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EFFECT OF FOLIAR APPLICATION WITH BIOFORMULATIONS ON MANGO ANTHRACNOSE INCIDENCE UNDER POT CULTURE CONDITION

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Abstract

The effect of foliar application with bioformulations on mango anthracnose incidence were studied under pot culture condition. In general the dosage level of 4g/litre was proved inadequate as it recorded significantly the maximum disease incidence in all the treatments. Among all the treatments, the combination of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) amended with chitin 6g/litre recorded the lowest disease incidence. In all the treatments, the dosage level of 5g/litre recorded statistically on par results with that of 6g/litre. Foliar application with combined application of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin 5g/litre of water showed 8.00 and 17.33 PDI at 7 and 15 days after inoculation. This was on par with Carbendazim (0.1%) which recorded 7.33 and 16.33 PDI at 7 and 15 days after inoculation with pathogen.
Key words: Bioformulation, chitin and mango anthracnose

Introduction

Mango (*Mangifera indica* L. Anacardiaceae) is one of the most popular fruit and grown throughout the tropical and sub-tropical regions of the world. Being highly perishable, mango fruits have to be marketed immediately after harvest. The anthracnose caused by *Colletotrichum gloeosporioides* is one of the most common and serious diseases of mango. The disease occurs at any stage of fruit growth. Occasionally anthracnose lesions are seen in young green fruit, more commonly in larger green fruit but most commonly in ripe fruit. The anthracnose fungus has the ability to produce latent or quiescent infections. It means that the fungus has the ability to penetrate green fruit where it may go into a dormant state until the fruit ripens. Then the anthracnose fungus gets reactivated in response to physiological changes associated with ripening, resulting in the development of lesions with subsequent spoilage of the fruit (Dodd *et al.,* 1997; Nelson, 2008).

As an alternative to chemical fungicides or to reduce the number of chemical sprays, it is essential to develop an effective, cheap and environmentally safe non chemical method for the control of anthracnose disease. 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 *Pseudomonas fluorescens* (Ardakani *et al.,* 2010) and *Bacillus 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). Chitin can be found in a variety of species in both the animal and plant kingdoms. The traditional source of chitin is shellfish waste from shrimp, antarctic krill, crab and lobster processing (Thirunavukkarasu *et al.,* 2011). 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, this study were formulated to assess effect

of foliar application with bioformulations on mango anthracnose incidence under pot culture condition.

Materials and Methods

Isolation of *C. gloeosporioides*

The pathogen causing anthracnose disease in mango was isolated from diseased leaf and fruit samples. The infected tissue bits were separated with a sterile blade and surface sterilized with 1 per cent sodium hypochlorite solution for 1 min. and subsequently washed three times with sterile distilled water. Then they were transferred into a sterile Petri dish containing Potato Dextrose Agar (PDA) medium (Ainsworth, 1961) amended with streptomycin. The plates were then incubated at room temperature (28 ± 2°C) for four days. The emerging colonies were sub cultured on to PDA slants. Single hyphal tip method was followed for making pure culture and maintained on PDA slants (Aneja, 2003).

Preparation of Inoculum

Conidial suspension was prepared from 7 days old culture of *C. gloeosporioides* grown on PDA medium. Concentration of conidia in the suspension was adjusted to 1×10^5 conidia ml^{-1} using haemocytometer (Martinez *et al.,* 2008).

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 and among them 30 were isolated from leaf surface, 6 were isolated from blossom and 16 were isolated from fruit skin and designated as BIL (Bacterial Isolate from Leaf), BIB (Bacterial Isolate from Blossom) and BIF (Bacterial Isolate from Fruit), respectively. Based on the observations on colony morphology and biochemical tests, only 22 isolates showed positive results. Out of this 22 bacterial isolates the biochemical tests confirmed, 12 isolates were identified as *Pseudomonas fluorescens* and 10 isolates were identified as *Bacillus subtilis*. Based on the dual culture technique and poison food technique *P. fluorescens* (BIB_2) and *B. subtilis* (BIL_8) strains were selected for the further studies.

Preparation of Commercial Formulation of Biocontrol Agents

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.

Chitin Amended Talc Based Formulations of Bacterial Antagonists

Preparation of Colloidal Chitin

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 become 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.

Effect of foliar application with bioformulation at different doses against mango anthracnose incidence in pot culture

Mango seedlings (Neelam) grown in a polyhouse was used for the study and spore suspension of *C. gloeosporioides* (1×10^5 spores ml^{-1}) was prepared as previously described. Talc based bioformulations with and without chitin amendment was sprayed at three different concentrations (4g, 5g and 6g per litre of water) on young leaves of mango seedlings and after 24 h mango seedlings were inoculated with the spore suspension of *C. gloeosporioides*. For comparison Carbendazim 50 WP @ 0.1% was used and control plants were sprayed with sterile water. The percent disease incidence was recorded at 15th day after inoculation. There were three replications in each treatment and the experiment was designed in a randomized block design (RBD).

Effect of foliar application with bioformulation on mango anthracnose

A pot culture experiment was conducted to test the efficacy of foliar application of bacterial bioformulations alone and in combination on the incidence of mango anthracnose. Three months old grafted mango seedlings (var. Neelam) were taken for the study. The talc based bioformulations with and without chitin amendment was sprayed @ 5g/litre of water on young leaves of mango seedlings and after 24 h. mango seedlings were spray inoculated with the conidial suspension of *C. gloeosporioides* with adequate cfu (1×10^5 conidia ml^{-1}). For comparison Carbendazim 50 WP @ 0.1% was used and plants sprayed with sterile water served as control. The inoculated seedlings were covered with transparent polyethylene bags of (100 gauge) for 48 h. and sterile distilled water was sprayed to ensure high humidity and favorable conditions for conidial germination and infection (Fitzell, 1979). The crop was maintained following standard agronomic practices and judicial watering. The experiment was conducted in a randomized block design with three replications for each treatment and the per cent disease incidence was recorded at 7th and 15th day after inoculation.

Results and Discussion

Effect of foliar application with bioformulation at different dosage levels (Pot culture)

In general the dosage level of 4g/litre was proved inadequate as it recorded significantly the maximum disease incidence in all the treatments. Among all the treatments, the combination of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) amended with chitin 6g/litre recorded the lowest disease incidence. In all the treatments, the dosage level of 5g/litre recorded statistically on par results with that of 6g/litre and hence the dosage level of 5g/litre was used in all the subsequent experiments. Amendment of chitin in the bioformulation enhance the disease suppression efficacy of individual as well as combination of biocontrol treatments when compare to treatments without chitin amendment (Table 1).

Effect of foliar application with bioformulation on mango anthracnose incidence (Pot culture)

The data presented in the table 2 revealed that all the three talc based chitin amended bioformulations recorded significant reduction in anthracnose incidence. However, foliar application with combined application of *P. fluorescens* (BIB_2) + *B. subtilis* (BIL_8) + chitin showed 8.00 and 17.33 PDI at 7 and 15 days after inoculation. This was on par with Carbendazim (0.1%) which recorded 7.33 and 16.33 PDI at 7 and 15 days after inoculation with pathogen. This was followed by application of *P. fluorescens* (BIB_2) + chitin which recorded 10.33 and 20.67% of disease incidence on 7 and 15 days after inoculation. The minimum reduction in disease incidence (23.00 and 39.00%) was observed in chitin @ 5g/l of water alone treatment.

Earlier studies indicated that several talc based formulations of biocontrol agents amended with chitin have been found effective against various plant diseases under greenhouse and field conditions (Vidhyasekaran and Muthamilan, 1995; Vidhyasekaran *et al.,* 1997; Nandakumar *et al.,* 2001; Ramamoorthy *et al.,* 2002). Bharathi *et al.* (2004) observed that *P. fluorescens* and *B. subtilis* were effective in increasing seed germination and seedling vigour and that the mixed bioformulation (*P. fluorescens* + *B. subtilis* + neem + chitin) was the best for reducing fruit rot incidence and increasing plant growth and yield of chilli. Bioformulation of *P. fluorescens* (Pf1) with chitin was effective in reducing the root rot incidence in green gram both under glasshouse and field conditions (Saravanakumar *et al.,* 2007). Application of talc based formulation of *P. fluorescens* TDK1 + Pf1 strain mixture (amended with or without chitin) through seed, soil and foliar spray effectively reduced the incidence of collar rot in groundnut compared to individual bioformulation both under glasshouse and field conditions (Senthilraja *et al.,* 2010). This might be due to the increased shelf life of the antagonists in the bioformulations, improved chitinolytic ability and better survival of the bacteria in the phylloplane of the mango seedlings in response to chitin amended bioformulation application.

Table 1 : Effect of foliar application with bioformulations at different doses against mango anthracnose incidence (Pot culture)

T. No	Treatments	Dosage in g/liter of water	Per cent diseases index (PDI) at 15 DAI
T_1	*P. fluorescens*	4	35.33
		5	30.67
		6	30.33
T_2	*B. subtilis*	4	38.33
		5	28.00
		6	27.33
T_3	*P. fluorescens* + *B. subtilis*	4	29.67
		5	22.00
		6	21.67
T_4	*P. fluorescens* + chitin	4	28.67
		5	17.00
		6	16.67
T_5	*B. subtilis* + chitin	4	30.33
		5	20.67
		6	19.00
T_6	*P. fluorescens* + *B. subtilis* + chitin	4	25.33
		5	15.67
		6	15.33
T_{10}	Carbendazim 50 WP @ 0.1%	0.1%	14.67
T_{11}	Control	-	58.67

Table 2 : Effect of foliar application with bioformulation on mango anthracnose incidence (Pot culture)

T. No	Treatments	Per cent disease index (PDI)			
		7 DAI	Per cent decrease over control	15 DAI	Per cent decrease over control
T_1	*P. fluorescens* @ 5g/litre	17.33^{d}	43.49	29.67^{d}	38.18
T_2	*B. subtilis* @ 5g/litre	18.67^{e}	39.12	33.33^{e}	30.56
T_3	$T_1 + T_2$	15.33^{d}	50.01	25.67^{c}	46.52
T_4	Chitin @ 5g/litre	23.00^{f}	25.00	39.00^{f}	18.75
T_5	T_1 + chitin amendment	10.33^{b}	66.31	20.67^{b}	56.93
T_6	T_2 + chitin amendment	13.00^{c}	57.61	22.00^{b}	54.16
T_7	T_3 + chitin amendment	08.00^{a}	73.91	17.33^{a}	63.89
T_8	Carbendazim 50 WP (0.1%)	07.33^{a}	76.10	16.33^{a}	65.97
T_9	Control	30.67^{g}	-	48.00^{g}	-

DAI- Days after inoculation

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

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