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RESEARCH ARTICLE

MASS PRODUCTION OF ENTOMOPATHOGENIC FUNGI *Trichoderma viridae, Pacilomyces liliaceae* AND *Verticilium lecani*

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ABSTRACT

Trichoderma viridae Pacilomyces liliaceae Verticilium lecani are the three Entamopathogenic fungi which are used to study the molasses as a source of their mass multiplication. Generally there are Fungi to control insects, weeds and to control other fungi. In nature every ecosystem exists in a balance. Growth and multiplication of each organism depends on the food chain, its predators, parasites, etc. In biological control system, these interrelations are exploited. The natural enemy of a pest, disease or weed is selected; its biology is studied for mass multiplication and utilizes the same to check the target pest. They are also specific in their action and perish once their feed (i.e. the pest) is exhausted. The result shows that mass production of fungus can be done widely by using molasses and shows that *Trichoderma viridae* shows Profused growth on molasses at 17th day. *Pacilomyces liliaceae* shows Profused growth on 22nd day after inoculation where as *Verticilium lecani* shows no growth after inoculation on molasses, but it shows Profused growth on PDA. The result indicates that molasses can used to grow fungal pathogens a s in India is a sugarcane belt where lot of sugarcane industries are located hence, molasses can be utilized to grow the same on large scale.

INTRODUCTION

Pest problem is one of the major constraints for achieving higher production in agriculture crops. India loses about 30% of its crops due to pests and diseases each year. The damage due to these is estimated to be Rs.60, 000crores annually. The use of pesticides in crop protection has certainly contributed for minimizing yield losses. The pesticides, which are needed to be applied carefully, only when the threshold limits of the pest population is exceeded. However, quite often the indiscriminate and unscientific use of pesticides has led to many problems, such as pests developing resistance, resurgence of once minor pest into a major, problem besides environmental and food safety hazards. However, excessive use of pesticides note only leave residues in soil, water and air but also have adverse effects on the non target organisms such as pollinators, parasitoids, predators and wild animals. Many fungi are use as biological agents, weeds and other fungi.

Fungi to control insects: Many fungi are capable of infecting and killing insects. Mass inoculation using insect fungal pests was initiated by Krassilstchik in 1888 (Glare, 2004). However, the advance in fungal insect biopesticides did not progress as quickly as initially thought. MThis was mainly due to the development of chemical pesticides. However, due to the environmental concerns of applying chemical pesticides, fungal insecticides are being revisited and explored. Some of the most widespread fungi used to control insects are Paecelomyces spp. Tricodermaviride&Verticilliumleccani. The application of fungal insecticides is usually done using fungal spores. However, fungal mycelia.

Fungi to control weeds: The use of fungi for the control of weeds termed as mycoherbicides is certainly an environmentally friendly control method. This is especially attractive alternative to controlling many weeds in range lands, turfs, and golf greens. Fungal herbicides are mass produced and then applied to the area needing control. Fungal spores are used in aerosol sprayers and applied to an area needing control (Boyetchko and Peng, 2004).

Fungi to control other Fungi: Several fungi are used to control the plant fungal pathogens. One example is the damping-off disease that affects seedlings. Other plant diseases form galls in and rots in plants while others infect the leaves. There are fungal organisms that actually attack these different plant pathogens and have been used to control them. Trichoderma sp. has been used to control Fusariumoxysporum, rhizoctoniasolani and Pithier on a variety of plants (Kim and Hwang, 2004).

Mode of action of Entamopathogenic fungi: Entamopathogenic fungi insect pests, usually insects, by a parasitism disease mechanism. The infection process consists of the following steps:

Attachment- The conidia of the entomogenic fungi spore is attached to the insect cuticle.

Germination- The conidia spore is germinated on the insect cuticle to form a germ tube.

Penetration- The germ tube penetrates directly into the cuticle. It is believed that the cuticular invasion involves both enzymatic and physical activities.

Growth- The fungus grows in the hemocoel as mycelium or blastospore. The fungi overcome the host by invasion of organs.

Saprophytic Growth- The fungi grows on the outside of the insect and produces aerial conidia spores.This has adverse effected the ecological balance resulting in pest resurgence, development of resistance in the pest species and environmental pollutions. Development of pest resurgence and resistance has resulted in high cost of production and low income especially to cotton farmers in Maharashtra and Andhra Pradesh. In view of the several

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disadvantages associated with the unscientific use of pesticides in agriculture, there is an urgent need for minimizing the use of chemical pesticides in the management of insect pests. Growing public concern over potential health hazards of synthetic pesticides and also steep increase in cost of cultivation/low profit making farmers has led to the exploration of eco-friendly pest management tactics such as integrated pest Management (IPM). IPM aims at suppressing the pest species by combining more than one method of pest control in the harmonious way with least emphasis on the use of insecticides. In simple terms "IPM is the right combination of cultural, biological and chemical measures which provides the most effective, environmentally sound and socially acceptable methods of managing diseases, pests and weeds". The major components of IPM are prevention, observation and intervention. The IPM seems to be the only answer to coulter some of the major pests of crops, which have become unmanageable in recent years. In nature every ecosystem exists in a balance. Growth and multiplication of each organism depends on the food chain, its predators, parasites, etc.

In biological control system, these interrelations are exploited. The natural enemy of a pest, disease or weed is selected; its biology is studied for mass multiplication and utilizes the same to check the target pest. They are also specific in their action and perish once their feed (i.e. the pest) is exhausted. Thus they are based on natural principles; do not leave any residue, safe and economical. Among the alternatives, biological control of pests is one of the important means for checking pest problems in almost all agro-ecological situations. Biopesticides are living organisms which can intervene the life cycle of insect pests in such a way that the crop damage is minimized. The agents employed as biopesticides, include parasites, predators and disease causing fungi, bacteria and viruses, which are the natural enemies of pests. Further, they complement and supplement other methods of pest control. Utilisation of naturally occurring parasites, predators and pathogens for pest control is a classical biological control. On the other hand, these bio agents can be conserved, preserved and multiplied under Laboratory condition for field release. Once these bio-agents are introduced in the field to build their population considerably, they are capable of bringing down the targeted pest' population below economic threshold level (ETL).Inherently less harmful than conventional pesticides. Suppress, rather than eliminate, a pest population .Biopesticides are effective and often quickly biodegradable and present no residue problems. Biopesticides are pest management tools that are based on beneficial microorganisms (bacteria, viruses, and fungi protozoa), beneficial nematodes or other safe, biologically based active ingredients. Benefits of biopesticides include effective control of insects, plant diseases and weeds, as well as human and environmental safety.

MATERIAL AND METHODS

Entamopathogenic fungi used

Trichoderma viridae, Pacilomyces liliaceae
Verticilium lecani

Materials: Molasses, Yeast extract, Dextrose, Sucrose, Water, Conical Flask, Weighing balance, Burner, LAF, Inoculating loop, Test tube, Cotton plug, Autoclave.

Table 1.

Name	Quantity Per litre
Molasses	30 g
Dextrose	5 g
Sucrose	15 g
Yeast extract	5 g
Peptone	5 g
Agar	8 g
Potato	50 g
Water	1000 ml

Procedure: consist of two phase

Mass multiplication on PDA

50gm peeled healthy potato was sliced and added with 500ml of distilled water and mixture was then boiled well. The extract was then filtered through a filter. To this filtrate 8g of agar was added and then cooked for 30 minutes with 500ml distilled water. 5g of dextrose was added and boiled for 5 minutes. The medium were poured in to the test tubes at the rate 20ml in each & plugged with non absorbent cotton plug. The tubes were autoclaved at 15lb pressure for 20 minutes. The tubes were then cooled and inoculated with *Trichoderma viridae, Pacelomyces liliace* and *verticillium lecani* separately and kept for incubation accordingly. Profused growth was observed in the tubes after 7-10 days which was use as starter culture for mass multiplication in molasses.

Mass multiplication on Molasses

30g of molasses (pure) was poured in to the aluminium vessel. Water was added to make the final volume 1000ml. 5g of dextrose along with 15g of sucrose & 5g peptone was added to above. The whole mixture was then mixed thoroughly with glass rod along with 6.0 maintained pH. Mixture was then boiled again for 30-60 minutes.30ml of media was poured in to the 250ml capacity conical flask and plugged with cotton. The flasks were then autoclaved at 15lb pressure for 15 minutes. The flasks was then inoculated with the *Trichoderma viridae, Pacelomyces liliace* and *verticillium lecani* separately each after two days and kept for incubation accordingly. After few days of incubation the growth of microorganism start the covering area of media i.e. Profused growth was observed. The lawn was then taken and grinded to make the fine powder. This fine powder can be used as a spray mixing with water for verticillium for rest other two they were mixed with talcum powder.

Observations: Table 2. On Potato dextrose agar

Date of inoculation	Name of species	Observations		% of area
		Day	Date	
22/02/2010	*Trichoderma viridae*	1	23/02/2010	10
		3	25/02/2010	40
		4	26/02/2010	60
		7	29/02/2010	97 (Profused)
24/02/2010	*Pacilomyces liliaceae*	1	24/02/2010	10
		3	27/02/2010	30
		5	29/02/2010	75
		7	22/02/2010	80 (Profused)
26/02/2010	*Verticilium lecani*	1	26/02/2010	10
		4	29/02/2010	30
		5	30/02/2010	40
		6	31/02/2010	55
		8	02/03/2010	75 (Profused)

RESULT

There are different methods for mass production of Entamopathogenic fungi *Trichoderma viridae, Pacilomyces liliaceae* and *Verticilium lecani which can use for* controlling sucking pest, disease and nematodes. The result shows that mass production of fungus can be done widely by using molasses and shows that *Trichoderma viridae* shows Profused growth on molasses at 17th day. *Pacilomyces liliaceae* shows Profused growth on 22nd day after inoculation where as *Verticilium lecani* shows no growth after inoculation on molasses, but it shows Profused growth on PDA. The result indicates that molasses can used to grow fungal pathogens a s in India is a sugarcane belt where lot of sugarcane industries are located hence, molasses can be utilized to grow the same on large scale.

Table 3. On molasses

Date	Species	Observations		% area
		Day	Date	
01/03/2010	*Trichoderma viridae*	1	02/03/2010	10
		3	04/03/2010	15
		4	05/03/2010	20
		5	06/03/2010	30
		8	09/03/2010	35
		10	11/03/2010	40
		12	13/03/2010	50
		14	15/03/2010	70
		15	16/03/2010	80
		17	18/03/2010	97(Profused)
03/03/2010	*Pacilomyces liliaceae*	1	04/03/2010	10
		3	06/03/2010	15
		5	08/03/2010	20
		6	09/03/2010	30
		7	10/03/2010	45
		9	12/03/2010	50
		12	15/03/2010	55
		15	18/03/2010	50
		17	20/03/2010	75
		22	25/03/2010	80(Profused)
05/03/2010	*Verticilium lecani*	1	06/03/2010	0
		3	08/03/2010	0
		4	09/03/2010	0
		5	10/03/2010	0
		7	12/03/2010	0
		10	15/03/2010	0
		13	17/03/2010	0
		15	19/03/2010	0

Conclusion

India loses about 30% of its crop due to pest and disease attack each year. The damage due to these is estimated to be 60,000 cores. The use of pesticides in drop protection has contributed for minimizing yield losses. Among the alternatives, biological control of the pest is one of the important means of checking pest problems in almost an ecosystem. The agent of bio pesticides fungi can acts as natural enemies threshold level (ETL) and also effective, quickly biodegradable and have no residual problem and environmentally safe. This can be employed in an area where pest are developing resistance, resurgence of minor pest into major one.

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