Journal of Biological Control, 36(2&3): 143-150, 2022, DOI: 10.18311/jbc/2022/21568

Research Article

Laboratory assessment on compatibility of entomopathogenic fungus, *Beauveria bassiana* Balsamo-vuillemin with imidacloprid 48% FS for bhendi (*Abelmoschus esculentus* L. moench) seed treatment

T. GAVYA[1*], K. PREMALATHA[1], C. CHINNAIAH[1] and N. REVATHY[2]

[1]*Department of Entomology, Agricultural College and Research Institute, Madurai – 625104, Tamil Nadu, India*

[2]*Department of Plant Pathology, Agricultural College and Research Institute, Madurai – 625104, Tamil Nadu, India*

**Corresponding author E-mail:* gavya95@gmail.com

ABSTRACT: *Beauveria bassiana* (Balsamo-Vuillemin), an effective entomopathogenic fungi is well positioned in the biological control of insect pests for more than ten decades around the world. Its potential can be attributed to the fungus's entry through several parts of the insect and its mode of action. But sometimes a virulent strain of *B. bassiana* may become ineffective because of xenobiotics and environmental factors. To enhance the efficacy of *B. bassiana,* which is necessary for placing it in Integrated Pest Management, the Colony Forming Unit (CFU) of *B. bassiana* should be compatible with xenobiotics used in crop production. The compatible concentrations of imidacloprid 48% FS (500 ppm) for *B. bassiana* was studied in the laboratory condition and results revealed low per cent growth inhibition (21.25%) and maximum radial growth (1.46 cm) at 15 Days After Inoculation (DAI). The inhibition of colony growth was reduced by treating the bhendi seeds with *B. bassiana* and imidacloprid 48 % FS at a time interval of four hours which showed high mean colony growth (51.12).

KEYWORDS: *Beauveria bassiana,* compatibility, imidacloprid 48% FS, seed treatment

(Article chronicle: Received: 19-12-2021; Revised: 14-07-2022; Accepted: 22-07-2022)

INTRODUCTION

Among the vegetable crops grown in India, bhendi, also known as lady's finger or okra (*Abelmoschus esculentus* L. Moench) belonging to the family Malvaceae and originated from South Africa is an important crop grown throughout the year. Bhendi fruits are used in culinary preparation as a vegetable and thickening agent for culinary gravies and soup making. In India, bhendi is grown in 5.07 lakh ha with the production and productivity of 58.53 lakh tones and 11.50 tonnes/ha, respectively (Welfare, 2016). Insect pests are one of the major limiting factors in bhendi production. As high as 72 species of insect pests have been recorded on bhendi (Srinivasa and Rajendran, 2003) among which the sucking pests comprises aphids (*Aphis gossypii* Glover), leafhopper (*Amrasca biguttula biguttula* Ishida), Whitefly (*Bemisia tabaci* Gennadius) and mite (*Tetranychus cinnabarinus* Boisduval) causes significant damage to the crop. To tackle this sucking pest menace, plenty of chemical insecticides are sprayed which leads to several problems like toxic residues, elimination of natural enemies, environmental disharmony and development of resistance. The biomagnification of pesticidal residues in the processed product may lead to the rejection of whole consignments during export. Fresh vegetables of bhendi are harvested at regular intervals for consumption is also another concern of residual effect.

Not only the newer insecticidal molecules, but it is also essential to have joint action of microbial pesticides (entomopathogens) and alternative application methods *viz.,* seed treatment which does not leave any pesticidal residue. Among the entomopathogens, *Beauveria bassiana* (Balsamo-Vuillemin) (Ascomycota: Hypocreales) is a significant natural pathogen of insects and can infect more than 700 species of arthropod species (Bermúdez *et al.*, 2011). It was found that there were excellent toxic effects of *B. bassiana* in combination with insecticides against different insect pests (Azevedo *et al.*, 2000). The integrated pest management programme may be bio-intensified by utilizing effective and compatible isolates of *B. bassiana* along with pesticides *viz.,* imidacloprid. Hence, the present study was carried out to study the compatibility of *B. bassiana* and imidacloprid.

MATERIALS AND METHODS

Source of fungus culture

The entomopathogen, *B. bassiana* culture from the Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore was used in this study. The mother culture was subcultured in Potato Dextrose Agar (PDA) medium (Moorhouse *et al.,* 1992) autoclaved at 121^{0}C (15 Psi) for 15-20 minutes and poured into sterilized Petri plates. The fungal discs were cored from 10 days old culture and used for further study.

Growth inhibition test

In vitro studies were undertaken to assess the compatibility of *B. bassiana* with seed treatment chemical imidacloprid 48% FS at different concentrations (500,640,800,960 and 1000 ppm) (Figure 1) by adopting the poisoned food technique (Moorhouse *et al*., 1992). The insecticide at the required concentrations was added to the Potato Dextrose Agar (PDA) medium and poured into Petri dishes after proper agitation and allowed to solidify. The ten-day old culture of *B. bassiana* was cut into 5mm discs with the help of a sterilized cork borer and was transferred to the centre of each plate containing the poisoned medium. The untreated check was maintained by placing the fungal disc in a medium without poison and the standard check was

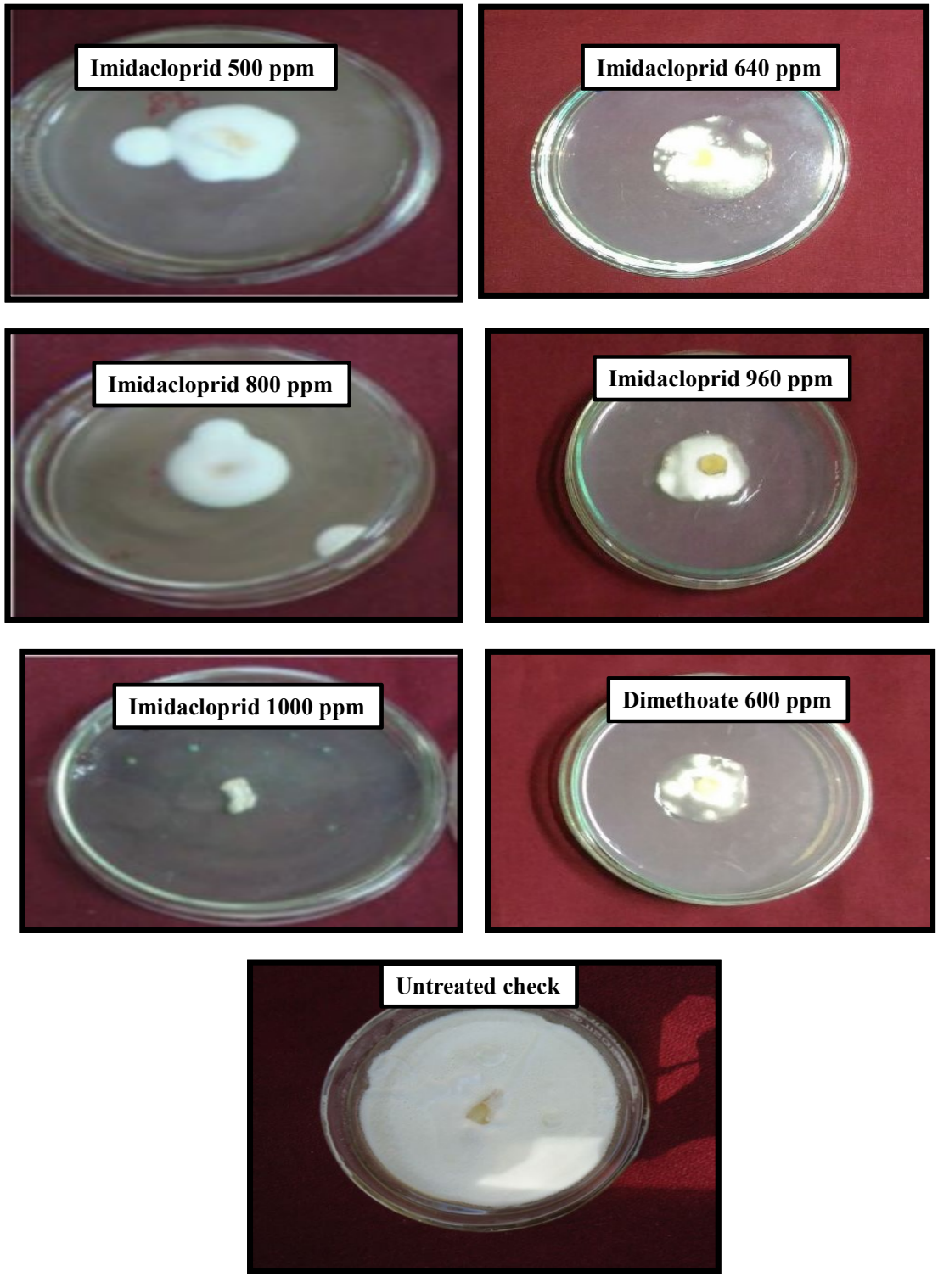

Figure 1. Studies on *in vitro* compatibility of different concentrations of imidacloprid 48% FS with *Beauveria bassiana* (Radial growth).

maintained by adding 600 ppm of Dimethoate 30% EC. Plates were allowed for 15 days to get maximum fungal growth. Each treatment was replicated four times. The observation diameter of the *B. bassiana* growth was measured at 3 days intervals of up to 15 days.

Two perpendicular straight lines were drawn on the bottom of each Petri dish. The crossing point coincided with the centre of the 5 mm initial fungi disc. Radial growth measurements were recorded at 3 days intervals from the edge of the initial inoculum until the extreme area of fungi mycelia development in all the four segments formed by the two perpendicular lines.

The data were expressed as percentage growth inhibition of *B. bassiana* by insecticide-treated PDA (Hokkanen and Kotiluoto, 1992).

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

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.

Post treatment test

Ten grams of commercial formulation of entomopathogen *B. bassiana* was added to 1 kg of seeds with adhesion agent teepol. After that one ml of 500 ppm of imidacloprid 48% FS was added in different time intervals *viz.,* 0 hr, 1hr, 2hr, 3hr and 4hr. The treated seeds were kept overnight. Two grams of treated seeds were placed into a PDA medium to assess the colonization potential of *B. bassiana* in combination with seed dressing chemical imidacloprid. The number of colonies was counted from each petri plates for up to 15 days. Each treatment was replicated 5 times. Seeds which were treated without imidacloprid was served as untreated check. The number of colonies was counted by using the colony counter.

Statistical analysis

Statistical analysis for lab studies was carried out by analyzing the available data in different experiments under a Completely Randomized Design (CRD). The data were subjected to square root and angular (Arcsin) transformation before analysis and the means were separated by DMRT (Gomez and Gomez, 1978).

RESULTS AND DISCUSSION

Studies on *in vitro* compatibility of *Beauveria bassiana* and Imidacloprid 48 % FS (Growth Inhibition)

Among the different concentrations, the lowest radial growth was observed in 1000 ppm of imidacloprid 48 % FS *ie* 0.16 cm at 3 Days After Inoculation (DAI) and 0.88 cm at 15 DAI. Whereas in the concentrations 960 ppm, 800 ppm and 640 ppm, the radial growth ranged from 0.19 cm to 1.17 cm, 0.22cm to 1.20 cm and 0.29 cm to 1.39 cm, respectively. The maximum radial growth was observed in 500 ppm of imidacloprid 48 % FS with 0.37 cm at 3 DAI and 1.46 cm at 15 DAI. In standard check, Dimethoate 30% EC at 600 ppm, the radial growth was neither maximum nor minimum which recorded 0.21 cm to 1.19 cm radial growth from 3 DAI to 15 DAI, respectively. In the untreated check, the recorded growth of *B. bassiana* was observed with 1.67 cm radial growth on 15 DAI (Table 1).

Table 1. Studies on *in vitro* compatibility of *Beauveria bassiana* and imidacloprid 48% FS

S.No	Treatment	Radial Growth (RG) and Per cent Growth Inhibition (% GI)											
		3 DAI		6 DAI		9 DAI		12 DAI		15 DAI		Mean	
		RG	% GI	RG	% GI	RG	% GI	RG	% GI	RG	% GI	RG	% GI
1.	T1 Imidacloprid 48 % FS 500 ppm	0.37 (3.48)^{b}	28.84 (32.48)^{a}	0.52 (4.13)^{b}	27.7 (31.75)^{a}	0.87 (5.35)^{b}	20.18 (26.69)^{a}	1.22 (6.34)^{a}	17.00 (24.35)^{a}	1.46 (6.94)^{a}	12.57 (20.76)^{a}	0.88 (5.24)^{a}	21.25 (27.20)^{a}
2.	T2 Imidacloprid 48 % FS 640 ppm	0.29 (3.08)^{bc}	44.23 (41.68)^{b}	0.42 (3.71)^{bc}	41.66 (40.20)^{b}	0.75 (4.96)^{b}	31.19 (33.95)^{b}	1.14 (6.12)^{b}	22.44 (28.27)^{b}	1.39 (6.77)^{b}	16.76 (24.16)^{b}	0.79 (4.92)^{a}	31.25 (33.65)^{b}
3.	T3 Imidacloprid 48 % FS 800 ppm	0.22 (2.68)^{bc}	57.69 (49.42)^{c}	0.39 (3.58)^{c}	45.83 (42.60)^{d}	0.057 (4.32)^{c}	47.7 (43.68)^{d}	1.01 (5.76)^{b}	31.29 (34.01)^{c}	1.20 (6.28)^{b}	28.14 (32.03)^{d}	0.67 (4.52)^{a}	42.13 (40.34)^{c}
4.	T4 Imidacloprid 48 % FS 960 ppm	0.19 (2.49)^{c}	63.46 (52.80)^{e}	0.30 (3.13)^{cd}	58.33 (49.79)^{e}	0.48 (3.97)^{c}	55.96 (48.42)^{e}	0.69 (4.76)^{c}	53.06 (46.75)^{e}	1.17 (6.20)^{b}	29.94 (33.17)^{e}	0.56 (4.11)^{a}	52.15 (46.18)^{e}
5.	T5 Imidacloprid 48 % FS 1000 ppm	0.16 (2.29)^{c}	69.23 (56.31)^{f}	0.25 (2.86)^{d}	62.66 (52.33)^{f}	0.43 (3.75)^{d}	60.55 (51.09)^{f}	0.65 (4.62)^{c}	55.78 (48.32)^{f}	0.88 (5.38)^{c}	47.3 (43.45)^{f}	0.47 (3.78)^{b}	59.10 (50.3)^{f}

Table 1. to be continued...

S.No	Treatment	Radial Growth (RG) and Per cent Growth Inhibition (% GI)											
		3 DAI		6 DAI		9 DAI		12 DAI		15 DAI		Mean	
		RG	% GI	RG	% GI	RG	% GI	RG	% GI	RG	% GI	RG	% GI
6.	T6 Dimethoate 30% EC 600 ppm	0.21 (2.62)^{b}	59.61 (50.54)^{d}	0.40 (3.62)^{b}	44.44 (41.80)^{c}	0.59 (4.40)^{c}	45.87 (42.63)^{c}	0.82 (5.19)^{c}	44.21 (41.67)^{c}	1.19 (6.26)^{b}	28.78 (32.44)^{c}	0.64 (4.41)^{a}	44.58 (41.81)^{d}
7.	T7 Untreated check	0.52 (4.13)^{a}	-	0.72 (4.86)^{a}	-	1.09 (5.99)^{a}	-	1.47 (6.96)^{a}	-	1.67 (7.42)^{a}	-	1.09 (5.87)^{a}	-
	SEd	0.257	6.12	0.264	5.93	0.256	4.84	0.399	5.02	0.282	3.66	0.904	4.12
	CD(0.05)	0.535	12.86	0.549	12.46	0.532	10.16	0.831	10.56	0.586	7.70	1.853	8.51

DAI = Days after inoculation.
*Each value is the mean of four replications
Figures in parenthesis are arc sine transformed values.
In a column, values denoted by common alphabets is/ are not significantly different by DMRT at P= 0.05%

The per cent growth inhibition derived from radial growth measurements was presented in Table 1. The maximum per cent growth inhibition was recorded at 1000 ppm of imidacloprid 48 % FS (47.3 on 15 DAI) followed by 960 ppm of imidacloprid 48 % FS (29.94 on 15 DAI). The concentration of 800 ppm of imidacloprid 48 % FS and Dimethoate 30% EC at 600 ppm were more or less on par with each other with per cent inhibition of 28.14 and 28.78 on 15 DAI respectively. The mean per cent growth inhibition showed that imidacloprid 48% FS at 500 ppm was found to be safe for *B. bassiana* with 21.25 per cent of growth inhibition. These results are in accordance with James and Elzen (2001) and Alizadeh *et al*. (2007) who reported that imidacloprid had no negative effect on *B. bassiana.* While comparing the toxicity of imidacloprid against *B. bassiana* at 3 days interval up to 15 DAI, the toxicity of different concentrations of imidacloprid was reduced at 15 DAI. 500, 640 and 800 ppm concentrations of imidacloprid were less harmful during 15 DAI. 960 and 1000 ppm concentrations of imidacloprid were relatively harmful to *B. bassiana* during 15 DAI. Any epizootics begins with germination and sporulation which is an essential parameter for pesticidal compatibility. The present study resulted in an insignificant effect of imidacloprid on *B. bassiana* which has been already reported by James and Elzen (2001). The positive interaction of imidacloprid and entomopathogenic fungi was reported by Furlong *et al.* (2001), Kaakeh *et al.* (1997), Lacey *et al.* (2001), Quintela and Mccoy (1997) Feng *et al.* (2004) and Usha *et al.* (2014). The growth of *B. bassiana* was affected by only the higher concentration of imidacloprid and it was concluded that the active ingredients of the insecticide, concentration used and nature of fungal isolates play a major role in the compatibility of insecticides and entomopathogenic fungus.

Generally, wettable powders and flowable formulations caused no inhibition and often increase colony counts (Anderson *et al.*, 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of conidia which would improve the field performance of *B. bassiana.* Although the different concentrations tested in the present study inhibited the growth of *B. bassiana* in the poisoned medium *in vitro,* the combined use of the fungus and insecticide cannot be completely ruled out. But imidacloprid may be combined at sub-lethal doses with *B. bassiana* for obtaining better control of the pest species. For instance, increased mortality due to mycosis of *Beauveria* by the addition of reduced doses of insecticides had been established in *Melolontha melolontha* L. (Ferron, 1971).

Studies on *in vitro* compatibility of Imidacloprid 48% FS with *Beauveria bassiana* at different time interval of seed treatment

There was a significant difference between post-treatment hours of *B. bassiana* with imidacloprid 48 % FS. The colony growth of *B. bassiana* observed on 1 hr post-treatment was 31.8 on 15 DAI. Similarly, the post-treatment at 2hr and 3hr recorded 34.2 and 37.6 colony numbers on 15 DAI. The maximum mean colony growth was recorded in 4 hr post-treatment (51.12 numbers) (Table 2; Figure 2).

The contemporaneous treatment of *B. bassiana* and imidacloprid 48 % FS (0 hr interval) showed minimum colony growth ranging from 24 to 30 numbers at 3 DAI to 15 DAI. But the colony growth was 48.2 number at four-hour interval seed treatment on 3 DAI. The chemical pesticides have the ability to inhibit the growth of CFU in *B. bassiana* colonies. But the post-treatment hours allow the colony growth which could not be disrupted by insecticide treatment. This was in accordance with Faraji *et al.* (2016) who reported that the effect of many insecticides on fungal growth declined gradually over time. In all the post-treatment hours, there was colony growth of *B. bassiana*. The fungi toxic effects of insecticide may vary with the different stages of fungus. The time interval may facilitate the fungi to enter the seed.

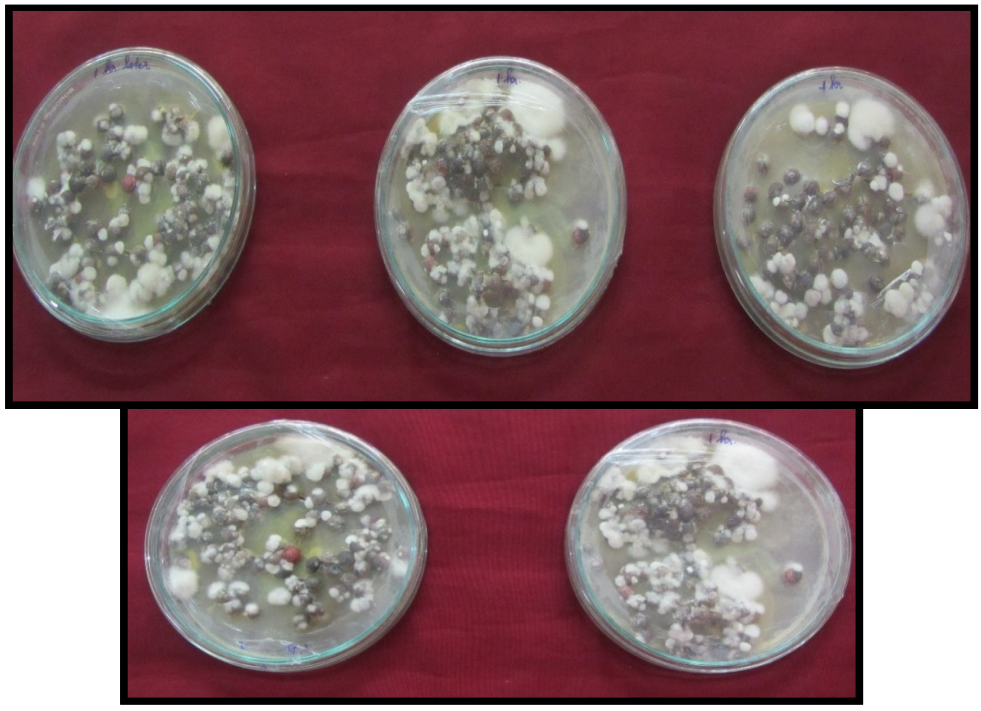

Figure 2a. Colony growth on treated seeds after 0 hour.

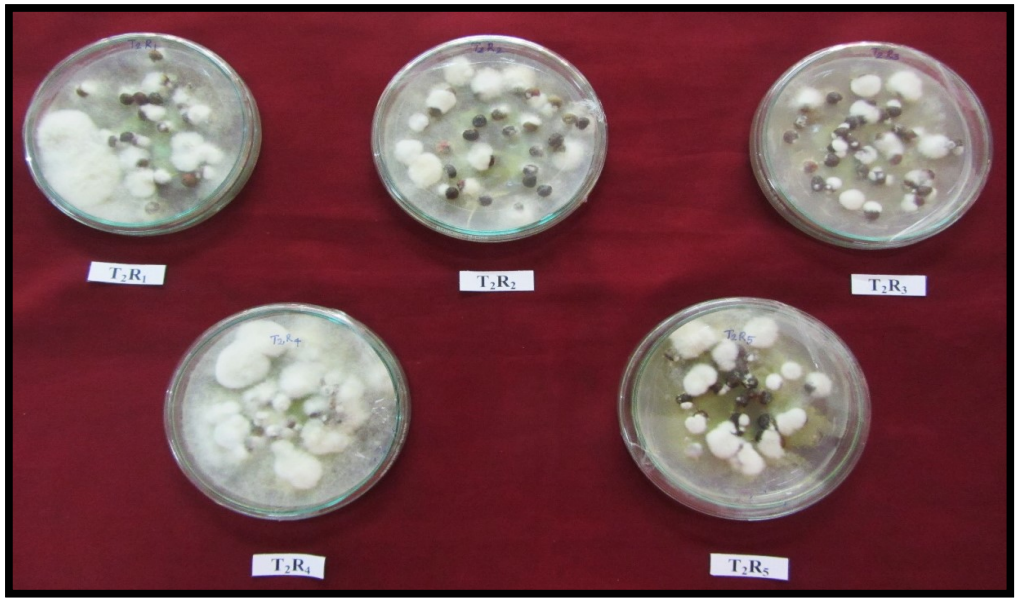

Figure 2b. Colony growth on treated seeds after 1 hour.

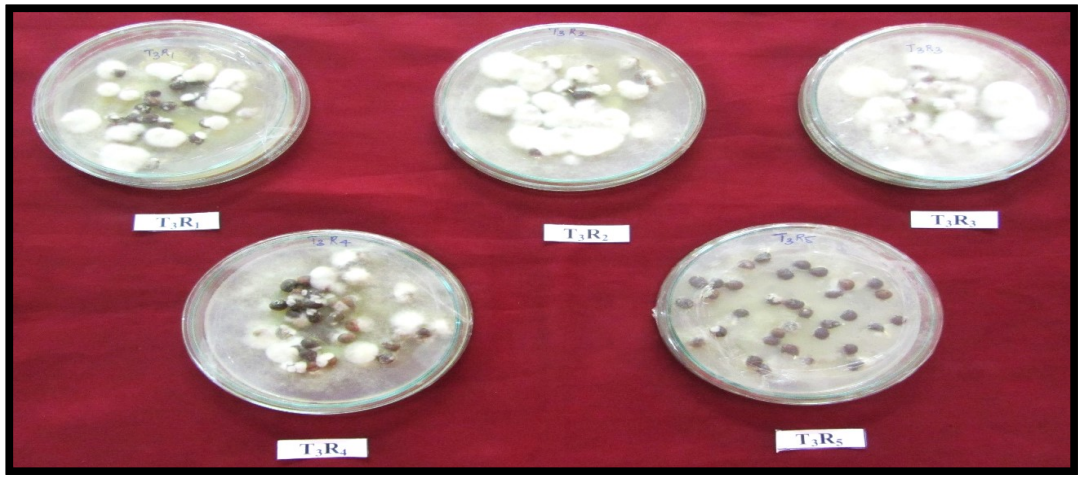

Figure 2c. Colony growth on treated seeds after 2 hours.

Figure 2d. Colony growth on treated seeds after 3 hours.

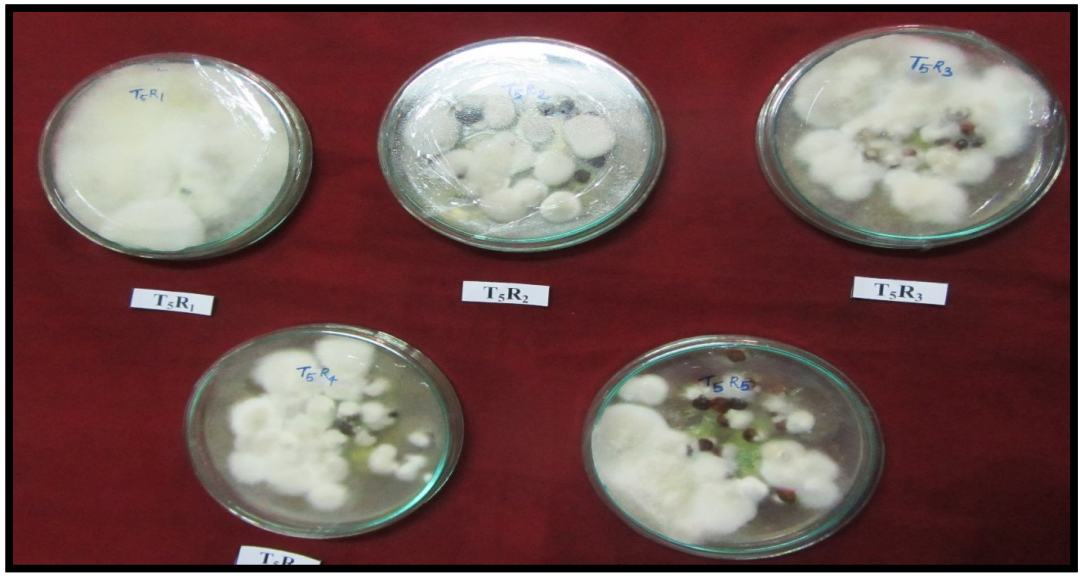

Figure 2e. Colony growth on treated seeds after 4 hours.

Figure 2. Studies on *in vitro* compatibility of imidacloprid 48% FS with *Beauveria bassiana* at different time interval of treatment (Colony growth).

Table 2. Studies on *in vitro* compatibility of *Beauveria bassiana* and imidacloprid 48% FS at different post treatment hours

S.No	Treatment	Number of colonies*					Mean number of colonies
		3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	
1.	T1 *Beauveria bassiana* 0 h + Imidacloprid 48 % FS 500 ppm at 0h	24 (29.33)[a]	26.2 (30.78)[a]	27.4 (31.56)[a]	28.4 (32.20)[a]	30 (33.21)[a]	27.2 (31.41)[a]
2.	T2 *Beauveria bassiana* 0 h + Imidacloprid 48 % FS 500 ppm at 1h	26 (30.65)[b]	27.8 (31.82)[b]	28.8 (32.45)[b]	30 (33.21)[b]	31.8 (34.32)[b]	28.88 (32.49)[b]
3.	T3 *Beauveria bassiana* 0 h + Imidacloprid 48 % FS 500 ppm at 2h	28.2 (32.07)[c]	29.8 (33.08)[c]	31.8 (34.32)[c]	32.2 (34.57)[c]	34.2 (35.79)[c]	31.24 (33.96)[c]
4.	T4 *Beauveria bassiana* 0 h + Imidacloprid 48 % FS 500 ppm at 3h	30 (33.21)[d]	32.4 (34.69)[d]	34 (35.66)[d]	36 (36.87)[d]	37.6 (37.82)[d]	34 (35.65)[d]
5.	T5 *Beauveria bassiana* 0 h + Imidacloprid 48 % FS 500 ppm at 4h	48.2 (43.96)[e]	49.8 (44.88)[e]	51.6 (45.91)[e]	52.8 (46.60)[e]	53.2 (46.83)[e]	51.12 (45.63)[e]
6.	Untreated check (*B. bassiana* alone)	79.8 (63.29)[f]	82 (64.89)[f]	83.2 (65.80)[f]	85 (67.21)[f]	86.8 (68.69)[f]	83.36 (65.97)[f]
	SEd	3.24	3.58	3.61	3.65	4.02	1.00
	CD(0.05)	6.69	7.38	7.46	7.53	8.31	2.07

The xenobiotics effect on *B. bassiana* also depends on the isolates and virulence of *B. bassiana.* This is also in tune with Bhattacharya *et al.* (2004). The minimum growth inhibition of *B. bassiana* at low concentrations of imidacloprid and maximum colony growth of *B. bassiana* at extended post-treatment hours may be due to the intermittent efficacy of toxic medium or xenobiotics on *B. bassiana* at both reproductive and vegetative growth.

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