Research Paper :

Suitability of different natural substrates for mass production of entomopathogenic fungus *Acremonium zeylanicum*

NAVEENKUMAR B. PATIL, C. P. MALLAPUR AND Y.H. SUJAY

International Journal of Plant Protection, Vol. 4 No. 2 (October, 2011) : 252-255

See end of the article for authors' affiliations

Correspondence to :
Y.H. SUJAY
A.I.C.R.P. on Soybean, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA
Email : morphosis77@gmail.com

SUMMARY

Evaluation of food grains for suitability as substrates for mass production of the fungus revealed that the spore count increased with increase in duration of incubation period after inoculation till harvesting. Rice and sorghums grains served as potential substrates for conidial production of *Acremonium zeylanicum* which yielded 9.15 x10^8 and 8.33 x 10^8 conidia/g of substrate, respectively, 15 days after inoculation. Maize and bajra were next best alternatives. However, the other materials like ragi, chickpea and wheat did not serve as efficient food source for mass production of the fungus.

Patil, Naveenkumar B., Mallapur, C.P. and Sujay, Y.H. (2011). Suitability of different natural substrates for mass production of entomopathogenic fungus *Acremonium zeylanicum*. *Internat. J. Plant Protec*., **4**(2): 252-255.

Key words : *Acremonium zeylanicum,* Entomopathogenic fungus, Mass production, Natural substrates

Received : *January, 2011*
Revised : *May, 2011*
Accepted : *July, 2011*

Interest in entomopathogenic fungus 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 fungus as biocontrol agents of insect pests, as part of a general movement towards integrated pest management (IPM) and away from dependence on chemical pesticides. The safety of entomopathogenic fungus towards humans, the environment and non-target organisms is an important criterion and offers 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 *Acromonium 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 chickpea 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^{0}C temperature for 30 minutes and cooled to room temperature. Then inoculation with conidial suspension of *A. zeylanicum* (1 x10^8 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

The results obtained from the present investigation as well as relevant discussion have been presented under following heads:

Suitability of substrates:

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 x 10^8 conidia/g) and sorghum (8.33 x 10^8 conidia/g) which were at par with each other. The next best substrates included bajra (4.05 x 10^8 conidia/g) and maize (3.97 x 10^8 conidia/g). The other substrates *viz.,* wheat, ragi and chickpea recorded significantly lower conidial production (2.67 to 3.17 conidia/g).

Table 1 : Conidial yield of *Acremonium zeylanicum* on different natural substrates at harvest

Sr. No.	Substrates used	Mean no. of conidia/g of substrate (x 10^8 conidia)
1.	Sorghum	8.33 a (2.89)
2.	Bajra	4.05 b (2.01)
3.	Ragi	2.90 c (1.70)
4.	Maize	3.97 b (1.99)
5.	Rice	9.15 a (3.02)
6.	Wheat	2.75 c (1.66)
7.	Chickpea	3.17 c (1.78)
S.E.±		0.04
C.D. (P=0.01)		0.18
CV (%)		2.93

Means followed by same letter in the column do not differ significantly by DMRT (P=0.01)
The figures in the parentheses are square root transformed values

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

The results pertaining to conidial yield of the entomopathogenic fungus on different substrates at different intervals have been presented in Table 2.

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 x 10^8 conidia/g of substrate at 16, 18, 20, 22 and 24 days after inoculation, respectively which proved superior over rest of the substrates. The next to follow were sorghum (5.33 to 8.00 conidia/g of substrate) and wheat (5.67 to 8.00 conidia/g of substrate). In the order of supremacy maize, bajra, chickpea and ragi produced 7.00, 5.67, 5.00 and 4.33 conidia/g of substrate, respectively at 24 days of inoculation.

Irrespective of substrates used for inoculation, as the days of inoculation advanced the conidial yield also increased. 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 x 10^8 conidia/g) followed by wheat (6.87 x 10^8 conidia/g) and sorghum (6.81 x 10^8 conidia/g). Whereas, the lowest mean conidial yield (3.20 x 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 and rice was found to be the superior treatment by yielding 13.00 x 10^8 conidia/g of substrate when harvested at 24 days after inoculation.

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 x10^9 per g of rice using *N. rileyi* after 18 days at 25$\pm$2^{0}C. During 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; Ajaykumar and Kanaujia, 2005; Nirmala *et al.,* 2005; Tamizharasi *et al.*, 2005 and Ayyasamy and Baskaran, 2006).

Table 2 : Conidial yield of *Acremonium zeylanicum* on different substrates at different intervals

Sr. No.	Substrates used	Conidial yield (x10^8 conidia / g of substrate)				
		16 DAI	18 DAI	20 DAI	22 DAI	24 DAI
1.	Sorghum	5.33 (2.31)	6.67 (2.58)	7.00 (2.64)	7.33 (2.71)	8.00 (2.82)
2.	Bajra	3.33 (1.82)	4.67 (2.16)	5.33 (2.31)	5.33 (2.31)	5.67 (2.38)
3.	Ragi	2.33 (1.52)	2.33 (1.52)	3.33 (1.82)	3.67 (1.91)	4.33 (2.08)
4.	Maize	4.67 (2.16)	5.67 (2.38)	6.00 (2.44)	6.67 (2.58)	7.00 (2.64)
5.	Rice	8.33 (2.89)	11.00 (3.31)	12.33 (3.51)	12.37 (3.51)	13.00 (3.60)
6.	Wheat	5.67 (2.38)	6.33 (2.51)	7.00 (2.64)	7.33 (2.71)	8.00 (2.82)
7.	Chickpea	2.67 (1.63)	3.67 (1.91)	4.33 (2.08)	4.67 (2.16)	5.00 (2.23)
Mean		4.67 (2.11)	5.90 (2.37)	6.43 (2.49)	6.71 (2.54)	7.29 (2.64)
Factors			S.E.±		C.D. (P=0.01)	
Substrate(S)			0.04		0.12	
Days(D)			0.04		0.10	
Interaction (SxD)			0.10		NS	

DAI-Days after inoculation NS=Non-significant

The figures in the parentheses are square root transformed values

Broken grains of sorghum enriched with yeast extract has been used to produce *N. rileyi* (Kulkarni, 1999; Vimaladevi, 1999; 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). These reports are in agreement with the present 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.

Acknowledgement:

The authors are thankful to the University of Agricultural Sciences, Dharwad for providing necessary facilities for the investigations.

Authors' affiliations:

NAVEEN KUMAR B. PATIL AND **C.P. MALLAPUR,** Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA

REFERENCES

Ajaykumar, P. and Kanaujia, K.R. (2005). Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* against *Spodoptera litura* larvae, *J. Biol. Control,* **19**(2): 129-133.

Anonymous (1995). Technology for production of natural enemies. Tech. Bull., PDBC, Bangalore, p. 22.

Ayyasamy, R. and Baskaran, P. (2006). Evaluation of various synthetic and grain media for radial growth, sporulation and infectivity of the entomopathogenic fungus *Paecilomyces farinosus. J. Ecobiol.*, **19** (5) : 229-233.

Bell, J.V. (1975). Production and pathogenicity of the fungus, *Spicaria rileyi* from solid and liquid media. *J. Invertebr. Pathol.*, **26** : 129-130.

Bhide, S.G. and Patil, P.D. (2005). Mass multiplication of *Metarhizium anisopliae* (Metsch.) Sorokin using grain media. *Pestology*, **29**: 40-41.

Coudron, J.A., Kroha, M.J. and El-Sayed, G.N. (1985). A novel semi-liquid for propagating entomopathogenic fungi. *J. Invertebr. Pathol.*, **46** : 335-336.

Garcia, I. and Pozo, E. (1999). *Nomuraea rileyi* (Farlow) Samson conidia isolation, production and their virulence on *Spodoptera frugiperda* (Smith) larvae. *Rev. Prot. Vegetal*, **14** : 95-100.

Goettel, M.S. and Hajek, A.E. (2000). Evaluation of non-target effects of pathogens used for management of arthropods. In *Evaluating indirect ecological effects of biological control* (Ed.) Wajnberg, E., Scott, J. K. and Quimby, P. C., CABI Publishing Wallingford, pp. 81-97.

Gopalkrishnan, C. and Mohan, K.S. (2000). A simple and cost effective in-vitro method for mass production of conidia of Nomuraea rileyi. *Pest Mgt. Hort. Ecosyst.,* **6**: 36-39.

Hegde, R. (2001). Exploitation of *Nomuraea rileyi* (Farlow) Samson against important lepidopterous pests of potato, cotton and chickpea. Ph.D Thesis, University of Agricultural Sciences, DHARWAD, KARNATAKA (India).

Kulkarni, N.S. (1999). Utilisation of fungal pathogen *Nomuraea rileyi* (Farlow) Samson in the management of lepidopterous pests. Ph. D. Thesis, University of Agricultural Sciences, DHARWAD, KARNATAKA (India).

Kulkarni, S., Shalini, D.S., Tippannavar, P.S. and Patil, S.B. (2006). A new fungal hyperparasite on woolly aphid of sugarcane. *J. Plant Dis. Sci.*, **1** (2) : 229.

Lopes, E., Lopes, M.I. and Barros, N.M. (1995). Virulence of stored conidia of *Nomuraea rileyi* against soybean caterpillar, *Anticarsia gemmatalis. Ciencia Rural*, **25** : 197-200.

Nirmala, R., Ramanujam, B., Rabindra, R.J. and Rao, N.S. (2005). Growth parameters of some isolates of entomofungal pathogens and production of dust free spores in rice medium. *J. Biol. Control*, **19** (2): 121-128.

Rachappa, V. (2003). Occurrence of entomopathogenic fungi and utilization of Metarhizium anisopliae in the management of selected crop pests in Northern Karnataka, Ph.D. Thesis. University of Agricultural Sciences, DHARWAD, KARNATAKA (India).

Silva, L. and Loch, L.C. (1987). Sporulation of the entomopathogenic fungus, *Nomuraea rileyi* on polished rice grain media. *Anais da Sociedade Entomologica da Brazil*, **16** : 213-222.

Steinhaus, E. A. (1949). *Principles of insect pathology*, McGraw Hill Book Co., New York, USA, 757 pp.

Tamizharasi, V., Srikanth, J. and Santhalakshmi, G. (2005). Molasses based medium requires no nitrogen suppliment for culturing three entomopathogenic fungi, *J. Biol. Control*, **19**(2): 135-140.

Vimaladevi, P.S. (1999). Isolation, identification, multiplication and maintenance of *Nomuraea rileyi.* In: Training programme on multiplication methodology and utilization of the insect pathogenic fungus. Nomuraea rileyi for pest control, 11-17 November, 1999, Directorate of Oilseed Research, Hyderabad, pp. 21-24.

****