Research Article

Suitability of different grain media for mass culturing of the fungal entomopathogen, *Acremonium zeylanicum* (Petch) W. Gams and H. C. Evans

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ABSTRACT: Evaluation of food grains for suitability as substrates for mass production of the fungus, *Acremonium zeylanicum* (Petch) W. Garns and H. C. Evans revealed that the spore count increased with increase in duration of incubation period after inoculation till harvesting. Rice and sorghum grain served as potential substrates for conidial production of *A. zeylanicum* which yielded 9.15×10^8 and 8.33×10^8 conidia/g of substrate, respectively, 15 days after inoculation. Maize and bajra were next best alternatives. However, the other materials like ragi, bengalgram and wheat did not serve as efficient food source for mass production of the fungus.

KEY WORDS: *Acremonium zeylanicum,* entomopathogenic fungi, mass production, **n**atural substrates.

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INTRODUCTION

Interest in entomopathogenic fungi dates back to over a hundred years. They cause regular and tremendous mortality of several pests in many parts of the world and constitute an efficient and extremely important natural control factor (Steinhaus, 1949). During the last 25 years, there has been a resurgence of interest in the use of entomopathogenic fungi as biocontrol agents of insect pests, as part of a general shift towards integrated pest management (IPM) and away from dependence on chemical pesticides. The safety of entomopathogenic fungi towards humans, the environment and non target organisms is an important criterion and offer a safer alternative for use in IPM over chemical insecticides (Goettel and Hajek, 2000).

The key factor which decides adaptability and success of a bioagent is its easy availability. *Acremonium zeylanicum* is a fungal parasite, which can grow and develop on natural host like sugarcane woolly aphid as a pathogen. The fungus readily sporulates on synthetic media like PDA (Kulkarni *et al.,* 2006), but the diet will be expensive. Hence, results of the present studies on mass production of the fungus using naturally and easily available substrates like grain media can provide economically feasible solution for large scale mass multiplication. Maltose released by the action of starch hydrolyzing enzymes present in the fungus induces sporulation (Coudron *et al.*, 1985). Since chitinase and exochitinase activities are low in conidia of *N. rileyi,* crushing of grains is necessary to increase the surface area of substrate available for hydrolyzing enzyme amylase.

MATERIALS AND METHODS

Broken grains of sorghum, bajra, ragi, maize, rice, wheat and bengal gram which are commonly used as dietary ingredients were assessed for their suitability as substrates to support the growth and development of the fungus in order to select the best substrate for mass production of the pathogen. Fifty grams of each of these grains were taken in saline bottle (500 ml capacity) containing 50 ml of 1 per cent yeast extract prepared by using distilled water. Three such bottles were maintained as replications for each substrate. Contents were thoroughly mixed and plugged with cotton. After soaking for 6 hours, saline bottles were autoclaved at 15 PSI pressure and 121°C temperature for 30 minutes and cooled to room temperature. Then inoculation with conidial suspension of *A. zeylanicum* (1×10^8 conidia/ml) @ 2 ml per bottle was done under aseptic condition and the bottles were incubated at room temperature. Conidial yield was assessed on 16, 18, 20, 22 and 24 day after inoculation. Digested substrate in each saline bottle was thoroughly mixed and taken out to dry the contents under aseptic condition. Samples were drawn randomly from each replication in each treatment (1 g each) and serially diluted with distilled water in 0.2 per cent Tween-80 and spore concentration in the suspension was estimated using haemocytometer.

RESULTS AND DISCUSSION

Conidial yield of *A. zeylanicum* on different substrates at different intervals

Different natural substrates were evaluated for their suitability to support the growth of the fungus. The results indicated significant difference among various substrates (Table 1). The maximum conidial production after 15 days of inoculation was recorded on rice (9.15×10^8 conidia/g) and sorghum (8.33×10^8 conidia/g) which were on par with each other. The next best substrates included bajra (4.05×10^8 conidia/g) and maize (3.97×10^8 conidia/g). The other substrates *viz.,* wheat, ragi and bengalgram recorded significantly lower conidial production (2.67 to 3.17 conidia/g).

The results pertaining to conidial yield of the entomopathogenic fungus on different substrates at different intervals have been tabulated in Table 1.

All the substrates showed significant difference in conidial yield at different intervals. Rice was found to be the best treatment by recording 8.33, 10.00, 12.33, 12.37 and 13.00×10^8 conidia/g of substrate at 16, 18, 20, 22 and 24 days after inoculation, respectively which proved superior over the rest of the substrates. Sorghum (5.33 to 8.00 conidia/g of substrate) and wheat (95.67 to 8.33 conidia/g of substrate) were next to follow. In the order of supremacy maize, bajra, bengalgram and ragi produced 7.00, 8.67, 5.00 and 4.33 conidia/g of substrate, respectively at 24 days of inoculation.

All the substrates recorded highest conidial yield when the fungus was harvested at 24 days after inoculation. Significantly highest mean conidial yield was observed in rice (11.40×10^8 conidia/g) followed by wheat (6.87×10^8 conidia/g) and sorghum (6.81×10^8 conidia/g). Whereas, the lowest mean conidial yield (3.20×10^8 conidia/g) was obtained in ragi at 24 DAI. Interaction of substrates when harvested at different intervals was found to be non-significant.

The present findings of superiority of rice with addition of yeast extract is in agreement with the findings of Gopalkrishnan and Mohan (2000) and Hegde (2001). They obtained conidial yield of 6×10^9 per g of rice using *Nomureau rileyi* after 18 days at 25±2°C. During

Table 1. Conidial yield of *Acremonium zeylanicum* grown on various grain media at different time intervals

Grain media	Conidial yield (x10^8 Conidia / g of grain media)					Mean yield of conidia/g of media
	16 DAI	18 DAI	20 DAI	22 DAI	24 DAI	
Sorghum	5.33 (2.31)	6.67 (2.58)	7.00 (2.64)	7.33 (2.71)	8.00 (2.82)	8.3 [a] (2.89)
Bajra	3.33 (1.82)	4.67 (2.16)	5.33 (2.31)	5.33 (2.31)	5.67 (2.38)	4.05[b] (2.01)
Ragi	2.33 (1.52)	2.33 (1.52)	3.33 (1.82)	3.67 (1.91)	4.33 (2.08)	2.90[c] (1.70)
Maize	4.67 (2.16)	5.67 (2.38)	6.00 (2.44)	6.67 (2.58)	7.00 (2.64)	3.97[b] (1.99)
Rice	8.33 (2.89)	11.00 (3.31)	12.33 (3.51)	12.37 (3.51)	13.00 (3.60)	9.15[a] (3.02)
Wheat	5.67 (2.38)	6.33 (2.51)	7.00 (2.64)	7.33 (2.71)	8.00 (2.82)	2.75[c] (1.66)
Bengal gram	2.67 (1.63)	3.67 (1.91)	4.33 (2.08)	4.67 (2.16)	5.00 (2.23)	3.17[c] (1.78)
CD between	SEm±	CD (P=0.01)				
Grain media	0.04	0.12				
Days after inoculation	0.04	0.10				
Grain media X days after inoculation	0.10	NS				

DAI-Days after inoculation
Figures in parentheses are square root transformed values

the present study ragi and wheat grains proved inferior for conidia production, perhaps due to less content of starch in wheat (53%) and amylose in ragi (6-18%). Besides, formation of clumping in ragi grain interfered in efficient harvest of spores and thus led to low productivity (Kulkarni, 1999).

Similarly, rice has been reported to be most suitable for *N. rileyi* production by several authors (Silva and Loch, 1987; Lopes *et al.,* 1995; Anonymous, 1995; Garcia and Pozo, 1999; Kulkarni, 1999, Rachappa, 2003; Bhide and Patil, 2005; Nirmala *et al.,* 2005; Tamizarasi *et al.,* 2005 and Ayyasamy and Baskaran, 2006).

Broken grains of sorghum enriched with yeast extract has been used to produce *N. rileyi* (Kulkarni, 1999; Vimaladevi, 1999 and Bhide and Patil, 2005). Multiplication on sorghum was developed as a low cost and rapid technique to produce the mycopathogen as compared to the use of expensive synthetic media which contains complex nutrients sources of nitrogen and carbon required for mycelial growth and sporulation (Bell, 1975 and Coudron *et al.,* 1985). Present findings are in agreement with the above findings.

Hence, results of the present studies on mass production of the fungus using naturally and easily available substrates like grain media can provide economically feasible solution for large scale mass multiplication.

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