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Research Article

Evaluation of the growth and sporulation of different entomopathogenic fungi in different liquid and solid media at varied concentrations

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Abstract: Entomopathogenic fungi like *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium lecanii* are used in biological control of agricultural insect pests. Their specific mode of action makes them an effective alternative to the chemical Insecticides. Virulent strains of Entomopathogenic fungi are effectively formulated and used as bio-insecticides world-wide. Amenable and economical multiplication of a virulent strain in a large scale is important for them to be useful in the field. Culture media plays a major role in the large-scale multiplication of virulent strains of Entomopathogens. Different substrates and media components are being used for this purpose. Yet, each strain differs in its nutritional requirements for the maximum growth and hence it is necessary to standardize the right components and their optimum concentrations in the culture media for a given strain of Entomopathogen. In the current study, three different nitrogen sources and two different carbon sources were tried to standardize the mass multiplication media for seven test isolates of Entomopathogenic fungi. A study was also conducted to determine the ideal grain media for the optimum conidial yields of the test isolates. Yeast extract was found to be the best Nitrogen source for the isolates. The isolates tested, differed in their nutritional requirements and showed variation in the best nitrogen and carbon sources necessary for their growth. Variation was also found in the optimum concentration of both the ingredients for the growth and sporulation of the isolates. In the solid-state fermentation study, rice was found to be the best grain for the growth of most of the fungi followed by barley. The significance of such a study in the development of an effective Myco-insecticide is vital and can be successfully employed in agriculture is discussed.

Keywords: Entomopathogenic fungi, Mass culturing, Liquid media, Nitrogen source, Carbon source, solid state fermentation.

Introduction

The use of microorganisms and its derivatives is an important component of the biological control of agricultural insect pests. It is an eco-friendly yet effective alternative to the chemical insecticides devoid of their adverse impact to man and his environment. The importance of the biological control of the insects occupies significance in the light of increase in the incidence of pest resistance to the chemical insecticides. The loss of beneficial non-target insects and predators due to the injudicious use of these chemical insecticides is also a major issue in both agricultural and ecological point of view. Due to their acceptability, Entomopathogenic fungal formulations form an important component in use of biological in plant protection.

Entomopathogenic fungi (EPFs) are a polyphyletic group of fungi consisting of approximately 750 species from different genera, able to infect, kill and grow saprophytically on insects (Khachatourians and Sohail, 2008). They can be found in diverse habitats and are known to cause natural epizootics thereby playing an important role in controlling the insect populations in nature. Several Entomopathogenic fungal species are found in the divisions Zygomycota, Ascomycota, Deuteomycota, Chytridiomycota and Oomycota of the fungal kingdom (Shah and Pell, 2003). Several species of the genera *Metarhizium, Beauveria, Nomuraea*, *lecanicillium* and *Cordyceps* are well characterized with respect to their insect pathogenicity and thus gain considerable agricultural importance. Several commercial mycopesticides have being developed based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* and used worldwide (Faria and Wraight, 2007). In India also, several products based on virulent local strains of different Entomopathogenic fungal species are commercially available.

Amenable and cost-effective mass production protocols form a basis through which commercially potential biopesticides can be mass produced and made available. Attempts of scientists to this end paved way for the development of numerous mass multiplication methods involving a wide variety of

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media and ingredients. These methods broadly fall into two categories (1) Liquid Submerged fermentation method which produces mycelium and yeast cell like spores called Blastospores and Chlamydospores and (2) Solid State Fermentation which results in the production of conidia – the primary infective propagule (Jaronski, 2013).

Media used for the mass multiplication of fungi generally consists of a carbon source, a nitrogen source and other growth factors. Different cheaply available agricultural and industrial waste products have been used as the raw materials for the production of biocontrol fungi. The growth and sporulation of the different Entomopathogenic fungal species and their strains vary depending on the nature and composition of the media used for their mass cultivation. (Gao *et al*., 2007; Mustafa and Kaur, 2009). The media in which a virulent strain is grown influences not only its survival but also its efficacy in the field conditions against the target pest (Jackson and Schisler, 1992; Fargues *et al*., 2001). It has been also found that the nutritional requirements of a particular strain can vary depending on its growth phase i.e., mycelial growth or conidial production (Shah *et al*., 2005).

Materials and Methods

In the present study, a series of experiments were conducted to standardize the media components and their optimum concentration for the mass multiplication of seven isolates of entomopathogenic fungi. Three different nitrogen sources Yeast extract, Peptone and Beef Extract were studied. All the three nitrogen sources used have many applications in the field of Microbiology. While, Peptone and Beef Extract are the products of enzymatic digestion of meat, Yeast extract is obtained from the autolysis of Yeast cells. The Entomopathogenic fungal isolates were grown in varying concentrations of each of the media ingredient and their biomass growth and sporulation were evaluated. A study was also conducted to evaluate the suitable grain media for a good growth of the isolates via solid state fermentation method.

The Study isolates

Seven isolates of Entomopathogenic fungi were used in the present study. Five of them were isolated from rhizosphere soils of different crops from the Telangana State of India. Of them, three isolates were identified as *Metarhizium anisopliae* and two were identified and *Aphanoascus terreus* by morphological identification keys and later confirmed using ITS DNA sequencing technique. One isolate each of *M. anisopliae* and *Lecanicillium lecanii* obtained from AICRP on Biological Control of Crop Pests were used in the study. All the isolates used were studied for their virulence against tobacco caterpillar, *Spodoptera litura* larvae for their insecticidal activity (Chandra Teja and Rahman Unpublished data)

Evaluation of different nitrogen sources and their concentrations for the optimum growth of the isolates in submerged liquid fermentation.

Different nitrogen sources of peptone, yeast extract and beef extract were evaluated at 1 percent concentration by taking molasses-3 percent as common carbon source in all the treatments. Each treatment was replicated five times for achieving statistical differences among the treatments. The following were the sets of treatments tested under the above experimentation.

Table 1. Media with different nitrogen source with molasses as common carbon source

S.No.	Treatmental Unit
1	Molasses alone – 3%
2	Molasses + 1% Peptone
3	Molasses + 1% Yeast extract
4	Molasses + 1% Beef extract

On the basis of the above experimentation, yeast extract was proven to be most promising with maximum dry weight and conidial concentration. In view of yeast extract being the most promising nitrogen source, different concentrations of yeast extract (above and below) one percent concentration which is commonly used, were tested for their efficiency in terms of dry weight as well as conidial concentration. The following are the details of the different concentrations of yeast extract taken up for the experimentation.

Table 2. Media with different varying concentrations of Yeast extract with molasses as common carbon source

S.No.	Treatment	Percentage of Yeast Extract
1	T1	0.25%
2	T2	0.5%
3	T3	1%
4	T4	2%
5	T5	4%
6	T6	8%

To confirm whether the standard concentration of three per cent molasses is good enough to support one per cent yeast extract as a nitrogen source, an experimentation was taken up to evaluate higher and lower concentrations of molasses to confirm the standard concentration of molasses to be used as carbon source in the production process. The following are the set of treatments undertaken for the said experimentation.

Each media was prepared using individual ingredients and poured 100 ml each into 1000 ml conical flasks so as to maximise the surface area of the media for optimum aeration. The flasks were closed with cotton plugs and wrapped with paper before autoclaving them at 15 lbs for 20 minutes. Later, the flasks were inoculated with 0.1 ml spore suspension (concentrations of 10^6 conidia per ml)

of their respective strains and incubated for 10 days undisturbed at room temperature. After incubation, the mycelia mat of the fungi was collected by filtering on to a double layered muslin cloth and the cloth along with the filtered mat was placed in a brown paper bag. The bags were kept in a hot air oven at 60^0C for 48 hrs for drying. The dried mycelia mat was weighed for its dry weight in an analytical balance. The conidial count of different isolates in different media was also estimated by dissolving the contents of each unit into 100ml of sterile 0.02% Tween80 solution and later counting the number of conidia per ml with the help of a haemocytometer.

Table 3. Media with different varying concentrations of molasses as carbon source with 1% Yeast extract as common nitrogen source

S.No.	Treatment	Percentage of Molasses
1	T1	0.25%
2	T2	0.5%
3	T3	0.75%
4	T4	1%
5	T5	2%
6	T6	3%
7	T7	4%
8	T8	5%
9	T9	6%
10	T10	7%

Evaluation of the growth of the test fungal isolates on different grain media under solid state fermentation

To evaluate commonly used grain media for its efficiency to be used in solid state fermentation, an experiment was planned with the following set of treatments. Each treatment was replicated thrice for statistical comparisons

Table 4. Different types of grains evaluated for the growth of the Entomopathogenic isolates

S. No.	Grains used
1	Sorghum
2	Rice
3	Wheat
4	Maize
5	Dehusked Barle
6	Brown rice

Each of the above grain commodities were washed twice and soaked for 2 to 3 hours in water. The excess water was strained off and the grains were dried under room temperature till the moisture was brought down below 10 per cent. 100 g of grains were weighed and filled in one litre conical flasks. The flasks were closed with cotton plugs and wrapped with paper. Five replications were kept for each strain and for each grain medium. The flasks were autoclaved twice with intermittent cooling for complete sterilization of the grains. The flasks were later inoculated 1 ml of their respective strains with concentrations of 10^6 conidia per ml and mixed thoroughly. One uninoculated control for each grain medium was also maintained. The flasks were kept incubated undisturbed for 10 days at room temperature. After incubation the conidial concentration was checked by thoroughly agitating the contents of the flask in 100 ml of 0.02% Tween 80 solution. The conidial load per 100ml was estimated by using a Haemocytometer. Flasks having profuse sporulation were serially diluted before checking the concentration

Results and Discussion

For their use in agriculture, the infective propagules of an effective Entomopathogen such as mycelia, conidia or microsclerotia have to be multiplied in large scale. The creation of avenues to grow the strains economically and efficiently with production of maximum number of the infective propagules in minimum amount of time is utmost necessary. Envisaging this need, different production methods have been devised for the mass cultivation of Entomopathogens efficiently using different ingredients (Jaronski, 2013). In the present study, a series of experiments to standardize the liquid and solid (grain) media needed for the maximum growth and sporulation of seven local isolates of entomopathogens. Franscisco *et al.,* (2006) postulated that the growth and sporulation of EPF strains was better in a complex nutrient rich undefined media compared to a chemically defined media. The different carbon and nitrogen sources evaluated in the current study are complex and undefined in nature.

Growth of different Entomopathogenic isolates in different nitrogen sources

The growth response to the treatments varied with the isolates (Table 5). All the other isolates gave the least biomass growth in the treatment having only Molasses (T4) without any nitrogen source. *A. terreus* isolates ArCo3 and PRg4 which did not show any growth in the treatment. In the treatment with peptone as the nitrogen source (T1), *M. anisopliae* LaMa1 (672.59mg) showed the maximum biomass dry weight of the isolates followed by *M. anisopliae* KoGn5 (527.51mg) and *L. Lecanii* LlAICRP (525.47mg). Isolate *A. terreus* ArCo3 had the least biomass (105.53mg) of all the test isolates. The isolate also gave the least in terms of the conidial yield (0.02 x 10^6/ml) among the isolates. The *M. anisopliae* isolate PaCo4 gave the highest conidial yield (640.67 x 10^6/ml) among the all isolates in peptone treatment followed *M. anisopliae* isolates KoGn5 (163.33 x 106/ml) and MaAICRP (40.67 x 106/ml). When Yeast extract is used as the nitrogen source (T2) highest biomass growth was found with *L. lecanii* LlAICRP (811.46mg) followed by *M. anisopliae* isolates LaMa1 (806.48mg), MaAICRP (691.22mg) and KoGn5 (660.33mg). In terms of the conidial yields, maximum yields were shown by *M. anisopliae* KoGn5 (165.33 x 10^6/ml) and LaMa1 (160.33 x 10^6/ml) followed by PaCo4 (139.33 x 10^6/ml) and *L. lecanii* LlAICRP (120 x 10^6/ml). The least both in terms of biomass dry

weight (17.50mg) and conidial yield (0.85 x 106/ml) was shown by *A. terreus* ArCo3. In the treatment having beef extract (T3) as the nitrogen source, *L. lecanii* isolate LlAICRP (902.54mg) showed the highest biomass followed by the *M. anisopliae* isolates MaAICRP (762.64mg) and LaMa1 (748.32mg). When the conidial yields are taken in to account, *M. anisopliae* PaCo4 (141.33 x 10^6/ml) showed that highest followed by LaMa1 (92.40 x 10^6/ml) and KoGn5 (32.50 x 10^6/ml). The *A. terreus* isolate ArCo3 again showed the least biomass (87.58mg) and conidial yield (0.04 x 10^6/ml) among all the isolates. The *A. terreus* isolate ArCo3 which gave lowest biomass of all the isolates also yielded negligible conidial yield. Interestingly, it was found that some isolates had different nitrogen source preference for each of the two growth parameters. For instance, isolate PaCo4 had low biomass growth but had the highest conidial yield in peptone. Comparing the three nitrogen sources, four out of the seven isolates tested showed the highest biomass growth in the treatment with Yeast extract (T2) whereas two isolates MaAICRP and LlAICRP showed highest growth in Beef extract. There was more variation among the isolates in terms of the conidial yields. Yeast extract (T2) gave high conidial yields for five of the seven isolates.

Table 5. Growth characteristics of the study isolates on the different nitrogen sources

	M + Pep T1		M + YE T2		M + BE T3		Molasses alone T4	
	DW	**CC**	**DW**	**CC**	**DW**	**CC**	**DW**	**CC**
Ma AICRP	418.48 ± 1.45	40.67 ± 2.52	691.22 ± 1.07	60.67 ± 1.53	762.64 ± 0.96	24.00 ± 1.73	128.00 ± 1.34	1.53 ± 0.10
KoGn5	527.51 ± 0.95	163.33 ± 1.15	660.33 ± 1.67	165.33 ± 0.58	600.44 ± 1.14	32.50 ± 0.5	140.40 ± 1.74	31.67 ± 3.06
LaMa1	672.59 ± 1.21	32.67 ± 3.51	806.48 ± 0.80	160.33 ± 4.51	748.32 ± 1.10	92.40 ± 0.96	141.61 ± 1.05	0.12 ± 0.01
PaCo4	507.03 ± 0.83	640.67 ± 5.03	649.48 ± 1.51	139.33 ± 4.04	626.51 ± 0.69	141.33 ± 3.21	180.56 ± 1.05	21.40 ± 1.18
Vl AICRP	525.47 ± 1.35	32.07 ± 0.40	811.46 ± 0.78	120 ± 4.58	902.54 ± 1.39	62 ± 0.36	102.70 ± 1.22	0.73 ± 0.01
ArCo3	105.53 ± 1.30	0.02 ± 0.01	17.50 ± 0.95	0.85 ± 0.01	87.58 ± 0.86	0.04 ± 0.01	No growth	No growth
PRg4	477.33 ± 1.05	5.13 ± 0.38	682.04 ± 0.83	3.27 ± 0.21	529.30 ± 0.99	0.51 ± 0.04	No growth	No growth
LSD @ 0.05	2.07	4.42	1.99	5.14	1.82	2.53	2.37	

DW: Mean Dry weights of biomass in mg
CC: No. of conidia X 10^6/ml

It is evident from the experiment with different protein sources that protein (Nitrogen) amendment is essential for optimum growth and sporulation of the Entomopathogenic fungi as seen with the low growth and sporulation of all the studied isolates in the media with only Molasses as the carbohydrate source. Although, all the three nitrogen sources commonly consist of peptides and amino acids, yeast extract and beef extract contain ingredients like organic acids, minerals, water soluble vitamins and other growth factors and this could be a nutritional advantage for the fungal growth. It can be inferred that Yeast extract is the preferred nitrogen source for the isolates tested followed by beef extract and peptone. Barnes *et al.,* (1975) observed good mycelial growth and sporulation of *M. anisopliae* with Yeast extract. Addition of Yeast extract also enhanced the growth and sporulation of some *M. anisopliae* isolates (Barmes *et al.,* 1975). Molasses yeast broth was found to contribute for the optimum growth of *L. lecanii* (Derakhsan *et al.*, 2008) and same was found for *M. anisopliae* by Masoud *et al.*, (2014).

The effect of increasing concentrations of Yeast extract in the media on the growth and sporulation of different Entomopathogenic fungal isolates

The growth and sporulation of the test isolates varied with the concentration of yeast Extract. An increase in the biomass of the isolates was observed with increase in the concentration of yeast extract in the treatments (Table - 6). The *M. anisopliae* isolates MaAICRP and KoGn5 highest biomass showed maximum biomass growth in media with 8 per cent yeast extract concentration (T6) and the rest showed highest at 4 per cent yeast extract concentration (T5). Considering the conidial yield per ml, 1 per cent yeast extract concentration (T3) was found to be optimum for all the *M. anisopliae* isolates and the *A. Terreus* isolate PRg4. The *L. lecanii* isolate LlAICRP gave maximum conidial yield (8.57 x 10^6/ml) in the highest tested yeast extract concentration (T6). The isolate ArCo3 though showed biomass growth produced negligible conidia evident from the very low conidial yields in all the treatments. It was interesting to note that the ingredient's optimum concentration for biomass growth of the isolates was different from that of their sporulation. For instance, isolates produced more biomass at the high Yeast extract concentrations but yielded high conidial production at 1 per cent concentration. In the mass multiplication of fungal entomopathogen, the conidial yields are of more importance as they are the infective propagules of a fungus and also have more shelf life than the mycelial biomass. Keeping this in mind and also considering the increase in cost of production if higher concentration of yeast extract is used, it would be rational to use yeast extract at the concentration of 1 per cent in the production media.

Table 6. Growth of the Entomopathogenic isolates on different varying concentrations of Yeast extract with molasses as common carbon source

	T1 (0.25%)		T2 (0.5%)		T3 (1%)		T4 (2%)		T5 (4%)		T6 (8%)	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
Ma AICRP	$398^{a} \pm 11.50$	$3.73^{b} \pm 0.25$	$608^{a} \pm 12.50$	$5.83^{a} \pm 0.76$	$785^{b} \pm 28.29$	$41.00^{a} \pm 4.58$	$1023^{b} \pm 11.06$	$27.33^{a} \pm 3.21$	$1347^{b} \pm 25.58$	$4.70^{a} \pm 0.75$	$1583^{a} \pm 39.67$	$0.32^{b} \pm 0.01$
KoGn5	$394^{a} \pm 63.63$	$12.63^{a} \pm 0.71$	$609^{a} \pm 6.11$	$6.13^{a} \pm 0.40$	$863^{a} \pm 14.19$	$26.67^{b} \pm 4.72$	$1089^{a} \pm 13.53$	$12.67^{b} \pm 2.08$	$1024^{e} \pm 12.22$	$0.05^{e} \pm 0.01$	$1398^{b} \pm 85.28$	$0.20^{bc} \pm 0.02$
LaMa1	$293^{b} \pm 8.62$	$1.57^{c} \pm 0.06$	$373^{e} \pm 20.55$	$1.63^{bc} \pm 0.12$	$797^{b} \pm 21.66$	$3.57^{c} \pm 0.15$	$710^{d} \pm 11.59$	$2.97^{c} \pm 0.40$	$1111^{d} \pm 15.28$	$2.27^{b} \pm 0.15$	$653^{d} \pm 20.95$	$0.04^{bcd} \pm 0.003$
PaCo4	$393^{a} \pm 17.16$	$1.10^{c} \pm 0.10$	$523^{c} \pm 11.68$	$1.00^{cd} \pm 0.20$	$563^{d} \pm 19.04$	$2.37^{c} \pm 0.25$	$986^{c} \pm 11.79$	$1.02^{cd} \pm 0.16$	$1409^{a} \pm 13.53$	$0.77^{cd} \pm 0.07$	$930^{c} \pm 20.53$	$0.03^{cd} \pm 0.001$
Vl AICRP	$93^{d} \pm 9.07$	$0.40^{d} \pm 0.51$	$428^{d} \pm 11.53$	$0.43d^{e} \pm 0.15$	$798^{b} \pm 22.03$	$1.13^{c} \pm 0.05$	$1011^{b} \pm 10.26$	$1.37^{cd} \pm 0.15$	$1183^{c} \pm 14.84$	$1.02^{c} \pm 0.05$	$663^{d} \pm 18.45$	$8.57^{a} \pm 0.42$
ArCo3	$159^{c} \pm 6.81$	$0.02^{d} \pm 0.0005$	$255^{f} \pm 7.81$	$0.08^{e} \pm 0.001$	$201^{e} \pm 13.43$	$0.02^{c} \pm 0.002$	$365^{e} \pm 7.57$	$0.06^{e} \pm 0.002$	$499^{g} \pm 39.51$	$0.01^{e} \pm 0.002$	$335^{f} \pm 10.54$	$0.03^{cd} \pm 0.006$
PRg4	$313^{b} \pm 4.36$	$0.48^{d} \pm 0.05$	$576^{b} \pm 10.69$	$2.10^{b} \pm 0.46$	$635^{c} \pm 8.50$	$2.23^{c} \pm 0.21$	$722^{d} \pm 14.01$	$0.11^{cd} \pm 0.01$	$832^{f} \pm 9.29$	$0.08^{e} \pm 0.005$	$436^{e} \pm 11.50$	$0.02^{cd} \pm 0.0006$
LSD @ 0.05	45.37	0.61	21.56	0.67	33.56	4.36	20.27	2.55	36.78	0.51	67.04	0.28

DW: Mean Dry weights of biomass in mg
CC: No. of conidia X 10^{6}/ml

Table 7a. Growth of the isolates on different varying concentrations of molasses as carbon source with 1% yeast extract as common nitrogen source

	T1 (0.25%)		T2 (0.50%)		T3 (0.75%)		T4 (1%)		T5 (2%)	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
Ma AICRP	$167^{bc} \pm 16.09$	0.21 ± 0.02	$239^{b} \pm 43.66$	0.54 ± 0.03	$330^{a} \pm 9$	0.52 ± 0.04	$394^{a} \pm 2$	0.52 ± 0.02	$570^{a} \pm 7.55$	1.36 ± 0.06
KoGn5	$135^{d} \pm 2.52$	0.51 ± 002	$167^{cd} \pm 4.58$	1.10 ± 0.18	$239^{c} \pm 14$	1.54 ± 0.25	$279^{d} \pm 17.01$	3.47 ± 0.15	$510^{b} \pm 15.72$	83 ± 2.77
LaMa1	$116^{e} \pm 6$	0.01 ± 0.01	$132^{e} \pm 12.50$	0.01 ± 0.01	$177^{e} \pm 11.68$	0.01 ± 0.01	$204^{f} \pm 5$	0.02 ± 0.01	$279^{e} \pm 14.11$	0.02 ± 0.01
PaCo4	$151^{cd} \pm 5.57$		$189^{c} \pm 5.57$	1.13 ± 0.15	$207^{d} \pm 9.54$	0.13 ± 0.16	$246^{c} \pm 4.58$	0.59 ± 0.04	$471^{c} \pm 5.03$	1.63 ± 0.06
Vl AICRP	$113^{e} \pm 2.52$	0.02 ± 0.01	$138^{de} \pm 6.51$	0.11 ± 0.03	$174^{e} \pm 0.58$	0.06 ± 0.01	$199^{f} \pm 5.58$	0.10 ± 0.01	$325^{d} \pm 3.51$	0.15 ± 0.04
ArCo3	$177^{b} \pm 9.07$	1.5 ± 0.10	$241^{b} \pm 6.11$	0.87 ± 0.06	$248^{c} \pm 8.74$	1.52 ± 0.03	$302^{e} \pm 7.51$	0.76 ± 0.01	$318^{d} \pm 6.51$	0.94 ± 0.07
PRg4	$223^{a} \pm 14.05$	0.04 ± 0.01	$275^{a} \pm 13.58$	0.05 ± 0.01	$303^{b} \pm 6.24$	0.43 ± 0.66	$334^{b} \pm 5.03$	0.11 ± 0.02	$512^{b} \pm 1.15$	1.03 ± 0.06
LSD @ 0.05	16.45	0.07	32.27	0.15	16.48	0.20	13.91	0.11	15.9	1.44

DW: Mean Dry weights of biomass in mg
CC: No. of conidia X 10^{6}/ml

Table 7b. Growth of the isolates on different varying concentrations of molasses as carbon source with 1% yeast extract as common nitrogen source

	T6 (3%)		T7 (4%)		T8 (5%)		T9 (6%)		T10 (7%)	
	DW	CC	DW	CC	DW	CC	DW	CC	DW	CC
Ma AICRP	$656^{b} \pm 34.08$	1.50 ± 0.03	$788^{b} \pm 3.06$	5.30 ± 0.17	$1{,}110^{a} \pm 12.50$	10.30 ± 0.26	$1{,}572^{a} \pm 16.50$	1363 ± 0.55	$1{,}351^{a} \pm 17.16$	18.23 ± 0.25
KoGn5	$761^{a} \pm 23.58$	438.3 ± 4.73	$976^{a} \pm 24.50$	7751 ± 9.54	$1{,}089^{ab} \pm 32.53$	4.46 ± 0.50	$1{,}516^{a} \pm 49.33$	12.50 ± 0.36	$1{,}265^{a} \pm 13.53$	18.33 ± 0.50
LaMa1	$499^{d} \pm 27.06$	0.01 ± 0.01	$567^{cd} \pm 6.51$	0.01 ± 0.01	$615^{e} \pm 66.25$	0.10 ± 0.01	$710^{e} \pm 22.72$	0.05 ± 0.01	$936^{c} \pm 123.45$	0.07 ± 0.01
PaCo4	$516^{cd} \pm 3.79$	1.70 ± 0.10	$820^{b} \pm 6$	1.23 ± 0.07	$999^{c} \pm 13.75$	0.52 ± 0.04	$1{,}270^{b} \pm 12.90$	0.81 ± 0.04	$1{,}090^{b} \pm 13.11$	0.92 ± 0.03
Vl AICRP	$371^{f} \pm 2$	0.25 ± 0.02	$557^{d} \pm 43.66$	0.09 ± 0.03	$623^{de} \pm 18.01$	1.69 ± 0.04	$738^{c} \pm 1.53$	1.61 ± 0.03	$764^{d} \pm 99.35$	2.67 ± 0.15
ArCo3	$408^{e} \pm 17.95$	0.35 ± 0.04	$487^{e} \pm 25.32$	1.27 ± 0.08	$638^{de} \pm 43.19$	0.47 ± 0.06	$375^{d} \pm 27.62$	0.13 ± 0.06	$220^{e} \pm 72.30$	0.04 ± 0.01
PRg4	$546^{c} \pm 7.02$	1.57 ± 0.06	$601^{c} \pm 2.65$	3.73 ± 0.20	$673^{d} \pm 12.12$	1.67 ± 0.12	$697^{c} \pm 15.08$	0.53 ± 0.06	$258^{e} \pm 6.51$	0.01 ± 0.01
LSD @ 0.05	35.27	3.13	37.69	6.32	59.67	0.39	65.49	0.44	116.59	0.39

DW: Mean Dry weights of biomass in mg
CC: No. of conidia X 10^{6}/ml

The effect of varying concentrations of Molasses used as sole carbon source on the growth and sporulation of different entomopathogenic fungal isolates

The carbon percentage and the ratio of carbon to nitrogen in the media play a key role in the mass multiplication of entomopathogenic fungal strains (Gao *et al*., 2007; Mustafa and Kaur, 2009). In the present study, Molasses as the carbon source was used in increasing concentrations to zero down on the optimum concentration of it along with the common yeast extract (1%) for the growth and sporulation of the test entomopathogens. Molasses is a viscous liquid obtained as a by-product of Sugar making process and is rich in sucrose sugar along traces of minerals and amino acids. It is often used as a substrate in the mass multiplication media for many fungal species and found applications in various industries.

From the study, it was found that the biomass of the isolates increased with the increase in the molasses concentration till an optimum concentration later which the growth either decreased or came to a plateau (Table - 7). Variations in growth were observed among the isolates in and each isolate varied in optimum concentration for their maximum biomass and conidial yields. The lowest concentration tested (0.25%) gave the least biomass of all the isolates under study. The isolates MaAICRP, KoGn5, PaCo4 and PRg4 gave the maximum biomass with 6 per cent (T9) concentration of Molasses and the isolates LaMa1 and LlAICRP produced maximum biomass in 7 per cent (T10) molasses concentration. The *A. terreus* isolate ArCo3 yielded its highest biomass at only 5 per cent (T8) concentration. In terms of conidial yields also, the isolates showed wide variations in response to the different concentration of Molasses. The highest conidial yield of all the isolates was by *M. anisopliae* isolate KoGn5 at the Molasses concentration of 4 per cent (T7) which is 7750 X 10^6 conidia/ml. The concentration of Molasses for maximum conidial yields for other isolates were 7 per cent (T10) for *M. anisopliae* MaAICRP, LaMa1 and *L. lecanii* isolate LlAICRP, 3 per cent (T6) for PaCo4, and 4 per cent (T7) for isolate PRg4. The *A. terreus* isolate ArCo3 fluctuated widely in its conidial yields at different molasses concentrations. Among the *M. anisopliae* isolates, MaAICRP and KoGn5 had more conidial yields than the other two particularly in the higher Molasses concentrations. The yields of isolate PaCo4 which ranged from 0.6 to 1.7 X 10^6/ml had less variation over the concentrations. Isolate LaMa1 in line with its biomass growth, showed very less conidial production in all the concentrations. The conidial yields of *L. lecanii* isolate LlAICRP increased with the increasing Molasses concentration from 0.02 to 2.7 X 10^6/ml whereas that of *A. terreus* isolate ArCo3 were high (1.5 X 10^6/ml) at low concentrations and decreased with the increasing concentration (0.04 X 10^6/ml at T10 treatment). Derakshan *et al.,* (2008) recommended 4 percent molasses concentration in Molasses Yeast extract broth for optimum growth of *L. lecanii*. Same was found true for *M. anisopliae* by Masoud *et al.,* (2014). Our results contradicted the previous findings and it was found that biomass of the isolates was high in higher concentration of Molasses like 5, 6 and 7 per cent. The variation among the isolates in terms of conidial yield is more profound than the biomass. As molasses is a raw material in liquor industry, its use is regulated by law in India which makes its use difficult for small scale industries and farmers to procure it and use to mass produce different microbial bioinoculants.

Table 8. Evaluation of growth of the isolates on different grains mean conidial concentration per ml (No. of conidia X 10^6/ml)

Strain	Sorghum T1	Rice T2	Brown Rice T3	Wheat T4	Maize T5	Barley T6	LSD @ 0.05
MaAICRP	$24.27^{b} \pm 0.61$	$243^{c} \pm 11.93$	$2^{b} \pm 0.15$	$0.29^{c} \pm 0.04$	$0.90^{d} \pm 0.03$	$111^{d} \pm 10.41$	11.51
KoGn5	$35.03^{a} \pm 1.65$	$96^{d} \pm 9.45$	$3.50^{a} \pm 0.50$	$15.23^{a} \pm 0.75$	$0.10^{e} \pm 0.03$	$195^{ab} \pm 13.23$	11.89
LaMa1	$35.47^{a} \pm 1.65$	$922^{b} \pm 4.93$	$0.80^{e} \pm 0.05$	$0.16^{e} \pm 0.08$	$0.93^{d} \pm 0.03$	$1.87^{f} \pm 0.12$	3.78
PaCo4	$21.87^{c} \pm 0.85$	$269^{c} \pm 6.03$	$2.07^{bc} \pm 0.80$	$0.04^{e} \pm 0.01$	$0.04^{e} \pm 0.02$	$184^{bc} \pm 3.51$	5.14
VlAICRP	$35.07^{a} \pm 0.45$	$1,011^{a} \pm 34.03$	$1.57^{d} \pm 0.21$	$0.92^{b} \pm 0.03$	$2.27^{b} \pm 0.25$	$218^{a} \pm 33.05$	34.45
ArCo3	$24.90^{b} \pm 0.95$	$37^{e} \pm 6.81$	$1.73^{cd} \pm 0.15$	$0.14^{e} \pm 0.02$	$1.73^{c} \pm 0.15$	$1.03^{f} \pm 0.06$	4.99
PRg4	$4.30^{d} \pm 1.08$	$48^{e} \pm 9.61$	$3.38^{a} \pm 0.13$	$0.15^{e} \pm 0.03$	$3.37^{a} \pm 0.21$	$37.10^{e} \pm 0.40$	7.03
LSD @ 0.05	1.97	26.39	0.66	0.50	0.24	24.66	

Evaluation of the growth of Entomopathogenic isolates on different grains through solid state fermentation

Solid state fermentation using natural and agricultural raw materials is an ideal method for the production of conidia of fungi. The undefined and complex nature of these media components contribute to the growth of asexual spores or conidia of most of the fungi. The selection of a particular raw material for the production of fungal conidia not only depends on the estimated yield of

the conidia but also on the cost of production process involving it. In the present study, the best grain type for high conidial yields of the test Entomopathogenic fungal isolates was evaluated (Table - 8). Rice among all the grains tested, gave the highest conidial yield for all the isolates except *M. anisopliae* KoGn5 which showed significantly high conidial yield on barley over rice. The lowest conidial yields were obtained with maize in the case of KoGn5 isolate and with wheat for the rest of the isolates. Our results were in contradiction to the reports of Moslim *et al.,* (2006) and Yujie shi *et al.,* (2009) where maize was found to be best for the optimum spore yield of *M. anisopliae* and *L. lecanii* respectively but were in agreement with Rachappa *et al.,* (2005) who reported high growth of *M. anisopliae* on broken rice. Rice also contributed for the high conidial yields of strains of *B. bassiana* and *M. anisopliae* (Mar and Lumyong, 2012). Barley gave the second higher conidial yield for isolates MaAICRP, PaCo4, LlAICRP and PRg4. Rice gave the second

highest conidial yield for the *M. anisopliae* isolate KoGn5 and sorghum for *M. anisopliae* LaMa1 and *A. terreus* ArCo3. Among the isolates, *Lecanicillium* isolate LlAICRP (1011 x 10^6/ml) and *A. terreus* isolate ArCo3 (37 X 10^6/ml) showed the highest and the lowest conidial yield respectively in rice. Karanja *et al.,* (2010) noted that

the moisture content of the grain and its surface to volume ratio is most important for the conidial yield than the actual nutritional composition of the grains. They reported high yields of *M. anisopliae* conidia on broken maize followed by rice. Similarly, it can be concluded that in the present study, rice and barley contributed for high conidial yields of Entomopathogenic fungal isolates by virtue of their high moisture content and increased surface to volume ratio. Among the two grain types, Rice is considered to be easily and cheaply available and so can be recommended as a raw material for efficient production of asexual conidia of the entomopathogenic fungal isolates. Our findings emphasize that isolates differ in their nutritional preference and physiology and the production process needs to be standardized accordingly.

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References

1. Barnes, G. L., D. J. Boethel, R. D. Eikenbary, J. T. Criswell, and C. R. Gentry. "Growth and sporulation of *Metarrhizium anisopliae* and *Beauveria bassiana* on media containing various peptone sources." *Journal of invertebrate pathology* 25, no. 3 (1975): 301-305.

2. de Faria, Marcos R., and Stephen P. Wraight. "Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types." *Biological Control* 43.3 (2007): 237-256.

3. Derakhshan, Ali, R. J. Rabindra, B. Ramanujam, and Mehdi Rahimi. "Evaluation of different media and methods of cultivation on the production and viability of entomopathogenic fungi, *Verticillium lecanii* (Zimm.) Viegas." *Pakistan journal of biological sciences: PJBS* 11.11 (2008): 1506-1509.

4. Francisco, Edimara Aparecida, Dinalva Alves Mochi, Antônia do Carmo Barcelos Correia, and Antonio Carlos Monteiro. "Influence of culture media in viability test of conidia of entomopathogenic fungi." *Ciência Rural* 36.4 (2006): 1309-1312.

5. Gao, Li, Man H. Sun, Xing Z. Liu, and Yong S. Che. "Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi." *Mycological research* 111.1 (2007): 87-92.

6. Jackson, Mark A., and David A. Schisler. "The composition and attributes of *Colletotrichum truncatum* spores are altered by the nutritional environment." *Applied and Environmental Microbiology* 58.7 (1992): 2260-2265.

7. Jaronski, Stefan. "Mass production of entomopathogenic fungi—state of the art." *Mass production of beneficial organisms: invertebrates and entomopathogens. Academic, San Diego* (2013): 357-413.

8. Karanja, L. W., N. A. Phiri, and G. I. Oduor. "Effect of different solid substrate on mass production of *Beauveria bassiana* and *Metarhizium amisopliae* entomopathogens." In *12th KARI Biennial Science Conference proceedings*. (2010): 789-97.

9. Khachatourians, George G., and Sohail S. Qazi. "Entomopathogenic fungi: biochemistry and molecular biology." In *Human and Animal Relationships*, pp. 33-61. Springer Berlin Heidelberg, 2008.

10. Latifian, Masoud, Bahar Rad, and Majid Amani. "Mass production of entomopathogenic fungi Metarhizium anisopliae by using agricultural products based on liquid-solid diphasic method for date palm pest control." *International Journal of Farming and Allied Sciences* 3, no. 4 (2014): 368-372.

11. Mar, Thet Thet. "Conidial production of entomopathogenic fungi in solid state fermentation." *Asia-Pacific Journal of Science and Technology* 17.5 (2017): 762-768.

12. Moslim, R. A. M. L. E., Mohd Basri Wahid, N. O. R. M. A. N. Kamarudin, Siti Ramlah Ahmad Ali, And Noor Hisham Hamid. "Research into the commercialization of *Metarhizium anisopliae* (Hyphomycetes) for biocontrol of the rhinoceros beetle, Oryctes rhinoceros (Scarabaeidae), in oil palm." *Oil Palm Journal of Oil Palm Research (Special Issue-April 2006)* (2006): 37-49.

13. Mustafa, Uzma, and Gurvinder Kaur. "UV-B radiation and temperature stress causes variable growth response in *Metarhizium anisopliae* and *Beauveria bassiana* isolates." *Journal of Microbiology* 7 (2009): 2114.

14. Rachappa, V., S. Lingppa, R. K. Patil, and P. S. Hugar. "Suitability of media and containers for mass production of *Metarhizium anisopliae*." *Karnataka Journal of Agricultural Sciences* 18.3 (2010).

15. Shah, Farooq A., Cheng S. Wang, and Tariq M. Butt. "Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae*." *FEMS Microbiology Letters* 251.2 (2005): 259-266.

16. Shah, P. A., and J. K. Pell. "Entomopathogenic fungi as biological control agents." *Applied microbiology and biotechnology* 61, no. 5-6 (2003): 413-423.

17. Shi, Yujie, Xiangqun Xu, and Yang Zhu. "Optimization of *Verticillium lecanii* spore production in solid-state fermentation on sugarcane bagasse." *Applied microbiology and biotechnology* 82.5 (2009): 921.

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