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B Nandini
Department of Entomology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

C Srinivas
Department of Entomology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

Correspondence
B Nandini
Department of Entomology, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana, India

Compatibility studies among different microbial pesticides commonly used for foliar application

B Nandini and C Srinivas

Abstract
The present investigation was carried out on compatibility between different microbial pesticides through Dual culture technique and Pot culture assays. In dual culture, the percent inhibition of radial growth of *B. bassiana* in treatment *B. bassiana* + *P. florescens* and *B. bassiana* + *L. lecanii* was found to be the 30.41 and 27.34 percent respectively, whereas, the percent inhibition of radial growth of *L. lecanii* in treatment *L. lecanii* + *P. florescens* was found to be 20.29 percent after 9 days after inoculation. In pot culture assay, *B. bassiana* in combination with *L. lecanii* was found to be most effective against *Spodoptera litura,* which recorded siginificantly lowest foliar damage of 14.43 percent compared to using alone *B. bassiana* with 15.06 percent foliar damage.

Keywords: Compatibility, microbial pesticides, *Spodoptera litura*

Introduction
Plant pests and diseases have a serious effect on food production. Global crop yields are reduced by 20-40 percent annually due to pests and diseases (FAO, 2012) [2]. Use of chemical pesticides and fertilizers have caused negative impact on the environment by affecting soil fertility, water hardness, development of insect resistance, genetic variation in plants, increase in toxic residue through the food chain and animal feed thus increasing health problems and much more. Therefore, an ecofriendly alternative is the need of the hour. Biopesticides or biological pesticides based on pathogenic microorganisms specific to a target pest offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health (Suman and Dikshit, 2010) [9].
Among the available methods of biological control, the pest management through microbial biopesticides started gaining momentum. Microbial pesticides are also known as Biological Control Agents. In this category, the active ingredient is a microorganism that either occurs naturally or is genetically engineered. The pesticidal action may be from the organism itself or from a substance it produces. They offer the advantages of higher selectivity and less or no toxicity in comparison to conventional chemical pesticides (MacGregor, 2006) [3].
Due to the popularization and increased usage of microbial pesticides across the crops for pest and disease management the issues pertaining to their compatibility with each other gains importance. The generation of information in this regard is the need of the hour. This type of situation makes it imperative to workout compatibility among the commonly recommended and used biopesticides.

Materials and Methods
The present investigations on "Compatibility and Virulence studies of Microbial pesticides commonly used in Telangana" were carried out at AICRP on Biological Control, Agricultural Research Institute, Rajendranagar, and Hyderabad during 2017-18. The materials used and the methods employed in these investigations are furnished here under. Completely Randomized Design (CRD) was followed for analyzing the data in different experiments. The data was subjected to angular transformation as per the requirement to improve homogeneity of error variances (Gomez, 1984) [1].
The microbial biopesticides commonly used for foliar applications viz., *Beauveria bassiana, Lecanicillium lecanii* and *Pseudomonas florescens* were taken up for the studies for compatibility among them. Compatibility were studied under laboratory conditions through dual culture assays. Colonization ability of the same were worked out under net house conditions through pot culture studies.

Dual culture Assay
Microbial Pesticides are eco-friendly in nature. However, there is a need to assess compatibility between different microbial pesticides that are being commonly used during recent times due to chances of synergism or antagonism or mutualism between organisms or their metabolites. Compatibility studies among microbial pesticides were done as per treatments:
T_1 = *Beauveria bassiana + Lecanicillium lecanii*
T_2 = *Beauveria bassiana + Pseudomonas florescnes*
T_3 = *Beauveria bassiana*
T_4 = *Lecanicillium lecanii + Pseudomonas florescnes*
T_5 = *Lecanicillium lecanii*

The test cultures were taken from AICRP on Biological Control, Agricultural Research Institute, Rajendranagar, Hyderabad and isolated, maintained in respective media.
Required amount of PDA media were weighed and dissolved in 100 ml of distilled water by thoroughly mixing using vertex mixer. After cotton plugging, wrapped with the paper, this media was kept in an autoclave at 121 degrees with 15 lbs pressure for 15 to 20 minutes. The media was allowed to cool to a tolerable temperature for handling after sterilization. The media was poured into Petriplates and allowed it for solidification. After solidification, test culture disc of 5 mm were placed on the peripheral side of 9 cm Petriplate with the PDA medium. Another test culture was placed on the opposite side of the 1st test culture by using sterile cork borer. In case of bacteria, streak opposite side with a sterile inoculation loop. Control plate was also maintained for comparison purposes. The plates were kept for incubation at 30 ˚C temperature for 3 to 5 days.
After the incubation period, the radial growth of each test organism was measured using a measuring scale at 5, 7, 9 days after inoculation and also inhibition zone were also worked out (Siddiqui and Shaukat, 2003) [6].
The compatibility was calculated by using following formula.

$$\% \text{ of Inhibition} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

Pot culture Assay
Plastic pots (1' dia) were filled with approximately 2.5 Kg sterile soil. Cotton seeds were sown in each pot and three replicates were maintained for each treatment. All the agronomic practices were followed for raising healthy plants. The experiment was conducted under net house conditions (Plate 2). Compatibility studies among microbial pesticides were done after 45 DAS (Days After Sowing) with following microbial pesticides as treatments:
T_1 = *Beauveria bassiana*
T_2 = *Lecanicillium lecanii*
T_3 = *Pseudomonas florescnes*
T_4 = *Beauveria bassiana + Lecanicillium lecanii*
T_5 = *Beauveria bassiana + Pseudomonas florescnes*
T_6 = *Lecanicillium lecani + Pseudomonas florescnes*
T_7 = *Beauveria bassiana + Lecanicillium lecanii +Pseudomonas florescnes*
T_8 = Control

The amount of 5 g of powder formulations of microbial pesticides were individually weighed and dissolved in 1000 ml of water by thoroughly mixing.
At 45 DAS (Days After Sowing), each potted plant were sprayed with equal load of microbial pesticides (@ 5 gL^{-1}) using a hand automizer as per the treatments. Control plants were maintained and sprayed with water. Potted plants were sprayed with water using hand automizer were maintained as a control. After administration of treatments, each plant were artificially infested with five third instar *S. litura* larvae. Observations on number of damaged leaves and area of damage was recorded at 7 DAT (Days After Treatment). The data were obtained and analysed statistically.

Results and Discussion
Dual culture Assay
Results (Table 1, Figure 1, Plate 1) on the radial growth of *B. bassiana* on PDA media with dual culture technique in treatment *B. bassiana + P. florescens* revealed that the culture plate recorded radial growth of 1.82 cm with 39.26 percent inhibition at 5 days after inoculation as against 3.00 cm in control, 2.29 cm with 35.83 percent inhibition at 7 days after inoculation as against 3.50 cm in control, 2.99 cm with 30.41 percent inhibition at 9 days after inoculation as against 4.30 cm in control.
The radial growth (Table 1, Figure 2, Plate 1) of *B. bassiana* on PDA media with dual culture technique in treatment *B. bassiana + L. lecanii* revealed that the culture plate recorded radial growth of 1.75 cm with 41.53 percent inhibition at 5 days after inoculation as against 3.00 cm in control, 2.43 cm with 30.39 percent inhibition at 7 days after inoculation as against 3.50 cm in control, 3.12 cm with 27.34 percent inhibition at 9 days after inoculation as against 4.30 cm in control.
The radial growth (Table 1, Figure 3, Plate 1) of *L. lecanii* on PDA media with dual culture technique in treatment *L. lecanii + P. florescens* revealed that culture plate recorded radial growth of 3.61 cm with 14.74 percent inhibition at 5 days after inoculation as against 4.23 cm in control, 4.62 cm with 20.68 percent inhibition at 7 days after inoculation as against 5.83 cm in control, 5.30 cm with 20.29 percent inhibition at 9 days after inoculation as against 6.66 cm in control.
The above results were in concurrence with findings of Sumalatha *et al*. (2017) [8] who reported effect of four Bio pesticides *viz*., *Beauveria bassiana, Metarhizium anisopliae*, *Pseudomonas florescens and Trichoderma viride* on the radial growth, conidial concentration and conidial viability of *V. lecanii.* The percent inhibition of radial growth over control was the lowest in *B. bassiana* (29.64 percent) followed by *M. anisopliae* (34.75 percent), *P. florescens* (36.28 percent) and *T. viride* (47.91 percent), respectively and all the above biopesticides were significantly different from each other. *B. bassiana* was found compatible with *V. lecanii* in laboratory condition.

Pot Culture Assay
In the present investigation, an attempt was made to study efficacy of microbial pesticides alone and in combinations at recommended doses by spraying on potted cotton plants against *S. litura*. Results presented in the table 2, Figure 4 revealed that all treatments were found significantly superior over control in reducing the foliar damage. Seven days after spraying, *B. bassiana + L. lecanii* has recorded siginificantly lowest foliar damage of 14.43 percent with an area of 37.11 mm^2 which was on par with *B. bassiana* recorded 15.06 percent foliar damage (96.92 mm^2).
The next best treatments in order of their efficacy was of *P. flourescnes* recorded 21.16 percent foliar damage (101.13 mm^2) and was on par with the other treatments like *B. bassiana + P. flourescnes* (102.33 mm^2), *L. lecanii + P. flourescnes* (129.40 mm^2) and *L. lecanii* (136.00 mm^2) which

recorded 23.13, 26.30, 26.96 percent foliar damage, respectively. The highest foliar damage of 36.86 percent was observed in *B. bassiana* + *L. lecanii* + *P. flourescnes* treatment (182.55 mm^2) which is statistically inferior than all other treatments.

The results obtained were in accordance with Suklova *et al*. (1989) [7] who reported that the combination of *V. lecanii* + Boverin (*Beauveria bassiana*) gave 98 percent control of whitefly compared to using alone. Sabbour and Sahab (2005) [5] suggested that *B. bassiana* (Balsamo) was highly effective against *P. xylostella* and *S. exigua* in the field and greenhouse, percentage of infestation was reduced up to 20- 21 percent after 90 days of treatment. Malekan *et al*. (2013) [4] tested the efficacy of entomopathogenic fungi against *Trialeurodes vaporariorum* and the beneficial effect was observed in using the two fungi *Beauveria bassiana* and *Lecanicillium muscarium* together compared to the separate application of *B. bassiana.*

Table 1: Variations in radial growth and percent inhibition in different test biopesticides and their combinations (Foliar application) through dual culture technique

Treatments	Days after inoculation					
	5		7		9	
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
B. bassiana + *P. florescens*	1.82^{d}	39.26^{ab} (38.75)	2.29^{de}	35.83^{a} (36.70)	2.99^{de}	30.41^{a} (33.41)
B. bassiana + *L. lecanii*	1.75^{de}	41.53^{a} (40.06)	2.43^{d}	30.39^{ab} (33.03)	3.12^{d}	27.34^{ab} (30.77)
B. bassiana	3.00c	0.00^{d} (0.00)	3.50^{c}	0.00^{d} (0.00)	4.30^{c}	0.00^{d} (0.00)
L. lecanii + *P. florescens*	3.61^{b}	14.74^{c} (22.07)	4.62^{b}	20.68^{c} (26.87)	5.30^{b}	20.24^{c} (26.72)
L. lecanii	4.23^{a}	0.00^{d} (0.00)	5.83^{a}	0.00^{d} (0.00)	6.66^{a}	0.00^{d} (0.00)
SE(m)±	0.08	1.59	0.11	1.99	0.14	2.11
CD(0.05%)	0.24	4.75	0.35	5.91	0.41	6.29

Values given in parentheses are angular transformed values

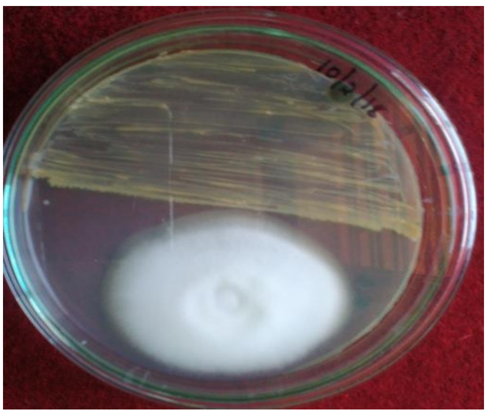
Figures indicated by same letter are not significantly different from one another as per DMRT

Table 2: Efficacy of different test biopesticides and their combinations in terms of extent of damage by *S. litura*

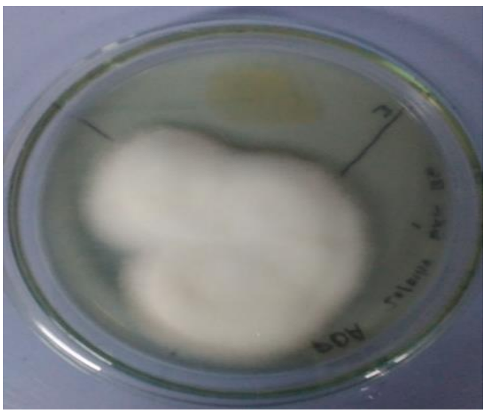
Treatments	Foliar damage (%)*#	Area of damage (mm^2) #
Beauveria bassiana	14.43^{a} (22.31)	37.11^{h}
Lecanicillium lecanii	26.96^{bc} (30.94)	136.00^{c}
Pseudomonas florescnes	21.16^{ab} (27.33)	101.13^{ef}
Beauveria bassiana + *Lecanicillium lecanii*	15.06^{ab} (22.09)	96.92^{fg}
Beauveria bassiana + *Pseudomonas florescnes*	23.13^{ab} (28.71)	102.33^{e}
Lecanicillium lecani + *Pseudomonas florescnes*	26.30^{b} (30.80)	129.40^{cd}
Beauveria bassiana + *Lecanicillium lecanii* + *Pseudomonas florescnes*	36.86^{c} (37.29)	182.55^{b}
Untreated	48.56^{d} (44.15)	388.91^{a}
SE(m)±	3.36	5.02
CD(0.05%)	10.17	19.59

*Values given in parentheses are angular transformed values

Figures indicated by same letter are not significantly different from one another as per DMRT

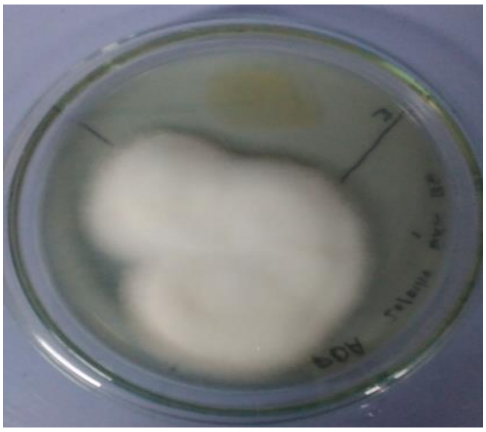


B. bassiana + *P. florescnes*

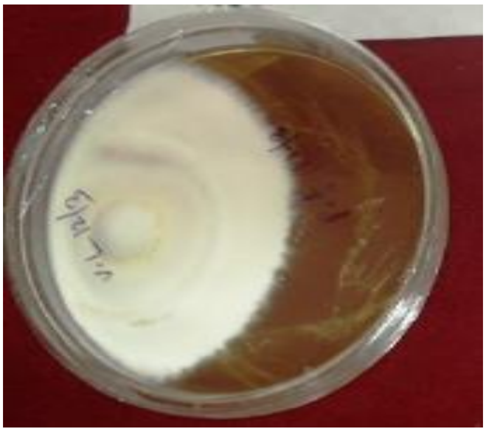


Control (*B. bassiana*)

B. bassiana + *L. lecani*



Control (*B. bassiana*)



L. lecani + *P. florescnes*

Control (*L. lecani*)

Plate 1: Extent of antagonism/compatibility among different test biopesticides (Foliar application) through dual culture test

Plate 2: Experimental setup for compatibility studies of microbial pesticides used for foliar application against *S. litura*

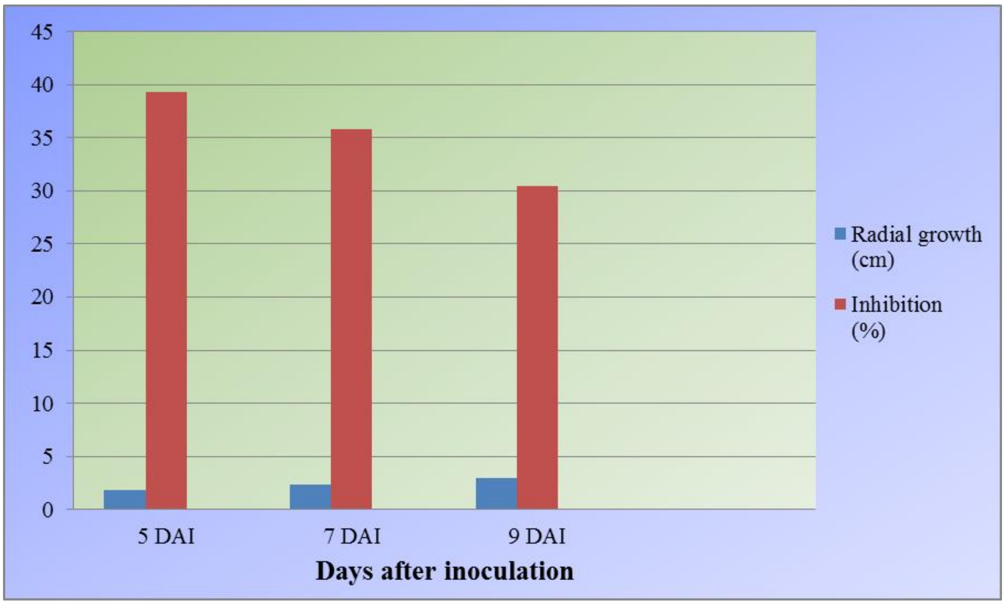


Fig 1: Radial growth and percent inhibition of *B. bassiana* with *P. florescnes*

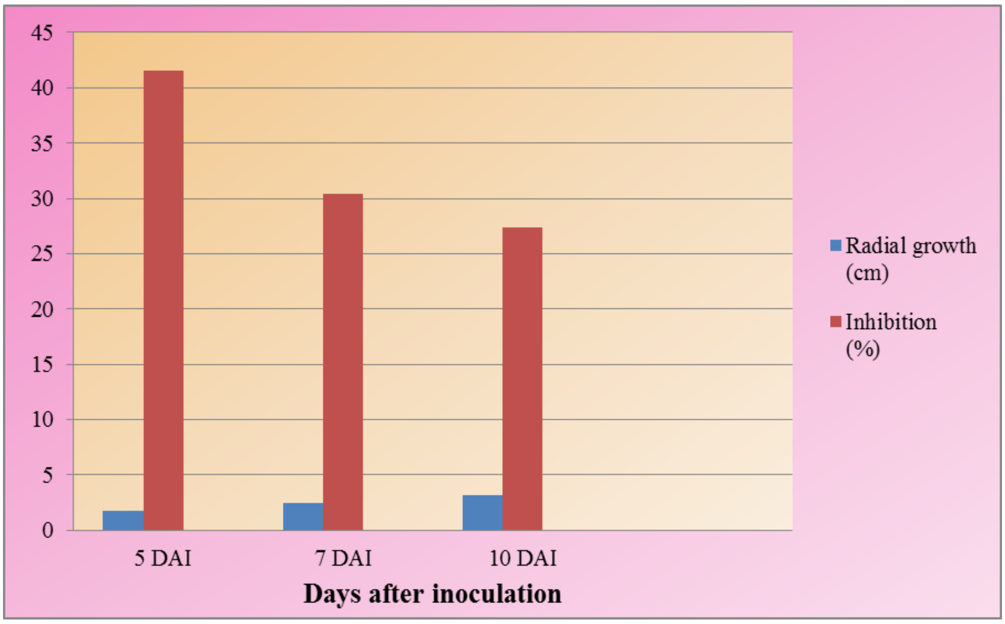


Fig 2: Radial growth and percent inhibition of *B. bassiana* with *L. lecanii*

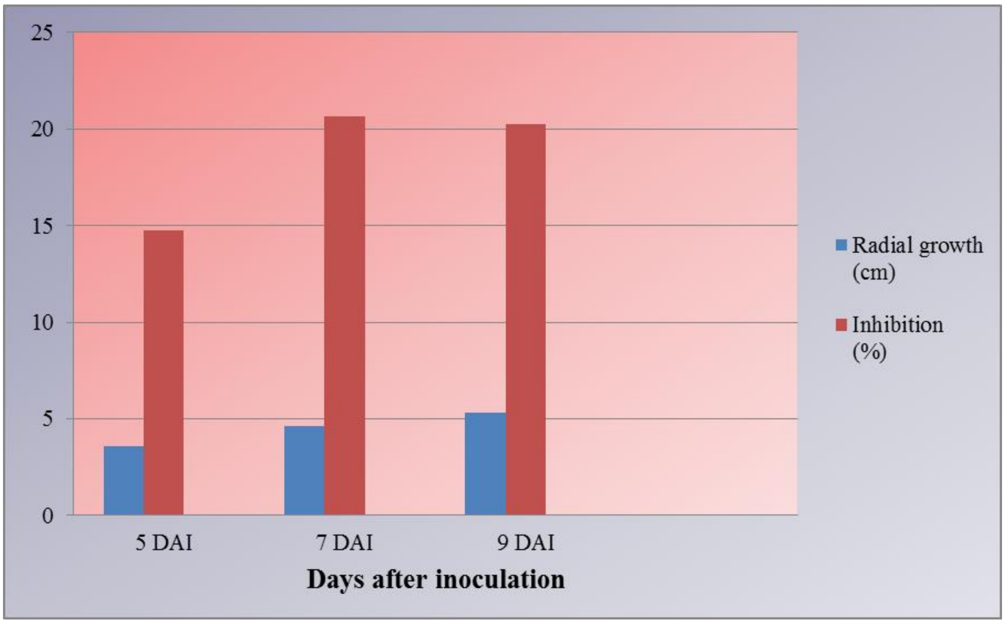
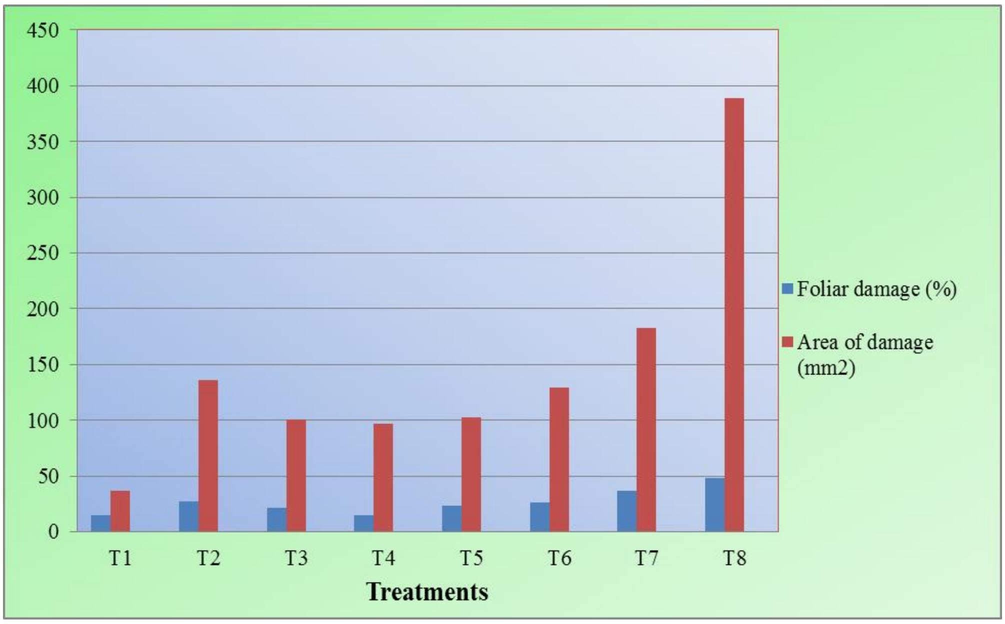


Fig 3: Radial growth and percent inhibition of *L. lecanii* with *P. florescens*



T_1: *Beauveria bassiana*, T_2: *Lecanicillium lecanii*, T_3: *Pseudomonas florescnes*, T_4: *Beauveria bassiana* + *Lecanicillium lecanii*, T_5: *Beauveria bassiana* + *Pseudomonas florescnes*, T_6: *Lecanicillium lecani* + *Pseudomonas florescnes*, T_7: *Beauveria bassiana* + *Lecanicillium lecanii* + *Pseudomonas florescnes*, T_8: Untreated

Fig 4: Efficacy of different test biopesticides and their combinations in terms of extent of damage by *S. litura*

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