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Effect of different culture media on radial growth of potential fungal endophytes isolated from clusterbean

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Abstract

Culture medium plays a crucial role in determining the growth and development of fungal endophytes under *in vitro* conditions. The present investigation was conducted to evaluate the influence of different culture media on the radial growth of seven potential fungal endophytes associated with cluster bean. The selected isolates were *Fusarium falciforme* JR-2, *Fusarium solani* BKNL-1, *Colletotrichum gloeosporioides* BMRL-3, *Trichoderma harzianum* JR-6, *Trichoderma asperellum* NR-1, *Alternaria alternata* PL-6 and *Curvularia plantarum* JS-1. Six culture media, namely Clusterbean Dextrose Agar (M1), Rose Bengal Agar (M2), Corn Meal Agar (M3), Oat Meal Agar (M4), V8-Juice Agar (M5) and Potato Dextrose Agar (M6), were evaluated, with Potato Dextrose Agar serving as the control. Radial mycelial growth was used as the principal criterion for assessing the response of each fungal isolate to the different nutritional environments. The study was designed to identify suitable culture media capable of supporting vigorous and consistent growth of the selected endophytes while accounting for isolate-specific nutritional requirements. Comparative evaluation of isolate × medium interactions provides useful information for optimizing the cultivation and maintenance of cluster bean-associated fungal endophytes and may facilitate their subsequent characterization and evaluation for potential beneficial applications.

Keywords: Cluster bean, fungal endophytes, culture media, radial growth, mycelial growth, *in vitro* evaluation, endophytic fungi

1. Introduction

Culture medium is an important factor governing the growth, development and recovery of fungi under *in vitro* conditions. The nutritional composition of a medium, particularly the availability and balance of carbon, nitrogen, minerals and other growth-supporting constituents, can considerably influence fungal mycelial development. This becomes particularly important when working with endophytic fungi because different taxa, and even different isolates belonging to the same taxon, may exhibit distinct nutritional requirements and growth rates. Consequently, the choice of culture medium can influence both the apparent growth potential of an isolate and its successful maintenance under laboratory conditions. Fungal endophytes associated with cluster bean represent a potentially useful group of microorganisms because some members can exhibit antagonistic, plant-growth-promoting and other beneficial characteristics. For their characterization and subsequent evaluation, it is therefore necessary to identify a suitable medium that supports vigorous and consistent mycelial growth. At the same time, dependence on a single culture medium may favour rapidly growing fungi while restricting comparatively slow-growing taxa. Evaluation of multiple media can therefore provide a more reliable understanding of the growth behaviour of selected endophytic isolates. In the present investigation, seven potential fungal endophytes, namely *Fusarium falciforme* JR-2, *Fusarium solani* BKNL-1, *Colletotrichum gloeosporioides* BMRL-3, *Trichoderma harzianum* JR-6, *Trichoderma asperellum* NR-1, *Alternaria alternata* PL-6 and *Curvularia plantarum* JS-1, were evaluated on six culture media. The media included Clusterbean Dextrose Agar (M1), Rose Bengal Agar (M2), Corn Meal Agar (M3), Oat Meal Agar (M4), V8-Juice Agar (M5) and Potato Dextrose Agar (M6), with PDA serving as the control. The objective was to determine the relative suitability of these media for radial growth of the selected fungal endophytes and to identify isolate × medium combinations supporting superior growth.

2. Materials and Methods

2.1 Experimental material

Seven potential fungal endophytes selected from the cluster bean endophyte collection were used for the study. The endophytes are tabulated below:

Table 1: List of endophytes along with Isolate code

S.No.	Name of the Isolate	Isolate Code
1.	<i>Fusarium falciforme</i>	JR-2
2.	<i>Fusarium solani</i>	BKNL-1
3.	<i>Colletotrichum gloeosporioides</i>	BMRL-3
4.	<i>Trichoderma harzianum</i>	JR-6
5.	<i>Trichoderma asperellum</i>	NR-1
6.	<i>Alternaria alternata</i>	PL-6
7.	<i>Curvularia plantarum</i>	JS-1

2.2 Culture media

Six different culture media, viz., Clusterbean Dextrose Agar (CDA), Rose Bengal Agar (RBA), Corn Meal Agar (CMA), Oat Meal Agar (OMA), V8-juice Agar and Potato Dextrose Agar (PDA), were evaluated for their suitability for the growth of selected fungal endophytes isolated from cluster bean. Potato Dextrose Agar was maintained as the control medium.

The required media were prepared according to their standard composition and the pH was adjusted to the recommended level before sterilization. The prepared media were dispensed into suitable containers and sterilized by autoclaving at 121°C and 15 psi pressure for 15–20 min. After sterilization, the media were allowed to cool to approximately 45–50°C and poured aseptically into sterile Petri plates. The plates were allowed to solidify under aseptic conditions.

Seven selected fungal endophytes mentioned in the table 1 were selected for the radial growth experiment on different medium. A 5-mm diameter agar disc was cut from the actively growing margin of each seven-day-old fungal culture using a sterile cork borer and transferred aseptically to the centre of each medium. The inoculated plates were incubated at 25 ± 2°C under laboratory conditions. Each treatment was maintained with three replications. Appropriate uninoculated plates were also maintained to check for contamination. The radial growth of each endophyte was recorded at regular intervals by measuring the colony diameter. Measurements were continued until the colony reached the edge of the Petri plate or until the predetermined observation period was completed. The radial growth was expressed in millimetres (mm).

2.3 Experimental design and statistical analysis

The experiment was analysed as a two-factor Completely Randomized Design (CRD), with fungal endophytes as Factor A and culture media as Factor B. The seven endophytes were

evaluated on six culture media. The analysis included the main effects of endophyte, medium and their interaction (A × B). The experiment has been conducted with three replicates.

3. Results

3.1 Radial growth of fungal endophytes

The radial growth of the seven fungal endophytes varied considerably across the six culture media. The overall mean colony diameter of the isolates ranged from 53.278 to 78.500 mm. Among the seven endophytes, *T. harzianum* JR-6 recorded the highest mean colony diameter (78.50 mm), followed by *F. falciforme* JR-2 (76.83 mm) and *C. plantarum* JS-1 (76.61 mm). *T. asperellum* NR-1 and *C. gloeosporioides* BMRL-3 recorded mean colony diameters of 75.50 and 75.06 mm, respectively. *F. solani* BKNL-1 recorded 61.89 mm, while *A. alternata* PL-6 exhibited the lowest mean colony diameter of 53.28 mm.

Thus, *T. harzianum* JR-6 exhibited the greatest overall radial growth among the evaluated endophytes, whereas *A. alternata* PL-6 showed comparatively slower growth.

3.2 Effect of culture media

Significant differences were observed among the culture media. PDA supported the highest overall radial growth, with a mean colony diameter of 86.10 mm. It was followed by Clusterbean Dextrose Agar (77.86 mm) and Corn Meal Agar (71.48 mm). Oat Meal Agar recorded 68.71 mm, whereas Rose Bengal Agar and V8-Juice Agar recorded comparatively lower mean colony diameters of 62.29 and 60.14 mm, respectively.

Therefore, PDA was the most favourable medium for overall radial growth, whereas V8-Juice Agar was the least favourable among the media tested.

3.3 Interaction between endophytes and culture media

A strong interaction was observed between fungal endophytes and culture media. The A × B interaction was highly significant, with an F-value of 149.836. This indicates that the response of the fungal isolates was not uniform across the media.

The maximum colony diameter of 90.00 mm was recorded by *C. gloeosporioides* BMRL-3, *T. harzianum* JR-6 and *T. asperellum* NR-1 on PDA. *C. plantarum* JS-1 and *F. falciforme* JR-2 recorded 89.33 and 89.00 mm, respectively, on PDA. In contrast, *A. alternata* PL-6 showed the minimum colony diameter of 20.67 mm on V8-Juice Agar, followed by 33.67 mm on Rose Bengal Agar. The results demonstrate that both the identity of the fungal endophyte and the culture medium substantially affected colony development, while the significant interaction confirms that the suitability of a medium depended on the particular fungal isolate (Table 1, Fig.1). The effect of different culture media on radial growth of potential endophytes isolated from cluster bean has been represented in the Figure 2.

Table 2: Effect of Different Culture Media on Radial Growth of Selected Fungal Endophytes Isolated from Cluster Bean

Treatments	Cluster bean dextrose agar	Rose Bengal agar	Corn meal agar	Oat meal agar	V8 juice agar	Potato dextrose agar (control)	Mean A
T ₁ (JR-2)	83.33	70.00	72.00	85.67	61.00	89.00	76.83
T ₂ (BKNL-1)	65.00	66.33	68.00	42.67	56.00	73.33	61.89
T ₃ (BMRL-3)	73.67	63.00	56.67	82.67	84.33	90.00	75.06
T ₄ (JR-6)	86.33	76.67	77.33	53.67	87.00	90.00	78.50
T ₅ (NR-1)	81.00	63.00	77.00	90.00	52.00	90.00	75.50
T ₆ (PL-6)	69.67	33.67	61.00	53.67	20.67	81.00	53.28
T ₇ (JS-1)	86.00	63.33	88.33	72.67	60.00	89.33	76.61
Mean B	77.86	62.29	71.48	68.71	60.14	86.10	
Factors	C.D.(p=0.05)			SE(m)±			
Factor(A) (Endophytes)	1.11			0.39			
Factor(B) (culture media)	1.03			0.36			
Factor (A X B)	2.72			0.96			

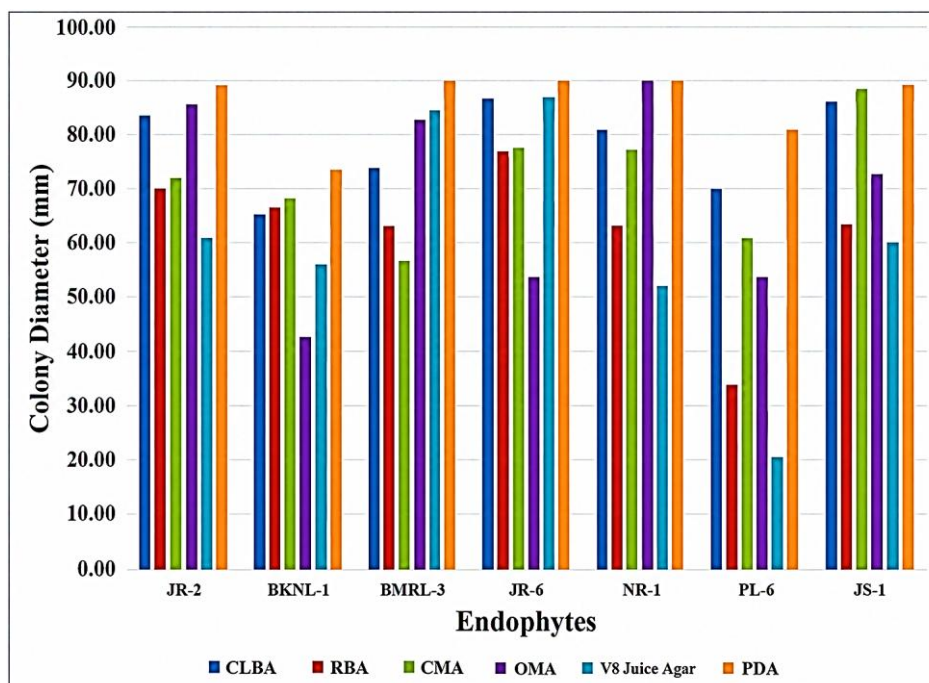


Fig 1: Growth of potential endophytes on different culture media

Fungal endophytes	PDA	Clusterbean dextrose Agar	Oat meal Agar	Corn meal Agar	V8 juice Agar	Rose Bengal Agar
T ₁ : <i>Fusarium falciforme</i>						
T ₂ : <i>Fusarium solani</i>						
T ₃ : <i>Colletotrichum gloeosporioes</i>						
T ₄ : <i>Trichoderma harzianum</i>						
T ₅ : <i>Trichoderma asperellum</i>						
T ₆ : <i>Alternaria alternata</i>						
T ₇ : <i>Curvularia plantarum</i>						

Fig 2: Graphical Representation of Different Culture Media Effect on the Growth of Endophytes

4. Discussion

The present investigation demonstrated considerable variation in the radial growth of the seven selected fungal endophytes when cultured on different media. The significant effects of fungal endophyte, culture medium and their interaction indicate that mycelial development was influenced by both isolate-specific characteristics and the nutritional environment provided by the culture medium. Such variability is expected in culture-dependent studies because fungal taxa differ in their ability to utilize particular carbon and nitrogen sources and respond differently to the physical and nutritional properties of culture media (Dos Reis *et al.*, 2022) ^[1].

Among the evaluated isolates, *T. harzianum* JR-6 recorded the highest overall mean radial growth of 78.50 mm. The high growth rate of this isolate may be associated with its vigorous vegetative development and efficient utilization of nutrients available in the media. *Trichoderma* species are generally characterized by rapid mycelial development and have considerable importance in agricultural biological control systems (Khan *et al.*, 2022) ^[3].

The relatively high growth of *F. falciforme* JR-2, *C. plantarum* JS-1, *T. asperellum* NR-1 and *C. gloeosporioides* BMRL-3 further indicates that these isolates were capable of effectively utilizing the nutrients supplied by the tested media. In contrast, *A. alternata* PL-6 exhibited the lowest overall mean colony diameter (53.28 mm), indicating comparatively slower growth under the conditions evaluated.

Among the media, PDA produced the highest overall mean colony diameter (86.10 mm). The superior performance of PDA can be attributed to its readily available nutrient and carbohydrate composition, which supports vegetative development of a broad range of fungi. PDA is also widely used for the isolation, maintenance and cultivation of fungi, including endophytic fungi (Dos Reis *et al.*, 2022) ^[1].

Clusterbean Dextrose Agar ranked second, with an overall mean colony diameter of 77.86 mm. This finding is particularly relevant to the present investigation because the medium was developed/evaluated in relation to cluster bean-associated fungi and supported relatively vigorous growth of the selected isolates. Nevertheless, its performance should be interpreted specifically within the conditions of the present experiment because nutritional requirements can vary among fungal species and isolates.

Corn Meal Agar and Oat Meal Agar supported intermediate levels of radial growth, whereas Rose Bengal Agar and V8-Juice Agar recorded lower overall growth. The relatively reduced growth on these media may be related to differences in nutrient availability and the ability of individual isolates to utilize the available substrates. The selective influence of culture media is an important consideration in culture-based endophyte studies because unsuitable media may favour particular taxa while limiting the development of others (Dos Reis *et al.*, 2022) ^[1].

The highly significant A × B interaction provides an important interpretation of the experiment. It demonstrates that there was no single nutritional response common to all seven endophytes. For example, *C. gloeosporioides* BMRL-3, *T. harzianum* JR-6 and *T. asperellum* NR-1 attained the maximum recorded diameter of 90.00 mm on PDA, whereas *A. alternata* PL-6 recorded only 20.67 mm on V8-Juice Agar.

This isolate-specific response has practical importance. Selection of an appropriate culture medium is necessary not only for obtaining rapid growth but also for maintaining and multiplying promising fungal endophytes. The strong growth of *T. harzianum* JR-6 is particularly noteworthy because this isolate also showed substantial antagonistic activity against *Macrophomina*

phaseolina in the broader investigation. Rapid mycelial colonization can be advantageous for a prospective biocontrol organism because it may improve substrate occupation and competition with a pathogen. *Trichoderma* spp. can suppress plant pathogens through several mechanisms, including competition, antibiosis, hydrolytic enzyme production and stimulation of plant defence responses (Khan *et al.*, 2022; Tiwari & Bae, 2022) ^[3, 4].

Similarly, *T. asperellum* NR-1 showed high radial growth, with an overall mean of 75.50 mm, and reached 90.00 mm on PDA. Its combination of vigorous growth and antagonistic potential makes it a suitable candidate for further evaluation. However, radial growth should not be considered independently when selecting a biocontrol agent. Antagonistic activity, enzyme production, secondary metabolites, ecological competence and plant-growth-promoting effects should also be considered.

Overall, the present findings demonstrate that PDA was the most suitable medium for supporting radial growth of the selected fungal endophytes under the experimental conditions, while V8-Juice Agar was comparatively less suitable. The significant interaction between fungal isolate and medium emphasizes that medium selection should be based on the individual isolate rather than assuming that one medium is equally suitable for all endophytes.

5. Conclusion

The evaluation of six culture media demonstrated significant variation in radial growth among the seven selected fungal endophytes. PDA recorded the highest overall mean colony diameter (86.10 mm) and was therefore the most suitable general-purpose medium among those evaluated. Clusterbean Dextrose Agar was the second-best medium (77.86 mm), indicating its potential for supporting growth of cluster bean-associated endophytes.

Among the isolates, *T. harzianum* JR-6 recorded the highest overall mean growth (78.50 mm), whereas *A. alternata* PL-6 showed the lowest (53.28 mm). The highly significant endophyte × medium interaction confirms that the growth response was isolate-dependent. *T. harzianum* JR-6 and *T. asperellum* NR-1 were particularly promising because of their vigorous growth and their broader potential as biological control candidates against *M. phaseolina*.

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