USE OF LOW COST SUBSTRATES IN MASS PRODUCTION OF *METARHIZIUM ANISOPLIAE* AND EVALUATION OF ITS INSECTICIDAL POTENTIAL AS MYCOPESTICIDE

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Abstract

Cultivation of *M anisopliae* on various grain substrates have shown that the sporulation of the fungus differs significantly among different substrate. Highest sporulation was recorded with broken rice (7 x10^4 spores per cm^3) and maize (6.6 x10^4 spores per cm^3), followed by broken jowar (5.4 x10^4 spores per cm^3).The lowest sporulation was recorded with broken wheat (4.6 x10^4 spores per cm^3). The results indicate high sporulation productivity and more spore count could be attained by use of low cost substrates as an economical means in mass scale production of this fungus. The significant finding was the potent insecticidal activity of this strain which caused killing of grasshoppers within fortnight application of conidial spores. The present study have shown that that broken grain substrates provide a simplest productive medium for mass production of *Metarhizium anisopliae* spores and 100% insecticidal action against grasshopper could be used as effective mycopesticide. **(INTERNATIONAL JOURNAL OF HIGHER EDUCATION AND RESEARCH, 5(2), 64-72, 2015)**

Keywords*: M anisopliae,* broken grains, mycopesticide and grasshoppers

1. Introduction

The increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also has resulted in adverse effects on the environment and

non-target organisms. In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Of the several microbial entomopathogens *viz.*, bacteria, fungi, viruses, protozoans and nematodes reported, only a few have been studied systematically for their usefulness. A careful evaluation of these beneficial pathogens can lead to gainful exploitation in microbial control programmes. (Bharathi H. Talwar 2005) and (Burges *et al* 1998). *Metarhizium anisopliae*, a widely distributed soil-inhabiting fungus is categorized as a green muscardine fungus due to the green color of the sporulating colonies. It has been reported to infect approximately 200 species of insects and other arthropods. It generally enters insects through spiracles and pores in the sense organs. Once inside the insect, the fungus produces a lateral extension of hyphae, which eventually proliferate and consume the internal contents of the insect. Hyphal growth continues until the insect is filled with mycelia. When the internal contents have been consumed, the fungus breaks through the cuticle and sporulates, which makes the insect appear "fuzzy." *M. anisopliae* can release spores (conidia) under low humidity conditions (<50%). The fungus can also produce secondary metabolites, such as destruxin, which have insecticidal properties on moth and fly larvae. (Raymond A. Cloyd 1999).

In past years, native species of grasshoppers have caused severe loss to crop system through seasonal outbreaks determined by environmental condition. There are at least 23 species of grasshopper considered potential pests. It seems that grasshopper outbreaks are mostly associated with the rainfall regime, especially from August to October, a critical period for the grasshopper cycle. Emphasis has been given to develop the fungus *Metarhizium anisopliae* as the most promising candidate found as bio-control agent against grasshopper. (B.P.Magalhaes, *et al*.2001) and against many insect pests including sucking pests. (*Sahayaraj* and *Borgio*, 2008).

A major motivation still exists for the exploitation of entomopathogenic fungi in the management of insect pests. (Bharathi H. Talwar, 2005). Mass production of the selected bacteria and fungi is a necessary prerequisite for any large-scale field application scale. To evaluate the utility of locally available substrates for mass multiplication of *M. anisopliae* substrates like broken rice grains, broken maize, broken jowar, broken wheat and broken ragi grains have been used.(Vimala Devi 1994), (Ramle Moslim *et al* 2005) & (Jagadeesh Babu. *et al*, 2008). The present investigation has been carried out to evaluate the utility of grain as substrates largely available in grain market that could be cost effectively used for Mass production of *Metarhizium anisopliae* and to evaluate the insecticidal activity of this fungal strain for grasshopper control as potent mycopesticide.

2. Materials and Methods

Low cost substrates such as broken rice, broken jowar, broken maize, broken wheat and Soybean oil used in the experiment were purchased from local market. Yeast extract and Triton X-100 a wetting agent was purchased from Hi-media laboratories, Mumbai.

2.1. Maintenance of *Metarhizium anisopliae* culture.

Culture procured from CMTRD, Wardha (M.S) was maintained on PDA agar slant at 28-30^{o}C. Potato dextrose agar (PDA) is the recommended medium for the isolation and enumeration of yeast & moulds and for stimulating sporulation to maintain stock culture.PDA was prepared by infusion method. About 200 gm of peeled potato was boiled in distilled water for one hour. To the decanted solution about 20 gm dextrose and agar powder was added and boiled for 1min. The solution was filtered through muslin cloth and the filtrate was used to prepare slants to maintain the fungus *M anisopliae.*

2.2. Production of inoculum culture.

The fungus *Metarhizium anisopliae* was grown at 28^{o} C for 10 days in plate containing PDA agar.

2.3. Media preparation for cultivation.

Medium for mass multiplication of *M. anisopliae* was prepared from broken rice grains, broken maize, broken jowar, and broken wheat grain. Thirty grams of each substrate was taken in 250ml conical flask containing 30 ml distilled water and yeast extract (1%), was added to all the substrates. After soaking over night, the medium was autoclaved at 121^{o}C for 15-20 minutes.

2.4. Growth of Conidia and harvesting

About 10 ml of sterile distilled water was added to PDA agar plates containing the fungus. The spores were scrapped off with the help of sterile inoculating needle. The spore suspension was then added to the flasks containing above sterile medium and were well agitated for proper distribution of the spores and incubated at 25±10^{o} C for 20 days. The conidia were superficially harvested by suspending them in 100ml of sterile distilled water containing 0.05 percent Triton-X-100 (wetting agent) with gentle shaking. The suspension was passed through muslin cloth to remove mycelial mat and solid medium.

2.5. Microscopic determination of conidial spores.

The counting of spores was done according to the method reported by (Bias Dorta et *al* 1990) using heamocytometer with slight modification. A drop of conidial suspension made from liquid culture (filtered through muslin cloth) was placed on the engraved grid and the preparation was allowed to stand for 1-2minutes to allow the conidia to settle at the bottom. A cover glass was placed over the grid carefully to avoid no air bubble enters between the slide and cover glass. The slide was focused until coloured rings were visible as the two surfaces of cover glass and

slide come into close contact. The conidia of fungus were counted in the middle square (V) which consists of 25 groups of 16 small squares, each group 0.2mm square. For larger spore or fewer spores, count spore in 4corner large square (I, II, III, IV) and in the middle one (V) to have a total count of 200-250.The number spore/cells per ml of suspension was calculated using the following formulas.

2.6. Demonstration of insecticidal activity

The determination of the bio-insecticidal property of *Metarhizium anisopliae*, was done by spraying of conidia on grasshoppers. For spray suspension was prepared from substrate which yielded high number of spores. Spraying of conidial spores was done with oil-based formulation (soybean oil) and water suspension on grasshopper. The bio-insecticidal activity was determined with death of grasshopper.

3. Results and Discussion

3.1. Microscopic determination of conidia.

Table 3.1 show the number of spores calculated which were mass multiplied on medium prepared from broken rice, wheat, jowar, and maize grains.

3.2. Total Spore Count

The result indicated in **(Table 3.2**) shows that the sporulation of the fungus differs significantly among different substrate. Highest sporulation was recorded with broken rice (7 $x10^4$ spores per cm^3) and maize (6.6 $x10^4$ spores per cm^3), broken jowar (5.4 $x10^4$ spores per cm^3).The lowest sporulation was recorded with broken wheat (4.6 $x10^4$ spores per cm^3). Our results show that more spores are produced with these substrates from mass cultivation of *M. anisopliae* in comparison to those earlier reported (Jagadeesh Babu, *et al* 2008). In their study they examined

that mass multiplication of *M. anisopliae* on, broken rice grains, broken maize, broken jowar, broken wheat, and broken ragi grains and have shown that, broken rice followed by broken jowar served as the most productive media for conidia production of the fungus, with a yield of $3.45x10^8$ and $3.2x10^8$spores per ml, respectively. The next best was broken maize ($2.2x10^8$ spores per ml), broken wheat ($1.94x10^8$ spores per ml). The spore productivity with this fungus was more with high spore counts which indicate that with same substrates as being used, the productivity and sporulation had differed significantly. This could predict that the strains may differ in the nutrient variability and conditions for productive sporulation with those reported earlier (B.P.Magalhaes, *et al* 2001) and (Jagadeesh Babu *et al* 2008).

3.3. Determination of Insecticidal Activity

Metarhizium bio-pesticide kills 70%–90% of treated locusts within 14–20 days, with no measurable impact on non target organisms Bio-insecticidal activity of spores of the fungus *Metarhizium anisopliae* was studied by spraying of conidia on grasshopper and death of grasshopper. The result was encouraging resulting in the death of grasshopper within fortnight of spraying. Water suspensions of spores were found to be ineffective in comparison to oil based formulations. The formulating pathogens in oil enhance their infectivity compared to conventional water-based formulations (*Agudelo & Falcon* 1983), (*Prior et al*. 1988), (Bateman *et al*. 1993) and (R.P. Bateman and R.T. Alves 2000). Oil can enable fungal pathogens to remain active under conditions of low humidity, and thus create opportunities for expanding the (presently limited) range of myco-pesticide applications. The principle of delivering microbial agent to their target sites for estimating the number of spores “packed” into each droplet size. From their point of view, large droplets may severely reduce the potential for environmentally benign biological agent activity.

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Table: 3. 1. Count of spores produced on various substrates

S. No	Substrates	Count of spores in five squares.					Total count of spore
		I	II	III	IV	V	
1	Rice	3	7	5	14	6	35
2	Wheat	8	6	3	1	5	23
3	Jowar	6	9	5	3	4	27
4	Maize	4	5	6	11	7	33

Table: 3.2 Spore productivity yields on different substrates

S. No	Substrate	Total No. of spore produced (spore per cm^3).
1	Broken rice +1%yeast extract	7.4×10^4
2	Broken wheat +1%yeast extract	4.6×10^4
3	Broken jowar +1%yeast extract	5.4×10^4
4	Broken maize +1%yeast extract	6.6×10^4