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RESEARCH ARTICLE :

Field evaluation of *Pseudomonas fluorescens* against the green bollworm, *Helicoverpa armigera* (Hubner)

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SUMMARY : Field experiments were conducted at Vanavarayar Institute of Agriculture Mankkadavu, Pollachi, Coimbatore Districtof Tamil Nadu and South Indian Millers Association (SIMA), Cotton Development and research Association, Ponnery, Udumelpet, Tirupur District of Tamil Nadu during 2014-15 and 2015-16. An investigation was carried out to assess the efficiency of *Pseudomonas flourescens* (PGPR) against cotton green bollworm, *Helicoverpa armigera*. The results revealed that the foliar application of *P. fluorescens* were found to be effective in reducing and *Beauveria basianna* @ 1% in reducing the larval population, square and boll damage percentage. The soil and foliar application of *P. fluorescens* @1% treated plots was recorded the highest seed cotton yield.

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KEY WORDS :
Helicoverpa armigera, Pseudomonas flourescens, Cotton

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BACKGROUND AND OBJECTIVES

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae), is a highly polyphagous pest, feeding on cotton (Malik *et al.*, 2002), pigeonpea (Sreekanth and Seshamahalakshmi, 2012), chickpea (Ahmad *et al.*, 1989), tomato (Sharma*et al.*, 2011), okra (Sarate *et al.*, 2012) and groundnut (Srivatsava *et al.*, 2009). The cotton bollworm is a pest of major importance in India in most agro-ecological zones ranging from Andaman & Nicobar Islands to Jammu and Kashmir (Singh *et al.*, 2002). Crop losses of 75–100% in chickpea (Lal, 1996) and 57–80% in cotton (Gupta, 1999) have been recorded. The estimated monetary loss in Tamil Nadu was Rs. 20.12 million USD on different crops (Jayaraj, 1990). In Punjab, Haryana and Rajasthan the damage due to the peston cotton was estimated at Rs. 296.93 million USD (Harish, 2002). Biological control is the conscious use of living beneficial organisms, called natural enemies, to control pests. Biological control should be an important part of any integrated pest management programme, an approach which combines a variety of pestcontrol methods to reduce pest levels below an economic threshold. Virtually allinsect and mite pests have some natural enemies. Managing these natural enemies inpositive perspective can effectively control many pests. Often the use of insecticidesor other practices can injure or kill natural enemies, thus increasing the survival of the remaining pest insects. *H. armigera* is known to be the most versatile insectpest that attacks more than 160 cultivated plant species including cereals, pulses,oilseeds, fibre crops, ornamental plants etc. However, the biological fitness andexceptional physiological dominance in detoxifying the insecticides as well as developing resistance to even higher doses of insecticides thrown a challenge to scientific community to control the pest.The role of *Pseudomonas* in suppressing the plant disease causing agents are quite evident, a few studies were so far undertaken to stabilize the significant impact asinsect pest control agent. The studies onpromisive effect of *Pseudomonas* on agricultural crop pests are very scanty (Otsu *et al.*, 2004; Campos *et al.*, 2009; Murat *et al.*, 2008; Blumer *et al.*, 2009 and Commare *et al.*, 2002). Ramamoorthy *et al.* (2001) reviewed some reports of influence of fluorescent pseudomonads on the growth and development of insects at all the stages of growth. The literature available on these lives clearly suggested that the insect control using *Pseudomonas* was mostly confined to control of mosquitoes and house flies (Pushpanathan *et al.*, 2009). *Pseudomonas fluorescens* having delta endotoxin gene of *Bacillus thuringiensis*, produced 4 times more toxin proteinand has the more potency to kill insect pests (Peng *et al.*, 2003). Although Bt has been widely used as biocontrol agent, particularly against lepidopter on insect, but many insect pests developed resistance against Bt endotoxins (Almin and Eriksson,1968 and Dmitri *et al.*, 2009). Therefore, it is the need of the how to search for new options for controlling the insect pests and use of pseudomonads for insect pest control could bea better option. The present study was conducted to evaluate the efficacy of *P. fluorescens* in two different areas against green bollworm, *H. armigera*.

RESOURCES AND METHODS

In order to evaluate the efficacy of *P. fluorescens* against green bollworm, *H. armigera* on cotton, two field experiments were conducted during 2014-15 and 2015-16 at Vanavarayar Institute of Agriculture Mankkadavu, Pollachi, Coimbatore district of Tamil Nadu and South Indian Millers Association (SIMA), Cotton Development and Research Association, Ponnery, Udumelpet, Tirupur district. The trials were laid out in a RBD design with seven treatments *viz.*, T_1- Foliar application of *P. fluorescens* @1%, T_2 - Soil application of *P. fluorescens 2.5 kg/ha*, T_3 - Soil and Foliar application of *P. fluorescens* @1%, T_4 - Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%, T_5 - Foliar application of *B. basianna* @ 1%, T_6 - Profenophos 50 EC @ 1 lit/ha and T_7 – control, replicated four times with the plot size of each experimental unit was 6 x 5 m. Row to row and plant to plant distance was maintain as 90 x 60 cm, respectively. The crop was raised following all standard agronomical practices. The surfactant, Teepol was added @ 1ml per litre of water to the treatments. Four rounds of sprays, were given using the hand operated Knapsac sprayer when the population of *H. armigera* exceeded the ETL of one larva/ two plant in any one replication.

The number of larvae, the total number of bolls and damaged bolls in each plot/replication were recorded on ten plants selected at random for the above observations. The observations were made at three stages *viz.*, pretreatment, third and seventh day after each spraying. The square damage, boll damage and the seed cotton yield per replication were recorded at harvest. The formula used to calculate the per cent infestation of bolls was

$$\textbf{Boll infestation (\%)} = \frac{\textbf{Number of infested bolls}}{\textbf{Total number of bolls}} \textbf{ x 100}$$

The per cent damage due to the bollworm was worked out and yield data were computed to hectare. The mean original data of percentage boll damage was calculated as percentage reduction over with the following formula (Abbott's, 1925).

$$\textbf{Per cent reduction} = \frac{\textbf{C} - \textbf{T}}{\textbf{C}} \textbf{ x 100}$$

where, C: Percentage square/boll damage of control or larval population on control

T : Percentage square/boll damage of treated plot or larval population on treatments

Statistical analysis :

The larval counts in the field experiments were transformed in to square root value and arcsine values asper the standard requisites (Gomez and Gomez, 1984). The analysis of variance in different experiments was carried out in AGRES ver. 7.01 and the means were separated by Duncan's new Multiple Range Test (DMRT) available in the package.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Trial I at Vanavarayar Institute of Agriculture (VIA)– 2014-15 :

Observations recorded on the larval population prior to treatments showed that the differences were not significant. After the second round of treatment on wards, significant differences in the larval population could be recorded fully. Table 1 revealed that all the treatments had significant effect in minimizing, recorded a pooled mean from 3.67 to 5.75 larvae/ten plants after four spraying as compared to 13.24 control.Among all the treatments the foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1% was found most effective, gave minimum population of 4.22 larvae/ten plants, with 63.29 per cent reduction over control, followed by soil and foliar application of *P. fluorescens* @ 1%. The trend was similar for square and boll damage, 11.27 and 9.22 per cent, respectively.

Trial II at South Indian Millers association (SIMA) – 2014-15 :

The statistically analyzed data presented in Table 2 showed that after four spray pooled mean number of *H. armigera* larvae ranged from 3.12 to 14.72 larvae/ten plants. The triazophos 0.05% was found highly effective among all the treatments with of 3.12 larvae/ten plants and 78.80 per cent reduction over control. The next treatments in order were foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1% (5.34 larvae/ten plant) and soil and foliar application of *P. fluorescens* @1%. (5.88 larvae/ten plants) were found effective. The trend was similar for square and boll damage.

Trial I at Vanavarayar Institute of Agriculture (VIA) – 2015-16 :

The data predicted in Table 3 revealed that after four spray pooled mean number of *H. armigera* larvae ranged from 4.97 to 6.01 larvae/ten plants foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%was observed most effective treatment by giving 4.97 larvae/ten plants with 60.68 per cent reduction over control.Followed by soil and foliar application of *P. fluorescens* @1%., foliar application of *B. basianna* @ 1%, Foliar application of *P. fluorescens* @1%, and Soil application of *P. fluorescens* 2.5 kg/ha were gave good results.The trend was similar for square and boll damage, 10.25 and 8.91 per cent, respectively.

Trial II at South Indian Millers association (SIMA) – 2015-16 :

The pooled mean number of larvae of *H. armigera*

Table 1 : Field efficacy of *P. fluorescens* against *H. armigera* on cotton (VIA- 2014-15)

Treatments	Number of larvae per 10 plants		Reduction over control (%)	Square damage (%) (pooled mean)**	Reduction over control (%)	Boll damage (%) (pooled mean)**	Reduction over control (%)
	Pre treatment count	Pooled mean**					
T_1- Foliar application of *P. fluorescens* @1%	12.23	5.31 (2.30)	59.89	12.72 (20.89)	31.83	11.01 (19.38)	31.74
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	11.61	5.75 (2.40)	56.57	13.28 (21.37)	28.83	11.64 (19.95)	27.84
T_3 – Soil and foliar application of *P. fluorescens* @1%	10.49	4.86 (2.20)	63.29	12.01 (20.28)	35.64	10.36 (18.77)	35.77
T_4 – Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	11.48	4.22 (2.05)	68.13	11.27 (19.62)	39.60	9.22 (17.68)	42.84
T_5 - Foliar application of *Beauveria basianna* @ 1%	9.54	5.27 (2.30)	60.20	12.66 (20.84)	32.15	10.94 (19.32)	32.17
T_6 – Triazophos 0.05%	12.67	3.67 (1.92)	72.28	9.21 (17.67)	50.64	6.94 (15.27)	56.97
T_7 – Untreated check	10.82	13.24 (3.64)	-	18.66 (25.59)	-	16.13 (23.68)	-
S.E. $\pm$		0.0124		0.1788		0.1507	
C.D. (P=0.05)		0.0261		0.3757		0.3167	

**Pooled mean after four rounds of spray

Table 2 : Field efficacy of *P. fluorescens* against *H. armigera* on cotton (SIMA- 2014-15)

Treatments	Number of larvae per 10 plants		Reduction over control (%)	Square damage (%) (pooled mean)**	Reduction over control (%)	Boll damage (%) (pooled mean)**	Reduction over control (%)
	Pre treatment count	Pooled mean**					
T_1- Foliar application of *P. fluorescens* @1%	13.26	6.22 (2.49)	57.54	12.85 (21.01)	34.27	9.91 (18.35)	35.40
T_2 - Soil application of *P. fluorescens* 2.5 kg/ha	9.92	6.79 (2.61)	53.87	13.49 (21.55)	30.99	10.33 (18.75)	32.66
T_3 – Soil and foliar application of *P. fluorescens* @1%	10.54	5.88 (2.42)	60.05	11.56 (19.88)	40.87	9.01 (17.47)	41.26
T_4 – Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	13.27	5.34 (2.31)	63.72	10.95 (19.33)	43.99	8.42 (16.87)	45.11
T_5 - Foliar application of *Beauveria basianna* @ 1%	12.64	6.06 (2.46)	58.83	11.67 (19.98)	40.31	9.77 (18.21)	36.31
T_6 – Triazophos 0.05%	14.51	3.12 (1.77)	78.80	8.34 (16.79)	57.34	5.68 (13.79))	62.97
T_7 – Untreated check	12.33	14.72 (3.84)	-	19.55 (26.24)	-	15.34 (23.06)	-
S.E.±		0.0145		0.0941		0.1435	
C.D. (P=0.05)		0.0305		0.1976		0.3016	

**Pooled mean after four rounds of spray

Table 3 : Field efficacy of *P. fluorescens* against *H. armigera* on cotton (VIA- 2015-16)

Treatments	Number of larvae per 10 plants		Reduction over control (%)	Square damage (%) (pooled mean)**	Reduction over control (%)	Boll damage (%) (pooled mean)**	Reduction over control (%)
	Pre treatment count	Pooled mean**					
T_1- Foliar application of *P. fluorescens* @1%	9.67	5.62 (2.37)	55.54	11.37 (19.70)	31.38	9.81 (18.25)	29.83
T_2 - Soil application of *P. fluorescens* 2.5 kg/ha	10.24	6.01 (2.45)	52.45	11.95 (20.22)	27.88	10.01 (18.45)	28.40
T_3 – Soil and foliar application of *P. fluorescens* @1%	10.46	5.31 (2.30)	57.99	10.78 (19.17)	34.94	9.23 (17.69)	33.97
T_4 – Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	10.32	4.97 (2.23)	60.68	10.25 (18.67)	38.14	8.91 (17.37)	36.27
T_5 - Foliar application of *Beauveria basianna* @ 1%	9.94	5.44 (2.33)	56.96	11.22 (19.57)	32.29	9.66 (18.11)	30.90
T_6 – Triazophos 0.05%	10.63	2.88 (1.70)	77.22	8.39 (16.84)	49.63	6.14 (14.34)	56.08
T_7 – Untreated check	9.82	12.64 (3.55)	-	16.57 (24.02)	-	13.98 (21.95)	-
S.E.±		0.0128		0.1721		0.1355	
C.D. (P=0.05)		0.0269		0.3615		0.2847	

**Pooled mean after four rounds of spray

was recorded after four spray showed in Table 4 foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1% (T_4) was found as best among all the treatments being 5.33 / ten plants and 59.86 per cent reduction overcontrol. The next effective treatments was soil and foliar application of *P. fluorescens* @1% which showed reduced 5.94 larvae/ten plants and 55.27 per cent reductionover control. The similar trend was observed in square and boll damage showed reduced 10.35 and 8.23 per cent, respectively.

Yield of seed cotton :

Observations on the yield of seed cotton showed that the soil and foliar application of *P. fluorescens* @ 1 % recorded the significantly maximum yield of 28.68 and 27.15 q/ha, respectively followed by the foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1% was next effective treatment.The trend was similar for both area at VIA and SIMA (Table 5).

The efficacy of *P. fluorescens* treatments against *H. armigera* was determined on the basis of number of larvae per ten plants, square and boll damage percentage. The data revealed that all the treatments were significantly superior over control. The minimum larval population and lowest square and boll damage percentage was observed in the foliar application of *P. fluorescens*

Table 4 : Field efficacy of *P. fluorescens* against *H. armigera* on cotton (SIMA- 2015-16)

Treatments	Number of larvae per 10 plants		Reduction over control (%)	Square damage (%) (pooled mean)**	Reduction over control (%)	Boll damage (%) (pooled mean)**	Reduction over control (%)
	Pre treatment count	Pooled mean**					
T_1- Foliar application of *P. fluorescens* @1%	10.82	6.99 (3.13)	47.36	11.52 (19.84)	35.79	9.27 (17.73)	35.13
T_2 - Soil application of *P. fluorescens* 2.5 kg/ha	9.48	7.08 (3.16)	46.69	12.01 (20.28)	33.05	9.66 (18.11)	32.40
T_3 – Soil and foliar application of *P. fluorescens* @1%	11.22	5.94 (3.04)	55.27	11.26 (19.61)	37.24	8.59 (17.05)	39.89
T_4 – Foliar application of *P. fluorescens* @ 1% and *Beauveria basianna* @ 1%	10.58	5.33 (2.98)	59.86	10.35 (18.76)	42.31	8.23 (16.67)	42.41
T_5 - Foliar application of *Beauveria basianna* @ 1%	10.13	6.58 (3.11)	50.45	11.36 (19.70)	36.68	9.01 (17.47)	36.95
T_6 – Triazophos 0.05%	10.05	2.91 (2.48)	78.09	7.64 (16.05)	57.41	5.94 (14.11)	58.43
T_7 – Untreated check	11.19	13.28 (3.74)	-	17.94 (25.06)	-	14.29 (22.21)	-
S.E.±		0.0159		0.1453		0.1214	
C.D. (P=0.05)		0.0335		0.3053		0.2550	

**Pooled mean after four rounds of spray

Table 5 : Yield of *P. fluorescens* in the control of *H. armigera* on cotton

Treatments	Seed cotton yield (q/ha)			
	VIA		SIMA	
	2014-15	2015-16	2014-15	2015-16
T_1-Foliar application of *P. fluorescens* @1%	23.90	24.37	24.61	25.22
T_2-Soil application of *P. fluorescens* 2.5 kg/ha	23.40	24.26	23.82	24.86
T_3-Soil and foliar application of *P. fluorescens* @1%	28.68	27.15	29.12	28.31
T_4-Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	26.18	25.27	27.33	27.01
T_5-Foliar application of *Beauveria basianna* @ 1%	24.70	24.08	25.44	25.75
T_6-Imidacloprid 200 SL @ 200ml/ha	27.03	25.56	27.64	26.25
T_7-Untreated check	18.78	18.25	18.94	17.34
C.D. (P=0.05)	1.49	0.65	1.0262	0.6957
S.E.±	0.71	0.31	0.4885	0.3311

@ 1 % and *Beauveria basianna* @ 1%. The highest seed cotton yield was recorded in the treatment of both soil and foliar application of *P. fluorescens* @ 1 %. The present experimental findings are supported by Rajendran *et al.* (2007) demonstrated the PGPR and endophytic bacteris mediated induction of defence response on cotton plants against bollworm (*H. armigera)* insect pest. In addition to *Pseudomonas* rhizobacteria have been reported to stimulate plant growth under field condition (Saravanakumar and Samiyappan, 2007). Further Vivekananthan *et al.* (2004) reported that application of fluorescent pseudomonads increased the fruit yield in mango. The results of two year trials revealed the potential of *P. fluorescens*as a microbial agent by causing significant reduction of *H. armigera* larval population and damage percentage. Therefore, either *P. fluorescens* treatments can beconsidered along with chemical control for developing environmentally safe, long lasting and effective IPM programme for the management of cotton bollworm in future.

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