DOI: 10.15740/HAS/AU/12.TECHSEAR(1)2017/97-104
Agriculture Update
Volume 12 | TECHSEAR-1 | 2017 | 97-104
Visit us : www.researchjournal.co.in
e ISSN-0976-6847

RESEARCH ARTICLE :

Efficacy of *Pseudomonas fluorescens* against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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ARTICLE CHRONICLE :
Received : *05.07.2017;*
Accepted : *22.07.2017*

SUMMARY : An investigation was carried out during 2014-15 and 2015-16 to evaluate the *Pseudomonas fluorescens* against the pink bollworm, *Pectinophora gossypiella* (Saunders), in Bt cotton at Vanavarayar Institute of Agriculture, Pollachi. Apart from the infestation, comparative cseed cotton yield was also assessed. The obtained results indicated that all treatments except control exhibited great reduction in pink bollworm infestation of both green boll damage and locule boll damage percentage and the larval population. The treatment could be arranged descendingly according to the general reduction of two seasons follows;Triazophos 0.05%, Soil and Foliar application of *P. fluorescens* @1%, Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%, Foliar application of *Beauveria basianna* @ 1%, Foliar application of *P. fluorescens* @1% and Soil application of *P. fluorescens* 2.5 kg/ha against pink bollworm.

How to cite this article : Manjula, T.R., Kannan, G.S. and Sivasubramanian, P. (2017). Efficacy of *Pseudomonas fluorescens* against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *Agric. Update*, **12**(TECHSEAR-1) **: 97-104; DOI: 10.15740/HAS/AU/12.TECHSEAR(1)2017/97-104.**

KEY WORDS :
Pectinophora gossypiella, *Pseudomonas flourescens*, Bt cotton

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BACKGROUND AND OBJECTIVES

Pink bollworm, *Pectinophora gossypiella* (Saunders), is one of the most serious pests of cotton occurring throughout most of the tropical and subtropical regions of the world (Ingram, 1994). The control of this pest depends largely on the application of pesticides, which has precipitated the development of resistance. As a result, in order to achieve effective control, more chemical applications per season are needed. Furthermore, control of this pest using insecticides becomes ineffective due to the concealed feeding habits of the larvae inside the cotton bolls. The continued application of insecticide to manage this pest also can lead to serious outbreaks of secondary pests species such as *Helicoverpa virescens* (tobacco budworm), *Helicoverpa zea* (bollworm), and *Bucculatrix thurberiella* (cotton leafperforator) (University of California, 1984).

World over, historically pink bollworm has become economically the most destructive insect pest of cotton. After hatching, the larvae are found in the flower, feeding on the anthers, pollens by living in a sort of web. Such flowers are characteristically twisted in the form of rosette. Later the larvae bore into the bolls, burrow through the lint penetrating deep into immature seeds. When one seed is destroyed, larvae then tunnel and enter through the developing lint and migrate to another seed and similarly to locules. The affected bolls rot and shed, while, those retained on plants open prematurely resulting in stained immature fibre (Agarwal *et al*., 1984), causing 0 per cent reduction in seed cotton yield and quality of lint (Henneberry *et al*., 1978). In North India, pink bollworm is considered as a key pest of cotton, causing a total crop failure in Punjob and Sindh during 1905, 1906 and 1911 (Khan and Rao, 1960). In peninsular region, Narayanan (1962) reported that 75 to 100 per cent bolls are liable to be damaged by pink bollworm in Karnataka. Pink bollworms spend the winter as diapausing larvae, then pupate and emerge as adults in spring and early summer (Bariola and Henneberry, 1980). After eclosion, moths disperse widely over large areas primarily from the previous years cotton fields, to find susceptible cotton or wild plants (Flint and Merkle, 1981). The pink bollworm under unprotected condition has been known to cause 2.81 to 61.87 per cent loss in seed cotton yield, 3.44 to 37.83 per cent loss in germination, 2.12 to 47.13 per cent loss in oil content and 10.66 to 59.15 per cent loss in normal opening of bolls (Patil, 2003).

Clearly, pink bollworm has been resistance to the Bt genes over time and these events also bring into sharp focus the subject of responsible use of Bt cotton technology. More specially, Indian cotton farmers have not been planting the prescribed ‘refuge’ area with non Bt cotton. Secondly, farmers have been ignoring pink bollworm specific pest management practices like cultivation of early /medium maturing cotton hybrids and strict avoidance of rejuvenation after harvest, especially in pink bollworm endemic areas, summer ploughing to destroy hibernating pink bollworm larvae and pupae; destruction of unopened bolls on stalks and in the soil, regular scouting of flowers and bolls and /or pheromone traps to decide on insecticide sprays and avoid storing of pink bollworm damaged cotton in homes. These time-tested pest management practices collectively suppress pink bollworm population in cotton fields, manage Bt resistance development and promote long term sustenance of Bt cotton technology.

The society is faced with the problem of increasing the use of pesticides to control pests in the absence of their predators or bioagents. On the other hand, there is an ever- increasing need for food and especially for improved crop production in the developing countries. Therefore some of the methods currently used to achieve higher yields, especially by pest and disease controls are environmentally undesirable. Also, manufacturing and application of conventional chemical pesticides has direct and indirect risks to man. Besides, many insects have developed market or complete resistance to many chemical insecticides. During the last few years, biologists have turned their attention to the possibility of using other organisms as biological control agents and the microbiologists contributing in the development of the efficacy of microbial substances (bacteria, fungi, virus and protozoa) for the control of many insect pests. Although a 100 or so bacteria cause diseases of insects, only few are used commercially as control agents. Some bacteria have been isolated from soil, insect habits, insect larvae or stored products.

The genus *Pseudomonas* makes commonly part of microbial communities of various insect species. Indeed, using culture-dependent and -independent approaches, pseudomonads were identified as common inhabitants of the intestinal tract or otherwise associated with field-collected or laboratory-raised larvae, pupae, and adults of representatives of the major insect orders. Examples include *Anopheles*, *Aedes* and *Culex* mosquitoes, the *Drosophila* fruit fly, and the Hessian fly *Mayetiola destructor* in the order Diptera (Corby-Harris *et al.*, 2007; Bansal *et al.*, 2011 and Osei-Poku *et al.*, 2012), *S. littoralis*, the cotton bollworm *Helicoverpa armigera*, and the gypsy moth *Lymantriadispar* in the Lepidoptera (Broderick *et al.*, 2004 and Tang *et al.*, 2012). Many of these insects feed on roots or aboveground parts of plants or spend a part of their life cycle in aquatic habitats, *i.e.*, in environments that are typically colonized by pseudomonads. It is therefore likely that pseudomonads are commonly acquired by insects via ingestion or contact. These highly versatile bacteria then may be very well-adapted to live inside or otherwise associated with their arthropod host, exploiting it as a shelter, vector, or food source.Second, the genomes of many *Pseudomonas* strains contain genetic loci with predicted function in insect interaction and insect toxicity. Third, following oral infection several *Pseudomonas* species are capable not only of colonizing insects but also of exhibiting significant pathogenicity toward insects. Besides the above-described plant-beneficial *P. protegens* and *P. chlororaphis* of the *P. fluorescens* group (Mulet *et al.*, 2012), currently only three pathogenic species are known to be capable of efficiently killing insects.

Until very recently, insecticidal activities in the *P. fluorescens* group had only been sparsely documented. Notably, strains of *P. fluorescens* were reported to exhibit insecticidal activity toward agricultural pest insects such as aphids (Hashimoto, 2002), phytophagous ladybird beetles (Otsu *et al.*, 2004), and termites (Devi and Kothamasi, 2009). In the same vein, a bioformulation of a combination of two *P. fluorescens* strains was demonstrated to simultaneously reduce the incidence of a herbivorous insect (the rice leafroller *Cnaphalocrocis medinalis*) and a phytopathogenic fungus (*Rhizoctonia solani*) in rice under greenhouse and field conditions (Commare *et al.*, 2002 and Karthiba *et al.*, 2010).The present study was carried out to assess the efficacy of *P. fluorescens* against pink bollworm *P. gossypiella* on cotton under field condition

RESOURCES AND METHODS

Two field experiments were conducted at Vanavarayar Institute of Agriculture, Pollachi, Coimbatore District during winter season of 2014 -15 and 2015-16 with Bt cotton under irrigated conditions. During 2014-15 and 2015-16, *P. fluorescens* was evaluated against the pink bollworm, *P. gossypiella*. The experiment was laid out in Randomized Block Design (RBD). There were six 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. along with a T_7 - control treatment. Recommended agronomic practices were followed for raising the crop. Each treatment was replicated four times. 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. Three sprays were given at 95 DAS, 110 DAS and 125 DAS. Pre treatment count was recorded before the spray and subsequent post treatment counts were considered as pre treatment count for subsequent spray. Hundred bolls were collected from each treatment and percentage of green boll damage, locule damage, number of larvae present and seed cotton yield were recorded.

The per cent infestation was calculated by the following formula :

$$\% \text{ infestation} = \frac{\text{No. of green bolls damaged}}{\text{Total no. of bolls}}$$

Biweekly pest scouting was carried out before and after the treatment spray upon attainment of economic threshold level (ETL) of both pink boll worm and spotted bollworm infestation (5 larvae/ 25 plants or 10% infestation of fruiting bodies). Treatments were sprayed according to their label recommended dose with the help of knapsack hand sprayer early in the morning using hollow- cone nozzle. Samples of 100 green bolls per treatment (25 bolls for each treatment) were taken at random and dissected. For each treatment, reduction percentages in bollworm infestation, bollworm larval content were calculated using Henderson and Titlon equation. (Henderson and Tilton, 1955) as follows.

% Reduction = [1- { (Control before* treatment after)/(Control after* treatment before) }]* 100.

The seed cotton yield for each plot was harvested and weighed then mean weight of seed cotton yield was compared among the treatment and the untreated check. Data were analyzed using analysis of variance followed by Tukey's multiple comparison test (Gomez and Gomez, 1984).

OBSERVATIONS AND ANALYSIS

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

Green boll damage :

Results in Table 1 the efficacy of *P. fluorescens* against the pink bollworm, *P. gossypiella* green boll damage percentage during three sprays in 2014 -16 season. The obtained results indicated that based on green boll damage percentage of pink bollworm was significant difference among the treatments. The mean green boll damage percentage was ranged between 8.8. to 20.44 percentage. The chemical treatment of Triazophos recorded the lowest green boll damage (8.80%) and the highest reduction over control (59.65%) followed by soil and foliar application of *P. fluorescens* was the most effective treatment as they reduce 39.48% and 12.37%

of green boll damage. While soil application of *P. fluorescens* alone was the least effective which reached to 25.34 percentage of reduction over control. In 2015-16 cotton season the green boll damage percentage by pink bollworm was less than 2014-15 cotton season after the three spray and could be arranged descendingly as follows, soil and foliar application of *P. fluorescens* (9.93%) followed by foliar application of *P. fluorescens* and *B. bassiana* (10.73%) and soil application of *P. fluorescens* was the least green boll damage reduction over control (32.45 %) (Table 4). According to general green boll damage percentage of two seasons, it was clear that the soil and foliar application of *P. fluorescens*was effective treatment than other treatments.

Locule damage :

Data present in Table 2 and 5 showed the effects of the same treatments of *P. fluorescens* against the pink bollworm, locule damage percentage were recorded during three sprays in 2014-15 and 2015-16 cotton season. The obtained results indicated that, based on the average mean of two season damage percentage, after three sprays. The soil and foliar application of *P. fluorescens* was most effective treatment recorded 19.87 % of locule damage followed by foliar application of both *P. fluorescens* and *B. bassiana* (21.55%). In the untreated check observed 32.38% of locule damage. While 2015-16 cotton season the treatments of soil and foliar application of *P. fluorescens* and foliar application of both *P. fluorescens* and *B. bassiana* were recorded 15.92 % and 16.91 % of locule damage, respectively. According to general average locule damage percentage of two seasons, the data was indicated that the soil and foliar application of *P. fluorescens* induced the highest effect than other treatments.

Larval population :

The number of larvae per 25 bolls was on par in all the treatments and less than the untreated check. The

Table 1 : Evaluation of *P. fluorescens* against *P.gossypiella* (2014-15)

Treatments	Green boll damage % 2014-15				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	11.64 (19.95)	15.98 (23.56)	16.52 (23.98)	14.71 (22.55)	28.03
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	12.39 (20.61)	16.07 (23.63)	17.32 (24.59)	15.26 (22.99)	25.34
T_3 - Soil and foliar application of *P. fluorescens* @1%	9.21 (17.67)	13.64 (21.67)	14.25 (22.18)	12.37 (20.59)	39.48
T_4 - Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	10.11 (18.54)	14.88 (22.69)	15.67 (23.32)	13.55 (21.60)	33.71
T_5 - Foliar application of *Beauveria basianna* @ 1%	10.98 (19.35)	15.34 (23.06)	15.94 (23.53)	14.09 (22.05)	31.07
T_6 - Triazophos 0.05%	6.82 (15.14)	8.57 (17.02)	11.01 (19.38)	8.80 (17.25)	56.95
T_7 - Untreated check	15.32 (23.04)	20.32 (26.79)	25.67 (30.44)	20.44 (26.88)	-
S.E.$\pm$	0.1486	0.2088	0.1956	0.1521	-
C.D. (P=0.05)	0.3123	0.4386	0.4110	0.3195	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values.

Table 2 : Evaluation of *P. fluorescens* against *P. gossypiella* (2014-15)

Treatments	Locule damage % 2014-15				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	16.01 (23.59)	26.28 (30.84)	27.43 (31.58)	23.24 (28.82)	28.23
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	16.98 (24.33)	26.94 (31.27)	27.98 (31.94)	23.97 (29.31)	25.97
T_3 – Soil and Foliar application of *P. fluorescens* @1%	12.48 (20.69)	22.46 (28.29)	24.66 (29.77)	19.87 (26.47)	38.63
T_4 -Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	14.66 (22.51)	24.01 (29.34)	25.97 (30.64)	21.55 (27.66)	33.45
T_5 - Foliar application of *Beauveria basianna* @ 1%	15.24 (22.98)	24.97 (29.98)	26.38 (30.91)	22.20 (28.11)	31.44
T_6 – Triazophos 0.05%	10.9 (19.28)	15.36 (23.08)	18.56 (25.52)	14.94 (22.74)	53.86
T_7 – Untreated check	22.75 (28.49)	35.84 (36.77)	38.54 (38.38)	32.38 (34.68)	-
S.E. $\pm$	0.2123	0.1781	0.1917	0.1918	-
C.D. (P=0.05)	0.4460	0.3742	0.4027	0.4029	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values.

mean population of after three sprays ranged from 2.48 larvae per 25 bolls in Triazophos chemical treatment which was significantly superior to other treatments. The next in order was soil and foliar application of *P. fluorescens* treatment was with a population of 4.83 per 25 bolls. The per cent reduction in larval population over control was maximum in Triazophos (78.66 %) followed by soil and foliar application of *P. fluorescens*(58.43 %).

Seed cotton yield :

There was significant difference among the treatment with respect to seed cotton yield. However,

Table 3 : Evaluation of *P. fluorescens* against *P. gossypiella* (2014-15)

Treatments	Larval population/20 bolls				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	5.66 (2.38)	6.84 (2.62)	6.24 (2.50)	6.25 (2.50)	46.21
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	6.24 (2.50)	6.99 (2.64)	6.87 (2.62)	6.70 (2.59)	42.34
T_3 -Soil and Foliar application of *P. fluorescens* @1%	4.21 (2.05)	5.31 (2.30)	4.97 (2.23)	4.83 (2.20)	58.43
T_4 - Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	4.94 (2.22)	5.97 (2.44)	5.28 (2.30)	5.40 (2.32)	53.52
T_5 - Foliar application of *Beauveria basianna* @ 1%	5.01 (2.24)	6.07 (2.46)	5.99 (2.45)	5.69 (2.39)	51.03
T_6 - Triazophos 0.05%	2.62 (1.62)	2.94 (1.72)	1.89 (1.37)	2.48 (1.58)	78.66
T_7 - Untreated check	10.67 (3.27)	12.5 (3.53)	11.67 (3.42)	11.62 (3.41)	-
S.E. ±	0.0104	0.0207	0.0143	0.0194	-
C.D. (P=0.05)	0.0218	0.0434	0.0301	0.0407	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values

Table 4 : Evaluation of *P. fluorescens* against *P.gossypiella* (2015-16)

Treatments	Green boll damage % 2015-16				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	11.36 (19.70)	13.64 (21.67)	11.07 (19.44)	12.02 (20.28)	37.59
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	12.74 (20.91)	13.97 (21.95)	12.31 (20.54)	13.01 (21.14)	32.45
T_3 – Soil and Foliar application of *P. fluorescens* @1%	8.33 (16.77)	11.91 (20.19)	9.54 (17.99)	9.93 (18.36)	48.44
T_4 – Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	9.67 (18.12)	12.65 (20.84)	9.88 (18.32)	10.73 (19.12)	44.29
T_5 - Foliar application of *Beauveria basianna* @ 1%	10.01 (18.44)	13.01 (21.14)	10.34 (18.76)	11.12 (19.48)	42.26
T_6 – Triazophos 0.05%	4.91 (12.80)	7.36 (15.74)	5.97 (14.14)	6.08 (14.27)	68.43
T_7 – Untreated check	14.68 (22.53)	21.61 (27.70)	21.49 (27.62)	19.26 (26.03)	-
S.E.±	0.1424	0.1475	0.1256	0.1787	-
C.D. (P=0.05)	0.2992	0.3098	0.2638	0.3755	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values.

Table 5 : Evaluation of *P. fluorescens* against *P.gossypiella* (2015-16)

Treatments	Locule damage % 2015-16				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	12.73 (20.90)	22.34 (28.21)	19.24 (26.02)	18.10 (25.18)	39.60
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	12.97 (21.11)	22.69 (28.44)	19.81 (26.43)	18.49 (25.47)	38.30
T_3 - Soil and Foliar application of *P. fluorescens* @1%	10.94 (19.32)	19.37 (26.11)	17.46 (24.70)	15.92 (23.51)	46.88
T_4 - Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	11.37 (19.71)	21.08 (27.33)	18.27 (25.31)	16.91 (24.28)	43.57
T_5 - Foliar application of *Beauveria basianna* @ 1%	11.99 (20.26)	21.94 (27.93)	18.94 (25.80)	17.62 (24.82)	41.21
T_6 - Triazophos 0.05%	6.28 (14.51)	11.34 (19.68)	10.55 (18.95)	9.39 (17.84)	68.67
T_7 - Untreated check	20.69 (27.06)	33.58 (35.41)	35.64 (36.65)	29.97 (33.19)	-
S.E.±	0.1383	0.2253	0.2169	0.1495	-
C.D (P=0.05)	0.2906	0.4734	0.4557	0.3141	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values.

Table 6: Evaluation of *P. fluorescens* against *P.gossypiella* (2015-16)

Treatments	Larval population/20 bolls				Reduction over control
	105DAS*	130DAS*	150DAS*	Mean	
T_1- Foliar application of *P. fluorescens* @1%	5.27 (2.30)	5.88 (2.42)	4.35 (2.09)	5.17 (2.27)	39.95
T_2 - Soil application of *P. fluorescens 2.5 kg/ha*	5.55 (2.36)	5.94 (2.44)	4.68 (2.16)	5.39 (2.32)	37.39
T_3 - Soil and Foliar application of *P. fluorescens* @1%	3.95 (1.99)	4.32 (2.08)	3.66 (1.91)	3.98 (1.99)	53.77
T_4 - Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	4.22 (2.05)	4.92 (2.22)	3.91 (1.98)	4.35 (2.09)	49.48
T_5 - Foliar application of *Beauveria basianna* @ 1%	4.97 (2.23)	5.34 (2.31)	4.05 (2.01)	4.79 (2.19)	44.36
T_6 - Triazophos 0.05%	1.88 (1.37)	2.61 (1.62)	1.94 (1.39)	2.14 (1.46)	75.14
T_7 - Untreated check	8.57 (2.93)	9.65 (3.11)	7.62 (2.76)	8.61 (2.93)	-
S.E.±	0.0143	0.0208	0.0140	0.0102	-
C.D. (P=0.05)	0.0300	0.0437	0.0293	0.0215	-

DAS: Days after spray
Figures in parentheses are arcsine transformed values

Table 7 : Average seed cotton yield of Bt cotton treated with *P. fluorescens*

Treatments	Seed cotton yield (q/ha)	
	2014-15	2015-16
T_1-Foliar application of *P. fluorescens* @1%	23.90	24.37
T_2-Soil application of *P. fluorescens* 2.5 kg/ha	23.40	24.26
T_3-Soil and Foliar application of *P. fluorescens* @1%	28.68	27.15
T_4-Foliar application of *P. fluorescens* @1% and *Beauveria basianna* @ 1%	26.18	25.27
T_5-Foliar application of *Beauveria basianna* @ 1%	24.70	24.08
T_6-Imidacloprid 200 SL @ 200ml/ha	27.03	25.56
T_7-Untreated check	18.78	18.25
C.D. (P=0.05)	1.49	0.65
S.E.±	0.71	0.31

numerically higher yield was recorded in the soil and foliar application of *P. fluorescens* which was one of effective treatment. Seed cotton yield of 28.68q/ha and 27.15q/ha were recorded in T_3 treatment in 2014-15 and 2015-16, respectively.

Earlier studies, which showed that application of *P. fluorescens* strain reduced aphid and bollworm incidence in cotton plants through altered feeding behaviour, which in turn resulted in reduced larval and pupalweight and increased mortality (Rajajandran, 2003 and Bhuvaneswari, 2005). Duraisamy *et al.* (2007) revealed that a combination of flurorescent pseudomonad strains affects the development of leaffolder pest by inducing defense molecules in rice plants which ii turn enhance resistance to leffolder attack. Histopathological studies showed that tissues of alimentary tract and body cavity of *Heterotermesindicola* were very susceptible to *P. fluorescens* (Kahalid *et al.*, 2008). In the present experiments in field condition the soil and foliar application of *P. fluorescens* performed better than other treatments. From the above evidence it is assumed that the reduced pink bollworm incidence in cotton plants by *P. fluorescens* bioformulation and this might be useful for developing a sustainable management strategy for pink bollworm pest.

Acknowledgement :

The authors acknowledge the Vanavarayar Institute of Agriculture and The Southern India Mills' Association (SIMA) for providing support for conducting the experiments successfully. And also thanks to Tropical Agro for providing the bio inoculants for the study.

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