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Attempts on mass production of *Nomuraea rileyi* on various agricultural products and byproducts

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ABSTRACT: **The suitability of various agricultural products and byproducts as substrates for mass culturing of *Nomuraea rileyi* was tested in the laboratory. Among the different concentrations (0.5, 1, 2, 3, 4, 5, and 6%) of sugarcane molasses tested maximum radial growth (4.65 cm); biomass production (1.55 g/100 ml) and spore production (5.0 x 10^{10} spores/ 100 ml) were noticed on molasses (6%). Maximum spore production of *N. rileyi* was noticed on sugarcane-spent wash (10%). The spore production decreased with increase in the concentration of spent wash and minimum spore production was noticed with 100 per cent concentration. Among the grains and tubers tested, carrot medium recorded greater radial growth, rice and tapioca extracts showed greater biomass production and rice and finger millet supported maximum spore production. There was no difference in the spore production observed on different agricultural byproducts like sugarcane bagasse, sugarcane press-mud, coconut- water and tapioca rind. Among the oil cakes tested, spore production of *N. rileyi* was maximum on groundnut cake (6.00 x 10^{10} spores/100g) followed by sesamum cake, neem cake and coconut cake.**

KEY WORDS: Agricultural products, byproducts, mass production, *Nomuraea rileyi*

INTRODUCTION

Although the entomopathogenic fungus *N. rileyi* was first described more than 100 years ago, no attempt was made to mass-produce and use it for biological control until 1955 (Samson, 1974). It infects economically important and polyphagous pests such as *Heliothis zea* (Boddie), *Heliothis virescens* (Fabricius) and *Trichoplusia ni* (Hübner) and several other species of caterpillar pests (Ignoffo and Boucias, 1992). In India, natural occurrence of this fungus has been reported on a variety of insects (Vimala Devi, 1999). *N. rileyi* is a slow-growing fungus with a preference for maltose as the carbon source (Glare, 1987). In the present study, an attempt has been made to develop a mass production medium for the fungus using agricultural products and by products.

MATERIALS AND METHODS

The stock culture of *N. rileyi*, an isolate of *Achaea janata* Linn. was obtained from Tamil Nadu Agricultural University. This culture was further

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sub-cultured and maintained on potato dextrose agar (PDA) slants at the Section of Entomology, Sugarcane Breeding Institute for conducting various experiments.

In order to find out a low cost and suitable medium for the multiplication of the fungus, the following substrates were tested.

1. Sugarcane molasses at different concentrations (0.5, 1, 2, 3, 4, 5 and 6 %),
2. Sugarcane spent wash (fortified with sucrose @ 10 g/l, urea @ 1 g) at different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 %),
3. Oil cakes - groundnut cake, cotton seed cake, neem cake, coconut cake and sesamum cake,
4. Grains-sorghum, finger millet, pearl millet and rice and tubers-potato, tapioca, beetroot and carrot.
5. Other agricultural byproducts namely, bagasse, sugarcane press mud, tapioca rind and coconut water.

The liquid media were tested as stationary culture.

Radial growth of the mycelium, biomass production and spore production were taken into consideration while determining the suitability of the media.

RESULTS AND DISCUSSION

Substrates

i. Molasses

Among different concentrations of molasses agar medium tested, 6 per cent was recorded the greatest mean diameter of growth circle (4.73 cm) after ten days of incubation. Molasses at 5 per cent and 4 per cent concentrations recorded 4.4 and 4.43 cm mean diameter of growth, respectively and were statistically on par with each other. Less fungal growth was recorded on 0.5, 1, 2 and 3 per cent molasses and standard potato dextrose agar medium (Table 1).

Maximum biomass production of 1.55 g / 100 ml was observed on 20th day after incubation in molasses (6%) followed by molasses (5%) (1.33g/ 100ml). The standard potato dextrose broth supported a biomass of only 0.76g/100 ml and it was better than only 2, 1 and 0.5 per cent molasses. There was a significant positive correlation ($r = 0.994$, $P<0.001$) between the concentration of molasses and biomass production.

Table 1. Radial growth, biomass production and spore production of *N. rileyi* on different concentrations of molasses

Substrate Molasses(%)	Radial growth on 10th day (cm)	Biomass production on 20th day (g)	Spore production on 20thday(X 10^{10} / 100 ml)
0.5	4.20	0.24	0.69
1.0	4.33	0.37	1.23
2.0	4.53	0.61	1.60
3.0	3.60	0.98	2.60
4.0	4.33	1.15	3.27
5.0	4.40	1.33	3.86
6.0	4.73	1.55	5.00
Potato dextrose broth	3.57	0.76	0.62
CD (P=0.01)	0.18	0.06	0.58

Production of aerial conidia was significantly higher on 6 per cent molasses broth (5 x 10^{10} spores/ 100ml). There was a gradual but significant reduction in the spore production with decrease in the concentration of molasses (Table 1). Least spore production was observed on 0.5 per cent molasses and potato dextrose broth, which were on par with each other.

In earlier studies, molasses yeast broth was selected as a medium for mass production of *Beauveria bassiana*, which produced 1 x 10^9 spores/ml (Sharma *et al.*, 1999). Easwaramoorthy *et al.* (2000) reported molasses (3%) as a suitable medium for the growth of *Beauveria brongniartii*, which is currently mass produced on molasses broth and used for the microbial control of sugarcane white grub, *Holotrichia serrata* F. Sharma *et al.* (1999) also reported that molasses yeast medium was suitable for the growth of *B. brongniartii* and *Metarhizium anisopliae*. Similar results were obtained in the present study with *N. rileyi*.

Table 2. Spore production of *N. rileyi* in different concentrations of spent wash

Spent wash (%)	Spore production (x 10^{10} per 100 ml)
10	1.48
20	0.53
30	0.75
40	0.23
50	0.19
60	0.24
70	0.15
80	0.15
90	0.12
100	0.16
Potato dextrose broth	1.12
CD (P=0.01)	0.39

ii. Spent wash

Among different concentrations of spent wash tested, maximum spore production was observed in 10 per cent spent wash (1.48 x 10^{10} spores/100ml) on 20th day. This was followed by spore production in potato dextrose broth. Moderate sporulation was noticed at 30 per cent followed by 20 percent spent wash (Table 2).

The spent wash, which has no economical value, supported the growth of *N. rileyi*. Tincilley (2002) reported no growth of *Beauveria bassiana* at 10-100% concentrations of spent wash. Daisy Leena (2002) observed meagre spore production of *Paecilomyces lilacinus* in 10 per cent spent wash and no growth of *P. farinosus*. This may be due to the fact that spent wash has high BOD and COD (30,000-40,000 ppm and 85,000-95,000 ppm, respectively). It also contains high amounts of dissolved salts and the high electrical conductivity (Gunjal and Hapase, 1995). So it is surprising that *N. rileyi*, which is a slow-growing fungus, could grow on such an apparently unsuitable medium and it is worthwhile to conduct further studies by the addition of different sources of nitrogen and carbon with low doses of spent wash. Spent wash is available in large quantities in all the sugar mills and it is normally diluted and used for irrigation, which is not safe to soil health. The possible diversion of spent wash for mass culturing of beneficial microorganisms, including entomopathogens, may minimize such damage to soil health.

iii. Grains and tubers

All the grain and tuber media tested except carrot medium were significantly inferior to standard potato dextrose agar medium. Next to carrot, rice and tapioca supported greater radial growth of the fungus. Moderate fungal growth was noticed on sorghum and pearl millet and least fungal growth was obtained with finger millet medium (Table 3).

Table 3. Radial growth, biomass production and spore production of *N. rileyi* on different grain and tuber media

Medium	Radial growth on 10th day (cm)	Biomass on 20th day (g)	Spore production on 20th day (X 10^{10} per 100 g)
Potato	2.87	0.37	1.43
Carrot	3.67	0.53	1.44
Beet root	2.83	0.51	1.34
Tapioca	3.03	1.13	1.25
Sorghum	2.70	0.60	1.03
Rice	3.07	1.21	4.09
Pearl millet	2.70	0.95	1.07
Finger millet	2.33	1.13	1.67
PDA / broth	4.07	0.78	0.77
CD (P=0.01)	0.13	0.20	0.06

Rice broth recorded the greatest biomass production (1.21g/ 100ml) followed by tapioca (1.13 x 10^{10}g/ 100ml) and finger millet broth (1.13x10^{10}g/ 100ml). Moderate biomass production was observed on pearl millet and sorghum both while least biomass production was observed on potato, carrot and beetroot extract (Table 3).

Rice grains gave significantly higher spore production (4.09x10^{10} spores/ 100g). Finger millet was the next best which supported 1.67x10^{10}spores/ 100g. Spore production on carrot (1.14x10^{10} spores/ 100ml) potato (1.43x10^{10}spores/100ml) and beetroot (1.34x10^{10}spores/100g) was not significantly different. Less spore production was observed on tapioca and potato dextrose broth while least sporulation was noticed on pearl millet and sorghum grains.

According to Silva and Loch (1987) polished rice grains particularly when boiled before sterilization supported maximum spore production of *N. rileyi* and this was confirmed in the present study. In the present study, sorghum medium yielded the least sporulation. But, addition of one per cent yeast extract to crushed sorghum was found to be an ideal medium (Vimala Devi, 1994; Kulkarni and Lingappa, 2002). Gopalakrishnan and Mohan (2000) reported carrot was found to be the cheapest and best suitable medium for large-scale multiplication of *N. rileyi*. Carrot – malt agar and oats – malt agar were found good for sporulation of *N. rileyi* (Balardin and Loch, 1989).

iv. Agricultural byproducts

Among the agricultural byproducts tested, *viz.* coconut water, tapioca rind, sugarcane press mud and sugarcane bagasse were on par with standard potato dextrose broth in respect of spore production (Table 4). Utilization of sugarcane waste products for the mass multiplication of other fungal biocontrol agents was reported earlier by Hari and Somasekhar (1998). According to Reji Rani and Mathai (1999), coconut water supports good sporulation of *Fusarium pallidoroseum*. Mani and Anandam (1991) reported that tapioca tuber and its peels supported only moderate spore production of *Beauveria bassiana*, which is in confirmation with the present study with *N. rileyi*.

Table 4. Spore production of *N. rileyi* on agricultural byproducts

Agricultural byproducts	Spore production ($X10^{10}$ per 100g)
Coconut water	0.73
Sugarcane bagasse	0.38
Tapioca rind	0.64
Sugarcane press mud	0.45
Potato dextrose broth	0.69
CD (P=0.05)	NS

v. Oil cakes

Spore production of *N. rileyi* was significantly higher on groundnut cake ($6x10^{10}$ spores/ 100g) (Table 5) and sesamum cake was next in the order of superiority and recorded $4.48x10^{10}$ spores/100g. Neem cake recorded a spore production of $3.67x10^{10}$ spores/100g. Moderate spore production was observed on coconut cake and poor spore production was observed on cotton seed cake and potato dextrose broth. This study clearly showed that all the oil cakes tested except coconut cake supported higher spore production than the standard medium. Spore production of *Beauveria bassiana* was significantly higher on sesamum cake (Tincilley, 2002). Reji Rani and Mathai (1999) observed maximum spore production of *Fusarium pallidoroseum* on cotton seed cake.

Table 5. Spore production of *N. rileyi* on different oil cakes

Oil cakes	Spore production ($X10^{10}$ per 100 g)
Neem cake	3.67
Coconut cake	2.73
Groundnut cake	6.00
Sesamum cake	4.48
Cotton seed cake	1.29
Potato dextrose broth	0.69
CD (P=0.01)	0.88

The present study clearly revealed that sugarcane molasses is the most economical medium (1 ton of molasses costs Rs. 600) for the mass production of *N. rileyi*. Since molasses is an excisable commodity, it may not be available readily to the private biocontrol laboratories involved in the mass production of fungal pathogens. Such laboratories can also select the agricultural byproducts or oil cakes, tubers and grains depending upon the availability and cost of production.

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