International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; SP-8(5): 163-165

Received: 22-07-2020
Accepted: 25-08-2020

Man Mohan Kumar
Department of Entomology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology SVPUAT, Meerut, Uttar Pradesh, India

Vishu Chaudhary
Department of Microbiology, College of Basic Sciences and Humanities, Punjab Agricultural University (PAU), Ludhiana, Punjab, India

Yogendra Kumar Mishra
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (JNKVV), Madhya Pradesh, India

Corresponding Author:
Man Mohan Kumar
Department of Entomology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology SVPUAT, Meerut, Uttar Pradesh, India

Assessment of media for mass culturing of *Lecanicillium lecanii*

Man Mohan Kumar, Vishu Chaudhary and Yogendra Kumar Mishra

DOI: https://doi.org/10.22271/chemi.2020.v8.i5c.10524

Abstract
Sustainable agriculture in the 21st century will increasingly rely on alternative for environmentally friendly pest management interventions. The specific micro-organisms are used as biocontrol agents against various agricultural pests worldwide. *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, *Lecanicillium lecanii* and *Hirsutella* spp. are the most important entomopathogenic-fungal pathogens used in the management of pests in agriculture. Many natural and synthetic substrates *viz*, sorghum grain, rice grain, farm manure, vegetable waste, vermicompost, sugarcane bagasse, neem seed kernel, sabouraud dextrose broth and potato dextrose broth were used during this study to estimate the sporulation of entomopathogenic fungi at temperatures of 26±2 ºC. Both solid and liquid media were tests of the *Lecanicillium lecanii* for mass multiplication. Among the various highest-evaluated substrates (13.34 spores / ml) was recorded on sabouraud dextrose broth, which was found to be significantly higher than all the substrates tested after potato dextrose broth spore (11.31 spores / ml), and the sugarcane bagasse medium was considered to be the least spore (1.36 spores / ml) of all the substrates tested.

Keywords: Entomopathogenic, *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, *Lecanicillium lecanii* and *Hirsutella thompsonii*

Introduction
Insect pests are a big factor in increasing crop productivity in tropical countries like India. Among all insect-like soil insects, borers, aphids, jassids and hoppers etc. India has a rich biodiversity of entomopathogenic and the use of these natural and renewable resources is important in a successful strategy for bio-control. Entomogenous fungi are potentially the most versatile biological control agents because of their wide range of hosting which often leads to natural epizootics. Entomopathogenic fungi, including genera with a broad geographic range, belong to the phylum deuteromycota. (Humber, 2000; Zimmermann, 1986) [4, 8]. Biological control agents are considered as substitutes or alternatives to conventional chemical insecticides believed to have toxic effects on nontarget organisms, including beneficial insects, livestock and other micro-organisms. Entomopathogenic fungi are essential regulatory factors of naturally occurring insect populations and attract attention as biocontrol agents for insect pests. Bioinsecticides, however, constitute a very small percentage of the overall demand for insecticides. But some major limitation to the development of bio-insecticides was that compared to chemical insecticides, since chemical insecticides have longer time and rapid effects on the pest population compared to after application bio-insecticides. Moreover, bioinsecticides have a short lifetime and a costly method of production compared with chemical insecticides. However, in agriculture, entomopathogenic fungi have good potential for control of insect-pests. The major issues involved in mass production and utilization of phytopathogens are a selection of effective strains, development of cost-effective methods for mass rearing, development of effective methods for storage and shipment, and creation of effective formulation.

Materials and Methods
Solid substrate *viz*, sorghum grain, rice grain, farm manure, vegetable waste, vermicompost, sugarcane bagasse, and neem seed kernel were used to estimate the sporulation of entomopathogenic fungi, at 26±2 ºC Table 3.4. 100 g of each substratum was washed and soaked overnight in water, and cooked until it was soft.

The excess water was drained by decanting and dry shade. The grains were packed separately into 250 ml conical flask and the flask's mouth was plugged with cotton and autoclaved for 20 minutes (min) at 15 pounds per square inch (psi) 120 ºC.After cooling, a 5 mm fungal disc of entomopathogenic fungi was inoculated into each flask under laminar airflow chamber by using the sterilized inoculation needle. Flasks were incubated in BOD incubator at 26 ± 2ºC. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grains and also to break the mycelia mat. Complete fungus growth appeared 10 -14 days after inoculation.

In 250 ml distilled water, 100 g of peeled and sliced potatoes were added, and the potatoes were boiled until they became soft. The beakers' contents were filtered through a muslin cloth and all liquid was squeezed out. 10 g of dextrose was dissolved in water and applied to the extract, up to 500ml of volume. Dispensed to each conical flask with 100ml and plugged with non-absorbent cotton. The flasks were sterilized at 15 psi pressure for 20 min in an autoclave. Following cooling, under laminar airflow chamber, a 5 mm fungal disk of entomopathogenic fungus was inoculated into each flask. Flasks had been incubated at 25ºC in BOD incubator. Two replications were maintained.

Taking 1000 ml of distilled water, inserting 10 g of dextrose and 2.5 g of peptone, distributing 100 ml of media into 250 ml of conical flask and plugging it with non-absorbent cotton. In an autoclave the flasks were sterilized at 15 psi pressure for 20 min. Upon cooling, under laminar airflow chamber, a 5 mm fungal disk of entomopathogenic fungus was inoculated into each flask. Flasks had been incubated at 25ºC in BOD incubator. It has maintained two replications.

After inoculation, observations on spore counting were made on the 7th, 14th, 21stand 28th days. A hemocytometer had been used to measure the spore of the fungus produced on various substrates. For this reason, 10g/10ml of homogenous grain / solution sample was taken from each duplicate of uniformly sporulated flasks and transferred in 250mL conical flasks to 100 ml sterilized distilled water containing Tween 80 (0.05 per cent) solution. The flasks were shaken for 10 minutes in a shaker. A double-layered muslin cloth was used to filter the suspension. After the serial dilution of the suspension, spores were counted using a Neubauer double-ruled hemocytometer to calculate the amount of conidia within 1 g/1ml of the substrate.

Results and Discussion

In this analysis, a variety of naturally available substrates in both solid and liquid media have been checked for *L. lecanii* mass multiplication. The success of insect pest microbial control depends not only on isolation, characterization, and pathogenicity, but also on the laboratory 's successful mass production of the microbial agents.

Large-scale pathogen availability is a prime requirement in the bio-control programmed. The agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication for a successful integrated pest management programmed.

Table 1: Media Substrates Spore concentration

Treat. No.	Media/Substrates	Spore concentration	Increase in growth of *V. lecanii* at different days after inoculation				
			7DAI	14DAI	21DAI	28DAI	Mean
	Solid Media						
T1	Sorghum grain +1% of YE	1 ×10^{7}spore / ml	3.49	6.25	11.32	17.39	9.61
T2	Rice grain +1% of YE	1 ×10^{7}spore / ml	3.20	5.94	8.65	8.73	6.63
T3	Farmyard manure (FYM) +1% of YE	1 ×10^{7}spore / ml	0.00	2.12	4.68	5.75	3.13
T4	Vegetable waste +1% of YE	1 ×10^{7}spore / ml	2.93	5.70	7.84	8.00	6.11
T5	Vermicompost +1% of YE	1 ×10^{7}spore / ml	0.00	1.60	2.80	2.86	1.81
T6	Sugarcane bagasse +1% of YE	1 ×10^{7}spore / ml	0.63	1.20	1.77	1.85	1.36
T7	Neem seed kernel (NSM) +1% of YE	1 ×10^{7}spore / ml	1.20	3.40	5.34	7.38	4.33
	Liquid Media						
T8	Sabouraud dextrose broth	1 ×10^{7}spore / ml	5.50	10.40	17.32	20.15	13.34
T9	Potato dextrose Broth	1 ×10^{7}spore / ml	4.53	8.70	14.32	17.71	11.31
	SEm$\pm$		0.12	0.09	0.14	0.09	0.11
	CD at 1%		0.35	0.26	0.43	0.27	0.33

DAI = Day after incubation YE = Yeast

The result showed that among the grain tested, *Lecanicillium lecanii* spore/ml production was significantly recorded higher 3.49, 3.20, and 2.93 spores/ml on sorghum grain +1% of YE, rice grain +1% of YE and vegetable waste+1% of YE (1X10^7 spore/ml). The spores/ml count recorded 1.20 and 0.63 at the respective temperatures on were Neem seed kernel (NSK) +1% of YE and Sugarcane bagasse +1% of YE slightly lesser. The different product s like Farmyard manure (FYM) +1% of YE, Vermicompost +1% of YE were recorded no spore/ml

The results showed that Lecanicillium lecanii spore / ml production was significantly increased on sabouraud dextrose broth and potato dextrose broth by 5.50 and 4.53 spores / ml among the liquid media tested.

The result showed that among the grain tested, *L. lecanii* spore/ml production was significantly recorded higher 6.25, 5.94, 5.70 and 3.40 spores/ml on sorghum grain +1% of YE, rice grain +1% of YE, vegetable waste+1% of YE, and neem seed kernel (NSK) +1% of YE, (1X10^7 spore/ml) respectively. The spore/ml count recorded 2.12, 1.60, and 1.20 at the respective temperatures on farmyard manure +1% of YE, vermin compost+1% of YE, and sugarcane bagasse1% of YE were slightly lesser.

The result showed that among the liquid media tested, *M. anisopliae* spore/ml production was significantly recorded higher 10.40 and 8.70 spores/ml on sabouraud dextrose broth and potato dextrose broth.

The result showed that among the grain tested, *L. lecanii* spore/ml production was significantly recorded higher 11.32, 8.65, 7.84, 5.34 and 4.68 spore/ml on sorghum grain +1% of YE, rice grain +1% of YE, vegetable waste+1% of YE, Neem seed kernel (NSK) +1% of YE and farmyard manure +1% of YE(1X10^7 spore/ml) respectively. The spore/ml count recorded 2.80 and 1.77 at the respective temperatures on,

vermin compost+1% of YE sugarcane bagasse1% of YE and was slightly lesser.

The result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 17.32 and 14.32 spore/ml on sabouraud dextrose broth and potato dextrose broth.

The result showed that among the grain tested, *L. lecanii* spore/ml production was significantly recorded higher 17.39, 8.73, 8.00, 7.38 and 5.75 spore/ml on sorghum grain +1% of YE, rice grain +1% of YE, vegetable waste+1% of YE, Neem seed kernel (NSK) +1% of YE and farmyard manure +1% of YE($1X10^7$ spore/ml) respectively. The spore/ml count recorded 2.86 and 1.85 at the respective temperatures on, vermin compost+1% of YE sugarcane bagasse1% of YE and was slightly lesser.

The result showed that among the liquid media examined, the output of *Beauveria bassiana* spore / ml on sabouraud dextrose broth and potato dextrose broth was significantly higher in 20.15 and 17.71 spore/ml.

Among the different substrates evaluated highest spores/ml 13.34 was observed on sabouraud dextrose broth which was significantly superior over all the substrates tested. Subsequent higher spore/ml load was recorded in 11.31 spores/ml followed by potato dextrose broth which was significantly better than other remaining substrates. The least spore/ml count was recorded on sugarcane bagasse medium 1.36 spores/ml.

The results showed that among the media tested, sorghum grain +1% of YE (9.61 spore/ml), rice grain +1% of YE (6.61 spore/ml), farmyard manure (FYM) +1% of YE(3.13 spore/ml), vegetable waste +1% of YE (6.11 spore/ml), vermicompost +1% of YE(1.81 spore/ml), sugarcane bagasse +1% of YE(1.36spore/ml), neem seed kernel (NSK) +1% of YE(4.33 spore/ml), sabouraud dextrose broth (13.34 spore/ml) and potato dextrose broth (11.31 spore/ml).Among the different substrates evaluated highest 13.34 spore/ml was observed on sabouraud dextrose broth which was significantly superior over all the substrates tested. The least spore/ml count was recorded on sugarcane bagasse medium 1.36 spore/ml. Lakshmi *et al*., (2001) [7] reported that sorghum is ideal cereal for mass production of *Lecanicillium lecanii* and Ibrahim and Low (1993)., Sharma *et al.* (2002) and Kumar M, Chaudhary V., (2019) [5, 6, 3] found rice, sorghum, groundnut, lentil and black gram as a suitable media for the mass culture of entomopathogenic fungus. According to Gopalakrishnan and Mohan (2000), Gopalakrishnan *et al*., (1999) and Tincilley *et al*., (2000) [1, 2, 7] reported that carrots were found to be the cheapest and best suited media for the large-scale production of deuteromycota fungi. The present study also supported this assumption, in which several naturally available substrates tested for mass multiplication of SDB, PDB, sorghum grain and rice entomopathogenic fungus are most suitable for growth and development.

Acknowledgements

The authors hardly thoroughly thank Dr. C. S. Prasad Professor, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut (U.P.) for his meticulous and exemplary guidance, unceasing and steadfast encouragement, and timely assistance provided during the investigation and manuscript preparation.

References

1. Gopalakrishnan C, Mohan SC. A simple and cost-effective *In vitro* method forthe mass production of conidia of Nomuraea rileyi. Insect Environ. 2000; 6:52-53.
2. Gopalakrishnan C, Anusuya D, Narayanan K. *In vitro* Production of Conidia of Entomopathogenic Fungus *Paecilomyces farinosus*. Entomology. 1999; 24:389-392.
3. Kumar M, Chaudhary V. Evaluation of media for mass culturing of *Metarhizium anisopliae*. Indian Journal of Entomology. 2019; 20:123-124.
4. Humber RA. Fungal pathogens and parasites of insects. In: Applied Microbial Systematics (Priest F, Goodfellow M, eds.), pp 199-227. Kluwer Acad. Publ., Dordrecht, 2000.
5. Ibrahim YB, Low W. Potential of Mass Production and Field Efficacy of Isolates of the Entomopathogenic Fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* on Plutella xylostella. Journal Invertebr Pathol. 1993; 39:222-232.
6. Sharma SP, Gupta RBL, Yadava CPS. Selection of a Suitable Medium for Mass Multiplication of Entomofungal pathogens. Indian Journal Entomology. 2002; 14:255-261.
7. Tincilley A, Easwaramoorthy G, Santhanalakshmi G. Attempts on mass production of *Nomuraea rileyi* on various agricultural products and by-products. Journal Biol Contro. 2000; 18(1):33-40.
8. Zimmermann G. The *Galleria* baits method for the detection of entomopathogenic fungi in soil. Journal of Applied Entomology. 1986; 102:213-215.