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RESEARCH ARTICLE :

Mass multiplication of *Beauveria bassiana* on substrates

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SUMMARY : Different solid substrates *viz.* sorghum, maize, chickpea, ragi,rice and Neem cakeand liquid media's *viz.*, potato dextrose broth(PDB), czapek dox broth (CDB) and sabouraud dextrose broth (SDB) were evaluated for mass multiplication of *Beauveria bassiana* (Balsamo) Vuillemin. Rice (T_5) were found to be best and exhibited maximum population (8.78×10^8cfu/ml) followed by PDB (T_7) with 7.44×10^8 cfu /ml. And significantly lowest production cost was recorded in rice (T_5) (Rs. 1.01).

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KEY WORDS :
Substrates,*Beauveria bassiana*, Spore load, Production cost

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BACKGROUND AND OBJECTIVES

Microbial control is the most promising approach to manage insect pest with little disturbance to environment. Entomopathogens offer several advantages *i.e*. no pollution problems and health hazards, more stable without any development of resistance in insect pest, and cause little or no disturbance in ecological balance. The white muscardine fungus, *Beauveria bassiana* is exploited in greenhouse and outdoor crops as a tool for control of many agricultural arthropod pests, including whiteflies, aphids, thrips, psyllids, weevils and mealybugs (Shah and Goettel, 1999). *B. bassiana* can have many effects on susceptible insects, including repellence, moulting disruption, growth reduction, interference with development and oviposition, and high mortality, particularly in immature insect (Mitchell *et al.*, 2004). Being facultative and amenable for easy multiplication on large scale, they offer great scope to develop as potent biopesticide (Lingappa and Patil, 2002).

For successful utilization of entomopathogens in management of insect pest, selection of virulent strain, production of large quantity of spores, pathogenicity against various pest species, selection of suitable media,formulation procedures and condition for storage are important (Gangwar,2013). Fungal spores are living organisms and their viability diminishes with time depending on environmental conditions and for the commercial production of fungal spores, there is need to obtain an ideal cheap and highly production culture medium (Moore *et al.*, 2000).

Therefore, mass production of entomopathogenic fungi is an essential component for their utilizationin the IPM. Major obstacle in the mass multiplication of *B. bassiana* is its slow growth rate and non-availability of suitable substrate. Therefore, thepresent study was carried out to determine the most suitable and locally available substrate and media for mass multiplication of *B. bassiana*.

RESOURCES AND METHODS

The present study was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka. The experiment was carried with Completely Randomized Block Design using nine different treatments including six solid substrates and three liquid media's for standardization a suitable economic medium for growth and sporulation of fungus substrates for mass production of *B. bassiana* (Table A).

Table A : Substrates selected for mass production of *Beauveria bassiana*

Treatment codes	Treatments
I	Solid substrates
T_1	Maize (*Zea mays* L.)
T_2	Chickpea (*Cicer arietinum* L.)
T_3	Sorghum (*Sorghum vulgaris* L.)
T_4	Ragi [*Eleusine coracana* (L.) Gaertn]
T_5	Rice (*Oryza sativa* L.)
T_6	*Neem* cake
II	Liquid media's
T_7	Potato dextrose broth (PDB)
T_8	Czapek dox broth (CDB)
T_9	Sabouraud dextrose broth (SDB)

Hundred grams of eachsubstrate *i.e.*,whole grains of rice, maize, sorghum, ragi and chickpea was washed and soaked in water for overnight except rice which was soaked for 3 hours before starting the experiment. The excess water was drained by decanting and further shade drying was done for half an hour to remove the excess moisture. For *Neem* cake, 100g of substrate was taken in a 250ml conical flask and to it 50ml of sterile distilled water was addedand were plugged with non-absorbent cotton. Each treatment was replicated thrice. The substrates were packed separately in individual 250 ml conical flask and were plugged with non-absorbent cotton and autoclaved at15 psi for 30 min. After cooling, 5 mm fungal disc was inoculated into each flask aseptically under laminar air flow chamber. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grains and to break the mycelial mat.

Artificial media's were obtained from Hi-Media company and were utilised for mass production of the fungus. The constituents and methodology for preparation of these medias are as follows:

Potato dextrose broth (PDB) :

100g of peeled and sliced potato were added in 250 ml distilled water in 1L beaker, the potatoes were boiled till they became soft. The contents of the beaker were filtered through muslin cloth to squeeze out all the liquid. 10g of dextrose was dissolved in water and added to the extract and the volume was made upto 500ml. 100ml of the extract was dispensed to each conical flask and was plugged with non-absorbent cotton.

Sabouraud's dextrose broth (SDB) :

250 ml of distilled water was taken, in which 10 g of dextrose and 2.5g of peptone was added, and 100 ml media was dispensed into 250 ml conical flask and was plugged with non absorbent cotton.

Czapek Dox Broth (CDB) :

Czapek Dox Broth is a semi-synthetic medium. The main ingredients were, Sucrose 30.00g, Sodium nitrate 3.00g, Dipotassium phosphate 1.00g, Magnesium sulphate 0.50g, Potassium chloride 0.50g and Ferrous sulphate 0.01g. 3.50 g from the mixture of the ingredients was suspended in 100 ml of distilled water.

These media containing flasks were sterilized at 15 psi pressurefor 30 min in the autoclave. After cooling, 5 mm fungal disc of *B. bassiana* was inoculated into each flask under laminar air flow chamber.Flasks were incubated in BOD incubator at 28ºC. Three replications were maintained.

Observation details :

Growth and sporulation was observed at 10^{th}, 20^{th} and 30^{th} days after inoculation. Sporulation was measured by suspending 1g colonized substrate from each replication. This suspension was serially diluted withadding 9 ml sterilized water to 1 ml conidial suspension. From $1x10^{8}$dilution, 1 ml suspension and 20 ml melted PDA was poured into Petri dishes and mixed

by rotating dishes. Three replications were maintained. Sporulation was observed as colony forming units (cfu) per gram colonized substrate.Method of Sahayaraj and Namasivayam (2008) and Rajnikanth *et al.* (2010) were followed with some little modification.

OBSERVATIONS AND ANALYSIS

All the tested substrates supported good growth of *B. bassiana*. Perusal of data in Table 1 and Fig. 1, revealed that highest conidial count was recorded on rice (T_5) (8.78×10^8 cfu/ml) followed by PDB (T_7) (7.44×10^8 cfu / ml) which was significantly superior spore load over all the other substrates tested.Subsequent higher spore load was recorded in chickpea (T_2) (6.44×10^8cfu/ml) and sorghum (T_3) (4.78×10^8cfu/ml) which were significantly better than other remaining substrates. The next group of substrates were CDB (T_8)(4.33×10^8 cfu/ml), Ragi (T_4) (3.67×10^8 cfu /ml), maize (T_1) (2.89×10^8 cfu/ml) and SDB (T_9) (2.89×10^8 cfu/ml), but all the treatments were at par with each other. The least spore count was recorded in neem cake (T_6) (0.89×10^8cfu/ml).

Cost of production of 1×10^8 spores/ml were calculated for all substrates and the data is presented in Table 1 and depicted in Fig. 1. The cost of production on different substrates/media significantly varied from each other. Significantly lowest production cost was recorded in rice (T_5) (Rs. 1.01).

For successful utilisation of microbial agents its mass multiplication plays a crucial role and faster, luxuriant growth of the fungus can only be obtained when grown on suitable substrate. All the substrates tested supported good growth of *B. bassiana*.

Among the different substrates evaluated ,rice recorded highest conidial count (8.78×10^8cfu/ml)followed by PDB (7.44×10^8cfu/ml) which were with significantly superior spore load over all the other substrates tested. These results were similar to the previous findings of Sharma *et al.* (2002) who reported that rice grains were best for sporulation of two *B. bassiana* isolates (9.7×10^7 and 7.56×10^7 conidia g^{-1}).While, Kalidas (2010) and Yadav *et al.* (2013) reported that rice powder broth was found more suitable than Potato Dextrose broth in terms of number of spores for carrying out mass multiplication.

Table 1 : Mass production of *Beauveria bassiana* on different substrates

Trt. code	Substrates	Spore count (1×10^8 spores/ml) at different DAI				Rate of increase in growth of *B.bassiana* (%) (DAI)		Cost of substrate per 100g (Rs.)	Cost of production of $1x10^8$ spores ml^{-1} (Rs.)
		10 DAI	20 DAI	30 DAI	Mean	10 to 20	20 to 30		
Solid substrates									
T_1	Maize	1.33	3.00	4.33	2.89	61.11	26.67	5.50	3.17
		(1.29)	(1.86)	(2.19)	(1.81)	(57.94)	(27.34)		
T_2	Chickpea	5.00	5.67	8.67	6.44	35.56	33.97	10.00	3.86
		(2.33)	(2.48)	(3.02)	(2.62)	(38.86)	(35.88)		
T_3	Sorghum	3.00	4.67	6.67	4.78	34.44	30.60	3.50	1.57
		(1.86)	(2.26)	(2.67)	(2.27)	(36.91)	(33.38)		
T_4	Ragi	1.67	3.33	6.00	3.67	50.00	44.29	4.20	2.22
		(1.46)	(1.95)	(2.54)	(1.99)	(46.27)	(41.99)		
T_5	Rice	6.33	8.33	11.67	8.78	23.15	26.32	3.00	1.01
		(2.61)	(2.97)	(3.48)	(3.02)	(28.11)	(30.46)		
T_6	*Neem* cake	0.33	1.00	1.33	0.89	50.00	33.33	3.5	3.09
		(0.88)	(1.17)	(1.34)	(1.16)	(46.45)	(32.70)		
Liquid (artificial) media									
T_7	PDB	2.67	7.33	11.00	7.00	55.56	50.00	479	193.86
		(1.77)	(2.79)	(3.38)	(2.66)	(48.53)	(33.53)		
T_8	CDB	1.67	3.67	7.67	4.33	72.22	36.67	525	265.86
		(1.46)	(2.04)	(2.85)	(2.12)	(63.43)	(45.28)		
T_9	SDB	1.00	3.00	4.67	2.89	61.11	36.67	350	208.95
		(1.17)	(1.86)	(2.27)	(1.79)	(57.94)	(37.24)		
	S.E.±	0.11	0.08	0.09	0.17	6.64	6.63	-	14.37
	C.D. (P=0.05)	0.22	0.17	0.19	1.36	NS	NS	-	29.79

Note: Values in parenthesis for spore count are square root transformation(x+0.5); NS=Non-significant
DAI-Days after inoculation;
() = Figure in the parentheses are arcsin transformed values

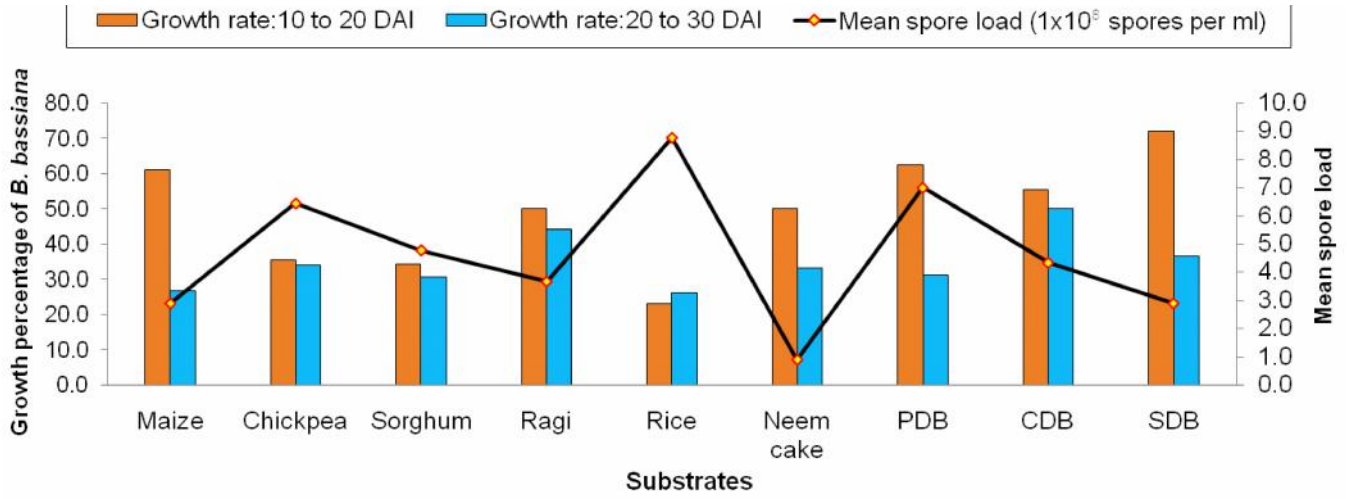

Fig. 1 : Spore load and rate of growth percentage of *Beauveria bassiana* on different substrates

Ricehas maximum spore production (8.13 x 10^9 conidia/g of substrate) for 10 days at 28±2°C, under natural day light (Babul *et al.*, 2016).

In addition to faster multiplication, highest conidial count and colony forming unitwere highest inrice substrate (Kaur and Joshi, 2014) reported that rice as the most suitable substrate as it yielded highest conidial count (31.0x 10^5conidia/g) and colony forming unit (30.5 x 10^5cfu/g).

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