0x10^4$	6.00 ± 0.0	$2.0x10^9$	7.00 ± 0.00	$3.1x10^5$	7.0 ± 0.00	$3.1x10^5$	11.6 ± 0.03	$4.2x10^7$	1.70 ± 0.00	$5.00x10^6$
B41	4.00 ± 0.0	$2.0x10^4$	5.00 ± 0.0	$2.0x10^4$	7.00 ± 0.00	$5.0x10^4$	8.5 ± 0.05	$2.0x10^8$	12.0 ± 0.00	$3.1x10^8$	1.53 ± 0.03	$2.10x10^8$
B42	-	-	6.00 ± 0.0	$4.0x10^4$	6.00 ± 0.00	$2.8x10^5$	8.0 ± 0.00	$1.6x10^7$	12.3 ± 0.03	$2.0x10^8$	1.43 ± 0.03	$3.20x10^7$

RG- Radial growth (mm), nm – no measurable growth/spore output, SP- Spore output (per ml)

Table 2: Radial growth and spore output of *Beauveria* isolates at higher temperatures

	20°C		25°C		30°C		33°C	
Isolate number	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10th day**	**RG 7th day**	**SP 10th day**	**RG 15h day**	**SP 15th day**
B6	1.33 ± 0.03	$4.25x10^8$	11.0 ± 0.01	$2.60x10^7$	20.3 ± 0.03	$1.37x10^9$	-	-
B7	1.40 ± 0.00	$2.30x10^8$	9.50 ± 0.02	$2.50x10^6$	19.0 ± 0.00	$1.52x10^7$	7.20 ±0.02	$4.10x10^8$
B8	1.50 ± 0.00	$5.25x10^7$	13.0 ± 0.00	$2.50x10^7$	2.20 ± 0.00	$1.75x10^7$		
B12	1.36 ± 0.05	$7.00x10^7$	8.00 ± 0.00	$3.70x10^7$	18.3 ± 0.00	$3.37x10^8$	7.00 ±0.00	$4.50x10^5$
B13	1.36 ± 0.03	$1.18x10^9$	10.0 ± 0.02	$8.75x10^8$	19.6 ± 0.03	$7.25x10^8$	9.60 ±0.02	$9.90x10^5$
B14	1.60 ± 0.05	$1.85x10^8$	8.00 ± 0.00	$1.30x10^7$	21.6 ± 0.00	$2.87x10^8$	-	-
B15	1.36 ± 0.03	$2.87x10^8$	10.0 ± 0.02	$9.60x10^7$	18.0 ± 0.00	$3.62x10^8$	-	-

B16	1.33 ± 0.03	$4.25x10^7$	8.00 ± 0.00	$1.00x10^7$	16.0 ± 0.00	$1.00x10^7$	-	-
B18	1.50 ± 0.00	$4.75x10^8$	8.50 ± 0.03	$3.20x10^7$	19.0 ± 0.00	$1.62x10^8$	-	-
B19	1.40 ± 0.00	$3.93x10^8$	8.00 ± 0.00	$4.80x10^7$	19.0 ± 0.00	$6.37x10^8$	6.60 ±0.02	$3.90x10^5$
B20	1.30 ± 0.00	$1.60x10^5$	7.50 ± 0.01	$5.00x10^6$	18.0 ± 0.00	$3.50x10^7$	-	-
B22	1.60 ± 0.00	$6.75x10^7$	10.5 ± 0.03	$6.00x10^8$	18.0 ± 0.00	$8.75x10^8$	6.60 ±0.08	$5.80x10^5$
B23	1.60 ± 0.00	$1.35x10^8$	9.00 ± 0.00	$3.10x10^7$	18.0 ± 0.00	$1.50x10^8$	-	-
B24	1.40 ± 0.00	$1.77x10^8$	8.00 ± 0.00	$2.50x10^6$	19.0 ± 0.00	$8.75x10^7$	-	-
B25	1.40 ± 0.00	$2.90x10^8$	11.5 ± 0.03	$6.00x10^8$	16.6 ± 0.03	$1.90x10^8$	7.00 ±0.00	$4.10x10^5$
B26	1.60 ± 0.00	$9.00x10^7$	13.0 ± 0.02	$7.00x10^8$	17.6 ± 0.03	$5.62x10^8$	6.40 ±0.05	$3.90x10^5$
B27	1.20 ± 0.00	$1.50x10^8$	9.00 ± 0.00	$1.10x10^7$	18.0 ± 0.00	$2.40x10^8$	-	-
B28	1.40 ± 0.00	$4.37x10^8$	9.00 ± 0.00	$9.00x10^7$	20.0 ± 0.00	$1.93x10^9$	-	-
B29	1.30 ± 0.00	$6.75x10^7$	8.00 ± 0.00	$2.50x10^7$	19.0 ± 0.00	$1.40x10^8$	-	-
B30	1.50 ± 0.00	$1.10x10^9$	7.00 ± 0.00	$4.60x10^7$	18.6 ± 0.03	$6.00x10^8$	7.00 ±0.05	$8.60x10^5$
B31	1.36 ± 0.03	$5.50x10^7$	9.00 ± 0.00	$7.00x10^7$	20.0 ± 0.00	$5.75x10^8$	-	-
B32	1.70 ± 0.00	$2.12x10^8$	9.00 ± 0.00	$1.10x10^7$	18.0 ± 0.00	$9.10x10^8$	4.00 ±0.03	$4.00x10^5$
B33	1.40 ± 0.00	$1.72x10^8$	7.50 ± 0.01	$2.00x10^7$	18.0 ± 0.00	$8.00x10^8$	-	-
B35	1.36 ± 0.03	$8.75x10^7$	8.00 ± 0.00	$1.20x10^6$	18.0 ± 0.00	$9.00x10^7$	-	-
B37	1.40 ± 0.00	$5.85x10^7$	8.00 ± 0.00	$1.30x10^7$	21.0 ± 0.00	$9.25x10^8$	5.00 ±0.02	$2.60x10^6$
B38	1.50 ± 0.00	$6.00x10^8$	8.00 ± 0.00	$7.50x10^6$	17.0 ± 0.00	$1.70x10^8$	-	-
B39	1.60 ± 0.00	$7.80x10^8$	8.50 ± 0.02	$4.50x10^6$	20.3 ± 0.03	$5.62x10^7$	-	-
B40	1.70 ± 0.00	$5.00x10^6$	7.50 ± 0.01	$9.70x10^7$	20.0 ± 0.00	7.50x106	-	-
B41	1.53 ± 0.03	$2.10x10^8$	11.0 ± 0.01	$1.10x10^7$	20.0 ± 0.00	$8.87x10^8$	-	-
B42	1.43 ± 0.03	$3.20x10^7$	12.0 ± 0.00	$7.23x10^8$	20.3 ± 0.03	$8.75x10^8$	-	-

RG- Radial growth (mm), nm – no measurable growth/spore output, SP- Spore output (per ml)

Table 3: Radial growth and spore output of *Beauveria* isolates on Dimethoate and Dichlorvos amended medium at 1x concentration

	Control		Dimethoate				Dichlorvos			
Isolate number	**CRG**	**CSP**	**RG 7^{th} day**	**SP 10^{th} day**	**% RG**	**% SP**	**RG 7^{th} day**	**SP 10^{th} day**	**% RG**	**% SP**
B6	12.2±0.05	$5.2x10^8$	3.80±0.05	$7.0x10^6$	30.0	1.30	4.90±0.03	$7.5x10^6$	39.0	1.40
B7	13.2±0.03	$7.5x10^7$	7.60±0.02	$1.7x10^7$	57.5	23.0	6.25±0.03	$2.5x10^7$	47.3	33.0
B8	7.50±0.00	$3.7x10^7$	5.40±0.05	$2.5x10^7$	72.0	17.0	-	-	-	-
B12	11.3±0.01	$1.5x10^8$	7.60±0.00	$2.5x10^7$	67.0	17.0	8.30±0.03	$5.0x10^6$	73.4	3.30
B13	12.2±0.02	$8.5x10^7$	5.90±0.01	$2.5x10^7$	48.3	48.3	5.12±0.02	$2.2x10^7$	41.9	26.0
B14	9.80±0.00	$1.8x10^8$	6.00±0.03	$1.2x10^8$	61.0	66.0	4.16±0.02	$2.4x10^7$	42.0	11.7
B15	10.0±0.02	$3.3x10^8$	7.40±0.05	$1.0x10^8$	74.0	30.0	5.25±0.02	$2.2x10^8$	73.0	30.0
B16	8.25±0.01	$3.7x10^7$	5.75±0.02	$2.0x10^7$	69.6	54.0	4.50±0.05	$1.7x10^7$	54.5	46.0
B18	8.50±0.03	$3.5x10^7$	4.60±0.05	$2.0x10^6$	54.0	5.40	4.00±0.00	0	47.0	0
B19	8.00±0.00	$4.9x10^7$	5.00±0.00	$3.0x10^7$	62.5	61.0	-	-	-	-
B20	7.50±0.00	$5.0x10^6$	8.00±0.00	$4.8x10^6$	106.6	96.0	-	-	-	-
B22	8.00±0.00	$5.0x10^7$	6.00±0.00	$4.6x10^5$	82.5	9.20	4.00±0.00	0	50.0	0
B23	9.00±0.00	$3.1x10^7$	7.60±0.03	$3.0x10^7$	84.4	96.7	-	-	-	-
B24	8.00±0.00	$7.2x10^8$	7.30±0.05	$1.2x10^8$	91.0	16.0	5.5±0.050	$2.2x10^8$	68.0	30.0
B25	8.50±0.04	$5.0x10^6$	8.30±0.01	$5.0x10^6$	97.6	100	7.00±0.00	$5.1x10^5$	82.3	10.2
B26	8.60±0.00	$1.5x10^8$	3.50±0.00	$2.2x10^7$	40.0	14.0	4.80±0.05	$1.0x10^7$	55.0	6.60
B27	8.83±0.00	$2.8x10^8$	6.75±0.03	$1.0x10^8$	76.0	35.0	6.50±0.08	$2.0x10^8$	73.0	71.0
B28	9.00±0.00	$9.0x10^7$	5.60±0.03	$7.0x10^5$	62.0	0.80	4.00±0.00	$7.5x10^6$	44.4	8.30
B29	8.00±0.00	$2.5x10^7$	5.60±0.05	$2.0x10^6$	70.0	8.00	7.60±0.05	$5.0x10^6$	95.0	20.0
B30	7.00±0.00	$4.6x10^7$	7.00±0.00	$5.0x10^7$	100	108	-	-	-	-
B31	9.00±0.00	$2.5x10^7$	6.60±0.07	$2.2x10^7$	73.3	88.0	-	-	-	-
B32	9.00±0.00	$2.0x10^7$	8.60±0.03	$4.9x10^6$	95.5	24.5	4.60±0.05	$1.7x10^7$	51.1	85.0
B33	4.00±0.00	$2.0x10^7$	5.60±0.01	$3.0x10^7$	140	150	4.60±0.05	$7.5x10^6$	115	37.5
B35	8.00±0.00	$5.0x10^6$	5.60±0.03	$5.6x10^5$	70.0	11.2	5.00±0.00	$5.0x10^6$	62.0	100
B37	8.00±0.00	$1.4x10^7$	6.60±0.03	$2.0x10^6$	82.5	14.2	-	-	-	-
B38	8.00±0.00	$7.5x10^6$	6.00±0.00	$6.8x10^5$	75.0	9.00	4.00±0.00	$7.0x10^4$	50.0	0.93
B39	8.50±0.03	$6.0x10^7$	5.30±0.03	$5.1x10^7$	62.3	85.0	-	-	-	-
B40	7.50±0.05	$6.0x10^7$	5.60±0.05	$6.0x10^6$	74.6	10.0	-	-	-	-
B41	110±0.00	$2.0x10^7$	5.60±0.03	$2.9x10^6$	51.0	14.5	4.00±0.00	0	36.3	0
B42	12.0±0.00	$7.2x10^7$	7.50±0.00	$6.1x10^6$	62.5	8.40	5.60±0.01	$7.0x10^6$	47.0	6.90

CRG-Control radial growth (mm), CSP- Control spore output (per ml), RG- Radial growth (mm), - no measureable growth/spore output SP- Spore output (per ml)

Table 4: Radial growth and spore output of *Beauveria* isolates on1x concentration of copper oxychloride and bavistin amended medium at 0.5x concentration

	Control		Copper oxychloride				Control		Bavistin			
Isolate number	**CRG 7^{th} day**	**CSP 10^{th} day**	**RG 7^{th} day**	**SP 10^{th} day**	**% RG**	**% SP**	**CRG 15^{th} day**	**CSP**	**RG 15^{th} day**	**SP 15^{th} day**	**% RG**	**% SP**
B6	14.0±0.0	$5.0x10^8$	8.30±0.05	$3.7x10^7$	59.0	7.40	-	-	-	-	-	-
B7	10.5±0.0	$2.5x10^8$	8.60±0.02	$5.0x10^7$	81.9	20.0	-	-	-	-	-	-
B8	4.00±0.0	$2.6x10^7$	11.0±0.00	$1.3x10^7$	78.5	50.0	-	-	-	-	-	-
B12	11.0±0.0	$4.5x10^7$	7.30±0.40	$2.5x10^6$	66.3	5.50	26.0±0.0	$4.30x10^9$	18.0±0.02	$3.0x10^9$	69.2	82.5
B13	11.0±0.0	$2.0x10^8$	8.60±0.01	$1.7x10^7$	78.0	8.50	-	-	-	-	-	-
B14	14.0±0.0	$6.0x10^7$	10.3±0.06	0	73.5	0	-	-	-	-	-	-
B15	13.0±0.0	$9.3x10^7$	10.6±0.12	$4.0x10^7$	81.5	44.0	-	-	-	-	-	-
B16	12.0±0.0	$3.0x10^7$	7.60±0.04	$2.5x10^6$	63.3	8.30	-	-	-	-	-	-
B18	13.0±0.0	$3.2x10^7$	8.60±0.07	0	66.0	0	-	-	-	-	-	-
B19	12.0±0.0	$4.8x10^7$	9.00±0.01	$2.1x10^7$	75.0	43.7	31.0±0.00	$4.30x10^9$	16.0±0.02	$2.7x10^9$	51.6	62.7
B20	12.0±0.0	$1.5x10^7$	12.0±0.00	0	71.6	0	-	-	-	-	-	-
B22	11.0±0.0	$1.5x10^7$	7.20±0.06	$1.2x10^6$	65.4	8.00	-	-	-	-	-	-
B23	14.0±0.0	$7.7x10^7$	10.6±0.09	$7.5x10^6$	75.4	9.70	33.0±0.00	$1.95x10^9$	13.4±0.03	$1.1x10^9$	40.6	60.0
B24	12.0±0.0	$5.4x10^8$	7.60±0.04	$6.2x10^7$	63.3		-	-	-	-	-	-
B25	14.0±0.0	$2.3x10^8$	10.3±0.41	$7.5x10^6$	73.5	3.20	-	-	-	-	-	-
B26	11.0±0.0	$2.5x10^8$	9.60±0.13	$2.0x10^7$	87.2	8.00	-	-	-	-	-	-
B27	11.0±0.0	$5.2x10^{10}$	8.00±0.73	0	72.7	0	-	-	-	-	-	-
B28	14.0±0.0	$3.0x10^8$	11.0±0.00	$1.1x10^8$	78.5	40.0	-	-	-	-	-	-
B29	12.0±0.0	$1.8x10^8$	10.0±0.09	0	83.3	0	23.0±0.00	$1.00x10^8$	8.00±0.02	$6.25x10^7$	34.6	62.5

B30	11.5±0.0	$4.6x10^7$	9.30±0.52	$1.1x10^7$	80.6	24.0	32.0±0.00	$3.80x10^9$	12.5±0.04	$2.87x10^8$	39.1	7.50
B31	14.0±0.0	$3.0x10^7$	11.6±0.08	$5.7x10^7$	82.8	190	27.0±0.00	$2.85x10^9$	11.0±0.03	$8.00x10^7$	40.7	2.80
B32	11.0±0.0	$7.2x10^7$	11.6±0.73	$4.0x10^6$	105.4	75.0	24.0±0.00	$8.60x10^9$	14.0±0.00	$2.70x10^9$	58.3	31.3
B33	13.0±0.0	$3.0x10^7$	9.60±0.90	0	73.8	0	-	-	-	-	-	-
B35	13.0±0.0	$4.0x10^7$	7.60±0.09	$8.7x10^6$	58.4	22.0	32.0±0.00	$3.60x10^9$	16.0±0.00	$2.50x10^8$	50.0	6.94
B37	12.0±0.0	$7.8x10^8$	10.3±0.15	$5.0x10^7$	85.8	6.40	-	-	-	-	-	-
B38	12.0±0.0	$5.0x10^7$	8.00±0.13	$3.8x10^7$	66.6	76.0	32.0±0.00	$8.30x10^9$	13.0±0.00	$1.25x10^9$	40.6	15.0
B39	12.0±0.0	$2.0x10^8$	9.60±0.14	$2.5x10^6$	80.0	1.20	32.0±0.00	$1.94x10^9$	14.5±0.00	$1.47x10^9$	45.3	75.7
B40	15.0±0.0	$1.5x10^8$	8.00±0.00	$7.0x10^7$	53.3	47.0	-	-	-	-	-	-
B41	12.0±0.0	$3.9x10^8$	8.60±0.20	$1.3x10^7$	71.6	3.30	33.0±0.00	$3.35x10^9$	14.0±0.00	$3.75x10^7$	42.4	1.10
B42	12.0±0.0	$7.2x10^7$	7.50±0.02	$4.6x10^5$	62.5	6.30	-	-	-	-	-	-

CRG -control radial growth. (mm), CSP- Control spore output., RG - Radial growth (mm), - no measureable growth/spore output SP- Spore output (per ml)

DISCUSSION

Results of temperature tolerance experiments facilitated categorization of *Beauveria* isolates into two groups a) Cold active (which were able to grow at lower temperatures), b) Heat active (which were able to grow at higher temperatures). Three out of thirty isolates however could not be categorized as they were able to grow at lower as well as higher temperatures. Hegedus and Khacharourians *et al*, (1996) observed that one of the *B. bassiana* strain was able to grow at both permissive as well as non permissive temperatures. A gradual decrease in growth rate among the isolates was observed from optimum temperature to extreme temperatures tested (lower and higher) in the present study. Moreover *Beauveria* isolates did not grow below 7ºC and beyond 33ºC. In contrast to this, Tong-K *et al*, (1989) reported that *B. bassiana* grew and sporulated at 5ºC but not at 35ºC while Luz and Fargues (1998) reported decreased germination rates in *B. bassiana* incubated at 30ºC to 35ºC. Growth rates of all the isolates increased as the temperatures increased from 7ºC to 30ºC but surprisingly, there was a decrease in radial growth in all isolates at 25ºC compared to that at 20ºC and 30ºC. Ilichova *et al*, (1976) expressed the opinion that favourable temperatures for *B. bassiana* were in the range of 20ºC-30ºC or for some strains even higher optimum temperature for growth under lab conditions and depended on the temperature of the district/place of the isolate origin. In contrast to our findings in the present study, Edelstein *et al*, (2004) reported that *Beauveria* isolates grew well at 25ºC, while Yeo *et al*, (2003) was of opinion that most of the *B. bassiana*, *M. anisopliae, V. lecanii and P. fumosoroseus* isolates were adversely affected at 10ºC-15ºC. Eleven out of the thirty isolates in the present study grew at 33ºC though the rate of growth was lesser when compared to 30ºC. Fargues *et al*, (1992) observed that various isolates of entomopathogenic fungi could grow at different extreme temperatures

Results of the pesticide tolerance studies revealed differences in terms of response to organophosphorous pesticides among 30 isolates of *Beauveria* tested and dimethoate proved to be more compatible than dichlorvos. Similar reports were also given by Ross *et al*, (1985). This differential response of *Beauveria* isolates in the two cases may be due to genotypic differences of the biocontrol agent. Few isolates did not show any traces of vegetative growth till the 10^{th} day of incubation in dichlorvos amended medium while some of the isolates grown in dimethoate amended medium could grow from 3^{rd} day onwards. Moreover slight to moderate stimulation in terms of growth and sporulation was found in few isolates on dimethoate amended medium. Chandrika and Padmaja (2004) reported that dimethoate stimulated spore output in few isolates of *M. anisopliae* though vegetative growth was less than that of controls. Results of the fungicide tolerance also revealed differential response among the *Beauveria* isolates to the test fungicides. Bavistin at 1x concentration proved to be much toxic, inhibiting growth of isolates moreover; similar results were also recorded by Vyas *et al*, (1990). Copper oxychloride on the other hand, permitted growth of all the isolates and stimulation in few isolates. Malo et al (1993) observed Mancozeb to be much more toxic to *B. bassiana*, *M. anisopliae* and *P. fumosoroseus*. In the present observation, bavistin proved to be fungicidal and also significant morphological changes were observed in the isolates grown on the Bavistin amended medium which was also in accordance to the results given by Waszkiewicz *et al*, (2003).

In conclusion, fungal growth at different temperatures in *in vitro* is not necessarily the same as that for growth in insects, and temperature ranges established according to *in vitro* experiments might be useful for selecting fungal candidates for microbial control. Moreover, the isolates which did not show any

signs of growth at 6ºC exhibited resilience in growth when transferred to optimal temperature whereas the isolates incubated at 34ºC did not restore normal growth when transferred to optimal temperature. The results of the present investigation strongly indicate that *Beauveria* isolates can grow at both lower as well as higher temperatures and 30ºC was the optimum temperature for maximum growth of isolates tested. At 1x concentrations of organophosphorous pesticides and fungicides, deleterious effects were apparent in terms of spore output but not in growth of the test organism. But compatibility between biocontrol agent and the chemical insecticide concentrations in *in vitro* conditions may not be a realistic estimate of their behaviour in field conditions. Since rapid evaporation of chemical and its wastage due to spread on non target sites prevailing in field conditions tend to reduce the effective concentration of the chemical.

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