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In vitro evaluation for compatibility of additives with *Beauveria bassiana* (Balsamo) Vuillemin

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Abstract

An in vitro evaluation was conducted for compatibility of 12 commonly used additives at three different concentrations of (0.1, 0.5, and 1.00%) with *Beauveria bassiana* through poisoned food technique. The results were expressed as radial growth and growth inhibition of *B. bassiana* on an additive treated medium. All the additives showed an inhibition in mycelial growth of *B. bassiana*, either partially or completely depending on their concentrations. On overall basis, carboxylmethyl cellulose (CMC) showed the highest radial growth with a least growth inhibition of (77.16 mm and 8.03%, respectively), followed by Kaolite (69.07 mm and 17.65%, respectively) and silica gel (65.20 mm and 22.25%, respectively). These findings concluded that CMC could be used in formulations of *B. bassiana* with the highest spore load of 4.67×10^8 spore's ml^{-1}.

Keywords: Compatibility, *Beauveria bassiana*, Additives, Radial growth and growth inhibition

Background

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has attracted significant interest as a biological control agent since it infects a wide range of insect pests in diverse agro-ecosystems (Ambethgar et al. 2009). *B. bassiana* is registered biopesticide that act on a broad host range of approximately 700 insect species used for management of several crop insect pests. Entomopathogenic fungi are usually applied in the form of spores, which need a stabilizing agent to facilitate application, stability, and enhancement of activity (Meikle et al. 2008). Bioactivity of *B. bassiana* has been established against several pests at the laboratory level, while efforts are underway to simulate these results in practical scenarios and under field conditions (Mishra et al. 2013). However, successful implementation of entomopathogenic activity shown at laboratory level to field scale necessitates the development of a suitable formulation (Amutha et al. 2010). The present investigation was carried out to study the compatibility of additives with *B. bassiana* under in vitro condition.

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Materials and methods

The present study was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. The experiment was carried out using a completely block design of 12 additives at three different concentrations (0.1, 0.5 and 1.00%) (Table 1) in three replicates and a control treatment.

Fungal isolate

B. bassiana was isolated from mulberry silk worm larvae, *Bombyx mori*. The fungus was grown on Potato dextrose agar slants and selected based on its virulence against tomato insect pest complex through laboratory bioassay studies with a standard concentration of 1×10^8 spore's ml^{-1}.

Experimental procedure

Effect of the additives was evaluated on the basis of radial growth and germination of *B. bassiana*. The additives including wetting agents and emulsifiers (T_1 to T_5); humectants'(T_6); desiccants (T_7 and T_8); crude/refined oils (T_9 to T_{11}); and detergent carrier (T_{12}) along with a control set were evaluated by poisoned food technique in Potato Dextrose Agar (PDA) medium (Moorhouse et al. 1992). Sterilized 20 ml PDA with the additives of

Table 1 Additives for compatibility studies of *B. bassiana*

Tr. Code	Category	Treatments	Importance	References
T_1	Wetting agents and Emulsifiers	Tween-20	Help to rehydrate spores stored dry and to disperse clumps	Burges (2012)
T_2		Tween-40		
T_3		Tween-60		
T_4		Tween-80		
T_5		Triton-X		
T_6	Humectants'	Glycerol	Delays the evaporation of the liquid and favors spore germination	Kubicek and Druzhinina (2007)
T_7	Desiccants	Kaolite	Regulate water availability to microorganisms and help in absorption of harmful metabolic by-products	Onions (1971)
T_8		Silica gel		
T_9	Oils	Sunflower oil	Improve spore survival and reduce sensitivity to UV radiations	Mishra et al. (2013)
T_{10}		Neem oil		
T_{11}		Pongamia oil		
T_{12}	Detergent carrier	Carboxylmethyl cellulose (CMC)	Enhances the ability of *B. bassiana* to reduce cellulolytic enzymes	Petlamul et al. (2017)
T_{13}	Control			

the concentrations (0.1, 0.5, and 1.0%) were incorporated into 25-mm diameter sterile petri dishes, and they were allowed to solidify under laminar flow cabinet. An agar disc along with mycelium mat of *B. bassiana* was cored with the help of cork borer and transferred onto the center of the PDA plate. Growth medium (PDA) without additive, but inoculated with mycelial disc, served as untreated check (control).The plates were sealed with parafilm and incubated at room temperature to allow maximum growth. The diameter of growing culture, i.e., the radial growth in excess of the plugs in each Petri dish, was measured on 10th day after inoculation (DAI). The data were expressed as percentage growth inhibition of *B. bassiana* by additive treated PDA (Hokkanen and Kotiluoto 1992).

$$X = \frac{Y\text{-}Z}{Y} \times 100$$

where *X*, *Y*, and *Z* stand for the percentage of growth inhibition, radial growth of fungus in untreated check, and radial growth of fungus in poisoned medium, respectively.

Results and discussion

All the additives showed significant differences relating to control in terms of all the observed parameters. Data on growth performance of *B. bassiana* 10DAI in different additives are presented in Table 2 and depicted in Fig. 1.

Radial growth and growth inhibition

Among the wetting agents and emulsifiers (T_1 to T_5) tested, Tween-80 @ 0.5% followed by Tween-80 @ 0.1% showed a maximum radial growth of 51.49 and 50.07 mm with percentage growth inhibition of 38.58 and 40.30%, respectively.

Among the different concentrations of humectants'(T_6), Glycerol @ 0.5%, followed by Glycerol @ 0.1%, presented maximum radial growth of 52.23 and 52.93 mm and growth inhibition percentage of 37.72 and 36.87%, respectively.

Among the desiccants (T_7 and T_8) tested, Kaolite @ 0.5%, followed by Kaolite @ 0.1%, showed a maximum radial growth of 73.83 and 66.48 mm and a growth inhibition percentage of 11.97 and 20.69%, respectively.

Among the tested oils (T_9 to T_{11}), sunflower oil @ 0.5%, followed by 0.1%, showed maximum radial growth of 55.45 and 45.78 mm and growth inhibition percentage of 33.98 and 45.42%, respectively.

The data further revealed that among different concentrations of the detergent carrier, i.e., CMC (T_{12}) @ highest concentration of 1.0%, showed a maximum radial growth of 81.29 mm and a growth inhibition percentage of 3.13%, respectively.

Thus, among the additives of various categories tested, CMC was relatively less toxic to *B. bassiana* at all the tested concentrations.

Spore load

Data on sporulation of *B. bassiana* in relation to additives treated media are presented in Table 2 and depicted in Fig. 1.

On the overall basis among the various additives tested at various concentrations, the highest mean spore load was recorded in control (T_{13}) (5.22×10^8 spores ml^{-1}) that was at par with CMC (T_{12}) (4.67×10^8 spores ml^{-1}). This was followed by Tween-80 (T_4) (3.44×10^8 spores ml^{-1}) which was at par with Kaolite (T_{10}) (2.89×10^8 spores ml^{-1}) and Glycerol (T_5) (2.78×10^8 spores ml^{-1}). Meanwhile, the least spore load was recorded in Tween-

Table 2 Compatibility of *Beauveria bassiana* with various additives on 10th day after inoculation

Trt. codes	Additives (Factor A)	Performance of *B. bassiana* in different additives at 3 different concentrations											
		Concentrations % (Factor B)											
		Radial growth (mm)				Growth inhibition (%)[a]				Mean spore count (1×10^8 spores ml^{-1})[b]			
		0.1	0.5	1.0	Mean	0.1	0.5	1.0	Mean	0.1	0.5	1.0	Mean
T_1	Tween-20	43.67	44.96	42.51	43.71	47.91 (43.77)	46.38 (42.91)	49.33 (44.60)	47.87 (43.76)	1.67 (1.46)	2.00 (1.56)	1.00 (1.17)	1.56 (1.43)
T_2	Tween-40	34.00	32.92	30.49	32.47	59.45 (50.43)	60.74 (51.20)	63.60 (52.87)	61.26 (51.50)	1.00 (1.17)	1.33 (1.34)	0.67 (1.05)	1.00 (1.22)
T_3	Tween-60	39.91	40.33	39.23	39.82	52.44 (46.39)	51.95 (46.09)	53.22 (46.82)	52.54 (46.43)	2.33 (1.68)	1.67 (1.46)	1.00 (1.22)	1.67 (1.46)
T_4	Tween-80	50.07	51.49	49.03	50.20	40.30 (39.35)	38.58 (38.16)	41.58 (40.14)	40.16 (39.29)	4.00 (2.11)	4.33 (2.20)	1.67 (1.46)	3.44 (1.96)
T_5	Glycerol	52.23	52.93	50.32	51.83	37.72 (37.84)	36.87 (37.32)	40.08 (39.25)	38.22 (38.15)	3.00 (1.86)	3.33 (1.95)	2.00 (1.58)	2.78 (1.80)
T_6	Triton-X	27.92	23.69	17.42	23.01	66.72 (54.76)	71.73 (57.86)	79.27 (62.90)	72.58 (58.50)	1.00 (1.17)	1.00 (1.17)	0.33 (0.88)	0.89 (1.16)
T_7	Neem oil	31.17	24.67	15.86	23.90	62.87 (52.44)	70.56 (57.14)	81.18 (64.32)	71.54 (57.92)	3.00 (1.86)	3.00 (1.86)	0.67 (1.05)	2.22 (1.61)
T_8	Pongamia oil	26.03	23.97	12.94	20.98	69.02 (56.16)	71.39 (57.71)	84.54 (66.89)	74.98 (60.20)	2.67 (1.77)	2.33 (1.68)	0.33 (0.88)	1.78 (1.46)
T_9	Sunflower oil	64.15	55.45	45.78	55.13	23.48 (28.84)	33.98 (35.60)	45.42 (42.35)	34.29 (35.63)	2.00 (1.56)	2.00 (1.56)	1.00 (1.17)	1.78 (1.50)
T_{10}	Kaolite	66.90	73.83	66.48	69.07	20.28 (26.70)	11.97 (19.74)	20.69 (26.82)	17.65 (24.63)	2.67 (1.74)	3.67 (2.04)	2.33 (1.68)	2.89 (1.83)
T_{11}	Silica gel	65.20	68.33	62.07	65.20	22.25 (27.97)	18.52 (25.37)	25.98 (30.56)	22.25 (28.06)	2.67 (1.77)	3.33 (1.93)	1.33 (1.34)	2.44 (1.70)
T_{12}	CMC	73.25	76.93	81.29	77.16	12.69 (20.72)	8.28 (16.29)	3.13 (9.34)	8.03 (15.86)	4.67 (2.26)	5.67 (2.48)	3.67 (2.04)	4.67 (2.27)
T_{13}	Control	83.95	83.95	83.95	83.95	–	–	–	–	5.33 (2.41)	5.33 (2.41)	4.67 (2.27)	5.22 (2.39)
	Mean	50.64	50.27	45.95		42.93 (40.44)	43.41 (40.45)	49.00 (43.90)		5.33 (2.41)	5.33 (2.41)	5.33 (2.41)	
	SEm±	0.80	0.89	0.91	1.53	0.83	1.01	0.95	1.31	0.10	0.09	0.09	0.09
	CD at 5%	1.67	1.84	1.88	3.16	1.73	2.10	1.98	2.71	0.20	0.18	0.18	0.20
Analyzed data for interactions													
		SEm±		CD ($P = 0.05$)		SEm±		CD ($P = 0.05$)		SEm±		CD ($P = 0.05$)	
	Factor A	0.87		2.44		0.93		2.64		0.09		0.25	
	Factor B	0.41		1.18		0.47		1.32		0.04		0.12	
	Factor A + B	1.50		4.23		1.62		4.58		0.15		NS	

[a]Arcsine transformed values

[b]Square root transformed values ($x + 0.5$)

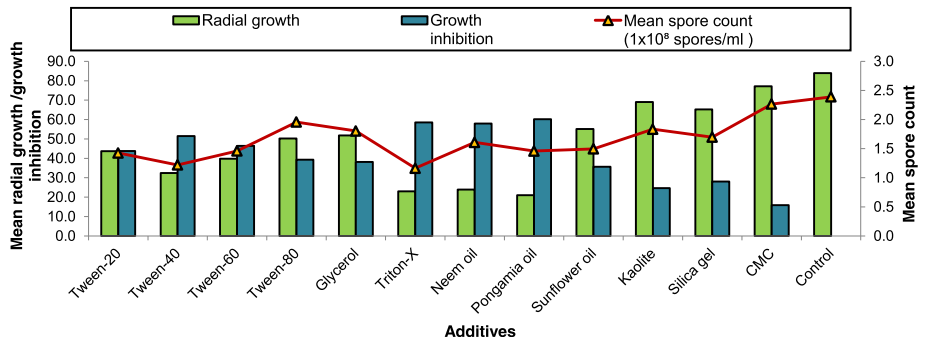

Fig. 1 Mean growth rate (mm), growth inhibition (%), and spore load of *B. bassiana* on various additives on the 10th day after inoculation

40(T_2) and Triton-X (T_6) with 1.00×10^8 spores ml^{-1} and 0.89×10^8 spores ml^{-1}, respectively.

Impact of additives and their tested concentrations on *B. bassiana*

Perusal of data in Table 2 revealed the following:

a) Radial growth and growth inhibition percentages

Factor A: additives

Different tested additives showed significant impact of the radial growth and growth inhibition percentages on *B. bassiana*.

Factor B: concentrations

Evaluation of additives at varied concentrations with *B. bassiana* revealed that they had significant impact on the radial growth and growth inhibition percentages of *B. bassiana*.

Interactions: additives × concentrations

The interaction effect of additives and concentrations had significant effect on the radial growth and growth inhibition percentages of *B. bassiana*.

b) Spore load

Factor A: additives

Different tested additives showed significant impact on the mean spore load of *B. bassiana*.

Factor B: concentrations

Evaluation of the additives at varied concentrations of *B. bassiana* revealed that they had significant impact on the mean spore load of *B. bassiana*.

Interactions: additives × concentrations

Different tested additives and concentrations had non-significant interaction effect on the spore load of *B. bassiana*.

In the present study, CMC at all the tested concentrations caused the highest mean radial growth (77.16 mm) with least inhibition percentage (8.03%) after control (83.95 mm); it was found to be relatively less toxic to *B. bassiana*. Results of the growth inhibition of *B. bassiana* in the additives were in accordance with those of Tanuja et al. (2010) who stated that the negative impact of surfactants to microorganism was probably due to increased cell permeability and amino acid leakage through inner membrane. Formulation of myco-insecticide must be compatible with the agent and must enhance its performance and, ideally, must maintain an adequate shelf-life of the agent in order to be successful (Derakhshan et al. 2008).

The highest mean spore load was recorded in T_{13} (5.22×10^8 spores ml^{-1}) which was at par with CMC (T_{12}) (4.67×10^8 spores ml^{-1}). The present results were in accordance with the findings of Petlamul et al. (2017) who revealed that *B. bassiana* had the ability to release cellulolytic enzymes on CMC for cellulose degradation to carbon source led to their growth.

The assessment of spores compatibility with surfactants is the primary requirement in successful development of surfactant based formulation, viz. emulsion. Particularly, fungi with hydrophobic conidia render the use of surfactants indispensable for laboratory bioassays and field trials (Jin et al. 2008).

Further studies need to be carried out for as a combination in order to achieve additive and develop or increase the efficacy of the fungus.

Conclusion

The findings conclude that CMC could be used in formulations of *B. bassiana* that helps to enhance its shelf life.

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Authors' contributions

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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