RESEARCH ARTICLE

RESPONSE OF THE ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* TO MOLASSES MEDIA FORTIFIED WITH SUPPLEMENTS

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Abstract

The comparative response of the entomopathogenic fungi (EPF) Beauveria bassiana (Balsamo-Criv.) Vuill. and Metarhizium anisopliae Metch. to calcium chloride, chitin, lactic acid and polyethylene glycol 6000 (PEG) used as supplements in molasses media was evaluated. The supplements were incorporated into broth or agar media at different concentrations and three parameters, viz. biomass, radial growth and spore output were assessed. At a concentration range of 0.5-3.0%, calcium chloride significantly enhanced biomass, indistinctly altered radial growth and significantly increased spore output of B. bassiana. The supplement indistinctly affected biomass and radial growth but significantly enhanced spore output of M. anisopliae. Chitin (0.1-0.6%) significantly reduced biomass, did not affect radial growth and significantly reduced spore output of B. bassiana. In the case of M. anisopliae, chitin significantly enhanced biomass and radial growth at the higher concentrations but did not affect spore output. Lactic acid (0.5-3.0%) significantly enhanced biomass, reduced radial growth at higher concentrations and reduced spore output at medium concentrations in B. bassiana. The supplement positively influenced biomass of M. anisopliae at higher concentrations, significantly reduced radial growth at all concentrations and indistinctly reduced spore output. PEG (1-6%) significantly enhanced biomass, favored radial growth and significantly, yet uniformly, promoted spore output in B. bassiana. In M. anisopliae too, PEG enhanced biomass at 2% and higher concentrations, did not affect radial growth and significantly favored spore output at the highest concentration. In correlation analysis of parameter response and supplement concentration, biomass showed significant positive correlations only for lactic acid and PEG in B. bassiana but for all supplements in M. anisopliae. Radial growth showed significant negative correlations for chitin and lactic acid in B. bassiana but lactic acid alone in M. anisopliae. Spore output showed significant negative correlation for chitin in B. bassiana but significant positive correlation for PEG in M. anisopliae. Based on the positive relationship and degree of response over that in control, calcium chloride and PEG emerged as the best supplements that can be used with molasses media for increased output of both B. bassiana and M. anisopliae in mass cultures.

***Key words**: Entomopathogenic fungi, Beauveria bassiana, Metarhizium anisopliae, media supplements, molasses media, spore output*

Introduction

Entomopathogenic fungi (EPF) constitute an important group of microbial biocontrol agents of sugarcane borers (Easwaramoorthy and Santhalakshmi 1991). For example, *Beauveria bassiana* (Balsamo-Criv.) Vuill. was recorded on shoot borer *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae) (Easwaramoorthy and Santhalakshmi 1987), and *Hirsutella nodulosa* Petch (Easwaramoorthy et al. 1997) and *Metarhizium anisopliae* Metch. (Easwaramoorthy et al. 2001) were reported on internode borer *Chilo*

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sacchariphagus indicus (Kapur) (Lepidoptera: Crambidae); pathogenicity of *B. bassiana* and *M. anisopliae* to their borer hosts was established in these studies. Field evaluation of *B. bassiana* against shoot borer was attempted (Mala and Solayappan 2001).

Field use of EPF is limited by, besides the hostile canopy, the large spore dosage required to achieve reasonable levels of infection as in the case of *Beauveria brongniartii* (Petch) against the white grub *Holotrichia serrata* (F.) (Coleoptera: Scarabaeidae) (Easwaramoorthy et al. 2004). At low dosage and as single application, the fungus, however, caused moderate long-term infection levels in this soil pest (Srikanth et al. 2010), apparently due to pathogen survival in the soil. On the other hand, frequent aerial applications in large dosages may be required to control borers. This, in turn, depends on the production of adequate quantities of conidia in the laboratory production systems. Laboratory mass production techniques that used low-cost molasses media (Sharma et al. 1999) produced moderate conidial output (Easwaramoorthy et al. 2002). Further, increasing concentration of molasses beyond 3% (Easwaramoorthy et al. 2002), economization of media (Tamizharasi et al. 2005) and improvisation of formulations (Srikanth et al. 2006) failed to enhance spore production significantly. Addition of supplements with spore enhancement properties provides an alternative to partly overcome this limitation in the mass production of EPF. For example, chitin used as a supplement generated a high percentage of submerged conidia (Hegedus et al. 1990), and chitin and lactic acid elicited strain-specific response in sporulation (Sun and Liu 2006) of *B. bassiana*. In solid media such as rice grain (Liu et al. 1990) and alginate pellet formulation with wheat bran (Gerding-González et al. 2007) too, chitin enhanced sporulation of *B. bassiana*. Other supplements such as polyethylene glycol (PEG) produced higher blastospore yield than chitin in nutrient media (Geetha and Balaraman 2001). In studies with *M. anisopliae*, additives such as lecithin, collagen, lactic acid or PEG in Adámek's medium generally enhanced blastospore yield (Kleespies and Zimmermann 1998); addition of up to 150 g l^{-1} PEG increased spore production of *M. anisopliae* var. *acridum* (Leland et al. 2005). Chitin peptone nutrient media enhanced colony growth but a high C/N ratio media produced maximum conidial yield (Wu et al. 2010).

The effect of supplements on growth of EPF has not been investigated for molasses media which emerged as a good low-cost material for the multiplication of *B. brongniartii*, besides *B. bassiana* and *M. anisopliae* (Easwaramoorthy et al. 2002). In a recent study with *B. brongniartii*, we evaluated four supplements added to molasses media vis-à-vis nutrient media and observed that calcium chloride and chitin improved spore productivity of the EPF in molasses media (Srikanth and Santhalakshmi 2011). As a continuation, we examined in the present study the effect of the same set of supplements in molasses media on the growth and sporulation of *B. bassiana* and *M. anisopliae*, the two other common EPF of sugarcane pests with the potential for exploitation against different pests.

Materials and methods

Fungal cultures

Cultures of *B. bassiana* and *M. anisopliae*, isolated earlier from shoot borer and internode borer, respectively, were maintained in slants on Sabourauds dextrose agar (SDA) (dextrose 40g; bactopeptone 10g; yeast 10g; agar-agar 18g; distilled water 1000 ml) and Emersson YPSS media (yeast extract 4g; starch 15.0g; dipotassium phosphate 1.0g; magnesium sulphate 0.5g; agar-agar 20g; distilled water 1000 ml). The cultures were passed through third instar larvae of their respective hosts once prior to the commencement of the tests. The inoculum required for all the tests was obtained from seven-day old cultures on SDA or YPSS media in petri plates.

Evaluation of supplements

Four supplements, namely calcium chloride (0.5-3.0%), chitin (0.1-0.6%), lactic acid (0.5-3.0%) and polyethylene glycol 6000 (PEG) (1-6%) were evaluated with 3% molasses broth (molasses 30ml; yeast extract 2.5g; potassium chloride 0.5g; magnesium sulphate 0.5g; distilled water 1000ml) or agar (18g) media evaluated earlier (Easwaramoorthy et al. 2002). The concentrations of supplements were selected on the basis of the response in other species of fungi observed in earlier

studies as well as our study with *B. brongniartii* (Srikanth and Santhalakshmi 2011). Usually 500 ml of broth was supplemented with the required quantity of each supplement, pH adjusted to 6.5 using dilute hydrochloric acid or sodium hydroxide and autoclaved at 120^{0}C and 15 psi for 20 minutes; agar was added selectively for preparing solid media.

The impact of supplements in molasses media on the two EPF was assessed using biomass production, radial growth and spore output as parameters following the procedure from our earlier study (Srikanth and Santhalakshmi 2011). For examining radial growth, about 25 ml of autoclaved agar media was poured in 10 cm diameter petri plates. The fungus was inoculated as 10 mm discs obtained from seven-day old pure cultures maintained on SDA or YPSS media in petri plates. The plates were incubated in the laboratory at 28.2 ± 0.7^{0}C maximum and 25.8 ± 1.6^0 C minimum temperatures for 10 days and radial growth of the fungus was measured as the mean diameter (cm) of the fungal mat. For biomass determination, 100 ml of autoclaved broth in 250 ml culture flasks was inoculated with 10 mm fungal discs. After incubating the flasks in the laboratory for 20 days, the broth was filtered through pre-weighed Whatman no.1 filter paper for 12 h and the mycelial mat was dried at 50-60^{0}C in hot air oven until constant weight. To assess spore output, an independent set of 250 ml culture flasks with 100 ml of autoclaved broth was inoculated with 10 mm fungal discs. After incubating the flasks for 20 days, the fungal mat and broth were homogenized in a blender for 2 min, filtered through muslin cloth and the filtrate made up to 1 liter suspension. After serial dilution, spore count was determined in a haemocytometer and the number of spores in 100 ml broth was computed.

Data analysis

Fungal growth and sporulation data from various tests were subjected to one-way analysis of variance (ANOVA) after suitable transformations and means compared using Duncan's Multiple Range Test (DMRT). Product-moment correlation coefficient was employed to establish the dosage-response relationship between supplement concentrations and growth parameter values for different supplements. Concentration vs. response scatter plots of various supplements were examined and unusual relationships presented. The statistical tests were performed as per Gomez and Gomez (1984).

Results

Effect of media supplements on the EPF

At a concentration range of 0.5-3.0%, calcium chloride significantly influenced the three growth parameters of *B. bassiana* in a variable manner (Table 1). Biomass was significantly higher at all concentrations than in control but showed greater effect at the medium concentrations. Radial growth

Table 1. Growth parameters of two entomopathogenic fungi in calcium chloride supplemented molasses media

Supplement concentration (%)	*Beauveria bassiana*			*Metarhizium anisopliae*		
	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)
0.0	0.8064 a[1]	4.38 b	0.69 a	0.6276 ab	5.38 ab[2]	0.51 a[3]
0.5	0.9931 c	3.22 a	1.42 bc	0.5799 a	5.18 abc	0.92 b
1.0	1.0454 d	4.15 b	1.28 b	0.6502 ab	4.60 abc	1.88 c
1.5	1.0651 d	4.43 b	1.05 ab	0.7071 b	5.45 a	2.01 c
2.0	0.9518 b	5.30 c	1.71 c	0.6688 b	4.53 bc	1.25 b
2.5	0.9598 b	3.68 ab	1.78 c	0.6924 b	4.65 abc	1.96 c
3.0	0.9952 c	4.28 b	1.28 b	0.7004 b	4.43 c	1.95 c

[1] Means followed by the same letter in a column are not significantly (P>0.05) different by DMRT

[2] Square root and [3] $\log_{10}$ transformed values analyzed

Table 2. Growth parameters of two entomopathogenic fungi in chitin supplemented molasses media

Supplement concentration (%)	*Beauveria bassiana*			*Metarhizium anisopliae*		
	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)
0.0	1.0453 a[1]	5.15 a	4.88 a[2]	0.6581 a[3]	5.88 a	1.76 a
0.1	0.9573 b	5.18 a	2.54 b	0.6742 a	5.93 ab	1.69 a
0.2	0.9599 b	4.90 a	1.77 bc	0.6817 a	5.95 ab	1.36 a
0.3	0.9456 b	4.88 a	1.55 bc	0.7007 ab	6.13 ab	1.70 a
0.4	0.9177 b	4.78 a	1.41 c	0.6729 a	5.90 ab	1.67 a
0.5	0.9621 b	4.93 a	1.61 bc	0.7005 ab	6.23 b	1.53 a
0.6	0.9374 b	4.80 a	1.56 bc	0.7358 b	6.05 ab	1.46 a

[1] Means followed by the same letter in a column are not significantly (P>0.05) different by DMRT
[2] $\log_{10}$ and [3]square root transformed values analyzed

varied among concentrations with indistinct differences. Spore output was significantly higher at all concentrations than in control but tended to show more pronounced effect at the higher concentrations. Both biomass and radial growth of *M. anisopliae* showed indistinct differences among different concentrations and control. As in the case of *B. bassiana*, spore output was significantly higher at all concentrations than in control with no clear-cut differences among the treatments.

Biomass of *B. bassiana* was significantly lower at 0.1-0.6% of chitin with no differences among the treatments (Table 2). Radial growth of the fungus was not affected by different concentrations of the supplement. Spore output was negatively and significantly affected which, however, did not differ among the concentrations. In the case of *M. anisopliae*, biomass and radial growth significantly increased only at the higher concentrations. Spore output of the fungus was not significantly affected by chitin at all concentrations.

Lactic acid (0.5-3.0%) significantly enhanced biomass of *B. bassiana* at 1.0% and higher concentrations (Table 3). Radial growth declined with concentration of the supplement but the differences were marginal and significant only at the higher concentrations. Spore output showed indistinct declining trend especially at medium

Table 3. Growth parameters of two entomopathogenic fungi in lactic acid supplemented molasses media

Supplement concentration (%)	*Beauveria bassiana*			*Metarhizium anisopliae*		
	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)	Biomass (g)	Radial growth (cm)	Spore output (x10^{10}/100ml)
0.0	0.8745 a[1]	5.80 ab	1.97 a	0.6025 a	5.05 a[2]	1.99 a
0.5	0.9275 a	5.95 a	2.09 a	0.5932 a	4.40 b	1.06 b
1.0	1.0353 b	5.55 ab	1.88 ab	0.6577 ab	4.03 c	1.16 ab
1.5	1.0199 b	5.78 ab	0.60 c	0.7543 bc	3.85 c	2.05 a
2.0	1.0412 b	5.63 ab	0.72 c	0.7357 bc	3.40 d	1.95 a
2.5	1.0747 bc	5.38 b	1.24 bc	0.6683 abc	3.28 de	1.93 a
3.0	1.1255 c	5.43 b	2.05 a	0.7694 c	3.15 e	1.19 ab

[1] Means followed by the same letter in a column are not significantly (P>0.05) different by DMRT
[2] Reciprocal transformed values analyzed

Table 4. Growth parameters of two entomopathogenic fungi in polyethylene glycol supplemented molasses media

Supplement concentration (%)	*Beauveria bassiana*			*Metarhizium anisopliae*		
	Biomass (g)	Radial growth (cm)	Spore output ($x10^{10}$/100ml)	Biomass (g)	Radial growth (cm)	Spore output ($x10^{10}$/100ml)
0	0.9871 a[1]	5.78 a	0.83 a	0.8569 a	6.05 a	0.68 a
1	1.0116 a	6.20 c	1.85 b	0.8913 a	5.88 a	0.58 a
2	1.1080 b	5.88 ab	1.62 b	1.0933 b	6.10 a	0.75 a
3	1.2048 c	6.10 bc	1.57 b	1.1515 b	6.75 b	0.90 ab
4	1.3272 d	6.23 c	1.93 b	1.1436 b	5.90 a	0.76 a
5	1.4067 e	5.93 ab	1.68 b	1.2427 bc	6.28 ab	0.84 ab
6	1.3969 e	5.70 a	1.85 b	1.3755 c	6.00 a	1.17 b

[1] Means followed by the same letter in a column are not significantly (P>0.05) different by DMRT

concentrations. The supplement significantly enhanced biomass of *M. anisopliae* at 1.5% and higher concentrations. Radial growth was significantly reduced by the supplement at all concentrations. As in the case of *B. bassiana*, lactic acid indistinctly reduced spore output, particularly at the lower concentrations.

Biomass of *B. bassiana* responded positively to PEG (1-6%) supplemented molasses media with significant differences in the 2-6% concentration range (Table 4). Radial growth increased indistinctly with significant differences at some concentrations. Spore output showed a clear-cut response with all concentrations being superior to control but on par with one another. In *M. anisopliae* too, PEG enhanced biomass at 2% and higher concentrations. Radial growth was largely unaffected by the supplement concentrations. Spore output was significantly higher at the highest concentration of 6% alone.

Comparative performance of supplements

Correlation coefficients between concentration of supplement and fungal response for different growth parameters showed variations among supplements and between the two EPF (Table 5). Biomass of *B. bassiana* showed non-significant correlations for calcium chloride and chitin but significant positive correlations for lactic acid and PEG. The correlations of radial growth were not significant for calcium chloride and PEG whereas they were negative and

Table 5 Correlation coefficients of growth parameters with supplement concentration for two entomopathogenic fungi cultured in molasses media

Supplement	Biomass (g)	Radial growth (cm)	Spore output ($x10^{10}$/100ml)
	Beauveria bassiana		
Calcium chloride	0.370 ^{ns}	0.210 ^{ns}	0.600 ^{ns}
Chitin	-0.681 ^{ns}	-0.810 *	-0.755 *
Lactic acid	0.946 **	-0.803 *	-0.313 ^{ns}
Polyethylene glycol	0.979 ***	-0.161 ^{ns}	0.628 ^{ns}
	Metarhizium anisopliae		
Calcium chloride	0.780 *	-0.711 ^{ns}	0.736 ^{ns}
Chitin	0.833 *	0.623 ^{ns}	-0.476 ^{ns}
Lactic acid	0.786 *	-0.970 ***	0.022 ^{ns}
Polyethylene glycol	0.968 ***	0.115 ^{ns}	0.816 *

^{ns} P>0.05; * P<0.05; ** P<0.01; *** P<0.001

significant for chitin and lactic acid. The negative correlation of spore output for chitin alone was significant among the four supplements. In slight contrast to this trend, biomass of *M. anisopliae* showed significant positive correlations for all the four supplements. Among the correlations of radial growth, lactic acid alone showed significant and negative relationship. The positive correlation of spore output for PEG alone was significant.

Some non-significant correlations between concentration and fungal response showed non-linear or unimodal relationships. In *B. bassiana*, calcium chloride drastically reduced radial growth at the lowest concentration, enhanced it at the next three concentrations and reduced it at the last two concentrations (Fig. 1a). In *M. anisopliae*, lactic acid similarly reduced spore output at the two lowest concentrations, enhanced it at the next three concentrations and reduced it at the highest concentration (Fig. 1b).

A comparison of the percent change in growth parameters due to the addition of supplements, based on the highest significant difference obtained for the range of concentrations used over control, indicated that PEG produced the highest level of positive response in biomass of both *B. bassiana* (+42.5%) and *M. anisopliae* (+45.0%). However, chitin showed a negative response (-8.4%) in biomass of *B. bassiana*. Radial growth, which showed either non-significant positive or significant negative correlations with concentration of supplement in both fungi, showed the highest enhancement for calcium chloride (+21.0%) in *B. bassiana* and PEG (+11.6%) in *M. anisopliae*. Chitin (-4.9%) in *B. bassiana*, and lactic acid (-35.0%) and calcium chloride (-3.7%) in *M. anisopliae* showed negative relationship for radial growth. Spore output produced positive response for calcium chloride and PEG with the former recording greater effect in both the fungi. The response values for calcium chloride and PEG were +105.7 and +89.2% in *B. bassiana* and +268.6 and +72.1% in *M. anisopliae*, respectively. In contrast, both chitin and lactic acid produced negative effect for both the fungi. While chitin showed greater response (-63.7%) than lactic acid (-37.1%) in *B. bassiana*, lactic acid (-46.7%) was stronger than chitin (-4.0%) in *M. anisopliae*.

Discussion

Media supplements are known to impact growth and sporulation of EPF differentially. The enhancement of biomass and spore output of both *B. bassiana* and *M. anisopliae* by calcium chloride in the present study derives support from similar effect of the supplement on *M. anisopliae* (Calderón et al. 1991) and *B. brongniartii* (Srikanth and Santhalakshmi 2011) as against the opposite effect of biomass reduction in *Nomuraea rileyi* (Farlow) Samson (Méndez et al. 2007). Similarly, the reduction in spore output of *B. bassiana* by chitin in the present study contrasted with enhanced conidial production of this fungus (Liu et al. 1990; Gerding-González et al. 2007) and *B. brongniartii* (Srikanth and Santhalakshmi 2011). The variable effects of supplements could partly be attributed to differences in EPF species, media composition, and nature and concentration of the supplement. However, in

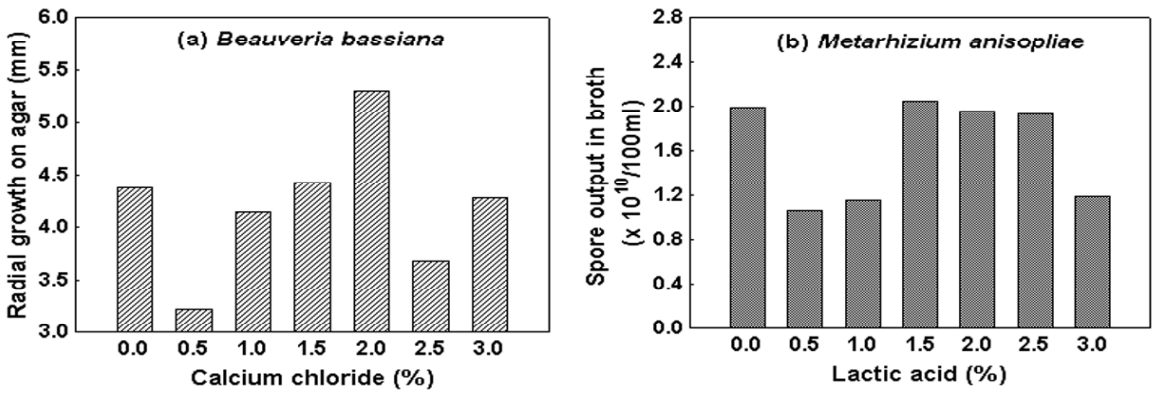

Fig. 1. Growth parameters of *Beauveria bassiana* and *Metarhizium anisopliae* in molasses media supplemented with different concentrations of calcium chloride and lactic acid

specific instances such as *B. bassiana* cultured in wheat bran media, spore output enhancement was indirectly related to the suppression of contamination by chitin (Gerding-González et al. 2007). Enhanced sporulation of *B. bassiana*, *B. brongniartii* and *M. anisopliae* by PEG in the present and earlier studies (Humphreys et al. 1989; Knudsen et al. 1991; Kleespies and Zimmermann 1992; Leland et al. 2005; Srikanth and Santhalakshmi 2011) indicated the general suitability of the supplement for a wide variety of EPF and media, apparently through regulation of water activity and the consequent prolonged fungal growth. Despite doubling *M. anisopliae* spore yield in one media (Kleespies and Zimmermann 1998), lactic acid failed to favor spore output of *B. bassiana*, *B. brongniartii* and *M. anisopliae* in the present and earlier studies (Sun and Liu 2006; Srikanth and Santhalakshmi 2011). These results proved lactic acid, with known inhibitory effect on germination of inoculated spores in the media (Barnes and Moore 1997), to be a poor supplement particularly in molasses media for mass production of EPF.

Concentration vs. response correlation coefficients highlighted some general trends for supplements and fungi in terms of trade-off between parameters. The predominantly positive correlations for biomass and neutral or negative trend for radial growth indicated accumulation of mycelial biomass in lieu of filamentous growth. The notable exception was chitin which negatively influenced both biomass and radial growth of *B. bassiana*. A similar general pattern was observed for biomass and radial growth of *B. brongniartii* cultured on molasses media with the same supplements in our earlier study (Srikanth and Santhalakshmi 2011), despite the opposite trend of increased filamentous growth and decreased mycelial pellet formation in *M. anisopliae* var. *acridum* under the influence of PEG (Leland et al. 2005). These results pointed to a distinct trade-off between biomass and radial growth in favor of the former under the stress or nutrient-rich conditions induced by the supplements. The positive correlations for spore output produced by calcium chloride and PEG in both *B. bassiana* and *M. anisopliae*, though significant only for PEG in *M. anisopliae*, with accompanying positive correlations for biomass indicated a lack of trade-off between the two parameters. However, trade-off in favor of either mycelial growth or spore production or none is not uncommon in EPF on supplemented media (Kleespies and Zimmermann 1998; Leland et al. 2005; Sun and Liu 2006; Srikanth and Santhalakshmi 2011). Nevertheless, from the economically important spore output point of view, correlation analysis supported ANOVA results in establishing the usefulness of calcium chloride and PEG as supplements for molasses media. However, with a general trade-off in favor of biomass, chitin and lactic acid proved ineffective as supplements. The contrasting suitability of chitin to *B. brongniartii* in molasses media (Srikanth and Santhalakshmi 2011) indicated species-specific response of EPF to similar supplemented media.

Unimodal or unusual responses in growth parameters, particularly spore production, were reported in EPF for a wide range of supplements including lactic acid on *M. anisopliae* (Kleespies and Zimmermann 1998), calcium chloride on *Cordyceps militaris* (Sehgal and Sagar 2006), PEG on *M. anisopliae* var. *acridum* (Leland et al. 2005) and chitin on *B. bassiana* (Gerding-González et al. 2007). In all these cases, the supplements enhanced fungal response up to some concentration but reduced it at higher or lower levels. In the present study with *B. bassiana* and *M. anisopliae* and our earlier study with *B. brongniartii* (Srikanth and Santhalakshmi 2011), both in molasses media, calcium chloride and lactic acid rapidly reduced the parameter below control at lower concentrations, enhanced at the medium concentrations bringing it on par with control and reduced at the higher concentrations. This pattern slightly contrasted with that in nutrient media wherein the same supplements enhanced the parameters above control up to some level but reduced it thereafter (Srikanth and Santhalakshmi 2011). These differential patterns of fungal response indicated the role of media, besides the inhibition of fungal growth and conidiogensis by induced compounds at higher concentrations of supplements (Gerding-González et al. 2007). Such differential patterns also suggested the prevalence of an optimum range of osmolality of media or nutrient availability and utilization produced by the osmotic or nutrient supplement, respectively (Srikanth and Santhalakshmi 2011).

The improvement in spore output produced by calcium chloride for *B. bassiana* (2.0 fold) and *M. anisopliae* (3.7 fold) in the present study ranks it as the best among the supplements tested. Similarly, the increase in spore output of *B. bassiana* (1.9 fold) and *M. anisopliae* (1.7 fold) at a lower concentration range (1-6%) of PEG in the present study is comparable to or higher than the 1.6 times spore yield enhancement of *M. anisopliae* var. *acridum* at a higher concentration range (5-30%) of the supplement (Leland et al. 2005). Nevertheless, the positive relationship of spore output with calcium chloride and PEG concentration for the two EPF in the present study and *B. brongniartii* earlier (Srikanth and Santhalakshmi 2011) suggested the possibility of greater response of the three EPF at higher supplement concentrations. The contrasting results for lactic acid and chitin discussed above, besides suggesting the interaction of media and fungal species, ruled out their role as media supplements. Lecithin, which enhanced the spore yield of *M. anisopliae* (Kleespies and Zimmermann 1998) and *B. brongniartii* (Srikanth and Santhalakshmi 2011) differentially at almost comparable concentrations, was not evaluated in the present study due to its concentration-dependent insolubility in the media and cost factor (Srikanth and Santhalakshmi 2011). The overall positive effect on spore output of the two EPF renders calcium chloride and PEG suitable supplements for their mass culture in molasses media. Although degeneration of *M. anisopliae* in terms of sporulation was not a serious problem in laboratory subcultures (Srikanth et al. 2011), lactic acid, which enhanced spore output of *B. brongniartii* in nutrient broth, could be a good supplement in nutrient media cultures of *B. bassiana* and *M. anisopliae* to avoid such problems; this aspect, however, needs confirmation.

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