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Compatibility studies of different insecticides with entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin

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Abstract
Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin was studied in the laboratory condition with ten widely used systemic insecticides with their three doses by poison food technique. The results showed that the dimethoate 30 EC was found the most compatible at lower, recommended, and higher doses, as it was categorized as harmless (Grade 1) to the fungus. Dinotefuran 20 SG at higher dose (0.005%) and carbosulphan 25 EC at all doses were categorized as harmful (Grade 4) to *B. bassiana.*

Keywords: *Beauveria bassiana* (Balsamo) Vuillemin, compatibility, entomopathogenic fungus, insecticides

Introduction
Several species of natural enemies including insects, mites, fungi, bacteria, virus and protozoa have been recovered from different ecosystem, only few are efficient in managing the pest population. Among them, the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin is a very potential bio-control agent for pest management. It is very effective and widely used bio-pesticide, which manages numerous pests of different crops. It can be developed in laboratory for use as mycoinsecticide agent. Earlier, for the first time, scientists have recorded this fungus occurring naturally in India [8]. This fungus has effect on more than 150 insect species [10]. Likewise, due to its wide host range, *B. bassiana* is referred to as "Magnificent pathogen" [3]. *B. bassiana* did not have an adverse effect on humans, livestock, birds, fish, beneficial insects, crops, waterways or groundwater resources [11].

To reduce the sole dependence on insecticides, there is a need to explore alternative methods of pest control, like Integrated Pest Management (IPM). Combined utilization of selective insecticides in association with fungal pathogens can increase the efficiency of control by reducing the amount of applied insecticides, minimalizing environmental contamination hazards and pest resistance [7, 9]. The use of insecticides with entomopathogenic fungus, *B. bassiana* has been selected for the management of insect pests. So, there is a need to study the compatibility between entomopathogenic fungi and pesticides used in crop protection. Common practice among farmers is to use only insecticides for insect pest management which has deleterious effects on environment and human beings. This study is based on necessity to reduce chemical dependency in crop protection by incorporating the biological agents such as *B. bassiana* with commonly used. Before recommendations could be made for such approach, a compatibility study between *B. bassiana* and most commonly applied insecticides was necessary. The comparable study with different insecticides has been done which shows greater compatibility of *B. bassiana* with insecticides [5]. These studies underline the possibility of application of less harmful insecticides in combination with entomopathogenic fungi for the most effective IPM programs.

Materials and Methods
The present study was conducted at Biocontrol Research Laboratory, Department of Entomology, College of Agriculture, Junagadh Agricultural University, Junagadh, and Gujarat, India during 2016-17. Ten insecticides at three different rates were assessed by poisoned food technique in Potato Dextrose Agar (PDA) medium [2]. The insecticide dose was calculated based upon, lower, recommended, and higher field application rate. Each treatment for each insecticide was repeated three times.

Twenty ml of PDA medium was sterilized in individual boiling tubes and the insecticide emulsions of required concentration (lower, recommended and higher) were incorporated into the melted sterile PDA aseptically, thoroughly mixed, poured into 9 cm diameter sterile petri dishes and allowed to solidify under laminar flow cabinet. An agar disc along with mycelium mat of *B. bassiana* was cored from the periphery of 10 days old colony of *B. bassiana* by 5 mm diameter cork borer and transferred in to the centre of the PDA plate. Growth medium (PDA) without insecticide but inoculated with mycelial disc served as untreated check.

When a full growth of *B. bassiana* was obtained in control (10^{th} day of inoculation), the growth of entomogenous fungi in all other treatments was recorded and following grades were assigned.

Chart 1: The growth observed on the medium

Grades	Percent growth
0	0%
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

The grades given after the observed growth was imparted for the calculation of the growth inhibition percentage of *B. bassiana* by using following formula.

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

Where,
X- Percent growth inhibition
Y- Grade obtained to the growth in control plate
Z- Grade obtained to the growth in treated plate

The fungal growth inhibition obtained from each treatment was further classified in evaluation categories as follows: 1- 4 scoring index in toxicity tests *in vitro* according to following Hassan's classification scheme [4].

Scoring Index	Toxicity
1	Harmless (<50% reduction in beneficial capacity)
2	Slightly harmful (50-79%)
3	Moderately harmful (80-90%)
4	Harmful (>90%)

Results and Discussion

Compatibility at lower dose

The results shown in Table 1 present percentage of growth inhibition in relation to the insecticide applied. Dimethoate 30 EC 0.020% at lower rate, showed, significantly, the lowest (20%) growth inhibition of *B. bassiana*. Imidacloprid 17.8 SL 0.003%, azadirachtin 0.15 EC 0.0003%, thiamethoxam 25 WG 0.005%, and spiromesifen 48 EC 0.007%, showed 23.33% of growth inhibition each. The treatments of dinotefuran 20 SG 0.005% (26.67%), flonicamid 50 WP 0.007% (36.67%), acetamiprid 20 SP 0.003% (36.67%) and diafenthiuron 50 WP 0.025% (36.67%) exhibited moderately growth inhibition of *B. bassiana*. The highest (100%) growth inhibition was recorded from carbosulfan 25 EC 0.025%.

Table 1: Effect of different low-rate insecticides on the radial growth of *B. bassiana*

Sr. No.	Treatments	Conc. (%)	Dose (g/ml)/liter	Lower dose	
				Growth inhibition (%)	*Grade
1.	Flonicamid 50 WP	0.007	0.15	36.02 (36.67)	1
2.	Acetamiprid 20 SP	0.003	0.15	37.28 (36.67)	1
3.	Imidacloprid 17.8 SL	0.003	0.15	26.88 (23.33)	1
4.	Dimethoate 30 EC	0.020	0.50	23.88 (20.00)	1
5.	Carbosulfan 25 EC	0.025	1.00	90.00 (100.00)	4
6.	Azadirachtin 0.15 EC	0.0003	2.50	26.88 (23.33)	1
7.	Thiamethoxam 25 WG	0.005	0.20	24.88 (23.33)	1
8.	Dinotefuran 20 SG	0.005	0.25	28.61 (26.67)	1
9.	Diafenthiuron 50 WP	0.025	0.50	36.02 (36.67)	1
10.	Spiromesifen 48 EC	0.007	0.15	25.62 (23.33)	1
11.	Untreated control	-	-	7.98 (0.00)	
S. Em.±				1.74	
C.D. at 5%				4.92	
C.V.%				12.52	

Data in parenthesis are original values, while outside values are arcsine transformed.
Local strain of *B. bassiana* @ 2×10^8 cfu/g was used.
*Grades: 1 = Harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50- 79%), 3 = moderately harmful (80-90%), 4 = Harmful (>90%)

The above results indicate that among the insecticides tested, flonicamid, acetamiprid, imidacloprid, dimethoate, azadirachtin, thiamethoxam, dinotefuran, diafenthiuron and spiromesifen were relatively harmless (Grade 1) to *B. bassiana* showing less than 50% growth inhibition at lower dose. In the same low-rate application category, Carbosulfan surfaced as the strongest growth inhibitor of *B. bassiana.*

Compatibility at recommended dose

The effects of insecticides on the growth of *B. bassiana* at recommended dose are shown in Table 2. The results clearly indicate that the lowest (33.33%) growth inhibition was observed due to thiamethoxam 25 WG 0.010% followed by dimethoate 30 EC 0.030% (36.67%), imidacloprid 17.8 SL 0.005% (43.33%), dinotefuran 20 SG 0.011% (43.33%) and diafenthiuron 50 WP 0.050% (43.33%). The insecticides, azadirachtin 0.15 EC 0.0007% (46.67%) and spiromesifen 48 EC 0.023% (56.67%) were found next in order to inhibit the growth of *B. bassiana*. Flonicamid 50 WP 0.015% (63.33%) and acetamiprid 20 SP 0.006% (70.00%) were moderately affecting the growth of the fungus. The results showed that carbosulfan 25 EC 0.050% caused the highest (100.00%) reduction in mycelial growth of *B. bassiana* with inhibition.

Table 2: Effect of different recommended rate of insecticides on the radial growth of *B. bassiana*

Sr. No.	Treatments	Conc. (%)	Dose (g/ml)/litre	Recommended Dose	
				Growth inhibition (%)	*Grade
1.	Flonicamid 50 WP	0.015	0.30	57.72 (63.33)	2
2.	Acetamiprid 20 SP	0.006	0.30	64.92 (70.00)	2
3.	Imidacloprid 17.8 SL	0.005	0.30	41.95 (43.33)	1
4.	Dimethoate 30 EC	0.030	1.00	38.02 (36.67)	1
5.	Carbosulfan 25 EC	0.050	2.00	90.00 (100.00)	4
6.	Azadirachtin 0.15 EC	0.0007	5.00	45.44 (46.67)	1
7.	Thiamethoxam 25WG	0.010	0.40	33.68 (33.33)	1
8.	Dinotefuran 20 SG	0.011	0.50	43.10 (43.33)	1
9.	Diafenthiuron 50 WP	0.050	1.00	43.10 (43.33)	1
10.	Spiromesifen 48 EC	0.023	1.00	51.90 (56.67)	2
11.	Untreated control	-	-	7.98 (00.00)	
S.Em.±				2.02	
C.D. at 5%				5.72	
C.V.%				11.15	

Data in parenthesis are original values, while outside values are arcsine transformed.
Local strain of *B. bassiana* @ 2×10^8 cfu/g was used.
*Grades: 1 = Harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50- 79%), 3 = moderately harmful (80-90%), 4 = Harmful (>90%)

The results of study on compatibility of *B. bassiana* with different insecticides at their recommended dose indicated that the insecticides imidacloprid, dimethoate, azadirachtin, thiamethoxam, dinotefuran and diafenthiuron were more compatible with tested fungus, as they were harmless (Grade 1) for the growth of the fungus. The growth inhibition was rated slightly harmful (Grade 2) to the insecticides flonicamid, acetamiprid and spiromesifen. Whereas, carbosulfan was harmful (Grade 4) and proved to be the strongest growth inhibitor of *B. bassiana.*

Compatibility at higher dose

All the insecticides tested have different compatible levels with *B. bassiana* at their higher dose (Table 3). Perusal of data on growth inhibition sign-posted that dimethoate 30 EC 0.045% recorded the lowest inhibition (56.67%). The treatments, azadirachtin 0.15 EC 0.0011% and diafenthiuron 50 WP 0.075% remained next in order, as both registered 60.00% growth inhibition. The growth inhibition was moderately increases in imidacloprid 17.8 SL 0.007% (73.33%), thiamethoxam 25 WG 0.015% (76.67%), spiromesifen 48 EC 0.033% (76.67%). The highest growth inhibition were obtained in the carbosulfan 25 EC 0.075% (100.00%) and dinotefuran 50 WP 0.075% as it showed (100.00%) growth inhibition of *B. bassiana*. The remaining insecticides, flonicamid 50 WP 0.022% (80.00%) and acetamiprid 20 SP 0.009% (83.33%) showed lower growth inhibition as compare to earlier insecticides.

Table 3: Effect of different higher -rate of insecticides on the radial growth of *B. bassiana*

Sr. No	Treatments	Conc. (%)	Dose (g/ml)/litre	Higher Dose	
				Growth inhibition (%)	Grade
1.	Flonicamid 50 WP	0.022	0.45	71.72 (80.00)	3
2.	Acetamiprid 20 SP	0.009	0.45	74.71 (83.33)	3
3.	Imidacloprid 17.8 SL	0.007	0.45	65.78 (73.33)	2
4.	Dimethoate 30 EC	0.045	1.50	53.90 (56.67)	2
5.	Carbosulfan 25 EC	0.075	3.00	90.00 (100.00)	4
6.	Azadirachtin 0.15 EC	0.0011	7.50	55.38 (60.00)	2
7.	Thiamethoxam 25 WG	0.015	0.60	70.12 (76.67)	2
8.	Dinotefuran 20 SG	0.016	0.75	90.00 (100.00)	4
9.	Diafenthiuron 50 WP	0.075	1.50	55.38 (60.00)	2
10.	Spiromesifen 48 EC	0.033	1.50	70.12 (76.67)	3
11.	Untreated control	-	-	7.98 (00.00)	
S.Em.±				1.89	
C.D. at 5%				5.36	
C.V.%				8.07	

Data in parenthesis are original values, while outside values are arcsine transformed.
Local strain of *B. bassiana* @ 2×10^8 cfu/g was used.
*Grades: 1 = Harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50- 79%), 3 = moderately harmful (80-90%), 4 = Harmful (>90%)

Conclusion

Compatibility at lower dose

Laboratory investigation on compatibility of *B. bassiana* fungus with ten different insecticides at lower dose revealed that dimethoate 30 EC 0.020% showed the lowest growth inhibition (20.00%), followed by imidacloprid17.8 SL 0.003%, azadirachtin 0.15 EC 0.0003%, thiamethoxam 25 WG 0.005% and spiromesifen 48 EC 0.007% with 23.33% of growth inhibition each. All of these insecticides at low-rate, were found harmless (Grade 1) to the tested fungus. Carbosulfan 25 EC 0.025%, with 95.83% growth inhibition, was found to be harmful (Grade 4) and incompatible with *B. bassiana*.

Compatibility at recommended dose

The same ten insecticides tested with *B. bassiana* at recommended dose, showed that the lowest (33.33%) growth inhibition occurred in thiamethoxam 25 WG 0.010%, followed by dimethoate 30 EC 0.030% (36.67%), imidacloprid17.8 SL 0.005% (43.33%), dinotefuran 20 SG 0.011% (43.33%), diafenthiuron 50WP 0.050% (43.33%). These fore mentioned insecticides were found to be more compatible with the *B. bassiana* at recommended rate. The insecticides azadirachtin 0.15 EC 0.0007% (46.67%), spiromesifen 48 EC 0.023% (56.67%) had higher rate of *B. bassiana* growth inhibition, placing them into the slightly harmful (Grade 2) category. At recommended dose, carbosulfan 25 EC 0.050% caused the highest growth inhibition with 95.83% and was found to be harmful (Grade 4) and incompatible with *B. bassiana*.

Compatibility at higher dose

The results at higher dose revealed that dimethoate 30 EC 0.045% showed 56.67% of inhibition, which was the lowest among all the insecticides. The azadirachtin 0.15 EC 0.0011% and diafenthiuron 50 WP 0.075% inflicted 60% growth inhibition. The Acetamiprid 20 SP 0.009% had the highest growth inhibition of 83.33% and was found to be harmful (Grade 4) and incompatible with *B. bassiana*.

The dimethoate 30 EC 0.045%, azadirachtin 0.15 EC, and diafenthiuron 50 WP 0.075%, were categorized as a slightly harmful (Grade 2); acetamiprid 20 SP 0.009% was classified as harmful (Grade 4).

The present study shows that dimethoate and thiamethoxam were the most compatible insecticide with *B. bassiana* at all rate-levels among all other insecticides studied. The insecticide carbosulfan has shown the strongest inhibition on the growth and sporulation of *B. bassiana* at lower, recommended field rates and at higher dose.

The insecticides, dimethoate and thiamethoxam were found slightly harmful in this study, which is in complete conformity with the results found by Kachot *et al.* [5] and Khan *et al.* [6] who stated that dimethoate were highly compatible with entomopathogenic fungus *B. bassiana* and thiamethoxam at sub-normal concentration were found to be slightly harmful to *B. bassiana* with 30.00% growth inhibition respectively. De-oliveira *et al.* [1] found that insecticide thiamethoxam was not found to cause radial growth.

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