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Rajendra Singh
Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India.

KD Verma
Department of Entomology, A. S. College, Lakhaoti, Bulandshahr, U.P, India

Joginder Singh
Department of Horticulture, Janta Vedic College Bauraut, Uttar Pradesh, India

Rashmi Nigam
Department of Plant Pathology, Janta Vedic College Bauraut, Uttar Pradesh, India

Mahendra Singh
Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India

Anant Kumar
Department of Horticulture, KVK, Ghaziabad, S. V Patel Univ. Agri. Tech., Meerut, Uttar Pradesh, India

Correspondence
Rajendra Singh
Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India.

Multiplication of entomopathogenic fungus *Beauberia bassiana* (Balsamo) on solid and liquid media

Rajendra Singh, KD Verma, Joginder Singh, Rashmi Nigam, Mahendra Singh and Anant Kumar

Abstract
The present studies according to findings, several naturally available substrates i.e. solid and liquid media were tested for mass multiplication of different *Beauveria bassiana* entomopathogenic fungus. The effect of different substrates for the mass production of *B. bassiana* spore/ml was significantly higher recorded were significantly producing spore per ml Data recorded on 7 day after the day after inoculation of various treatments in the T_{10} Savoured dextrose broth (SDB), was the best treatment by bringing down the *B. Bassiana* production up to (48.3) spore/ml in liquid medium. Overall finding showed that different days among the liquid and solid media tested, for *B. bassiana* spore/ml production was significantly higher recorded 224.59 and 193.06 spore/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB). evaluated most appropriate medium for the production of *B. bassiana* potatoes, wheat flour, rice flour, corn flour and sugar cane molasses and solid phases includes sugar cane, corn, barley, rice, millet and sorghum. Among different media, sugar cane molasses extract and rice showed maximum growth of *B. bassiana* Different solid substrates i.e maize, bran, cotton seed, rice husk, wheat and liquid media such as coconut water were content and yeast extract concentration for mass production of entomopathogenic fungi: substrate for spore production. Collect the substrate for cost effective of *B. bassiana* spore/ml ($1x10^6$) cost incurred for the production of spores T_{10} Savoured dextrose broth (SDB) was the best low cost substrate and high production (Rs 5.43 and 149.35 spore/ml), T_{11} Potato dextrose broth (PDB) (Rs 6.96 and 127.47 spore/ml), In the mass production of *B. bassiana* on different substrate solid and liquid medium "Savoured dextrose broth (SDB), Potato dextrose broth (PDB) and solid substrate "Vermi compost + 1% YE + 1.0 g Dextrose" the best low cost substrate and high production found to be the best substrate in maximum spore production in minimum time as well as in cost of production of spore.

Keywords: *Beauveria bassiana*, maize, bran, cotton seed, rice husk, wheat and liquid media

Introduction
Day to day indiscriminate use of a great many chemical pesticides (e.g. organochloride insecticides, methyl bromide etc.) cause human health risks, environmental pollution, Soil health and effects on non-target organisms of the development of pest resistance. resulted, have many drawbacks including pest resistance, resurgence of pests, emergence of secondary pests due to the loss of activity of parasitoids and predators, effects on non target organisms, contamination of the environment (soil, water, air polution), and the presence of chemical residues on agricultural produce. These drawbacks forced to use more selective and compatible methods which may be safer to environment. Biological control is one of the promising methods of insect- pest management and constitutes an eco friendly alternative strategy (Sajad *et.al*, 2007). *Beauveria bassiana* is an entomopathogenic fungus that grows naturally in soils throughout the world and acts as a parasite on various insect species, causing white muscardine disease. It is being used as a biological insecticide to manage a number of pests such as termites, whitefly, different beetles and its use in the control of the malaria-transmitting mosquitos is under investigation (Donald G. & McNeil Jr, 2005). The spores are tiny, measuring only a few microns. The hyphae and spores are with white mycelium bearing masses of powdery spores burst out through the body parts of infected insects. Conidium or spore is an infectious stages which attached to the insect cuticle, germination of the conidium and penetration of the insect cuticle by a germ tube from the conidium, growth of the *B. bassiana* fungus inside the insect body (Hemocoel) and eventual death of the insect, penetration of the fungus to the surface of the dead insect and formation of conidia under conditions of high relative humidity, dispersal of the conidia to locations where they may encounter susceptible insects and start the process again.
Agricultural crops i.e. sugarcane, vegetables trees etc roots are damaged by grubs and termites,

yellowish appearance and after that at last die. Most of this research has been undertaken with *B. bassiana* (Latifian and Rad, 2012). However, the production of fungi in suitable media for large scale application has not yet been studied therefore, the present study was undertaken to evaluate g sorghum and liquid media such as Potato Dextrose agar Dextrose Broth for the mass production of *B. bassiana.* Masoud Latifian *et al.* (2013) [13]. Study, fungi, the most appropriate medium for the production of *B. bassiana* in liquid and solid phases to produce clamidospore and conidiosopre were elected. For the liquid phase of plant foods, including potatoes, wheat flour, rice flour, corn flour and sugar cane molasses, and for the solid phase of plant materials, including sugar cane, corn, barley, rice, millet and sorghum were evaluated. The performance characteristics of the liquid phase compared by spore concentration and germination percent of clamidospores. The performance characteristics of the solid phase compared by wet weight, dry weight, conidia concentration and germination. Results showed that between different plant extracts used as liquid and solid medium to produce clamidospores and conidia of *B. bassiana* were significantly different at and 1% probability. Masoud Latifian *et.,al*., (2013) [13].

Among 85 genera of entomopathogenic fungi only six species are commercially available for field application. However, comparatively few have been investigated as potential mycoinsecticides. Fungal pathogens particularly *B. bassiana*, Paceliomces- fumosorosea and M. anisopliae are being evaluated against numerous agricultural and urban insect pests. Several species belonging to order Isoptera (Hussain *et. al*, 2011) [11], Lepidoptera, Coleoptera (Ansari *et. al*, 2006) [2], Hemiptera (Leite *et. al*, 2005) [11]. This has led to a number of attempts to use entomopathogenic fungi for pest control with varying degrees of success. The majority of fungal production systems consist of two stages system in which fungal inoculum of hyphal bodies is produced in liquid culture and then transferred to a solid substrate for production of aerial spores (Devi, 1994) [5]. For practical use of entomopathogenic fungi as bio-insecticides at each stage, it is necessary to develop culture medium and method that produce high concentrations of viable and virulent propagules at low cost (Jackson *et. al*., 1997) [8]. These goals can be achieved by using the most favorable inexpensive components for fungal growth at the lowest concentration that produce high yield. Most common compounds for fungal entomopathogens include agro-industrial products and by-products such as corn steep liquor (Zhao *et. al*., 2010) [23], sugarcane molasses (Hussain *et al*., 2011) [11]. The major issues involved in mass production developing of cost effective methods for mass production and rearing. Keeping in view the above facts, the present study was carried out with the following objective: (i) To the growth medium and their physical growth conditions. (ii) To study the *B. bassiana* strains (iii) To the mass production of the *B. bassiana* production techniques.

Materials and Methods

The present study was carried out in Bio-control Laboratory, Department of Entomology Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.) India in 2016-17. The experiment was conducted in a laboratory to test the effect on solid and liquid substrates of mass production and economics of *Beauveria bassiana* entomopathogenic fungi (EPFs). The details of material used experimental procedure followed and techniques adopted in the present investigation are described in the follows:

Experimental details

To evaluate the different substrate for Mass production of entomopathogenic fungus *Beauberia bassiana* (Balsamo) on solid and liquid substrates a plan of field experiment was carried out for the year *20*16-17 are details 11 treatments, replication three and design RBD.

Table 1: Treatments along with of solid and liquid substrate

Treatments	Substrates A. Solid medium	Spore concentration
T_1	Farm yard manure (FYM)	$1x10^7$ spores/ ml
T_2	Vermi compost	$1x10^7$ spores/ ml
T_3	Dung (Fresh)	$1x10^7$ spores/ ml
T_4	Farm yard manure (FYM) + 1% YE	$1x10^7$ spores/ ml
T_5	Vermi compost + 1% YE	$1x10^7$ spores/ ml
T_6	Dung (Fresh) + 1% YE	$1x10^7$ spores/ ml
T_7	Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml
T_8	Vermi compost + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml
T_9	Dung (Fresh) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml
	B. Liquid medium	
T_{10}	Savoured dextrose broth (SDB)	$1x10^7$ spores/ ml
T_{11}	Potato dextrose broth (PDB)	$1x10^7$ spores/ ml

Nucleus culture

The nucleus culture of *B. bassiana*, was thereafter maintained on Sabouraud Dextrose Broth (SDB) medium as per the procedure of Prasad *et al*. Briefly, SDB was prepared and sterilized at 121ºC (15 lbs) for 20 minutes. Then cooled and poured in presterilized Petri plates and *B. bassiana* from stock culture was inoculated aseptically. The Petri plates were incubated at 25 ± 1ºC in BOD incubator for two weeks to harvest the inoculums culture.

Substrates and *in vitro* production

Nine mass production substrates were evaluated for the conidial production of *B. bassiana*, under controlled conditions of 25 ± 1oC in BOD incubators for 3 weeks. The substrates were i. Farm yard manure (FYM), ii. Vermi compost, iii. Dung (Fresh), iv. Farm yard manure (FYM) + 1% YE, v. Vermi compost + 1% YE, vi Dung (Fresh) + 1% YE, vii. Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose, viii. Vermi compost + 1% YE + 1.0 g Dextrose, ix. Dung (Fresh) + 1% YE + 1.0 g Dextrose, x. Savoured dextrose broth (SDB) and xi. Potato dextrose broth (PDB).

The last six medium were supplemented with dextrose. There were nine treatments in three replications. A quantity of 130 g dehydrated SDB was suspended in 2000 ml distilled water, heated to dissolve the medium (pH 5.6) and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. The lukewarm liquid media was poured in conical flask. Then *B. bassiana*, were inoculated aseptically and then conical flasks were incubated in BOD. The each substrate cleaned with fresh water and 100 g of each was put in separate Conical flasks (250 ml capacity) supplemented with dextrose (1.0 g), plugged with non-absorbent cotton and autoclaved. Upon cooling of media, *B. bassiana*, were inoculated aseptically and incubated in BOD incubator.

Spore counting

A drop of conidial suspension of *B. bassiana*, (obtained from the growing media by filtering through muslin cloth) was placed on the hemocytometer. The cover glass was put over the grid carefully so that no air bubble entered between cover

glass and slide. The conidia of entomopathogenic fungi were counted under Olympu.

Preparation of fungi

The cultured in SDA medium and kept 10 days at 25±1 ºC, 100% RH and 12:12 (L:D) photoperiod, Then conidia were harvested and suspended in Tween 80 (0.2 ml/l) in sterile distilled water and vortexes for 3 min to produce a homogenous suspension. Then the suspension was filtered through several layers of cheesecloth to remove mycelia and debris. By using a Haemocytometer, the spore concentration was determined and adjusted 104 conidia/ml-1.

Liquid Media: Potato dextrose broth (PDA)

100g of peeled and sliced potato was added in 250 ml distil water the potatoes were boiled till they became soft. The contents of the beakers were filtered through muslin cloth and squeezed out all liquid 10g dextrose was dissolved in water and added to the extract and made the volume to 500ml. Dispensed 100ml to each conical flask and plugged with non-absorbent cotton. The flasks were sterilized at 15 psi pressure for 20 mim in an autoclave. After cooling, 5 mm fugal disc of entomopathogeni fungus was inoculated 25^0C three replications were maintained.

Savoured dextrose broth (SDB)

1000 ml of distilled water was taken, in which 10 g of dextrose and 2-5g of peptone was added, and dispensed 100ml media into 250 ml conical flask and plugged with non-absorbent cotton. Sterilized the flasks at 15 psi pressure for 20 min in a autoclave. After cooling, 5 mm fungal disc of entomopathogenic fungus was inoculated into each flask under laminar air flow chamber. Flasks were incubated in BOD incubator at 25^0C.Two replications were maintained.

Spore harvesting and drying

The spores were harvested at 3 and 12 days after the inoculation for liquid and solid media respectively, to evaluate spore yield. To harvest the spores as powder it was necessary to dry the fungus to reduce moisture content and allow the spores to separate from the substrata.

To dry the cultures the plastic bags were opened in a room with a temperature of 20 ± 5ºC and an average relative humidity of 50±5% and allowed to air dry.

Statistical analysis of the different insecticides treatments

The data recorded during the course of investigation were subjected to statistical analysis by using analysis of variance technique (ANOVA) for randomized block design as suggested by Panse and Sukhatme (1978).

Findings

Beauveria bassiana, sabouraud dextrose borth (SDA) colonies are generally white at the edge becoming cream to pale yellow, occasionally reddish. Conidiophores are abundant rising from vegetative hyphae, 1-2 µm wide, bearing groups of clustered conidiogenous cells 3 – 6 ×3- 5 µm which may branch to give rise to further conidiogenous cells, globular to flash shaped With a well-developed rachis up to 20 µm long by1 µm wide, geniculate with denticles up to 1 µm long. Chlamydospore is absent.

Multiplication of entomopathogenic fungi on different agricultural products

The present studies, several naturally available substrates of both solid and liquid media were tested for mass multiplication of different *Beauveria bassiana* entomopathogenic fungus on agriculture products. The successfully microbial management of insect pests on the crops and successful mass production of the microbial agents in the laboratory on media/substrates. Large-scale availability of the media/substrates is a primary requirement in the bio-control program. The growth and development of microorganisms is responsible for the mycelia growth and spore yield. Although, the saprophytic fungi utilize a range of nutrient sources but for mass production and commercialization, simple and cheap media are needed. However, the production technique of *Beauveria bassiana* on suitable media prepare studied, thus the present study was under laboratory.

Mass production of *Beauveria bassiana*

Solid and Liquid media

Seventh Day after inoculation

The effect of different substrates for the mass production of *Beauveria bassiana* spore/ml was significantly higher recorded showed that in (Table -2 and Figure-1) The results revealed that all the treatments were significantly producing spore per ml and thus increasing the yield significantly as compared to other substrates. The results revealed that all the treatments were significantly higher effective in producing spore/ml as compared to other substrates.

Data recorded on 7 day after the day after inoculation of various treatments in the (Table -2 and Figure-1). T_{10} Savoured dextrose broth (SDB), was the best treatment by bringing down the *B. Bassiana* production upto (48.3) spore/ml in liquid medium during the 2016-17 years. The other treatments in order of spore producing was with T_{11} Potato dextrose broth (PDB) (37.42), in liquid medium and solid medium in the treatment and T_8 Vermi compost + 1% YE + 1.0 g Dextrose (27.73), followed by with T_7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose 20.72 spore/ml, T_5 Vermi compost + 1% YE (16.43 spore/ml), T_4 Farm yard manure (FYM) + 1% YE (13.67 spore/ml), T_2 Vermi compost (8.4 spore/ml), T_1 Farm yard manure (FYM) (6.23 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose (16.43 spore/ml), T_6 Dung (Fresh) + 1% YE (1.12 spore/ml), and T_3 Dung (Fresh) respectively.

Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 48.30 and 37.42 spore/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB).

Similar trend was recorded on fourteenth day after inoculation. T_{10} Savoured dextrose broth (SDB) again was the most effective treatment (85.03 spore/ml) producing. The second most effective treatment was T_{11} Potato dextrose broth (PDB) (72.64spore/ml) producing and solid medium T8 Vermi compost + 1% YE + 1.0 g Dextrose (67.24 spore/ml) producing during both the years followed by T_7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose (57.05 spore/ml), T_5 Vermi compost + 1% YE (45.08 spore/ml), T_4 Farm yard manure (FYM) + 1% YE (36.01spore/ml), T_2 Vermi compost (22.83 spore/ml), T_1 Farm yard manure (FYM) (14.37 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose(13.76 spore/ml), T_6 Dung (Fresh) + 1% YE (6.47 spore/ml), and T_3 Dung (Fresh) (2.04 spore/ml) during both the year 2016-17 respectively.

Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly

recorded higher 85.03 and 72-64 spore/ml were recorded on sabouraud dextrose broth (SDB) and potato dextrose broth (PDB).

The observation data recorded twenty one days after inoculation trend during the year 2016-17 of observation basis T_{10} Savoured dextrose broth (SDB) again was the most effective treatment (151.64 spore/ml) producing. The second most effective treatment was T_{11} Potato dextrose broth (PDB) (116.59 spore/ml) producing and solid medium T8 Vermi compost + 1% YE + 1.0 g Dextrose (99.22 spore/ml) producing during both the years followed by T_7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose (81.64 spore/ml), T_5 Vermi compost + 1% YE (71.78 spore/ml), T_4 Farm yard manure (FYM) + 1% YE (68.61 spore/ml), T_2 Vermi compost (53.56 spore/ml), T_1 Farm yard manure (FYM) (45.92 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose(36.11 spore/ml), T_6 Dung (Fresh) + 1% YE (29.59 spore/ml), and T_3 Dung (Fresh) (23.33 spore/ml) during both the year 2016-17 respectively.

Overall result showed that 21 days among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 151.59 and 116.59 spore/ml were recorded on sabouraud dextrose broth (SDB) and potato dextrose broth (PDB).

Our finding showed that twenty eight days after inoculation trend during the year 2016-17 of observation basis on all substrates i.e. T_{10} Savoured dextrose broth (SDB) again was the most effective treatment (224.59 spore/ml) producing. The second most effective treatment was T_{11} Potato dextrose broth (PDB) (193.06 spore/ml) producing and solid medium T8 Vermi compost + 1% YE + 1.0 g Dextrose (152.84 spore/ml) producing during both the years followed by

T_7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose (123.89 spore/ml), T_5 Vermi compost + 1% YE (99.43 spore/ml), T_4 Farm yard manure (FYM) + 1% YE (88.22 spore/ml), T_2 Vermi compost (73.85 spore/ml), T_1 Farm yard manure (FYM) (62.28 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose (47.3 spore/ml), T_6 Dung (Fresh) + 1% YE (35.58 spore/ml), and T_3 Dung (Fresh) (25.26 spore/ml) during both the year 2016-17 respectively.

Our finding overall result showed that on 21 days among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 224.59 and 193.06, spore/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB).

Among the different solid and liquid media on thirty five days after inoculation slow growth of *Beaubaria bassiana* spore/ml count witch different significantly from the substrate T_{10} Savoured dextrose broth (SDB) again was the most effective treatment (237.17 spore/ml) producing. The second most effective treatment was T_{11} Potato dextrose broth (PDB) (217.62 spore/ml) producing and solid medium T8 Vermi compost + 1% YE + 1.0 g Dextrose (163.25 spore/ml) producing during both the years followed by T7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose (138.36 spore/ml),

Table 2: Cost effective of multiplication of *Beauveria bassiana* on different media

Treatments	Substrates	Spore concentration	Effect of substrates on the inoculation Increase growth on *Beauveria bassiana* at different days after inoculation				
			7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
	Solid medium						
T_1	Farm yard manure (FYM)	$1x10^7$ spores/ ml	6.23 (2.68)	14.37 (3.92)	45.92 (6.85)	62.28 (7.95)	74.99 (8.71)
T_2	Vermi compost	$1x10^7$ spores/ ml	8.4 (3.06)	22.83 (4.88)	53.56 (7.38)	73.85 (8.65)	84.62 (9.25)
T_3	Dung (Fresh)	$1x10^7$ spores/ ml	1.45 (1.56)	2.04 (1.74)	23.33 (4.92)	25.26 (5.12)	32.33 (5.77)
T_4	Farm yard manure (FYM) + 1% YE	$1x10^7$ spores/ ml	13.67 (3.83)	36.01 (6.08)	68.61 (8.34)	88.22 (9.45)	96.08 (9.85)
T_5	Vermi compost + 1% YE	$1x10^7$ spores/ ml	16.43 (4.17)	45.08 (6.79)	71.78 (8.53)	99.43 (10.02)	105.9 (10.33)
T_6	Dung (Fresh) + 1% YE	$1x10^7$ spores/ ml	1.12 (1.45)	6.47 (2.73)	29.59 (5.51)	35.58 (6.04)	44.66 (6.75)
T_7	Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	20.72 (4.65)	57.05 (7.62)	81.64 (9.09)	123.89 (11.17)	138.36 (11.80)
T_8	Vermi compost + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	27.73 (5.35)	67.24 (8.26)	99.22 (10.01)	152.84 (12.40)	163.25 (12.81)
T_9	Dung (Fresh) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	2.72 (1.92)	13.76 (3.84)	36.11 (6.08)	47.3 (6.95)	55.39 (7.50)
	Liquid medium						
T_{10}	Savoured dextrose broth (SDB)	$1x10^7$ spores/ ml	48.3 (7.01)	85.03 (9.26)	151.64 (12.35)	224.59 (15.02)	237.17 (15.43)
T_{11}	Potato dextrose broth (PDB)	$1x10^7$ spores/ ml	37.42 (6.19)	72.64 (8.58)	116.59 (10.84)	193.06 (13.93)	217.62 (14.78)
SEm ±			0.097	0.107	0.123	0.121	0.071
CD at 5%			0.288	0.317	0.364	0.361	0.211

Figures are parenthesis in squire root value

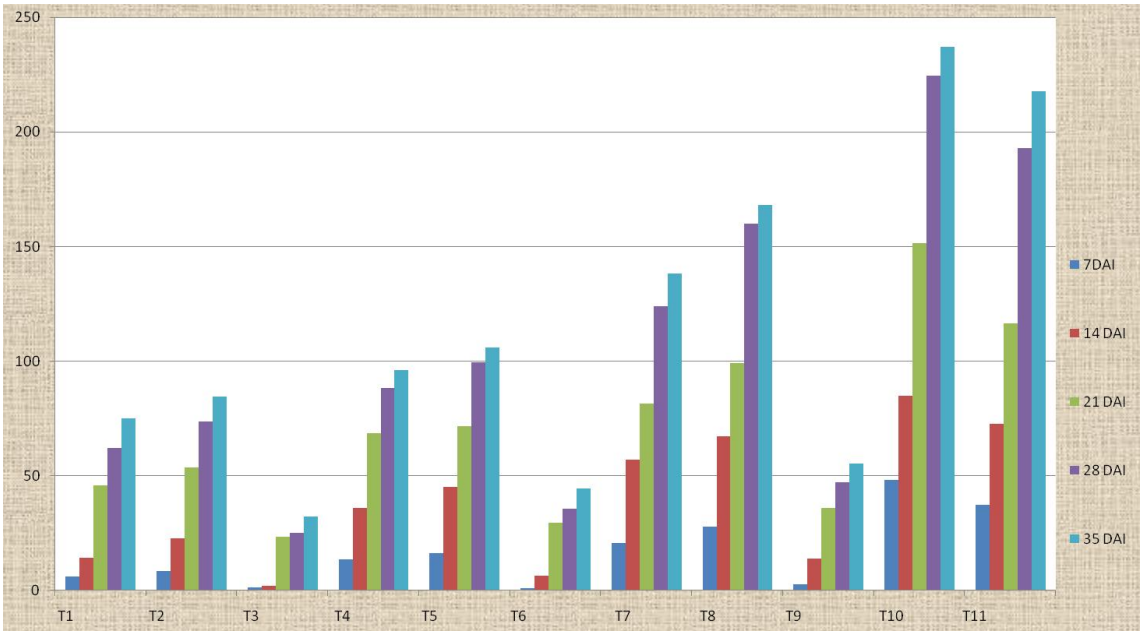

Fig 1: Multiplication of *Beauveria bassiana* on different media

T_5 Vermi compost + 1% YE (105.9 spore/ml), T_4 Farm yard manure (FYM) + 1% YE (96.08 spore/ml), T_2 Vermi compost (84.62 spore/ml), T_1 Farm yard manure (FYM) (84.99 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose (55.39 spore/ml), T_6 Dung (Fresh) + 1% YE (44.66 spore/ml), and T_3 Dung (Fresh) (32.33 spore/ml) during both the year 2016-17 respectively. Overall finding showed that in the table no. at 35 days among the liquid and solid media tested, for *Beauveria bassiana* spore/ml production was significantly higher recorded 224.59 and 193.06 spore/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB). The present results are in conformity with the findings of Mondal and Battacharya (2004) [14] they reported that conidial count PI (Pantnagar) isolates of *B.bassiana* was highest, Latifian *et al.* (2013) [13] evaluated most appropriate medium for the production of *Beauveria bassiana* potatoes, wheat flour, rice flour, corn flour and sugar cane molasses and solid phases includes sugar cane, corn, barley, rice, millet and sorghum. Among different media, sugar cane molasses extract and rice showed maximum growth of *Beauveria bassiana* Different solid substrates *i.e* maize, bran, cotton seed, rice husk, wheat and liquid media

Such as coconut water were content and yeast extract concentration for mass production of two entomopathogenic fungi: Vuellemin and *Metarhizium anisopliae* substrate for spore production and their viabil 2013). Sahayaraj *et al.* (2008) [19] reported that, wheat supported maximum spore production for recorded maximum spore production in different grains. Sivakalai *et al.* available products such as vegetables (bitter gourd, drumstick, green banana, potato), oil-cakes (coconut oil cakes, coconut cakes, groundnut cakes, sunflower cakes) and agro wastes such as rice grain, boiled bran, raw bran, rice husk, powder and whey for mass production of two entomopathogenic fungi such as *Metarhizium anisopilae.* Results showed that rice grain supporting maximum spore production for both entomopathogenic fungi.

Economics of mass production of *Beauveria bassiana* spore/ml

Collect the substrate for cost effective of *Beauveria bassiana* spore/ml ($1x10^6$) cost incurred for the production of spores T_{10} Savoured dextrose broth (SDB) was the best low cost substrate and high production showed in the table 3 and Figure 2 (Rs 5.43 and 149.35 spore/ml), T_{11} Potato dextrose broth (PDB) (Rs 6.96 and 127.47 spore/ml), and solid substrate T_8 Vermi compost + 1% YE + 1.0 g Dextrose (Rs 6.11 and 104.46 spore/ml) and T_7 Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose (Rs 6.69 and 84.33 spore/ml) followed by T_5 Vermi compost + 1% YE(Rs 7.22 and 67.72 spore/ml), T_4 Farm yard manure (FYM) + 1% YE(Rs 7.77 and 60.52 spore/ml), T_2 Vermi compost(Rs 8.05 and 48.65 spore/ml), T_1 Farm yard manure (FYM) (Rs 8.84 and 40.76 spore/ml), T_9 Dung (Fresh) + 1% YE + 1.0 g Dextrose (Rs 9.85 and 31.06 spore/ml), T_6 Dung (Fresh) + 1% YE (Rs 10.13 and 23.48 spore/ml), and T_3 Dung (Fresh) (Rs 10.55 and 16.88 spore/ml). The methodology evaluated in this study allows for the production of high quality *B. Bassiana* fungal spores, but the quantity produced are only suitable for small-scale laboratory and field trials. Conformity this findings Prasad, *et al.* (2014) [4] revealed production was recorded in sugarcane biogases (Rs. 1.14, 2.18 and 1.92) followed by press mud for and *V. lecanii.* experiment.

Conclusion

In the mass production of *B. bassiana* on different substrate solid and liquid medium "Savoured dextrose broth (SDB), Potato dextrose broth (PDB) and solid substrate "Vermi compost + 1% YE + 1.0 g Dextrose" the best low cost substrate and high production found to be the best substrate in maximum spore production in minimum time as well as in cost of production of spores.

Table 3: Cost effective of multiplication of *Beauveria bassiana* on different media

Treatments	Substrates	Spore concentration	Production and cost effective of of *B. bassiana* on different substrates.	
			Average Production	Cost of each substrate
	Solid medium			
T_1	Farm yard manure (FYM)	$1x10^7$ spores/ ml	40.76	8.84
T_2	Vermi compost	$1x10^7$ spores/ ml	48.65	8.05
T_3	Dung (Fresh)	$1x10^7$ spores/ ml	16.88	10.55
T_4	Farm yard manure (FYM) + 1% YE	$1x10^7$ spores/ ml	60.52	7.77
T_5	Vermi compost + 1% YE	$1x10^7$ spores/ ml	67.72	7.22
T_6	Dung (Fresh) + 1% YE	$1x10^7$ spores/ ml	23.48	10.13
T_7	Farm yard manure (FYM) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	84.33	6.69
T_8	Vermi compost + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	104.46	6.11
T_9	Dung (Fresh) + 1% YE + 1.0 g Dextrose	$1x10^7$ spores/ ml	31.06	9.85
	Liquid medium			
T_{10}	Savoured dextrose broth (SDB)	$1x10^7$ spores/ ml	149.35	5.43
T_{11}	Potato dextrose broth (PDB)	$1x10^7$ spores/ ml	127.47	6.96

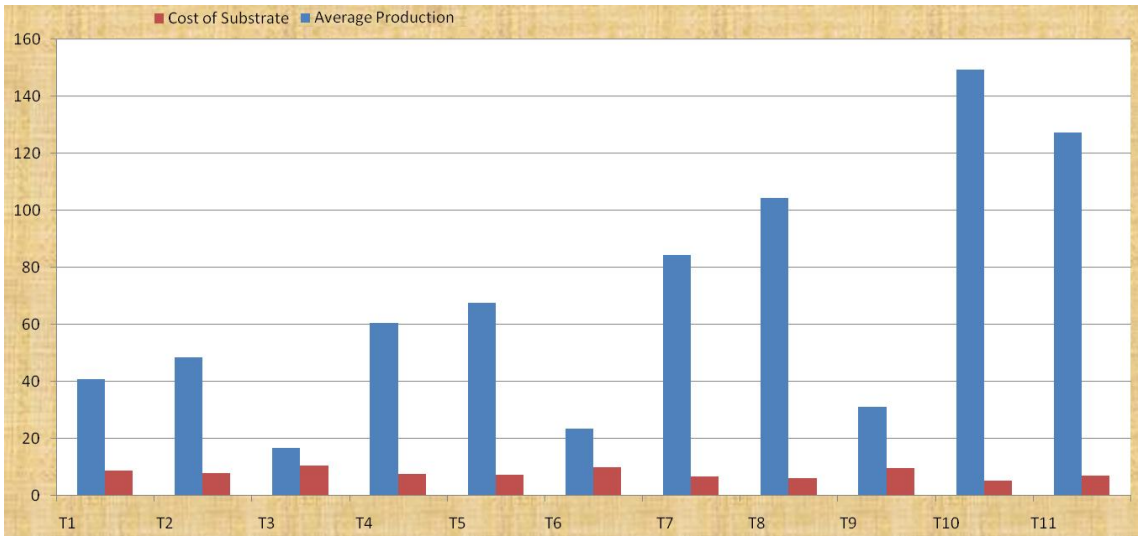

Fig 2: Cost effective of multiplication of *Beauveria bassiana* on different media

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