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Effect of various adjuvants on growth and development of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT

Studies on the effect of various adjuvants on growth and development of *Beauveria bassiana* (Balsamo) Vuillemin was undertaken with a view to select suitable adjuvants for developing aqua suspension formulation. It was revealed that the *B. bassiana* formulations with combination of adjuvants helped in increasing production of fungal biomass at 10 DAI. Based on the results it is concluded that the overall performance of the adjuvants for growth and development of *B. bassiana* in series of lab experimentation out of 62 test formulations 8 formulations comprising 1) *B.b.*+ SFO (1%), 2) *B.b.*+ GNO (1%), 3) *B.b.*+ GLY (2%) + BA (2%), 4) *B.b.*+ GLY (2%) + BA (2%) + TW (0.5%) 5) *B.b.*+ SFO (1%) + CMC (0.5%), 6) *B.b.*+ GLY (2%) + CMC (0.5%), 7) *B.b.*+ GLY (2%) + HO (1%), 8) *B.b.*+ SFO (1%) + HO (1%) were emerged out as most promising and advanced stage formulations of *B. bassiana*

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INTRODUCTION

Microbial control is the biological suppression of insect pests employing microbial world. It has advantage of higher host specificity, virulence, safety to natural enemies, ease in mass production, multiple benefit in bioefficacy due to accelerating and spreading epizootics in pests, shelf-life and compatibility with other methods. Among the pathogens used in microbial control, entomopathogenic fungi have played an important role in the history of insect pathology and microbial control of insects (Sundarababu, 1992). More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (Hajek and Leger, 1994). The entomopathogenic fungi causing diseases to the insects and are practically more significant as they are epizootic in nature. Also they have the advantage of ease of production and contact action which allow direct penetration of the host cuticle without ingestion.

Beauveria bassiana, the white muscardine fungus

belongs to division: Eumycota, Subdivision: Deuteromycotina, Class: Deuteromycetes, Order: Moniliales and Family: Moniliaceae, Genus: *Beauveria* and species: *bassiana*. It is globally occurring soil born mycelia fungus. Bassi (1835) was the first to demonstrate that entomopathogenic fungus; *B. bassiana* could cause an infectious disease in silkworm and suggested the concept that, an infectious micro-organism might be used to control insect pests. Steinhaus (1965) reported that *B. bassiana* causes mycosis in 175 host insects from order Lepidoptera, Coleoptera and Hemiptera.

B. bassiana is cosmopolitan fungus useful for the control of various insect pests of different crops. Gopalakrishnan and Narayanan (1990); Parmar (2001); Udar (2002) and Vimaladevi and Hari (2009) reported the pathogenicity of *B. bassiana* to *H. armigera* larvae. This fungus also found useful for the control of various sucking pests of important field crops. Aphids, *Aphis craccivora* Koch, *A. gossypii* and *Rhapalosiphum maidis* were found to be attacked by *B. bassiana* causing 16-80 % mortality (Nirmala *et al*., 2006). Srivastava and Fasih (1988) and Haseeb *et al*. (1998) found that *B. bassiana* was pathogenic to mango mealy bug, *Drosicha mangiferae*.

Efficiency of entomopathogens in the field depends upon virulence towards target pest, coverage and persistence on target site. However, major constraints for successful use of such bio-agents are their difficulties in use of pure cultures, survival on crop after application due to short shelf-life, loosing virulence by ultra violet (UV) rays and dependability on the prevailing environmental conditions are the problems reported by Kaur *et al.* (1999). The foregoing problem can largely be overcome by developing suitable WP, suspension, granules etc. formulations. The performance and shelf-life can be improved by adding suitable adjuvants leading to growth, development and viability of the fungus that may act as nutrient, adhesive, UV protectants or wetting agents etc.

Chocking of nozzles, hesitation of customers and exporters to purchase fruits and vegetables with WP spots resulted in strong demand for aqua suspension formulation of entomopathogens including fungi. Presently, crude suspensions of the fungi with short shelf-life of around one to two months are marketed. More aged preparations loss their viability due to submerging the floating fungi. For developing aqua suspension formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before formulating the object. There are some examples were fungi have been formulated with various adjuvants. Kaur *et al.* (1999) found that the edible oils of sunflower and groundnut accelerated the spore germination and growth rate of *B. bassiana* in vitro. According to Verhaar *et al*. (1999) *in vivo* arachnid (0.5%) in *V. lecanii* formulation resulted in best development of the fungus on mildewed cucumber leaves.

Hence, in the present study a range of adjuvants *viz.,* chemical adjuvants, vegetable oils and honey were screened for their growth and development of *B. bassiana* on culture medium with a view to select suitable adjuvants for developing the formulations of entomopathogen.

MATERIAL AND METHODS

The present investigation was carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra State, India during year 2009-2011.

Fungus culture :

The pure fungus culture of *B. bassiana* was available at Biocontrol Research Laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Medium :

The medium used for multiplication and growth of the fungus was potato dextrose broth.

Maintenance of culture :

Sabouarauds Dextrose Agar (SDAY) used by Pandey and Kanaujia (2005) and Potato Dextrose agar (PDA) suggested by Nirmala *et al.* (2005) was utilized to maintain the culture.

Methodology:

Effect of various adjuvants with inoculum on growth and development of B.bassiana:

Various formulations with adjuvants were made and tested for growth and development of *B.bassiana.*

Effect of the combinations of chemical adjuvants :

Optimum concentration of promising chemical

adjuvants comprising glycerol (2 %), Tween 80 (0.5 %), Triton-X100 (0.03%), boric acid (2%) and carboxymethyl cellulose (CMC) (0.5 %) were selected along with their individual concentrations and combined with each other in optimum concentration of aqua suspension 40 % v/v of *B. bassiana* and 8 liquid formulations with multiple adjuvants and 5 liquid formulations with single adjuvants along with control (*B.b.* alone) were prepared. These 14 formulations were tested for their effect on growth and development of *B. bassiana.*

Effect of combinations of chemicals and edible oils as adjuvants :

Optimum concentration of promising chemical and oils adjuvants comprising glycerol (2%), Tween 80 (0.5 %), Triton-X 100 (0.03 %), boric acid (2%) and carboxymethyl cellulose (CMC) (0.5%) and oils as sunflower oil (1 %), groundnut oil (1%) and *Ghee* (0.5%) were selected along with their individual concentrations and combined with each other in optimum concentration of aqua suspension 40 per cent v/v of *B. bassiana* and 18 liquid formulations with multiple adjuvants and 8 liquid formulations with single adjuvants along with control (*B.b.* alone) were prepared. These 27 formulations were tested for their effect on growth and development of *B. bassiana*.

Effect of combination of chemicals, edible oils and nutrient substrates as adjuvants :

Optimum concentrations of promising chemical adjuvants, edible oils and nutrient substrates comprising glycerol (2 %), Tween 80 (0.5 %), Triton-X 100 (0.03 %), boric acid (2 %) and carboxymethyl cellulose (CMC) (0.5 %) and oils as sunflower oil (1 %), groundnut oil (1 %), *Ghee* (0.5 %) and honey (1 %) were selected along with their individual concentrations and combined with each other in optimum concentration of aqua suspension 40 per cent v/v of *B. bassiana* and 13 liquid formulations with multiple adjuvants and 9 liquid formulations with single adjuvants along with control (*B.b.* alone) were prepared. These 23 formulations were tested for their effect on growth and development of *B. bassiana.*

The requisite quantities as per the test concentrations of adjuvants were added in aqua suspension 40 per cent v/v *B. Bassiana* to get 100 ml preparation. The bottles with the experimental formulations were kept at 28 $\pm$ 2^{0}C. One ml of the formulated aqua suspensions per respective treatment was added to autoclave and cooled 40 ml PDB medium filled in 750 ml capacity glass bottles and closed with tight cotton wool plug to ensure aeration for the floating fungus. Whole process was carried out in laminar flow cabinet. The observations on medium surface coverage (%) by the fungus on 3, 7 and 10 days and fungal biomass at 10 days after inoculation (DAI) were noted. The experimental data was subjected to statistical analysis. These experiments were carried out in CRD with three replications.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Influence of combination of multiple adjuvants with inoculum on growth and development of *B. bassiana:*

The combination of chemical adjuvants :

The data on the influence of combinations of chemical adjuvants glycerol (2 %), Tween 80 (0.5 %), Triton-X 100 (0.03 %), boric acid (2%) and Carboxymethyl cellulose (CMC) (0.5 %) along with their individual concentrations on growth and development of *B. bassiana* at 3, 7 and 10 DAI are presented in Table 1.

Medium surface coverage :

At 3 DAI, T_6-*B.b.*+ GLY + BA recorded highest (71.67 %) surface coverage which was at par with T_7-*B.b.*+ GLY + BA + TW and T_{11}- *B.b.*+ GLY recording 68.33 per cent surface coverage against the 23.33 per cent in the control. The growth in the rest of the treatment ranged from 5.00 to 53.33 per cent except T_2- *B.b.*+ TW + TX, T_4- *B.b.*+ GLY + TX, T_8- *B.b.*+ TW + GLY + TX, T_9- *B.b.*+ TW and T_{10}- *B.b.*+ TX which completely prevented the growth of the fungus.

At 7 DAI, the growth of fungus was 8.33 to 100.00 per cent in all treatments except T_2- *B.b.*+ TW + TX and T_4- *B.b.*+ GLY + TX which completely restricted the growth of fungus. T_6-*B.b.* + GLY + BA, T_{11}-GLY, T_{12}-*B.b.*+ BA recorded the cent per cent surface coverage. These were at par with T_{13}-CMC (96.67 %). Later showed at par growth to T_7- *B.b.*+ GLY + BA + TW (93.33 %). At 10 DAI the surface coverage by the fungus ranged from 11.67 to 100 per cent except T_2- *B.b.*+ TW

+ TX and T_4- *B.b.*+ GLY + TX which did not allow the fungal growth. The T_6-*B.b.* + GLY + BA, T_7- *B.b.*+ GLY + BA + TW, T_{11}- *B.b.*+ GLY, T_{12}- *B.b.*+ BA, T_{13}- *B.b.*+ CMC and control recorded cent per cent surface coverage.

Biomass development (g 40 ml^{-1} medium) at 10 DAI and effect of pH :

The T_6-*B.b.* + GLY + BA gave significantly highest biomass of 8.20 g which was at par with T_7- *B.b.*+ GLY + BA + TW recording 8.07 g fungal biomass. The T_{11}- *B.b.*+ GLY recorded 6.33 g biomass followed by T_9- *B.b.*+ TW (6.05 g) against 6.07 g in control. All rest of the treatments recorded biomass of 0.87 to 5.70 g 40 ml^{-1} liquid medium except T_2- *B.b.*+ TW + TX, T_4- *B.b.*+ GLY + TX which showed consistency in inhibition of the growth of fungus.

The pH of the medium before inoculation was 8.29.The pH of the fungal culture developed from 14 treatments 3.07 to 7.92. The pH of the combination treatments was acidic (3.07 to 5.94) except *B.b* +TW+TX (7.43). Triton X 100 (TX) tended to increase the pH in combination (T_2, T_4 and T_8) when it was 3.06 in TX alone and 7.92 in control. It indicated that *B. bassiana* in presence and absence of adjuvants reacted differently and tried to decline the pH.

Considering overall performance of the combinations of chemical adjuvants T_6- *B.b.*+ GLY + BA and T_7- *B.b.*+ GLY + BA + TW were emerged as most promising for aqua suspension formulation of *B. bassiana* .

There is no published work on effect of combination of chemical adjuvants Glycerol + Boric acid and glycerol + Boric acid + Tween 80 in aqua suspension formulation of *B. bassiana.* In case of liquid formulation of *V. lecanii,* Chavan (2005) found glycerol and Tween 80 to be effective for the growth and development of the white hollow fungus. The results could not be compared due to lack of published literature.

The combination of chemicals and edible oils as adjuvants :

The data on the influence of combinations of chemical adjuvants glycerol (2 %), Tween 80 (0.5 %), Triton-X 100 (0.03 %), Boric acid (2 %), CMC (0.5 %) and edible oils sunflower oil (1%), groundnut oil (1 %) and *Ghee* (0.5%) along with their individual concentrations on growth and development of *B. bassiana* at 3, 7 and 10 DAI are presented in Table 2.

Medium surface coverage :

At 3 DAI the medium surface covered was 5 to

Table 1 : Effect of combinations of chemical adjuvants with inoculum in 40 per cent (v/v) on growth and biomass of the mycoagent

Tr. No.	Treatments**	Conc. (%) of adjuvants	Surface area covered (%) on			Biomass (g)	pH
			3 DAI	7 DAI	10 DAI	10 DAI	
T_1	*B.b* +TW + GLY	0.5+2.0	8.33 (16.59)*	10.00 (13.43)	21.67 (27.71)	0.87	4.30
T_2	*B.b* +TW + TX	0.5+0.03	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	7.43
T_3	*B.b* +TW + CMC	0.5+0.5	10.00 (18.43)	36.67 (37.22)	43.33 (41.15)	3.13	4.46
T_4	*B.b* +GLY+TX	2.0+0.03	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	5.94
T_5	*B.b* +TX + CMC	0.03+0.5	5.00 (12.92)	8.33 (16.59)	11.67 (19.88)	0.83	3.07
T_6	*B.b* +GLY+BA	2.0+2.0	71.67 (57.85)	100.00 (90.00)	100.00 (90.00)	8.20	4.36
T_7	*B.b* +GLY+BA+TW	2.0+2.0 +0.5	68.33 (55.76)	93.33 (77.71)	100.00 (90.00)	8.07	4.00
T_8	*B.b* +TW+GLY+TX	0.5+2.0 +0.03	0.00 (0.00)	15.00 (22.59)	25.00 (29.92)	2.77	4.80
T_9	*B.b* +TW	0.50	0.00 (0.00)	35.00 (36.23)	100.00 (90.00)	6.05	3.98
T_{10}	*B.b* +TX	0.03	0.00 (0.00)	30.00 (33.21)	100.00 (90.00)	4.21	3.06
T_{11}	*B.b* +GLY	2.0	68.33 (55.76)	100.00 (90.00)	100.00 (90.00)	6.33	3.72
T_{12}	*B.b* +BA	2.0	53.33 (46.92)	100.00 (90.00)	100.00 (90.00)	5.70	4.40
T_{13}	*B.b* +CMC	0.5	50.00 (45.00)	96.67 (83.85)	100.00 (90.00)	5.03	5.74
T_{14}	Control (*B.b.* alone)	-	23.33 (28.85)	66.67 (54.74)	100.00 (90.00)	6.07	7.92
	S.E. ±		1.32	2.56	1.86	0.05	-
	C.D. (P=0.05)		3.82	7.41	5.39	0.16	-

* Figures in the parentheses indicate arcsin transformed values

** *B.b.*= *Beauveria bassiana*, TW = Tween 80, GLY = Glycerol, TX = Triton-X 100 CMC = Carboxymethyl cellulose, BA = Boric acid, DAI = Days after inoculation

91.67 per cent except T_{18}- *B.b.*+ TW + GLY + TX + GH, T_{19}- *B.b.*+ TW + GLY + TX + GH + SFO and T_{20}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC, which completely inhibited the growth of the fungus. The highest (91.67 %) surface coverage was recorded in T_8- *B.b.*+ GH + CMC, T_9- *B.b.*+ SFO + CMC, T_{12}- *B.b.*+GLY + CMC + GH and T_{13}- *B.b.*+ GLY + GNO.It was followed by T_{26}- *B.b.*+ SFO and T_{27}- *B.b.*+ GNO recording 81.67 per cent surface growth in both the treatments.

Significant differences in the surface growth in single, dual or multiple adjuvant treatments were observed at 7 and 10 DAI. The medium surface coverage at 7 and 10 DAI was 3.33 to 100.00 per cent and 10.00 to 100.00 per cent, respectively. At 10 DAI all the single (T_{19} - T_{27}) adjuvant combinations; all dual adjuvant combination comprising glycerol; GH + CMC, SFO + CMC, GLY +GH + TW, GLY + CMC

Table 2 : Effect of combinations of chemicals and edible oils as adjuvants with inoculum in 40 per cent (v/v) on growth and biomass of the mycoagent

Sr. No.	Treatments**	Conc. (%) of adjuvants	Surface area covered (%) on			Biomass (g)	pH
			3 DAI	7 DAI	10 DAI	10 DAI	
T_1	*B.b* +TW + GH	0.5+0.5	10.00(18.43)*	18.33(25.30)	50.00(45.00)	3.17	3.20
T_2	*B.b* +TW + SFO	0.5+1.0	13.33(21.14)	50.00(45.00)	86.67(68.85)	3.07	3.01
T_3	B.b +GLY + GH	2.0+0.5	33.33(35.21)	93.33(77.71)	100.00(90.00)	6.00	5.08
T_4	*B.b* +GLY + SFO	2.0+1.0	73.33(59.00)	100.00(90.00)	100.00(90.00)	6.40	4.72
T_5	*B.b* +GLY + CMC	2.0+0.5	71.67(57.85)	95.0079.54)	100.00(90.00)	8.50	5.30
T_6	*B.b* +TX + GH	0.03+0.5	5.00(12.92)	10.00(18.43)	16.67(24.04)	1.07	4.06
T_7	*B.b* +TX + SFO	0.03+1.0	5.00(12.92)	8.33(16.59)	11.67(19.88)	0.77	7.46
T_8	*B.b* +GH + CMC	0.5+0.5	91.67(73.40)	100.00(90.00)	100.00(90.00)	6.00	5.24
T_9	*B.b* +SFO + CMC	1.0+0.5	91.67(73.40)	100.00(90.00)	100.00(90.00)	9.33	4.20
T_{10}	*B.b* +GLY+SFO+TW	2.0+1.0+0.5	18.33(25.30)	28.33(32.14)	55.00(47.87)	4.33	3.12
T_{11}	*B.b* +GLY+GH+TW	2.0+0.5+0.5	35.00(36.23)	68.33(55.85)	100.00(90.00)	6.87	4.00
T_{12}	*B.b* +GLY+CMC+GH	2.0+0.5+0.5	91.67(73.40)	100.00(90.00)	100.00(90.00)	4.87	5.38
T_{13}	*B.b* +GLY+GNO	2.0+1.0	91.67(73.40)	100.00(90.00)	100.00(90.00)	7.50	4.24
T_{14}	*B.b* +GNO+TW	1.0+0.5	25.00(29.92)	75.00(60.07)	91.67(73.40)	6.87	3.72
T_{15}	*B.b* +GNO+GLY+TW	1.0+2.0+0.5	18.33(25.30)	88.33(70.11)	100.00(90.00)	5.80	4.58
T_{16}	*B.b* +TW+GLY+TX+GH	0.5+2.0+0.03+0.5	0.00(0.00)	3.33(8.61)	10.00(18.04)	1.27	6.56
T_{17}	*B.b* +TW+GLY+TX+GH +SFO	0.5+2.0+0.03+0.5+1 .0	0.00(0.00)	8.33(8.61)	15.00(22.59)	2.27	7.06
T_{18}	*B.b*+TW+GLY+TX+GH +SFO+CMC	0.5+2.0+0.03+0.5+1 .0+0.5	0.00(0.00)	10.00(18.04)	18.33(25.30)	3.63	7.48
T_{19}	*B.b* +TW	0.50	0.00(0.00)	33.33(35.21)	100.00(90.00)	6.07	3.98
T_{20}	*B.b* +TX	0.03	0.00(0.00)	30.00(33.21)	100.00(90.00)	4.13	3.06
T_{21}	*B.b* +GLY	2.0	68.33(55.76)	100.00(90.00)	100.00(90.00)	6.33	3.72
T_{22}	*B.b* +BA	2.0	56.67(48.84)	100.00(90.00)	100.00(90.00)	5.80	4.40
T_{23}	*B.b* +CMC	0.5	53.33(46.92)	96.67(83.85)	100.00(90.00)	5.03	5.74
T_{24}	*B.b* +SFO	1.0	81.67(64.69)	100.00(90.00)	100.00(90.00)	8.40	4.23
T_{25}	*B.b* +GNO	1.0	81.67(64.69)	100.00(90.00)	100.00(90.00)	8.47	4.42
T_{26}	*B.b* +GH	0.5	78.33(62.29)	100.00(90.00)	100.00(90.00)	5.17	5.10
T_{27}	Control (*B.b*. alone)	-	21.67(27.71)	68.33(55.85)	100.00(90.00)	6.13	7.92
	S.E. ±		1.43	2.4	1.73	0.06	--
	C.D. (P=0.05)		4.07	7.06	4.91	0.17	--

* Figures in the parentheses indicate arcsin transformed values

** *B.b.*= *Beauveria bassiana*, TW = Tween 80 GLY = Glycerol, TX = Triton-X 100, BA = Boric acid, CMC = Carboxymethyl cellulose, SFO = Sunflower oil, GH = Ghee, GNO = Groundnut oil, DAI = Days after inoculation

+GH and GNO + GLY + TW recorded cent per cent coverage. However, control also recorded cent per cent growth of the mycoagent; when rest of the treatments showed 18.33 to 91.67 per cent surface coverage due to more or less adverse effect of the combinations.

Biomass development (g 40 ml^{-1} medium) at 10 DAI and effect of pH :

Among the various treatments of adjuvants T_9- *B.b.*+ SFO + CMC recorded the significantly highest biomass of 9.33 g. The next promising and at par treatments were T_5- *B.b.*+ GLY + CMC (8.50 g), T_{25}- *B.b.*+ GNO (8.47 g) and T_{26}- *B.b.*+ SFO (8.40 g). These were followed by the treatments in their descending order of superiority for development of fungal biomass over control (6.13 g) were T_{13}- *B.b.*+ GLY+ GNO, T_{11}- *B.b.*+ GLY + GH + TW, T_{14}- *B.b.*+ GNO + TW, T_4- *B.b.*+ GLY + SFO and T_{23}- *B.b.*+ GLY which produced 7.50, 7.40, 6.87, 6.87, 6.40 and 6.33 g biomass, respectively. The biomass developed in control was 6.13 g. When, T_7- *B.b.*+ TX + SFO recorded lowest (0.77 g) biomass due to detrimental effect of Triton-X 100.

The pH of medium before inoculation was 8.29. It was lowered (3.01 to 5.74) by fungal activities in the presence of different adjuvants except comparatively higher pH of TX comprising treatments T_7, T_{16}, T_{17} and T_{18} (6.56 to 7.48).The pH in control decline from 8.29 to 7.92 in absence of adjuvants.

Considering the overall performance of the

Table 3 : Effect of combinations of chemicals, oils and honey as adjuvants with inoculumin 40 per cent (v/v) on growth and biomass of the mycoagent

Tr. No.	Treatment details	Conc. (%) of adjuvants	Surface area covered (%) on			Biomass (g)	pH
			3 DAI	7 DAI	10DAI	10DAI	
T_1	*B.b.*+ TW + HO	0.5+1.0	11.67 (19.88)	50.00 (45.00)	93.33 (75.24)	4.20	378
T_2	*B.b.* + GLY + HO	2.0+1.0	86.67 (68.85)	100.00 (90.00)	100.00 (90.00)	8.93	4.58
T_3	*B.b.*+ TX + HO	0.03+1.0	5.00 (12.92)	8.33 (16.59)	11.67 (19.88)	1.00	7.52
T_4	*B.b.*+ GH + SFO	0.5+1.5	11.67 (19.88)	25.00 (29.92)	58.33 (49.80)	2.17	4.70
T_5	*B.b.*+ GH + HO	0.5+1.0	86.67 (68.85)	100.00 (90.00)	100.00 (90.00)	5.77	4.55
T_6	*B.b.*+ SFO + HO	1.0+1.0	91.67 (73.40)	100.00 (90.00)	100.00 (90.00)	8.60	5.19
T_7	*B.b.*+ HO + CMC	1.0+0.5	35.00 (36.23)	100.00 (90.00)	100.00 (90.00)	6.30	4.84
T_8	*B.b.*+ GLY + GH +HO	2.0+0.5+1.0	73.33 (59.00)	95.00 (79.54)	100.00 (90.00)	5.93	5.56
T_9	*B.b.* + GLY+HO+TW	2.0+1.0+0.5	28.33 (32.14)	93.33 (77.71)	100.00 (90.00)	7.20	5.52
T_{10}	*B.b.* + GLY+CMC+HO	2.0+0.5+1.0	23.33 (28.85)	76.67 (61.22)	100.00 (90.00)	7.17	4.78
T_{11}	*B.b.*+TW+GLY+TX+GH+ SFO+CMC+HO	0.5+2.0+0.03+0.5 +1.0+0.5+1.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	4.99
T_{12}	*B.b.*+ TW+ GLY+ TX+ GH + SFO+ MC+ HO+BA	0.5+2.0+0.03+0.5 +1.0+0.5+1.0+2.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	5.02
T_{13}	*B.b.*+TW+GLY+TX+GH+ SFO+CMC + HO + BA+GNO	0.5+2.0+0.03+0.5+ 1.0+0.5+1.0+2.0+1.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	4.78
T_{14}	*B.b.*+ TW	0.50	0.00 (0.00)	36.67 (37.22)	100.00 (90.00)	6.13	3.98
T_{15}	*B.b.*+ TX	0.03	0.00 (0.00)	31.67 (34.23)	96.67 (83.85)	4.17	3.06
T_{16}	*B.b.*+ GLY	2.0	68.33 (55.76)	100.00 (90.00)	100.00 (90.00)	6.33	3.72
T_{17}	*B.b.*+ BA	2.0	56.67 (48.84)	100.00 (90.00)	100.00 (90.00)	5.80	4.40
T_{18}	*B.b.*+ CMC	0.5	53.33 (46.92)	96.67 (83.85)	100.00 (90.00)	5.03	5.74
T_{19}	*B.b.*+ SFO.	1.0	81.67 (64.69)	100.00 (90.00)	100.00 (90.00)	8.50	4.23
T_{20}	*B.b.*+ GNO.	1.0	81.67 (64.69)	100.00 (90.00)	100.00 (90.00)	8.35	4.42
T_{21}	*B.b.*+ GH	0.5	78.33 (62.29)	100.00 (90.00)	100.00 (90.00)	5.20	5.10
T_{22}	*B.b.*+ HO	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	6.50	5.71
T_{23}	Control (*B.b.* alone)	-	21.67 (27.71)	66.67 (54.74)	100.00 (90.00)	6.10	7.92
	S.E. ±		1.37	2.40	1.38	0.06	--
	C.D. (P=0.05)		3.91	6.85	3.94	0.17	--

* Figures in the parentheses indicate arcsin transformed values* * *B.b.=Beauveria bassiana*, B = Boric acid, TW = Tween 80, SFO = Sunflower oil, GLY = Glycerol GH = Ghee, TX = Triton-X100, GNO = Groundnut oil, CMC = Carboxymethyl cellulose, HO = Honey,DAI = Days after inoculation

combinations of chemicals and oils as adjuvants T_9- *B.b.*+ SFO + CMC, T_5- *B.b.*+ GLY + CMC, T_{27}- *B.b.*+ GNO and T_{26}- *B.b.*+ SFO besides cent per cent growth resulted in 9.33, 8.50, 8.47 and 8.40 g biomass production, respectively. These emerged as highly promising adjuvant combinations for of *B. bassiana* aqua suspension.

There is no published work on effect of combination of chemical adjuvants and edible oils (*B.b.*+ SFO + CMC, *B.b.*+ GLY + CMC, *B.b.*+ GNO and *B.b.*+ SFO) in aqua suspension formulation of *B. bassiana*. In case of liquid formulation of *V. lecanii,* Chavan (2005) found glycerol and Tween 80 to be effective for the growth and development of the white hollow fungus.

The combinations of chemicals, edible oils and honey as adjuvants :

The data on influence of combinations of chemicals, oils and honey as adjuvants on growth and development of *B. bassiana* at 3, 7 and 10 DAI are presented in Table 3.

Medium surface coverage :

At 3 DAI the medium surface coverage by the mycoagent was 5.00 to 100 per cent except that completely inhibited in T_{11}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC + HO, T_{12}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC + HO + BA, T_{13}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC + HO + BA + GNO, T_{14}- *B.b.*+ TW and T_{15}- *B.b.*+ TX. The highest (100.00%) surface coverage was observed in T_{22}- *B.b.*+ HO. The next promising and significantly superior at par treatments to rest of the treatments (35 to 81.67%) were T_6- *B.b.*+ SFO + HO (91.67 %), T_5- *B.b.*+ GH + HO (86.67 %), and T_2- *B.b.*+ GLY + HO (86.67 %). The lower (5.00 %) surface coverage was noticed in T_3- *B.b.*+ TX + HO than control (21.67%) confirmed detrimental effect of TX.

At 7 and 10 DAI the medium surface covered in the treatments was 8.33 to 100.00 and 11.67 to 100.00 per cent, respectively,except T_{11}- *B.b.*+ TW + GLY + TX + GH+ SFO + CMC + HO, T_{12}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC + HO + BA and T_{13}- *B.b.*+ TW + GLY + TX + GH + SFO + CMC + HO + BA + GNO which completely inhibited the growth of fungus due to the TX factor in long chain multiple combination treatments. At 10 DAI, most of the treatments recorded cent per cent surface coverage except T_1- *B.b.*+ TW + HO (93.33 %), T_3-*B.b.*+ TX + HO (11.67 %), T_4- *B.b.*+ GH + SFO (58.33 %) and T_{15}- *B.b.*+ TX (96.67 %).

Biomass development (g 40 ml^{-1} medium) at 10 DAI and effect of pH :

At 10 DAI, T_2- *B.b.*+ GLY + HO gave significantly highest biomass of 8.93 g 40 ml^{-1} liquid medium. The next promising and at par treatments for the biomass production were T_6- *B.b.*+ SFO + HO and T_9- *B.b.*+ SFO recording 8.60 and 8.50 g biomass, respectively. The later treatment was at par with T_{20}- *B.b.*+ GNO (8.35 g). The next promising treatments in descending order of their potential for the biomass were T_9- *B.b.*+ GLY + HO + TW (7.20 g) and T_{10}- *B.b.*+ GLY + CMC + HO (7.17 g). The biomass in the rest of the treatments was 1.00 to 6.50 g per 40 ml^{-1} except T_{11}, T_{12} and T_{13} which completely inhibited the growth and development of the fungus due to adverse effect of TX.

The pH of culture medium of multiple combination treatments with oils were higher (4.23 to 5.56) as compared to those with chemicals (3.046 to 4.40) except CMC (5.74). The pH of the culture medium of the promising treatments for biomass production (T_2- *B.b.*+ GLY, T_6- *B.b.*+ SFO, T_{19}- *B.b.*+ SFO and T_{20}- *B.b.*+ GNO) yielding 8.35 to 8.93 g biomass was 4.23 to 5.19 against the pH of 4.78 to 5.02 in T_{11} to T_{13} yielding zero biomass. It indicated that at too much combination of adjuvants created lot of pressure and inoculum which tried to appropriate pH but could not develop.

The pH of the culture medium of the chemical adjuvant comprising treatments were lower than the oil comprising and multiple adjuvants treatments and *B. bassiana* try to optimize the pH between 4- 6. Young jun *et al*. (2011) observed that pH 4 to 6 was found to be the most suitable for growth and sporulation of *B. bassiana* supports the results of present finding.

Considering overall performance of the combinations of chemicals, edible oils and honey T_2- *B.b.*+ GLY (2%) + HO (1%), T_6- *B.b.*+ SFO (1%) + HO (1%), T_{19}- *B.b.*+ SFO (1%) and T_{20}- *B.b.*+ GNO (1%) were emerged as the most promising combinations of bioactive ingredient and adjuvants for aqua suspension formulation of *B. bassiana*. Hence these were subjected to further studies on growth, development, viability, bioefficacy and UVC rays protectability.

There is no published work on effect of combination of chemical adjuvants, edible oils and honey (*B.b.*+

GLY + HO, *B.b.*+ SFO + HO, *B.b.*+ SFO and *B.b.*+ GNO) in aqua suspension formulation of *B. bassiana*. In case of liquid formulation of *V. lecanii*, Chavan (2005) found glycerol and Tween 80 to be effective for the growth and development of the white hollow fungus. The results could not be compared due to lack of published literature.

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