

Mass Production of *Paecilomyces Lilacinus* by using Different Cultivation Media as an Alternative of Incubator

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Received:- 01 September 2021/ Revised:- 11 September 2021/ Accepted:- 15 September 2021/ Published: 30-09-2021

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Abstract— *Paecilomyces lilacinus* is a common saprophytic, filamentous fungus. Morphological characters of *Paecilomyces lilacinus* were separate mycelium, hyaline, conidia white to pink colored and formation of phialides. The growth of *Paecilomyces lilacinus* carried out on SDA media at room temperature was better than incubator. Various solid substrates like Rice, Wheat bran, and Sorghum were evaluated for the mass multiplication of fungus *Paecilomyces lilacinus*. Added dextrose and antibiotics in solid media for mass multiplication at room temperature. Among all the substrate Wheat bran recorded the maximum spore count of 7.1×10^8 spore/ml followed by Sorghum 5.4×10^8 spore/ml and Rice 5.1×10^8 spore/ml after 20 days. Also dry mycelia weight or biomass of fungus *Paecilomyces lilacinus* without an incubator was more than using an incubator.

Keywords— *Paecilomyces lilacinus*, filamentous fungus, phialides, biomass of fungus, incubator.

I. INTRODUCTION

In recent years, few environmental issues have aroused the concern of the public as much as pesticides, especially in relation to the health of children. In spite of the many published studies on the subject of pesticides and human health, there remains deep controversy surrounding these crops. They are in a dilemma to either sacrifice a significant share of their crops to pests or use highly toxic pesticides that can harm human health and the environment. Bio pesticides are key elements of incorporated insect management programs, and are receiving much practical attention as a means to reduce the fill of artificial chemicals being used. After twenty years it was found that the level of synthetic pesticides were building and were not biodegradable and their harmful effects started coming out. There is a need to create bio pesticides which are effective, eco-friendly and do not leave any harmful effect on the environment. 'Bio pesticides' are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Bio pesticides also play an important role in providing pest management tools in areas where pesticide resistance, niche markets and environmental concerns limit the use of chemical pesticide products. The most widely Known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or BT, which can Control certain insects in cabbage, potato, and other crops.

Bio pesticides can be considered as dividing into three major classes:

1. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that can control certain weeds, and other fungi that can kill specific insects.
2. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, which interfere with mating, as well as various scented plant extracts that attract insect pests to traps.
3. Plant-Incorporated-Protectants are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the B.T.pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the B.T.bacterium, manufactures the substance that destroys the pest.

They impede the take up of water and nutrients and weaken the stand ability of affected plants. The nematode species involved are worldwide in their distribution and collectively cause billions of dollars of crop damage every year. The plant parasitic nematodes, the hidden enemies of farmers, cause an average annual loss of about 8. Plant nematodes are one of the most important and difficult pests to control in agriculture. Vegetative hyphae are branched and septate. *P.lilacinus* occurs naturally in soil, in egg clusters contained in the gelatinous egg mass of root-knot nematodes, and in cysts of *Globodera* spp. and *Heterodera* spp. In this respect *Paecilomyces lilacinus*, a facultative fungal parasite on eggs and females of root-knot nematodes, is a promising tool. Carrier used in the mass production system and application technology determines the successful use of bio agents against nematodes. It has been found in nematode eggs and occasionally from females of root knot and cyst nematodes. In addition, it has been frequently detected in the rhizosphere of many crops. It has a wide pH tolerance and can grow on a variety of substrates. *P.lilacinus* has shown promising results as a biocontrol agent to control the growth of destructive root knot nematodes.

II. REVIEW OF LITERATURE

Lysek first reported the association of the fungus *Paecilomyces* with the eggs of *Meloidogyne* spp. was affected by naturally occurring *P.lilacinus* and *Verticillium* sp. in soils of peanut collected from fields of Alabama, USA. Stirling and West reported that the considerable numbers of *Meloidogyne* eggs were parasitized by *P.lilacinus* in tropical and subtropical soils in Australia. The fungus *P.lilacinus* was reported to parasitize eggs of root knot nematode *M.incognita* (Jatala et al., 1979, Jatala, 1982, Gintis et al., 1983; Goodey et al., 1983). They also studied the effect of temperature on their growth and bio efficacy and reported that maximum growth as determined by dry weight of mycelium has occurred from 26-30o C where as it was least at 12- 36 C. Jatala reported that application of *P.lilacinus* to rhizosphere of oranges and lemons in Peru and reduced the damage caused by *M.incognita* and *Tylenchus semipenetrans*. Stephan and Al Din, standardised the optimum temperature requirement for the growth of *P.lilacinus* and reported it to be between 20-250 C and sporulation between 10-300 C. Mani et al. indicated that wheat, bajra, rice and jowar were suitable substrates for multiplication of *P.lilacinus* and also reported reduction of *Tylenchus semipenetrans* population with increased levels of *P.lilacinus*. Daisy worked on mass production of nematophagous fungi, *Paecilomyces farinosus* and *P.lilacinus*. Among different media tested for the mass production of *P.lilacinus*, 6% molasses medium supported significantly less mycelial growth compared to PDB whereas the biomass production and spore production were significantly high on 6% molasses. Among the oil cakes, *P.lilacinus* produced significantly more spores on cotton seed cake followed by groundnut and coconut cake. Sugarcane press mud, an Agricultural waste, supported the growth as well as significantly greater spore production of *P.farinosus* and *P.lilacinus* (10. Prabhu et al. mass produced *P.lilacinus* in different liquid media and evaluated them for growth and spore production. Viability of the spores was tested at frequent intervals and shelf life was up to 120 days in talc and flyash. Amala evaluated various solid substrates like rice bran, wheat bran, gingelly oil cake, coir pith and neem cake for the mass multiplication of *Paecilomyces lilacinus*. Mojumder et al. reported the application of neem and biocontrol agents *P.lilacinus* and *Verticillium chlamydosporium* singly or in combination reducing the reniform Nematode population on egg plant. Sixty days after sowing, the growth of okra plants was greater and the root knot nematode population was reduced in all the treatments compared to untreated control. Raja and Ranganathan evaluated the biocontrol potential of *Paecilomyces lilacinus* in field conditions in two seasons during 2005-2008 at Annamalainagar district of Tamilnadu. In the field evaluation of different doses and application methods of *P.lilacinus* viz., seed treatment, seedling treatments, soil application treatments and the integration treatments produced mixed results on the growth of okra. Rao et al. studied the bio-efficacy of a bio-nematicide *P.lilacinus* (*Purpureocillium lavenderum* Luangsa-Ard) for the management of *Meloidogyne incognita* on tomato at IIHR Bangalore and Kanpur.

III. MATERIAL AND METHOD

3.1 Material

- Glassware: - petri plate, test tube, beaker, flask, spreader, inoculating loop, funnel.
- Other: what man paper, aluminum foil, micro pipette, tips
- Equipment: - Autoclave, Laminar air flow, Incubator, weighing balance, burner.

3.2 Media

3.2.1 Solid media

- SDA: Composition for 1 liter: Peptone- 10 g, Dextrose- 40 g, Agar- 30 gm.
- Wheat bran
- Sorghum powder & sorghum seed
- Rice powder

3.2.2 Antibiotic

Chloramphenicol, Tetracycline, Amoxicillin, Gentamicin, Mancozeb+Metalaxyl, Fluconazole.

3.3 Methods

- Isolation Method
- Subculture Method
- Mass Multiplication Method
 1. Multiplication of *P.lilacinus* on sorghum powder with incubator
 2. Multiplication of *P.lilacinus* on sorghum powder without incubator
 3. Multiplication of *P.lilacinus* on wheat bran with incubator
 4. Multiplication of *Paecilomyces lilacinus* on wheat bran without incubator
 5. Multiplication of *P.lilacinus* on rice (poa) with incubator
 6. Multiplication of *P.lilacinus* on rice (poa) without incubator
 7. Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *P.lilacinus* growth on different content of moisture:
 1. Take 20 gm of A media powder in each 4 flask.
 2. Add different content of *P.lilacinus* suspension in each four A media flask.
 3. Add 4ml, 6ml, 8ml, 9ml suspension of *P.lilacinus*. In each 20gm of A media flask under laminar air flow and cap with cotton plug and apply aluminum foil paper.
 4. After 25°C, 7 days of incubation, the fungal biomass of *P.lilacinus* along with A media.

3.4 Parameters

3.4.1 CFU count by Hemocytometer

- Loading the Hemocytometer
- Counting cells in a hemocytometer:

$$Total\ cells/ml = Total\ cells\ counted \times \frac{dilution\ factor}{number\ of\ squares} \times 10,000\ cells/ml$$

So, for example, if you diluted your sample 1:1 with Trypan blue, and you counted 325 cells in 4 corner square plus the central big square.

$$Total\ cells/ml = 325\ cells \times \frac{2(dilution\ factor)}{5\ squares} \times 10,000\ cells/ml = 130 \times 10^4\ cells/ml$$

If you want to know how many cells you have in your original sample, just multiply the cell concentration by total sample volume. For example, if your original sample volume is 5 ml, than your sample has a total of:

$$130 \times 10^4\ cells/ml \times 5ml = 650 \times 10^4\ cells$$

3.4.2 Microscopic Examination

3.4.3 Biomass calculation of *Paecilomyces lilacinus*:

3.4.3.1 *Paecilomyces lilacinus* growth with incubator:

The dry weight of the fungus was calculated by using the following formula:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

3.4.3.2 *Paecilomyces lilacinus* growth without incubator:

The dry weight of the fungus was calculated by using the following formula:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

IV. RESULT

4.1 Zone of inhibition on different antibiotics dose on *Paecilomyces lilacinus*:

Gentamicin, Mancozeb, Mancozeb + Metalaxyl, and fluconazole which are antibacterial antibiotics which reduce the contamination of bacteria in culture plates during incubation periods.

TABLE 1
ZONE OF INHIBITION ON DIFFERENT ANTIBIOTICS DOSE ON *PAECILOMYCES LILACINUS*

Antibiotics	Zone of inhibition for 10 ml (mm)	Zone of inhibition for 100 ml (mm)
Mancozeb + metalaxyl	0.05	0.1
Gentamicin	0.02	0.3
Fluconazole	0.01	0.1
Mancozeb	0.5	0.8

[Zone of inhibition of different antibiotic dose on *P.lilacinus* on culture plate using Disc Diffusion method day 3 results]

- **100 ml and 10 ml concentration of different antibiotic dose combination :**

Zone of inhibition on *P.lilacinus* culture plate by using 100ml and 10ml concentration of different antibiotic dose combinations did not appear but inhibited contamination of bacteria.

4.2 Biomass calculation of *Paecilomyces lilacinus* with incubator and without incubator:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

4.3 Effect of different grain substrate medium on sporulation of *Paecilomyces lilacinus* with incubator:

P.lilacinus maximum spore production on wheat bran 3.4×10^{-8} . Rice and sorghum also produce good spore production.

TABLE 2
DIFFERENT GRAIN SUBSTRATE MEDIUM ON SPORULATION OF *PAECILOMYCES LILACINUS* WITH INCUBATOR

Grain (20g)	Spore count ($\times 10^{-4}$) (Series 1)	Spore count ($\times 10^{-6}$) (Series 2)	Spore count ($\times 10^{-8}$) (Series 3)
Rice	4.1	3.6	2.8
Wheat bran	5.9	4.02	3.4
Sorghum	4.8	4.01	3.2

4.4 Effect of different grain substrate medium on sporulation of *Paecilomyces lilacinus* without incubator:

P.lilacinus maximum spore production on wheat bran 7.1×10^{-8} which are more than with incubator method. On rice and sorghum spore production is also more than with the incubator method.

TABLE 3
DIFFERENT GRAIN SUBSTRATE MEDIUM ON SPORULATION OF *PAECILOMYCES LILACINUS* WITHOUT INCUBATOR

Grain (20g)	Spore count (10^{-4}) (Series 1)	Spore count (10^{-6}) (Series 2)	Spore count (10^{-8}) (Series 3)
Rice	6.5	5.8	5.1
Wheat	9	8.4	7.1
Sorghum	7.3	6.6	5.4

4.5 Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *P.lilacinus* growth on different content of moisture:

Various concentration volume of suspension in A media and measured growth of *P.lilacinus*.

TABLE 4
***P.LILACINUS* GROWTH ON DIFFERENT CONTENT OF MOISTURE**

Volume of Suspension	Growth of <i>P.lilacinus</i>
4 ml	60%
6 ml	69%
8 ml	86%
9 ml	90%

V. DISCUSSION

Recently, Paecilomyces lilacinus based different formulations are used for the control of nematode diseases, which is economical, eco friendly and sustainable in the long run. Among the microorganisms, bioagents are having great promise with the dual advantage of plant growth promotion and plant disease suppression. First, observed Zone of inhibition of different antibiotics 100 ml and 10 ml concentration for reducing contamination of bacteria. Different antibiotics did not reduce the growth of fungus P.lilacinus, only inhibited bacterial contamination. Evaluated Biomass of P.lilacinus without incubator results were better than with incubator. Also find out that the growth of P.lilacinus appeared earlier on without incubator culture plates. For reducing contamination of bacteria using different combinations of antibiotics and measuring biomass of P.lilacinus fungus on SDA media plates. Various solid substrates like Rice, Wheat bran and Sorghum were evaluated for the mass production of fungus P.lilacinus. Various solid substrates like Rice, Wheat bran and Sorghum were evaluated for the mass production of fungus P.lilacinus. Added dextrose and combination of antibiotics, without incubator production, among all substrate Wheat bran recorded maximum spore count of 7.

VI. SUMMARY AND CONCLUSION

The present investigation "Mass production of Paecilomyces lilacinus by different methods given by vise organization" was carried out at the Department of Microbiology, Aspee Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, and Gujarat, India during 2018. The results obtained from the present study can be summarized and concluded here. Nematophagous fungus Paecilomyces lilacinus infect nematodes with their spores which either adhere to the surface of nematodes or are swallowed by them. Ultimate result of nematode infection in any way is always the death of the host. Different antibiotics did not inhibit the growth of fungus P.lilacinus, only reduced contamination of bacteria, which helped in the mass multiplication of P.lilacinus without an incubator. Various solid substrates like Rice, Wheat bran, and Sorghum were evaluated for mass multiplication and observed without incubator growth of P.lilacinus was better than incubator. Also observed without incubator growth of fungus P.lilacinus starts earlier than with the help of incubator. From this data concluded that without incubator also carried out mass multiplication of P.lilacinus fungus, which very helpful to industry and also farmers can easily produce biopesticides. Which helps in reduced cost of biopesticide

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