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STUDIES ON THE PERFORMANCE OF DIFFERENT SOLID MEDIA FOR SPORULATION OF *Metarrhizium anisopliae* UNDER LABORATORY CONDITION

Nirmala D. Wayal, S. K. Mehendale, P. P. Raut, V. S. Desai and Kumud V. Naik

Department of Agril. Entomology, College of Agriculture,
Dr. Balasaheb Konkan Krishi Vidyapeeth, Dapoli. Dist. Ratnagiri (M.S.)

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Abstract: *The present research was carried out to standardize the method for mass culturing the green muscardin fungus, M. anisopliae under konkan conditions and to test the efficacy of M. anisopliae against Aphis craccivora (Koch) under laboratory conditions during 2015-17. Study indicated that treatment T_8-Banana psuedostem was the most suitable solid medium for multiplication of the fungus under Konkan condition. Mean spore production of T_8-Banana psuedostem after 20 days of inoculation was 951.3 x 10^8 spores per gram which was at par with T_2-Tea waste (930.67 x 10^8) and was second best media. Whereas, Sugarcane baggase produced lowest number of spores (41.33 x 10^8) at 20 days of inoculation of the fungus.*

Key words*: M. anisolpae, Aphid, Solid medium.*

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, Inglis *et al.* (2001).

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, Nirula (1957) 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. Steinhaus (1949) 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

- Refrigerator.
- Hot air oven
- Electronic Digital balance.
- Autoclave.
- Laminar air flow bench.
- Incubator.

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 (solid)

The treatments details given in Table No.1

Table 1: Composition of solid medium

Treatment No.	Media	Weight (g)
T1	Sorghum grain (Standard medium)	50
T2	Tea waste (without sugar and milk)	50
T3	*Nagli* husk	50
T4	Soybean chunks	50
T5	Sugarcane baggase	50
T6	Wheat grain	50
T7	Tea waste (with sugar and milk)	50
T8	Banana psuedostem	50

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.

Experiment details:

Statistical Design: - CRD (Complete randomized design)

No. of Repetition: - 3

No. of Treatments: - 8

Method of preparation and application of fungal suspension of solid medium

Five g of each solid medium along with fungal spores was thoroughly agitated in 100 ml of sterilized distil water in conical flask. The suspension simply filtered with muslin cloth to separate out the debris of substrate, and collected in same conical flask again. This suspension was filled in 1 saloon spray and was calibrated 5 times by spraying the material once which, was measured as 0.5 ml. Thus uniform application of spray material was achieved.

Mass multiplication on solid media

For the multiplication of *M. anisopliae*, different solid media were used as mentioned above. In this experiment, dry grains of Wheat, Sorghum, Soya chunks were purchased from local market. Agricultural residues like Banana pseudo stem, *Nagli* husk, and Sugarcane baggase as well as hotel Tea waste were collected from college campus. The grains (150 g) were taken and boiled in tap water for 25 minutes to hold the maximum moisture for better growth and sporulation of *M. anisopliae*. The same were cooled and kept as 50 g boiled grains per three different plastic polypropylene bags having capacity 500 gm. Banana psuedostem, *Nagli* husk, Sugarcane baggase and Tea waste were soaked in water for 3 h to hold maximum moisture for growth and sporulation of *M. anisopliae*. The excess water was drained out from the media. The material was placed in polypropylene bag, closed by putting non-absorbent cotton plug and then sterilized in autoclave at 121^0C, 15 psi for 1 h. After sterilization, bags were kept for cooling. With the help of cork (size 5 mm), abit of PDA containing *Metarhizium* was removed and inoculated in each bag in aseptic condition. After inoculation, the bags were incubated at room temperature (28^0C). After 3 days of inoculation, mycelial growth developed on grains and organic residues was mixed thoroughly in the bag to enhance faster growth of the fungus.

Method of spore count:

After 20 days of inoculation, the spore mass developed on grains and also other media was taken at 1 g from each polypropylene bag and crushed properly in small amount of sterilized water and filtered with the muslin cloth. Further, the volume was adjusted to 10 ml in each of the test tube and all test tubes were well shaken. Then eight test tubes, each containing 9 ml sterilized distilled water were arranged serially. With the help of a micropipette (1000 µl), 1 ml spore suspension was taken from the test tube containing 10 ml stock suspension and was added in the 1^{st} test tube containing 9 ml sterilized distilled water. In this way, the total volume in the first test tube became 10 ml and it was the first dilution of spore suspension. This way 1 ml quantity of spore suspension from first test tube was transferred to another test tube containing 9 ml sterilized distilled water to make volume of second test tube 10 ml and this was the second dilution of spore suspension. After repeating the same procedure of dilution next dilutions were made up to 8^{th} test tube. In this way, eight dilutions *viz.* 1:10, 1:100, 1:1000, 1: 10000, 1:100000, 1:1000000, 1:10000000, 1:100000000 were available in 1 to 8 test tubes, respectively. From 8^{th} test-tube, 0.5 ml representative suspension was taken in amicropipette aseptically and transferred at the center of sterilized Petri plate. Three such Petri plates were prepared. The sterilized PDA medium was melted, cooled to 40^0C and one pinch (500 mg)crystals of antibiotic,

streptomycin was added aseptically to prevent the bacterial growth. The plates were shaken gently to mix the medium and spore suspension. The plates were incubated at room temperature and fungal growth was monitored after 24 h. Colonies from each plate were counted by digital colony counter within 24-36 h from inoculation.

Results and Discussion

During the present investigation the efforts were made to obtain maximum sporulation of *M. anisopliae* within a shortest period and also a cheaper medium which will suffice the said purpose. For the purpose of which, the grains like, Sorghum, Wheat and also some agricultural residues like Banana pseudostem, Sugarcane baggase, *Nagli* husk, Tea waste and Soybean chunks, were tested as a solid media. The results of a statistically designed laboratory experiment are summarized in Table 2.

The result indicated that there were significant differences among various treatments. The higher conidia production after 20 days of inoculation was recorded in the treatment T_8- banana pseudo stem with 951.33 x 10^8spores per g which, was at par with T_2-Tea waste (930.67 x 10^8) which also supported good growth of the fungus. Among other treatments, treatment T3-*Nagli*husk produced 528.67 x 10^8spores followed by T_1- Sorghum grain (454.67 x 10^8) which at par with T_7-Hotel tea waste (397.33 x 10^8) while other treatments *viz.,* T_6- Wheat grain and T_4-Soybean chunks produced 327.33 x 10^8, 172 x 10^8spores, respectively. The minimum spore production was recorded in sugarcane baggase (41.33 x 10^8).The results in general indicated that amongst various solid media tested, the banana pseudo stem was found to be the most suitable medium for mass multiplication of *M. anisopliae* followed by Tea powder waste. Among these two media banana pseudo stem contains rich cellulose. Similarly, tea powder waste contains dried tea leaves which, also represents cellulose material. This might have favored mycelial growth and more number of spores. Banu (2012) obtained maximum sporulation of fungus *Lecanicilliumlecanii*(Zimmerman) on solid state fermentation with rice grains (9.84 x 10^8 spores g^{-1}) followed by wheat grains (9.12 x 10^8 spore's g^{-1}). Sharma *et al.* (1997) and Bhide (2001) obtained the maximum sporulation of *M. anisopliae* on sorghum grains.Thus the results of present investigation are in line with above.

Conclusion

During present investigations, studies on the mass production of green muscardin fungus, *M. anisopliae* on solid media indicated that treatment T_8-Banana psuedostem was the most suitable solid medium for multiplication of the fungus under Konkan condition. Mean spore production of T_8-Banana psuedostem after 20 days of inoculation was (951.3 x 10^8) spores per gram which was at par with T_2-Tea waste (930.67 x 10^8) and was second best media. Whereas; Sugarcane baggase produced lowest number of spores (41.33 x 10^8) at 20 days of inoculation of the fungus.

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