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Compatibility of imidacloprid with plant pathogenic antagonistic microorganisms, *Trichoderma viride* (Pers) and *Pseudomonas fluorescens* (Migula) in cotton

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In cotton, the compatibility of imidacloprid as seed treatment was evaluated with plant pathogenic antagonistic microorganisms, *Trichoderma viride (*Pers) and *Pseudomonas fluorescens* (Migula). The radial growth of *T. viride* was less at higher concentration of 7000 ppm (12.5mm) compared to the untreated check (15.1mm) at 24 h. The radial growth was less in treatments compared to untreated check at 48 and 72 h of incubation. Similarly the spore yield was less in treatments compared to untreated check. Regarding the bioefficacy of *T. viride,* the radial growth of the pathogen, *Macrophomina phaseolina* was 20 mm in the treatment of *T. viride* alone while it was 38 and 40 mm in the combination treatments of imidacloprid 70 WS at 7 g and 5 g kg-1 with *T. viride* 4 g kg-1while in the untreated check it was 45 mm. In the pot culture studies, it was found that the population of *T. viride* was higher in the treatment of *T. viride* 4 g kg-1alone compared to the combination treatment of imidacloprid 70 WS and *T. viride*. Similar trend was also observed in case of *P. fluorescens*. It was concluded that imidacloprid was having lesser impact on *T. viride* and significant inhibitory effect on *P. fluorescens*.

Key words: Cotton, *Trichoderma viride*, *Pseudomonas fluorescens*, imidacloprid

INTRODUCTION

Cotton is one of the most important cash crops of India, which accounts for about 50 per cent of the total fibre consumption of the world. The sucking pests, aphids, jassids, thrips, whiteflies and the bollworm complex are considered to be the key pests causing severe damage leading to loss in yield of seed cotton. A new insecticide molecule imidacloprid 1-(6-chloro-3-pyridinyl) methyl 4, 5-dihydro-N-nitro-) H-imidazole-2-amine was developed by Nihon Bayer Ltd. and it belongs to the chloronicotinyl group. It has a superior performance against sucking pests. To combat the seed and soil borne diseases, the antagonistic microorganisms *Trichoderma viride* (Pers.) and *Pseudomonas fluorescens* (Migula) were reported to be effective (Mirhuta -Grim and Rose, 1986; Vidyasekaran and Muthamilan, 1995). The present study was undertaken to evaluate the compatibility of imidacloprid with *T. viride* and *P. fluorescens* in cotton.

MATERIALS AND METHODS

Effect of imidacloprid on the growth of Trichoderma viride (Pers) :

A laboratory experiment was conducted to study the effect of imidacloprid on the growth of *T. viride*, a potential biocontrol agent used against root rot causing organism, *Macrophomina phaseolina* (TassiGoid) under *invitro* conditions. The treatments were imidacloprid 70 WS at 3000, 5000 and 7000 ppm and an untreated check. There were five replications.

To the l00 ml of sterilized Potato Dextrose Agar medium (PDA) 3, 5 and 7 ml ofimidacloprid 70 WS 1,00,000 ppm (of the formulation) was added and mixed thoroughly under aseptic condition and imidacloprid unamended PDA medium served as control. The amended and unamended media were poured to the sterilized Petridishes under aseptic condition and allowed to solidify. After solidification, an 8 mm circular disc of 5 day old *T. viride* was transferred to the centre of the plate using inoculation needle under aseptic condition and the plates

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were incubated at 32 ±2^{0}C in an incubator. The radial growth of *T. viride* was measured at 24, 48 and 72 h after inoculation and the results are expressed in mm.

Effect of imidacloprid on the sporulation of T. viride: Sporulation efficacy of *T.viride* was assessed under different levels of imidacloprid concentration under *in vitro* conditions. The treatments were as in section A. To l00 ml of sterilised yeast molasses broth 1, 5 and 7 ml of imidacloprid (1,00,000 ppm) was added under aseptic condition to obtain 3000, 5000 and 7000 ppm respectively. A small circular disc of *T. viride* (8 mm) was transferred aseptically to imidacloprid- amended medium. The imidacloprid unamended medium served as control. The tubes were incubated at 32 ±2^{0}C for 7 days. After seven days, spore count was taken with haemocytometer and the results were expressed in number per ml.

Effect of imidacloprid on the bioefficacy of T. viride: An experiment was conducted to study the effect of imidacloprid 70 WS on the bioefficacy of *T.viride* on cotton (variety MCU5). *T.viride* talc formulation obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, containing 280 x 106 colony forming units (cfu)/g was used in this study. The treatments included imidacloprid 70 WS 5 g and 7 g kg-1 + *T.viride* 4 g kg-1 and compared with *T.viride* alone and untreated check. There were five replications. To the sterile Petriplates, PDA supplemented with streptomycin sulphate (100 ppm) was transferred and allowed to solidify. The treated seeds were placed on the PDA at four places near the periphery of the Petridish. At the centre of the plate an 8 mm disc of root rot causing organism *Macrophomina phaseolina* was placed in all the treatments and plates were kept for incubation at 32 ±2^{0}C. The untreated seeds served as control. The growth of the pathogen was measured after 72 h and expressed in mm.

Effect of imidacloprid on the growth of P. fluorescens: A laboratory experiment was conducted to study the effect of imidacloprid on the growth of *P. fluorescens*, a Biocontrol agent used against the root rot causing organism, *Rhizoctonia solani* (Kuhnn.) The treatments were, imidacloprid 70WS at 3000, 5000 and 7000 ppm along with untreated check. There were five replications. Kings B agar medium (100ml) was amended with 3, 5 and 7ml imidacloprid 70WS (1,00,000ppm) to obtain a final determination of 3000, 5000 and 7000ppm respectively. To the amended broth, 1ml of *P. fluorescens* broth culture was inoculated and 20ml was transferred to each Petridish and incubated for 72hrs at 32 ±2^{0}C.The unamended medium served as control. After incubation, population count was taken and expressed as number of colonies formed per ml.

Compatibility of imidacloprid with antagonistic fungi and bacteria :
A pot culture experiment was conducted to study the effect of imidacloprid on the antagonist's *viz*., *T. viride* and *P. fluorescens* with test crop cotton (variety-MCU5). A combination treatment of *T. viride* (talc formulation) 4 g kg^{-1}with imidacloprid 70 WS at 5 and 7 g kg^{-1}, *P. fluorescens* (talc formulation) 4 g kg^{-1}with imidacloprid 70 WS at 5 and 7 g kg^{-1}and compared with *T. viride* and *P. fluorescens* alone along with untreated check. There were four replications. Sterilized garden soil was taken in earthem pots and treated cotton seeds were sown in the pots. Untreated seeds served as control. Rhizosphere population of *T viride* and *P. fluorescens* was assessed on 15 and 30 days after sowing (DAS) using the PDA and Kings B agar medium, respectively. One g of rhizosphere soil was taken and diluted 4 times (10^{-4}). From this, 1 ml was transferred to respective medium and kept for incubation for 72 h and after incubation; the radial growth of *T. viride* and population number of *P. fluorescens* was recorded. The data were subjected to statistical analysis and the critical difference values were calculated at 5 per cent probability level and the treatment mean values of the experiment were compared using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion

Effect of imidacloprid on the growth of T. viride :
The radial growth of T viride at higher concentration (imidacloprid 70 WS - 7000 ppm) was 12.5, 24.2 and 32.4 mm after 24, 48 and 72 h of incubation, respectively. At 5000 ppm, it was 14, 28.4 and 38 mm and 15.2, 30.4 and 43.8 mm at 3000 mg kg^{-l} compared to the untreated check, which recorded 15.1, 30.1 and 43.6mm. The radial growth was less in treatments compared to untreated check (Table 1).

Effect of imidacloprid on the sporulation of T. viride: The results on the effect of imidacloprid 70 WS on *T.viride* (Table 1) revealed that the spore yield was 412, 380 and 300 (xI0^6/ml) at 3000, 5000 and 7000 ppm, respectively. The spore yield was less in treatments compared to untreated check, which were 492 (x 10^6/ml).

Table 1. Effect of imidacloprid on the growth of *Trichoderma viride* and sporulation of *Pseudomonas fluorescens*

Sl. No.	Treatments	Radial growth of *T. viride* (mm)			Spore yield (x 10^6/ml)**
		24 hr**	48 hr**	72 hr**	
1.	Imidacloprid 70 WS – 3000 ppm	15.2 a	30.4 a	43.8 a	412 b
2.	Imidacloprid 70 WS – 5000 ppm	14.0 b	28.4 b	38.0 b	380 c
3.	Imidacloprid 70 WS – 7000 ppm	12.5 c	24.2 c	32.4 c	300 d
4.	Untreated check	15.1 a	30.1 a	43.6 a	492 a

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT
** - Significant at P=0.01

Table 2. Effect of imidacloprid on the bioefficacy of *T. viride*

Sl. No.	Treatments	Radial growth of pathogen (mm)**
1	Imidacloprid 70 WS @ 5 g/kg + *T. viride* @ 4 g/kg	40.0 c
2.	Imidacloprid 70 WS @ 7 g/kg + *T. viride* @ 4 g/kg	38.0 b
3	*T. viride* @ 4g/kg	20.0 a
4	Untreated check	45.0 d

In a column means followed by a common letter are not significantly different (P=0.05) by DMRT
** - Significant at P=0.01

Effect of imidacloprid on the bioefficacy of T. viride :
The bioefficacy of *T. viride* was studied in combination with imidacloprid on the growth of pathogens and the results are furnished in Table 2. It was found that the radial growth of the pathogen *Macrophomina phaseolina* was 20 mm in the treatment of *T.viride* alone while it was 38 and 40 mm in the treatments of imidacloprid 70 WS 7 g kg-l + *T.viride* 4 g kg^{-1} and imidacloprid 70 WS 5 g kg^{-1} + *T.viride* 4 g kg^{-1}, respectively. The radial growth of the pathogen in the untreated check was 45 mm.

Effect of imidacloprid on the growth of P. fluorescens:
The number of colonies of *P.fluorescens* was 27.4, 15.6 and 0.4 at 3000, 5000 and 7000 ppm respectively. As the concentration increased the growth decreased compared to the untreated where the number of colonies was 356.6 (Fig.1).

Effect of imidacloprid on the plant pathogenic antagonists :

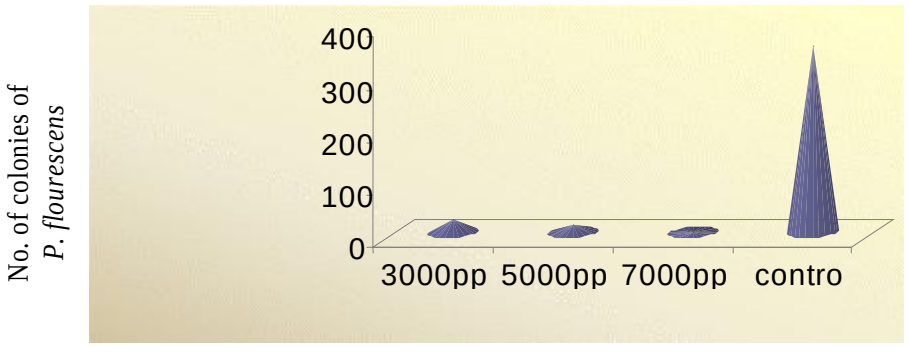

Fig.1 Effect of imidacloprid on the growth of *Pseudomonas fluorescens*

The results of the laboratory experiments were confirmed in the pot culture studies. The population of *T.viride* and *P.fluorescens* was assessed in the rhizosphere soil. The population of *T.viride* was higher, 92 x 10^4 colony forming units (cfu) in *T.viride* 4 g kg^{-1} alone as against 18 x 10^4 and 30 x 10^4 in *T.viride* 4 g kg^{-1} + imidacloprid 70 WS 7 g kg^{-1} and *T.viride* 4 g kg^{-1} + imidacloprid 70 WS 5 g kg^{-1}, respectively at 15 DAS. At 30 DAS the growth was 42 x 10^4 and 80 x 10^4 in *T.viride* 4 g kg^{-1} + imidacloprid 70 WS 7 g kg^{-1}, *T.viride* 4 g kg^{-1} + imidacloprid 70 WS 5 g kg^{-1}, respectively, while it was 154 x 10^4 in *T.viride* 4 g kg^{-1} alone. In the case of *P.fluorescens* the population number was 110 x 10^4, 13 x 10^4 and 1.67 x 10^4 in *P.fluorescens* 4 g kg^{-1} alone, *P.fluorescens* 4 g kg^{-1} + imidacloprid 70 WS 5 g kg^{-1} and *P.fluorescens* 4 g kg^{-1} + imidacloprid 70 WS 7 g kg^{-1} respectively at 15 DAS. At 30 DAS, the population was highest in *P.fluorescens* alone (175 x 10^4) as against 27 and 6 in insecticide mixture treatments (Table 3). In the present study, it was found that imidacloprid 70 WS found that there was an increase in population of 133.33 per cent from 15 to 30 days in the combination of .. .. $^{-1}$ + *T. viride* 4 g kg^{-1} while it was 67.39 per cent in *T. viride* alone. This shows that there is a possibility that the rhizosphere population of *T. viride* will be normal after sometime due to the decrease in activity of imidacloprid. In case of *P. fluorescens*, after 30 days also a significant inhibitory effect was observed. Mote *et al*. (1994) reported that imidacloprid 70 WS seed treatment at 15 g kg^{-1} is compatible with seed treating fungicides like Captan and Thiram in Okra. Tu (1995) indicated that imidacloprid produced only short-lived inhibitory effect on fungal organisms and enzymes and the soil indigenous microbes can tolerate the application of imidacloprid in the seed. Steinhaus and Tugwell (1997) reported that a combination of imidacloprid with insect pathogenic fungi, *Beauveria bassiana* (Bals.) resulted in 97.9 per cent mortality of *Lygus lineolaris* (Palisot de Beauvois) in rape and the mortality was more than when

Table 3. Compatibility of imidacloprid on biocontrol agents of plant pathogens

Sl. No.	Treatments	Rhizosphere population (cfu's) Days after sowing	
		15**	30**
1	Imidacloprid 70 WS @ 5 g/kg + *T. viride* @ 4 g/kg	30.0 c	80.0 c
2	Imidacloprid 70 WS @ 7 g/kg + *T. viride* @ 4 g/kg	18.0 d	42.0 d
3	*T. viride* @ 4 g/kg	92.0 b	154.0 b
4.	Imidacloprid 70 WS @ 5 g/kg + *P. fluorescens* @ 4 g/kg	13.0 e	27.0 d
5.	Imidacloprid 70 WS @ 7 g/kg + *P. fluorescens* @ 4 g/kg	1.67 f	6.0 e
6.	*P. fluorescens* @ 4 g/kg	110.0 a	175.0 a
7.	Untreated check	0.0 f	0.0 f

In a column means followed by a common letter are not significantly different by DMRT (P=0.05)
** Significant at P=0.01 cfu – Colony forming units.

had suppressed the growth of *T. viride*. The inhibitory effect increased as the dosage increased and the reduction in radial growth was only 25.68per cent at 72 h at 7000 ppm. This indicated that there was no drastic reduction of radial growth at 7000 ppm which is recommended for field application while the sporulation was affected to an extent of 39.02 per cent at 7000 ppm compared to untreated check. It was observed that the chemical had not completely inhibited the sporulation of *T.viride*. The studies on the bioefficacy of *T. viride* on the pathogen, *Macrophomina phaseolina* indicated that there was a reduction in efficacy of *T.viride* as the concentration of the chemical was increased and the reduction was to an extent of 47 .36 per cent. In the pot culture studies, it was they are applied alone. Quintela and Mccoy (1998) reported that the active ingredient of formulated imidacloprid had no effect on conidial attachment, but both the inert ingredient and the formulated product reduced conidial attachment significantly. It was concluded that in the present study the insecticide imidacloprid is found to have an inhibitory effect on the plant pathogen antagonistic organisms, *T. viride* and so on *P. fluorescens*.

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