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Field evaluation of *Pseudomonas fluorescens* against the sucking pest complex and impact on natural enemies in Bt cotton

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Abstract
Pseudomonas fluorescens was evaluated in 2013-14 and 2014 -15 cotton growing season against the sucking pest complex infested in Bt cotton, aphid, *Aphis gossypii*, leafhopper, *Amrasca devastans* and thrips, *Thrips tabaci*. Reduction in the infestation and pest population was determined after three successive sprays with 15 days interval. The effect of *P. fluorescens* on seed cotton yield was considered and compared with the untreated check. Side effect on natural enemies, ladybird beetle, *Menochilus sexmaculatus*, green lacewing, *Chrysoperla carnea*, hover fly, *Syrpus* sp. and spiders population were also considered. Data obtained from the present study revealed that the soil and foliar application of *P. fluorescens* was found to be effective in reducing the aphid, leafhopper and thrips population more than 58, 77 and 40 % respectively. Imidacloprid was found to be the most effective chemical treatment which records the least population of aphid, 13.86/3 leaves, leafhopper, 2.90/3 leaves and thrips, 13.80/ 3 leaves and was followed by soil and foliar application of *P. fluorescens*, 17.98, 3.63 and 19.45/3 leaves of aphid, leafhopper and thrips respectively. The imidacloprid treatment was associated with the greatest reduction in the population of the natural enemies. In contrast *P. fluorescens* treatments had the minimum side effect on the natural enemies. Significantly higher seed cotton yield of 27.64q/ha was harvested with soil and foliar application of *P. fluorescens* plots.

Keywords: *Pseudomonas fluorescens*, Bt cotton, sucking pest complex, natural enemies

1. Introduction
Cotton is an important cash crop unanimously designated as king of fiber crops playing a key role in the economy of Indian farmers. In India 45% of the pesticides are applied in cotton alone (David 2008). Pesticide load in crop ecosystem has culminated in many undesirable effects such as resistance, resurgence, residues etc., disturbing the agro ecosystem. With the changes in agronomical practices after the introduction of Bt cotton genotype for commercial cultivation. The Bt toxin can effectively control specific lepidopteron species but lack resistance against sucking pests, aphid, leafhopper, thrips and whitefly. These sucking pests occur at all the stages of crop growth and responsible for indirect yield losses. A reduction of 22.85% in seed cotton yield due to sucking pests has been reported by Satpute *et al* 1990 [22] and Dhawan *et al* 1988 [8]. In impact assessment of transgenic cotton a little attention has been given on the changing dynamics of sucking pests and other non-target organism with Bt cotton it has been experienced that reduction in usage of insecticides lead to increased population of sucking insect pest (Men *et al* 2005) [13]. The sucking pests causes great damage indirectly to cotton by secreting honey dew and transmitting viral disease. The sucking insects ingest phloem sap from the plants thus the plants reduce vigor, severe infestations destroy terminal buds and infested plant produce excessive branching (Bohnfalk *et al*., 1996) [6]. The aphid and whitefly secrete honey dew on cotton lint which creates problem during lint processing at textile mills (Bellows et. al., 1994; Bohnfalk *et al*., 1996, Bi *et al*., 2001) [4, 6, 5]. Moreover the deposition of honeydew droplets on leaves provides a suitable substrate for sooty mold development, which inhibits foliar photosynthesis and reduce yield and quality (Bohnfalk *et al*., 1996; Bi *et al*., 2001) [6, 5].
A huge number of synthetic pesticides are used annually for the control of these insects. Some pesticides have active ingredients that act as hormone disruptors and may cause infertility, carcinogenesis and mutagenesis to most crops has meant that pesticides are present in the ecosystem aquifers and water system of most agricultural areas. In the long term this could have repercussions on both environment and human health. Therefore there is an urgent need

to replace pesticides with alternative means of control that are safe, low in cost, local in production and also environment friendly. Bio pesticides or biological pesticide on pathogenic microorganisms specific to a target pest offers an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health.

The predators associated with cotton pests include beetles, lacewing, spiders and predatory mites (Hoffmann and Frodsahm, 1993) [10]. The most abundant predatory inhabitants in the field are lady bird beetles, syrphids, lacewing and spiders (Azad *et al*., 2010) [3]. Based on many years of research it is stated that bacterial biological preparation at rates recommended for use in agriculture show low toxicity to the predators *Coccinella septempunctata* and *Chrysoperla carnea* (Mikul'skaya, 2000) [14]. The green lacewing, *C. carnea* is one of the most common arthropod predators (Tauber *et al*., 2000; McEwen *et al*., 2001) [25, 12] with a wide prey range including aphids egg, neonates, mites and other soft bodied insects (New 1995; McEwen *et al*., 2001) [12]. There is an increasing interest in the ecology of polyphagous predators in agriculture spiders are important natural enemies of many insect pests, as they are generalist predators and comprise a large part of the beneficial arthropod community in the Agricultural field (Nyffeler, 1982; Richert and Lockley, 1984; Sunderland *et al*, 1986) [18, 20, 24]. However spiders are also easily affected by pesticides (Volkmar 1995) [27].

Pseudomonas fluorescens as seed treatment against cotton leaf hopper, *Amrasca devastans* (Murugesan and kavitha, 2009) [15]. *P. fluorescens* significant impact on whitefly, *Bemisia tabcii* (Soundarajan and Chitra, 2011) [23] and foliar application of *P. fluorescens* induced resistance in onion thrips, *Thrips tabci* (Sanjay and Sivasubramanian, 2012) [21]. The mechanism of plant disease controlled by *P. fluorescens* like production of antibiotics, siderophores, volatile compounds like HCN and ammonia, induction of systemic resistance and competition for nutrients may be the cause of the reduction in leaf hopper population in cotton (Muthusamy, 1999 and Vidhyasekaran,1999) [16, 26]. *P. fluorescens* attack on various species of termites and moreover *P. fluorescens* is safer than *P. aeruginosa*. Kahalid *et al.* 2008 [11] and Amsalingam *et al.* 2011 [2] reported that the mortality of red spider mites caused by *P. fluorescens.* The present study was carried out to assess the efficacy of *P. fluorescens against* sucking pests on cotton under field condition.

2. Materials and Methods

Field experiments were carried out during winter season in 2013- 14 to 2014-15 at Vanavarayar Institute of Agriculture, Mankkadavu, Pollachi, Coimbatore District of Tamil Nadu. The experiments were configured with seven treatments which were replicated four times. Cotton hybrid RCH 20 Bt was sown in a plot size 5 x 4 m with a spacing of 90 x 60 cm. The crop was raised following all standard agronomical practices. The treatments were imposed as and when sucking pests crossed ETL viz. 2 nymphs of jassids, ten nymphs or adults of thrips and aphids per leaf.

The spray materials were prepared at their recommended doses and spray the bioinaculants by knapsack sprayer. The population of sucking pests viz. aphids, leafhopper. thrips and natural enemies, ladybird beetle, *Menochilus sexmaculatus*, green lacewing, *Chrysoperla carnea*, hover fly, *Syrpus* sp. and spiders from each plot were recorded24 hrs. before and after spray of bioinaculants from ten randomly selected plants. The populations of aphids, leafhoppers, thrips as well as their natural enemies were recorded from top, middle and bottom leaves of the plants and averaged as per plant of the insects.

Observations were subjected to statistical analysis to assess the impact of *P. fluorescens* application on pest incidence. Seed cotton yield was recorded for each treatment and finally expressed in quintal/ha.

2.1 Statistical analysis

The data collected were transformed into square root value as per the standard requisites (Gomez and Gomez, 1984) [9]. The experiments were subjected to statistical scrutiny following the method of Panse and Sukhatme (1989) [19] and the means were compared with Least significant Difference.

3. Results and Discussion

The field trial conducted during 2013-14, before the imposition of treatment, population of sucking pests was quite uniform and above the economic threshold level. One week after the application, aphid, leafhopper and thrips population reduced considerably and registered 19.55, 2.75 and 23.0 /3 leaves respectively in the plots treated with soil and foliar application of *P. fluorescens* @ 1% which was statistically on par with standard chemical check imidocloprid 200 SL @ 200ml/ha (16.4. 2.20and 17.95 /3 leaves). Significantly higher seed cotton yield of 26.6 q/ha was obtained from T3 treatment, soil and foliar application of *P. fluorescens* @ 1% and comparable to imidocloprid 200 SL @ 200ml/ha recorded 25.60 q/ha. The next best treatment was T4, combination of *P. fluorescens* @ 1% and *Beauveria bassiana* @ 1 % and statistically on par with standard chemical check imidocloprid 200 SL @ 200ml/ha. (Table.1).

During 2014-15 also prior to the application of bio inoculant treatment, population of all sucking pest complex was quite uniform and also above ETL. (Table 2). However the standard chemical checks imidocloprid 200 SL @ 200ml/ha record a significantly least number of aphid, leafhopper, thrips, 11.33, 3.6. 9.65 /3 leaves respectively and was found to be significantly lower population of sucking pest complex 16.4, 4.5 and 15.10 /3 leaves observed in soil and foliar application of *P. fluorescens* @ 1%. The seed cotton yield was significantly highest in the same T3 treatment recorded 28.68 q/ha and followed by T4 treatment, combination of *P. fluorescens* @ 1% and *Beauveria bassiana* @ 1 % applied plot recorded 26.18q/ha.

Pooled observations of 2013-14 to 2014-15 revealed that significantly lower sucking pest complex, aphid, leafhopper and thrips were noticed in T3 treatment, soil and foliar application of *P. fluorescens* @ 1% recorded 17.98, 3.63 and 19.45 /3 leaves which was found to be quite effective and par with standard chemical check imidocloprid 200 SL @ 200ml/ha (13.86, 2.90 and 13.80 /3 leaves respectively) (Table.3). The seed cotton yield obtained from the different treatments of *P. fluorescens* was significantly higher compared to the untreated check (18.98q/ha). The soil and foliar application of *P. fluorescens* @ 1% registered higher seed cotton yield of 27.64 q/ha and was statistically comparable with standard chemical check imidocloprid 200 SL @ 200ml/ha (26.31q/ha) and combination of *P. fluorescens* @ 1% and *Beauveria bassiana* @ 1 % treated plot (25.34q/ha) were next best options.

Mean number of predators counted in bioinaculants treatments, standard pesticide and untreated check treatments

is recorded in (Table.4 & 5 and Fig.1). The natural enemies included, ladybird beetle, *Menochilus sexmaculatus*, green lacewing, *Chrysoperla carnea*, hover fly, *Syrpus* sp. and spiders population. Mean number of predators averaged 1.20 to 4.25 in the pre spray count. After the three successive sprays the general average of predators declined to ≤ 1.1 in pesticide treatemnts. In contrast the bioinaculnts, *P. fluorescens* and *B. bassiana* treatments showed a mean number of predators (2.8) was statically similar to that in the control (3.7). However, was significantly higher than the conventional pesticide treatment.

Several earlier workers also reported that the *P. fluorescens* were best ones which were better control of cotton leafhopper, *Amrasca devastans* than dimethoate (Murugasen and Kavitha 2009) [15]. Soundarajan and chitra 2011 [23] reported that *P. fluorescens* had significant impact on sucking pest complex in urdbean. The lowest thrips population per plant was recorded in *P. fluorescens* treated onion plots reported by Sanjoy and sivasubramanian 2012 [21]. Kahalid 2008 [11] reported that *P. fluorescens* attack on various species of termites. The mortality of red spider mites caused by *P. fluorescens* reported by Amsalingam, 2011 [2]. Abdelrahman *et al*., 2007 suggested to use gamma cyhalothrin in rotation with bioinaculants to minimize the side effect on natural enemies.

The results of two year trials revealed the potential of *P. fluorescens* as a microbial agent by causing significant mortality of sucking pest complex in Bt cotton and can be best utilized for the ecofriendly IPM programme of either BT cotton or conventional cotton cropping system.

Table 1: Bio efficacy of *P. fluorescens* against sucking pest complex in Bt cotton

Treatment	2013-14						
	No. of Aphid/3 leaves		No. of Leaf hopper / 3 leaves		No. of Thrips/3 leaves		Seed cotton yield (q/ha)
	BS	7DAS	BS	7DAS	BS	7DAS	
T1- Foliar application of *P. fluorescens* @1%	51.00(7.09)	26.38(5.13)	11.33(3.35)	3.50(1.86)	49.85(7.06)	28.40(5.33)	23.20
T2 - Soil application of *P. fluorescens 2.5 kg/ha*	49.00(6.92)	26.60(5.15)	9.63(3.10)	3.80(1.95)	44.05(6.64)	28.95(5.38)	22.80
T3 – Soil and Foliar application of *P. fluorescens* @1%	45.00(6.67)	19.55(4.42)	11.48(3.38)	2.75(1.66)	52.63(7.25)	23.80(4.87)	26.60
T4 – Foliar application of *P. fluorescens* @1% & *Beauveria basianna* @ 1%	47.50(6.87)	21.45(4.63)	10.83(3.28)	3.15(1.76)	48.35(6.95)	25.40(5.04)	24.50
T5 - Foliar application of *Beauveria basianna* @ 1%	45.50(6.71)	23.05(4.80)	9.65(3.10)	3.40(1.84)	43.83(6.62)	27.70(5.26)	23.83
T6 – imidacloprid 200 SL @ 200ml/ha	44.75(6.66)	16.40(4.05)	10.98(3.31)	2.20(1.48)	46.30(6.80)	17.95(4.22)	25.60
T7 – Untreated check	47.00(6.78)	57.15(7.53)	10.55(3.25)	13.90(3.71)	47.20(6.87)	53.50(7.31)	19.18
CV%	13.85	5.57	6.49	11.93	2.76	4.66	5.28
CD (p=0.05)	1.40	0.42	0.31	0.36	0.28	0.37	1.86
SEm±	0.67	0.20	0.15	0.17	0.13	0.18	0.88

No. of sprays: Three Hybrid: RCH 20 BS: Before spray 7DAS: 7 days after spray
Figures in parentheses are square root transformed values.

Table 2: Bio efficacy of *P. fluorescens* against sucking pest complex in Bt cotton

Treatment	2014-15						
	No. of Aphid/3 leaves		No. of Leaf hopper /3 leaves		No. of Thrips/3 leaves		Seed cotton yield (q/ha)
	BS	7DAS	BS	7DAS	BS	7DAS	
T1- Foliar application of P. fluorescens @1%	40.61(6.37)	22.90(4.78)	12.50(3.53)	6.6(2.57)	41.58(6.45)	20.45(4.52)	23.90
T2 - Soil application of P. fluorescens 2.5 kg/ha	35.60(5.97)	22.00(4.69)	13.70(3.70)	6.7(2.59)	35.18(5.93)	21.00(4.58)	23.40
T3 – Soil and Foliar application of P. fluorescens @1%	40.88(6.39)	16.40(4.05)	14.63(3.82)	4.5(2.11)	42.25(6.50)	15.10(3.87)	28.68
T4 – Foliar application of P. fluorescens @1% & Beauveria basianna @ 1%	35.05(5.92)	18.60(4.31)	13.30(3.65)	5.4(2.31)	32.00(5.66)	17.04(4.12)	26.18
T5 - Foliar application of Beauveria basianna @ 1%	37.30(6.11)	20.80(4.56)	14.80(3.85)	5.9(2.43)	31.90(5.65)	19.25(4.38)	24.70
T6 – imidacloprid 200 SL @ 200ml/ha	33.53(5.79)	11.33(3.36)	14.30(3.78)	3.6(1.89)	33.20(5.76)	9.65(3.10)	27.03
T7 – Untreated check	36.53(6.04)	40.15(6.33)	12.90(3.59)	14.8(3.85)	35.83(5.98)	46.25(6.80)	18.78
CV%	1.94	4.22	3.18	6.61	2.49	3.99	4.08
CD (p=0.05)	0.18	0.29	0.18	0.25	0.22	0.27	1.49
SEm±	0.08	0.14	0.08	0.12	0.11	0.13	0.71

No. of sprays: Three Hybrid: RCH 20 BS: Before spray 7DAS: 7 days after spray
Figures in parentheses are square root transformed values.

Table 3: Bio efficacy of *P. fluorescens* against sucking pest complex in Bt cotton

Treatment	2013-14 & 2014-15						
	No. of Aphid/3 leaves		No. of Leaf hopper /3 leaves		No. of Thrips/3 leaves		Seed cotton yield (q/ha)
	BS	7DAS	BS	7DAS	BS	7DAS	
T1- Foliar application of P. fluorescens @1%	45.81 (6.87)	24.64 (4.96)	11.91 (3.32)	5.05 (2.24)	45.71 (6.76)	24.43 (4.94)	23.55
T2 - Soil application of P. fluorescens 2.5 kg/ha	42.30 (6.42)	24.30 (4.93)	11.66 (3.41)	5.25 (2.29)	39.61 (6.29)	24.98 (5.00)	23.10
T3 – Soil and Foliar application of P. fluorescens @1%	42.94 (6.36)	17.98 (4.24)	13.05 (3.58)	3.63 (1.90)	47.44 (6.89)	19.45 (4.40)	27.64

T4 – Foliar application of P. fluorescens @1% & *Beauveria basianna* @ 1%	41.28 (6.54)	20.03 (4.47)	12.06 (3.61)	4.28 (2.06)	40.18 (6.34)	21.22 (4.61)	25.34
T5 - Foliar application of Beauveria basianna @ 1%	41.40 (6.44)	21.93 (4.68)	12.23 (3.52)	4.65 (2.15)	37.86 (6.15)	23.48 (4.84)	24.26
T6 – imidacloprid 200 SL @ 200ml/ha	39.14 (6.17)	13.86 (3.72)	12.64 (3.60)	2.90 (1.70)	39.75 (6.30)	13.80 (3.71)	26.31
T7 – Untreated check	41.76 (6.16)	48.65 (6.96)	11.73 (3.44)	14.35 (3.78)	41.51 (6.44)	49.88 (7.06)	18.98
CV%	6.20	3.8	1.68	7.78	2.09	3.83	2.77
CD (p=0.05)	0.59	0.27	0.09	0.27	0.20	0.28	0.99
SEm±	0.28	0.13	0.14	0.13	0.10	0.13	0.47

No. of sprays: Three Hybrid: RCH 20 BS: Before spray 7DAS: 7 days after spray
Figures in parentheses are square root transformed values.

Table 4: Impact of *Pseudomonas fluorescens* on field population of Lady bird beetles and Syrphid on Bt cotton during 2013-14 & 2014-15

Treatments	No. of Lady bird beetles/ plant			No. of Syrphids/ plant		
	BS	7DAS	15 DAS	BS	7DAS	15 DAS
T1- Foliar application of *P. fluorescens* @1%	4.25	2.39	2.80	2.38	2.20	2.80
T2 - Soil application of *P. fluorescens 2.5 kg/ha*	3.20	2.61	3.00	3.15	2.43	2.90
T3 – Soil and Foliar application of *P. fluorescens* @1%	2.95	2.00	2.60	2.80	2.45	2.80
T4 – Foliar application of *P. fluorescens* @1% & *Beauveria basianna* @ 1%	3.85	2.20	2.60	2.88	2.00	2.70
T5 - Foliar application of *Beauveria basianna* @ 1%	4.00	2.20	2.90	2.78	2.20	2.58
T6 – imidacloprid 200 SL @ 200ml/ha	3.60	1.40	1.62	3.00	0.90	1.10
T7 – Untreated check	3.78	4.20	4.40	3.10	3.60	3.70
CV%	14.14	15.07	14.15	15.26	19.53	17.23
CD (p=0.05)	0.77	0.54	0.59	0.65	0.65	0.68
SEm±	0.37	0.26	0.28	0.31	0.31	0.32

No. of sprays: Three Hybrid: RCH 20
BS: Before spray 7DAS: 7 days after spray, 15 DAS: 15 days after spray

Table 5: Impact of *Pseudomonas fluorescens* on field population of *Chrysoperla* and Spiders on Bt cotton during 2013-14 & 2014-15

Treatments	No. of *Chrysoperla*/ plant			No. of Spiders/ plant		
	BS	7DAS	15 DAS	BS	7DAS	15 DAS
T1- Foliar application of *P. fluorescens* @1%	1.88	1.35	1.48	1.84	1.48	1.78
T2 - Soil application of *P. fluorescens 2.5 kg/ha*	1.90	1.39	1.50	1.71	1.62	1.85
T3 – Soil and Foliar application of *P. fluorescens* @1%	1.13	1.30	1.45	2.05	1.45	1.74
T4 – Foliar application of *P. fluorescens* @1% & *Beauveria basianna* @ 1%	1.79	1.23	1.43	1.82	1.44	1.69
T5 - Foliar application of *Beauveria basianna* @ 1%	1.20	1.16	1.24	1.92	1.43	1.66
T6 – imidacloprid 200 SL @ 200ml/ha	1.80	0.70	0.70	2.16	0.86	0.92
T7 – Untreated check	1.84	2.15	2.03	1.67	2.05	2.23
CV%	21.91	13.55	16.85	13.42	16.97	20.06
CD (p=0.05)	0.54	0.27	0.35	0.37	0.36	0.50
SEm±	0.26	0.13	0.17	0.18	0.17	0.24

No. of sprays: Three Hybrid: RCH 20
BS: Before spray 7DAS: 7 days after spray 15 DAS: 15 days after spray

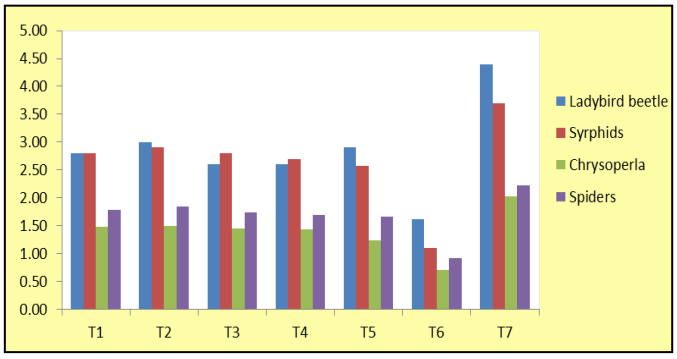

Fig 1: Impact of *Pseudomonas fluorescens* on natural enemies of sucking pests in Bt cotton

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