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EFFICACY OF DIFFERENT CARBON AND NITROGEN SOURCES AGAINST MYCELIAL GROWTH AND SPORULATION OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE*

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ABSTRACT

Different carbon and nitrogen sources were evaluated at BTC, College of Agriculture and Research Station, (IGKV) Bilaspurin the year 2018-19 to find out the best source for mass production of entomopathogenic fungi and found that maximum mean mycelial growth of *B. bassiana* was recorded in sucrose (66.30 mm) and dextrose (62.85 mm) carbon source. Least growth was observed in cellulose (52.67 mm). Isolates of *B. bassiana* Bb3 (75.22 mm) most favoured by all carbon sources and least favoured for Bb11 (26.82 mm). Maximum mean sporulation was recorded in dextrose ($14.5x10^7$) and least in cellulose ($3.24x10^7$conidia ml^{-1}). Similarly mycelial growth and sporulation of *Metarhizium anisopliae* were recorded maximum in sucrose (85.17 mm). Maximum mean sporulations were recorded by dextrose ($45.84x10^7$).

Among the nitrogen sources potassium nitrate (62.47 mm) best suited the growth, while maximum mean sporulation was recorded in sodium nitrate ($8.70 x10^7$) by all isolates of *B. bassiana, M. anisopliae* were recorded maximum growth and sporulation by ammonium sulphate (67.33 mm, $32.72x10^7$) and potassium nitrate (47.17 mm, $30.68 x10^7$).

(Key words : *Beauveria bassiana*, *Metarhizium anisopliae,* carbon and nitrogen sources sporulation)

INTRODUCTION

In the recent time intensive use of chemicals in agriculture leads to improve yields of crops but side by side development of pests resistance, contaminate ground water, slow or non- degradable thereby great losses to environments, harmful for beneficial microorganism, dangerous to human health by entering food chain and some are extremely carcinogenic. On the view of above side effects it is necessary to produce crops in sustainable manners through eco friendly management system. Microbial control of crop pests offers environmentally acceptable strategies with lower cost and longer run effect. In recent years, more emphasis has been given to the integrated approach for managing the pest. Biointensive IPM is most relevant approach in agricultural production system (Saxena and Ahmad, 1997). Among the several components of integrated pest management, biological control gained increasing acceptance because of public awareness for environmental quality and consumer consciousness to health risk associated with use of synthetic chemical insecticides.Entomopathogens are naturally occurring organisms, such as bacteria, viruses and fungi for the control of crop pests, which can act as a parasite of insects and kills or seriously disables them. In recent years, microbial pathogens like viruses, bacteria, fungi and protozoa have been recognized for the biological suppression of many insect pests. About 1.5 million species of fungi alone are known to occur worldwide out of which nearly half of the species have been identified. Amongst these, several asexual stages of fungi are associated with insect infection.

Beauveria bassisna (Balsamo) Vuill is a successful known insect pathogenic agent, parasite on various arthropods and appear as dried and milky liquid, effective against diverse groups of insects i.e. leaf feeding insect, leaf folder and stem borer of rice, potato beetle, silk worm, termites, sweet potato weevil and white grubs.*Metarhizium anisopliae* (Metchn.) Sorokin appear as mummified,hard and covered olive green powdery mass of spores. The fungus has wide host range and over two hundred insect hosts viz., Grubs of Coconut Rhinoceros Beetle, Grasshopper, Rice BPH, Sugarcane Pyrilla, Bollworm, termites etc. (Vijayavani *et al.*, 2010).

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Commercialization of such entomopathogenic fungi are important steps considering identification of new virulent isolates, quality product formulation and wide protection. For large scale commercial production its need to evaluate the best sources for growth and sporulation as cheaper rate for farmers community benefits and market competition.

MATERILAS AND METHODS

Different carbon sources namely Dextrose, Cellulose, Maltose, Fructose, Sucrose, Glucose, Mannitol, Lactose, Starch and nitrogen sources i.e. Potassium nitrate, Sodium nitrate, Ammonium sulphate, Urea and (Di-ammonium phosphate) DAP were evaluated. PDA basal medium compositions replaced with the carbon 20 gl^{-1} and nitrogen 5 gl^{-1} (Mehta *et al.*,2012). Inoculated media plates were incubated at 25±2^{0}C. Three replicated plates were maintained for each source. For their effect on mycelial growth and sporulation of both the fungi. Radial growth of mycelium was recorded on 7th and 72 hrs, while spore ml^{-1} was calculated on 15thand 5th day after inoculation as per the formula described.

PDA composition

Peeled Potato - 200g

Dextrose- 20g (replace with other carbon and nitrogen sources)

Agar-Agar - 20g

Distilled water - 1 lt.

Determination of number of spores

Counting of spores was made at 15th day after inoculation. Total mycelial content of conical flask was poured in a sterilized mixer grinder and grind for one minute. One ml of grind suspension was added to 9 ml of sterilized distilled water in a sterilized test tube using serial dilution technique. From this test tube, 1 ml suspension was added to 9 ml of sterilized distilled water in another sterilized test tube. One drop of suspension was placed on the engraved grid of haemocytometer and was allowed to stand for 1-2 minutes to settle the spores at the bottom, cover glass was then placed over the grid. The number of spores present ml^{-1} of suspension was calculated by using the following method described earlier workers Soundarapandian and Chandra (2007); Amandeep and Neelam (2014).

No. of Conidia ml^{-1} or cm^2 = Average number of spores X Dilution factor X 4000 X 1000

RESULTS AND DISCUSSION

Effect of different carbon sources on mycelial growth and sporulation of *Beauveria bassiana*

Efficacy of nine different carbon sources viz., Cellulose, Mannitol, Sucrose, Fructose, Lactose, Maltose, Glucose, Starch and Dextrose was evaluated for the mycelial growth and sporulation of *B. bassiana* isolates under laboratory condition (Table 1). The result showed that maximum mean mycelial growth was recorded in Sucrose carbon source (66.30 mm) followed by Dextrose (62.85 mm) and Mannitol (62.19 mm). Sucrose was significantly superior over all other isolates where as Dextrose and Mannitol was at par with each other. Other carbon sources Maltose (59.76 mm), Glucose (58.68 mm) and Glucose and Starch (57.47 mm) were at par with each, while Maltose was differ with Starch. Least growth was observed in Cellulose (52.67 mm) followed by Lactose (55.64 mm). Isolates of *B. bassiana* Bb3 (75.22 mm) most favoured by all carbon sources followed by Bb1 (70.21 mm), least favoured by Bb11 (26.82 mm) followed by Bb2 (30.33 mm). Isolates Bb6, Bb7, Bb9, Bb13 and Bb16 were at par with each other and appear equally responsive for all carbon sources. Mycelial growth of *B. bassiana* isolates was most favoured by Dextrose (Bb3, Bb1, Bb7, Bb13 and Bb16), Maltose favoured (Bb3, Bb1, Bb9, Bb14, Bb7), Mannitol favoured (Bb9, Bb10, Bb3, Bb14, Bb16), Fructose favoured (Bb3, Bb14, Bb6, Bb1 and Bb16) and Starch favoured (Bb3, Bb15, Bb13, Bb1 and Bb14). Among all isolates and carbon sources, Bb3 was appeared as most responsive isolates for utilization of carbon sources taken in the study (Table 1).

Maximum mean sporulation was recorded in Dextrose (14.5x10^7) followed by Starch (10.61x10^7), Fructose (8.24x10^7) and Sucrose (7.76x10^7).Cellulose was least favoured carbon sources (3.24x10^7) followed by Lactose (4.25x10^7) by all isolates. Among the isolates, maximum sporulation was recorded in Bb11 (21.55x10^7) followed by Bb3 (14.88x10^7) and Bb1 (12.27x10^7).Least sporulating isolatewas found Bb2 (0.56x10^7) followed by Bb4 (0.57). The highly virulent isolates Bb3, Bb1, Bb6,Bb10 and Bb8 and Bb16 recorded high sporulation (Table 2). Maximum sporulation was recorded at dextrose by Bb11 (56.08) followed by Bb3 (22.69x10^7) isolates.

Effect of different nitrogen sources on mycelial growth and sporulation of *Beauveria bassiana*

Among the five nitrogen sources tested *viz.*, Ammonium Sulphate, Potassium Nitrate, Sodium Nitrate, Urea and DAP for the mycelial growth and sporulation of *B. bassiana* isolates were expressed that maximum mean mycelial growth was supported by Potassium nitrate (62.47 mm) followed by Sodium nitrate (61.51 mm) and Ammonium sulphate (47.85) and both Potassium nitrate and Sodium nitrate were statistically at par, while Ammonium sulphate was differ statically with these two nitrogen sources. Least growth was observed in Urea (31.87 mm) followed by DAP (39.35 mm). Among the all Nitrogen sources tested most favoured isolates was Bb3 (66.90 mm) followed by Bb1 (57.20 mm) Bb5 (55.63 mm). Least mycelial growth was in Bb11 (17.96 mm) followed by Bb2 (27.50 mm). Isolates Bb6, Bb7, Bb13 and Bb15 were recorded statistically at par with each other. There was no significant difference between Ammonium suphate, Potassium Nitrate, Sodium Nitrate and DAP for isolates Bb1 and Bb3 (Table 3).

Maximum mean sporulation was recorded in Sodium nitrate (8.70 x10^7) followed by Potassium nitrate (7.62 x10^7) and DAP (7.26 x10^7), least sporulation was recorded in Urea nitrogen sources (5.89x10^7) by all isolates. Among the sixteen isolates of *B. bassiana* tested maximum mean sporulation was found by all nitrogen sources in Bb11 (22.00 x10^7) followed by Bb16 (10.78 x 10^7), Bb3 (8.88 x10^7) and Bb8 (9.62 x10^7). Maximum sporulation in individual isolates was recorded in Bb11 (29.50 x10^7) in DAP followed by Bb4 (14 x10^7) in Sodium nitrate and Bb8 (13.80 x10^7) in DAP. No sporulation was observed in Bb2 isolate in Ammonium nitrate, Bb4 in Urea and Potassium nitrate (Table 4).

Effect of different carbon and nitrogen sources on mycelial growth and sporulation of *Metarhizium anisopliae*

Nine carbon sources were evaluated for mycelial growth and sporulation of *Metarhizium anisopliae* isolates and found that maximum mycelial growth was recorded in Sucrose (85.17 mm) followed by Glucose (85.00 mm), Fructose and Maltose (84.75 mm) and showed that all carbon sources were equally effective against *M. anisopliae* as they statistically at par with each other. The two isolates of *M. anisopliae* were at par with each other over all carbon sources. Isolates Ma2 favoured higher growth (84.85 mm) followed by Ma1 (84.11). Maximum mean sporulation of *M. anisopliae* were recorded by Dextrose (45.84x10^7) followed by Sucrose (42.75x10^7) and Fructose (41.77x10^7). Least sporulation was recorded by Maltose (32.92x10^7) followed by Cellulose (38.25x10^7) by these two isolates. Ma2 isolates recorded higher sporulation (40.43x10^7) by all carbon sources followed by Ma1 (39.69x10^7) (Table 5).

Among the five nitrogen sources, maximum mycelial growth and sporulation were recorded by Ammonium sulphate (67.33 mm, 32.72x10^7) followed by Potassium nitrate (47.17 mm, 30.68x10^7) and Sodium nitrate (46.17 mm , 16.47x10^7), least growth and sporulation were recorded by Urea (35.65 mm, 14.97x10^7). Ammonium sulphate was found superior and significantly differ with Potassium and Sodium all other nitrogen sources. Potassium and Sodium nitrate were significantly at par with each other, while Urea and DAP was found differ with nitrates and at par among each others (Table 6). Findings of various researchers agreed with findings of present study. Tamizharasi *et al.*(2005); Mehta *et al.*(2012); Yadav *et al.*(2013) found radial growth and sporulation were most favoured by Sodium nitrate (4.08 cm, 6.90x10^{10}) and (4.80 cm, 2.90 x10^{10}) and on Potassium nitrate (4.0 cm, 6.20 x10^{10}) and (4.65 cm, 8.10 x10^{10}), least effective was Urea (3.85 cm, 4.20 x10^{10}) and (3.80 cm, 8.56 x10^{10}), respectively for both entomopathogenic fungi *B. bassiana* and *M. anisopliae* and also suggested that among carbon sources Fructose was higher biomass producers (24.16 g) followed by Dextrose (21.53g). Other researchers *viz.*, Yadav *et al.*(2013); Sekher *et al.* (2017) gets similar types of finding and support our results as they concluded that Starch supported highest growth (8.12g) biomass followed by Sucrose (7.0g) and Fructose (5.73g) and least by Lactose (5.0g).Lower radial growth by Urea could be due to differential sensitivity of the fungi to impurities present in this form of Urea.Nitrate is better than Ammonium because they acidify the medium and up to certain level encourages the growth of fungi but beyond that limit, it is probably responsible for induction of sporulation (Rath *et al*.,1995).One of the key factors in enhancing the population of mycopathogens propagules in the inoculums is the availability of nutrients. The availability of nutrients also influences the survival of these organisms in nature. Carbon and nitrogen are the most vital nutrients required for growth and sporulation (Campbell *et al.,* 1983). For continuous growth and extension of the hyphae and to prevent autolysis, exogenous nitrogen source is required (Smith and Grula, 1981). Therefore, the effect of different carbohydrates and nitrogen on the growth of *M. anisopliae* and *B.bassiana* was evaluated.

Among the different carbon sources evaluated maximum mean mycelial growth of *B. bassiana* was recorded in Sucrose and Dextrose, while, maximum mean sporulation was recorded in Dextrose and Cellulose. Mycelial growth and sporulation of *Metarhizium anisopliae*was found maximum in Sucrose, while, maximum mean sporulation was recorded by Dextrose. Similarly among the nitrogen sources tested, maximum mean mycelial growth was supported by Potassium nitrate and maximum mean sporulation was recorded in Sodium nitrate by *B. bassianan.*Maximum mycelial growth and sporulation of *M. anisopliae* were recorded by Ammonium sulphate.

Table 1.Effect of different carbon sources on mycelial growth of *Beauveria bassiana*

Carbon sources	Isolates of *Beauveria bassinna*																Mean
	Bb1	Bb2	Bb3	Bb4	Bb5	Bb6	Bb7	Bb8	Bb9	Bb10	Bb11	Bb12	Bb13	Bb14	Bb15	Bb16	
	Mycelial growth (mm) at 7th day																
Cellulose	58.00	24.50	63.33	28.83	51.80	50.17	58.33	57.83	68.83	53.00	25.50	51.00	63.67	63.00	61.67	63.17	**52.67**
Mannitol	69.67	25.50	75.17	33.17	55.83	67.17	65.27	69.67	75.17	75.17	26.83	68.00	70.83	74.83	68.67	74.17	**62.19**
Sucrose	72.33	35.83	80.33	39.50	69.00	74.00	71.83	66.50	76.00	76.17	29.17	69.00	77.00	75.00	71.17	78.00	**66.30**
Fructose	71.67	32.17	76.00	41.67	70.17	74.83	66.50	46.00	47.33	39.83	29.83	63.33	38.33	75.00	69.33	70.00	**57.00**
Lactose	63.67	31.83	73.33	38.33	69.33	65.00	69.33	58.83	61.67	57.50	23.83	66.00	67.50	61.67	32.83	49.50	**55.64**
Maltose	72.67	32.50	74.00	46.67	54.00	66.50	69.17	58.67	73.67	62.50	26.33	60.67	59.33	73.17	67.00	59.33	**59.76**
Glucose	71.00	27.17	73.33	37.00	56.27	71.33	70.17	54.83	71.83	66.17	26.33	60.50	71.83	64.00	46.33	70.83	**58.68**
Starch	71.67	27.50	80.00	21.67	60.67	62.33	52.17	52.50	73.50	59.83	26.33	56.33	71.00	69.50	79.33	55.17	**57.47**
Dextrose	81.23	36.00	81.50	23.50	66.83	67.67	85.00	53.90	56.60	67.36	27.18	53.27	80.25	64.00	78.33	83.00	**62.85**
Mean	**70.21**	**30.33**	**75.22**	**34.48**	**61.54**	**66.56**	**67.53**	**57.64**	**67.18**	**61.95**	**26.82**	**60.90**	**66.64**	**68.91**	**63.85**	**67.02**	**59.17**

	SEm±	CD (0.05)
A (Carbon sources)	0.52	**1.45**
B (Isolates)	0.69	**1.94**
A x B	2.09	**5.82**
CV		**6.14**

Table 2.Effect of different carbon sources on sporulation of *Beauveria bassiana*

Carbon sources	Isolates of *Beauveria bassinna*																Mean
	Bb1	Bb2	Bb3	Bb4	Bb5	Bb6	Bb7	Bb8	Bb9	Bb10	Bb11	Bb12	Bb13	Bb14	Bb15	Bb16	
	No. of conidia X10^7																
Cellulose	4.2	0.0	6.5	0.0	3.2	5.2	6.2	4.0	0.5	1.6	8.0	5.2	0.80	0.20	0.20	6.00	**3.24**
Mannitol	8.2	0.50	8.6	0.2	3.2	7.8	4.6	7.6	2.0	6.0	14.0	6.8	1.80	0.40	0.40	5.64	**4.86**
Sucrose	15.50	0.80	18.50	0.3	6.2	9.6	7.2	9.50	4.4	8.54	18.4	12.6	3.00	1.00	0.40	8.20	**7.76**
Fructose	16.8	0.6	19.8	0.4	8.2	9.42	7.8	9.6	4.8	9.46	19.8	8.80	3.40	1.50	0.80	10.64	**8.24**
Lactose	6.6	0.4	10.8	0.6	3.6	6.8	2.2	4.5	0.60	6.0	16.0	4.3	0.80	0.90	0.40	3.50	**4.25**
Maltose	12.4	0.5	15.8	0.8	6.2	7.85	6.56	8.63	3.2	8.80	14.0	8.5	0.60	0.40	0.20	9.40	**6.49**
Glucose	12.0	0.2	12.6	0.6	6.60	7.20	5.20	6.5	3.10	8.0	12.30	7.63	1.20	0.80	0.40	8 .80	**5.27**
Starch	16.6	0.5	18.60	0.50	7.20	18	9.4	7.2	8.0	15.2	35.4	12.5	3.50	2.60	2.10	12.46	**10.61**
Dextrose	18.12	1.50	22.69	1.75	14.25	17.2	15.2	14.8	09.4	16.1	56.08	14.5	5.20	4.20	6.20	15.80	**14.56**
Mean	**12.27**	**0.56**	**14.88**	**0.57**	**6.52**	**9.90**	**7.15**	**8.04**	**4.00**	**8.86**	**21.55**	**8.98**	**2.26**	**1.33**	**1.23**	**7.96**	

Table 3.Effect of different nitrogen sources on mycelial growth of *Beauveria bassiana*

Nitrogen sources	Isolates of *Beauveria bassinna*																Mean
	Bb1	Bb2	Bb3	Bb4	Bb5	Bb6	Bb7	Bb8	Bb9	Bb10	Bb11	Bb12	Bb13	Bb14	Bb15	Bb16	
	Mycelial growth (mm) at 7th day																
Ammonium Sulphate	59.50	24.83	70.17	61.67	33.50	38.83	32.33	52.00	35.33	49.50	19.50	46.83	59.50	60.83	69.17	52.17	**47.85**
Potassium Nitrate	58.67	26.00	66.83	74.33	65.33	68.83	65.67	63.17	73.17	63.17	25.00	64.67	74.50	75.17	70.17	64.83	**62.47**
Sodium Nitrate	59.33	27.33	73.00	71.50	66.33	66.83	67.67	58.83	64.67	65.33	24.67	62.67	71.50	72.67	70.50	61.33	**61.51**
Urea	50.00	28.33	55.17	9.50	56.67	50.33	52.67	23.00	19.67	25.17	8.67	26.83	29.67	25.83	27.00	21.33	**31.87**
DAP	58.50	31.00	69.33	8.33	56.33	48.83	49.83	38.67	26.83	46.50	12.00	52.17	28.67	25.83	27.00	49.83	**39.35**
Mean	**57.2**	**27.5**	**66.9**	**45.06**	**55.63**	**54.73**	**53.63**	**47.13**	**43.93**	**49.93**	**17.96**	**50.63**	**52.76**	**52.06**	**52.76**	**49.90**	**48.61**

	SEm±	CD (0.05)
A (Nitrogen sources)	0.51	1.43
B (Isolates)	0.92	2.56
A x B	2.05	5.72
CV	7.36	

Table 4.Effect of different nitrogen sources on sporulation of *Beauveria bassiana*

Nitrogen sources	Isolates of *Beauveria bassinna*																Mean
	Bb1	Bb2	Bb3	Bb4	Bb5	Bb6	Bb7	Bb8	Bb9	Bb10	Bb11	Bb12	Bb13	Bb14	Bb15	Bb16	
	No. of conidia X10^7																
Ammonium Sulphate	7.40	0.00	9.50	10.8	5.80	6.00	8.40	5.20	3.20	6.60	12.50	4.30	4.40	3.50	0.40	11.60	**6.23**
Potassium Sulphate	8.90	1.00	10.0	13.60	8.56	8.50	8.60	8.50	3.60	7.20	17.00	5.60	4.60	2.50	1.20	12.50	**7.62**
Sodium Nitrate	9.25	1.50	13.5	14.00	9.30	8.40	9.50	13.0	4.50	7.60	26.60	8 .20	4.50	2.50	1.50	13.60	**8.70**
Urea	5.00	0.60	8.20	0.00	4.60	0.60	1.40	7.60	6.40	7.40	24.40	8.40	6.40	3.80	1.20	8.20	**5.89**
DAP	0.40	4.60	3.20	0.00	4.40	8.40	5.20	13.80	8.60	10.30	29.50	8.30	5.20	5.40	0.80	8.00	**7.26**
Mean	**6.19**	**1.54**	**8.88**	**7.68**	**6.53**	**6.38**	**6.62**	**9.62**	**5.26**	**7.82**	**22.00**	**5.32**	**5.02**	**3.54**	**1.02**	**10.78**	**22.00**

Table 5. Effect of different carbon sources on mycelial growth and sporulation of *Metarhiziumanisopliae*

Carbon Sources	Mycelial growth (mm) at 72 hrs		Mean	No. of Conidia X10^5		Mean
	Ma1	Ma2		Ma1	Ma2	
Cellulose	83.83	84.11	**83.97**	38.21	38.29	**38.25**
Mannitol	83.00	84.83	**83.92**	39.43	40.17	**39.80**
Sucrose	85.33	85.00	**85.17**	42.23	43.27	**42.75**
Fructose	84.50	85.00	**84.75**	41.41	42.12	**41.77**
Lactose	83.83	85.00	**84.42**	38.44	39.48	**38.96**
Maltose	84.50	85.00	**84.75**	32.55	33.29	**32.92**
Glucose	85.00	85.00	**85.00**	40.12	41.28	**40.70**
Starch	83.00	84.67	**83.83**	39.29	39.88	**39.59**
Dextrose	84.00	85.00	**84.50**	45.56	46.12	**45.84**
Mean	**84.11**	**84.85**	**84.48**	**39.69**	**40.43**	
		SEm±	CD (0.05)			
	A (carbon sources)	2.19	6.13			
	B (Isolates)	1.03	2.89			
	AxB	3.09	8.67			
	CV		6.18			

Table 6. Effect of different nitrogen sourceson mycelial growth and sporulation of *Metarhizium anisopliae*

Nitrogen Sources	Mycelial growth (mm) at 72 hrs		Mean	No. of Conidia X10^5		Mean
	Ma1	Ma2		Ma1	Ma2	
Ammonium Sulphate	68.33	66.33	**67.33**	32.14	33.29	**32.72**
Potassium Nitrate	48.00	46.33	**47.17**	30.14	31.22	**30.68**
Sodium Nitrate	47.00	45.33	**46.17**	16.12	16.82	**16.47**
Urea	42.00	36	**39.00**	15.72	14.22	**14.97**
DAP	36.97	34.33	**35.65**	16.29	16.01	**16.15**
Mean	**48.46**	**45.67**	**47.06**	**22.08**	**22.31**	
		SEm±	CD (0.05)			
	A (Nitrogen sources)	1.29	3.59			
	B (Isolates)	0.81	2.27			
	AxB	1.82	5.07			
	CV		**6.29**			

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