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Field Evaluation of Microbial Consortia Mediated Resistance against Okra Shoot and Fruit borer, *Earias vittella*

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A field experiment was conducted during *Kharif* 2009 to evaluate the microbial consortia mediated resistance against okra shoot and fruit borer, *Earias vittella* (Fab.) in okra variety, Arka Anamika and hybrid, CoBhH1. The microbial consortium *Pseudomonas fluorescens* Pf1 + *Beauveria bassiana* B2 isolate (Pf1 + B2) was effective in reducing the shoot and fruit borer infestation through seed treatment, soil application and foliar spray. Pf1 + B2 recorded minimum shoot damage during vegetative and reproductive phases (3.99 and 14.33 %) as against untreated check (13.58 and 24.16 %). Fruit infestation was less in Pf1 +B2 consortia treatments on weight and number basis (4.22 and 2.83%), whereas it was 13.59 and 17.96 per cent in untreated check. Phenol content was high in Pf1+B2 consortia treated plants in Arka Anamika (0.94 mg/g) and CoBhH1 (1.39 mg/g), as against untreated check (0.20 and 0.41 mg/g, respectively). There was significant negative correlation between the shoot and fruit borer infestation and phenols (r=-0.937), defensive enzymes viz., peroxidase (r=-0.909*), poly phenol oxidase (r=-0.993**), and phenyl alanine ammonia lyase (r=-0.960**).**

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

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Okra, *Abelmoschus esculentus* L. (Moench), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. The crop is infested by 72 species of insects. Among them, the shoot and fruit borer, *Earias vittella* (Fab.) is the most destructive pest. The larva starts damaging two to three weeks old plants and reaches a peak of 8.5 per cent shoot damage before fruiting. Fruit infestation starts at the initiation of fruiting, increases progressively up to harvesting and reaches a peak of 41.25 per cent, before harvesting (Abishek Shukla *et al.,* 1997). Thus, *E. vittella* is a limiting factor for successful production. In order to reap the full genetic potential of okra, it is imperative that the crop should be protected from shoot and fruit borer.

At present, management of the okra insect pest relies largely upon pesticides application. Frequent and extensive use of pesticides has led to severe ecological consequences like destruction of natural enemy fauna and harmful effect on non target organisms. Research during the previous decade indicated that biocontrol agents have the potential for the management of insect pests. Induced resistance has been considered as a potential strategy for insect pest control in plants (Allen, 2001). But little work has been done on induced resistance against insects. Studies made on *Pseudomonas fluorescens* Migula and *Beauveria bassiana* (Bals.) Vuill. indicated that they were able to induce the defense mechanism in host plants through alterations in the secondary plant compounds and thus enhance the resistance in plants against insect pests (Sivasundaram *et al.,* 2008; Karthiba *et al.,* 2010). *Beauveria* was found toxic to insects by producing feeding deterrents or antibiotics (Hardy *et al.,* 1985). The present study was aimed at understanding the role of Plant Growth Promoting Rhizobacteria, *Pseudomonas fluorescens*, Pf1 strain and entomopathogenic fungus, *Beauveria bassiana,* B2 strain in inducing resistance against shoot and fruit borer in okra.

Materials and Methods

The performance and reaction of microbial consortia on okra variety/hybrid against shoot and fruit borer was evaluated in the experimental field, under drip fertigation system at Agricultural College and Research Institute, Madurai during Kharif, from August to November 2009 (34.0±0.7°C and 79.2 ± 8.0 % RH). The experiment was laid in split plot design with five treatments and three replications. The effects of treatments were tested on Arka Anamika (Ruling variety) and CoBhH1 (hybrid released by TNAU). The details of the treatments were *Pseudomonas fluorescens* Pf1 (Talc - based formulation), *Beauveria bassiana* B2 isolate (Talc-based formulation), Pf1 + B2 (1:1) consortia, Endosulfan 35 EC 2ml/lit (Treated check) and Untreated check. *P. fluorescens* (Strain Pf1) (containing 2.5 to 3.0 x 10^8 cfu/g) and *Beauveria bassiana* (B2 isolate) (1 x 108 spores/ml) were obtained from Department of Plant Pathology, Tamil

Nadu Agricultural University, Coimbatore. The microbials were applied as seed treatment, soil application and foliar spray.

The okra seeds were treated with respective microbial talc-based formulations (10g/kg of seed) by wet seed treatment method, 24h before sowing. Untreated seeds were used for treated and untreated check. The treated and untreated okra seeds of variety/ hybrid were sown in the respective treatmental plots. They were sown in raised bed of 4.3 x 4.5 m plot at 60x20 cm spacing for variety and 60x30 cm spacing for hybrid. Recommended agronomic practices were adopted uniformly for all the treatments.

Thirty days after sowing (DAS), respective talc-based bioformulation (2.5 kg/ha) was mixed with 50 kg of FYM and applied to soil by broadcasting. The talc based bio products were dissolved in water (20 g/lit), soaked overnight and filtered through muslin cloth and the filtrate was sprayed using high volume hand sprayer on 30 DAS (Saravanakumar *et al.,* 2008; Sivasundaram *et al.,* 2008). It was followed by two sprayings at 15 days interval upto 60 DAS. Foliar spray of endosulfan 35 EC (2ml/l) was given at 30, 45 and 60 DAS. Foliar spraying of water was done in untreated check.

Shoot infestation was observed on 15 after seed treatment and five days after each spraying. Observations were made on total number of healthy and infested shoots in 25 randomly selected plants and the per cent shoot damage was estimated. Number and weight (g) of total fruits and infested fruits were recorded five days after each spraying at fortnightly interval, in 25 randomly selected plants. The fruit infestation was expressed as mean percentage on number and weight basis.

To study the activation of microbial induced phytochemical defense mechanism, similar set of treatments were given to plants in pot culture maintained at screen house, which was kept free of insect infestation. Samples were taken on five days after third spraying (50 DAS), to determine the phenol content (Malick and Singh, 1980) and defense herbivore enzymes *viz*., peroxidase (Putter, 1974), phenylalanine ammonia lyase (Brueske, 1980) and poly phenol oxidase (Augustin *et al.,* 1985).

The data were transformed and analysed statistically. The analysis of variance was done and the means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1995). These data were also subjected to correlation analysis with biochemical parameters.

Results and Discussion

The data revealed that the shoot borer incidence was less during vegetative phase when compared to reproductive phase. During vegetative phase, Pf1 +B2 consortia recorded the minimum per cent shoot damage (3.99 %), followed by *B. bassiana,* B2 (5.33%). Their per cent reduction over untreated check was 70.61 and 60.75 per cent, respectively. The percent shoot damage was maximum in untreated check (13.58 %) (Table 1). During reproductive phase, among the microbial bioformulation treatments, Pf1+B2 consortia (14.33%) was significantly superior in inducing resistance against the shoot damage followed by *B. bassiana,* B2 (16.99%) as against untreated check (24.16%). The per cent reduction over the untreated check was high in these treatments (40.68 and 29.67 %, respectively) (Table 2). The reduction in shoot infestation was higher during vegetative phase.

Table 1. Effect of microbial consortia mediated resistance on shoot damage by *E. vittella* in okra in vegetative phase

Treatment	Mean shoot damage (%) *				Mean (% shoot damage)	% Reduction over control
	15 days after seed treatment		5 days after soil application and 1st foliar spray			
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1		
P.fluorescens, Pf1 (ST,SA,FS)	8.00 (16.42)f	0.00 (0.52)a	12.00 (20.26)f	10.66 (19.06)e	7.66 (14.02)C	43.59
B. bassiana, B2 (ST,SA,FS)	6.66 (14.96)e	0.00 (0.52)a	8.00 (16.42)d	6.66 (14.96)c	5.33 (11.67)B	60.75
Pf1 + B2 (1:1) (ST,SA,FS)	5.33 (13.34)d	0.00 (0.52)a	5.33 (13.34)b	5.33 (13.34)b	3.99 (10.10)A	70.61
Endosulfan 35 EC (FS alone)	14.00 (21.90)g	3.40 (10.62)b	4.00 (11.53)a	4.00 (11.53)a	6.35 (14.10)C	53.24
Untreated check	14.33 (22.23)h	4.00 (11.53)c	20.00 (26.55)h	16.00 (23.57)g	13.58 (20.97)D	
Variety Mean	9.66 (17.70)B	1.58 (5.02)A	9.86 (17.62)B	8.53 (16.49)A		
SE_d	T=0.140	V=0.103	T=0.3346	V=0.043	T=0.188	
$CD_{0.05}$	T=0.298**	V=0.443**	T=0.709**	V=0.187**	T =0.382**	
T=treatment; V=variety						

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

The grand mean fruit infestation on weight basis indicated that Endosulfan 35 EC (treated check) recorded the lowest per cent fruit damage (2.98%) and was followed by Pf1 +B2 consortia (4.22%). The maximum fruit infestation was recorded in the untreated check (13.59%) on weight basis. The per cent reduction over the untreated check was high (78.07%) in Endosulfan 35 EC followed by in Pf1 +B2 consortia (68.94%) (Table 3). Among the microbials, per cent fruit infestation on number basis was less in Pf1 +B2 consortia (2.83%) treated plots followed by *B.bassiana,* B2 (4.16%) and it was high in the untreated check (17.96%). The per cent reduction over untreated check was high in these treatments (84.24 and 76.83%, respectively) (Table 4). This is in conformity with Murugan (2003), who reported that the combined application of P. fluorescens (ST + FS) with NSKE 5 per cent reduced *E. vittella* fruit damage in okra. Mohana Sundaram and Dhandapani (2009) also stated that application of *P.fluorescens* in combination with GA3 reduced *P. xylostella* damage in cauliflower. Combination of Pf1 + TDK1 with *Beauveria* induced systemic resistance in groundnut plants, which reduced the leaf miner incidence followed by *Beauveria* infection, led to death of the insect (Senthilraja *et al.,* 2010). Karthiba *et al.* (2010) reported the increased efficacy of bioformulation when the *P. fluorescens* (Pf1 + AH1) was applied in combination with *Beauveria*, B2 rather than the individual strains. This combination of Pf1+ AH1+ B2 effectively reduced the incidence of leaffolder in rice (Karthiba *et al.,* 2010). When B.

Table 2. Effect of microbial consortia mediated resistance on shoot damage by *E. vittella* in okra in reproductive phase

Treatment	Mean shoot damage (%) (5 days after)*				Mean (% shoot damage)	% Reduction over control
	2nd foliar spray		3rd foliar spray			
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1		
P. fluorescens, Pf1	17.33	16.00	23.00	20.33	18.83	22.06
(ST,SA,FS)	(24.59)e	(23.57)d	(28.65)f	(26.79)de	(25.90)D	
B. bassiana, B2	14.66	13.33	21.33	18.66	16.99	29.67
(ST,SA,FS)	(22.51)bc	(21.40)c	(27.50)e	(25.59)d	(24.25)C	
Pf1 + B2 (1:1)	12.00	10.66	18.66	16.00	14.33	40.68
(ST,SA,FS)	(20.26)b	(19.06)ab	(25.59)d	(23.57)c	(22.12)B	
Endosulfan 35 EC	10.66	9.33	13.33	10.66	10.99	54.51
(FS alone)	(19.06)ab	(17.78)a	(21.40)b	(19.06)a	(19.32)A	
Untreated check	22.66	20.66	28.00	25.33	24.16	
	(28.42)g	(27.03)f	(31.94)h	(30.21)g	(29.40)E	
Variety Mean	15.46	14.00	20.86	18.20		
	(22.97)B	(21.77)A	(27.01)B	(25.04)A		
SEd	T=0.425	V=0.077	T=0.5031	V=0.099	T= 0.310	
CD0.05	T=0.901**	V=0.333**	T=1.066**	V=0.428**	T =0.630**	
T= Treatment; V= Variety						

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

Table 3. Effect of microbial consortia mediated resistance on okra fruit infestation on weight basis

Treatment	Mean Fruit infestation (%) (5 days after)*				Mean (% fruit infestation)	% Reduction over control
	2nd foliar spray		3rd foliar spray			
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1		
P. fluorescens, Pf1	6.10	5.80	11.90	6.28	7.52	44.66
(ST,SA,FS)	(14.29)e	(13.93)e	(20.18)f	(14.51)d	(15.69)D	
B. bassiana,B2	5.60	5.10	7.51	3.75	5.46	60.26
(ST,SA,FS)	(13.68)d	(13.04)cde	(15.91)e	(11.17)cd	(13.31)C	
Pf1 + B2(1:1)	4.86	4.40	5.61	2.03	4.22	68.94
(ST,SA,FS)	(12.73)bc	(12.10)b	(13.69)d	(8.20)b	(11.87)B	
Endosulfan 35 EC	3.50	3.10	4.26	1.06	2.98	78.07
(FS alone)	(10.77)a	(10.13)a	(11.90)c	(5.92)a	(10.02)A	
Untreated check	11.33	8.90	20.94	13.22	13. 59	
	(19.66)g	(17.35)f	(27.22)h	(21.31)g	(20.30)E	
Variety Mean	6.27	5.46	10.04	5.27		
	(14.23)B	(13.31)A	(17.78)B	(12.22)A		
SEd	T=0.254	V=0.046	T=0.304	V=0.067		
CD0.05						
T= treatment;	T=0.540**	V=0.201**	T=0.644**	V=0.292**		
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) bassiana was applied to plants by foliar application or by injection, it colonized, translocated, and persisted within the maize plants (Bing and Lewis, 1993) and provided season-long suppression of *Ostrinia nubilalis* and *Sesamia calamistis* (Cherry *et al.,* 2004).

The data on mean shoot damage in Arka Anamika and CoBhH1, showed that CoBhH1 had significantly lower damage and it ranged between 1.58 and 18.20 per cent, whereas, Arka Anamika had 9.66 to 20.86 per cent shoot damage. It infers that *E. vittella* shoot damage was significantly lower in CoBhH1 than Arka Anamika. The mean fruit infestation both on number and weight basis was also less in CoBhH1 than Arka Anamika. It was in corroboration with the findings of Jalgoankar *et al.* (2002), who reported that Arka Anamika was susceptible to *E.vittella.*

Both Arka Anamika and CoBhH1, exhibited induced resistance against *E. vittella* when treated with microbial talc-based bioformulations individually and in combinations (Pf1+ B2). Comparatively, the induced resistance developed due to microbial consortia treatment was high in CoBhH1 than Arka Anamika.

Table 4. Effect of microbial consortia mediated resistance on okra fruit infestation on number basis

Treatment	Mean Fruit infestation (%) (5 days after)*				Mean (% fruit infestation)	% Reduction over control
	2nd foliar spray		3rd foliar spray			
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1		
P. fluorescens, Pf1	6.72	2.52	13.60	7.18	7.50	58.24
(ST,SA,FS)	(15.02)g	(9.13)d	(21.64)f	(15.53)e	(15.33)D	
B. bassiana, B2	2.92	2.46	7.51	3.75	4.16	76.83
(ST,SA,FS)	(9.84)e	(9.02)d	(15.91)e	(11.17)c	(11.48)C	
Pf1 + B2 (1:1)	3.58	1.08	4.91	1.78	2.83	84.24
(ST,SA,FS)	(10.90)f	(5.98)b	(12.79)d	(7.66)b	(9.33)B	
Endosulfan 35 EC	1.71	0.50	3.13	0.78	1.52	91.53
(FS alone)	(7.51)c	(4.05)a	(10.18)c	(5.07)a	(6.70)A	
Untreated check	16.28	9.12	28.48	17.97	17.96	
	(23.79)i	(17.57)h	(32.25)h	(25.08)g	(24.67)E	
Variety Mean	6.24	3.13	11.53	6.29		
	(13.41)B	(9.15)A	(18.55)B	(12.90)A		
SEd	T=0.235	V=0.039	T=0.341	V=0.055		
CD0.05	T=0.499**	V=0.170**	T=0.724**	V=0.239**		
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)

The phenol content was enhanced in Pf1+ B2 consortia inoculated Arka Anamika (0.94 mg/g) and CoBhH1 (1.39 mg/g), when compared to untreated plants of Arka Anamika (0.20 mg/g) and CoBhH1 (0.41 mg/g). There was 3.4 fold increase in phenol content in CoBhH1, 4.7 fold increase in Arka Anamika, due to microbial consortia inoculation. These findings are similar to the earlier reports given by several authors. Application of *P. fluorescens,* induced tomato plants and recorded enhanced phenol content (Murugan, 2003). *B.bassiana,* B2 isolate inoculated rice plants also recorded increased phenol content (Sivasundaram *et al.,* 2008).

Similarly, plants treated with Pf1+ B2 consortia recorded higher peroxidase activity in both Arka Anamika (3.75/min/g) and CoBhH1 (5.03/min/g) as against untreated check (0.50 and 0.75/min/g, respectively). Similar trend was observed in polyphenol oxidase and phenyl alanine ammonia lyase activity. Increase in defense enzymes due to inoculation of microbial consortia was higher in CoBhH1 than Arka Anamika. This is in line with findings of Karthiba (2008) who reported that the defense enzymes viz., PO, PPO and PAL were enhanced in rice plants by the combined application of microbial consortia, Pf 1+ AH +B2.

Shoot and fruit borer infestation was negatively correlated with phenols (r= -0.937**) and herbivore defense enzymes viz., peroxidase (r= -0.909*), polyphenol oxidase (r= -0.993**), phenylalanine ammonia lyase (r= -0.960**). It is evident from the present study that the phenol content and herbivore defense enzymes were highly increased in microbial consortia treatments when compared to untreated check. This might have induced resistance which led to the reduced infestation of shoot and fruit borer.

It is concluded that the microbial consortia of talc-based bioformulation (Pf1 +B2) was effective in reducing shoot and fruit borer incidence by accumulation of phenols and inducing defense enzyme activity. Hence it can be included as one of the components in Integrated Pest Management for *E. vittella* in okra.

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