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Evaluation of the bio-efficacy and phytotoxicity of Bio Cure B (*Pseudomonas fluorescens* 1.50%-Liquid formulation) against blast (*Magnaporthe grisea*) disease in paddy

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Abstract
Rice blast caused by *Magnaporthe grisea* is one of the most devastating diseases which account for yield loss up to 65% in susceptible cultivars of rice. In an attempt to biologically manage the disease, six strains of Pseudomonas viz., Bio-cure – B(MTCC 5671), CbePf, AuPf, DhrPf, KriPf and SalPf were tested against *M. grisea* by dual culture and roll paper towel techniques. Among the strains tested, Biocure B (Strain - MTCC 5671) recorded the highest percent inhibition of the test pathogen and enhanced plant growth parameters of rice. In field studies application of liquid formulation of Bio-Cure-B (*Pseudomonas fluorescens* 1.50 %) as Seed treatment @ 4.5 ml/kg for 1 hour + three foliar sprays @ 6.0 litters/ha from 45 days after transplanting at 10 days interval effectively suppressed the blast disease to the minimum and enhanced the plant growth and yield of rice to the maximum during both the seasons tested. Also, application of Bio-Cure-B showed no phytotoxic symptoms and was found safe to the environment as it did not affect the population of beneficial insects and microbes in the soil during both the seasons tested.

Keywords: Rice, Bio Cure B (*Pseudomonas fluorescens* 1.50%-Liquid formulation and *Magnaporthe grisea*.

Introduction
Rice (*Oryza sativa* L.) is the most widely cultivated food crop and contributing approximately 23% of the per capita energy for six billion people worldwide. Global rice production was 468.1M.T (million tons) during the year 2010 and it rose to a record 480M.T in 2011. According to Agriculture ministry, India harvested a record 103.41 M.T of rice in the 2011-2012 crop years (July- June) as against 95.98 M.T in the previous year (Anonymous. 2012) [1]. More than 90% of rice produced in Asia, where China and India being the lead producer (Kumar *et al.* 2011). It is widely affected More than 70 diseases are caused by fungi, bacteria, viruses and mycoplasma that results in significant yield losses (Ou, S. H. 1985) [15]. Rice blast disease, caused by *Pyricularia grisea* (Cooke) Sacc [teleomorph *Magnaporthe grisea* (Hebert) Barr], has long been recognized as the most destructive disease of wheat, rice, barley and millet (Chadha, S., and T. Gopalakrishna. 2005) [4] The disease occurs in almost all rice growing areas of the world and is the most serious in temperate and tropical area of non irrigated environments. In temperate regions, the blast problem is perpetuated by the high pathogenic variability of the fungus while in the upland environments blast exacerbated with drought. Though, this disease being managed through fungicides, their adverse effects on environment and beneficial soil microorganisms are quite evident. Biocontrol approach for managing these diseases is considered to be a practical and economical alternative (Prasanna Reddy *et al.* 2009) [16]. Many microorganisms from the rhizosphere can positively influence plant growth and plant health and are referred to as PGPR. These microbes can act as biocontrol agents in several ways, including niche exclusion, bio antagonism and induction of induced systemic resistance (ISR) against infection by fungal, bacterial and viral pathogens in different plant species (Compant *et al.* 2005) [5]. Since biocontrol is a key component of integrated disease management, it is important to search for rhizobacteria active against blast and to evaluate the antagonists for application in field conditions.

2. Methodology
In vitro screening of *P. fluorescens* (MTCC 5671) against *Magnaporthe grisea.* The *in vitro* efficacy of *P. fluorescens* (T-Stanes Strain - MTCC 5671) against *Magnaporthe grisea* was

assessed following the standard dual culture assay (Dennis and Webster, 1971). A nine mm mycelial disc of the rice blast pathogen *P. oryzae* was placed in the centre of the Petri plate containing PDA medium. One cm long streak was gently made on to medium using two days old culture *P. fluorescens* (MTCC 5671) culture just opposite to the pathogenic culture at equidistance and incubated at room temperature (28 ± 2^0C). After incubation, the mycelial growth of *Magnaporthe grisea* and the zone of inhibition (mm) was measured and recorded. The per cent inhibition of mycelial growth was calculated according to Vincent (1927).

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where I = Inhibition per cent
C = Radial growth in control
T = Radial growth in treatment

Treatment details: Seven
An experiment was conducted using paper roll towel technique to assess the efficacy of seed treatment with *P. fluorescens* stanes (Biocure - B liquid formulation) on the seed germination and seedling vigour of rice. The product was treated to the seeds @ 10ml/kg of seeds and shade dried. Three replications per treatment were maintained and twenty five seeds were randomly selected per replication from each treatment. The data on root length, shoot length and dry weight of seedlings were collected after 14 days of treatment from ten seedlings selected random per replication. The seedling vigour index was calculated using the following formula:

Seedling vigour index = (Root length + Shoot length) × percent seed germination

Treatment No.	Treatments	Dosage and Methods of application
T1	*P. fluorescens* 1.50% LF (Bio Cure B)	Seed treatment @ 4.5 ml/kg seed for 1 hour
T2	*P. fluorescens* 1.50% LF (Bio Cure B)	Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval
T3	*P. fluorescens* 1.50% LF (Bio Cure B)	Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval
T4	*P. fluorescens* 1.50% LF (Bio Cure B)	Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval
T5	*P. fluorescens* 1.50% LF (Bio Cure B)	Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval
T6	Hexaconazole 5% EC	Foliar spray @1000 ml/ha
T7	Control	-

Methods of application of bio-formulation
Seed Treatment:
The seeds were spread uniformly on a flat surface and the product, as per the dosage, was applied to the seeds ensuring uniform coating. The treated seeds were kept for 24 h in shade and then sown.

Foliar spray
Foliar sprays were given using a Knapsack sprayer fitted with hollow cone nozzle and a spray volume of 500 lit./ha. The sprays were initiated at first appearance of the disease viz., 45 days after transplanting as per the dosage mentioned in the treatment schedule and repeated twice at 10 days interval.

Assessment of biometrics and blast disease incidence
Germination percentage and Vigour index
The germination percentage was assessed at seven days after sowing and calculated by using the following formula

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The observations on shoot length and root length were assessed at the time of transplanting and the seedling vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973)

Vigour index = (Mean root length + Mean shoot length) × Germination (%)

Percent disease incidence
Bio-efficacy of the antagonist was evaluated by assessing the PDI after 7 & 15 days of final treatment using 0-9 scale as followed in IRRI (International Rice Research Institute). The data regarding the occurrence of the blast disease was collected one week after the last application of fungicides by using the disease rating scale of 0-9 developed by International Rice Research Institute (IRRI. 1996) and then converting into per cent disease by using the formula.

$$\text{Disease \%} = \frac{\text{Sum of the scores}}{\text{Number of observation}} \times \frac{100}{\text{Highest number in rating scale}}$$

Disease rating scale:

Score	Description
0	No lesions
1	Small brown specks of pinhead size without sporulating centre
2	Small roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter with a distinct brown margin, lesions are mostly found on the lower leaves
3	Lesions type is the same as in scale 2, but significant number lesions are on the upper surface
4	Typical sporulating blast lesions, 3mm or longer, infecting less than 2% of the leaf area
5	Typical blast lesions infecting 2-10 % of the leaf area
6	Blast lesions infecting 11-25 % leaf area
7	Blast lesions infecting 26-50 % leaf area
8	Blast lesions infecting 51-75% leaf area
9	More than 75 % leaf area affected

Growth and yield parameters
The growth parameters like plant height, no. of tillers etc and yield attributes like no. of grains, grain yield etc would be recorded following standard procedures.

Grain yield and straw yield
The crop was harvested at maturity and sun dried. The harvested plants were thrashed, grains separated and cleaned

by winnowing. The grains and straw were weighed separately. The yield per hectare was calculated and recorded.

Evaluation of Phytotoxicity

To know the crop tolerance and extent of phytotoxicity, ten plants were randomly selected from each plot and the total number of leaves showing phytotoxic symptoms if any like leaf injury on tips/surface, Wilting, Vein clearing, Necrosis, Epinasty and Hyponasty after were counted on 1, 3, 5, 7 and 10 days after spraying and grading was done as per CIB guidelines adopting 0 -10 scale. The standard dose along with double the dosage was assessed for phytotoxicity.

3. Results and Discussion

Experimental Results

The bio efficacy trial of *P. fluorescens* strain Bio-cure-B (T-Stanes Strain - MTCC 5671) against rice blast disease were conducted during Rabi season (September 2014 — February 2015 first season) and Kuruvai season (May-2015 second season) using the rice variety ADT, 43

Six strains of *Pseudomonas viz.,* Bio-cure – B, Cbe*Pf,* Au*Pf,* Dhr*Pf,* Kri*Pf* and Sal*Pf* were tested by dual plate against *M. grisea.* The results revealed that Biocure B (T-Stanes Strain - MTCC 5671) recorded the highest percent inhibition of 44.55% when compared to control.

Growth promotion activity of bio formulations

An experiment was conducted to test the growth promotion efficacy of six strains of *Pseudomonas viz* Cbe*Pf,* Au*Pf,* Dhr*Pf,* Kri*Pf* and Sal*Pf* obtained from culture collection section, Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram along with Bio-cure - B obtained from M/s. T. Stanes and company Ltd., Coimbatore (MTCC 5671) following roll paper towel technique. The results revealed that the Rice seeds treated with the different strains of PGPR's showed enhanced plant growth when compared to untreated seeds. Among the various strains tested, the strain obtained from M/s. T. Stanes and company Ltd., Coimbatore viz., Bio-cure - B significantly recorded the maximum seed germination 100 per cent and enhanced the growth parameters of rice to the maximum with a root and shoot length of 20.76 cm and 7.99 cm respectively and vigor index of 2875.00 table 2.

Blast incidence

The results depicted in table 4 (Rabi and Kuruvai season) revealed that among the different treatments with liquid formulations, Bio cure B- as Seed treatment @ 4.5 ml/kg for 1 hour + three foliar spray @ 6.0 litters/ha from 45 days after transplanting at 10 days interval (T5) recorded the least blast incidence of first season PDI of 7.68% and second PDI of 7.50% which accounted for 74.77 and 75.41 per cent decrease in the incidence of blast over control. This was followed by the treatment with Bio cure B- as Seed treatment @ 4.5 ml/kg for 1 hour + three foliar spray @ 4.5 litters/ha from 45 days after transplanting at 10 days interval (T4.10.23% and-10.20%). The chemical treatment with Hexaconazole recorded the minimum blast incidence of 4.33% and 5.98% which accounted for 85.78 and 80.39 per cent decrease in the incidence of blast over control whereas the untreated control plots recorded a blast incidence of 30.45 and 30.50 per cent (Table 3).

Biometrics of paddy

The data pertaining to the growth parameters due to treatment with Bio-Cure-B are presented in table 4&5(Rabi and Kuruvai season). In general, the various treatments with Bio-Cure-B treated plots showed increased biometric values when compared to control. Among the various treatments with Bio-Cure-B, the treatment T5 viz., Seed treatment 4.5 ml/kg for 1 hour + three foliar spray @ 6.0 litters/ha from 45 days after transplanting at 10 days interval recorded the highest plant height (62.10 cm, 62.11 cm), highest number of tillers (27.00, 26.00) and maximum grain and straw yields with (4565, 4560 kg/ha) and (6820, 6821 kg/ha) respectively. The treatment T4 (Seed treatment 4.5 ml/kg for 1 hour + three foliar spray @ 4.5 litters/ha from 45 days after transplanting at 10 days interval) came next in the order of merit with increased biometrics of paddy. The comparison chemical treatment recorded a plant height of (52.74, 52.70 cm), number of tillers (27.00 26.00) and grain and straw yields with (44.23, 44.20 kg/ha) and (67.00, 67.01 kg/ha) respectively. The untreated control plots recorded the least biometric values with a plant height of (47.95, 47.95 cm), number of tillers (20.00 19.00) and grain and straw yields with (3700, 3699 kg/ha) and (5700, 5701 kg/ha) respectively.

Phytotoxicity

No phytotoxic symptoms *viz.,* leaf tip injury, leaf surface injury, vein clearing, necrosis, epinasty and hyponasty were observed during the entire cropping period in both season and in all the concentration of the test product (Bio-Cure-B) treated plots (Table 6).

Effect on the population of natural enemies

The results presented in table 7&8 (Rabi and Kuruvai season) showed that the population of natural enemies *viz.,* spiders, mirid bug and coccinellids increased appreciably in Bio-Cure-B treated plots when compared to chemical treated plots. Especially, the spider population showed maximum increase in Bio-cure B liquid formulation treated plots as against the chemical treated plots. Also appreciable number of mirid bug and coccinellids were observed in the Bio-Cure-B treated plots when compared with the chemical treated plots.

Effect on soil beneficial micro organisms

The results of field experiments (Rabi and Kuruvai season) demonstrated that application of liquid formulation of Bio-Cure-B *(Pseudomonas fluorescens* 1.50 %) did not have any adverse impact on the population of soil beneficial micro organisms in rice fields. However, application of chemical (Hexaconazole) showed reduced beneficial micro organism population in the soil when compared to control (Table 9).

Discussion

In the present study, bacterial antagonistic Bio Cure B (*Pseudomonas fluorescens* (T-Stanes Strain - MTCC 5671) 1.50%-Liquid formulation) was studied under laboratory and field test. The T-Stanes Strain - MTCC 5671 was found to be very effective and significantly inhibited the rice blast pathogen *Magnaporthe grisea* and when treated to the seeds, enhanced the biometrics of paddy seedlings. Further, application of liquid formulation of Bio-Cure-B *(Pseudomonas fluorescens* 1.50 %) as Seed treatment @ 4.5 ml/kg for 1 hour + three foliar spray @ 6.0 liters/ha from 45 days after transplanting at 10 days interval effectively controlled the blast disease and enhanced the plant growth and

yield of rice. Also, application of Bio-Cure-B showed no phytotoxic symptoms and was found safe to the environment as it did not affect the population of beneficial insects and microbes in the soil. Similar findings were made by earlier workers. The inconsistent performance of the introduced agents on aerial plant parts need to modify the delivery system or supplementation of additives (Guetsky *et al.*, 2002; Ting *et al.*, 2009) [10, 19]. At present, the most economical method for producing most economical biocontrol agents is using liquid culture fermentation (suspension) (Ashofteh *et al.*, 2009) [2]. Recent study also pointed out that talc-based formulation of *Pseudomonas* strains (Talc-Bl and Talc-B2) were effective against sugar beet disease (Jorjani *et al.*, 2012) [11]. The *Pseudomonas fluorescens* and fungicides were the effective management of rice blast disease (kishanlal, *et al*., 2017). Mishra *et al.*, (2010) [14] reported that Plant growth promoting *Pseudomonas* strains increased 27.6% productivity in *Pelargonium graveolens* L. *herit. Pseudomonas* MR-18 increased dry weight, height of *Mucuna pruriens* by 84 and 24% respectively (Deshwal *et al.*, 2011) [8]. In the previous study, it was reported that several bacterial isolates could suppress the growth of pathogenic fungi P. oryzae (Suryadi *et al.*, 2011) [18]. Vleesschauwer *et al.*, 2012 demonstrated that treatment of rice plant with *P.fluorescens* induced resistance against *P.oryzae.* Development of biocontrol formulation can besuccessful if microbial agents could survive during storage, as well as competitive and aggressiveness after inoculation process (Beatty and Jensen, 2002; Selim *et al*., 2005) [3, 17].
All the above literatures supported that plant growth promoting *Pseudomonas* increased plant growth.

Table 1: Effect of different strains *oi Pseudomonas fluorescens* strains against *Magnaporthe grisea* under *in vitro* conditions

Tr. No.	Treatments	Mycelial Growth (cm)	Per cent inhibition (C-T/C*100)
1	**Bio-cure - B**	4.99	44.55
2	Cbe*Pf*,	5.40	40.00
3	*AuPf*,	5.51	38.77
4	*DhrPf*,	5.75	36.11
5	Kri*Pf*	5.55	38.33
6	Sal*Pf*	5.55	38.33
7	Control	9.00	--
	SEd	0.01	--
	CD (p=0.05)	0.03	

Table 2: Growth promoting activity of fluorescent pseudomonas strains in rice seedling (Roll towel method) under *in vitro* conditions

S. No	Treatments	Germination %	Root length (cm)	Shoot length (cm)	Vigour Index
1	Bio-cure - B	100.0	20.76	7.99	2875.00
2	Cbe*Pf*	100.0	16.12	7.60	2372.00
3	Au*Pf*	100.0	14.50	7.84	2234.00
4	Dhr*Pf*	96.0	13.98	7.40	2052.48
5	Kri*Pf*	100.0	12.42	7.55	1997.00
6	Sal*Pf*	96.0	12.68	6.92	1881.60
7	Control	80.0	08.99	5.80	1183.20
	SEd	0.01	0.02	0.01	--
	CD (p=0.05)	0.02	0.05	0.02	

Table 3: Effect of Bio-Cure —B 1.50% liquid formulation against rice blast (First season: September 2014 — February, 2015) second

Tr. No.	Treatments	First season		Second season	
		Per cent disease index (PDI)	% decrease over control	Per cent disease index (PDI)	% decrease over control
T_1	*P. fluorescens* 1.50% LF(Bio Cure B) Seed treatment @ 4.5 ml/kg seed	19.66 (26.32)	35.43	19.69 (26.34)	35.44
T_2	*P. fluorescens* 1.50% LF(Bio Cure B) Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	15.45 (23.14)	49.29	15.55 (23.22)	49.01
T_3	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	12.70 (20.87)	41.70	12.71 (20.88)	58.32
T_4	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	10.23 (18.65)	66.40	10.20 (18.63)	66.56
T_5	*P. fluorescens* 1.50% LF(Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	7.68 (16.08)	74.77	7.50 (15.89)	75.41
T_6	Hexaconazole 5% EC@1000 ml/ha	4.33 (12.01)	85.78	5.98 (14.15)	80.39
T_7	Control	30.45		30.50 (33.52)	--
	SEd	0.88	--	0.82	--
	CD (p=0.05)	1.92		1.96	

Table 4: Effect of Bio-Cure —B 1.50% liquid formulation on the biometrics of rice (First season: September 2014 — February, 2015)

Tr. No.	Treatments	Plant height (cm)	No. of tillers per hill	Grain yield (kg/ha)	Straw yield (kg/ha)
T_1	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg seed	53.10	24.00	3898	6290
T_2	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	54.71	25.00	3935	6315
T_3	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	55.45	24.00	4185	6410
T_4	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	55.61	25.00	4200	6525
T_5	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	62.10	27.00	4565	6820
T_6	Hexaconazole 5% EC@1000 ml/ha	52.74	27.00	4423	6700
T_7	Control	47.94	20.00	3700	5700
	SEd	0.23	0.06	5.12	8.64
	CD (p=0.05)	0.82	0.14	12.65	18.92

Table 5: Effect of Bio-Cure —B 1.50% liquid formulation on the biometrics of rice ((Second season: May-2015)

Tr. No.	Treatments	Plant height (cm)	No. of tillers per hill	Grain yield (kg/ha)	Straw yield (kg/ha)
T_1	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg seed	53.11	23.50	3900	6200
T_2	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	54.70	24.00	3934	6300
T_3	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	55.40	23.00	4180	6411
T_4	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	55.60	24.00	4199	6520
T_5	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	62.11	26.00	4560	6821
T_6	Hexaconazole 5% EC@1000 ml/ha	52.70	26.00	4420	6701
T_7	Control	47.95	19.00	3699	5701
	SEd	0.31	0.12	10.25	12.56
	CD (p=0.05)	0.63	0.25	25.34	30.45

Table 6: Phytotoxicity evaluation of Bio-cure —B 1.50 % on rice during Sep. 2014 — Feb., 2015 (First season: September 2014 — February, 2015)

Tr. No.	Treatments	Phytotoxicity Symptoms / Days after application First season					Phytotoxicity Symptoms / Days after application Second season				
		Chlorosis/ Vein clearing	Necrosis	Scorching	Epinasty	Hyponasty	Chlorosis/ Vein clearing	Necrosis	Scorching	Epinasty	Hyponasty
T_1	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg seed	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T_2	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T_3	*P. fluorescens* 1.50% LF (Bio Cure B) Three foliar spray @ 6.0 litre/ha from 45 days after transplanting	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

T_4	at 10 days interval *P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T_5	*P. fluorescens* 1.50% LF (Bio Cure B) Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T_6	Hexaconazole 5% EC@1000 ml/ha	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
T_7	Control	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 7: Effect of Bio-cure —B 1.50 % on the population of natural enemies (First season: September 2014 — February, 2015)

Tr. No.	Treatments	Spiders (Nos.)				Mirid bug (Nos.)				Coccinellids (Nos.)			
		Before I spray	After I spray	After II spray	After III spray	Before I spray	After I spray	After II spray	After III spray	Before I spray	After I spray	After II spray	After III spray
1	Bio-cure - B - Seed treatment @ 4.5 ml/kg of seed for 1 hour	6.33	6.45	7.12	6.62	4.05	3.63	3.90	3.81	5.82	5.63	6.55	5.71
2	Bio-cure - B - Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	5.90	6.10	6.15	6.11	3.45	3.39	4.19	4.15	6.40	6.00	6.25	5.70
3	Bio-cure - B - Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	6.80	6.50	5.90	6.77	3.27	3.10	3.52	4.10	5.73	6.20	5.77	5.30
4	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @4.5 litre/ha from 45 days after transplanting at 10 days interval	7.33	6.90	7.25	7.20	3.28	3.14	3.90	4.34	6.50	6.37	5.82	5.63
5	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	7.26	7.14	6.95	7.48	3.05	3.10	3.67	4.55	5.90	6.71	6.68	6.50
6	Hexaconazole 5% EC - Foliar spray @1000 ml/ha	7.10	5.64	4.35	3.10	3.65	0.66	0.00	0.00	6.12	2.50	2.15	1.96
7	Control	6.05	6.61	7.10	7.25	3.17	3.87	4.04	4.68	5.47	6.54	6.20	6.00
	SEd	0.01	0.02	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.21	0.03	0.04
	CD (p=0.05)	0.02	0.05	0.03	0.05	0.02	0.07	0.03	0.03	0.03	0.43	0.06	0.08

Table 8: Effect of Bio-cure —B 1.50 % on the population of natural enemies (Second season: May-2015)

Tr. No	Treatments	Spiders (Nos.)				Mirid bug (Nos.)				Coccinellids (Nos.)			
		Before I spray	After I spray	After II spray	After III spray	Before I spray	After I spray	After II spray	After III spray	Before I spray	After I spray	After II spray	After III spray
1	Bio-cure - B - Seed treatment @ 4.5 ml/kg of seed for 1 hour	6.34	6.46	7.11	6.60	4.07	3.65	3.91	3.82	5.83	5.64	6.56	5.70
2	Bio-cure - B - Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	5.86	6.11	6.16	6.12	3.40	3.40	4.20	4.15	6.39	6.11	6.24	5.69
3	Bio-cure - B - Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	6.79	6.50	5.91	6.78	3.28	3.11	3.53	4.11	5.74	6.21	5.78	5.29
4	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @4.5 litre/ha from 45 days after transplanting at 10 days interval	7.32	6.91	7.26	7.21	3.29	3.15	3.91	4.35	6.51	6.38	5.83	5.64
5	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	7.27	7.15	6.96	7.50	3.47	3.11	3.70	4.56	5.91	6.72	6.70	6.51
6	Hexaconazole 5% EC - Foliar spray @1000 ml/ha	7.11	5.65	4.36	3.11	3.60	0.70	0.00	0.00	6.13	2.51	2.16	1.95
7	Control	6.05	6.61	7.10	7.25	3.17	3.87	4.04	4.68	5.47	6.54	6.20	6.00
	SEd	0.01	0.11	0.01	0.02	0.01	0.02	0.03	0.01	0.04	0.01	0.06	0.08
	CD (p=0.05)	0.03	0.23	0.02	0.04	0.03	0.05	0.07	0.03	0.09	0.02	0.13	0.17

Table 9: Effect of Bio-cure-B 1.50 % liquid formulation on the soil beneficial micro-organisms (First & Second season)

Tr. No	Treatments	First Season				Second Season			
		Trichoderma viride	*Rhizobacterium*	*Azotobacter*	*Azospirillum*	*Trichoderma viride*	*Rhizobacterium*	*Azotobacter*	*Azospirillum*
1	Bio-cure -B - Seed treatment @ 4.5 ml/kg seed for 1 hour	0.8×10^4	1.1×10^6	1.4×10^6	1.0×10^6	0.7×10^4	1.2×10^6	1.3×10^6	1.1×10^6
2	Bio-cure - B - Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	0.9×10^4	1.2×10^6	1.5×10^6	0.9×10^6	1.0×10^4	1.1×10^6	1.5×10^6	1.8×10^6
3	Bio-cure - B - Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	0.7×10^4	0.9×10^6	1.3×10^6	1.2×10^6	0.8×10^4	1.0×10^6	1.4×10^6	1.3×10^6
4	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 4.5 litre/ha from 45 days after transplanting at 10 days interval	1.0×10^4	1.1×10^6	1.6×10^6	1.9×10^6	1.1 x Ą	1.3×10^6	1.7×10^6	2.0×10^6
5	Bio-cure - B - Seed treatment @ 4.5 ml/kg for 1 hour + Three foliar spray @ 6.0 litre/ha from 45 days after transplanting at 10 days interval	0.9×10^4	1.4×10^6	1.8×10^6	1.9×10^6	1.0×10^4	1.5×10^6	1.9×10^6	2.0×10^6
6	Hexaconazole 5% EC - Foliar spray @1000 ml/ha	0.1×10^4	0.6×10^6	0.4×10^6	0.7×10^6	0.2×10^4	0.7×10^6	0.5×10^6	0.8×10^6
7	Control	1.4×10^4	$1.5 \times 10^{5-}$	2.1×10^6	1.2×10^6	1.4×10^4	$1.5 \times 10^{5-}$	2.1×10^6	1.2×10^6

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