Mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi* using agricultural products and agro wastes

Mamta Thakre*, Mahendra Thakur, Nagesh Malik and Suman Ganger

ABSTRACT

Various agricultural products like rice, sorghum and wheat and agrowastes namely refuse raw potatoes and refuse raw bananas were evaluated for mass scale cultivation of entomopathogenic fungus *Nomuraea rileyi*. Among the grains, rice (5.53 x 10^7spore/g) supported the maximum spore production of fungus followed by refuse raw bananas (4.2 x 10^7spores/g) and sorghum (4.01 x 10^7spores/g) on the 11^{th} day after inoculation of spore suspension. whereas, wheat (3.55 x 10^7spores/g) and refuse potato chips (3.1 x 10^7spores/g) supported less spore load than other substrates on the 11^{th} day and on the 15^{th} day after inoculation of spore suspension respectively. According to the references based on *N. rileyi*, use of raw banana as dry chips form for cultivation of fungus was carried out for the first time in this study. This study suggests alternative nutrient sources for mass scale cultivation of fungus and that the biotechnological potential of agro refuses could be employed in biopesticide development.

Key words: Biopesticide, entomopathogenic fungus, *Nomuraea rileyi*, solid state fermentation, refuse raw banana.

INTRODUCTION

Among the several existing entomogenous fungi, *Nomuraea rileyi* is a cosmopolitan species infecting many noctuids such as *Helicoverpa armigera, Spodoptera litura, Tricoplusia ni, Anticarsia gammatalis, Pseudoplusia includes* and has a potential for development into mycoinsecticide (Vimala Devi, 1994; Shanthakumar *et al*., 2010). A successful microbial insecticide should be able to produce high quantities of inoculums (Goettle and Roberts, 1992). Lack of reliable and cost effective substrate limits the mass cultivation and commercialization of these mycoinsecticides. The standard medium used the world over for their multiplication is Sabouraud's Maltose Agar Yeast medium (SMAY). High cost of SMAY ingredients is a serious limitation in scale-up of *N. rileyi* (Bell, 1975; Bell *et al*., 1982).

Several attempts have been made to multiply the fungus in general (for example Sahayaraj and Karthick Raja, 2008) and *Nomuraea rileyi* in particular (Sonai Rajan and Muthukrishnan, 2010) using semisynthetic media and solid substrates in order to cut down the cost of production . Cost-effective and rapid multiplication of *N. rileyi* is reported by Vimala Devi *et al.* (2000); Sonai Rajan and Muthukrishnan (2010) and is found that the spore yields in semi-synthetic media and liquid diet, respectively are comparable to or significantly higher than the standard medium. Because of the specific carbon source requirements of *N. rileyi*, attempts to develop cost-effective, rapid multiplication protocols have met with limited success. Although, *N. rileyi* maximum growth on crushed sorghum and 1% yeast extract has been standardized by Vimala Devi (1994), it would be essential to make it highly cost effective and easily adaptable with locally available indigenous substrates as well as agro wastes. Standardization of mass cultivation methods with locally available resources favors local entrepreneurship to ensure availability of the *N. rileyi* as a biocontrol agent and also its successful usage.

In the present study, efforts were made to grow the *N. rileyi* on agro wastes. The agricultural wastes are produced in large quantities in agricultural fields. During the harvesting of fruits, they get damaged due to improper cutting and handling practices. Such damaged fruits are not accepted in market and are considered as waste. Hence, these damaged fruits were collected from the field of potato and banana cultivation at the time of harvesting and cut into chips form, sun dried and stored in dry place so that they can be used throughout this study. Both potato and banana have water content of 79.34g and 74.91g and sugar content of 0.78g and 12.23g per 100g of fruit respectively, which are major growth requirements of fungus. At the same time, this is natural and biodegradable. Hence, media was optimized using such wastes and used as alternatives to standard medium (SMAY).

MATERIALS AND METHODS

The fungal isolate used in this study was obtained from Department of Plant pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra. The culture was originally isolated from field collected *N. rileyi* infected larvae of *Spodoptera litura* on soybean crops in Dr. PDKV experimental plot.

Preparation of spore suspension

Culture grown on steril SMAY slants incubated at 25-28^0 C for 7- 8 days was used for preparation of spore suspension. Spore culture was washed twice with steril distilled water containing a wetting agent tween 80 at 0.1%. Washed spores were reconstituted in fresh sterile distilled water containing 0.1% tween 80 to prepare inoculums. This suspension was then charged in Neubauer's hemocytometer slide, to count the number of spores present. The number of spores was adjusted to 1x10^{7}spores/ml, and this suspension was used as inoculums for further experiments.

Spore production on different grain media

To evaluate the utility of locally available substrates for mass scale cultivation of *N. rileyi* substrates (grains) like rice, wheat and sorghum were used. All grains used were of very low grade quality (20 Rs/kg). The protocol given by Vimala Devi (1994) was followed for this experiment. Dry grains were crushed in the household mixer for about 30 sec. to make small pieces. Crushed grains (30 g) with 1% yeast extract were added to 250 ml capacity flask containing 30 ml distilled water and soaked overnight and autoclaved for 20 min at 121^{0}C. After cooling, clumps of grains were broken using sterile glass rod and each flask was then inoculated with 1 ml of spore suspension containing 1 x 10^{7}spores/ml and shaken well to disperse the inoculum. The flasks were incubated at room temperature for 15 days.

Spore production on refuse raw potato (RP) and refuse raw banana (RB)

A great alternative to achieve a satisfactory price is the utilization of agro wastes. Hence, potatoes and banana refuse was collected from field and then cut into chips form, dried in sunlight and stored in dry place. Such dried chips of approximately 4 - 5 cm of diameter were used for spore production of *N. rileyi*. A five g of dry chips of RP and RB were taken in Petri plates (90 mm). 10 ml of distilled water was added into each plate. These plates were then packed carefully and kept in upward position in a container one above the other and then autoclaved for 20 min at 121^{0}C. After cooling plates were inoculated with 1ml spore suspension containing 1x10^{7}spores/ml and then incubated at room temperature.

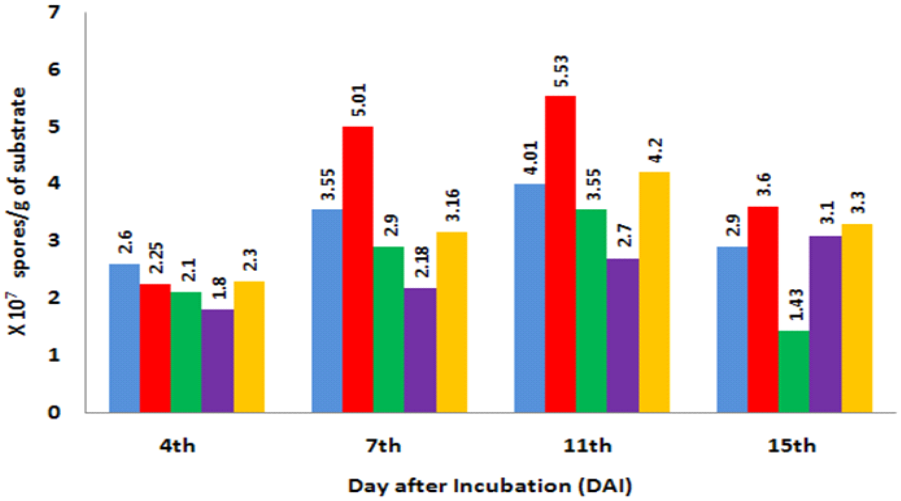
Observation recording

Conidia were harvested in D/W containing 0.1% tween 80 after 4, 7, 11 and 15 days of incubation. The suspension was filtered through double layered muslin cloth and the number of conidia was determined microscopically with the help of Neubauer's haemocytometer.

RESULTS AND DISCUSSION

The results indicated that the sporulation of the fungus differed significantly among various substrates. The results as seen in Figure 1 are average of three replications. Among

Figure 1. Spore count of *N. rileyi* on sorghum, rice, wheat, refuse potato chips and refuse banana chips (%) after 4th , 7th , 11th and 15th day of incubation (DAI)



the substrates tested, rice (5.53 x 10^7 spore/g) was found to support the production of maximum spore load of *N. rileyi* followed by refuse raw banana chips (4.2 x 10^7 spores/g) and sorghum (4.01 x 10^7spores/g) on 11^{th} day after incubation (DAI), whereas, wheat (3.55 x 10^7spores/g) and refuse potato chips (3.1 x 10^7 spores/g) supported least spore load than other substrates on 11^{th} and 15^{th} DAI, respectively (Figure 1). Grains are cheap, easily available and good. They provide more surface area for growth and multiplication (nutritive media for the mass scale cultivation of many microorganisms). Rice contains higher proportion of starch and amylase. Hydrolysis of starch in rice resulted in the release of glucose and maltose depending on clarification (Preen *et al.*, 1985). Maltose released by the action of starch hydrolysis enzymes present in the fungus induces sporulation (Coudron *et al.*, 1985). Rice has also been found to be the most suitable substrate for quicker and better mass multiplication of *N. rileyi* as observed by Gopalakrishnan and Mohan (2000); Lingappa *et al.* (2002). Similar results were obtained in the present study.

But based on cost incurred for production of spores, agro wastes i.e. raw banana recorded lowest cost compared to agricultural products. Though rice recorded highest spore yield among the substrates, the production cost was higher as compared to agro wastes. Upon the knowledge that agro - waste, refuse raw banana provide high spore production rates in case of this *N. rileyi* strain. This natural media could be employed hereafter in mass scale cultivation evaluations on this and other related fungi with potential for biological control. In order to achieve low-cost and yield high concentrations of viable fungal spores and to make good use of agricultural wastes produced in large quantities in fields, this study suggests alternatives for nutrient sources aiming *N. rileyi* mass scale cultivation. The biotechnological potential of these agro refuses could be employed in byproducts development and improvement for biocontrol programs establishment. Results obtained in this study inferred the possibility of using these substrates for mass scale cultivation of fungus but work need to be done on the optimization of media with respect to moisture content of substrate.

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REFERENCES

Bell, J. V. 1975. Production and pathogenicity of the fungus *Spicaria rileyi* from solid and liquid media. *Journal of Invertebrate Pathology*, **26**: 129-130.

Bell, J. V., Hamalle, R. J. and Ignoffo, C. M. 1982. Methods and costs of producing *Nomuraea rileyi* conidiospores. *Advances in Agricultural Technology*, **24**: 1-7.

Coudron, T. A., Kroha, M. J. and Sayed, G. N. 1985. A novel semi liquid for propagating entomopathogenic fungi. *Journal of Invertebrate Pathology*, **46**: 335-336.

Gopalakrishnan, C. and Mohan, S. C. 2000. A Simple and cost effective *in vitro* method for the mass Production of conidia of *Nomuraea rileyi*. *Insect Environment*, **6**: 52-53.

Goettle, M. S. and Roberts, D. W. 1992. Mass Production, formulation and field application of entomopathogenic fungi. In: *Biological control of lLocusts and Grasshoppers* (Lomer, C. J. and Prior, C. ed.), CAB International; Wallingford, Oxon, UK. 230-238 **PP**.

Lingappa, S., Patil, R. K., Rachappa, V., Hegde, R. and Navi, S. S. 2002. Large-scale demonstration of efficacy of *Nomuraea rileyi* (F) Samson on soybean. *In: Biological control of lepidopteran pests.* Proceedings of the Symposium of Biological Control of Lepidopteran Pests, (Tandon, P. L. ed.), Bangalore, India. 251–253 **PP**.

Preen, J. C., Jong, F. D., Botes, P. J. and Lategon, T. M. 1985. Fermentation alcohol from grain sorghum starch. *Biomass*, **8**: 101-117.

Sahayaraj, K. and Karthick Raja, Namasivayam, S. 2008. Mass production of entomopathogenic fungi using agricutlural products and by products. *African Journal of Biotechnology*, **7** (12): 1907 - 1910.

Shanthakumar, S. P., Murali, P. D., Malarvannan, S., Prabavathy, V. R. and Sudha Nair. 2010. Laboratory evaluation on the potential of entomopathogenic fungi, *Nomuraea rileyi* against tobacco caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) and its safety to *Trichogramma* sp. *Journal of Biopesticides*, **3**(1): 132-137.

Sonai Rajan, T. and Muthukrishnan, N. 2010. Influence of various health drinks media on growth and sporulation of *Nomuraea rileyi* (Farlow) Samson isolates. *Journal of Biopesticides*, **3**(2): 463-465.

Vimala Devi, P. S. 1994. Conidial production of entomopathogenic fungus *Nomuraea rileyi* and its

evaluation for control of *Spodoptera litura* (Fab.) on *Ricinus communis*. *Journal of Invertebrate Pathology*, **63**: 145 - 150.

Vimala Devi, P. S., Chowdary, A. and Prasad, Y. G. 2000. Cost-effective multiplication of entomopathogenic fungus *Nomuraea rileyi* (F) Samson. *Mycopathologia*, **151**:35-39.

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