

Mass Production of entomopathogenic fungi *Beauveria bassiana* (Metsch.) on Liquid and Solid Media

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ABSTRACT

The present investigation entitled “Mass Production of entomopathogenic fungi *Beauveria bassiana* (Metsch.) on Liquid and Solid Media” was carried out at Bio Control Laboratory, Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut during 2020-21. In all there were eight treatments including control replicated thrice in a complete block design (CRD). The substrate for spores constrictive effective of *Beauveria bassiana* spore/ml (1×10^6) production incurred for the production of spores significantly superior of various treatments in showed the treatments Savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. Bassiana* production on 7, 14, 21, 28 and 35 days (47.28, 84.12, 147.23, 219.21 and 258.31 spores constrictive) in liquid medium followed by second most effective other treatments in order of spore producing was with the treatment in Potato dextrose broth (PDB) on 7, 14, 21, 28 and 35 days (41.23, 77.86, 114.27, 191.36, and 211.65) spores constrictive), in liquid medium and solid medium is the most effective treatment Black gram + Molasses + 1% YE + 1.0 g Dextrose on 7,14,21,28 and 35 days (27.32, 63.11, 93.32, 152.31 and 161.64 spores constrictive), superior in solid medium.

KEYWORDS

Mass Production, Entomopathogenic Fungi, *Beauveria bassiana* (Metsch.) Liquid, Solid Media

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Entomopathogenic fungi *Beauveria bassiana* and which can be used as a biological control agent of several insect and pest. Among 85 genera of entomopathogenic fungi only six species are commercially available for field application. However, comparatively few have been investigated as potential mycoinsecticides. Particular interest is the ability displayed by various genera of entomopathogenic fungi to colonize wide plant species in different families, both naturally and artificially following inoculation, ensuring protection against not only insect pests but also

plant pathogens (Jaber and Ownley, 2018). Mode of action of *B. bassiana* against insects. The infection cycle of *B. bassiana* in invertebrates bodies has been depicted by (Mascarin and Jaronski, 2016). Asexual spores (conidia) are dispersed by wind, rain splashing or even by arthropod vectors facilitating the fungus to establish infection on susceptible hosts (Ortiz- Urquiza and Keyhani, 2013). Several studies revealed the insecticidal potential of *B. bassiana* as mycopesticides and commercial endophytic fungi (Jaber and Ownley, 2018).

All the studies involved direct application of the entomopathogen to target pests or indirect application by inoculation of the pest host plant. In the last case, *B. bassiana* is considered as an endophyte of the host plant. In the endophytic colonization strategy, methods of inoculating plant species consist of seed coating and seedlings injection (Brownbridge *et al.*, 2012).

Materials and Methods

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.*, 2014) briefly, SDB was prepared and sterilized at 121⁰C (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 ± 1⁰C in BOD incubator for two weeks to harvest the inoculums culture.

Procedure of *B. bassiana* Production

Procedure involving soaking the solid media (sorghum grains) overnight (24 hours) and decanting the water. Then transfer the soaked grains in conical flask @ 100 g/250 ml of flask and autoclaved these flasks at 15 lbs/psi for 15 minutes then with the help of sterilized cork borer cut 5 mm disk of fungal pathogen, from 10 days old grown Entomopathogenic fungi on SDA petri dish is bored out and transfer such 5-6 disk per conical flask in aseptic conditions and polythene also.

Substrates and *In Vitro* Production

Eleventh substrate solid and liquids Nine mass production substrates were evaluated for the conidial production of *B. bassiana*, under controlled conditions of 25 ± 1⁰C 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 + 1.0g Dextrose, viii. Vermi compost + 1% YE + 1.0g Dextrose, ix. Dung (Fresh) + 1% YE + 1.0g 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 (121⁰C) 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. 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.

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 mm in an autoclave. After cooling, 5 mm fungal disc of entomopathogenic fungus was inoculated 25⁰C 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 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 25⁰C. Three replications were maintained.

Results and Discussion

The effect of different substrates for the mass production of *Beauveria bassiana* spore/ml was significantly higher recorded showed that in (table 1 and fig 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 taken after on seventh days after inoculation of various treatments in showed the (table 1 and fig 1). T₁₀ savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. bassiana* production up to (47.28) spores/ml in liquid medium during the 2020-21 years. The second most effective other treatments in order of spore producing was with T₁₁ Potato dextrose broth (PDB) (41.23), in liquid medium and solid medium in the treatment T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (27.32), superior in solid medium, followed by with T₇ Green gram + Molasses + 1% YE + 1.0 g Dextrose (23.64 spores/ml, T₅ Rice + Molasses + 1% YE (19.69 spores/ml), T₄ Wheat + Molasses + 1% YE (13.19 spores/ml), T₂ Maize + Molasses (11.31 spores/ml), T₁ Bajra + Molasses (9.17 spore/ml), T₉ Gram + Molasses + 1% YE + 1.0 g Dextrose (4.32 spore/ml), T₆ Barley+ Molasses + 1% YE (3.22 spores/ml), and T₃ Sorghum + Molasses (2.27) Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 47.28 were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB).

Similar trend was recorded on T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (27.32), superior in solid medium respectively. The observations data recorded on fifteenth days after inoculation of various treatments in the (table 1 and fig 1). T₁₀ savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. bassiana* maximum spores production up to (84.12 spores/ml) in liquid medium during the 2012-21 years. The second most effective other treatments in order of spore producing was in T₁₁ Potato dextrose broth (PDB) (77.86 spores/ml), in liquid medium and solid medium in the treatment T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (63.11 spores/ml), significantly superior in solid medium, followed by with T₇ Green gram + Molasses + 1% YE + 1.0 g Dextrose (59.83 spores/ml, T₅ Rice + Molasses + 1% YE (47.34 spores/ml), T₄ Wheat + Molasses + 1% YE (36.21

spores/ml), T₂ Maize + Molasses (25.31 spores/ml), T₁ Bajra + Molasses (17.19 spore/ml), T₉ Gram + Molasses + 1% YE + 1.0 g Dextrose (16.12 spore/ml), T₆ Barley+ Molasses + 1% YE (8.32 spores/ml), and T₃ Sorghum + Molasses (4.14) Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 84.12 spores/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB) 77.86 spores/ml. Similar trend was recorded on T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (63.11), superior in solid medium respectively. Similar trend was recorded in recorded on twenty-one days after the inoculation of various treatments in the (table 1 and figure 1). T₁₀ savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. bassiana* maximum spores production up to (147.23 spores/ml) in liquid medium during the 2020-21 years.

The second most effective other treatments in order of spore producing was in T₁₁ Potato dextrose broth (PDB) (114.27 spores/ml), in liquid medium and solid medium in the treatment T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (93.32 spores/ml), significantly superior in solid medium, followed by with T₇ Green gram + Molasses + 1% YE + 1.0 g Dextrose (81.23 spores/ml, T₅ Rice + Molasses + 1% YE (77.38 spores/ml), T₄ Wheat + Molasses + 1% YE (68.79 spores/ml), T₂ Maiz + Molasses (57.36 spores/ml), T₁ Bajra + Molasses (49.17 spore/ml), T₉ Gram + Molasses + 1% YE + 1.0 g Dextrose (38.11 spore/ml), T₆ Barley+ Molasses + 1% YE (33.19 spores/ml), and T₃ Sorghum + Molasses (26.13) Similar trends the experiments finding were recorded on 28th day after the inoculation of various treatments in the (table 1 and figure 1).

T₁₀ Savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. Bassiana* maximum spores production up to (219.21 spores/ml) in liquid medium during the 2020-21 years. The second most effective other treatments in order of spore producing was in T₁₁ Potato dextrose broth (PDB) (191.36 spores/ml), in liquid medium and solid medium in the treatment T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (152.31 spores/ml), significantly superior in solid medium, followed by

with T₇ Green gram + Molasses + 1% YE + 1.0 g Dextrose (131.32 spores/ml), T₅ Rice + Molasses + 1% YE (111.98 spores/ml), T₄ Wheat + Molasses + 1% YE (87.64 spores/ml), T₂ Maize + Molasses (75.82 spores/ml), T₁ Bajra + Molasses (67.21 spore/ml), T₉ Gram + Molasses + 1% YE + 1.0 g Dextrose (51.49 spore/ml), T₆ Barley+ Molasses + 1% YE (36.76 spores/ml), and T₃ Sorghum + Molasses (26.13). Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 219.21 spores/ml were recorded on savoured dextrose broth (SDB) and potato dextrose broth (PDB) 114.27 spores/ml. Similar trend was recorded on T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (151.31 spores/ml), superior in solid medium compared to other than respectively. Similar trends the experiments finding were recorded on thirty-five days after the inoculation of various treatments in the (table 1 and fig 1).

T₁₀savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. bassiana* maximum spores production up to (258.31 spores/ml) in liquid medium during the 2020-21 years. The second most effective other treatments in order of spore producing was in T₁₁ Potato dextrose broth (PDB) (211.65 spores/ml), in liquid medium and solid medium in the treatment T₈ Black gram + Molasses + 1% YE + 1.0 g Dextrose (161.64 spores/ml), significantly superior in solid medium, followed by with T₇ Green gram + Molasses + 1% YE + 1.0 g Dextrose (142.39 spores/ml), T₅ Rice + Molasses + 1% YE (136.67 spores/ml), T₄ Wheat + Molasses + 1% YE (95.35 spores/ml), T₂ Maize + Molasses (84.22 spores/ml), T₁ Bajra + Molasses (81.14 spore/ml), T₉ Gram + Molasses + 1% YE + 1.0 g Dextrose (59.15 spore/ml), T₆ Barley+ Molasses + 1% YE (46.17 spores/ml), and T₃ Sorghum + Molasses (34.17).

Overall result showed that among the liquid media tested, *Beauveria bassiana* spore/ml production was significantly recorded higher 258.31 spores/ml were recorded on Savoured dextrose broth (SDB) and potato dextrose broth (PDB) 211.65 spores/ml. Overall finding showed that in the table no.2 at 7 to 35 days among the liquid and solid media tested, for *Beauveria bassiana* spore/ml production was significantly higher recorded in the

liquid substrates 258.31 and 211.64 spore/ml were recorded on Savoured dextrose broth (SDB) and potato dextrose broth (PDB) and solid substrate significantly superior with black gram 161.64 spores/ml. The present results are in conformity with the findings of (Rajnish Rai *et al.*, 2021) study on entomopathogenic fungi (EPF) *Beauveria bassiana* (Bals.- Criv.) Vuill. is one of the important microbial agents used against insect pests. Biological control using such agents depends on the mass multiplication with suitable media. This study explores a reliable protocol with suitable media to achieve maximum production of biomass, conidial count, and germination. It was observed that broken rice is the best substrate giving maximum biomass, conidia and germination (0.62 g, 10.92x 10⁷ conidia/ml and 86.94%) followed by sorghum (0.54g, 7.35x 10⁷ conidia/ ml and 77.43%) and maize (0.37g, 6.05x 10⁷ conidia/ ml and 72.44%).Supported this finding by (Rajendra Singh *et al.*, 2017).

B. bassiana spore/ml was significantly higher recorded were significantly producing spore per ml data recorded on 7 days after the day after inoculation of various treatments in the T₁₀ 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. Niemczyk Marzena *et al.* (2019) patch as a pathogen of cockchafers occurred at 41% of sites, but often at densities below the threshold values for infection, and it infected only 1.3% of cockchafer grubs. Our results suggest that *B. brongniartii* genotype isolated from cockchafers in forest soils can potentially expand the pool of BCAs in this environment. Punia Gudia *et al.* (2016) studied was undertaken to evaluate grains, pulses, oilseeds and liquid media such as Potato Dextrose Broth and Sabouraud's Dextrose Broth for the mass production of *B. bassiana*. Studies revealed that dry weight of Meerut (UP) isolate varied from 0.623 g to 0.811 g on different media.

Table 1. Mass multiplication of *Beauveria bassiana* on different agricultural substrat

Treatments	Substrates	Spore Concentration	Effect of substrates on inoculation increase growth on <i>B. bassiana</i> at different days after inoculation				
			7 DAI	14 DAI	21 DAI	28 DAI	35 DAI
Solid Medium							
T ₁	Bajra + Molasses	1x10 ⁷ spores/ ml	9.17 (4.11)	17.19 (6.24)	49.17 (9.14)	67.21 (10.21)	81.14 (9.85)
T ₂	Maize + Molasses	1x10 ⁷ spores/ ml	11.31 (6.25)	25.31 (6.54)	57.36 (9.11)	75.82 (10.25)	84.22 (10.13)
T ₃	Sorghum + Molasses	1x10 ⁷ spores/ ml	2.27 (3.21)	4.14 (3.24)	26.13 (5.97)	36.76 (8.18)	34.17 (7.36)
T ₄	Wheat + Molasses + 1% YE	1x10 ⁷ spores/ ml	13.29 (6.27)	36.21 (8.14)	68.79 (10.21)	87.64 (12.24)	95.35 (113.11)
T ₅	Rice + Molasses + 1% YE	1x10 ⁷ spores/ ml	19.69 (7.36)	47.34 (9.67)	77.38 (12.35)	111.98 (13.40)	136.67 (19.34)
T ₆	Barly + Molasses + 1% YE	1x10 ⁷ spores/ ml	3.22 (2.41)	8.32 (4.13)	33.19 (7.19)	42.78 (7.44)	46.17 (7.39)
T ₇	Greengram + Molasses + 1% YE + 1.0 g Dextrose	1x10 ⁷ spores/ ml	23.64 (6.98)	59.83 (9.33)	81.23 (12.13)	131.32 (14.38)	142.39 (118.32)
T ₈	Black gram + Molasses + 1% YE + 1.0 g Dextrose	1x10 ⁷ spores/ ml	27.32 (7.15)	63.11 (10.22)	93.32 (12.69)	152.31 (19.28)	161.64 (19.35)
T ₉	Gram + Molasses + 1% YE + 1.0 g Dextrose	1x10 ⁷ spores/ ml	4.32 (3.27)	16.12 (5.80)	38.11 (8.22)	51.49 (8.22)	59.15 (9.57)
Liquid Medium							
T ₁₀	Savoured dextrose broth (SDB)	1x10 ⁷ spores/ ml	47.28 (7.83)	84.12 (9.88)	147.23 (12.86)	219.21 (14.23)	258.31 (16.75)
T ₁₁	Potato dextrose broth (PDB)	1x10 ⁷ spores/ ml	41.23 (8.22)	77.86 (10.19)	114.27 (12.19)	191.36 (16.11)	211.65 (16.53)
SEm ±			0.118	0.153	0.149	0.071	
CD at 5%			0.386	0.388	0.362	0.352	

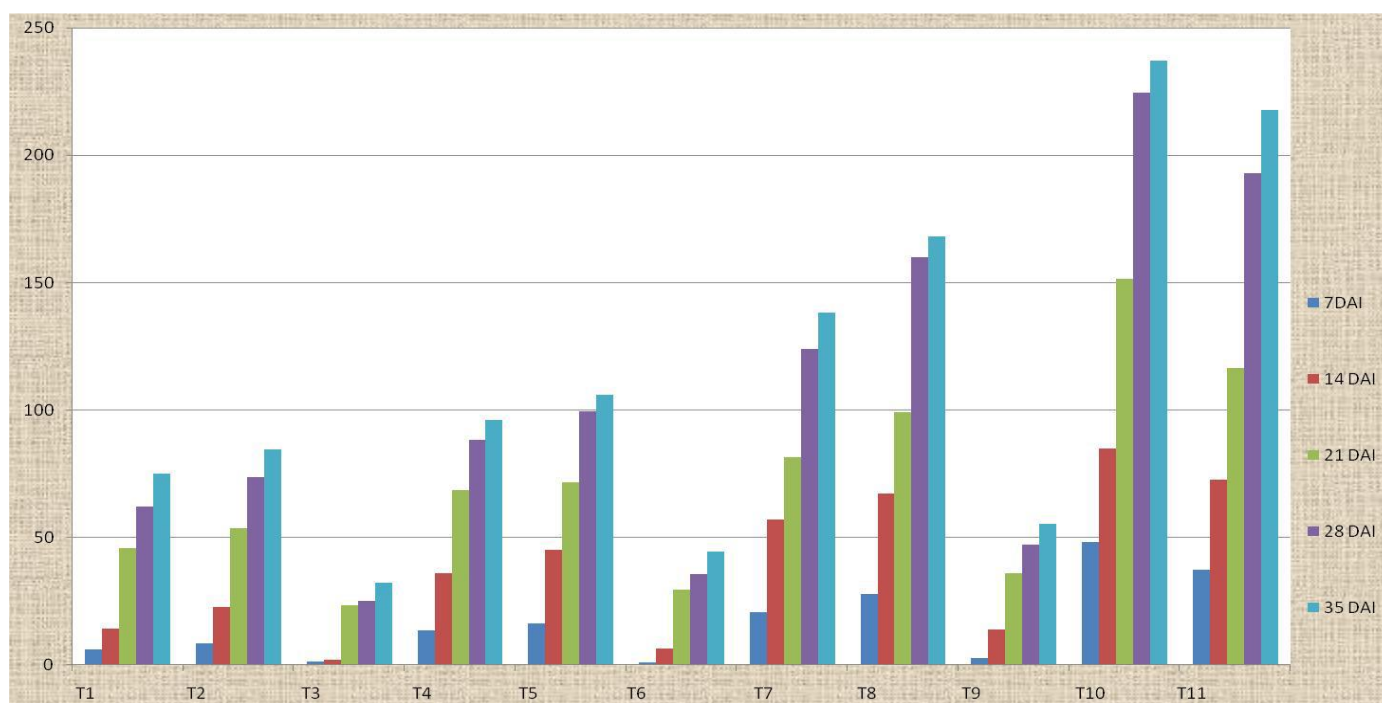


Fig 1. Multiplication of *Beauveria bassiana* on different media

Growth on broth media indicated maximum dry matter production in chickpea (0.811 g) followed by SDB (0.802 g) > pea (0.796) > soybean (0.751 g) > cowpea (0.715 g) > PDB (0.711) > urd (0.663 g) > lentil (0.623 g) and groundnut (0.582 g). Significant differences in conidial count were observed on maize, paddy, sorghum, rice and chickpea. Yadav Seema, Tandan Neeraj and Kumar Krishan (2013) different solid substrates *i.e.* such as grains, vegetable wastes, maize, bran, cotton seed, rice husk, wheat and liquid media such as coconut water were evaluated at variable moisture content and yeast extract concentration for mass production of two entomopathogenic fungi: *Beauveria bassiana* (Bals.).

Thet Thet Mar and Saisamorn Lumyong (2012) and Latifian *et al.* (2014) 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: (Sachin Kumar *et al.*, 2011, Prasad C. S. *et al.*, 2016 and Singh Rajendra *et al.*, 2017).

Mass production of *Beauveria bassiana* on different substrates, *Beauveria bassiana* substrate for spore production and their viability. Sahayaraj *et al.* (2008) 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 anisopiliae*. Results showed that rice grain supporting maximum spore production for both entomopathogenic fungi.

Conclusion

The constriction spores constriction effective of *Beauveria bassiana* spore/ml (1×10^6) production incurred for the production of spores significantly superior of various treatments in showed the treatments Savoured dextrose broth (SDB), significantly superior was the best treatment by bringing down the *B. Bassiana* production followed by second most effective other treatments in order of spore producing was with the treatment in Potato dextrose broth (PDB) and effective treatment Black gram + Molasses + 1% YE + 1.0 g Dextrose respectively.

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