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Use of Plant Water Extracts and Bio-Control Agents for Post-Harvest Management of Mango Anthracnose Caused by *Colletotrichum Gloeosporioides*

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ABSTRACT

Mango anthracnose caused by *Colletotrichum gloeosporioides* Penz. with its perfect stage as *Glomerella cingulata* is one of the most important and serious disease in all the mango growing regions of India including Himachal Pradesh. It causes 40 to 50 per cent post- harvest losses that sometimes increased up to 100 per cent under wet or very humid conditions. Efficacy of plant water extract and bio-control agents was tested against development of anthracnose on mango fruit cv. Dashehari. Fruit dip in leaf water extract of *Azadiracta indica* (15%) and solution of *Trichoderma harzianum* ($1.2x10^4$cfu/ml) for five minutes was found most effective and controlled the disease upto 70 to 80 per cent.

Key Words: Mango, *Colletotrichum gloeosporioides,* Plant water extract, Bio-control agents, Post-harvest management.

INTRODUCTION

Mango (*Mangifera indica* L.) is known as the king of fruits, belongs to the family Anacardiaceae. The major mango producing countries include India, China, Thailand, Indonesia, Philippines, Pakistan, Brazil, Bangladesh, USA, Africa and Mexico. In India, it is grown over an area of 25.16 lakh hectare with total production of 1.83 lakh tonnes (Anon, 2014). In Himachal Pradesh, the total area under mango cultivation is 41,105 ha with production to the tune of 47,612 MT (Anon, 2015). Mango production is, however, constrained by several biotic and abiotic factors. Among the biotic factors, the successful cultivation of this crop is being hindered due to its proneness to large number of diseases at all stages of its growth and development starting from nursery up to fruit production and storage conditions. Mango, anthracnose caused by *Colletotrichum gloeosporioides* is one of the most important fungal diseases prevalent in all mango producing areas of the world and is favoured by high rainfall and relative humidity. Fruits infected at pre-harvest stage carry the incipient fungus causing fruit rot thereby resulting in considerable losses during transit, storage and marketing. In India, losses due to this disease in field have been estimated to the tune of 2-39 per cent whereas, post-harvest losses during storage varied between 47.9 - 51.7 per cent (Prabakar, 2005). However, a large number of fungicides have been reported to be effective against this particular pathogen worldwide and its application is also one of the easiest and most effective methods to control various diseases, but it has number of drawbacks like development of resistant strains of pathogen, more expensive and polluting environment. Therefore, use of plant water extract and native bio-control agent extracts has recently been exploited in management of plant diseases.

Hence, keeping all these points in view, the present study was undertaken to evaluate the efficacy of plant water extract and bio-control agents against

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mango anthracnose to reduce the post-harvest losses caused by C*olletotrichum gloeosporioides.*

MATERIALS AND METHODS

Periodic survey in different mango growing areas in the districts of Kangra, Mandi, Sirmour and Solan of Himachal Pradesh was done. During the survey, mango leaves and fruits infected with anthracnose were collected from these areas and were kept in polythene bags and brought to the laboratory for the isolation and confirmation of associated pathogen.

Isolation of the pathogen

Pathogen was isolated from infected leaves and fruits by taking small bits of 1 to 2 mm size from the junction of diseased and healthy portions with the help of sterilized blades. These bits were then surface sterilized with sodium hypochlorite solution (1%) for 3 minutes, washed twice with sterilized distilled water and subsequently transferred to already sterilized potato dextrose agar (PDA) medium in Petri plate. These were incubated in Biological Oxygen Demand (BOD) incubator at a temperature of 28 ± 2^0C. It was further purified by following hyphal tip method. The pure culture was kept in refrigerator for further use in different experiments. The purified cultures of different isolates of fungus were identified based on its mycelial and conidial characteristics as per the standard mycological keys (Barnett, 1972).

Evaluation of different plant water extracts on mango fruit under laboratory conditions

Leaves of Azadirachta indica (Neem), *Melia azedarach* (Darek), *Aloe barbedens* (*Aloe vera*) and fruit of *Emblica officinalis* (Amla), weighing 200 g of each were separately taken and then washed under tap water and grind for 5m in blender by adding small quantity of distilled water. Thereafter, 200 ml of distilled water was added and homogenized in orbital shaker at the rate of 2000 rpm for half an hour to get an extract of 100 per cent concentration. The plant material was then filtered through double-layered muslin cloth. These were sterilized in an autoclave at 5 psi pressure for one hour and were tested at 10, 25 and 50 per cent concentrations. Thus, *in vitro* effective botanicals were further evaluated on mango fruit.

Mango fruits of cv. Dashehari of uniform size and shape were procured from local market for evaluation plant water extracts against development of anthracnose. Fruits were dipped separately in the different plant extract solution (15%), these fruits were then air dried by keeping them apart on newspaper sheets spread on the table. Sticker namely Mixin was added @ 0.2 per cent in the plant extract solution to ensure the proper and uniform sticking of the solution on the surface of the fruit. In control treatment, fruits were only dipped in pure water taken from (RO) installed in the laboratory. Each treatment was replicated thrice. Thereafter, the treated fruits were inoculated with the test pathogen by pin prick method and stored at room temperature (25-28^{0}C). Observations on efficacy of these treatments against mango anthracnose were recorded after 7, 15, 21, 28 days of inoculation

Table1. Disease rating scale.

Grade/ Rating	Per cent area of infection		
	Inflorescence	Leaves	Fruits
0	No infection observed	0	No infection
1	1-10	1-20	Upto 5
2	10.1-15.0	21-40	6-10
3	15.1-25	41-60	11-20
4	25.1-50	61-80	21-50
5	> 50	>80	>50

by adopting 0-5 disease rating scale as described by Sundravadana *et al* (2007) as described below (Table1).

Per cent disease index (PDI) Mckinney (1923) and Decay reduction index (DRI) Sharma and Bhardwaj (2000) was calculated by adopting the formula as described as below.

$$\text{Disease index (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of samples observed x Maximum disease grade}} \times 100$$

$$\text{Decay Reduction Index (DRI)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Evaluation of different bio-control agents on mango fruit under laboratory conditions

Six bio-control agents viz., *Trichoderma harzianum, Trichoderma virens, Bacillus subtilis, Pseudomonas fluorescens, Pichia anomala* and *Rhodotorula phylloplana*, as found effective under *in vitro* conditions, were further evaluated for their comparative efficacy on mango fruit cv. Dashehari against mango anthracnose. Fungal bio-control agents namely *T. harzianum* and *T. viride* were mass multiplied by using molasses yeast extract broth. The liquid medium was prepared by mixing molasses, yeast and water (30:50:1000) in flasks and autoclaved at 1.5 kg/cm^2 pressure for 20m. After cooling, the medium was inoculated with 10d old pure culture of two fungal bio-control agents separately under aseptic conditions. These were then incubated at 25 ± 1^{0}C in BOD incubator shaker and bacterial bio-control agents namely *Pseudomonas fluorescens* and *Bacillus subtilis* were grown in nutrient broth and yeast antagonists *Pichia anomala* and *Rhodotorula phylloplana* were prepared in malt extract broth at 28 ±1°C in BOD incubator shaker for three days.

The respective solution of above bio-control agents of different origin i.e fungal, bacterial and yeast were prepared each containing 1.2x10^{4}cfu/ml, 1.2x10^{8}cfu/ml and 1.2x10^{8}cfu/ml, respectively. Fruits were dipped in 5 per cent of these bio-control agents solutions for 5m and then air dried in the laboratory by keeping on working tables on newspaper sheets. Sticker namely Mixin (0.2%) was added in the bio-control agent's solution to ensure the uniform adhesion of bio-control agents on the fruit surface. The efficacy of bio-agents was compared with fruit dipped in distilled water. These fruits were inoculated with the test pathogen by pin prick method and stored at room temperature (25-28^{0}C). Observations on efficacy of these treatments against mango anthracnose were recorded after 7, 15, 21, 28 days of inoculation by adopting 0-5 disease rating scale as described above. Per cent disease index and decay reduction index for each treatment were calculated. The data recorded in different experiments as conducted both under laboratory and field conditions were subjected to statistical analysis by following the methods of variance described by Gomez and Gomez (1984). Critical differences (CD) amongst the treatments in various experiments at 5 per cent level were also calculated to find out the least significant differences.

RESULTS AND DISCUSSION

Evaluation of different plant water extracts on mango fruit under laboratory conditions

Data recorded on evaluation of four *in vitro* effective plant water extracts (Table 2) under *in vivo* conditions against anthracnose (*C. gloeosporioides*) revealed that *A. indica* was highly effective in checking the development of anthracnose infection with Disease Reduction Index of 74.82 per cent. Fruit water extract of *E. officinalis* and *M. azedarach* were the next best in order with 71.11 and 66.67 per cent DRI, respectively. Faiz *et al* (2016) reported that spray with leaf water extract of garlic, eucalyptus, neem and akk lowered the disease severity of anthracnose upto an extent of 3.25, 4.0, 3.75 and 4.25 per cent, respectively. The higher efficacy of leaf water extract of *A.indica*

Table2. Evaluation of *in vitro* effective plant water extract against development of anthracnose (*C. gloeosporioides*) on mango fruit cv. Dashehari.

Botanical Name	Concentration (%)	Disease index (%)	Decay reduction index DRI (%)
Azadiracta indica	15	25.18(30.10)	74.82
Emblica officinalis	15	28.89(32.49)	71.11
Melia azedarach	15	33.33(35.24)	66.67
Aloe barbedens	15	40(39.21)	60
Control	Distilled Water	100(90.00)	-
C.D0.05	-	2.05	

Figures in parentheses were angular transformed values

in the present study against mango anthracnose was due to its antimicrobial effect because of the presence of diterpenoids, nimbolinin and nimbonone. Further, antimicrobial activity of aonla fruit water extract had also been reported against post harvest fungal pathogens in apple and it was attributed due to the presence of high phenolic contents like lupeol, B-sitosterol and phyllembin. Ethanolic extract of *M. azedarach* fruits possesses both fungistatic and fungicidal activities due to presence of various organic molecules reported as vanillin, hydroxyl-3-methoxcinnamaldehyde and pinoresinol (Mishra *et al*, 2013). Jasso *et al* (2005) found antifungal activities of pulp extract of *Aloe vera* on the mycelium development of *Rhizoctonia solani, Fusarium oxysporum* and *Collectotrichum coccodes. A. vera* leaves contain specific antimicrobial compounds such as anthraquinones (Dabai *et al*, 2007) and dihydroxyanthraquinones (Wu *et al*, 2006) as well as saponins.

Evaluation of different bio-control agents on mango fruit under laboratory conditions

The perusal of data (Table 3) revealed that *T.harzianum* was the most effective with 81.67 per cent Disease Reduction Index and significantly superior over all the other bio-control agents followed by *T. virens* (79.96% DRI) and *P. anomala* (78.52 % DRI). *B. subtilis* was the next best effective treatment with DRI of 77.78 per cent. Prabakar *et al* (2008) have also reported that fruit dip in *T. harzianum* and *P. fluorescens* were effective in lowering anthracnose caused by *C. gloeosporioides* on mango fruit by 40.0 and 43.3 per cent, respectively. Growth inhibition of *C. gloeosporioides* by the *Trichoderma sp.* could be attributed mainly due to the production of antibiotics, such as trichodermin, trichodermol, trichotoxin and harzianolide which are inhibitory to fungal growth and sporulation (Yonas and Amare, 2008). Begum *et al* (2008) observed that the *Trichoderma* isolates coiled around the hyphae of *C. truncatum* which further restricts its growth and spread. Effectiveness of *B. subtilis* can be substantiated to the production of antimicrobial peptide substances such as subtilin, bacilysin, mycobacillisyn, and iturin which caused changes in hyphal morphology including hyphal swelling, distortion and cytoplasm aggregation of *C. acutatum* and *C. gloeosporioides* (Yoshida *et al*, 2001). Further support to the present finding can be ascribed to the findings of Rahman *et al.* (2007) wherein it was established that antibiotic metabolites produced by bacterial antagonists penetrate protoplasm resulting its dissolution and disintegration finally causing malformation of mycelium. *Pichia guilliermondii, Candida musae, Issatchenkia orientalis* and *Candida quercitrus* have been used to control *Colletotrichum capsici* in *Capsicum annuum* in Thailand (Chanchaichaovivat *et al,* 2008). Yeast bio-control agents are considered as suitable potential bio-control agent because they grow more quickly and colonize the fruit surface

Table 3. Evaluation of *in vitro* effective bio-control agents against development of anthracnose (*C. gloeosporioides*) on mango fruit cv. Dashehari

Bio-control agent	Disease index (%)	Decay Reduction Index DRI (%)
Trichoderma harzianum	18.33 (21.36)	81.67
Trichoderma virens	20.04 (24.36)	79.96
Bacillus subtilis	22.22 (28.09)	77.78
Pseudomonas fluorescens	26.67 (31.07)	73.33
Pichia anomala	21.48 (27.58)	78.52
Pseudomonas phylloplana	33.33 (35.24)	66.67
Control	100 (90.00)	-
$C.D_{0.05}$	2.49	

Figures in parentheses were angular transformed values

efficiently, thus limit the availability of nutrient and space to pathogen.

CONCLUSION

Evaluation of plant water extracts and native bio-control agents against development of anthracnose on mango fruit cv. Dashehari revealed that fruit dip in leaf water extract of *A. indica* (15%) and solution of *Trichoderma harzianum* ($1.2x10^4$cfu/ml) for five minutes was most effective and provided DRI up to an extent of 74.82 and 81.67 per cent, respectively. Thus, use of plant water extracts and native bio-control agents was effective and cost-effective alternative to fungicides for their use in post-harvest management of mango anthracnose

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