Effect of pre harvest application of bioformulations on the anthracnose disease incidence and biometrics of mango

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ABSTRACT

The effect of pre harvest application of bioformulations on the disease incidence of leaves, inflorescence, fruits and biometrics of mango was tested in this study. Foliar spraying of *Pseudomonas fluorescens* (BIB_2) + *Bacillus subtilis* (BIL_8) + chitin on 20 days before flowering, flowering stage, 20 and 60 days after flowering plus Carbendazim (0.1%) at 40 days after flowering recorded the minimum incidence of leaf anthracnose at pre flowering, flowering, fruit set and fruit maturity stages *viz*., 8.67, 12.33, 13.00 and 13.00 per cent, respectively. Similarly nil infection on inflorescence and fruit was recorded in above treatment. The same treatment recorded maximum number of panicle/tree (142.00), fruits/tree (748.67), fruit weight (221.76g/fruit) and fruit yield/tree (166.02 kg). *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin recorded highest number of bacterial population (16.67×10^4 cfu g^{-1} of leaf) at 16 days after spray.

Keywords: *Pseudomonas fluorescens*, *Bacillus subtilis*, chitin, mango anthracnose

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INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruit crops in the world. It belongs to the family Anacardiaceae and is called the King of fruits (Hayes, 1953). India has the richest collection of mango cultivars and mango regarded as the "National fruit" of the country. Cultivation of mango is believed to have originated from South East Asia. Though India is the house of about 1000 varieties, only a few are commercially grown in different states. In Tamil Nadu, mango is cultivated in an area of 20.20 lakh ha and varieties like Neelam, Bangalora, Alphonso and Banganapalli are commercially grown. Mango cultivation is an important agribusiness in India. Even though it has the largest area, the productivity is very low due to a number of diseases. Mango trees are affected by several fungal, bacterial and viral diseases, of which mango anthracnose caused by *Colletotrichum gloeosporioides* Penz. is a highly destructive pathogen that causes considerable damage, inflicting severe qualitative and quantitative losses (Sangeetha and Rawal, 2010; Sayiprathap *et al.,* 2018). Anthracnose can render the tree completely unproductive (up to 100%), as it destroys the developing or developed fruits both in field and in storage conditions. It causes leaf, blossom blight and tree die-back in the orchard and can subsequently give rise to rottened fruits during storage and thus poses several problems (Ploetz and Freeman, 2009; Manasa *et al*., 2018).

Normally fungicides are the primary means of controlling plant diseases. But the fungicides are under special scrutiny for posing potential oncogenic risks (Eckert and Ogawa, 1985). They are used alone, combined in mixtures, or applied separately in sequence (Prabakar *et al.,* 2008; Johnson and Hofman, 2009). Even if acceptable fungicides are applied the pathogen often develops resistance and produces new biotypes (Eckert *et al.,* 1994). Moreover, concerns have been raised about the health risk

involved in the use of synthetic fungicides on fresh fruits and vegetables.

The increased consumer preference for healthy agricultural products and environmental risks associated with chemical residues in food are the major driving forces for the search of new safer control methods. Over the past few decades, biological control has emerged as an effective strategy to combat the decay of fruits. Plant growth promoting rhizobacteria (PGPR) especially *P. fluorescens* (Ardakani *et al.,* 2010) and *B. subtilis* are promising candidates as bioprotectants (Ramamoorthy *et al.,* 2001; Mahadtanapuk *et al.,* 2007). Though remarkable success has been achieved in this direction through the use of antagonistic microorganisms, the information generated on the performance of the introduced antagonists into the ecosystem under varying field conditions still remains inadequate constituting a major obstacle in the large scale adoption of this technology. Recently more emphasis has been laid on supplementing various nutrients with bioprotectants, which is better than either alone (Janisiewicz and Bors, 1995). It implies several good attributes by enhancing the antagonist's multiplication, survival rate for effective establishment in the field and subsequent fruit rot control.

Recently a potential approach in biocontrol involves the use of the natural bioactive substances which inhibits fungal growth and also activates the biological efficiency of the antagonistic microorganisms. Chitin is a naturally occurring high molecular weight linear homo polysaccharide composed of N-acetyl-D glucosamine residues in α (1-4) linkage. Chitin and its derivatives are biodegradable and biocompatible natural polymers with a wide range of uses in cosmetology, food industry, biotechnology, medicine and agriculture (Li *et al.,* 1997). Involvement of chitin adjuvant in improving the efficacy of various antagonists and triggering the plant originated ISR either alone or in combination with biocontrol agents has been demonstrated in various crops (Vivekananthan *et al.,* 2004; Viswanathan and Samiyappan, 2008; Loganathan *et al.,* 2010). With this background, these studies have been formulated to bring out an effective strategy for the management of mango anthracnose.

METRIALS AND METHODS

Isolation of bacterial antagonists

Antagonistic bacteria were isolated from leaf surface, fruit skin and blossom of mango collected from major mango growing areas of Tamil Nadu using leaf washing technique (Gould *et al.,* 1996). A small plant material was mixed with 5 ml of sterile distilled water in a flask which was shaken on a shaker for 30 min. Then 1 ml of suspension was added to a Petri plate containing nutrient agar medium and incubated at room temperature (28 ± 2°C) for 48 h. The growing colony was subcultured on nutrient agar (NA) using single colony isolation. The slant was kept at 10°C in refrigerator and used as stock culture. Totally 52 isolates of bacteria were isolated from leaf surface, blossom and fruit skin. Based on the dual culture technique and poison food technique *P. fluorescens* (BIB_2) and *B. subtilis* (BIL_8) strains were selected for further studies.

Preparation of commercial formulation

A loopful of effective bacterial isolate was inoculated into the sterile King's B broth for *P. fluorescens* (BIB_2), Nutrient agar for *B. subtilis* (BIL_8) and incubated in a rotary shaker at 150 rpm for 72 h. at room temperature (28 ± 2°C). After 72 h. 400 ml of bacterial suspension containing 9×10^8 cfu ml^{-1}, one kg of the carrier material (talc powder), 15 g calcium carbonate and 10 g CMC were thoroughly mixed, shade dried to reduce the moisture content below twenty per cent and packed in polythene bags (Nandakumar *et al.,* 2001). For testing the combination effect of the talc based formulation of *P. fluorescens* (BIB_2) and *B. subtilis* (BIL_8) the individual formulations with the adequate cfu were mixed thoroughly at 1:1 (w/w) ratio just before application and sprayed.

Preparation Chitin amended talc based formulations

Five grams of crab shell chitin was slowly added into 100 ml of cold 0.25 N HCl with

vigorous stirring and kept overnight at 4°C. The mixture was filtered through glass wool into 200 ml of ice cold ethanol at 4°C with continuous stirring. The resultant chitin suspension was centrifuged at 1000 rpm for 20 min. and the chitin pellets were washed repeatedly with distilled water until the pH becomes neutral. The conc. of colloidal chitin was adjusted to 10 mg ml^{-1}.

Incorporation of colloidal chitin into broth medium and formulation development

Colloidal chitin was prepared as described earlier and incorporated into broth medium (1%, v/v) and the mixture was autoclaved at 15 psi for 30 min. Then the cultures were inoculated individually into their respective broth and kept in a shaker for 72 h. at room temperature (28 ± 2°C). After 72 h. of incubation, the broth containing 9×10^8 cfu ml^{-1} was used for the preparation of talc based formulation. To the 400 ml of bacterial suspension, 1 kg of the purified talc powder (sterilized at 105°C for 12 h.), calcium carbonate 15 g (to adjust the pH to neutral) and carboxy methyl cellulose (CMC) 10 g (adhesive) were mixed under sterile conditions. The product was shade dried to reduce the moisture content (less than 20%) and then packed in polypropylene bags and sealed. At the time of application, the population of biocontrol strains in talc formulation was found to be 2.5 to 3×10^8 cfu g^{-1}. Chitin at 1% alone amended media without any inoculation of antagonistic bacteria was mixed with talc powder and used for the chitin alone treatment.

Disease incidence

The field trial was conducted in Vedasandur village, Dindigul District of Tamilnadu. The talc based bioformulations with and without chitin amendment was sprayed @ 5g/litre of water. Five sprays at 20 days interval were given from pre flowering stage to fruit maturity stage @ 20 litre/tree. Besides, integration of Carbendazim 50 WP @ 0.1 % was tried by spraying the chemical at 40 days after flowering (Most susceptible stage) along with bioformulations spray schedule. The experiment was laid out in randomized block design with three replications and each replicate consisted of nine trees. Carbendazim 50 WP @ 0.1% was used for comparison and a suitable control was also maintained without any treatment. In field trial, about 13 years old mango trees (var. Neelam) with uniform growth were selected and standard agronomic practices and fertilizer application were followed as per the State Horticultural Department recommendations. The disease incidence was recorded on leaves (0-5 scale of Suharban *et al.,* 1985) at pre flowering stage, flowering stage, fruit set and fruit maturity stage. Whereas the disease incidence on inflorescence was recorded at flowering stage (0-9 scale of Jamadar and Desai, 1997) and fruit infection (0-4 scale of Prabakar *et al.,* 2008) was recorded at fruit maturity stage.

Biometric observations

The number of panicles were counted at the end of flowering season and the number of fruits harvested from each tree was recorded and the total number of fruits per tree was arrived at by addition. Twenty five fruits from each tree were randomly selected and weighed individually and the average was worked out and expressed in gram. The total weight of fruits harvested from each tree from first to final harvest was recorded in kilograms.

Phylloplane population of bacterial antagonists

The survival of biocontrol agents on sprayed mango leaves was assessed by taking leaf samples at 2, 4, 8 and 16 days after the final foliar application. Leaf samples (1g) were transferred to a test tube containing 100 ml of sterile distilled water. After thorough shaking, the populations of *P. fluorescens* (BIB_2) and *B. subtilis* (BIL_8) in the suspension were estimated by serial dilution plating, using King's B medium for *P. fluorescens* and Nutrient agar for *B. subtilis*.

Statistical analyses

The data generated were subjected to statistical analysis using computer aided IRRISTAT Version 92 software developed by the International Rice Research Institute, Philippines.

RESULTS AND DISSCUSION

Mango anthracnose incidence

Studies on the effect of bioformulations under field condition revealed that foliar spraying of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin on 20 days before flowering, flowering stage, 20 and 60 days after flowering plus Carbendazim at 40-days after flowering recorded the minimum incidence of leaf anthracnose at pre flowering, flowering, fruit set and fruit maturity stages (Table 1). This was followed by application of *P. fluorescens* ($B1B_2$) + *B. subtilis* (BIL_8) + chitin on 20 days before flowering, flowering stage, 20, 40 and 60 days after flowering. Spraying of Carbendazim at 20 days before flowering, flowering stage, 20, 40 and 60 days after flowering recorded on par disease incidence with T_7. In general maximum anthracnose incidence was observed in control treatment. This might be due to the increased multiplication of the PGPR, enhanced chitinase activity and induced systemic resistance in mango plants in response to chitin amended bioformulation treatment.

Incidence of anthracnose of mango (Field study)

The data depicted in table 2 revealed that pre harvest application of bioformulations either alone or in combination showed significant influence on the incidence of inflorescence and fruit anthracnose. Among the various treatments, nil infection on inflorescence and fruit was recorded in T_6 - *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin at 20 days before flowering, flowering stage, 20, 40 and 60 days after flowering, T_7 - *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin on 20 days before flowering, flowering stage, 20 and 60 days after flowering plus Carbendazim (0.1%) at 40 days after flowering and T_8- Carbendazim (0.1%). It was followed by application of *P. fluorescens* (BIB_2) + chitin which recorded 3.33 per cent of inflorescence and 0.67 per cent of fruit

anthracnose. The maximum disease incidence was observed in control. Anthracnose has become a major challenge to mango researchers in general. The routine control measure involving chemical pesticide application leads to toxicity, residual effect and resistance development by pathogens (Koomen and Jeffries, 1993). Since all the commercial mango varieties are susceptible to the disease, biological control provides an effective, persistent and durable protection.

Besides, the results revealed that amendment of chitin in the bioformulations enhanced the disease suppression when compared to the bioformulation treatment without chitin amendment. Radja Commare *et al.* (2002), observed that the foliar application of Pfl (*P. fluorescens*) with chitin, FP7 (*P. fluorescens*) with chitin recorded lower per cent disease index (PDI) and enhanced yield of rice. Saravanakumar *et al.* (2007) reported that PGPR strain Pfl (*P. fluorescens*) amended with chitin significantly reduced the dry root rot of green gram in field conditions. Biocontrol efficiency of antagonists might have been stimulated by chitin resulting in a significant antagonistic effect against *C. gloeosporioides* as observed by Rajkumar *et al.* (2008) against *R. solani*.

It was also observed that the combination of the chitin amended antagonists recorded better results in reducing the incidence of anthracnose when compared to treatment with individual antagonists. The better performance of the combination of the antagonists might be due to the combined action of different mechanisms and better performance of *P. fluorescens* and *B. subtilis.* Integration of Carbendazim @ 0.1% at 40 DAF in the spray schedule further enhanced the disease suppression and resulted in the lowest incidence of anthracnose. Enhancement of the efficacy of bioformulations in combination with chemical fungicides has been reported earlier. Biocontrol agents for the control of foliar diseases are available, but inconsistent performance of the introduced agents on aerial plant parts has been a limitation for their extensive adoption (Andrews, 1992). In several disease management strategies, the addition of fungicide at reduced rates in combination with biocontrol agents has significantly enhanced disease control,

Table1. Effect of pre harvest application of bioformulations on anthracnose disease incidence in mango leaves

	Treatments	Per cent disease index (PDI) on leaf at different stages				Mean	Per cent decrease over control
		Pre flowering	Flowering	Fruit set	Fruit maturity		
T_1	*P. fluorescens* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	8.00 [b]	18.67 [e]	24.67 [e]	28.33 [d]	19.91	33.63
T_2	*B. subtilis* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	8.33 [c]	19.33 [f]	27.33 [f]	30.67 [e]	21.41	28.63
T_3	$T_1 + T_2$	8.67 [d]	16.00 [d]	22.00 [d]	25.67 [d]	18.08	39.73
T_4	T_1 + chitin	7.67 [a]	15.00 [c]	19.33 [c]	21.00 [c]	15.75	47.50
T_5	T_2 + chitin	8.33 [c]	15.33 [c]	20.67 [d]	24.33 [c]	17.16	42.80
T_6	T_3 + chitin	8.00 [b]	14.67 [b]	17.33 [b]	19.67 [b]	14.91	50.26
T_7	T_6 on 20 DBF, FS, 20, 60 DAF plus spraying Carbendazim on 40 DAF	8.67 [d]	12.33 [a]	13.00 [a]	13.00 [a]	11.75	60.83
T_8	Carbendazim 50 WP @ 0.1% on 20 DBF, FS, 20, 40, 60 DAF	8.00 [b]	11.33 [a]	11.67 [a]	12.00 [a]	10.75	64.16
T_9	Control	8.33 [c]	32.33 [g]	36.67 [g]	42.67 [f]	30.00	-

DBF- Days before flowering, DAF-Days after flowering, FS- Flowering stage; Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

Table 2. Effect of pre harvest application of bioformulations on the disease incidence of inflorescence and fruits of mango

T.No	Treatments	Per cent disease index (PDI)			
		Inflorescence infection (at flowering stage)	Per cent decrease over control	Fruit infection (at fruit maturity stage)	Per cent decrease over control
T_1	*P. fluorescens* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	11.33 [e]	55.84	3.00 [d]	50.00
T_2	*B. subtilis* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	17.00 [f]	33.74	3.00 [d]	50.00
T_3	$T_1 + T_2$	7.67 [d]	70.10	1.67 [c]	72.16
T_4	T_1 + chitin	3.33 [b]	87.02	0.67 [b]	88.00
T_5	T_2 + chitin	6.67 [c]	74.00	1.67 [c]	72.16
T_6	T_3 + chitin	0.00 [a]	100.00	0.00 [a]	100.00
T_7	T_6 on 20 DBF, FS, 20, 60 DAF plus spraying Carbendazim on 40 DAF	0.00 [a]	100.00	0.00 [a]	100.00
T_8	Carbendazim 50 WP @ 0.1% on 20 DBF, FS, 20, 40, 60 DAF	0.00 [a]	100.00	0.00 [a]	100.00
T_9	Control	25.65[g]	-	6.00[e]	-

DBF- Days before flowering, DAF-Days after flowering, FS- Flowering stage
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

compared to treatments with biocontrol agent alone (Buck, 2004; Prafulkumar and Mane, 2017).

Investigation on the efficacy of *P. fluorescens* integrated with a reduced dose of fungicide to a half of the normal dose effectively reduced the incidence of anthracnose and powdery mildew in chilli (Anand, 2010). Similarly, Kishore *et al.* (2005) found that the combination of *P. aeruginosa* and chlorothalonil reduced the severity of late leaf spot in groundnut. *P. fluorescens* and *B. subtilis*, in combination with reduced dose of fungicides, were equally as effective as the standard fungicides alone in control of *Penicillium expansum* on pear fruits (Frances *et al.,* 2002) and damping off in tomato plants (Kondoh *et al.,* 2001). These earlier reports are in line and lend support to the results observed in the present study.

Biometrics of mango under field condition

Generally all the treatments with bioformulations showed increased growth and yield attributes when compared to control (Table 3). Of these, the treatment consisting of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin on 20 days before flowering, flowering stage, 20 and 60 days after flowering plus Carbendazim (0.1%) at 40 days after flowering recorded the maximum number of panicle/tree, fruits/tree, fruit weight and fruit yield/tree. This was on par with the treatment T_8 - Carbendazim (0.1%). The untreated control recorded the minimum number of biometrics.

The enhanced disease suppression due to integration with Carbendazim might also be attributed as the reason for the improved yield observed in the present study. Kloepper *et al.* (1980) have indicated that PGPR application was often associated with increased plant growth. The trees sprayed with FP_7 (*P. fluorescens*) + chitin bioformulation at 15 days intervals recorded maximum panicle number and yield (Vivekananthan *et al.,* 2004).The PGPR strains promote plant growth by synthesizing phytohormones like gibberellins, cytokinins and indole acetic acid which might be responsible for the increased yield (Malhotra and Srivastava, 2006).

The increased yield of celery (Bell *et al.,* 1998), cucumber (Zehnder *et al.,* 1997), sugarcane (Viswanathan and Samiyappan, 1999) and tomato (Murphy *et al.,* 2000) due to PGPR treatment has also been reported under field conditions. Saravanakumar *et al.* (2007) reported that the highest yield was recorded by Pfl + chitin in green gram. *P. fluorescens* (FP7) + chitin treated Alphonso trees recorded significantly high yield attributes of mango *viz*., mean number of fruits and fruit yield (Vivekananthan *et al.,* 2004). The PGPR mixed bioformulation Pf1 + *B. subtilis* + neem + chitin were found to be increasing the plant growth and yield parameter of chilli under both green house and field conditions (Bharathi *et al.,* 2004). The above results lend support to the present findings.

Phylloplane population of antagonistic bacteria at different days after spraying

The survival of *P. fluorescens* (BIB_2) and *B. subtilis* (BIL_8) on mango phylloplane was assessed and the results are presented in table 4. Among the treatments, T_6- *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin recorded highest number of bacterial population at 16 DAS, followed by T_4- *P. fluorescens* (BIB_2) + chitin @ 5g/litre of water and T_5- *B. subtilis* (BIL_8) + chitin @ 5g/litre of water. The lowest number of bacterial population was recorded in T_5 - Carbendazim (0.1%) applied treatment.

Biocontrol efficiency of *Pseudomonas* spp. may be stimulated by chitin resulting in a significant increase in their population density and antagonistic effect against *R. solani* (Rajkumar *et al.,* 2008). The bacterium *P. putida* was able to colonize in the lamina, leaf stalk, pericarp and pulp of papaya and strongly inhibit ten kinds of phytopathogens (Shi *et al.,* 2010). Similarly, several workers reported that presence of chitin resulted in a better bacterial population in phylloplane (Manjula and Podile, 2001; Kishore *et al.,* 2005). The above results lend support to the present findings.

Table 3. Effect of pre harvest application of bioformulations on biometrics of mango

T.No	Treatments	No. of panicles / tree	Mean no. of fruits/tree	Fruit weight (g/fruit)	Fruit yield (kg/tree)
T_1	*P. fluorescens* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	118.00 ^{d}	583.67 ^{e}	194.38 ^{e}	113.45 ^{c}
T_2	*B. subtilis* @ 5g/liter on 20 DBF, FS, 20, 40, 60 DAF	109.67 ^{e}	551.00 ^{f}	189.88 ^{e}	104.62 ^{d}
T_3	$T_1 + T_2$	120.00 ^{c}	607.33 ^{d}	199.81 ^{d}	121.35 ^{c}
T_4	T_1 + chitin	125.00 ^{b}	668.67 ^{c}	204.80 ^{c}	136.94 ^{b}
T_5	T_2 + chitin	122.33 ^{c}	660.00 ^{c}	201.16 ^{d}	132.76 ^{b}
T_6	T_3 + chitin	129.33 ^{b}	684.00 ^{b}	208.06 ^{b}	142.31 ^{b}
T_7	T_6 on 20 DBF, FS, 20, 60 DAF plus spraying Carbendazim on 40 DAF	142.00 ^{a}	748.67 ^{a}	221.76 ^{a}	166.02 ^{a}
T_8	Carbendazim 50 WP @ 0.1% on 20 DBF, FS, 20, 40, 60 DAF	137.00 ^{a}	725.67 ^{a}	217.31 ^{a}	157.69 ^{a}
T_9	Control	98.00 ^{f}	474.00 ^{g}	172.73 ^{f}	81.87 ^{e}

DBF- Days before flowering, DAF-Days after flowering, FS- Flowering stage
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

Table 4. Phylloplane population of antagonistic bacteria at different days after spraying

T.No	Treatments	Bacterial population (cfu×10^4/ g of leaf)				Mean
		2DAS	4DAS	8DAS	16DAS	
T_1	*P. fluorescens* @ 5g/litre	26.33 ^{e}	24.67 ^{d}	17.33 ^{e}	8.67^d	19.25
T_2	*B. subtilis* @ 5g/litre	23.00 ^{f}	20.67 ^{d}	16.67 ^{e}	5.00 ^{e}	16.33
T_3	$T_1 + T_2$	29.00 ^{d}	26.00 ^{c}	21.00 ^{d}	9.00 ^{d}	21.25
T_4	T_1 + chitin	43.00 ^{b}	33.67 ^{b}	28.00 ^{b}	14.00 ^{b}	29.66
T_5	T_2 + chitin	30.33 ^{c}	28.00 ^{c}	23.33 ^{c}	10.33 ^{c}	22.99
T_6	T_3 + chitin	49.33 ^{a}	38.00 ^{a}	30.33 ^{a}	16.67 ^{a}	33.58
T_7	Carbendazim 50 WP @ 0.1%	02.00 ^{g}	01.67 ^{e}	02.00 ^{f}	02.00 ^{f}	01.91
T_8	Control	02.00 ^{g}	02.33 ^{e}	02.00^f	02.67 ^{f}	02.25

DAS- Days after spraying, DBF- Days before flowering, DAF-Days after flowering, FS-Flowering stage

Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT)

REFERENCE

Anand, T., Chandrasekaran A., Kuttalam, S. Senthilraja, S. and Samiyappan, R. 2010. Integrated control of fruit rot and powdery mildew of chilli using the biological agent Pseudomonas fluorescens and a chemical fungicide. *Biocontrol*, **52**: 1-7.

Andrews, J.H. 1992. Biological control in the phylloplane. *Annual Review of Phytopathology,* **30**: 603-635.

Ardakani, S.S., Heydari, A., Khorasani, N. and Arjmandi, R. 2010. Development of new bioformulations of *Pseudomonas fluorescens* and evaluation of these products against damping-off of cotton seedlings. *Journal of Plant Pathology*, **92**: 83-88.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A. and Samiyappan, R. 2004. Rhizobacteria based bioformulations for the management of fruit rot infection in chillies. *Crop Protection,* **23**: 835-843.

Buck, J.W. 2004. Combination of fungicides with phylloplane yeasts for improved control of Botrytis cinerea on geranium seedlings. *Phytopathology*, **94**:196-202.

Eckert, J.W. and Ogawa, J.M. 1985. The chemical control of post-harvest diseases: subtropical and tropical fruits. *Annual Review of Phytopathology,* **23**: 421-454.

Eckert, S.A., Crouse, D., Crowder, L.B., Maceina, M. and Shah, A. 1994. Review of the Kemp's ridley sea turtle head start program. NOAA Tech. Memo. U. S. Dept. Commerce. **PP** 10.

Frances, J., Vilardell, P., Bonaterra, A., Badosa, E. and Mantesinos, E. 2002. Combination of *Pseudomonas fluorescens* EPS288 and reduced fungicide dose for control of *Penicillium* rot during postharvest storage of pear. *Acta Horticulturae*, **596**: 883-886.

Gould, A.B., Kobayashi, D.Y. and Bergen, M.S. 1996. Identification of bacteria for biological control of *Botrytis cinerea* on petunia using a petal disk assay. *Plant Disease,* **80**: 1029-1033.

Hayes, W.B. 1953. Fruits Growing in India, Kitabistan, Allahabad, India, **PP** 14.

Janisiewicz, W.J. and Bors, B. 1995. Development of a microbial community of bacterial and yeast antagonists to control wound invading post-harvest pathogens of fruits. *Applied and Environmental Microbiology,* **61**: 3261-3267.

Johnson, G.I. and Hofman, P.J. 2009. Postharvest technology and quarantine treatments. In.Litz, R.E. (ed.) *The mango: Botany, Production and Uses.* 2nd edition. CABI.

Kishore, G.K., Pande, S. and Podile, A.R. 2005. Chitin supplemented foliar application of *Serratia marcescens* GPS5 improves control of late leaf spot disease of groundnut by activating defence-related enzymes. *Journal of Phytopathology,* **153**: 109-173.

Kloepper, J.W., Schroth, M.N. and Miller, T.D. 1980. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. *Phytopathology,* **70**: 1078-1082.

Kondoh, M., Hirai, M. and Shoda, M. 2001. Integrated biological and chemical control of damping-off caused by *Rhizoctonia solani* using *Bacillus subtilis* RB14-C and flutolanil. *Journal of Bioscience and Bioengineering,* **91**: 173-177.

Koomen, L. and Jeffries, P. 1993. Effects of antagonistic microorganisms on the post-harvest development of *Colletotrichum gloeosporioides* on mango. *Plant Pathology,* **42**: 230-237.

Li, Q., Dunn, E. T., Grandmaison E. W. and Goosen, M. F. A. 1997. Applications and properties of chitin and chitosan. In applications of chitin and chitosan, Ed. Mattheus F.A. Goosen, 3-29. Pennsylvania: technomic publishing company. chitin recovery. *Process Biochemistry,* **37**: 1359-1366.

Loganathan, M., Sible, G.V., Maruthasalam, S., Saravanakumar, D., Raguchander, T., Sivakumar, M. and Samiyappan, R. 2010. *Trichoderma* and chitin mixture based bioformulation for the management of head rot (*Sclerotinia sclerotiorum*) root-knot (*Meloidogyne incognita*) complex diseases of cabbage. *Archives of Phytopathology and Plant Protection,* **43**: 1011-1024.

Mahadtanapuk, S., Sanguansermsri, M., Cutler, W.R., Sardsud, V. and Anuntalabhochai, S. 2007. Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alismatifolia* using antagonistic *Bacillus* spp. *American Journal of Agricultural Biological Sciences,* **2**: 54-61.

Malhotra, M. and Srivastava, S. 2006. Targeted engineering of *Azospirillum brasilense* SM with indole acetamide pathway for indoleacetic acid over-expression, *Canadian Journal of Microbiology,* **52**: 1078.

Manasa, B., Jagadeesh, S.L., Thammaiah, N., Jagadeesh, R.C., Gangadharappa, P.M. and Nethravathi, 2018. Pre-harvest application of azoxystrobin minimised anthracnose of mango (cv. 'Alphonso') both at field and postharvest level enhancing yield and quality of fruits. *Journal of Pharmacognosy and Phytochemistry*, **7(3):** 2962-2967.

Manjula, K. and Podila, A.R. 2001. Chitin supplemented formulations improve biocontrol growth promoting efficiency of *Bacillus subtilis* AFI. *Canadian Journal of Microbiology,* **47**: 618-625.

Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polston, J.E. and Kloepper, J.W. 2000. Plant growth promoting rhizobacterial mediated protection in tomato against tomato mottle virus. *Plant Disease,* **84**: 779-784.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T. and Samiyappan, R. 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens. Soil Biology and Biochemistry,* **33**: 603-612.

Ploetz, R.C. and Freeman, S. 2009. Foliar, floral and soil-borne diseases. In.Litz, R.E. (ed.) *The mango: Botany, Production and Uses.* 2nd edition. CABI.

Prabakar, K., Raguchander, T., Saravanakumar, D., Muthulakshmi, P., Parthiban, V.K. and Prakasam, V. 2008. Management of post harvest disease of

mango anthracnose incited by *Colletotrichum gloeosporioides*. *Archives of Phytopathology and Plant Protection,* **41**: 333-339.

Prafulkumar and Mane, S.S. 2017. Studies on the compatibility of biocontrol agents with certain fungicides. *International Journal* of *Current Microbiology and applied Sciences,* **6(3):** 1639-1644.

Radja Commare, R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T. and Samiyappan, R. 2002. *Pseudomonas fluorescens* based bioformulation for the management of sheath blight and leaf folder in rice. *Crop Protection,* **21**: 671-677.

Radja Commare, R., Venkatesan, S. and Samiyappan, R. 2010. Biological control of phytopathogenic fungi of vanilla through lytic action of *Trichoderma* species and *Pseudomonas fluorescens*. *Archives of Phytopathology and Plant Protection,* **43**: 1-17.

Rajkumar, M., Lee, K.L. and Freitas, H. 2008. Effect of chitin and salicylic acid on biological control activity of *Pseudomonas* spp. against damping off of pepper. *South African Journal of Botany,* **74**: 268-273.

Ramamoorthy, V., Viswanthan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. *Crop Protection,* **20**: 1-11.

Sangeetha, C.G. and Rawal, R.D. 2010. Temperature requirement of different isolates of *Colletotrichum gloeosporioides* isolated from mango. *African Journal of Biotechnology,* **9**: 3086-3090.

Saravanakumar, D., Harish, S., Loganathan, M., Vivekananthan, R., Rajendran, L., Raguchander, T. and Samiyappan, R. 2007. Rhizobacterial bioformulation for the effective management of *Macrophomina* root rot in mung bean. *Archives of Phytopathology and Plant Protection,* **40**: 323-337.

Sayiprathap, B.R., Ekabote, S.D., Narayanaswamy, H., Ravindra, H. and Adivappar, N.2018. Survey for the Severity of Anthracnose Disease of Mango (*Mangifera indica* L.) on Nurseries in Karnataka State during Kharif 2015-16. *International Journal* of *Pure and* Applied *Bioscience*, **6(1):** 1139-1141.

Shi, J., Liu, A., Li, X., Feng, S. and Chen, W. 2010. Identification of endophytic bacterical strain MGP1 selected from papaya and its biocontrol effects on pathogens infecting harvested papaya fruit. *Journal of the Science of Food and Agriculture,* **90**: 227-232.

Viswanathan, R. and Samiyappan, R. 1999. Induction of systemic resistance by plant growth promoting rhizobacteria against red rot disease caused by *Colletotrichum falcatum* went in sugarcane. *Proceedings of Sugar Technology Association of India,* **61**: 24-39.

Viswanathan, R. and Samiyappan, R. 2008. Bioformulation of fluorescent *Pseudomonas* spp. induces systemic resistance against red rot disease and enhances commercial sugar yield in sugarcane. *Archives of Phytopathology and Plant Protection,***41**: 377-388.

Vivekananthan, R., Ravi, M., Saravanakumar, D., Kumar, N., Prakasam, V. and Samiyappan, R. 2004. Microbiology induced defense related proteins against post-harvest anthracnose infection in mango. *Crop Protection,* **23**: 1061-1067.

Zehnder, G., Kloepper, J.W., Yao, C. and Wei, G. 1997. Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Chrysomelidae) by plant growth promoting rhizobacteria. *Journal of Economic Entomology,* **90**: 391-396.

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