Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium

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ABSTRACT

The two culture media tested both Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) recorded maximum mycelial growth in *Beauveria bassiana* isolates. Maximum growth was observed in the isolates of VpNlKKL 2121 (65.22mm), BbMtKKL 2107 (65.16mm), BbMdKKL 2106 (63.44mm) and FmNvKKL 2121 (62.78mm) and minimum growth was noticed in VpPmKKL 2120 (37.33mm) in PDA media. With SDA media maximum growth was observed in BbMtKKL 2107 (68.02mm) and BbMdKKL 2106 (66.91). Slowest growth was noticed in VpPmKKL 2120 (32.18 mm) .Highest spore count was noticed in *B. bassiana* isolates BbMtKKL 2107 (8.90 X 10^8 spores / ml) and BbMdKKL 2106 (8.77 X 10^8 spores / ml) isolates and are on par with FmNvKKL 2124 and VpPmKKL 2120 with PDA media. All other isolates had similar effect. Similarly, BbMdKKL 2106 isolate of *B. bassiana* in SDA media showed maximum spore count of 8.95 X 10^8 spores / ml and other isolates were on par with each other, except FpEvKKL 2119 which recorded least spore count of 0.52 X 10^8 spores / ml. Of the various fungi tested in (Potato Dextrose Broth)PDB and(Sabouraud Dextrose Broth) SDB (liquid broths) revealed that isolate BbMtKKL 2107 produced highest yield of (1.87g) followed by FpCmKKL 1526 (1.66g) and BbMdKKL 2106 (1.40g) and they were on a per with each other and remaining cultures four cultures were on par with each other. With reference to SDB media tested, highest yield was noticed in FpCmKKL 1526 (2.10g).

KEY WORDS: Entomopathogenic fungi, mycelial growth, sporulation, biomass and Broth

INTRODUCTION

During the recent years there has been a resurgence of interest in entomopathogenic fungi caused by factors such as increasing insecticide resistance and environmental concerns over pesticide use. All insects orders are more or less susceptible to fungal diseases. Several techniques for the mass production of entomogenous fungi were mostly designed to yield infective conidia in large numbers. Wadyalkar *et al.* (2003) reported that potato dextrose broth was found to be the best in spore production for *M. anisopliae.* Sharma *et al.* (2002) observed *M. anisopliae* produced maximum sporulation on molasses yeast broth, the highest conidia of *Beauveria* species was obtained with molasses yeast broth . In the present study laboratory tests were undertaken to determine the suitability of media for mass culturing various fungi under laboratory conditions .

MATERIALS AND METHODS

Growth on solid media

Two solid media Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were used for the study. Twenty ml of autoclaved solid media were poured into a sterilized petri plates. A 10 mm actively grown culture of selected entomopathogenic fungi was placed individually in the centre of the respective medium. The inoculated plates were incubated at 25^{0}C for 10 days. Three replications were maintained. The diameter of the fungal colony was measured following Daggupati (1988). For assessing sporulation of selected pathogens, 5 mm mycelial disc was cut from the 7 day old culture and then they were transferred to distilled water containing Tween-80 solution.

Growth on liquid media

Test broth (PDB and SDB) for each 100 ml medium were prepared in 250 ml Erlenmeyer flask and final pH was adjusted to 6.5. They were later inoculated aseptically with a 10 mm actively grown culture disc of the fungus. Three replications were maintained. The entire set up was incubated for 10 days at 25^{0}C to attain maximum growth and sporulation. The growth of various fungi *viz.*, BbMdKKL 2106, BbMtKKL 2107, FmNvKKL 2124, FpCmKKL 1526, FpEvKKL 2119, VpNlKKL2121 and VpPmKKL 2120 were studied on two media (PDA and SDA) and the mycelial growth of fungi was observed on 3rd, 5th and 7th day. A clear mycelial mat was obtained by filtering on pre- weighed filter paper (Whatman No.1) dried in hot

air oven at 70^0C until a constant weight was obtained. The difference in the weight gave the biomass produced (Hall and Bell 1961).

RESULTS AND DISCUSSION

Over all mean mycelial growth revealed that maximum growth was observed in the isolates of VpNlKKL 2121, BbMtKKL 2107, BbMdKKL 2106 and FmNvKKL 2121 and minimum growth was noticed in VpPmKKL 2120 in PDA media. With SDA media maximum growth was observed in BbMtKKL 2107 and BbMdKKL 2106. Slowest growth was noticed in VpPmKKL 2120 (Table 1). In general, highest mycelial growth was observed in *B. bassiana* isolates and *V. psalliotae* BPH isolate and lowest mycelial growth was noticed in *V. psalliotae* isolate on (PDA). Similar trend was noticed with test pathogens on medium SDA . This finding was in confirmity with the earlier workers that PDA medium was found to be suitable for the growth of *F. pallidoroseum* (Hareendranath *et al*., 1986). Pandit and Som (1988) recommended PDA for *B. bassiana*. Similarly, Sharma *et al*. (2002) also reported that PDA was best for the growth of *Metarhizium* isolates. The data furnished in Table 2 revealed that highest spore count was noticed in *B. bassiana* isolates BbMtKKL 2107 (8.90 X 10^8 spores / ml) and BbMdKKL 2106 (8.77 X 10^8 spores / ml) isolates and were on a par with FmNvKKL 2124 and VpPmKKL 2120 with PDA media. All other isolates had similar effect. Similarly, BbMdKKL 2106 isolate of *B. bassiana* in SDA media showed maximum spore count of 8.95 X 10^8 spores / ml and was on a par with other isolates except FpEvKKL 2119 which recorded least spore count of 0.52 X 10^8 spores / ml.

Higher spore count of *B. bassiana* was witnessed in *B. bassiana* isolates with PDA media .This was in line with Sharma *et al.* (2002) who reported that tapioca potato dextrose and potato dextrose agar supported better growth of fungus, *M. anisopliae, B. bassiana* and *B. brongniartii* over carbohydrate rice synthetic media (Richards, Zapek's and Sabouraud's). According to Manisegarane and Letchoumanane (1996) PDA was considered to be best next to Richard's medium for culturing *F. pallidoroseum.*

Similar trend was noticed with SDA media also. Amongst the two *B. bassiana* isolates, BbMdKKL 2106 isolate recorded maximum spore count and was on a par except FpEvKKL 2119 had recorded least spore count . This was in confirmity with Sharma *et al.* (2002) who reported that molasses yeast and Sabouraud's media were found to be suitable for sporulation. He also reported Richard's media supported excellent growth of fungi in all the *Metarhizium* isolates studied but proved poor sporulation. *Hirsutella thomsonii* was able to grow and sporulate profusely in PDA as well as SDA media (Kumar and Anuroop, 2004).

Of the various fungi tested in PDB and SDB (liquid broths) revealed that isolate BbMtKKL 2107 produced highest yield of (1.87g) in PDB followed by FpCmKKL 1526 (1.66g) and BbMdKKL 2106 (1.40g) which were all on par and better than all other fungi. With reference to SDB media tested, highest yield was noticed in FpCmKKL 1526 (2.10g) and it was on a par with BbMtKKL 2107 (1.81g), BbMdKKL 2106

Table 1. Effect of culture media on the diameter of mycelial growth (mm) of entompathogenic fungi under *in vitro* condition

Fungal isolate	3rd Day		5th Day		7th Day		Over all mean radial growth (mm)	
	PDA	SDA	PDA	SDA	PDA	SDA	PDA	SDA
BbMdKKL 2106	37.33^a	41.00^a	69.00^a	71.33^a	84.00^a	87.46^a	63.44^a	66.91^a
BbMtKKL 2107	39.66^a	41.16^a	71.83^a	75.50^a	84.00^a	86.66^a	65.16^a	68.02^a
FmNvKKL 2124	31.00^b	29.33^c	68.33^a	66.33^b	89.00^a	86.00^{ab}	62.78^a	60.28^b
FpCmKKL 1526	27.66^b	32.66^b	48.00^c	61.66^c	81.66^a	81.66^{ab}	52.44^b	57.13^b
FpEvKKL 2119	28.33^b	27.33^c	55.00^b	55.66^d	84.33^a	82.33^{ab}	55.89^b	56.09^b
VpNlKKL 2121	42.00^a	24.66^d	72.33^a	49.66^e	81.33^a	79.16^b	65.22^a	50.87^c
VpPmKKL 2120	26.00^b	16.66^e	33.66^d	25.66^f	52.33^b	50.66^c	37.33^c	32.18^d
CD	0.41	0.16	0.44	0.21	0.54	1.07	0.26	0.25
SE	0.14	0.05	0.15	0.08	0.18	0.36	0.09	0.09

PDA- Potato dextrose agar medium; SDA- Sabouraud's dextrose agar medium; Mean of three replications; In a column, mean followed by a common letter are not significantly different at (P=0.05) 5 % level by DMRT.

BbMdKKL2106- *B. bassiana* coccinellid , FpCmKKL 1526- *F. pallidoroseum* leaf folder, BbMtKKL 2107- *B. bassiana* spotted pod borer, VpNlKKL2121- *V. psalliotae* BPH, FpEvKKL 2119- *F. pallidoroseum* bhendi fruit borer VpPmKKL2120- *V.psalliotae* skipper, FmNvKKL 2124- *F. moniliforme* GLH

Table 2. Effect of culture media on the sporulation of entomopathogenic fungi under *in vitro* condition

Fungal isolate	Agar media Mean table X 10^8 spores / ml	
	PDA	SDA
BbMdKKL 2106	8.77 [a]	8.95 [a]
BbMtKKL 2107	8.90 [a]	6.21 [ab]
FmNvKKL 2124	3.33 [ab]	3.21 [ab]
FpCmKKL 1526	0.28 [b]	3.31 [ab]
FpEvKKL 2119	0.53 [b]	0.52 [b]
VpNlKKL 2121	3.08 [b]	3.31 [ab]
VpPmKKL 2120	5.33 [ab]	5.92 [ab]
CD	0.51	0.68
SE	0.17	0.23

Mean of three replications; In a column , means followed by a common letter are not significantly different at (P=0.05) 5% level by DMRT.

PDA- Potato dextrose agar, SDA- Sabouraud's dextrose agar, BbMdKKL2106- *B. bassiana* coccinellid FpCmKKL 1526- *F. pallidoroseum* leaf folder, BbMtKKL 2107- *B. bassiana* spotted pod borer, FpEvKKL 2119- *F. pallidoroseum* bhendi fruit borer, FmNvKKL 2124- *F. moniliforme* GLH, VpNlKKL2121-*V. psalliotae* BPH, VpPmKKL2120-*V.psalliotae* skipper

Table 3. Effect of culture media on the biomass production of entomopathogenic fungi under *in vitro* condition

Fungal isolate	PDB	SDB
	Dry Wt (g)	
BbMdKKL 2106	1.40[a]	1.80[ab]
BbMtKKL 2107	1.87[a]	1.81[ab]
FmNvKKL 2124	0.95[b]	1.36[b]
FpCmKKL 1526	1.66[a]	2.10[a]
FpEvKKL 2119	0.61[b]	1.50[ab]
VpNlKKL 2121	0.94[b]	1.39[b]
VpPmKKL 2120	0.80[b]	1.36[b]
CD	0.14	0.17
SE	0.05	0.06

Mean of three replications; In a column , mean followed by a common letter are not significantly different at (P=0.05) 5 % level by DMRT

PDB- Potato dextrose broth medium, SDB- Sabouraud's dextrose broth medium, BbMdKKL2106 - *B. bassiana* coccinellid FpCmKKL, 1526- *F. pallidoroseum* leaf folder, BbMtKKL 2107 - *B. bassiana* spotted pod borer, FpEvKKL 2119- *F. pallidoroseum* bhendi fruit borer, FmNvKKL 2124 - *F. moniliforme* GLH, VpNlKKL2121- *V. psalliotae* BPH , VpPmKKL2120- *V.psalliotae* skipper,

(1.80g) and FpEvKKL 2119 (1.50g). Whereas equally lower biomass was recorded in VpNlKKL 2121 (1.39g), VpPmKKL 2120 (1.36g) (Table 3). *B. bassiana* coccinellid, spotted pod borer isolates and *F. pallidoroseum* leaf folder isolate recorded maximum yield of biomass and lowest production was noticed in *V. psalliotae* isolates on PDB on dry weight basis. This was in consonance with Wadyalkar *et al.* (2003) that Emersons YPSS medium recorded highest spore count of 1.71 X 10^7 spores and biomass production of 4.80g / 100ml followed by PDB with 1.03 X 10^7 spores / ml and 4.50 g / 100ml biomass production.

Isolates of *B. bassiana* and *F. pallidoroseum* leaf folder isolate recorded highest mycelial weight and lowest weight was noticed in all other isolates with respect to PDB. With SDB, maximum mycelial weight was obtained in *Fusarium pallidoroseum* leaf folder isolate and have similar effect with *B. bassiana* isolates, *F. pallidoroseum* bhendi fruit borer isolate on dry weight basis (Table 3). Previous workers had reported that good growth and sporulation of *Beauveria* spp. with Sabouraud's liquid medium might be due to presence of peptone as a source of nitrogen (Sharma *et al.,* 2002). Manisegarane and Letchoumanane (1996) reported that Richard's medium showed the highest mycelial growth (2.43g) followed by potato dextrose liquid broth (2.20g) and found suitable for the multiplication of *F. pallidoroseum*. Sharma *et al.,* (2002) observed maximum production of *M. anisopliae* and *Beauveria* spp. on molasses yeast broth. However, Parthasarathy (1998) observed that maximum biomass production and infectivity of *Z. radicans* with Sabouraud's maltose agar (SMA) medium when supplemented with prawn exoskeleton powder.

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