Madras Agric. J., 98 (7-9): 273-276, September 2011
https://doi.org/10.29321/MAJ.10.100295

Antixenosis Induced by Talc-based Bioformulation *Pseudomonas fluorescens* against Okra Shoot and Fruit Borer, *Earias vittella*

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In studies undertaken to assess the antixenotic resistance induced by talc-based bioformulations, the microbial consortia *Pseudomonas fluorescens* Pf1 + *Beauveria bassiana* B2 isolate (Pf1 + B2) treated plants were least preferred by the *Earias vittella* (Fab) moths for settling (3.11 and 6.00 nos.) and oviposition (18.36 and 21.62 nos.) in variety, Arka Anamika and hybrid, CoBhH1 respectively as against untreated check (9.00, 11.00 nos. of moths and 56.66, 80.66 nos. of eggs, respectively) The Pf1 + B2 treated plants had high Repellency index (43.11 %) and negative relative ovipositional index (-59.72%). The variety, Arka Anamika was less preferred for the settling of moths compared to the hybrid, CoBhH1. Among the different plant parts, egg laying was significantly greater on the terminal bud with 37.33 eggs on Arka Anamika and 53.00 eggs in CoBhH1.

Key words: Okra, *Earias vittella*, *Pseudomonas fluorescens*, *Beauveria bassiana*, antixenosis.

Induced protection of plants against various insect pests and pathogens by biotic or abiotic elicitors has been reported since 1930s. Various studies indicated *Pseudomonas fluorescens*, (Migula) Pf1 and *Beauveria bassiana* (Bals.)Vuill. B2 were able to induce the defense mechanism in host plants through alterations in the secondary plant compounds and thus enhancing the resistance in plants against challenging insect pests, pathogens and nematodes (Saravanakumar *et al.,* 2007; Sivasundaram *et al.,* 2008; Karthiba *et al.,* 2010). The chemical composition of the plant is of fundamental significance in the acceptance or rejection of host plant for shelter, oviposition and feeding by insects. *P. fluorescens* treated plants deterred *Plutella xylostella* (L.) moths from oviposition on cauliflower (Mohana Sundaram *et al.,* 2006) and *Helicoverpa armigera* (Hubner) on tomato (Murugan *et al.,* 2007). Fungal metabolites produced by *B. bassiana* reduced infestation due to feeding deterrence rather than direct fungal infection (Vega *et al.,* 2008). Preliminary field study also showed that okra plants, on application of microbial talc-based formulation exhibited resistance against okra shoot and fruit borer, *Earias vittella* (Fab). Laboratory studies were hence carried out to assess the mechanism underlying the antixenosis mechanism of resistance due to microbial induction in okra plants on the behaviour of *E. vittella.*

Materials and Methods

Pot culture studies were carried out in screen house at Insectary, (35±1.5°C and 72±3% RH), Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai during the year 2009. Okra shoot tips were collected from plants and utilized for laboratory experiments to assess the settling and oviposition preference.

Induction of resistance in pot culture okra plants

Okra variety, Arka Anamika and a hybrid, CoBhH1 were used for the experiment. Five treatments, two variety/hybrid with three replications were maintained. Microbials were applied as seed treatment, soil application and foliar spray. Endosulfan 35 EC (treated check) 0.07% was given only as foliar spray.

Okra seeds were treated with talc based bioformulations at the rate of 10 g/ kg of seed, by wet seed treatment method. Untreated seeds were used for treated and untreated check. Treated and untreated seeds were sown in respective treatment pots at the rate of three seeds per pot (5 kg soil capacity). On 30 days after sowing (DAS), five gram of talc-based bioformulations mixed with FYM (100 g) was applied per pot as soil application. The talc based bio formulations were dissolved in water (20g / lit), soaked overnight, filtered through muslin cloth and the filtrate was sprayed using knapsack hand sprayer on potted plants on 30 DAS and 45 DAS (Saravanakumar *et al.,* 2008). Endosulfan 35 EC (2ml/lit) was applied only as foliar spray on 30 and 45 DAS. It served as treated check. Foliar spray with water was given on 30 and 45 DAS in the untreated check. The plants were maintained free of insect

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infestation. The shoot tips were excised after five days of second spraying and utilized for settling and ovipositional preference studies.

Mass culturing of E. vittella

Infested okra fruits were collected from the field and brought to the Insectary, for mass culturing of *E. vittella*. Larvae were released individually on the okra fruit blocks kept in multicavity tray and allowed to grow. Pupae were collected and kept in adult emergence cage (44 x 45 x 43 cm). Moths were provided with ten per cent honey solution as artificial food. On emergence, moths were paired and allowed for mating and oviposition at the rate of ten pairs per cage. Eggs were collected daily in gada cloth and disinfected with 0.05 per cent formaldehyde on the second day. Upon hatching, the neonate larvae were released individually, using soft camel hairbrush, onto the okra fruit blocks kept in multicavity tray (32 cells/ tray). The spent fruit blocks were replaced with fresh ones daily, until pupation. Pupae were collected and kept in adult emergence cage to continue mass culturing. Moths were utilized for the conduct of laboratory experiment (Shanthi, 2000).

Evaluation of settling and ovipositional preference of E. vittella moths

Settling and ovipositional preferences of *E. vittella* moths towards treated okra shoot tips were studied under free-choice conditions. Shoot tips (20 cm long) were excised at flowering phase (5 days after second spraying) from treated and untreated okra (variety/ hybrid) plants grown in pots under screen house condition and placed immediately in conical flask containing sugar solution (3%) to maintain leaf turgidity (Sharma, 2008). They were arranged equi-distantly in circular manner at random in insect cage (44x45x43 cm) without touching each other (Dhillon and Sharma, 2004). Thirty pairs of freshly emerged moths were released inside the cage for each replication. Moths were provided with (honey solution 10%). The setup was maintained for three days. The experiment was laid out in Completely Randomized Design (CRD) with three replications.

Number of moths settled on each shoot tip was recorded at 24 hours interval for three days. It was expressed as number of moths settled per shoot. Adult repellency was estimated with the formula suggested by Roman Pavela and Gerhard (2007) and expressed as Repellency Index (RI) (%).

In the same experiment, moths were allowed to oviposit for three days. Shoot tips were removed after third day and the number of eggs laid on different plant parts were recorded individually (Dhillon and Sharma, 2004) and total number of eggs laid was estimated. Using the above data, relative ovipositional preference (ROP) was estimated (Mehta and Saxena, 1970).

Statistical analysis

The data were transformed by using square root and arcsine transformation and analysed statistically. The analysis of variance was done and the means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Results and Discussion

In the present study, application of talc-based bioformulation either individually or as consortia on the okra plants significantly reduced both the settling and oviposition of *E. vittella* under laboratory conditions (Tables 1-4).

Settling of E. vittella moths

Settling of moths was affected by microbial talc based formulations and also by insecticide treatment. Chemical (Endosulfan 35 EC) treatment proved maximum deterrent towards moths from settling on treated shoot tip. Among the microbial talc based formulation, consortia (Pf1+B2) treated shoot tips were the least preferred (Table 1).

Table 1. Effect of microbial consortia on settling behaviour of *E. vittella* moths in okra

Treatment	Mean number of moths settled#						Mean	
	24 h		48 h		72 h			
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1
P.fluorescens,	5.66	8.66	5.33	8.00	5.33	7.00	5.44	7.88
Pf1 (ST,SA,FS)	(2.38)e	(2.94)h	(2.30)d	(2.82)e	(2.30)de	(2.64)f	(2.33)e	(2.86)h
B. bassiana,	5.00	6.66	4.00	7.33	5.00	6.33	4.66	6.77
B2 (ST,SA,FS)	(2.23)d	(2.58)g	(2.00)c	(2.70)e	(2.23)c	(2.51)def	(2.15)c	(2.66)g
Pf1 + B2 (1:1)	3.00	6.00	3.00	5.66	3.33	6.33	3.11	6.00
(ST,SA,FS)	(1.73)b	(2.44)f	(1.73)b	(2.37)d	(1.82)b	(2.51)def	(1.73)b	(2.40)f
Endosulfan 35 EC	1.00	4.66	2.00	5.00	1.33	4.33	1.44	4.66
(Foliar)	(1.00)a	(2.16)c	(1.41)a	(2.23)d	(1.15)a	(2.08)c	(1.27)a	(2.21)d
Untreated check	8.66	10.66	9.00	10.66	9.33	11.66	9.00	11.00
	(2.94)h	(3.26)i	(3.00)f	(3.26)g	(3.05)g	(3.41)h	(2.97)i	(3.26)j
Variety Mean	4.66	7.33	4.66	7.33	4.86	7.13	4.73	7.26
	(2.15)A	(2.68)B	(2.15)A	(2.70)B	(2.20)A	(2.68)B	(2.07)A	(2.68)B
SEd	T=0.069	V=0.044	T=0.050	V=0.032	T=0.050	V=0.032	T=0.023	V=0.015
CD	T=0.145**	V=0.091**	T=0.105**	V=0.066**	T=0.105**	V=0.066**	T=0.047**	V=0.030**

T= Treatment; V= Variety # Mean of three replications; ST – Seed treatment; SA – Soil application; FS – Foliar spray. Figures in parentheses are square root transformed values. In a column means followed by similar letters are not significant different by DMRT (P = 0.05) **Highly significant

Maximum of 54.50 per cent reduction in settling was observed in plants treated with microbial consortia formulation, which was next to endosulfan 35 EC (69.50% reduction). Consortia exhibited the highest RI of 43.11, which was next to endosulfan 35 EC (58.31%) (Table 2).

Table 2. Repellent effect of microbial consortia on *E. vittella* adults

Treatment	Mean Repellency Index (RI) (%)#		
	Arka Anamika	CoBhH1	Treatment Mean
P. fluorescens, Pf 1	25.22	19.15	22.18
(ST,SA,FS)	(30.13)e	(25.93)f	(28.03)D
B. bassiana, B2	35.29	26.50	30.89
(ST,SA,FS)	(36.43)d	(30.96)e	(33.69)C
Pf1 + B2 (1:1)	51.91	(46.10)b	34.32
(ST,SA,FS	(35.84)d	43.11	(40.97)B
Endosulfan 35 EC	71.67	(57.86)a	44.95
(Foliar)	(42.09)c	58.31	(49.98)A
Untreated check	-	-	-
Variety mean	46.02	31.23	
	(42.63)B	(33.71)A	
SEd	T=0.990	V=0.700	TxV=1.400
CD	T=2.099**	V=1.484**	TxV=2.968*

T= Treatment; V= Variety # Mean of three replications; ST - Seed treatment; SA - Soil application; FS - Foliar spray. Figures in parentheses are arc sine transformed values In a column means followed by similar letters are not significant different by DMRT (P = 0.05) **Highly significant *Significant

When the moths were offered a choice among treated shoot tips of the same variety (Arka Anamika) or hybrid (CoBhH1), the preference was significantly lower for chemical and microbial consortia treated shoots than for the untreated shoots (Table 1 and 2). In microbial consortia inoculated plants, maximum reduction in settling of moths was recorded in variety (65.44%) followed by hybrid (45.45%). It can be inferred that between the variety and hybrid, Arka Anamika was less preferable for moths settling (4.73 nos.).

There was no noteworthy variation in moths settling response over time from 24 hours to 72 hours. During different periods of observation, only 3.00 to 3.33 moths settled on Arka Anamika and 5.66 to 6.33 moths on CoBhH1 in the microbial consortia treated shoot tips, as against 8.66 to 11.66 moths in untreated check (Table 1). Arka Anamika exhibited higher RI (25.22 to 71.67%) compared to CoBhH1 (19.15 to 44.95%) (Table 2). This is in line with the findings of Murugan *et al.* (2007) who reported that volatiles released by the *P. fluorescens* treated plants affected the adult settling and egg laying behaviour of *Bemisia tabaci* (Gennadius) and *H.armigera* in tomato. *P. fluorescens* also possibly induces the JA dependent octadeconoid pathway mediated by jasmonic acid (JA) (Van Loon *et al.,* 1998). This stimulates enhanced activities of some enzymes required for volatile synthesis critical for terpenoid biosynthesis that may distract the insects from settling.

Oviposition of E.vittella moths

Volatiles also have indirect role in repelling females and reducing oviposition (Kessler and Baldwin,2001). Some phenolics and sesquiter penes along with volatiles can repel herbivores from oviposition on host plants. In the present study, the data on total number of eggs laid revealed that oviposition behaviour was greatly influenced by the microbial bioformulation inoculation. The Pf1+B2 consortia treated shoot tip was the least preferred for oviposition among the bioformulations. Endosulfan 35 EC (14.14 eggs) was the least preferred among the treatments. The untreated check supported 68.66 eggs. In Arka Anamika, Pf1 +B2 consortia (18.36 eggs) and *B. bassiana*, B2 (20.66 eggs) treatments were equally effective in reducing the oviposition. *B. bassiana*, B2 treated Arka Anamika was on par with microbial consortia treated CoBhH1 which supported 21.62 eggs. With regard to untreated plants, CoBhH1 was highly preferred for oviposition (80.66 eggs) than Arka Anamika (56.66 eggs) (Table 3).

Relative ovipositional preference (ROP) results also revealed that the microbial consortia inoculated shoot tips were the least preferred with -62.87 and -56.50 per cent ROP, in CoBhH1 and Arka Anamika, respectively. The decreasing order of relative preference for oviposition was endosulfan 35 EC, Pf1 +B2 consortia, *B. bassiana*, B2 and P. fluorescens, Pf1. The present findings are in close conformity to the reports of Murugan (2003), who observed that *P. fluorescens* induced plants recorded enhanced concentration of acyl sugar, which is known to reduce oviposition and feeding by *Liriomyza trifolii* (Burgess).

Table 3. Effect of microbial consortia on oviposition behaviour of *E. vittella* in okra

Treatment	Total number of eggs laid*#		Relative ovipositional preference (%)*$	
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1
P. fluorescens, Pf1	23.33	39.33	- 41.50	-31.69
(ST,SA,FS)	(4.82)c	(6.26)e	(40.14)	(34.25)
B. bassiana, B2	20.66	26.66	-47.99	-50.14
(ST,SA,FS)	(4.54)bc	(5.16)d	(43.85)	(45.08)
Pf1 + B2 (1:1)	18.36	21.62	-56.50	-62.87
(ST,SA,FS)	(4.28)b	(4.64)c	(48.79)	(52.47)
Endosulfan 35 EC	13.60	14.68	-66.40	-73.5
(Foliar)	(3.68)a	(3.83)a	(54.60)	(59.10)
Untreated check	56.66	80.66	-	-
	(7.52)f	(8.97)g		
Variety Mean	26.52	36.59	-53.13	-54.5
	(4.97)A	(6.04)B	(46.84)	(47.73)
SEd	T=0.097	V=0.061	T=1.158	V=0.818
CD	T=0.203**	V=0.128**	T=2.45**	V=1.73NS

T= Treatment; V= Variety * Mean of three replications; ST - Seed treatment; SA - Soil application; FS - Foliar spray # Figures in parentheses are square root transformed values $Figures in parentheses are arc sine transformed values. In a column means followed by similar letters are not significant different by DMRT (P = 0.05) ** Highly significant NS-Non-Significant

In untreated check, among the plant parts, preference for egg laying was significantly greater on the terminal bud, with 37.33 eggs in Arka Anamika and 53.00 eggs in CoBhH1 (Table 4).Terminal bud supported only minimum eggs in Pf1+B2 consortia treated shoot tip of Arka Anamika and CoBhH1, which

Table 4. Effect of microbial consortia on oviposition of *E. vittella* on different plant parts in okra

Treatment	Terminal bud#		Petiole#		Leaf#		Stem#	
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1
P. fluorescens, Pf1	16.00	32.33	6.00	4.00	0.66	2.60	0.66	0.33
(ST,SA,FS)	(3.99)d	(5.68)f	(2.44)d	(1.99)b	(1.08)b	(1.78)e	(1.08)b	(0.91)b
B. bassiana, B2	16.33	23.00	1.33	1.33	3.00	2.33	0.00	0.00
(ST,SA,FS)	(4.03)d	(4.79)e	(1.15)a	(1.15)a	(1.87)f	(1.68)d	(0.70)a	(0.70)a
Pf1 + B2 (1:1)	12.76	14.00	5.60	5.00	0.50	2.29	0.00	0.33
(ST,SA,FS)	(3.57)c	(3.74)c	(2.36)d	(2.23)c	(0.70)a	(1.67)d	(0.70)a	(0.91)b
Endosulfan 35 EC	6.30	8.13	4.00	4.16	3.30	2.00	0.00	0.39
(Foliar)	(2.50)a	(2.85)b	(1.99)b	(2.03)b	(1.94)g	(1.58)c	(0.70)a	(0.94)b
Untreated check	37.33	53.00	7.33	14.00	4.66	5.66	7.33	8.00
	(6.10)g	(7.27)h	(2.70)e	(3.74)f	(1.94)g	(2.48)h	(2.79)d	(2.91)e
Variety Mean	17.74	26.09	4.85	5.69	2.32	2.99	1.60	1.81
	(4.04)A	(4.86)B	(2.13)A	(2.23)B	(1.51)A	(1.83)B	(1.20)A	(1.27)B
SEd	T=0.081	V=0.051	T=0.0398	V=0.025	T=0.025	V=0.016	T=0.021	V=0.0135
CD	T=0.169**	V=0.107**	T=0.083**	V=0.052*	T=0.053**	V=0.033**	T=0.044**	V=0.028**

T= Treatment; V= Variety # Mean of three replications; ST - Seed treatment; SA - Soil application; FS - Foliar spray. Figures in parentheses are square root transformed values In a column means followed by similar letters are not significant different by DMRT (P = 0.05) ** Highly significant * Significant

was 65.82 and 73.58 per cent less than untreated check, respectively. The next preferred part was petiole followed by leaf and stem. With regard to petiole, maximum number of egg was recorded in untreated CoBhH1 (14.00 eggs), but less number of eggs was laid in *B. bassiana*, B2 (1.33 eggs) and Pf1 (4.00 eggs) treated shoot tips, which contributed 90.50 and 71.43 per cent reduction over untreated check, respectively.

It is concluded that the microbial talc-based bioformulation (Pf1+B2) is effective in inducing antixenotic resistance against shoot and fruit borer, the biochemical parameters and volatiles released from microbial bioformulation treated plants greatly affect the settling and oviposition behaviour of *E. vittella.*

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Received: July 10, 2011; Accepted: November 22, 2011