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Mass production of *Metarhizium anisopliae* (Mets.) dust formulation using Jack fruit seed powder and testing its pathogenecity against *Corcyra cephalonica* Stainton

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Abstract
The major objective of this study is to mass produce *Metarhizium anisopliae* (Mets.) in a cheap substrate like jack fruit seed and to ascertain spore quality by evluating the effect of their powder formulation against the life stages stored grain pest, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). The production of conidiospores in jack fruit seed medium was high. The 16 days old culture was fully covered with the conidiospores. The conidiospore production recorded after 16 days of incubation was 21.2x 10^4 spores/ ml and the viable count was 41.4x10^7 cfu/ ml. The dust formulation of *M. anisopliae* containing the adjuvants like charcoal, kaolin, wheat flour and ash was prepared to contain 3, 6 or 9 percent dry fungal powder preparations of different concentrations were very effective against all the larval instars of *C. cephalonica*. The mortality rate increased with the increasing concentration of dust formulation and days of exposure. Third instar larvae were more susceptible to the fungal formulation than the older ones. The culture media and adjuvants used for the preparation are ideal, since the cost of materials is very low and the entomopathogenecity high.

Keywords: Conidiospores, *Metarhizium anisopliae* (Mets), *Corcyra cephalonica* Stainton, jack fruit seed, viable count

Introduction

Most of the developing countries in the world are giving importance to increase their agricultural yield and safe storage of grains to meet the food requirements of the growing human population. For the last few decades increased production of crops is accompanied by the application of large quantities of chemical pesticides, which cause hazardous effects to the environment and living organisms. Recently global attention has been diverted towards the use of biological control agents like bacteria, viruses and fungi. Among them entomopathogenic fungi, formulated as mycopesticides are found to be ideal biocontrol agents. The field level performance of most of the mycopesticides available in the market containing entomopathogenic fungi like *Metarhizium anisopliae* Mets. (Biogene, GreenGuard and Bio cane TM), *Beauveria bassiana* Balsomo- Criv. (Gmax bioguard), *Verticillium lecanii* Zimmerman (Ecocill, Verticel) and *Paecilomyces fumosoroseus* (PFR-97) is extremely effective and the only inhibitory factor is the high cost of the preparations. Most of the farmers do not prefer microbial pesticides because the high cost factor.

Successful mass production of viable conidiospores of entomopathogenic fungi depends on the fungus selected and substrate used. Fungal strains mainly utilize a wide range of nutrient sources but for the mass production and commercialization, simple and cheap media are needed [1]. The standard medium used their mass multiplication of entomopahogenic fungi is Potato Dextrose Agar (PDA) and Sabourauds Maltose Agar Yeast Medium (SMAY). High cost of PDA and SMAY ingredients is a serious handicap in the scale up of entomopathogenic fungi [2]. In order to cut down the cost of production several attempts have been made to multiply the fungus using solid substrates, developed from freely available cheap organic materials.

Several authors have explained about the mass production of entomopathogenic using different cheap substrates and the pathogenecity of the fungi thus produced. The common substrates used for solid state fermentation of *Trichoderma* sp. were grain bran [3], coconut water amended coir pith [4], shelled maize, cob powder and black gram shell powder [5] and tea waste [6]. Variety of substrates have been reported for the mass production of *V. lecanii* like sorghum, pearl millet and maize [7], almond mesocarp [8] and sugar beet molasses [9].

This paper explores the possibility of making use of jack seed powder as a cheap substrate for the mass propagation of *M.anisopliae* and use the dried substrate along with the fully grown fungus for the preparation of a dust formulation with adjuvants like charcoal, ash, wheat flour and kaolin powder and test the preparation against *Corcyra cephalonica* Stainton larvae.

2. Materials and Methods

2.1 Microbial culture and preparation of conidial suspension

A pure culture of *Metarhizium anisopliae* (Mets.) var Sorokin was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The spores were transferred from pure culture slants to PDA in 9.0 cm diameter petriplates. A conidial suspension of the fungal isolate was prepared by adding 10 ml of sterile distilled water to the petriplate containing the fungus. The suspension at the surface of the plate containing *M. anisopliae* conidiospores was gently collected in a conical flask using micropipette under the laminar air flow cabinet and uniformly mixed in a shaker.

2.2 Preparation of Jackfruit seed media

The seed coat of jack fruit seed was removed and boiled in distilled water until the seed become soft. After boiling, the inner seed coat was removed and the boiled seed was smashed well. The smashed seeds were dried and powdered well in an electrical blender. To 10g of jack fruit seed powder, 1 g of anhydrous dextrose was added and moistened well by sprinkling sterile water and mixed well. The entire mixture was sterilized for 15 minutes under pressure. After cooling this sterilized medium was poured into sterile petriplates, allowed to solidify and inoculated with 5 ml each of conidial suspension of *M.anisopliae*. After inoculation this medium was incubated at 26 ^{0}C for 8 days. Spores obtained from this culture were either stored as dry powder or as suspension in a preservative.

2.3 Assessment of spore quality

To quantify the wet biomass production in *M. anisopliae* from the jack fruit seed substrates, the fungal spores along with the bed were carefully removed from the culture after different days of incubation with the help of sterile foreceps and scalpal and the weight was determined. After quantifying the wet biomass, the fungal bodies were oven dried at 40-45 °C [10] and weighed in a sensitive electronic balance (Schmidsu LC: 0.001 g) to obtain the dry biomass. The conidiospore production and viable count were determined by following the standard methods [11].

2.4 Preparation of dry fungal formulation

The whole fungal body was isolated along with the culture medium and oven dried at 40 ^{0}C. After 12 hrs, the dried biomass was powdered well and different quantities of the fungal biomass were mixed with measurd quantities of ash, charcoal, chalk powder and wheat flour mixture.

2.5 Administration of powder form of fungal formulation to *C. cephalonica*

Third, fourth and fifth instar larvae of *C. cephalonica* larvae were selected for the experiment and were introduced into petriplates containing 3, 6 and 9 percent of fungal formulation separately. Individual insects were gently introduced into the dry preparation with a fine brush and the mortality was checked for a maximum of 9 days of exposure.

3. Results and Discussion

The factors determining spore quality like wet and dry biomass, conidiospore production and viability were assessed. The highest wet biomass of 25.2±1.96 and dry biomass of 1.15±0.09 were recorded after 16 days of incubation. It was observed that the mycelial growth and production of conidia increased with increase in incubation time upto 16 days (21.2x10^4 spores/ ml), and the entire surface of the media was almost completely covered with hyphae (table1). This indicated that jack fruit seed powder is an ideal medium promoting excellent fungal growth.

Table 1. Spore quality of *M. anisopliae* spores produced in the jack fruit seed powder medium after different days of incubation

Sl. No	Days of incubation	Spore qulality			
		Wet biomass (in gm/100ml)	Dry biomass (in gm/100ml)	Conidiospore production (X10^4 spores/ml)	Viability of Conidiospore (X10^7 cfu/ml)
1	6	7.49± 0.66	0.31±0.08	13.4±1.07	21.4±1.13 (2nd day)
2	8	12.7±1.09	0.52±0.03	18.9±1.09	35.4±2.14 (3rd day)
3	10	20.68±1.14	0.81±0.06	19.7±1.32	38.3±2.19(4th day)
4	12	22.7±1.11	0.97±0.07	20.4±1.33	39.1±1.98(5th day)
5	14	23.9±1.93	1.01±0.07	20.8±1.33	39.8±2.11(6th day)
6	16	25.2±1.96	1.15±0.09	21.2±1.29	41.4±2.14(7th day)

Table 2: Response of *C.cephalonica* larvae to *M. anisopliae* dust formulaitons

	Concentration (in %)	No. of *C.cephalonica* larvae (dead/ 10)				
		5	6	7	8	9
Third	3	1± 0.54	2±0.54	5±1.14	8±1.14	9±1.14
	6	1±0.54	2±0.70	5±0.44	8±0.70	9±0.83
	9	1±.83	3±0.54	6±0.54	9±0.83	10±0.44
Fourth	3	-	1±0.54	2±0.54	5±1.14	8±0.88
	6	1±0.54	2±0.54	4±0.83	7±1.14	9±0.54
	9	1±0.54	1±0.53	2±0.44	6±0.83	8±0.70
Fifth	3	1±0.54	1±0.54	2±0.89	4±0.83	7±0.83
	6	1±0.54	2+0.54	4±0.54	6±0.83	8±0.54
	9	-	1±0.54	2±0.70	5±0.83	8±0.44

n=10, X±SD: All the deviations are significant at≤0.05 (t-test)

The viable count recorded after 7 days of incubation was 41.4x10^7 cfu/ml. This high count is presumably due to the presence of high amount carbohydrates in the jack fruit seed. Li and Holdom [12] examined the effects of a range of carbon and nitrogen sources and vitamins on colony formation, mycelial growth and sporulation of two isolates and concluded that soluble starch was best among different carbon sources tested for growth of *M. anisopliae* and also found that nitrogen sources rich in amino acids showed stimulatory effect on the growth and germination of conidiospores. Rath *et al.* [13] examined the utilization of 49 carbohydrates by 134 isolates of *M. anisopliae* and concluded that carbohydrate utilization was a useful and biologically relevant taxonomic criteria and tool for the separation of *Metarhizium* strains and other entomogenous fungi.

Mass production of conidiospores of *M. anisopliae* - Jack fruit seed medium

3 days old culture

16 days old culture

The three formulations (3, 6 and 9% w/w) of *M. anisopliae* were tested against the third, fourth and fifth instar *C. cephalonica*. The mortality rate of *C. cephalonica* larvae increased with the increasing concentration of *M. anisopliae* formulation and prolonged time of exposure. In 9 percent concentration, 10±0.44 third instar larvae died after 9 days of exposure. In the fourth instar larvae, the corresponding mortality rate was 8±0.70 and fifth instar larva, 8±0.44. Which is about 20 percent less compared to the third instar larvae (table 2). Batta [14] who studied the control of rice weevil with various formulations of *M. anisopliae*, found that the mixture of *M. anisopliae* conidiospores with charcoal and oven ash at a rate of 2% or 2.8 mg resulted in 73.3- 86.7% mortality of *Sitophilus oryzae* L. after 7 days, when treatments were gives before or after pest infestation. The mortality caused by fungal isolates was confirmed by external sporulation of mycelia on the insect cadavers.

The results also indicated that the third instar larvae were more susceptible to the fungal formulation than the later instars. This may due to the incresed detoxicative metabolism in the mature larvae. Jbilou *et al*. [15] reported that early instar larvae of *Tribolium castaneum* are more prone to four medicinal plant extracts than the final instar and adults.

Jack seeds that support the luxuriant growth of *M. anisopliae*, are cheap, compared to commercial synthetic culture media. These cheaper substrates are convenient to handle for large scale use because labour and energy requirements are low. The formulation of *M. anisopliae* conidiospores with ash, charcoal, chalk powder and wheat flour is found to be very effective in the control of *C. cephalonica* larvae and the production cost was very cheap when compared to other mycopesticides. This preparation can be kept open in storage areas as ovitraps, so that mated *C.cephalonica* females would be attracted to lay eggs into the mixture resulting in the death of the emerging larvae. Such ovitraps would considerably reduce the population of *C. cephalonica* in ware houses where rice and wheat are stored.

4. Conclusion

Jack fruit seed powder is comparatively cheaper than the other synthetic media available in the market. This cheap media and low-tech mass production methods used to supply the fungal pesticides at an affordable cost. The dry fungal powder preparations of different concentrations were very effective against all the larval instars of *C. cephalonica*.

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