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Pre-harvest application of a new biocontrol formulation induces resistance to post-harvest anthracnose and enhances fruit yield in mango

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Summary. Pre-harvest application of biocontrol formulations consisting of 1. the two plant growth promoting rhizobacterial strains FP7 and Pf1 of *Pseudomonas fluorescens*; 2. a strain of *Bacillus subtilis* Bs-1; and 3. a strain (Sc-1) of the yeast *Saccharomyces cerevisiae,* given at fortnightly or monthly intervals and with or without a chitin amendment, were evaluated in two trials for their ability to reduce anthracnose in mango caused by *Colletotrichum gloeosporioides*. Growth of *C. gloeosporioides in vitro* was significantly reduced by strain FP7 and in both field trials the bacterial strain in combination with chitin significantly reduced infection. Pre-harvest application of these formulations at fortnightly intervals also significantly improved flower initiation, yield parameters (mean number of fruits and fruit yield) and fruit quality (total soluble solids, ascorbic acid, free acidity, total, reducing and non- reducing sugar content). The delay in latent symptom expression increased by 15 days under stored conditions. The highest levels of phenolic content, peroxidase and polyphenol oxidase on mango leaves, flowers and fruits were achieved with FP7 +chitin.

Key words: biocontrol formulations, chitin, PGPR, yeast, induced systemic resistance.

Introduction

Mango (*Mangifera indica* L.) is one of the top five fruit crops in the world. It is adaptable to a wide range of climates, ranging from wet tropical to dry subtropical. In India, the area under mango cultivation and production have both increased manifold in the past 30 years, but productivity is still low when compared to that of other countries. Among the various constraints, the most important is anthracnose caused by *Colletotrichum gloeosporioides* Penz. and Sacc. (teleomorph *Glomerella cingulata*). This disease can render the tree completely unproductive as it destroys the developed or developing fruits both in the field and in storage. Anthracnose-affected trees produce completely unmarketable fruit by disrupting carbohydrate sugar synthesis, accumulation and translocation. Although the extensive use of chemical pesticides remains the main means of control, they are now being superseded by non-fungicidal protectants that are more specific towards the target patho-

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gen and hence more ecofriendly. Plant growth promoting rhizobacteria (PGPRs), especially *Pseudomonas fluorescens, Bacillus subtilis* and some yeast antagonists, are promising candidates as bioprotectants. They compete with pathogens for nutrients and space, trigger antibiotic and siderophore production and induce a resistance reaction in the plant. Bioprotectants effective against post-harvest anthracnose are concentrated mainly under stored conditions (Wisniewski and Wilson, 1992). However, this strategy was here ineffective due to the prolonged latent existence of the pathogen from the field onwards.

Colletotrichum gloeosporioides normally invades the flower during the pre-flowering stage and persists in dormant form (latent infection). Hence bioprotectants applied in the field may reduce pathogen infection by colonizing the plant parts before the pathogen can do so. Because of the longer interaction time between the bioprotectant and the pathogen, the inoculum load in storage is reduced. Recently more emphasis has been laid on adding various nutrients to the bioprotectants in order to enhance their performance. The addition of chitin or chitosan adjuvant to improve the efficacy of the antagonists and to induce systemic resistance in the plant either alone or in combination with other biocontrol agents has been successful against *C. gloeosporioides* in various crops (Bell *et al*., 1998; Radja Commare *et al*., 2002). The present investigation was undertaken to determine the efficacy of some biocontrol agents in controlling anthracnose of mango both *in vitro* and *in vivo*, and whether these biocontrol formulations induced accumulation of defense-related enzymes enhancing disease resistance and improving fruit quality and yield.

Materials and methods

Biocontrol micro-organisms

Two strains of *P. fluorescens,* Pf1 and FP7, isolated from the soil rhizosphere of rice (Nandakumar *et al*., 2001), one of *B. subtilis* (Bs-1) isolated from greengram leaves (Saravanakumar, 2002), and one of *Saccharomyces cerevisiae* (Sc-1) isolated from tobacco leaves (Loganathan, 2002) were included in this study. Strains of *P. fluorescens* were cultured on King's medium B (KB), *B. subtilis* on nutrient agar (NA), and *S. cerevisiae* on yeast-extract-glucose agar (YEGA). Stock cultures were stored at 4°C.

Isolation of *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides was isolated from infected mango fruits and identified according to Ekbote *et al*. (1997). Pathogenicity was tested on mango seedlings and a highly virulent isolate was selected for further study.

Efficacy of biocontrol strains under *in vitro* conditions

For the *in vitro* screening of the antagonistic activity of the biocontrol agents against *C. gloeosporioides*, the biocontrol agents were streaked on one side of a Petri dish (1 cm from the edge of the dish) containing potato dextrose agar (PDA), and a mycelial disc (8 mm diameter) of a seven-day-old culture of the highly virulent *C. gloeosporioides* isolate was placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Nandakumar *et al*., 2001). The dishes were incubated at room temperature (28±2°C) for 4 days and the zone of inhibition was measured.

Preparation of biocontrol formulations

To prepare the biocontrol agents, a talc-based formulation with colloidal chitin was developed (Nandakumar *et al*., 2001). The PGPR and yeast strains were grown on King's B (KB) broth (*P. fluorescens*), NA (*B. subtilis*), and yeast extract glucose agar (YEGA) (*Saccharomyces cerevisiae*) in shake culture at 150 rpm for 48 h at room temperature (28±2°C). Cells were harvested by centrifugation at 8,000 *g* for 15 min and were resuspended in phosphate buffer (0.01 M, pH 7). The cells were adjusted with a spectrophotometer to approximately 10^8 cfu ml^{-1} (OD_{595}=0.3) and used as inoculum (Thompson, 1996).

Five grams of crab shell chitin (Sigma, St. Louis, Missouri, USA) was slowly added to 100 ml of 0.25 N HCl with vigorous stirring and kept overnight at 4°C. The mixture was filtered through glass wool into 200 ml of ethanol at 4°C with rapid stirring. The resultant chitin suspension was centrifuged at 12,000 g for 20 in and the chitin pellets were washed repeatedly with distilled water until the pH became neutral. The concentration of colloidal chitin was adjusted to 10 mg per ml.

The colloidal chitin preparation was incorporated into a broth medium (KB, NA or YEGA) (1%, v:v), the mixture autoclaved at 150 psi for 30 min, inoculated with 1 ml of the biocontrol agents in

phosphate buffer, and grown at room temperature in shake culture (28±2°C). After 48 h of incubation, 1 kg of sterile talc powder (sterilized at 105°C for 12 h), 15 g calcium carbonate (to adjust the pH to neutral) and 10 g carboxymethyl cellulose (adhesive) were mixed under sterile conditions with 400 ml of microbial suspension containing about 9×10^8 cfu ml^{-1}. The mixture was shade-dried to reduce the moisture content (less than 20 per cent) and then packed in a polypropylene bag and sealed. At the time of application, the population of the biocontrol strains in the talc formulation was 2.5–3×10^8 cfu g^{-1}.

Efficacy of pre-harvest application of biocontrol formulations under field conditions

To study the efficacy of the biocontrol formulations on anthracnose development and the relation of these formulations to yield parameters, two field experiments were conducted: one at Bodinaikkanur (South Tamil Nadu - Trial I) and one at Coimbatore (West Tamil Nadu - Trial II). In two selected mango fields (cv. Neelum), the anthracnose disease ranged from 40 to 50% that of the previous year. The experiments were laid out with a randomized block design, three replications were maintained in each treatment, and each replicate consisted of five trees. Treatments included *P. fluorescens* Pf1 and FP7, *B. subtilis* (Bs-1), *S. cerevisiae* (Sc-1), with or without chitin; the synthetic fungicide carbendazim (0.1%); chitin alone (1%) and an unsprayed control. The biocontrol formulations were sprayed until run-off at the rate of 5 g l^{-1} at either 15- or 30-day intervals. Sprays were applied from the pre-flowering stage (second week of November 2001) to the fruit maturity stage (second week of April 2002). Each spray volume consisted of 20 litres per tree (approximately 15 year-old trees of the anthracnose-susceptible cultivar Neelum). In total, 12 sprays (15-day intervals) and 6 sprays (30-day intervals) were applied.

The efficacy of the biocontrol agents was evaluated by measuring the mean number of panicles m^{-2} (= mango tree foliage area) (Rangaswamy, 1995) at the time of flowering; the percentage of anthracnose-affected fruits prior to harvest; the mean number of fruits, mean fruit weight and the fruit yield at harvest stage. Data recorded in each replication were extrapolated to values per hectare.

Induction of defence compounds and enzymes in mango trees

Sprayed and unsprayed mango leaves, flowers and fruit samples (3 replications per treatment; 15 samples per replication) were collected after the second and the tenth spraying and analyzed for phenol content, polyphenol oxidase and peroxidase content.

Phenol content

Mango tissues (1 g) were homogenized in 10 ml of 80% methanol and shaken for 15 min at 70°C (Zieslin and Ben-Zaken, 1993). One ml of methanolic extract was added to 5 ml of distilled water and 250 ml of Folin Ciocalteau reagent (1 N) and the solution was kept at 25°C. After 3 min, 1 ml of saturated solution of Na_2CO_3 and 1 ml of distilled water were added and the reaction mixture and incubated for 1 h at 25°C. The absorption of the developed blue colour was measured using a Beckman DU64 spectrophotometer at 725 nm. The content of total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reaction with phenol, and expressed as phenol equivalents in μg g^{-1} fresh weight.

Polyphenol oxidase (PPO)

Mango tissues (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 *g* for 15 min at 4°C.

The supernatant was used as the enzyme source. The reaction mixture consisted of 200 μl of enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 μl of 0.01 M catechol was added and PPO activity was expressed as changes in absorbance at 495 nm min^{-1} g^{-1} fresh weight (Mayer *et al.,* 1965).

Peroxidase (PO)

Mango tissues (1 g) were homogenized in 2 ml of 0.1 M phosphate buffer (pH 7) at 4°C and centrifuged at 16,000 *g* and at 4°C for 15 min, and the supernatant was used as the enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28±2°C) and the changes in absorbance at 420 nm were recorded at 60 s intervals for 3 min. Enzyme activity was expressed as changes

in the absorbance per minute per g fresh weight (Hammerschmidt *et al*., 1982).

Latent symptom development and quality of harvested fruits

Representative sprayed and unsprayed fruit samples (three replications per treatment; 50 fruit samples per replication) were collected at random (Rangaswamy, 1995). Latent symptom expression and the percent disease index (Rose, 1974) 2, 5, 10 and 15 days after storage at room temperature (28±2°C) were recorded. For the assessment of fruit quality, harvested mango fruit samples from the different treatments were assessed for total soluble solids (TSS), acidity, ascorbic acid, total sugars, and reducing and non-reducing sugar content following standard procedures (Somogyi, 1952; Freed, 1966).

Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared with Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute Biometric Unit, Metro Manila, Philippines.

Results

Efficacy of biocontrol strains against *in vitro* growth of *C. gloeosporioides*

Among the biocontrol isolates evaluated for their efficacy in suppressing mycelial growth, *P. fluorescens* FP7 caused the widest inhibition zone. This isolate also exhibited the greatest pathogen suppression (62.7%), followed by *S. cerevisiae* (52.3%) (Table 1, Fig. 1).

Table 1. Antagonistic effect of biocontrol strains against *Colletotrichum gloeosporioides* under *in vitro* conditions

Biocontrol stain	Radial growth (cm)[a]	Percent inhibition over control
Pseudomonas fluorescens (Pf1)	5.05 c	37.9
Pseudomonas fluorescens (Fp7)	3.30 a	59.0
Bacillus subtilis	4.70 c	42.2
Saccharomyces cerevisiae	3.88 b	52.3
Control	8.13 d	-

[a] Values are mean of four replications. In a column, means followed by the same letter are not significantly different at 5% level by DMRT.

A **B**

Fig. 1. Effect of *Pseudomonas fluorescens* strain FP7 on *in vitro* growth of *Colletotrichum gloeosporioides*. A. Control (*C. gloeosporioides*). B. FP7+*C. gloeosporioides*.

Efficacy of pre-harvest application of biocontrol formulations on panicle initiation and yield parameters

In the field trials, flower initiation in both PGPR and yeast-treated plants was greater than that in the untreated control (Fig. 2). The chitin-amended rhizobacterium FP7 sprayed at 15-day intervals showed a significant increase in the number of panicles (28 m^{-2}) compared to the untreated control (20 m^{-2}). Similarly in the other field, the greatest number of panicles was in trees sprayed with FP7 (24 m^{-2}), followed by trees sprayed with *B. subtilis*+chitin (23 m^{-2}) .

In trial I, the average number of fruits (60,560 ha^{-1}) and fruit yield (11,628 kg ha^{-1}) recorded with FP7+chitin was significantly higher than the fruit number (36,166 ha^{-1}) and yield (4,665 kg ha^{-1}) in the untreated control. The percent increase over the control was 67.4% for mean number of fruits ha^{-1}, and 149.2% for mean yield ha^{-1} (Fig. 3) . The *S. cerevisiae* bioformulation sprayed at fortnightly intervals caused a greater fruit weight (0.195 kg) than the untreated control (0.129 kg) (Fig. 4) . Similarly in trial II the average number of fruits and fruit yield recorded with FP7+chitin, 28,950 ha^{-1} and 4,806 kg ha^{-1}, was higher than that in the un-

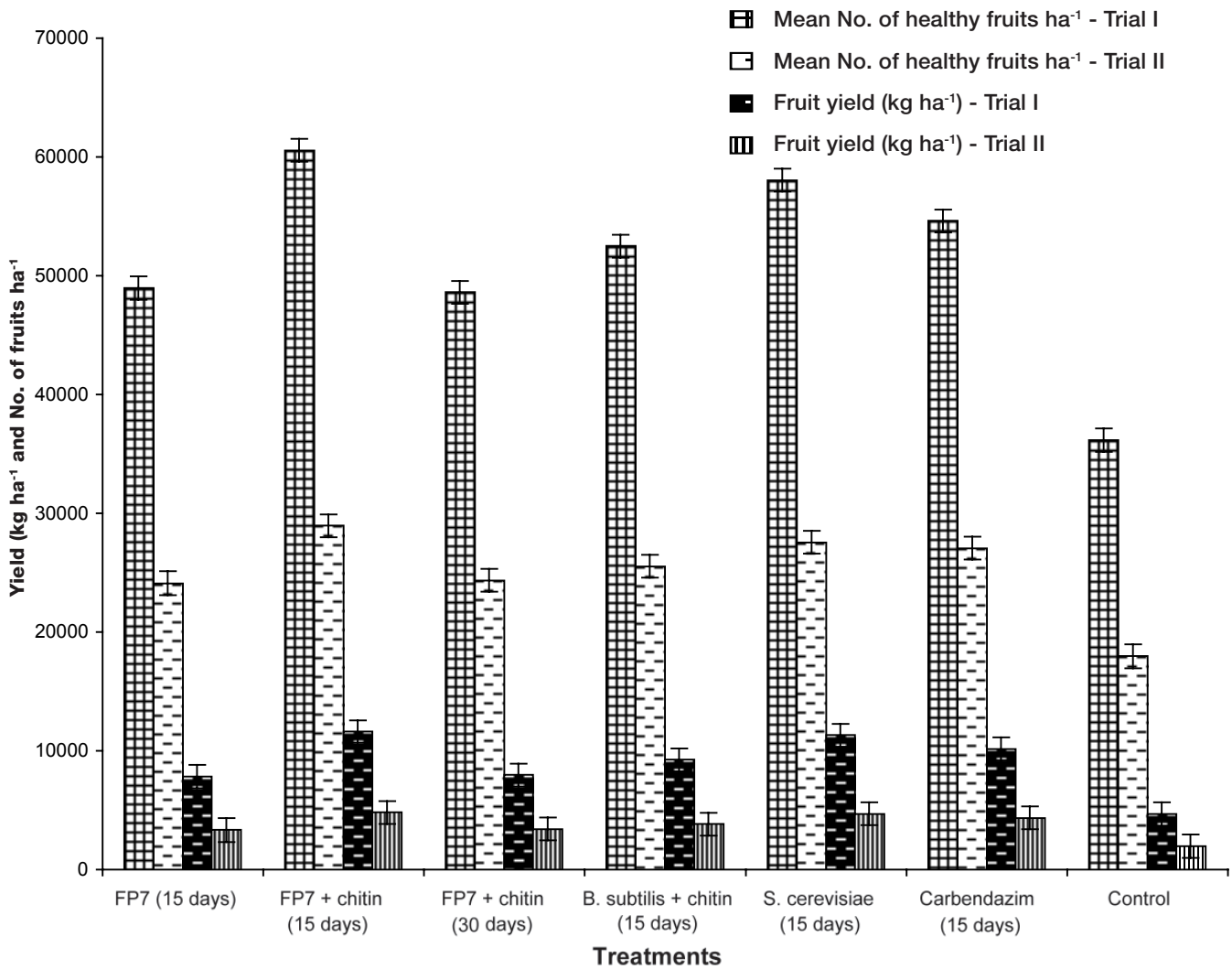

Fig. 2. Effect of pre-harvest application of *Pseudomonas fluorescens* strain FP7 with chitin biocontrol formulation on flower initiation and anthracnose incidence in mango.

treated control (17,960 ha^{-1} and 1,976 kg ha^{-1}) (Fig. 3). The percent increase over the control was 61.2% (fruit number) and 143.3% (yield ha^{-1}).

Effect of pre-harvest application of biocontrol formulations on anthracnose incidence

In both field trials, anthracnose incidence was assessed at fruit maturity. In trial I, the mean percentage of infected fruits with the bioformulation treatments ranged from 4 to 30%, compared with 45% in the control group. Anthracnose infection of fruits treated with FP7+chitin was 4%, a significant reduction of 39% compared with the control . In trial II, the mean percentage of infected fruits ranged from 4.5 to 39% with PGPR, while it was 46.5% in the control. Strain FP7 + chitin produced the lowest rate of infection (4.46%), significantly lower than that with the fungicide (17.02%) .

Induction of defence-related enzymes and phenolic compounds

Mango trees sprayed with the biocontrol formulations were assessed for induction of multiple defence proteins and accumulation of phenolic substances at various days after application. The samples were collected after the second and the tenth spraying with the bioformulations or the fungicide. PGPR-treated mango tissues had higher levels of defence proteins than the unsprayed control. Cells of FP7 + chitin when sprayed at fortnightly intervals had a significantly greater phenolic content and defence-related enzyme activity in all sprayed

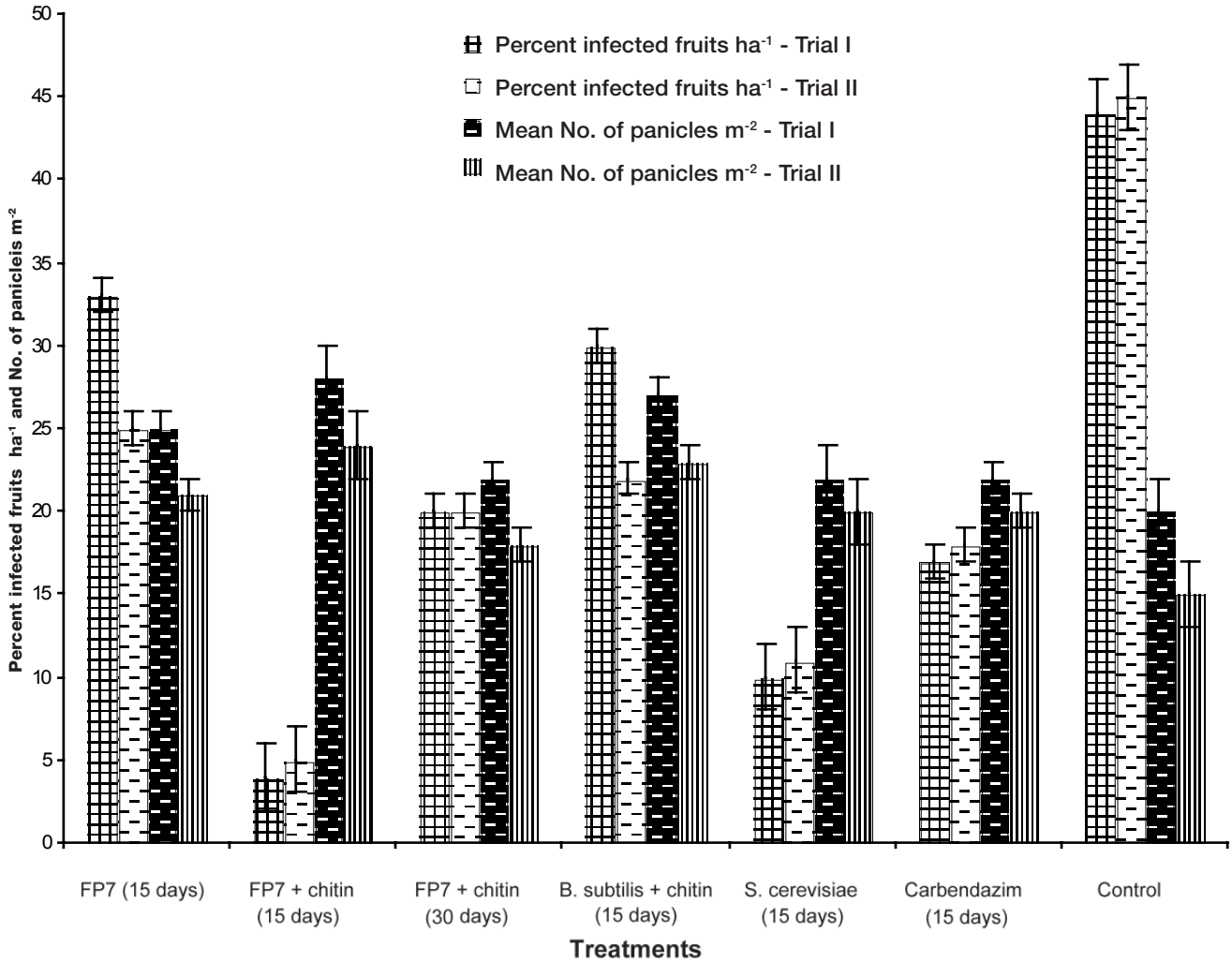

Fig. 3. Effect of pre-harvest application of biocontrol formulations on yield parameters of mango.

tissues: leaves, flowers and fruits in both field trials (Fig. 5, 6 and 7). Generally, enzyme activity was greater in plants sprayed at 15-day-intervals than in plants sprayed at 30-day-intervals. In trial I, the mango leaves sprayed with FP7+chitin at fortnightly intervals had a greater increase in phenolic content (96.6% increase over control) (Fig. 5) and in peroxidase activity (58.3% increase over control) (Fig. 6) than in the other defence proteins. Significant increases in polyphenol oxidase activity (68.4% increase over control) (Fig. 7) were also observed in treated flower tissues. In trial II, likewise, mango leaves treated with FP7+chitin had a greater increase in phenolic content (99.9% increase over control) (Fig. 5) and in peroxidase activity (68.75% increase over control) (Fig. 6) than in the other defence components. The flower tissues also revealed a significant increase in polyphenol oxidase activity (68.4% and 71.2% increase over control) (Fig. 7) compared to the other enzymes.

Effect of pre-harvest application of biocontrol formulations on latent symptom expression and fruit quality

Symptom expression was delayed in fruits stored after bioformulation treatment. FP7 with chitin sprayed at fortnightly intervals led to the lowest percent disease index (PDI) even after 15 days of storage (28±2°C) in both field trials (Table 2). Symptoms remained latent for up to 5 days in both trials. In trial I, the latent symptom expression took significantly longer with fortnightly spraying (3.13 and 6.25 PDI on the 10th and 15th day respectively) than with monthly spraying (25 and 28.13 PDI on the 10th and 15th day respectively) and than in the untreated control (25, 56, 71, 81 PDI on the 2nd, 5th, 10th and 15th days respectively). The same trend was found in trial II. In this

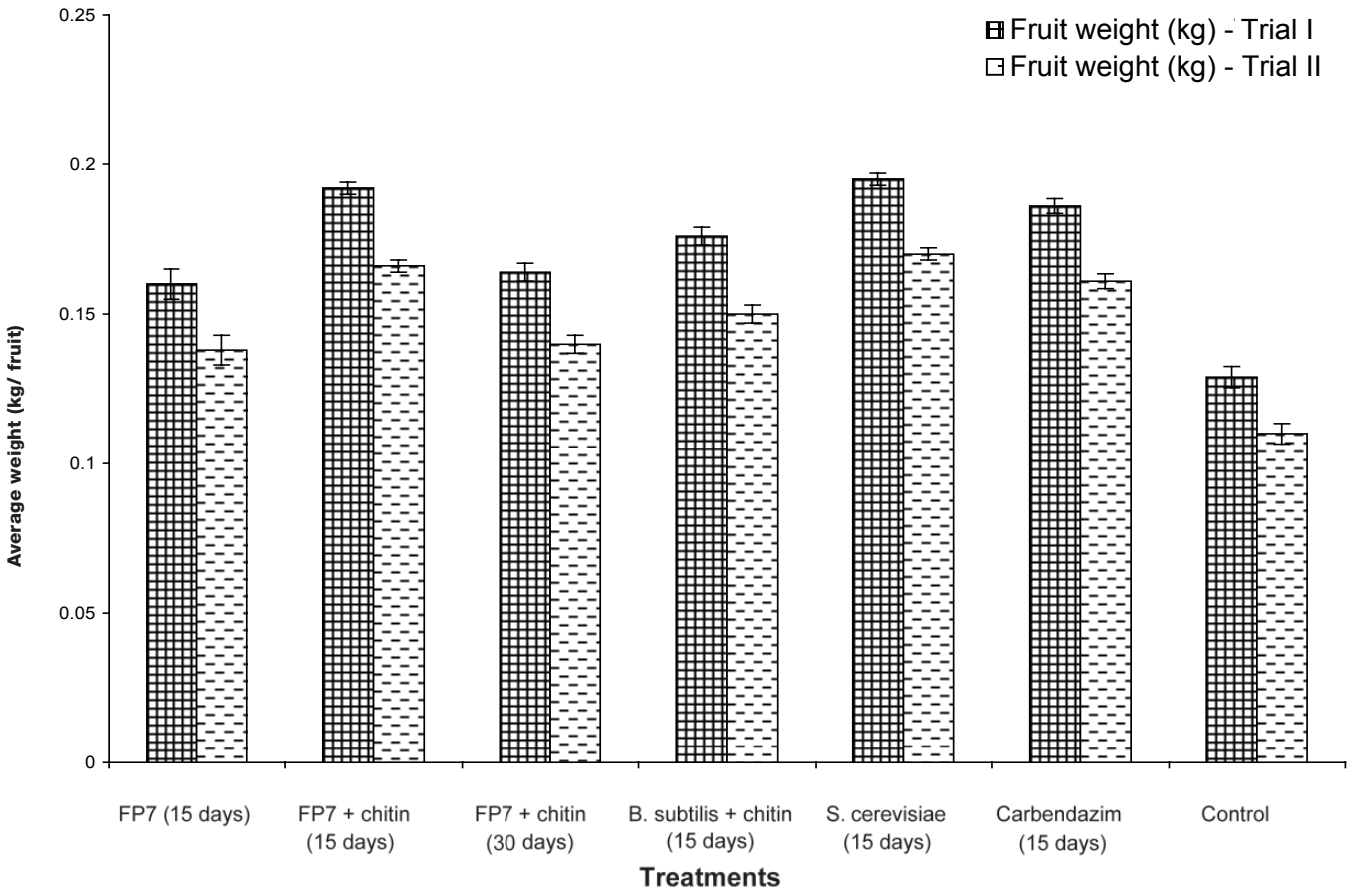

Fig. 4. Effect of pre-harvest application of biocontrol formulations on fruit weight in mango.

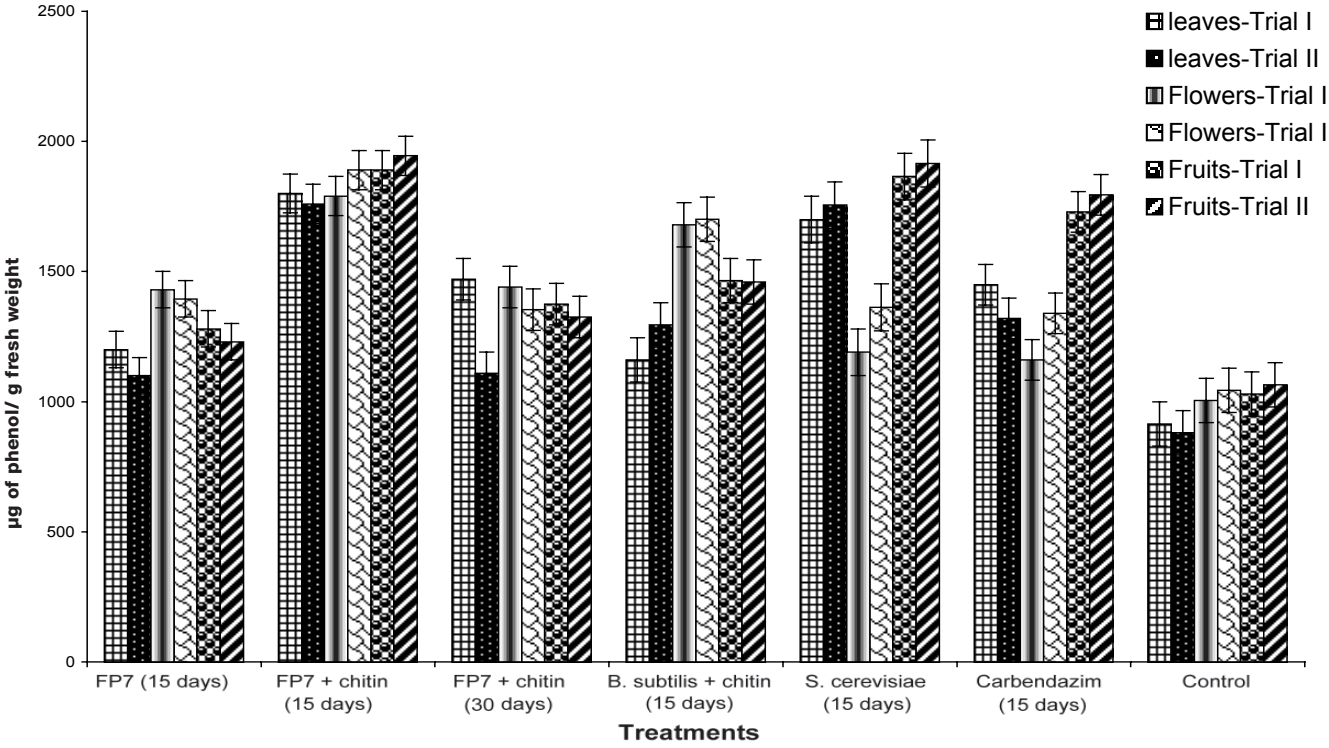

Fig. 5. Effect of pre-harvest application of biocontrol formulations on phenol accumulation in mango trees.

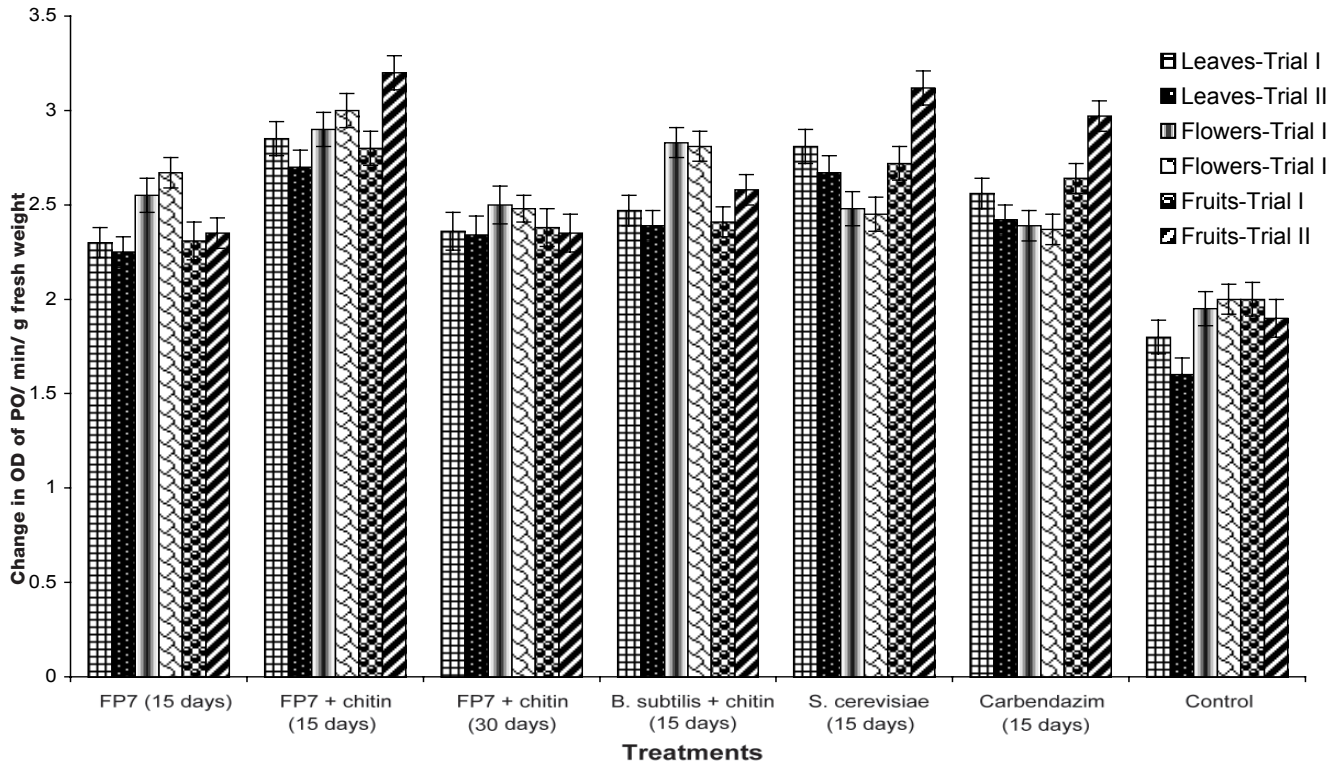

Fig. 6. Effect of pre-harvest application of biocontrol formulations on induction of peroxidase in mango trees.

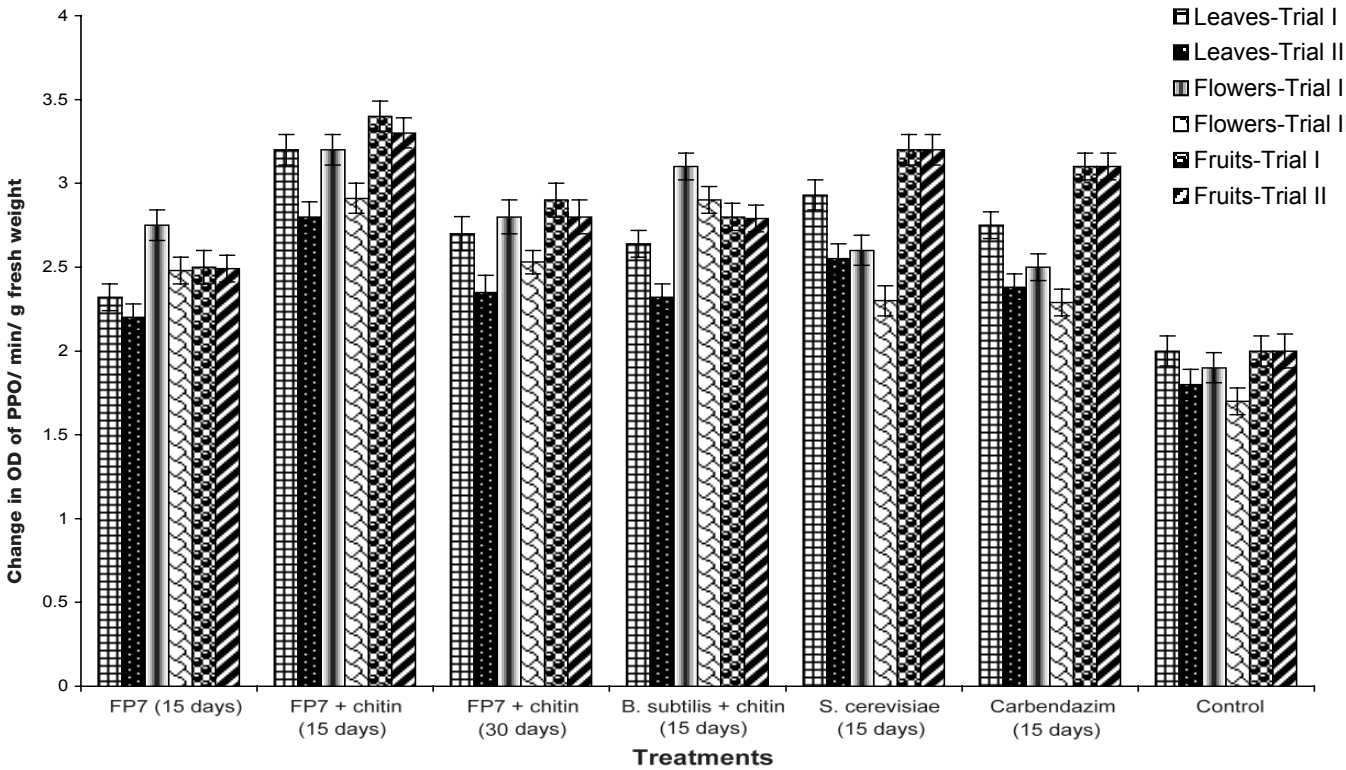

Fig. 7. Effect of pre-harvest application of biocontrol formulations on induction of PPO in mango trees.

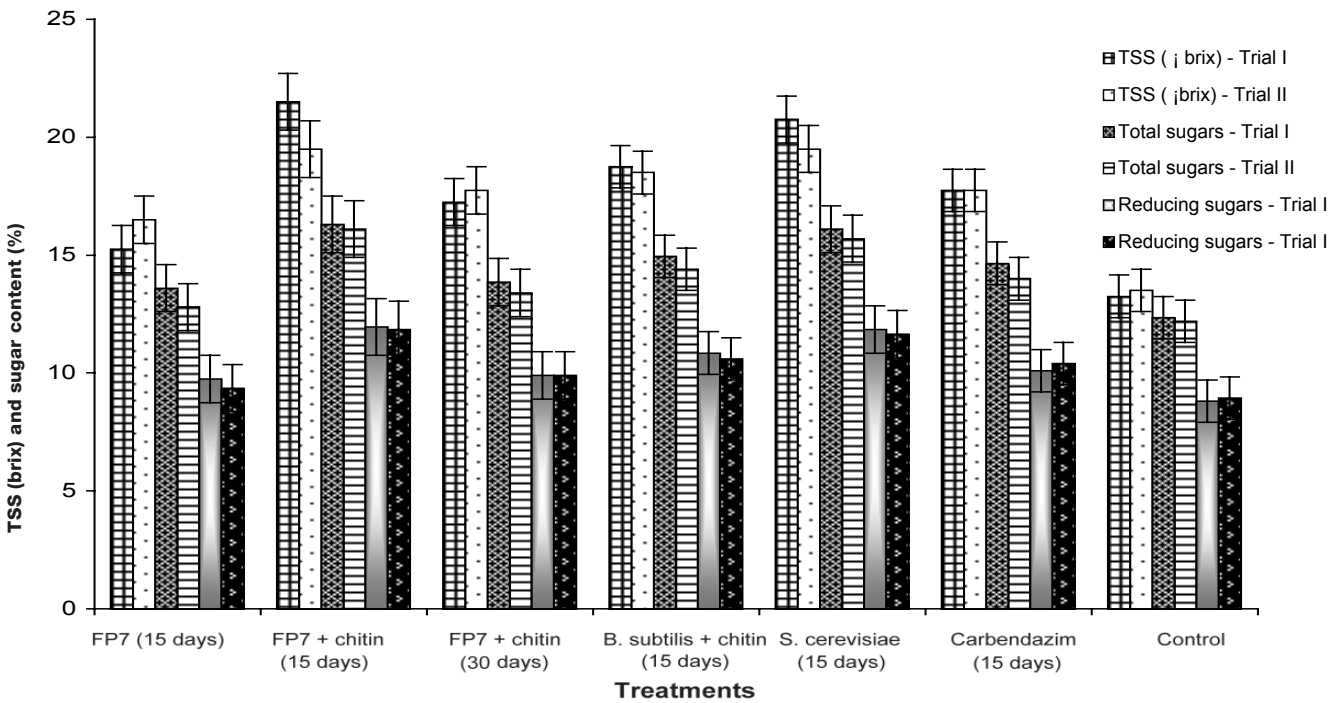

Fig. 8. Effect of pre-harvest application of biocontrol formulations on total soluble solids (TSS), total sugars and reducing sugars content in mango fruits.

trial, symptom expression was significantly slower (3.13, 12.5 PDI on the 10th and 15th day respectively) with fortnightly than with monthly spraying (25, 28.13 PDI on the 10th and 15th day respectively) and than with the untreated control (25, 56, 75, 90 PDI on the 2nd, 5th, 10th and 15th days respectively). In both field trials, fruits treated with *S. cerevisiae* (Sc-1), *P. fluorescens* (Pf1)+chitin, *B. subtilis* (Bs-1)+chitin and carbendazim exhibited no symptoms for up to 2 days, but after that symptom expression gradually increased (Table 2).

Mango quality parameters were assessed on harvested fruit pulp samples from both field trials. In trial I, fruit pulp from crops sprayed fortnightly with FP7+chitin had higher levels of total soluble solids (21.5 °brix), ascorbic acid (10.3 mg/100 g pulp), free or titrable acidity (0.45%), total sugars (16.3%) and reducing and non reducing sugars (11.95 and 4.35%) (Fig. 8, 9 and 10) . Similarly in trial II, the FP7 with chitin treatment was associated with significantly higher levels of total soluble solids (TSS) (19.5 °brix), ascorbic acid (9.51 mg/100 g pulp), free or titrable acidity (0.5%), total sugars (16.1%) and reducing and non-reducing sugars (11.8 and 4.2%) (Fig. 8, 9 and 10) . The yeast formulation was the second most effective treatment after the FP7+chitin treatment.

Discussion

In India, talc based bioformulations have been reported to be effective in the management of several crop diseases (Nandakumar *et al*., 2001). The present investigation clearly indicated that PGPR strain FP7+chitin significantly suppresses endemic anthracnose. *P. fluorescens* FP7 without chitin had the greatest inhibitory effect on *C. gloeosporioides in vitro,* but was only moderately effective

Table 2. Efficacy of pre-harvest application of biocontrol formulations on latent symptom development in mango fruits under stored conditions (28±2°C) as percent disease index (PDI) 2, 5, 10, 15 days after storage.

Spraying interval	Treatment	PDI							
		Trial I				Trial II			
		2 d	5 d	10 d	15 d	2 d	5 d	10 d	15 d
15 days	*Pseudomonas fluorescens* (Pf1)	25 e	34 gh	56 lm	65 op	25 d	34 fg	43 h	62 kl
	P. fluorescens (FP7)	25 e	31 fg	59 mn	71 qr	25 d	31 ef	53 ij	75 no
	Bacillus subtilis	25 e	25 e	50 kl	53 k	25 d	31 ef	50 i	56 j
	Saccharomyces cerevisiae	0 a	3 ab	12 c	12 c	0 a	3 a	12 b	18 c
	Pf1 +chitin	0 a	18 d	25 e	34 gh	0 a	25 d	25 d	31 ef
	FP7+chitin	0 a	0 a	3 ab	6 b	0 a	0 a	3 a	12 b
	B. subtilis+chitin	0 a	25 e	28 ef	50 k	0 a	25 d	34 fg	50 i
	S. cerevisiae+chitin	25 e	31 fg	53 kl	75 r	25 d	31 ef	53 ij	75 no
	Carbendazim	0 a	12 c	12 c	18 d	0 a	18 c	18 c	25 d
	Chitin	25 e	31 fg	59 mn	75 r	25 d	31 ef	62 k	84 q
30 days	*Pseudomonas fluorescens* (Pf1)	25 e	37 hi	59 mn	75 r	25 d	37 g	62 k	78 op
	P. fluorescens (FP7)	25 e	37 hi	62 no	75 r	25 d	37 g	62 k	81 pq
	B. subtilis	25 a	37 hi	59 mn	75 r	25 d	37 g	62 k	71 mn
	S. cerevisiae	0 a	25 e	37 hi	40 ij	0 a	25 d	37 g	37 g
	Pf1+chitin	0 a	25 e	50 k	62 no	0 a	25 d	50 i	62 k
	FP7+chitin	0 a	25 e	25 e	28 f	0 a	25 d	25 d	28 de
	B. subtilis+chitin	0 a	37 hi	62 no	68 pq	0 a	37 g	62 k	68 lm
	S. cerevisiae+chitin	25 e	37 hi	62 no	84 s	25 d	37 g	62 k	84 q
	Carbendazim	0 a	25 e	43 j	53 kl	0 a	25 d	43 h	50 l
	Chitin	25 e	37 hi	75 r	81 s	25 d	37 g	75 no	90 r
	Control	25 e	56 lm	71 qr	81 s	25 d	56 j	75 no	90 r

[a] Values are means of three replications. Each replication contained 15 fruits per treatment. In a column, means followed by the same letter/s are not significantly different at 5% level by DMRT. d, days, i.e. storage duration.

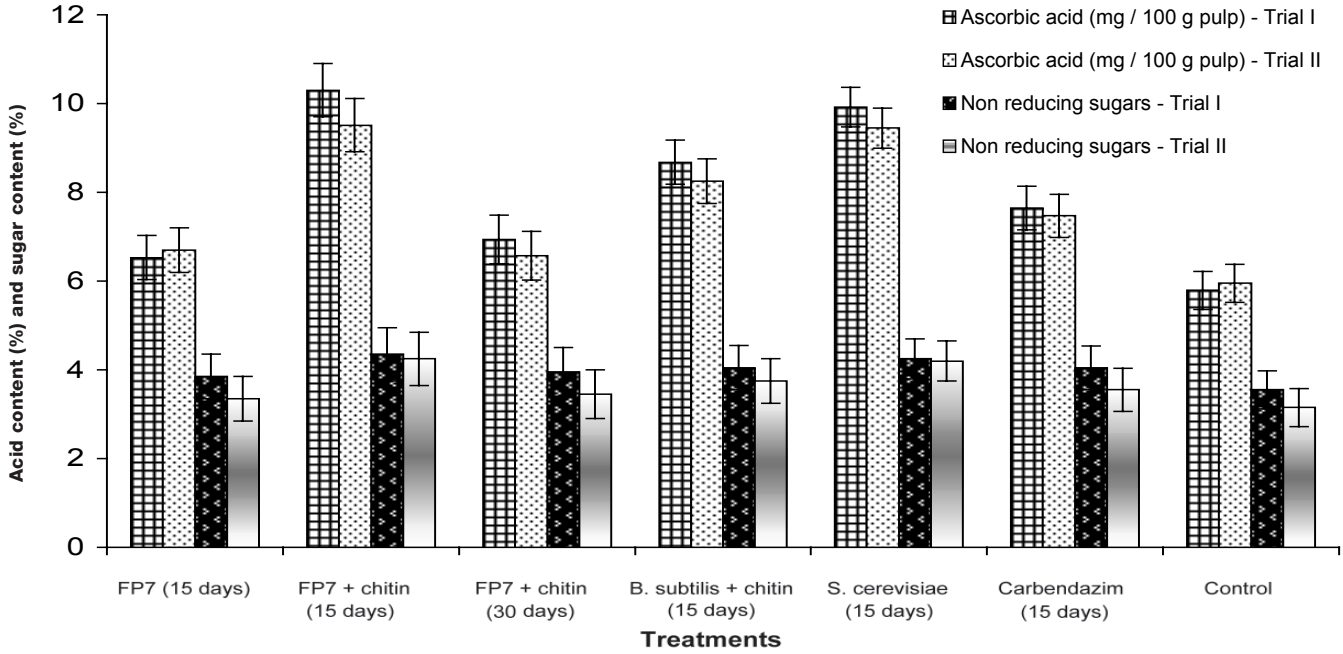

Fig. 9. Effect of pre-harvest application of biocontrol formulations on ascorbic acid and non reducing sugars content in mango fruits.

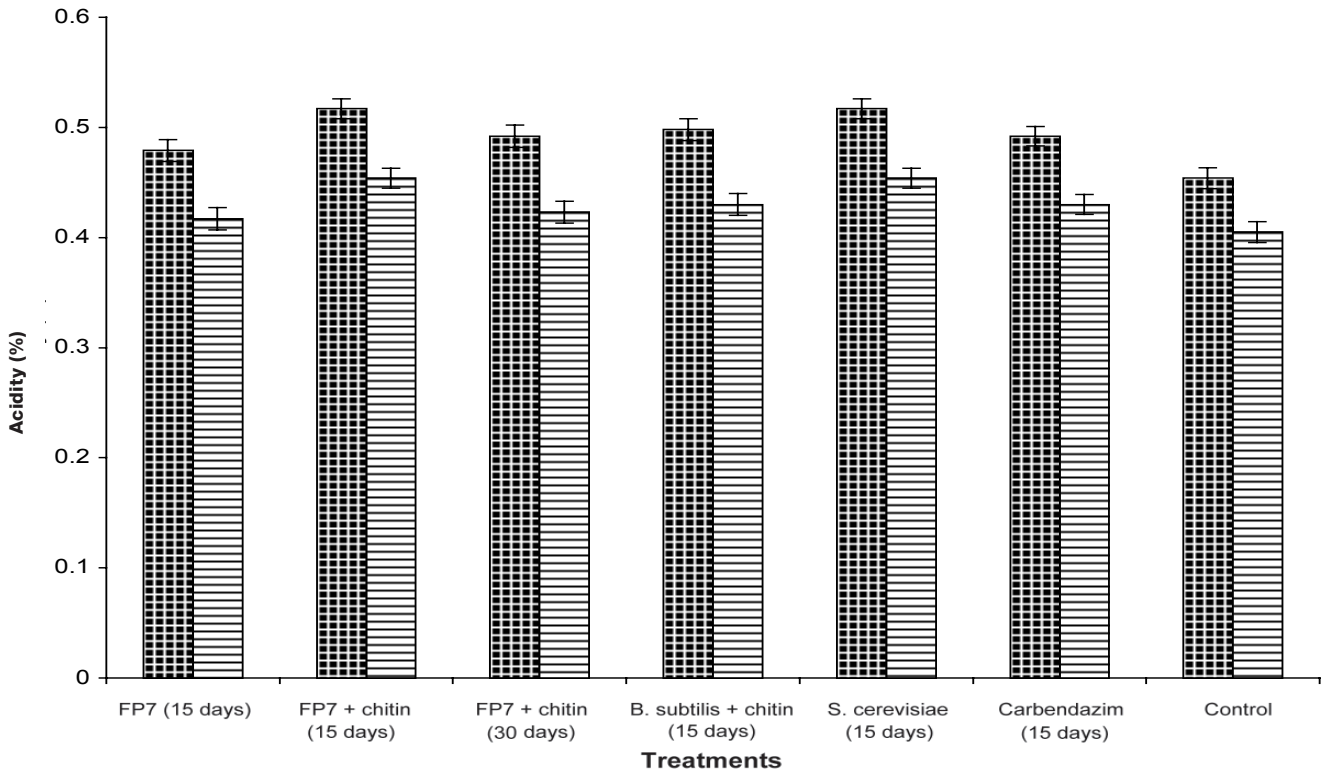

Fig 10. Effect of pre-harvest application of biocontrol formulations on free acidity content in mango fruits.

in the field. The addition of chitin enhanced FP7 effectiveness in the field. This finding is consistent with Radja Commare *et al*. (2002) who reported a very low incidence of sheath blight in rice treated with PGPR + chitin. A similar type of enhanced biocontrol efficacy when chitin was added to fluorescent pseudomonads against *Colletotrichum falcatum* in sugarcane was reported by Viswanathan and Samiyappan (2001), a result attributed to the increased multiplication of the PGPR due to the chitin. Isolate FP7+chitin induced durable systemic resistance throughout the post-harvest period, unlike the synthetic fungicide carbendazim which gradually lost its effectiveness over time. Chitin amendment of the *S. cerevisiae* formulation resulted in moderate effectiveness under field conditions when compared to *S. cerevisiae* alone. One possible explanation for this only modest increase might be that yeast is a poor user of chitin as a nutrient.

It is well known that anthracnose symptoms and its depressing effects on yield are most severe when trees become infected in the early growth stages. In this context, a pre-flowering application of biocontrol agents in the orchard can reduce these early infections, and hence storage rot. The present study found that *P. fluorescens* FP7+chitin drastically reduced anthracnose when it was applied at the pre-flowering stage. A similar trend was observed on strawberry (Peng *et al*., 1992) against *Botrytis cinerea*, on sweetcherry (Wittig *et al.,* 1997) against *Monilinia laxa* and on peach (Smilanick *et al*., 1993) against brown rot infections The biocontrol agents *Aureobasidium pullulans, Candida oleophila, Epicoccum purpurascens, Pseudomonas corrugata* and *P. cepacia* also successfully controlled those post-harvest pathogens.

Panicle formation was greatly enhanced with certain treatments (FP7+chitin; *B. subtilis*+chitin) in this study. Other treatments showed increased flowering due to PGPR action. Mango trees treated with FP7+chitin increased flower induction and the number of bearing fruit. This could be associated with the synthesis of phytohormones like gibberellins, cytokinins and indole acetic acid by PGPR and yeast antagonists (Dubeikovsky *et al*., 1993; Ramamoorthy and Samiyappan, 2001). Increased yield in cucumber (Zehnder *et al.,* 1997), sugarcane (Viswanathan and Samiyappan, 1999) and tomato (Murphy *et al.*, 2000) due to treatment with PGPR strains under field conditions has already been reported. Although *B. subtilis*+chitin induced maximum panicle formation, it was not very effective in controlling anthracnose as compared with FP7+chitin.

The present study suggests that the pre-harvest application of biocontrol agents may help overcome pre and post-harvest infection by increasing levels of defence-related enzymes and phenolic substances. Mango trees treated with FP7+chitin at 15 day intervals showed maximum increases in phenol, peroxidase, polyphenol oxidase and PAL enzymes. The association between higher levels of defence-related enzymes and greater disease resistance has been reported by several workers. Higher levels of phenol (Ramamoorthy *et al.,* 2002), peroxidases (Viswanathan and Samiyappan, 1999; Nandakumar *et al.,* 2001) and PPO (Chen *et al*., 2000) have been reported to be effective against various fungal diseases.

Levels of total soluble solids in the pulp were lower in unsprayed fruits (control treatment), in which post-harvest *Colletotrichum* infection was more common than it was in fruits treated with FP7+chitin. This has been well documented by Hussey *et al*. (1969) and Willink and Moore (1988), who suggested that the reduction in fruit quality was due to pathogen infection. Latent infections disrupt the normal physiological processes of food manufacturing, conversion and accumulation, and hence lead to the production of poor quality fruits.

In conclusion, the pre-harvest application of FP7 plus chitin was durably effective against anthracnose both in the field and in post-harvest storage. Higher levels of phenolics, peroxidase and polyphenol oxidase may have contributed collectively to induced resistance in mango trees against anthracnose, which eventually resulted in better mango fruit quality and greater yields.

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