Journal of Plant & Agriculture Research

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Research Article

STUDIES ON THE PERFORMANCE OF DIFFERENT LIQUID MEDIA FOR SPORULATION OF METARRHIZIUM ANISOPLIAE UNDER LABORATORY CONDITION

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Received January 20, 2018; Accepted February 22, 2018; Published March 29, 2018;

Cite This Article: Wayal, N., Mehendale, S., Raut, P., Naik, K., Joshi, M.(2018). Studies on the performance of different liquid media for sporulation of Metarrhizium anisopliae under laboratory condition. Journal of Plant & Agriculture Research, 3(1).1-4

ABSTRACT

The present research was carried out to standardize the method for mass culturing the green muscardin fungus, *M. anisopliae* under konkan conditions under laboratory conditions during 2015-17. Study indicated that the mass multiplication of *M. anisopliae* on liquid / broth medium showed that the T-6 Czeapeks broth medium was the most suitable medium for mass multiplication of *M. anisopliae* (average mat weight of dry fungus 1554 mg), after 20 days of inoculation which was at par with T_5-corn flour medium (1545.67 mg)., which also supported good growth of the fungus.

KEY WORDS: *M. anisolpae,* Aphid, liquid medium.

INTRODUCTION

The use of pesticides has gradually become a part of our modern agricultural practices and its consumption has increased remarkably in the past, causing serious health and environmental problems in developing countries including India. Chemical control is generally practiced by farmers for higher gains, but its injudicious use has created many problems. Sole reliance on chemical control leads to problem of pesticide resistance, resurgence of pests, pesticide residues, destruction of beneficial fauna and environmental pollution.

Microbial control has been considered as an important tool in IPM to conventional chemical control. The microorganisms like bacteria, virus, fungi, protozoa, rickettsia and nematodes have the capacity to affect the pest. *Bacillus thuringiensis* (bacteria) is very effective in controlling many lepidopterous larvae like cabbage worm, sugarcane stem borer etc. Nuclear polyhedrous viruses (NPV) have the effective control over *Spodoptera liturra* (Fabricius) and *Helicoverpa armigera* (Hubner). Entomopatogenic fungi are employed as biocontrol agents reducing pest population and consequently their damages in different agro-ecosystem [1].

The most important species of fungus, *M. anisopliae* and *Beauveria bassiana* (Balsamo) Vuillemin are insect pathogenic fungi which have to meet several host challenges like producing enough new infectious spores in each operation for maintaining viable population. The green muscardin fungus *M. anisopliae* (Deuteromycotina: Moniliales) is already reported to be very useful fungus for the management of many insect pests. In India, [2] first reported the said fungus inhabiting the breeding site of *Oryctes rhinoceros* L. After great exploratory surveys and pathogencity studies, many workers have suggested that the fungus could be effectively used in microbial control of some other pest. Soil is the main reservoir for many entomopathogenic fungi, but only a few strains obtained from soil have been used against insect pest. [3] found it to have a wide distribution as that of the white muscardine fungus, *B. bassiana.M. anisopliae* is an important candidate among the entomopathogenic fungi, for use in bio-intensive pest management strategies.

The key factor which decides the success and adaptability of a bio-agent is its easy availability in time and space at affordable cost. *M. anisopliae* being a facultative fungal pathogen, which can grow on organic material and readily, sporulate on semi synthetic media like PDA or carrot malt agar. Natural media, which are invariably rich in Carbon and nitrogen were, proved to support the growth and sporulation of the fungus. The most convenient and durable development stage of hypomycetes fungi is the dusty spores (conida) which are easy for application and storage and also a natural distributive stage.

In Konkan region, agricultural residue or waste material like *Nagli* husk, rice husk, banana pseudo-stem and hotel waste tea powder, sugarcane baggase etc. are found in large amount. Coconut water is also available in abundant quantity. These raw waste materials are available in market at cheaper cost. With a view to generate more information on different aspects of the efficacy of different media on sporulation of the fungus, *Metarhizium* and its effectiveness as an biological control agent of the pest aphid, *Aphis craccivora* (Koch) present study were undertaken.

MATERIALS AND METHODS

The present investigation was carried out in Quarantine laboratory of "Plant Pathology Department and Agricultural Entomology Department, Dr.BalasahebSawant Konkan Krishi Vidyapeeth, Dapoli, Dist: Ratnagiri (M.S.) during the academic year 2015-2017. The details of the various laboratory chemicals used in the present investigation for media preparation are given below:

Chemicals for media preparation

i. Sucrose as a energy source
ii. Agar-agar as a solidifying agent

Chemicals for surface sterilization

i. Mercuric chloride ($HgCl_2$)
ii. Ethyl alcohol (70 %)

Glass wears

i. Conical flasks of capacity 250, 500 ml
ii. Beakers of capacity 500 ml
iii. Petri plates of size 100 x 20 mm
iv. Pipettes of capacity 10 ml
v. Micropipettes of capacity 100-1000 µ
vi. Measuring cylinders of capacity 10 and 1000 ml

Laboratory Equipments

i. Refrigerator.
ii. Hot air oven
iii. Electronic Digital balance.
iv. Autoclave.
v. Laminar air flow bench.
vi. Incubator.
vii. Others

Trays, caps, Polypropylene bags, Aluminium foil, Non-absorbent cotton, Spirit lamp or Gas burner, Forceps, Bacterial needle, and Cork were usedfor maintaining the aseptic culture.

Experimental Conditions

All *In vitro* studies were carried out aseptically in laminar air flow chamber. The Experiments were conducted under well-defined conditions of culture room maintained at 25 ± 2^0C temperature, uniform light (1600 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 16/8 hours.

Culture medium (liquid)

The treatments details given in Table No.1

Table 1: Composition of liquid medium (For 100 ml).

Treatment No.	Broth/liquid Medium	Quantity for 100 ml
T1	Coconut water	100 ml
T2	Carrot broth	
	Carrot	25 g
	Dextrose	2.0 g
	Water	100 ml
T3	Potato dextrose broth	
	Potato	25 g
	Dextrose	2.0 g
	Water	100 ml
T4	Coon's (broth) medium	
	Sucrose	0.72 g
	Dextrose	0.36 g
	Magnesium sulphate	0.12 g
	Potassium nitrate	0.20 g
	Distilled water	100 ml
T5	Starch (Corn flour) + water	
	Corn meal	2 g
	Peptone	2 g
	Dextrose	2 g
	Distilled water	100 ml
T6	Czeapek'sDox medium	
	Sucrose	3 g
	Sodium nitrate	0.2g
	Potassium diphosphate	0.1 g
	Magnesium sulphate	0.05 g
	Ferrous sulphate	0.05 g
	Distilled water	100 ml
T7	Molasses + yeast + water	

Table 2: Mass multiplication of M. anisopliae on liquid/broth medium.

Treat. No.		Mycelial (Mat) Production
	Treatment	RI
1	Coconut water	1014
		(31.84)*
2	Carrot broth	1167
		-34.16
3	Potato dextrose broth	998
		-31.59
4	Coon's medium	697
		-26.4

METHODOLOGY

Standardization of media for mass multiplication of *M. anisopliae*.

A master culture of the test fungus, *M. anisopliae* was obtained from Biocontrol laboratory, Department of Agricultural Entomology college of Agriculture, Dapoli and used for mass multiplication. From this, inoculated test tubes were maintained at $26^0C \pm 2^0C$ in an incubator till sporulation and the master culture was maintained in refrigerator. Mass multiplication of *M. anisopliae* using different solid and liquid media is discussed below.

Mass multiplication on broth liquid/media

For the multiplication of *M. anisopliae*, different broth/liquid media were used as mentioned below.

Experiment details

Statistical design: - CRD (Complete randomized design)
No. of repetition: - 3
No. of treatments: - 9

Table 3: Details of various liquid media for sporulation of *M. anisopliae.*

Treat. No.	Broth/liquid Medium	Volume (ml)
T1	Coconut water	100
T2	Carrot broth	100
T3	Potato dextrose broth	100
T4	Coon's (broth) medium	100
T5	Starch (Corn flour) + water	100
T6	Czeapek'sDox medium	100
T7	Molasses + yeast + water	100
T8	Gram flour + Water	100
T9	Control (Water)	100

Coconut water: The tender coconut water (100 ml) was drawn out in 250 ml conical flask aseptically. The flask was plugged with non-absorbent cotton and sterilized in autoclave at 121^0C at 15 psi for 1 h and the same was used as medium.

Potato dextrose broth: Peeled potato (25 g) was mixed in 100 ml water and boiled to have extract. Only extract of potato and dextrose (20 g) were mixed and filled in 100 ml conical flask. Volume adjusted to 100 ml by adding required distil water, flask were then autoclaved as usual.

Carrot broth medium: Peeled carrot (25 g) was mixed in 100 ml water and boiled to have extract. Only extract of carrot and dextrose were mixed and filled in 100 ml conical flask. Volume adjusted to 100 ml by adding required distil water, flask were then autoclaved as usual.

Molasses + yeast + water, Gram flour + Water, Czeapek's broth medium and Coon's medium: known quantity as mentioned earlier was mixed in distilled water (100 ml), poured in conical flask (100 ml), plugged with non-absorbent cotton and sterilized in autoclave at 121^0C for 1 h. After sterilization, flasks were kept for cooling. With the help of cork (size 5 mm) one bit of PDA containing *Metarhizium* was inoculated in each flask in aseptic condition. After inoculation, the flasks were incubated at room temperature 28^0C. After 3 days of inoculation mycelial growth was developed on broth media.

Method of determining mat weight

After 3-4 days, the mycelial mat of *Metarhizium* was monitored on surface of the liquid media. After sufficient development of the mat (20 days after inoculation), the media was filtered through funnel fitted with filter paper (What man # 2). Each filter paper disc was initially weighed. Mat was collected along with filter paper and remaining suspension was stored in the conical flask. The mat was dried in hot air oven at 100^0C for 5 min and then the weight of the mass taken by deducting filter paper weight.

RESULTS AND DISCUSSION

Mass multiplication of *M. anisopliae* on liquid/broth medium.

In order to obtain maximum growth of the *M. anisopliae* within shortest period, the efforts were made to multiply it on various liquid or broth media. The results of a statistically designed laboratory experiment are presented in Table 2. The maximum mycelium mat weight after 20 days of inoculation was recorded in T_6- Czeapek's broth medium (1554 mg) which, was at par with T_5- corn flour medium (1545 mg). Further, next best treatment T_8-Gram flour medium (1196 mg) was at par with T_2- Carrot broth (1121 mg) and T_1- Coconut water (1064 mg). Also T_2- Carrot broth was at par withT_1-Coconut water andT_7- Molasses (985 mg). Further, Molasses was at par withT_3- potato dextrose broth (938mg). In control treatment T_9- Sterilized distilled water no fungal mat was recorded. However, 558 mg weight of the bit of agar which was initially inoculated in the sterile distilled was recorded. Among the liquid media tested, T_4- Coon's medium was found to be the weakest medium which produced lowest number of spores (661mg) at 20 days of inoculation.

The results in general revealed that the Czeapeks medium was found to be the best suitable liquid medium for mass multiplication and was found at par with corn flour medium. Further, two flour media and coconut water also emerged as promising media for mass production of *M. anisopliae*. [4] Reported that the aseptically drawn out coconut water was the superior and cheapest medium for mass production of *M. anisopliae*. During present investigation also coconut water gave better fungal development. Further, [5] revealed that Sabouraud's Dextrose Yeast Broth medium was the best with regards to biomass production, conidial count (4.80×10^7 conidia ml^{-1}) of *M. anisopliae*and *B. bassiana.* Thus the present findings are in accordance with that of above findings.

CONCLUSION

During present investigations, Studies on the mass multiplication of *M. anisopliae* on liquid / broth medium showed that the T-$_6$Czeapeks broth medium was the most suitable medium for mass multiplication of *M. anisopliae* (average mat weight of dry fungus 1554 mg), after 20 days of inoculation which was at par with T_5-corn flour medium (1545.67 mg). Further, Treatment T_8-Gram flour medium (1196 mg) was at par with T_2-Carrot broth (1121 mg) and T_1- Coconut water (1064 mg), which also supported good growth of the fungus.

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