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Screening of Different Media and Substrates for Cultural Variability and Mass Culture of *Arthrobotrys dactyloides* Drechsler

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Variability in growth and sporulation of five isolates of *Arthrobotrys dactyloides* was studied on five agar, 6 bran and 5 grain media. Potato dextrose agar (PDA) supported maximum growth of isolate A, C and E, while growth of isolate B and D was significantly lower on this medium. On Czapek's agar and yeast glucose agar media the differentiation in the isolates in relation to growth was poor than PDA. The other two media showed much poorer differentiation. On Czapek's agar medium, sporulation was recorded in isolate B only, whereas other isolates showed rare sporulation. Among the bran media, pea bran agar medium supported maximum growth of all the isolates except isolate B. Gram and rice bran agar media were next best. However, the growth of isolate B on the gram bran agar medium was more or less equal as other isolates. On pigeon pea bran agar medium, isolate E failed to grow while other isolates recorded poor growth. On lentil bran agar medium, only isolate B and D recorded little growth, whereas other isolates failed to grow. All the isolates recorded good sporulation on bran agar media except pigeon pea and lentil bran agar media. The grain agar media supported moderate to very good growth of all the isolates. In general isolate B remained slow growing on these media except gram grain and sorghum grain agar media on which growth of this isolate was comparable to other isolates. Sporulation in general, was good on all the grain agar media. Among different substrates screened, barley grain and pea bran were found superior to others for mass culture of isolate A of *A. dactyloides*.

KEYWORDS: ***Arthrobotrys dactyloides*, Constricting ring, Nematode-trapping fungus, Biocontrol**

Arthrobotrys dactyloides is a well known nematode-trapping fungus with high biocontrol potential against root knot nematodes. It bears two celled elongated conidia in open capitate arrangement on its respective conidiophores. This fungus produces three celled constricting rings on its growing hyphae for capturing and killing of nematodes. In addition to constricting rings on hyphae, the spores of *A. dactyloides* frequently form conidial traps in response to soil fungistasis (Mankau, 1962; Cook, 1964; Barron, 1977, 1981; Persmark and Nordbring-Hertz, 1997). These conidial traps also capture and kill the live nematodes.

Since the observation of Fresenius (1852) on first nematode-trapping fungus *A. oligospora* and establishment of its biological relationship with nematodes by Zoph (1888), substantial work on predacious behaviour of nematode trapping fungi has been carried out by several workers (Drechsler, 1937; Cooke, 1963; Cooke and Pramer, 1968; Monoson, 1968; Belder and Jansen, 1994; De Gives *et al.*, 1994; Heintz, 1978). Galper *et al.* (1995) reported that the constricting ring-forming fungus *A. dactyloides* was more effective in reducing the population of second stage juveniles of *Meloidogyne javanica* in soil than the fungi forming adhesive networks. Stirling *et al.* (1998) and Stirling and Smith (1998) reported that formulation of *A. dactyloides* successfully reduced the severity of root knot disease of tomato in pot and field experiments. In this continuation, recently Kumar and Singh (2005) reported that application of *A. dactyloides* at the rate of 4×10^6 colony forming unit (CFU) per kg of soil successfully reduced the number of root knot up to 66% in tomato.

Despite its high predacity, *in vitro*, (Kumar and Singh, 2005) and biocontrol potential, the large scale application of this fungus in problem soil is only possible if the fungus can be grown with heavy sporulation on the easily available substrates. In view of these facts, the aim of present investigation was to screen several media/substrates for growth and sporulation of *A. dactyloides* to find out the best substrates for its mass culture. The other objective of this work was to select some media/substrates for differentiation of isolates collected from different locations. The observations of the same are described in this paper.

Materials and Methods

Isolation, purification and maintenance of cultures. Five isolates of *A. dactyloides* were isolated during October 1998 to March 1999 from different locations: Vegetable Research Farm of the Banaras Hindu University, Varanasi (Isolate-A), Agricultural field soil from Ghazipur (Isolate-B), Chunar (Isolate-C), Mirzapur (Isolate-D) in Uttar Pradesh state and Ranchi (Isolate-E) in Jharkhand state, India by the method described by Duddington (1955) with

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slight modification (Bandyopadhyay and Singh, 2000). Pure cultures of all the isolates of *A. dactyloides* were made by picking spores from the conidial heads of individual isolate by a fine needle and inoculating the same on the maize meal agar medium (maize-20 g, agar-20 g, distilled water-1000 *ml*). Further single spore cultures of all the isolates were made by the method given by Singh *et al.* (2004) and cultures were maintained on maize meal agar medium at 28 ± 1°C.

Radial growth and sporulation of *A. dactyloides* on different media. The radial growth and sporulation of all the isolates of *A. dactyloides* were studied on some general media: Potato dextrose agar (PDA; peeled potato - 250 g, dextrose - 20 g, agar - 20 g, distilled water-1000 *ml*); Yeast glucose agar (YGA; yeast extract - 4.0 g, glucose - 20 g, K_2HPO_4 - 20 g, Mg $SO_4 \cdot 7H_2O$ - 0.5 g, agar - 20 g, distilled water-1000 *ml*); Czapek's agar (CA; $NaNO_3$ - 3.0 g, K_2HPO_4 - 1.0 g, $MgSO_4 \cdot 7H_2O$ - 0.5 g, KCL - 0.5 g, $FeSO_4 \cdot 7H_2O$ - 0.01 g, sucrose - 30 g, agar - 20 g, distilled water - 1000 *ml*); Emerson YpSs agar (Y_pS_sA; yeast extract - 4.0 g, soluble starch - 15 g, $MgSO_4$ - 0.5 g, agar - 20 g, distilled water - 1000 *ml*); Sabouraud's dextrose agar media (SDA; neopeptone - 10 g, dextrose - 40 g, agar - 20 g, distilled water - 1000 *ml*). These media were prepared, sterilized and 20 *ml* of each medium was poured into each of Petri dishes. Four Petri dishes were taken as replicates for each treatment. A 5 mm fungal disc was taken from the periphery of 15 day old culture of *A. dactyloides* and inoculated into each Petri dish. The inoculated Petri dishes were incubated at 29 ± 1°C. Radial growth of the fungus was measured at the interval of three days up to 21 days of inoculation. For observation on sporulation of each isolate in each medium, each Petri dish was flooded with 5 *ml* sterile water, agitated well for separation of spores and collected separately. The spores were counted using heamocytometer and the number of spores per ml of water was calculated. Isolate B which produced smaller conidia along with two celled elongated conidia were counted separately and these longer and smaller conidia were designated as macro and microconidia. Necessary photographs were taken and data were statistically analyzed.

Radial growth and sporulation of *A. dactyloides* on different bran and grain agar media. To study the growth and sporulation of *A. dactyloides* on some bran and grain agar media, brans of pea (*Pisum sativum*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), gram (*Cicer arietinum*), lentil (*Lens esculentum*) and pigeon pea (*Cajanas cajan*) (each bran powder - 20 g, agar - 20 g, distilled water - 1000 *ml*) and splitted grains of maize (*Zea mays*), wheat, sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*) and gram (each grain - 20 g, agar - 20 g, distilled water - 1000 *ml*) were used. The media were prepared and poured in to Petri dishes. The method of inoculation and observation of radial growth and sporulation of each isolate in each bran and grain agar medium were similar as described earlier.

Mass culture of *A. dactyloides* on different substrates. Different substrates viz; brans of pea, wheat, rice, pigeon pea, gram and lentil, grains of wheat, barley, maize, and sorghum and straw of wheat and rice were tested for the growth of *A. dactyloides* in mass culture. Brans and straw were powdered and grains were splitted in warring blender before addition of desired amount of water. Substrates and water were taken as follows: pea bran 10 g + 50 *ml* water, wheat bran 10 g + 50 *ml* water, rice bran 10 g + 30 *ml* water, pigeon pea bran 10 g + 50 *ml* water, gram bran 10 g + 50 *ml* water, lentil bran 10 g + 50 *ml* water, wheat straw 5 g + 40 *ml* water, rice straw 5 g + 40 *ml* water, wheat grain 20 g + 35 *ml* water, barley grain 20 g + 35 *ml* water, maize grain 20 g + 35 *ml* water, sorghum grain 20 g + 35 *ml* water. Each substrate was taken into a 150 *ml* conical flask and moistened with desired amount of water as mentioned above. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. A 10 mm fungal disc was cut from the periphery of the 15 day old culture of isolate A by a sterilized cork borer and inoculated in the centre of a substrate contained in a flask with the help of sterilized inoculation needle. One fungal disc was inoculated into each flask. Four replications were maintained for each treatment. The inoculated flasks were incubated at 25 ± 1°C. Visual ratings were made to assess the growth of *A. dactyloides* after 25 days of inoculation.

Results

Radial growth and sporulation of *A. dactyloides* on different media. The radial growth and sporulation of five isolates of *A. dactyloides* on used media are presented in Fig. 1 and Table 1. Of the five agar media tested, PDA supported better growth of three isolates (A, C and E) than other media. The growth of isolates D and B remained significantly lower on this medium. On SDA also the radial growth of isolate B was significantly lower than other isolates. On CA, lowest radial growth was recorded for isolate D followed by isolate B. Isolates did not differ significantly in growth on Emerson Y_pS_sA and YGA medium. PDA and CA media appeared to be useful for studying variability in isolates of *A. dactyloides*.

Of the five media used, Emerson Y_pS_sA and SDA supported rare sporulation of all the isolates. CA supported excellent sporulation of isolate B only while other isolates (A, C, D and E) recorded rare sporulation on this medium. PDA and YGA favoured sporulation of all the isolates, however, the degree of sporulation varied (Table

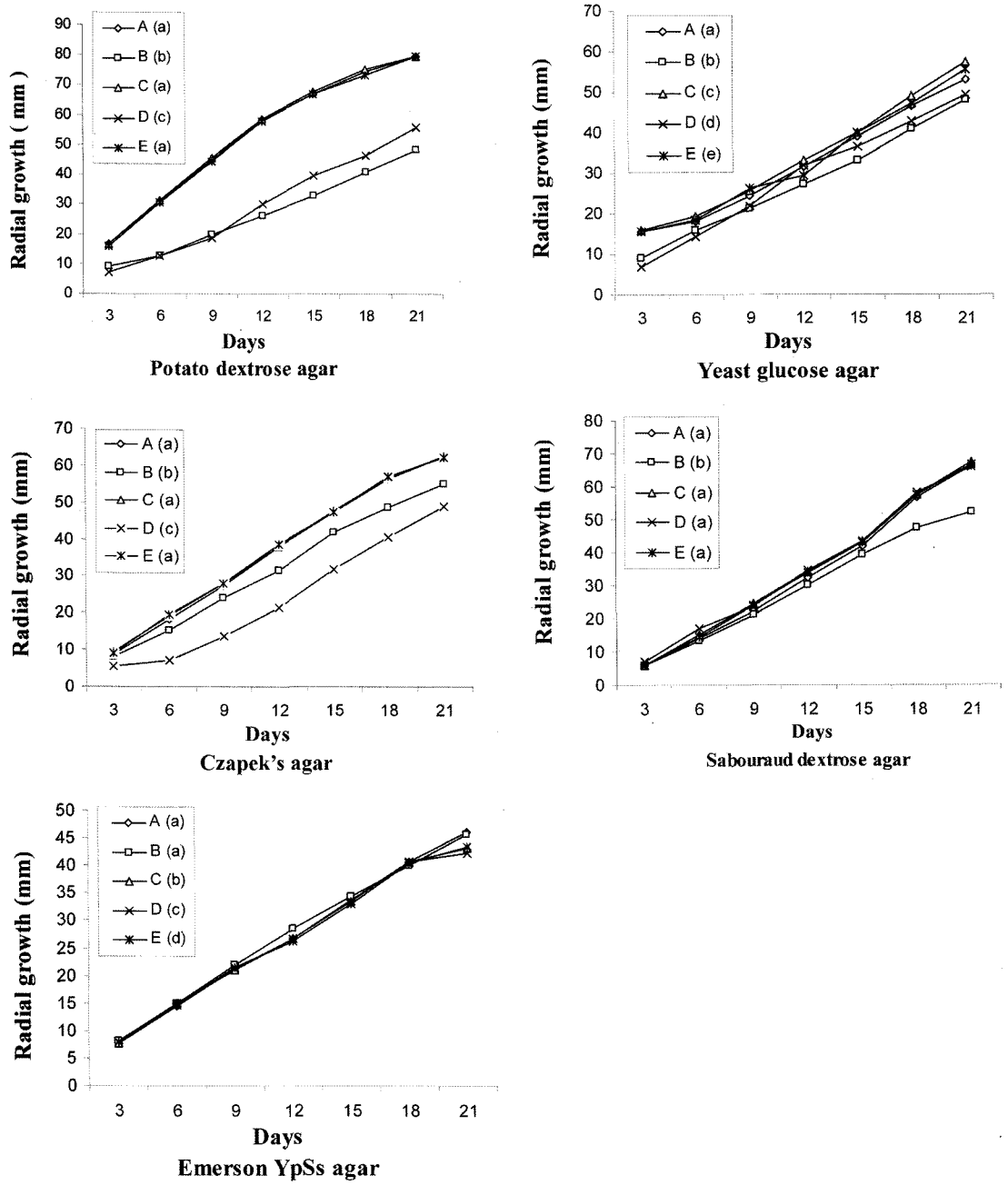

Fig. 1. Effect of different agar media on growth of the five isolates of *Arthrobotrys dactyloides*. Isolates with different letters in parenthesis show significant difference in radial growth on 21st day of observation by CRD test at p = 0.01.

Table 1. Effect of different media on sporulation of the five isolates of *Arthrobotrys dactyloides*

Isolates		Spore/*ml* in different media				
		Potato dextrose agar	Yeast glucose agar	Czapek's agar	Sabouraud dextrose agar	Emerson Y_PS_S agar
A		0.81×10^4 a (A)	0.53×10^4 a (A)	Rare	Rare	Rare
B	Macroconidia	1.1×10^4 a (A)	0.72×10^4 a (A)	2×10^4 b (A)	Rare	Rare
	Microconidia	3.2×10^4 a (B)	1.8×10^4 b (B)	6.5×10^4 c (B)	Rare	Rare
C		1.2×10^4 a (A)	0.63×10^4 b (A)	Rare	Rare	Rare
D		0.61×10^4 a (A)	0.59×10^4 a (A)	Rare	Rare	Rare
E		1.2×10^4 a (A)	0.68×10^4 b (A)	Rare	Rare	Rare

Data with different small letters show significant difference of row data by CRD test at P = 0.01.
Data with different capital letters in parenthesis show significant difference of column data by CRD test at P = 0.01.

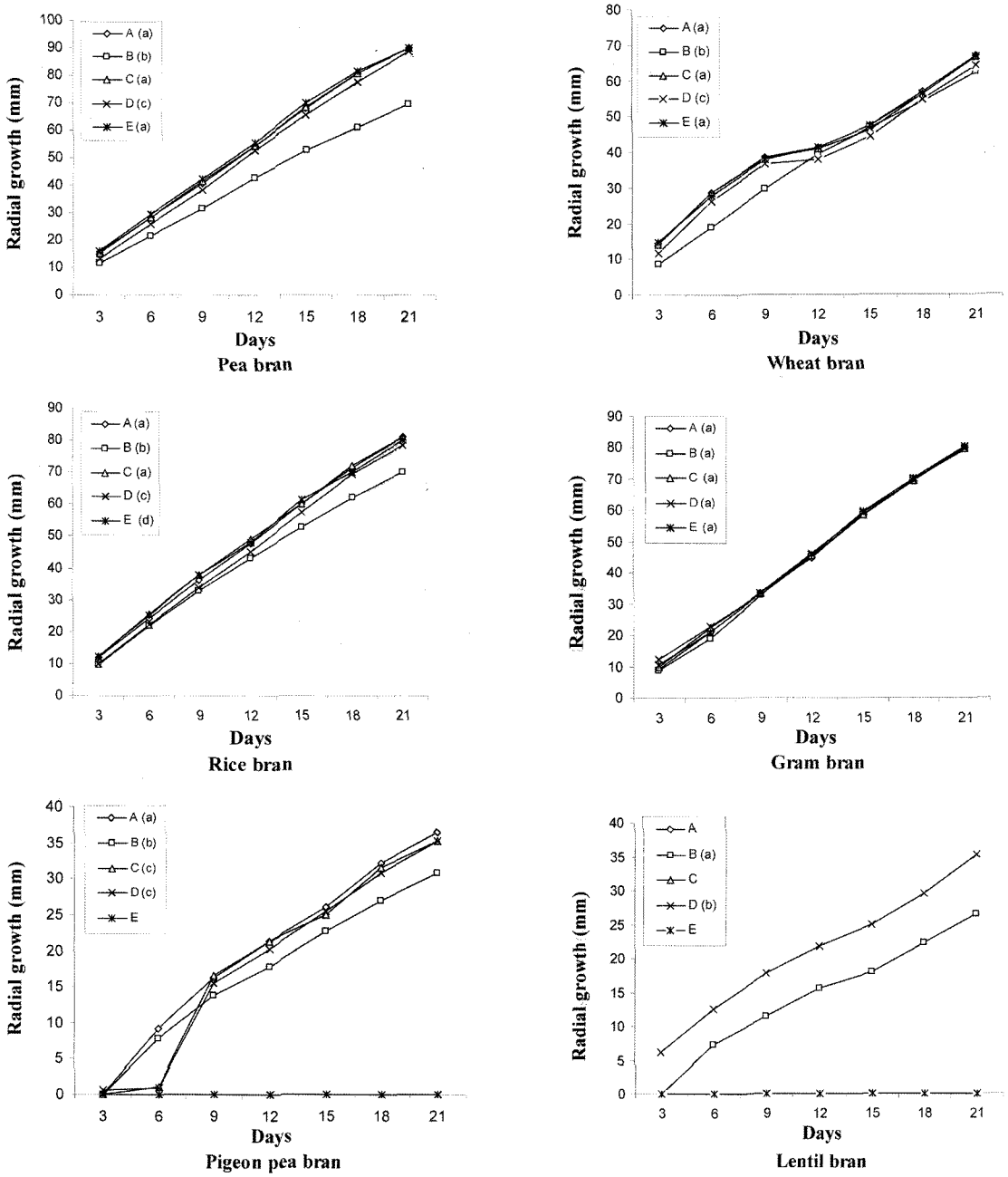

Fig. 2. Effect of different bran agar media on growth of five isolates of *Arthrobotrys dactyloides*. Isolates with different letters in parenthesis show significant difference in radial growth on 21st day of observation by CRD test at p = 0.01.

Table 2. Effect of different bran agar media on sporulation of the five isolates of *Arthrobotrys dactyloides*

Isolates		Spore/*ml* in different bran agar media					
		Pea bran	Wheat bran	Rice bran	Gram bran	Pigeon pea bran	Lentil bran
A		3.1×10^4 a (A)	12×10^4 b (A)	15×10^4 c (A)	8×10^4 d (A)	0.73×10^4 e (A)	0
B	Macroconidia	4.8×10^4 a (A)	7.6×10^4 b (B)	7.5×10^4 b (B)	20×10^4 c (B)	0.81×10^4 d (A)	0.9×10^4 d (A)
	Microconidia	9.3×10^4 a (B)	11×10^4 b (A)	75×10^4 c (C)	15×10^4 d (C)	1.3×10^4 e (A)	1.5×10^4 e (A)
C		4.1×10^4 a (A)	20×10^4 b (C)	22×10^4 b (D)	8.1×10^4 c (A)	0.8×10^4 d (A)	0
D		3.8×10^4 a (A)	1.6×10^4 a (D)	28×10^4 b (E)	60×10^4 c (D)	0.92×10^4 d (A)	0.92×10^4 d (A)
E		5.7×10^4 a (C)	18×10^4 b (E)	14×10^4 c (A)	4×10^4 d (E)	0	0

Data with different small letters show significant difference of row data by CRD test at P = 0.01.
Data with different capital letters in parenthesis show significant difference of column data by CRD test at P = 0.01.

1). Of all the isolates, isolate B recorded maximum sporulation in CA followed by PDA and YGA. The number of microconidia was three times more of the macroconidia in CA. In this medium, isolate B also produced spontaneous rings on the aerial mycelia which were not observed in other isolates (Fig. 4d).

Radial growth and sporulation of *A. dactyloides* on different bran agar media. Among bran agar media, irrespective of the isolates, maximum growth of *A. dactyloides* was supported by pea bran followed by rice and gram bran agar media. Pigeon pea bran agar medium supported very poor growth and sporulation of some isolates (A, B, C and D) however, growth of isolate E on this medium did not occur at all (Fig. 2). Lentil bran agar medium supported poor growth and sporulation of only isolate B and D while the other isolates (A, C and E) failed to grow.

On pea bran agar medium the isolates could be divided into two categories: fast growing (isolate A, C, D and E)

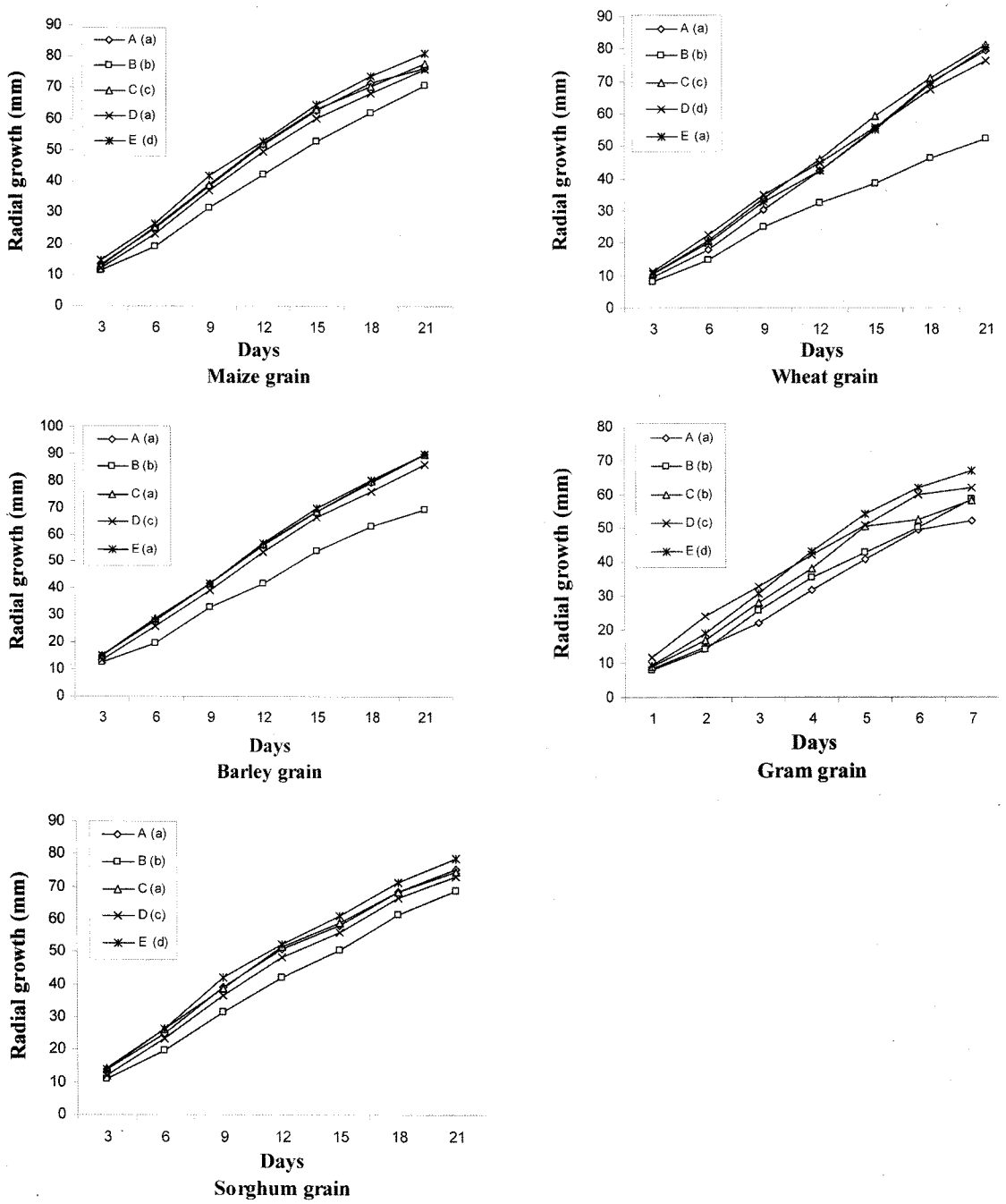

Fig. 3. Effect of different grain agar media on growth of the five isolates of *Arthrobotrys dactyloides*. Isolates with different letters in parenthesis show significant difference in radial growth on 21st day of observation by CRD test at $p = 0.01$.

and slow growing (isolate B). On rice bran agar medium, radial growth of isolate B was similar to pea bran agar. However, growth of other fast growing isolates on rice bran agar was significantly lower than on pea bran agar medium. It was interesting to note that number of microconidia was 10 times more of the macroconidia in isolate B in rice bran agar medium (Table 2). On gram bran agar medium, growth of all the isolates was similar including isolate B as there was no significant difference in the radial growth. Wheat bran agar medium showed moderate radial growth in all the isolates, however, lower growth of isolate B was also recorded on this medium. Pigeon pea and lentil bran agar media, which recorded reduced growth of some isolates only, appeared to be useful for differentiation of isolates. On pigeon pea bran, isolate E did not grow, while other isolates recorded reduced growth as compared to other bran media. Lentil bran agar medium was found to be more toxic, as it supported very poor growth of two isolates (B and D) only and the other three isolates (A, C and E) showing fast growth on pea bran agar medium did not grow at all. This clearly indicates that these media can be useful for differentiation of isolates.

Irrespective of isolates, maximum sporulation was recorded in rice bran agar medium followed by gram and wheat bran agar media. Pigeon pea and lentil bran agar media, which supported poor growth of few isolates, recorded minimum sporulation in these isolates. Among

Table 3. Effect of different grain agar media on sporulation of the five isolates of *Arthrobotrys dactyloides*

Isolates		Spore/*ml* in different grain agar media				
		Maize grain	Wheat grain	Barley grain	Gram grain	Sorghum grain
A		3.2×10^4 a (A)	1.2×10^4 b (A)	2.9×10^4 a (A)	7.2×10^4 c (A)	1.9×10^4 b (A)
B	Macroconidia	10×10^4 a (B)	1.7×10^4 b (A)	20×10^4 c (B)	7.1×10^4 d (A)	5.5×10^4 e (B)
	Microconidia	24×10^4 a (C)	4.9×10^4 b (B)	16×10^4 c (C)	30×10^4 d (B)	2.3×10^4 e (A)
C		3.1×10^4 a (A)	1.6×10^4 b (A)	5.7×10^4 c (D)	7.5×10^4 d (A)	2.3×10^4 a (A)
D		7.4×10^4 a (D)	1.4×10^4 b (A)	3×10^4 c (A)	16×10^4 d (C)	3.9×10^4 c (C)
E		7.4×10^4 a (D)	1.5×10^4 b (A)	5.9×10^4 c (D)	16×10^4 d (C)	2.8×10^4 e (A)

Data with different small letters show significant difference of row data by CRD test at P = 0.01.
Data with different capital letters in parenthesis show significant difference of column data by CRD test at P = 0.01.

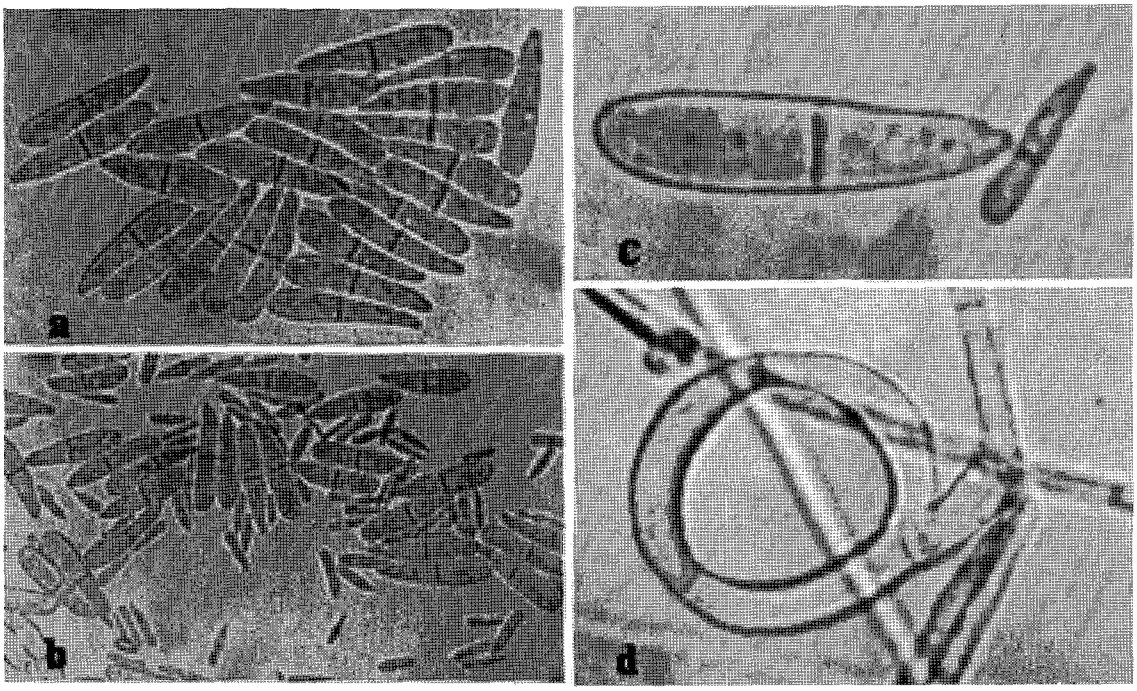

Fig. 4. Conidial variations in different isolates of *Arthrobotrys dactyloides* and constricting ring. (a) Two celled conidia of *A. dactyloides* produced by isolate A, C, D and E (×500). (b) Two celled macro and microconidia of isolate B of *A. dactyloides* (×300). (c) Enlarged view of macro and microconidia of isolate B of *A. dactyloides* (×1200). (d) Enlarged view of three celled constricting ring of isolate B of *A. dactyloides* produced on the aerial hyphae in Czapek's agar medium (×1450).

the five isolates, isolate B recorded maximum sporulation, which also varied in different media. In all the bran agar media, ratio of microconidia was higher than the macroconidia except gram bran agar medium, which recorded higher number of macroconidia than the microconidia.

Radial growth and sporulation of *A. dactyloides* on different grain agar media. The observations on radial growth and sporulation of five isolates of *A. dactyloides* on different grain agar media are presented in Fig. 3 and Table 3. Of the five grain agar media tested, barley grain medium showed the best growth of almost all the isolates except isolate B, which recorded significantly lower growth than other isolates. Growth of all the isolates on this medium was comparable with pea bran agar medium. On wheat grain agar medium also the growth of isolate B was slow; however, on maize grain agar medium the difference in radial growth was narrower. On gram grain agar medium, minimum radial growth was recorded for isolate A, which otherwise was fast growing on barley grain and pea bran agar media. This showed that nature of growth of the isolates varied with different media also. Growth of different isolates on sorghum grain agar medium was more or less similar to maize grain agar medium.

In general grain agar media supported moderate to heavy sporulation in all the isolates of *A. dactyloides* (Fig. 4 a, b & c). Irrespective of the isolates, maximum number of spores recorded on gram grain agar followed by barley and maize grain agar media. Among the isolates, maximum number of spores was recorded in isolate B. The ratio of macroconidia and microconidia also varied in grain media, the number of macroconidia was more of the microconidia in barley and sorghum grain agar media, whereas the number of microconidia was about 2.4 to 4.2 times more of the macroconidia in other grain agar media. Least sporulation in isolate B was recorded on wheat grain agar medium.

Table 4. Mass culture of *Arthrobotrys dactyloides* on different substrates

Substrates	Visual rating[a]
Pea bran	++++
Barley grain	++++
Maize grain	+++
Sorghum grain	+++
Wheat grain	+++
Rice bran	+++
Wheat straw	++
Wheat bran	+
Gram bran	+
Pigeon pea bran	+
Rice straw	+
Lentil bran	-

[a]++++ : Excellent, +++ : Good, ++ : Fair, + : Poor, - : No growth.

Mass culture of *A. dactyloides* on different substrates. Observations on mass culture of isolate A of *A. dactyloides* on different substrates showed excellent growth on pea bran and barley grains on the basis of visual rating (Table 4). However, the good growth of the fungus was observed in rice bran, wheat, sorghum and maize grain. Only fair growth of *A. dactyloides* was observed in wheat straw. Brans of wheat, gram and pigeon pea and straw of rice supported poor growth of the fungus. No growth of the fungus was observed on lentil bran.

Discussion

The variability in radial growth and sporulation of different isolates of *A. dactyloides* on different types of media may be attributed to differences in the nutritional status of the media. However, difference in the growth and sporulation of isolates on a specific medium may be attributed to nature of growth and sporulation of isolates and their respective nutritional requirements.

For the better differentiation of isolates of *A. dactyloides* two media viz: PDA and CA were found useful. PDA was useful in differentiating the fast growing isolates (A, C and E) and slow growing isolates (B and D) while CA was useful in differentiating the poorly sporulating isolates (A, C, D and E) and heavily sporulating isolate (B) (Table 1).

Among the bran agar media, pea bran agar medium could be used to differentiate fast growing isolates A, C, D and E and isolate B as slow growing one. The pigeon pea and lentil bran agar media supported growth of only isolates A, B, C and D and isolates B and D, respectively. These media could, therefore, be also used for differentiation of the isolates of *A. dactyloides*. Complete inhibition in growth and sporulation of some isolates in pigeon pea and lentil bran agar media may be attributed to toxic chemicals in these brans. It appears that the relative tolerance of the isolates differed to the toxic chemicals present in brans of pigeon pea and lentil. Slow growth of isolate B in maize, wheat, barley and sorghum grain agar media during entire growth period indicate that these grain agar media could also be used for differentiation of fast growing and slow growing isolates.

Excellent growth of *A. dactyloides* on pea bran and barley grain in mass culture may be attributed to availability of all the necessary nutrition for the growth of this fungus, which is also confirmed from the results obtained on the effect of brans and grain agar media on the growth and sporulation of this fungus. However, the poor growth of the fungus for mass culture on wheat and gram bran may be attributed to their higher concentrations used. Similar observations on reduced growth of *Catenaria anguillulae* at higher concentrations of wheat bran in media and mass culture were made by Vaish and Singh,

2002 and Vaish, 1998. Vaish and Singh (2002) reported that a ratio of 12 : 88 of wheat bran and sand along with 0.5% linseed oil cake resulted in good growth of *C. anguillulae* . Thus there is need to modify the concentrations of these brans by adding sand or other inert materials for bioformulation of mass culture of *A. dactyloides*.

To the author's knowledge, variability in growth and sporulation in isolates of *A. dactyloides* has not been studied earlier. Based on the excellent growth of the fungus pea bran and barley grain could be used for mass culture of *A. dactyloides*.

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