ASSESSMENT OF SUITABLE GROWTH MEDIA FOR MAJOR ENTOMOPATHOGENIC FUNGI

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ABSTRACT

As the demand for sustainable agriculture grows, the use of non-toxic chemicals to manage insect pests is on the rise. Entompathogenic fungi (EPF) like *Beauveria* and *Metarhizium* have been extensively studied and their growth has been well optimized in commonly known growth media. However, for novel EPF species, the optimization of growth media is still limited. Fungi rely on essential nutrients for their development, and various culture media can be used to supplement these nutrients, providing carbohydrates, minerals and moisture to support their growth. In this study, we tested six different growth media to assess their efficacy for cultivating some well-known as well as potential EPFs such as *Beauvaria bassiana, Clonostachys rosea, Talaromyces muroii* and *Fusarium keratoplasticum*. The results revealed that the fungi showed robust growth on PDA and CDA, while CWM supported the least growth of the EPF species.

Keywords: *Beauveria bassiana, Clonostachys rosea, Talaromyces,* PDA, SDAY, SMAY

INTRODUCTION

The need for sustainable pest management is pressing due to the increasing rates of pesticide consumption, leading to soil, water, and environmental contamination, along with hazards to human health. As a potential biological control agent, entomopathogenic fungi have undergone extensive investigation (Freimoser *et al.,* 2003). These fungi have been naturally found in over 750 host species and have been developed into microbial insecticides in various countries (Shah and Pell, 2003). EPFs like *Beauveria bassiana, Metarhizium anisopliae, Nomurea rileyii, Lecanicillium lecanii,* and *Paecillomyces* have been extensively studied among EPF for their bioefficacy, leading to the development of numerous commercial products (de Faria and Wraight, 2007).

However, with recent advances in isolating and identifying novel entomopathogenic fungi, it is essential to find optimal artificial growth media for laboratory culturing. This step is crucial for facilitating mass culturing, enabling further use in pest management. Culture medium refers to a blend of various constituents in appropriate proportions, providing essential nutrients needed for fungal growth and multiplication. A wide range of media types

is utilized, and they influence fungal growth, texture, pigmentation, and sporulation, which depend on the composition of media, pH, temperature, and water constituents (Northolt and Bullerman, 1982; Kumara and Raval, 2010). Different culture media have varying effects on fungal growth.

The objective of this study was to assess the effect of various culture media on the growth and sporulation of entomopathogenic fungi. The research underscores the importance of optimizing growth media for both novel and well-known EPF species. By identifying suitable culture conditions, it becomes feasible to scale up the production of these fungi, providing a sustainable and environmentally friendly approach to managing insect pests in agriculture.

MATERIALS AND METHODS

The experiment was conducted at Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Using soil baiting technique, few entomopathogenic fungal were isolated. These cultures include *Beauveria bassiana, Clonostachys rosea, Talaromyces muroii and Fusarium keratoplasticum.*

Six different culture media, namely PDA- Potato dextrose agar, SDAY- Sabouraud's dextrose agar with yeast extract, SMAY- Sabouraud's maltose agar with yeast extract, BDA- Beetroot dextrose agar, CDA- Carrot dextrose agar, CWM- Tender coconut water medium utilized for this study (Table 1). All the media were supplemented with streptomycin sulphate at a concentration of 100mg/l, and a pH value of 5.5 was maintained (Arti and Kalpana, 2016). A fungal disc with a diameter of 6 mm was placed at the centre of 9 cm Petri plate and then incubated at a temperature of 25±5°C for a period of 15 days. The experiment was conducted in triplicates to ensure reliability and accuracy of the results.

Table 1: Composition of media (1 litre)

Media	Components	Quantity (g/ml)	Media*	Components	Quantity (g/ml)
PDA	Peeled potato	200 g	BDA	Beetroot	250 g
	Dextrose	20 g		Dextrose	20 g
	Agar	15 g		Agar	20 g
	Distilled water	1000 ml		Distilled water	1000 ml
SDAY	Dextrose	40 g	CDA	Carrot	250 g
	Peptone	10 g		Dextrose	20 g
	Yeast extract	5 g		Agar	20 g
	Agar	15 g		Distilled water	1000 ml
	Distilled water	1000 ml	CWM	Tender coconut water	500 ml
SMAY	Maltose	40 g		Agar	20 g
	Peptone	10 g		Distilled water	500 ml
	Yeast extract	5 g			
	Agar	15 g			
	Distilled water	1000 ml			

The radial growth of fungi in various media was measured and recorded in this study according to Afifah and Saputro (2020). Morphological characteristics and sporulation of fungi were observed for each type of media. Statistical analysis was performed using SPSS 29.0 software to analyze the obtained data.

RESULTS AND DISCUSSION

The growth of fungi in the experiment was notably affected by the various culture media utilized, as presented in Table 2. For isolate BNPR-Bb1, the PDA media exhibited the most significant growth, with a radial growth of 50mm and high sporulation. Similarly, for other isolates like CCPR-Bb2, the CDA media resulted in optimal fungal growth. For isolate IW-Bb1, the SDAY media promoted the highest growth of fungi.

Overall, the PDA media proved to be the most suitable growth medium, showing excellent results, followed closely by the CDA media. These findings indicate the importance of selecting appropriate culture media to achieve optimal growth and sporulation of the studied entomopathogenic fungi.

Table 2: Pattern of fungal growth on different media

Fungi	Culture media	Radial growth (mm)** (Mean ± SE)	Sporulation
Beauveria bassiana (BNPR-Bb1)	*PDA	50.00±2.52	High
	SDAY	25.67±0.33	Moderate
	SMAY	44.33±2.33	High
	BDA	53.00±3.06	Moderate
	CDA	26.33±1.45	Low
	CWM	48.00±1.53	Low
Beauveria bassiana (CCPR-Bb2)	PDA	36.00±0.58	Moderate
	SDAY	41.67±2.85	High
	SMAY	52.33±4.91	High
	BDA	57.00±1.15	High
	*CDA	60.67±0.67	High
	CWM	43.33±0.88	High
Beauveria bassiana (IW-Bb1)	PDA	37.67±1.76	High
	*SDAY	50.00±1.15	High
	SMAY	31.00±1.15	High
	BDA	40.67±1.20	High
	CDA	30.67±0.88	Moderate
	CWM	35.67±2.73	Moderate
Clonostachys rosea (SNPT-Cr2)	*PDA	75.67±0.33	High
	SDAY	58.67±1.20	High
	SMAY	59.33±0.67	High
	*BDA	75.67±1.20	High
	CDA	69.67±0.33	Moderate
	CWM	62.67±0.88	Moderate
Talaromyces muroii (SNPT-Tam1)	*PDA	76.00±1.15	High
	SDAY	51.67±2.60	Low

	SMAY	43.00±1.53	Low
	BDA	57.67±1.45	Moderate
	CDA	60.67±0.67	High
	CWM	64.33±0.33	High
Fusarium keratoplasticum (CCPR-Fk1)	PDA	80.67±0.33	High
	SDAY	80.67±0.67	High
	*SMAY	81.67±0.33	High
	BDA	61.00±1.15	Moderate
	CDA	73.00±1.15	Low
	CWM	62.00±1.53	Low

*Highest growth and sporulation of individual isolate of EPF
**Radial growth observed after 15 days (Mean of 3 replications)

The radial growth of the entomopathogenic fungi (EPF) isolates was found to be different across all six culture media. Likewise, distinct differences in colony morphology and sporulation were observed with different media. Specific details about the colony diameter of each EPF isolate can be found in Table 3.

Among the culture media tested, the lowest growth was observed in CWM, followed by SMAY (except for isolate CCPR-Fk1). This suggests that CWM and SMAY may not be the most favorable media for promoting the growth and development of the EPF isolates, at least in comparison to the other tested media.

Table 3: Colony diameter (mm) of EPF on different media upto 15 days

Fungi	**Media**	**Mean colony diameter (mm)***		
		3 DAI	**9 DAI**	**15 DAI**
Beauveria bassiana (BNPR-Bb1)	PDA	10.33	27.33	50.00
	SDAY	7.33	22.33	25.67
	SMAY	5.67	20.33	44.33
	BDA	14.00	34.67	53.00
	CDA	8.33	21.33	26.33
	CWM	12.33	31.67	48.00
Beauveria bassiana (CCPR-Bb2)	PDA	14.33	33.00	36.00
	SDAY	17.33	37.00	41.67
	SMAY	18.00	37.33	52.33
	BDA	16.00	33.33	57.00
	CDA	19.67	36.67	60.67
	CWM	21.00	40.33	43.33
Beauveria bassiana (IW-Bb1)	PDA	10.00	22.33	37.67
	SDAY	16.00	32.67	50.00
	SMAY	6.67	17.67	31.00
	BDA	9.67	22.33	40.67
	CDA	7.67	20.67	30.67

	CWM	10.33	22.33	35.67
Clonostachys rosea (SNPT-Cr2)	PDA	27.33	46.33	75.67
	SDAY	15.33	34.67	58.67
	SMAY	16.33	34.67	59.33
	BDA	27	46.00	75.67
	CDA	30.67	51.00	69.67
	CWM	33.67	53.33	62.67
Talaromyces muroii (SNPT-Tam1)	PDA	25.67	45.67	76.00
	SDAY	16.33	26.00	51.67
	SMAY	10.00	18.67	43.00
	BDA	14.67	24.67	57.67
	CDA	24.33	42.00	60.67
	CWM	30.67	47.33	64.33
Fusarium keratoplasticum (CCPR-Fk1)	PDA	40.33	60.00	80.67
	SDAY	44.33	71.33	80.67
	SMAY	46.33	72.67	81.67
	BDA	20.00	39.00	61.00
	CDA	37.00	53.33	73.00
	CWM	27.67	50.00	62.00

*Mean of 3 replications; DAI-Days after inoculation

In a study conducted by Senthamizhlselvan et al. (2010), they investigated the growth of *Beauveria bassiana* using two culture media, PDA and SDA. And, found that maximum mycelia growth occurred on both of the culture media. This result aligns with the findings of Afifah and Saputro (2020), who also observed that PDA was the most suitable medium for the growth of various Beauveria bassiana strains, with an average colony diameter of 90 mm.

Also, the outcomes from other earlier research conducted by Xu et al. (1984); Maheshwari et al. (1999); Saha et al. (2008); and Arti and Kalpana (2016), were in accordance with the present study, where PDA consistently yielded the best results compared to other media.

CONCLUSION

Various factors indeed play a crucial role in influencing the growth of entomopathogenic fungi, including temperature, nutrients, pH, relative humidity, and other environmental conditions. The study mentioned also highlighted the significant impact of different media on the growth and sporulation of fungal isolates. Among the media tested, PDA (Potato Dextrose Agar) was found to be the most suitable for the growth of most fungal isolates, followed by CDA (Carrot Dextrose Agar). However, *Fusarium keratoplasticum* showed the best growth on SMAY media.

The results underscore the importance of developing fungus-specific artificial media for mass culturing and field use studies of novel entomopathogenic fungi. Each fungus appears to have specific preferences for growth conditions, and identifying the optimal medium is crucial for their successful cultivation. These findings also show the importance of

carefully selecting the appropriate culture media to support the growth and sporulation of entomopathogenic fungi for effective pest management strategies. In conclusion, further research is necessary to better understand the requirements of each entomopathogenic fungus and to formulate fungi specific culture media to achieve optimal growth and ultimately enhance their potential use as biocontrol agents in pest formulating the management strategies in sustainable agriculture.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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