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Compatibility of entomopathogenic fungus *Beauveria bassiana* (Bals.) with selected chemical insecticides

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Abstract

In vitro investigation was carried out to examine the compatibility of *Beauveria bassiana* (Bals.) (Bb-5a) with ten insecticides through poisoned food technique. The insecticides were tested at three different doses viz., field recommended dose (RD), half of the recommended dose ($0.5 \times RD$) and double the recommended dose ($2 \times RD$). Among all the chemical insecticides tested for their compatibility, only diafenthiuron was not compatible with *B. bassiana* and it inhibited the growth. While, imidacloprid, thiamethoxam, acetamiprid, clothianidin, acephate, fipronil, buprofezin, flonicamid and azadirachtin were showed compatible at 14th and 30th day after inoculation. Consequences of the present investigation recommended that aside diafenthiuron, rest of the insecticides examined can be safely used along with *B. bassiana* in integrated pest management.

Keywords: *Beauveria bassiana*, compatibility, entomopathogenic fungi, insecticides, integrated pest management

Introduction

Entomopathogenic fungi have significant potential for effective suppression of a various arthropod pests. Natural pest control specifically achieved by entomopathogens and use of entomopathogens is a strategy that should be considered in IPM programs as a significant reduction factor of pest population density. There are various examples where utilization of specific insecticides has increased the effectiveness of fungal growth against various insect pests [1]. The widely used entomofungal agent *B. bassiana* is an imperfect fungi belongs to the subdivision Deuteromycotonia and also it is an enlisted bio pesticide with a scope of broad host range of nearly 700 insect species and utilized for insect pest management in different crops. The combined application of microbial pesticides with chemical insecticides pest management practices needs to understand the compatibility of entomopathogens and their additive effect in controlling insect pests. Information from *in vitro* compatibility studies would help the farmers to select proper compatible mixtures of fungal pathogens and chemical pesticides treatments so that profits by compatible sets can be explored and advocated to the farmers with adoption of IPM programs in different crops [2].

On the other hand, utilization of incompatible insecticides may decrease the vegetative growth and spore reproduction of the pathogens and negative effect on IPM [3]. Though, impacts of chemicals on conidial spore germination, that have been frequently disregarded, are the at most significant aspect to judge insecticide compatibility [4]. The present experiment was carried out to study the various effects of different insecticides on conidial germination and radial growth of *B. bassiana* in *in vitro* conditions.

2. Material and methods

The present investigation was conducted at Bio-control laboratory, Main Agricultural Research Station, University of Agricultural Sciences Raichur, Karnataka, India. The analysis was done by using factorial completely randomized design. The chemical insecticides chose for *in vitro* compatibility experiment were among those frequently utilized for the management of sunflower sucking pests. The impact of these chemical insecticides on the vegetative growth and spore germination of *B. bassiana* was assessed.

2.1.1 Maintenance of pure culture of *B. bassiana*

Pure fungus culture of *B. bassiana* (Bb - strain 5a) was collected from ICAR-NBAIR was used for the present studies. For maintenance of pure culture, a loopful of inoculum from mother culture slants of *B. bassiana* were transferred to PDA slants and Petri plates and maintained as a pure culture. The PDA medium was sterilized at a pressure of 15 psi and temperature 121 °C for 30 minutes in an autoclave, autoclaved PDA media poured to sterilized Petri plates, cooled and inoculated with a pure culture of the *B. bassiana* under aseptic condition. The plates were then incubated at room temperature (26 ± 2 °C) for fourteen days. After complete sporulation, pure cultured slants and Petri plates were stored in the refrigerator at 4 °C before using for further studies. Suspension of spores was made using distilled water with Tween - 80 (0.2 %) and filtered through a double-layered muslin cloth. Spore count was made using Neubauer's haemocytometer under the microscope after necessary serial dilutions. From the stock solution, further dilutions were

made to obtain the required concentrations for further studies.

2.1.2 Preparation of test chemical insecticide concentrations

For compatibility study ten insecticides were selected shown in table 1. The quantity of insecticides was calibrated with the help of micropipette in case of liquid formulation and in case of granular or powder formulation using electronic weighing balance. To decide the compatibility of the selected insecticides with *B. bassiana* the recommended dose of selected insecticides were calibrated for 200 ml of PDA media. For example, the recommended dose of imidacloprid 17.8 % SL was 0.3 ml per litre, imidacloprid 60 µl was added to 200 ml of PDA media utilizing micropipette. All ten selected insecticide concentrations were calibrated for 200 ml of PDA media, in laminar air flow cabinet. Increase or decrease of radial growth of *B. bassiana* significantly differed with the three different dose such as RD, 0.5 × RD and 2 × RD of selected insecticides.

Table 1: List of insecticides used for compatibility studies with *B. bassiana*

S. No.	Insecticides	Dosage g or ml/l	Chemical group	Trade name	Manufacturing company
1	Imidacloprid 17.8 % SL	0.30 ml	Neonicotinoid	Victor	Insecticides (India) Limited
2	Thiamethoxam 25 % WG	0.20 gm	Neonicotinoid	Tagxone	Tropical Agro system (India) Pvt. LTD
3	Acetamiprid 20 % SP	0.20 gm	Neonicotinoid	Dyken	Heranba industries LTD
4	Clothianidin 50 % WDG	0.07 gm	Neonicotinoid	Dantop	Sumitomo chemical co. LTD
5	Acephate 75 % SP	1.00 gm	Organophosphate	Goldstar	Heranba industries LTD
6	Fipronil 5 % SC	1.00 ml	Phenyl pyrazole	Agent	Tropical Agro system (India) Pvt. LTD
7	Diafenthiuron 50 % WP	1.00 gm	Thiurea derivative or IGR	Apollo	Agastya Agro Limited
8	Buprofezin 25 % SC	1.00 ml	Thiadiazinone	Heralaud	Heranba industries LTD
9	Flonicamid 50 % WG	0.40 gm	Amide group	Ulala	UPL Limited
10	Azadirachtin 0.15 % W/W	5.00 ml	Botanical	Neemdhan	Anu products Limited

2.1.3 Germination inhibition test: Each insecticide based on recommended dose was put into water (100 ml) in flask to get desired insecticide suspension. The insecticides effect on the germination of the conidia was determined by placing 10 micro litre drop of each insecticide suspension containing fungal spores at concentration of 2×10^8 cfu/g on a thin film of PDA medium in a Petri plate. The conidia in distilled water suspension served as control for comparison. Petri plates were incubated at 27 ± 1 °C, 80 ± 5 % relative humidity in the dark for 24 hours. After staining with lacto-phenol cotton blue, germination was checked under microscope. Only conidia with a germ tube as long as the conidia widths were considered to have germinated. Inhibition of conidial germination in comparison with untreated control was examined for individual insecticide at three different doses (RD), 0.5 x RD and 2 x RD.

2.1.4 Mycelial growth test

The nine insecticides and one botanical insecticide was evaluated for their compatibility with *B. bassiana* at three concentrations: average recommendation utilized for application in the field (FR), 0.5 x FR (FR - 50 %) and 2 x FR (FR + 100 %) by poisoned food technique [5] in potato dextrose agar (PDA) media as follows.

1. Required concentrations of each test insecticides were incorporated into 200 ml of melted sterile PDA media under aseptic condition, mixed thoroughly, poured into 9 cm diameter sterile petri plates and allowed to solidify under laminar air flow cabinet.
2. Mycelium mat of *B. bassiana* along with agar disc was cored from the periphery of fourteen - day old colony of *B. bassiana* using 10 mm diameter cork borer and placed

in to the centre part of the PDA plate. PDA media without insecticides however inoculated with mycelial disc treated as untreated control.

3. Parafin sealed Petri dishes were incubated at 25 ± 1 °C temperature and 70 ± 5 % relative humidity with 12:12 hours' photo: dark phase for fourteen days to allowed extreme growth. Individual treatment was three times replicated. The breadth of developing culture in excess of the plugs in individual Petri plates was estimated on 14 days after inoculation (DAI) (When radial growth in the control plate fully covered the media) and furthermore on 30 days after inoculation. The information was indicated as *B. bassiana* per cent growth inhibition by insecticide treated PDA media [6].

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

Where, X = Percentage of growth inhibition

Y = Radial growth of fungus in untreated check

Z = Radial growth of fungus in poisoned PDA media

4. Compatibility ratings for test insecticides were classified in evaluation categories of 1 - 4 scoring index according to Jayasing's classification [7].

Sl. No	The average reduction in growth over an untreated control	Compatibility status
1	< 20 % reduction in growth	Highly compatible
2	20 - 50 % reduction in growth	Compatible
3	50 - 80 % reduction in growth	Partially compatible
4	> 80 % reduction in growth	Incompatible

3. Results

Conidial germination

A significant conidial germination reduction of *B. bassiana* was seen in all tested insecticides doses and their impact was dependent on dose (Table 2). Highest germination percentage

was observed in acetamiprid, which indicated 84.76 per cent and it was significantly differed from other insecticides. Highest inhibition of spore germination was seen in diafenthiuron (72.87 %) at three different doses which was incompatible with *B. bassiana* (Table 5)

Table 2: Effect of selected chemical insecticides and their different doses on spore germination of *B. bassiana*

Sl. No.	Treatments	% Germination				% Germination inhibition over untreated check			
		RD**	HRD	2RD	Mean	RD	HRD	2RD	Mean
1	Imidacloprid 17.8 % SL	85.87	91.02	62.23	79.70	14.13	8.98	37.77	20.29
2	Thiamethoxam 25 % WG	86.12	93.74	68.08	82.64	13.88	6.26	31.92	17.35
3	Acetamiprid 20 % SP	88.25	95.63	70.42	84.76	11.75	4.37	29.58	15.23
4	Clothianidin 50 % WDG	72.34	80.74	65.61	72.89	27.66	19.26	34.39	27.10
5	Acephate 75 % SP	70.45	81.27	59.03	70.25	29.55	18.73	40.97	29.75
6	Fipronil 5 % SC	65.00	76.28	55.62	65.63	35.00	23.72	44.38	34.36
7	Diafenthiuron 50 % WP	27.11	34.17	20.09	27.12	72.89	65.83	79.91	72.87
8	Buprofezin 25 % SC	65.45	72.23	58.41	65.36	34.55	27.77	41.59	34.63
9	Fonicamid 50 % WG	76.37	83.54	64.23	74.71	23.63	16.46	35.77	25.28
10	Azadirachtin 0.15 % W/W	78.51	86.12	67.12	77.25	21.49	13.88	32.88	22.75
11	Untreated check	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00

**RD: Recommended dose, HRD: Half of the recommended dose, 2RD: 2 times of the recommended dose

Table 5: Effect of insecticides on germination and vegetative growth of *Beauveria bassiana*

Sl. No.	Treatments	Concentration	Germination		Radial growth in mm at 14 th DAI		Compatibility status
			Germination (%)	% reduction over control	Growth in mm	% reduction over control	
1	Imidacloprid 17.8 % SL	0.5 × RD	91.02	8.98	73.00	18.88	C
		RD	85.87	14.13	72.00	20.00	C
		2 × RD	62.23	37.77	63.60	29.33	C
2	Thiamethoxam 25 % WG	0.5 × RD	93.74	6.26	88.50	2.33	HC
		RD	86.12	13.88	87.90	1.60	HC
		2 × RD	68.08	31.92	78.30	13.00	C
3	Acetamiprid 20 % SP	0.5 × RD	95.63	4.37	90.00	0.00	HC
		RD	88.25	11.75	90.00	0.00	HC
		2 × RD	70.42	29.58	86.40	4.00	HC
4	Clothianidin 50 % WDG	0.5 × RD	80.74	19.26	83.50	7.20	HC
		RD	72.34	27.66	75.00	16.66	C
		2 × RD	65.61	34.39	74.50	17.22	C
5	Acephate 75 % SP	0.5 × RD	81.27	18.73	84.00	6.66	HC
		RD	70.45	29.55	75.60	16.00	C
		2 × RD	59.03	40.97	71.10	21.00	C
6	Fipronil 5 % SC	0.5 × RD	76.28	23.72	82.50	8.33	HC
		RD	65.00	35.00	72.60	19.33	C
		2 × RD	55.62	44.38	60.60	32.66	C
7	Diafenthiuron 50 % WP	0.5 × RD	34.17	65.83	17.50	80.55	IC
		RD	27.11	72.89	15.70	82.55	IC
		2 × RD	20.09	79.91	14.80	83.55	IC
8	Buprofezin 25 % SC	0.5 × RD	72.23	27.77	81.20	9.77	HC
		RD	65.45	34.55	57.90	35.66	C
		2 × RD	58.41	41.59	57.40	36.22	C
9	Fonicamid 50 % WG	0.5 × RD	83.54	16.46	87.90	2.33	HC
		RD	76.37	23.63	83.20	7.55	HC
		2 × RD	64.23	35.77	62.30	30.77	C
10	Azadirachtin 0.15 % W/W	0.5 × RD	86.12	13.88	66.40	26.22	C
		RD	78.51	21.49	64.70	28.11	C
		2 × RD	67.12	32.88	56.60	37.11	C
11	Untreated check	-	100.00	00	90.00	00	HC

RD: Recommended field dose, 0.5 × RD: Half of the field recommended dose, 2 × RD: Double the field recommended dose, C: Compatible, HC: Highly compatible, IC: Incompatible.

Growth performance of *B. bassiana* in PDA media treated with different insecticides at 14th and 30th day after inoculation

The data on influence of selected chemical insecticides at three different doses on radial growth of the *B. bassiana*

observed at 14th and 30th days after inoculation are presented in table. 3, figure 1 and figure 2. The results showed that increase in the concentration of the chemical insecticides decreased the spore germination and radial growth of the *B. bassiana*

Table 3: Growth performance of *B. bassiana* in PDA media treated with different Insecticides at 14th and 30th day after inoculation

Sl. No.	Treatments	Radial growth in mm at 14 th DAI				Radial growth in mm at 30 th DAI			
		RD**	HRD	2RD	Mean	RD	HRD	2RD	Mean
1	Imidacloprid 17.8 % SL	72.00 (58.05)*	73.00 (58.69)	63.60 (52.89)	69.53 (56.49)	76.10 (60.73)*	78.50 (62.37)	75.00 (60.00)	76.53 (61.02)
2	Thiamethoxam 25 % WG	87.90 (69.64)	88.50 (70.17)	78.30 (62.23)	84.90 (67.13)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)
3	Acetamiprid 20 % SP	90.00 (71.56)	90.00 (71.56)	86.40 (68.35)	88.80 (70.44)	90.00 (71.56)	90.00 (71.56)	89.60 (71.18)	89.86 (71.43)
4	Clothianidin 50 % WDG	75.00 (60.00)	83.50 (66.03)	74.50 (59.67)	77.66 (61.79)	79.90 (63.36)	84.70 (66.97)	77.90 (61.95)	80.83 (64.03)
5	Acephate 75 % SP	75.60 (60.39)	84.00 (66.42)	71.10 (57.48)	76.90 (61.27)	78.30 (62.23)	84.40 (66.73)	73.90 (59.27)	78.86 (62.26)
6	Fipronil 5 % SC	72.60 (58.43)	82.50 (65.27)	60.60 (51.11)	71.90 (57.98)	76.20 (60.80)	85.80 (67.86)	64.30 (53.30)	75.43 (60.28)
7	Diafenthiuron 50 % WP	15.70 (23.34)	17.50 (24.72)	14.80 (22.62)	16.00 (23.57)	19.80 (26.42)	28.20 (32.07)	17.70 (24.87)	21.90 (27.90)
8	Buprofezin 25 % SC	57.90 (49.54)	81.20 (64.30)	57.40 (49.25)	65.50 (54.02)	60.30 (50.94)	90.00 (71.56)	58.50 (49.89)	69.60 (56.53)
9	Flonicamid 50 % WG	83.20 (65.80)	87.90 (69.64)	62.30 (52.12)	77.80 (61.88)	87.90 (69.64)	88.60 (70.26)	73.30 (58.88)	83.26 (65.84)
10	Azadirachtin 0.15 % W/W	64.70 (53.54)	66.40 (54.57)	56.60 (48.79)	62.56 (52.27)	78.30 (62.23)	81.80 (64.74)	67.50 (55.24)	75.86 (60.57)
11	Untreated check	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)
	Mean	71.32 (57.62)	76.77 (61.19)	65.05 (53.76)	71.05 (57.44)	77.78 (61.87)	78.47 (62.35)	70.70 (57.23)	75.64 (60.43)
	Treatments (T)	S. E. ±		C.D at 1%		S. E. ±		C.D at 1%	
	Doses (D)	0.34		1.29		0.26		0.99	
	Interaction (T×D)	0.18		0.66		0.13		0.51	
		0.59		1.88		0.46		1.69	

DAI: day after inoculation, **RD: Recommended dose, HRD: Half of the recommended dose, 2RD: 2 times of the recommended dose *Figures in parenthesis are arcsine transformed values

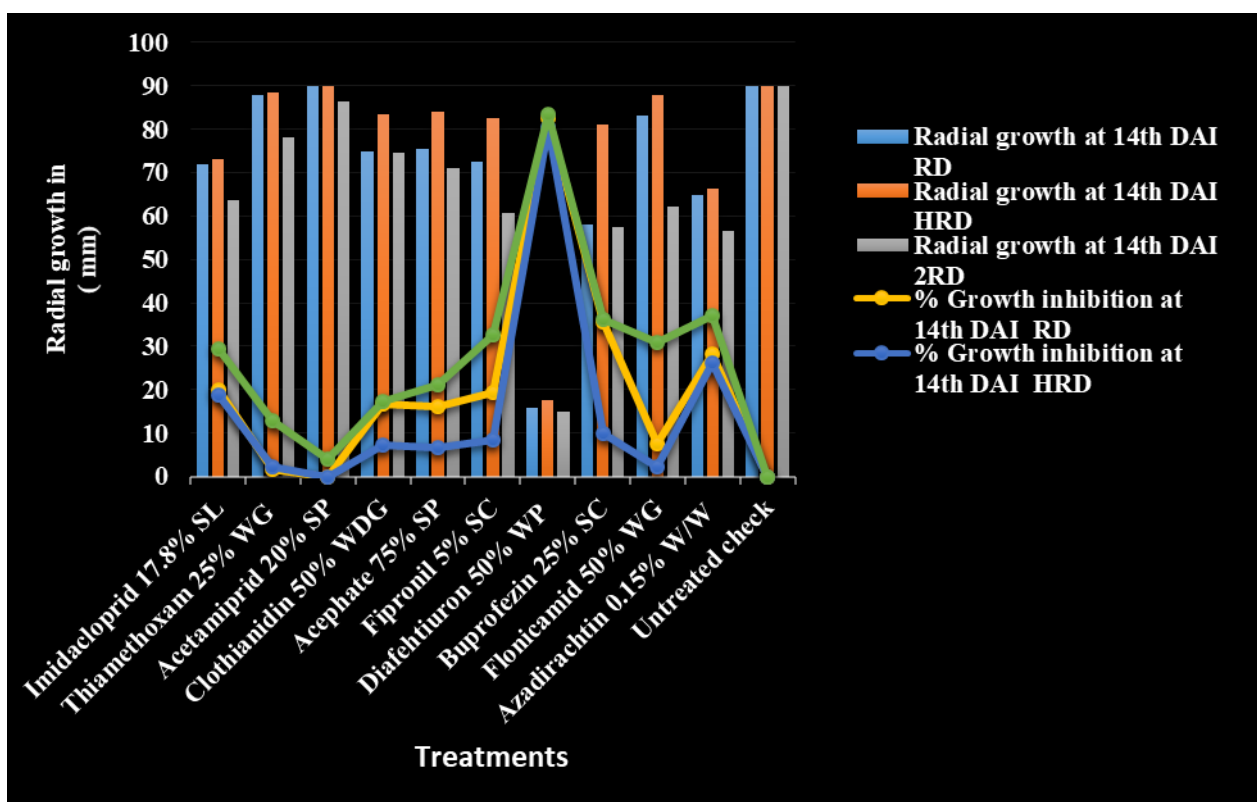


Fig 1: Effect of selected chemical insecticides and their different doses on radial growth and per cent inhibition of *B. bassiana* at 14th day after inoculation

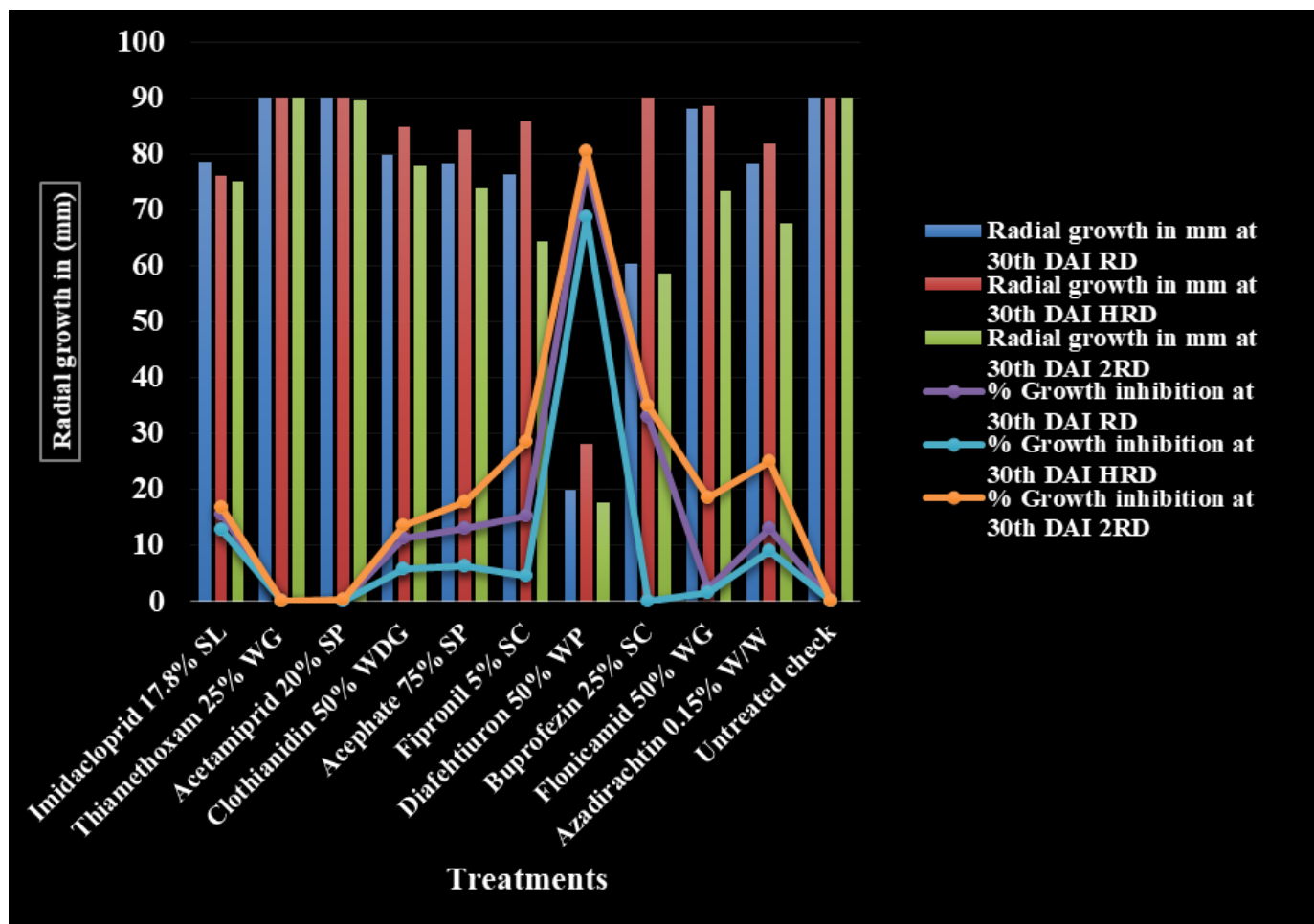


Fig 2: Effect of selected chemical insecticides and their different doses on radial growth and per cent inhibition of *B. bassiana* at 30th day after inoculation

At 14th day after inoculation, significantly highest radial growth was recorded in acetamiprid with 88.80 mm average radial growth over untreated check, at three doses the radial growth was RD (90 mm), 0.5 × RD (90 mm) and 2 × RD (86.40). The radial growth at RD and 0.5 × RD of acetamiprid was on par with untreated check which recorded 90.00 mm and it was followed by thiamethoxam which showed an average radial growth of 84.90 mm and the radial growth observed at RD, 0.5 × RD and 2 × RD were 88.50, 87.90 and 78.30 mm respectively. Average radial growth of 77.80 mm was recorded in flonicamid with 83.20, 87.90 and 62.30 mm radial growth observed at RD, 0.5 × RD and 2 × RD respectively. Clothianidin showed 77.66 mm average radial growth with 75.00, 83.50 and 74.50 mm radial growth at RD, 0.5 × RD and 2 × RD respectively. The next best chemical treatment was acephate with average radial growth of 76.90 mm with radial growth of 75.60, 84.00 and 71.10 mm respectively at RD, 0.5 × RD and 2 × RD. Average radial growth of 71.90 mm was recorded in fipronil with radial growth of 72.60, 82.50 and 60.60 mm at RD, 0.5 × RD and 2 × RD respectively. Imidacloprid showed average radial growth of 69.53 mm and 72.00, 73.00 and 63.60 mm radial

growth recorded at RD, 0.5 × RD and 2 × RD respectively. Azadirachtin recorded 64.70, 66.40 and 56.60 mm radial growth at RD, 0.5 × RD and 2 × RD respectively with an average radial growth 62.56 mm.

At 30th day after inoculation, thiamethoxam showed 90 mm significantly highest average radial growth and was on par with the untreated check (90 mm) with no per cent inhibition. The next best average radial growth was 89.86 mm observed in acetamiprid with 90, 90 and 89.60 mm radial growth recorded at RD, 0.5 × RD and 2 × RD respectively. RD, 0.5 × RD of acetamiprid on par with untreated check. Flonicamid showed 83.26 mm average radial growth followed by clothianidin showed 80.83 mm average radial growth. Acephate and imidacloprid recorded average radial growth with 78.86 mm and 76.53 mm respectively. 75.86, 75.43 and 69.60 mm of average radial growth was recorded in azadirachtin, fipronil and buprofezin respectively. The poorest growth observed in diafenthiuron with an average radial growth of 21.90 mm with average radial growth of 19.80, 28.20 and 17.70 mm recorded at RD, 0.5 × RD and 2 × RD respectively with maximum per cent inhibition 75.66 per cent (Table 4).

Table 4: Effect of selected chemical insecticides and their different doses on per cent growth inhibition of *B. bassiana* over untreated check at 14th and 30th DAI

Sl. No.	Treatments	% Growth inhibition over untreated check at 14 th DAI				% Growth inhibition over untreated check at 30 th DAI			
		RD*	HRD	2RD	Mean	RD	HRD	2RD	Mean
1	Imidacloprid 17.8 % SL	20.00	18.88	29.33	22.73	15.44	12.77	16.66	14.95
2	Thiamethoxam 25 % WG	1.60	2.33	13.00	5.64	0.00	0.00	0.00	0.00
3	Acetamiprid 20 % SP	0.00	0.00	4.00	1.33	0.00	0.00	0.40	0.13
4	Clothianidin 50 % WDG	16.66	7.20	17.22	13.69	11.22	5.88	13.44	10.18
5	Acephate 75 % SP	16.00	6.66	21.00	14.55	13.00	6.22	17.88	12.36
6	Fipronil 5 % SC	19.33	8.33	32.66	20.10	15.33	4.66	28.55	16.18
7	Diafenthiuron 50 % WP	82.55	80.55	83.55	82.21	78.00	68.66	80.33	75.66
8	Buprofezin 25 % SC	35.66	9.77	36.22	27.21	33.00	0.00	35.00	22.66
9	Flonicamid 50 % WG	7.55	2.33	30.77	13.55	2.33	1.50	18.55	7.46
10	Azadirachtin 0.15 % W/W	28.11	26.22	37.11	30.48	13.00	9.10	25.00	15.70
11	Untreated check	-	-	-	-	-	-	-	-

DAI: day after inoculation, **RD: Recommended dose, HRD: Half of the recommended dose, 2RD: 2 times of the recommended dose

4. Discussion

There are several reports on compatibility of neonicotinoid insecticides with *B. bassiana* strains, present results are in accordance with [7, 8] they opined that almost all the neonicotinoid insecticides viz., imidacloprid, thiamethoxam and acetamiprid were compatible with different strains of *B. bassiana*. In general, the reasons for this compatibility of *B. bassiana* with neonicotinoid insecticide may be due to

- ❖ Physiological mechanism of fungi to metabolize the neonicotinoides and these may liberate compounds that act as a secondary nutrient for entomopathogenic fungi.
- ❖ Apart from physiological mechanism, substances present in the insecticide formulations might have provided nutrients for the development of vegetative growth and conidial production.

The present results are contradicts with some of the authors [9] who pointed that imidacloprid, buprofezin and thiamethoxam had 89.13, 91.92 and 93.04 per cent inhibition of mycelial growth over an untreated check. The variations may be due to inherent variability of chemical insecticides to entomopathogens [10, 11] who disclose the in-consistent interaction between fungal pathogens and insecticides. Toxic nature of such chemical insecticide to fungal entomopathogens may vary with different species and strains of entomopathogens, chemical nature of the active ingredient, mode of action, product formulation and recommended label rate [12].

Present study revealed that acephate which belongs to organophosphate group is compatible with *B. bassiana* and results are in agreement with some findings [13] who studied *in vitro* compatibility of different isolates of *B. bassiana* with organophosphate compounds (acephate, triazophos, dimethioate and phosalone) were compatible with different isolates of *B. bassiana*. Difference in the response of the different isolates to a particular chemical insecticide stipulate differential tolerance limit of entomopathogenic fungal isolates.

The compatibility of *B. bassiana* with azadirachtin was in confirmaty with many authors [7] and stated that the compatibility may be due to the chemical nature of azadirachtin and other active ingredients present in the formulations. reported that 1.0 % neem (Azadirachtin 0.3 % EC) was amended with *B. bassiana* (isolate Bb 62), the reason may be due to the neem oil having below 5 % emulsible concentration did not cause significant fungal toxicity effects and the enhancing effect of some pesticide formulation on

fungal growth is due to the presence of adjuvants in the insecticides formulations. Adjuvants act as mild abrasives and break up conidial agglomerations, which increase the number of propagules, thereby promoting better vegetative growth [14].

5. Conclusion

B. bassiana isolate Bb-5a proved to be more promising for development as fungal pesticides and for application along with insecticides like imidacloprid, thiamethoxam, acetamiprid, clothianidin, acephate, fipronil, buprofezin, flonicamid and azadirachtin in integrated pest management Programme. However, field investigation of the interactions between *B. bassiana* and insecticides should be under taken to examine their impact on pests and beneficial insects.

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