

ACQUINTANCE OF MASS MULTIPLICATION OF IMPORTANT ENTAMOPATHOGENOIC FUNGI

With the ever increasing awareness of the harmful effects of the chemical pesticides on man and his environment the immediate need for sustainable, eco-friendly pest management has been felt very strongly providing an impetus to research and development of microbial pesticides. Among the different microbial agents developed and tested, bacteria, viruses and fungi are considered promising for the control of insect pests.

Microbial agents that kill insects can be exploited successfully only after a reliable method of production is developed. Problems that are to be addressed are identification of virulent isolates/strains, retention of pathogenicity, process development for productivity in terms of yield in the commercial production and storage mechanism of ensuring retention of the desirable features. Various basic techniques to be employed for the utilization of entomopathogenic fungi in insect pest control are discussed.

1. Multiplication on media

Beauveria bassiana can be easily multiplied on Potato dextrose agar and Saboraud's dextrose agar (SDAY) while *Nomuraea rileyi* has specific growth requirements and can grow well on rich media like Saboraud's maltose agar (SMAY).

(A) Semisynthetic media

Potato Dextrose gar (PDA)	Saboraud's dextrose agar (SDAY)	Saboraud's maltose agar (SMAY)
Potato – 200g	Agar – 15 g	Agar – 15g
Dextrose – 20 g	Peptone – 10 g	Peptone – 10 g
Agar – 20 g	Dextrose – 40 g	Maltose – 40 g
Distilled water – 100 ml	Yeast extract – 5 g	Yeast extract – 5 g
	Distilled water – 1000 ml	Distilled water – 1000 ml

For preparation of PDA, potatoes are peeled and sliced. 20 g of the potato pieces are boiled in water for 10 minutes and the extract obtained by filtration is made up with water to 1000 ml after cooling. The dextrose and agar are added to the extract, melted and dispensed into glass tubes (10 ml/tube), 25 ml conical flask and autoclaved at 15 psi for 20 minutes.

For SDAY and SMAY media, add all the medium components to the water and dissolve by heating. Add chloramphenicol @ 80 mg/l. Dispense into glass tubes (10 ml/tube), 25 ml/250 ml conical flask, Autoclave.

B. Sorghum

Crush the sorghum grains in a mixer to get broken pieces. Weigh 25 g of the crushed sorghum in a 250 ml conical flask and add 22.5 ml of 1% yeast extract solution. Soak overnight at 25°C. Plug the flasks and autoclave. Immediately after cooling, break the clump of sorghum aseptically using a blunt forceps. Add dry spore of *Nomuraea rileyi* to the sorghum using a micro-spatula. Shake well to disperse the spore evenly in the medium and incubate the flasks at 25°C in the dark.

C. Barley

5 g of crushed barley is taken in a 250 ml conical flask and 45 ml of distilled water containing 0.125 g of yeast extract. The flask is plugged with non-absorbent cotton and autoclaved. After cooling, the flasks are inoculated aseptically with dry spore of *N. rileyi*, mixed well using a sterile spatula. The flask is plugged and incubated at 25°C in the dark till mycelia growth and sporulation is complete.

II. Mass Multiplication

Take 250 g of wheat bran in a polythene cover, add 250 ml of distilled water containing 10 g of molasses and 1.25 g yeast extract. Autoclave at 15 psi for 20 minutes. After cooling, cut one end of the cover (corner) in a laminar airflow, add aseptically dry powder of *B. bassiana* from a PDA slope, insert a sterile tube containing non-absorbent cotton in the cut end and seal it. Incubate at 25°C. Mycelial growth appears on 3rd day and continues for 3 days. The substrate with the mycelium is transferred to a sterile plastic tray/tub and covered tightly with a polythene sheet using a rubber band. Aeration is given everyday in the laminar airflow. The sporulation starts on 6th and continues till 10 days. The tray is transferred to the refrigerator and kept for the week. The tray is then removed, the cover is opened and the tray kept in shade for drying. The substrate is dried and the dry spore is harvested by sieving through a muslin cloth. The spore is stored in the refrigerator and used when required.

III. Multiplication on larvae

Prepare a suspension of the fungal conidia (2×10^8 conidia / ml) in 0.02% sterile tween-80 solution containing 0.05% streptomycin sulphate. Wash the leaves and allow the moisture on the leaf surface to dry keeping only the stalks dipped in water. After drying keep the leaf stalk in moist cotton plugs to keep the leaves fresh for the least 48 h. Apply/spread the spore suspension on the upper leaf surface and when still wet, release 6-7 days old host insect larvae. Freshly molted larvae should be preferred. Alternately, 2-3 leaves can be kept in a small conical flask or bottle with the stalks dipped in water and can be sprayed with the spore solution. Transfer the leaves along with the larvae to a plastic tub/container that has been thoroughly disinfected. Allow the larvae to feed on the treated leaves for 48 h and then transfer to fresh castor leaves. In case of *H. armigera* larvae, they should be maintained individually. Here, infection can be caused by surface treatment of diet pieces with the spore solution.

Mortality starts on 6th day generally. Take out the dead larvae (cadavers) and wash them with tap water followed by sterile tap water containing 0.05% streptomycin sulphate. Blot them dry on a blotting / tissue paper and transfer to a petri plate containing a thin layer of moist cotton covered by tissue paper. Incubate in the dark at 25^oC. Mycelial growth starts after 24 hrs and sporulation starts after 48 hrs. After 2-3 days, transfer the petri plates to the refrigerator and store. The larvae can be placed for sporulation in plastic tub on sterile moist mud covered with a news paper and cover the tub with a polythene sheet.

Record Work:

Collect insect cadavers infected by entomopathogenic fungi.

ACQUAINTANCE OF MASS MULTIPLICATION TECHNIQUE OF NPV

Pathogens used in insect control include viruses, bacteria, fungi, protozoans and nematodes. Their utilization for insect control requires their mass production either on natural host insects or using synthetic media.

Viruses :

Among viruses of the group baculoviridae, nuclear polyhedrosis viruses are utilized for the successful suppression of various insect pests of many agricultural and horticultural crops.

Nuclear polyhedrosis viruses of *Helicoverpa armigera* and *Spodoptera litura* are highly specific to their respective live hosts for multiplication. So production of viruses for use as insecticides needs mass production of their hosts as a first step. Basic steps in the production of nuclear polyhedrosis viruses of any insect are

1. Mass culturing and maintenance of host insects
2. Host inoculation with viruses
3. Harvesting of viruses
4. Purification
5. Storage

Host insects can be reared either on their natural host plants (foliage, pod, fruit etc) or on artificial diet. Since natural host plants cannot be found throughout the year, maintenance of host insects on an artificial diet has the advantages of rearing under sterile conditions, avoiding contamination, saving space, time and labour. Thus the use of an artificial diet for mass production of host insects is economical and easy.

Mass culturing of host insects can be started either from field collected adults using light traps or from field collected larvae reared to adult stage in the laboratory. Production starts with the availability of males and females of the host insects that are allowed in a oviposition jar having 10% honey and water soaked cotton wads placed separately inside in lids as adults food and its top covered with muslin cloth pinned with few muslin cloth strips hanging inside the jar.

Eggs laid on cloth are collected daily and surfaces sterilized by soaking in 0.15 % sodium hypochlorite for 5-10 minutes. Eggs settling at the bottom of the container are collected and incubated in petri dishes.

Rearing of newly hatched *H.armigera* larvae is done by transferring individually (since the larvae are cannibalistic) into rearing vials containing artificial diet. In case of *S.litura*, since the eggs are laid in masses and larval stages are not cannibalistic, first two instars can be reared in groups or castor leaves.

Preparation of artificial diet:

<i>Ingredients</i>	<i>Quantity</i>
Kabuli gram flour	: 105.00 g
	: 1.00 g
<i>Sorbic acid</i>	
Methyl para hydroxyl benzoate	: 2.00 g
Ascorbic acid	: 3.25 g
Yeast tablets	: 10.00 g
Agar-agar	: 12.75 g
Formalin (10%)	: 2.00 ml
Vitamin E	: 2 capsules
Multivitaplex	: 2 capsules
Streptomycin sulfate	: 0.25 g
Water	: 780.00 ml

Gram flour yeast tablets, methyl parahydroxy benzoate and sorbic acid are added to half the quantity of water in a blender and mixed for 2-3 minutes. Simultaneously agar-agar is boiled with remaining quantity of water and cooled down to 70⁰C. Hot agar liquid is added to the blender and mixed with other ingredients. Finally multivitaplex, vitamin E, ascorbic acid and formalin are added and blended for about 2 minutes.

Hot liquid diet is dispensed into rearing vials/tubes and allowed for solidification at room temperature for about 20 minutes. Tubes/vials are arranged in plastic/aluminium trays. Single larvae should be introduced in each vial on the diet surface and closed with cotton plug. The trays can then be stacked on racks. While 20% of the larval population can be allowed to pupate for continuous maintenance of host insect culture, the rest can be used for virus production.

Mass production of viruses :

Infestation of 8 to 9 day old larvae is done by inoculating the surface of the diet poured in plastic cups (as against in vials/tubes used for healthy host insect culture), with a virus dose of 1.1×10^4 polyhedroinclusion bodies per larva. Larvae are placed individually on virus contaminated diet in cups and capped. The containers are arranged in plastic trays and stacked on racks.

Observations on larval mortality is made daily and the moribund/dead larvae with viral symptoms (cuticle of the larvae becomes fragile and ruptures easily when touched; body colour changes to blue or bluish purple) are harvested and placed in conical flask.

The diseased/dead larvae are macerated in a mixture using sterile distilled water. The contents are allowed as such in conical flasks for several days at room temperature, during which time the polyhedra settle down as a white layer. Alternatively, macerated contents can be sieved through a double layer of muslin cloth and the filtrate is standardized for the PIB/ml using a haemocytometer for field use. 250 larval equivalents (LE)/Ha; One LE = 6×10^9 PIB