Microbial and herbivore induced phytochemical changes in okra against Shoot and fruit borer, *Earias vittella* (Fab.)

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ABSTRACT

Microbial and herbivore induced phytochemical changes were studied in okra variety, Arka anamika and hybrid, CoBhH1, against shoot and fruit borer, *Earias vittella*. Induction was done either by microbial alone (microbial talc-based bioformulation *Pseudomonas fluorescens* (Pf1), *Beauveria bassiana* B2 isolate, Pf1+B2 consortia) or by both microbial and herbivore (*E. vittella* infestation). Herbivore alone infested plants served as infested control and undamaged plants left as absolute control. Biochemical pathways encompassing induced resistance *viz*., phenols and oxidizing defense enzymes such as peroxidase (PO), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) were assessed to envisage the development of induced resistance. Accumulation of phenol was high in Pf1+B2 consortia inoculated plants as against absolute control in variety, Arka anamika and hybrid, CoBhH1. Inoculation with both microbial consortia and *E. vittella* caused 9.8 times increased accumulation of phenolics in Arka Anamika and 8.6 times in CoBhH1. Defense enzymes activity was also significantly higher in Pf1 + B2 consortia treated plants as against absolute control. Further, infestation by *E. vittella* resulted in the enhancement of accumulation of phenols, induction of PO, PAL and PPO activity. In absolute control, CoBhH1 had higher phenolic accumulation and increased activity of defense enzymes than Arka Anamika.

Keywords: *Beauveria bassiana*, defense enzymes, *Earias vittella*, *Pseudomonas fluorescens*, phenol

INTRODUCTION

Okra, *Abelmoschus esculentus* L. (Moench) (Malvaceae), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world and infested by 72 species of insects. Shoot and fruit borer, *Earias vittella* (Fab.) is the most destructive pest causing 8.5 per cent shoot damage before fruiting and reaches a peak of 41.25 per cent, before harvesting (Abishek Shukla *et al*., 1997). Plants have developed a multitude of inducible defense mechanisms against aggressive biotic and abiotic agents (Agrawal and Fishbein, 2006). Plants are able to respond to herbivore attack by defensive mechanisms which directly affect the herbivore (Devendra Kumar, 2008). Herbivory-induced plant responses can negatively affect herbivore's physiology directly by stimulating the synthesis of toxic metabolites (Kahl *et al.*, 2000).

Phenols are extremely abundant plant allelochemicals, often associated with feeding deterrence or growth inhibition. Phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO), and peroxidase (POD) are enzymes involved in phenol oxidation and correlated with plant defense mechanisms (Tomas Barberan and Espin, 2001). Induction of defense enzyme activities in plants because of herbivore damage has received great attention in recent years (Jin Yin Peng *et al.*, 2005; Maffei *et al.*, 2006; Shize Zhang *et al.*, 2008). Studies made on the *Pseudomonas fluorescens* and *Beauveria bassiana* indicated that they 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 (Sivasundaram *et al.*, 2008; Karthiba *et al.*, 2010). Therefore this investigation was aimed to study the induction of phenolics and defense enzymes in microbial consortia inoculated and *E. vittella* infested plants.

MATERIALS AND METHODS

To assess the microbial and herbivore induced phytochemical changes, pot culture studies under screen house were carried out at Insectary (35±1.5°C and 72±3% RH). Okra variety, Arka Anamika and hybrid, CoBhH1 were used for the experiment. The details of the treatments were *Pseudomonas fluorescens* (Pf1-containing 2.5 to 3.0 x 10^8 cfu/g) (Talc - based formulation), *Beauveria bassiana* (B2- containing 1 x 10^8 spores/mL) (Talc - based formulation), Pf1 + B2 (1:1) consortia, endosulfan (35 EC 2 ml/lit) and uninoculated control. The microbials were applied as seed treatment (ST), soil application (SA) and foliar spray (FS) (Karthiba *et al.*, 2010).

The okra seeds were treated with talc based bioformulations @ 10g/kg of seed by wet seed treatment method, 24h before sowing. The untreated seeds were used for endosulfan and uninoculated control. Three seeds were sown in a pot (5 kg soil capacity). Five gram of talc-based bioformulations mixed with FYM (100 g) was applied per pot on 30 DAS as soil application. The talc based bio formulations were dissolved in water (20g /L), soaked overnight, filtered through muslin cloth and the filtrate was sprayed using Knapsack hand sprayer on potted plants from 30 DAS to 45 DAS, at fortnightly interval (Saravanakumar *et al.*, 2008). Endosulfan 35 EC (2mL/ L) was applied only as foliar spray on 30 and 45 DAS. Foliar spray with water was given from 30 to 45 DAS at fortnightly interval in the absolute control

For each treatment, two set of plants were maintained, to study the induction of phytochemical defense in microbial bioformulation inoculated plants and in shoot and fruit borer, *E. vittella* infested plants. One set of plants were inoculated with microbials alone, up to 45 DAS as per treatment schedule. Another set of plants were inoculated with microbials as per schedule and also infested by second instar larvae of *E.vittella* for 24 hrs @ five larvae per plant on four days after second spraying of microbials. Before release, the larvae were starved for two hrs (but water satiated). Two sets of uninoculated control were maintained *viz*., plants infested by *E.vittella* alone served as infested control and undamaged plants left as absolute control. In absolute control, plants were neither inoculated with bioformulation nor infested with *E. vittella*, which were maintained for comparison (Saravanakumar *et al.*, 2008; Sivasundaram *et al.*, 2008). All the plants were maintained free from insect infestation until 45 DAS.

Total phenol content (Malick and Singh, 1980), peroxidase (Putter, 1974), Phenylalanine ammonia lyase (PAL) (Brueske, 1980) and poly phenol oxidase (PPO) (Augustin *et al.*, 1985) was determined as per the standard procedure. Five days after second spraying (50 DAS), samples were collected from different treatments, to assess the phenolic content and activity of defense enzymes in the plants inoculated with microbials alone. To study the activation of herbivore induced phytochemical changes, 24 hrsafter release of larvae (on 5th day after second spraying), samples were collected from different treatments. 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).

RESULTS AND DISCUSSION

Phytochemical changes due to microbials inoculation alone

All the treatments had significantly higher phenol content than absolute control (Table 1). Phenol content was high in Pf1 +B2 consortia inoculated plants which was followed by Arka Anamika and hybrid, CoBhH1. Absolute control recorded the lowest phenol content both in variety. While comparing the variety and hybrid, the phenol content was high in hybrid, CoBhH1. This result is in line with the findings of earlier

Table 1. Phenols and defense enzymes activity in microbial consortia inoculated okra plants before *E. vittella* damage

	Treatment	Phenol content mg g^{-1} fresh weight*		Peroxidase (min^{-1}g^{-1} tissue)*		Phenylalanine ammonia lyase (μMmin^{-1}g^{-1} tissue)*		Poly phenol oxidase (unit min^{-1}g^{-1} tissue)*	
		Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1
	P. fluorescens, Pf1 (ST,SA,FS)	0.37^{c}	0.66^{c}	2.55^{c}	2.52^{c}	90.33^{c}	94.33^{c}	11.30^{c}	13.63^{c}
	B. bassiana, B2 (ST, SA, FS)	0.59^{b}	1.02^{b}	3.53^{b}	3.23^{b}	112.33^{b}	116.00^{b}	19.60^{b}	22.40^{b}
	Pf1 + B2 (1:1) (ST, SA, FS)	0.94^{a}	1.39^{a}	3.75^{a}	5.03^{a}	123.66^{a}	124.66^{a}	28.66^{a}	31.26^{a}
	Chemical control (FS alone)	0.30^{d}	0.52^{d}	1.20^{d}	1.50^{d}	51.66^{d}	51.33^{d}	10.03^{d}	11.00^{d}
	Absolute control	0.20^{e}	0.41^{e}	0.50^{e}	0.75^{e}	49.33^{e}	51.00^{d}	9.80^{d}	10.23^{e}
SE_d		0.017	0.018	0.008	0.008	0.003	0.027	0.024	0.006
$CD_{0.05}$		0.038**	0.040**	0.018**	0.018**	0.083**	0.061**	0.053**	0.015**

*Mean of three replications; ST – Seed treatment; SA – Soil application; FS – Foliar spray
In a column means followed by similar letters are not significant different by DMRT (P = 0.05)

reports. *P. fluorescens* induced plants recorded the enhanced level of phenol content in tomato plants (Murugan, 2003), leaf tissues of banana (Thangavelu *et al.*, 2003), chickpea (Vinod Kumar *et al.*, 2007). The rice plants inoculated with *Beauveria*, B2 isolate recorded the increased phenol content (Sivasundaram *et al.*, 2008).

Among the microbial bioformulations, the defense enzyme activity was significantly higher in microbial consortia treated plants (Pf1+B2) when compared to individual strains (Table 1). Application of microbial consortia (Pf1 +B2) recorded higher PO activity in variety, Arka Anamika and hybrid, CoBhH1 (3.75 and 5.03 per min/g, respectively) which was inoculated with microbial alone. It was followed by *B. bassiana*, B2 (3.53 and 3.23 per min/g, respectively). The activity was lower in the absoulte control (0.50 and 0.75 per min/g, respectively) in variety and hybrid (Table 1).

The results revealed that there was an increase in PAL activity due to the microbial bioformulation treatments (Table 1). The enzyme activity was less in Pf1+B2 consortia treated plants followed by B2, whereas in the absoulte control, the activity was comparatively lesser in variety, Arka Anamika and hybrid, CoBhH1. Similarly, Pf1 +B2 consortia treated plants induced the higher activity of PPO in variety, Arka Anamika and hybrid, CoBhH1) (Table 1). In the present study, the higher activity of defense related enzymes, PO, PPO and PAL was recorded in okra plants treated with microbial consortia (Pf1 +B2) compared with individual and uninoculated control. Due to the inoculation of microbials, peroxidase activity was enhanced by 7.5 times. These findings are supported by several authors. PAL and PO activity reached maximum at the fourth day, while activity of PPO reached maximum at the fifth day after challenge inoculation of *P. fluorescens*, Pf1 in tomato plants (Ramamoorthy *et al.*, 2002). The defense enzymes *viz.*, PO, PPO and PAL were enhanced by the combined application of Pf1+TDK1+PY15 in rice plants (Saravanakumar *et al.*, 2008) and microbial consortia, Pf 1+ AH +B2 treated rice plants (Karthiba, 2008).

Phytochemical changes due to microbials and *E. vittella*

Generally**,** the phenol content and the activity of defense enzymes *viz.*, peroxidase, phenyl alanine ammonia lyase and poly phenol oxidase were increased in the plants inoculated with microbials and *E. vittella* larvae (Table 2), when compared to plants treated with microbials alone (Table 1). While comparing variety and hybrid, the phenol content and enzyme activity was found to be the maximum in hybrid, CoBhH1.

Enhanced phenol content was recorded in Pf1 +B2 consortia inoculated plants (0.98 and 1.71 mg/g) after *E. vittella* infestation, the increase was 4.7 and 3.3 times, respectively. It was followed by B2, whereas, the infested control had significant increase in phenol content than absolute control. It indicates that *E. vittella* infested plants exhibited defense response by significant increase in phenol content.

Table 2. Phenols and defense enzymes activity after *E. vittella* infestation in microbial consortia inoculated okra

Treatment	Phenol content mg g^{-1} fresh weight*		Peroxidase ($min^{-1}g^{-1}$ tissue)*		Phenylalanine ammonia lyase (μMmin^{-1}g^{-1} tissue) *		Poly phenol oxidase (unit $min^{-1}g^{-1}$ tissue)*	
	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1	Arka Anamika	CoBhH1
P. fluorescens, Pf1 (ST,SA,FS)	0.41^c	0.71^c	14.03^b	16.16^b	94.33^c	97.23^c	16.53^c	17.13^c
B. bassiana, B2 (ST, SA, FS)	0.81^b	1.22^b	15.16^a	18.06^a	114.00^b	119.26^b	23.46^b	26.36^b
Pf1 + B2 (1:1) (ST, SA, FS)	0.98^a	1.71^a	16.03^a	18.70^a	125.33^a	128.00^a	32.66^a	37.20^a
Chemical control (FS alone)	0.31^d	0.61^d	8.20^c	11.13^c	53.00^d	52.00^d	13.50^d	13.90^d
Infested control	0.21^e	0.49^e	7.03^d	10.10^d	51.00^e	54.00^e	12.20^e	12.50^e
Absolute control	0.10^e	0.21^f	5.78^e	8.97^e	48.50^f	50.61^f	11.00^f	11.50^f
SE_d	0.035	0.027	0.065	0.035	0.024	0.023	0.051	0.049
$CD_{0.05}$	0.077**	0.060**	0.142**	0.077**	0.052**	0.051**	0.111**	0.108**

*Mean of three replications; ST – Seed treatment; SA – Soil application; FS – Foliar spray
In a column means followed by similar letters are not significant different by DMRT (P = 0.05)

PO activity was significantly higher and lesser in Pf1 +B2 consortia inoculated Arka Anamika and CoBhH1 after *E. vittella* infestation and absolute control, respectively. Infested control had significantly higher PO activity than absolute control. Similarly, PAL activity was maximum in Pf1 +B2 consortia inoculated *E. vittella* infested plants. Absolute control had comparatively lesser PAL activity in Arka Anamika and CoBhH1 than infested control. PPO activity was higher in Pf1 +B2 consortia treated plants as against control in Arka Anamika and CoBhH1. Due to the inoculation of microbials and *E. vittella* infestation, PAL activity was increased by 2.25 times and PPO activity by 3.33 times. This is in collaboration with the reports given by several authors. PO activity was induced by the *Helicoverpa armigera* damage in tomato plants (Lin lin *et al.*, 2008). There was higher induction of PPO, when the banana plants challenge inoculated with viruliferous aphids (Harish, 2005). Murugan (2003) reported the defense enzymes PO, PPO, PAL were increased in *P. fluorescens* induced tomato plants; in addition the activity was enhanced when the plants were challenged with *Liriomyza trifolii* and B*emisia tabaci*.

It is concluded from the findings of the present investigation that microbial inoculation (Pf1 +B2) in okra plants enhanced induction of phenols and enzyme activity. When the microbial inoculated plants were infested with *E. vittella*, the accumulation of phenols and enzyme activity were further enhanced.

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