

# Probiotics as a Biocontrol Agent in Management of Post Harvest Diseases of Mango

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## Authors' contributions

This work was carried out in collaboration among all authors. Author KG carried out the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CDD and KSR managed the analyses of the study. Author VKB managed the literature searches. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/CJAST/2020/v39i230501

### Editor(s):

(1) Dr. Sangita Sahni, Assistant Professor, Department of Plant Pathology, Tirhut College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, India.

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Complete Peer review History: <http://www.sdiarticle4.com/review-history/54905>

Original Research Article

Received 11 December 2019  
Accepted 17 February 2020  
Published 28 February 2020

## ABSTRACT

Mango (*Mangifera indica* L.) is the most important horticultural crop in Maharashtra as well as in India. However, this crop is well acclimatized to tropical and subtropical climatic regions. Post harvest losses caused by micro organisms are reported as from 30 to 35% losses with reduction in quality and quantity of marketable fruits and accounts for millions of dollars in perishable produce every year. Over the years, the plant protection was achieved by use of synthetic chemicals. However, use of probiotics for control of post harvest diseases has taken momentum in recent years in management of post harvest plant pathogens. The post harvest pathogens like *Colletotrichum*, *Alternaria*, *Aspergillus*, can be checked by post harvest probiotic sprays. Which include use of commercial probiotics (Prowel, Flora and Vbact) and Probiotic isolates, which are isolated and cultured from curd, fermented dosa material, fermented jowar and bajra flour. The studies on use of probiotics in the management of post-harvest diseases of mango conducted at Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar during the period 2017-2019. suggest that the post-harvest pathogens particularly *Colletotrichum*, *Alternaria* can be managed as a post-harvest pathogen by sprays of probiotics under *in-vitro* conditions when the load of inoculums of

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these post-harvest pathogens are less on the fruits and the probiotic spray resulted in increase in the TSS (Total Soluble Sugars) brix (0.8-3.2) in the fruits and also be used to increase the shelf life of the fruits and to keep them as fresh for long time. The efficacy of probiotics against post-harvested pathogens indicates that probiotics can be used in management of post harvest diseases and they can form an integral part of organic farming system.

**Keywords:** Mango; post harvest disease; probiotics; management; biocontrol.

## 1. INTRODUCTION

Fruits are the important component of human diet. They are considered as perfect food as they are the rich source of vitamins, minerals, enzymes and fiber. They play a vital role in human nutrition only. Fruits along with vegetables are rich sources of vitamins (A, B complex and C) and minerals (calcium, iron and phosphorus) in diets to keep human health in condition and are termed as 'protective foods'. Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is one of the most popular of all tropical fruits. Mango fruits are rich in amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins and vitamins. A fully ripe mango rich in vitamin A (beta carotene is considered a cancer-fighting agent. It is also high in vitamin C (27.7 mg) and is a good source of carbohydrates (17 gm) and fibre too. Mangoes are good for the kidneys, aid digestion, provide excess body heat and favour relieve clogged skin pores and reduce cysts [1].

In India, Mango is cultivated over an area of 2262.77 thousand mha. with a productivity of 8.7mt (horticulture statistics division, 2018) and total production of 19686.93 thousand mt. Maharashtra state occupies 157.07 thousand ha area with production of 514.87 thousand million tonne and ranks tenth in production of various mangoes but stands unique in production of Alphonso in the regions of Ratnagiri, Raigad, Sindhudurg. Devgad and Pune is also playing an important role in production of rich mangoes on the sides of Sholapur highway.

Fungal pathogens play a major role in post harvest rotting of mangoes. Especially, Mango anthracnose caused by *Colletotrichum gloeosporioides*, Alternaria fruit rot caused by *Alternaria alternata* and *Alternaria tenuissima*, *Botryodiplodia theobromae*, *Lasiodiplodia theobromae* and *Dothiorella species* responsible for stem end rot Dodd et al. [2]; Kuos [3]; Okigbo and Osuinde [4]. Among them in India, *C.gloeosporioides* causing large spreading lesions on the fruit surface. Losses in the field

due to this disease have been estimated to be 2 - 39% [5]. During storage, losses extended up to 51.70%. *L. theobromae* causes black lesions around the base of the pedicel [5]. Losses due to stem end rot of mango were reported to be around 7% and transit rot was 2% the losses due to post harvest pathological disease were found from producer to consumer was 27% [6]. Mango post-harvest diseases include anthracnose (*C. gloeosporioides*), stem end rot (*Botryodiplodia theobromae* Pat.), *Aspergillus* rot (*Aspergillus niger* (L.) Van Tieghem) and *Alternaria* rot and the incidence was found in range of 40.00-90.00 per cent, 16.00-86.60 per cent, 3.33-53.30 per cent and 16.76 per cent respectively [7].

Microorganisms causing post-harvest diseases resulting in losses accounts for millions of dollars in perishable produce every year [8]. To minimize these losses it is very necessary to control the post-harvest diseases by biological, chemical and physical treatments [9]. The chemical control which includes application of therapeutical fungicides as pre-harvest treatment under field conditions, poses a serious threat of residues of these chemicals on consumable product. Therefore a novel method of control of these post-harvest diseases is a need of the day to save our produce. The use of probiotic in the management of human, animal and aquaculture diseases has gained momentum in recent years; however, their use in the management of crop plant disease is growing in recent years with increase in health consciousness of people.

In 2002, an FAO/WHO joint panel defined probiotics as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). Most probiotics are bacteria, the most common type among is lactic acid bacteria (LAB) but a few molds and yeasts can also be used as probiotics [10].

A good probiotic must be an organism that is capable of exerting a beneficial effect on the human hosts, increase growth or resistance to disease; it must be nonpathogenic and non-toxic;

it must be capable of surviving and metabolizing in the gut environment by resisting the low pH of the stomach, enzymes, organic and bile acids present in the intestine and it should be stable under storage and field conditions [11]. Genera commonly used as probiotics include the bacterial- *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Carnobacterium*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Propionibacterium*, *Leuconostoc*, and *Bacillus* species, *Saccharomyces* yeasts and *Aspergillus* molds [12]. They are beneficial organisms because they can provide beneficial effects on human such as altering the intestinal microflora balance, inhibiting the growth of pathogenic bacteria, synthesizing and enhancing the bioavailability of nutrients, promoting good digestion, reducing the effect of allergens, boosting immune function, lowering cholesterol, stimulating the immune system, [13]. Probiotics occur naturally with in the fermented food product such as yoghurt, sauerkraut, kefir, soybean-based miso and natto and cabbage kimchee. As the probiotics are consumed orally and beneficial to the human health their presence on the consumable fruits wont have any harmful affect on the human. As the probiotics are known to control pathogenic infections in human and are safe microbial formulations, there use to control pathogenic infection in post-harvest food produce is interesting to study and therefore the present investigation is taken up.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Pathogen

The collected diseased samples of mango were surface sterilized with 1% sodium hypochloride solution and dried for 2-3 minutes and partly diseased portion with some partly healthy portion was cut with the help of sterilized blade under aseptic conditions in laminar air flow and the sterilized bits were kept on potato dextrose media plates. The inoculated Petri plates were then incubated at  $25\pm 2^{\circ}\text{C}$  for 3 days. The fungal growth obtained was purified by mycelial tip isolation method and further it was identified upto genus level based on their characteristics.

### 2.2 Probiotic Isolates

Probiotic isolates were isolated from respective samples such as fermented jowar, bajra, dosa flour on Malt Extract Agar and From curd on Nutrient Agar media by streaking, they are named as Probiotic isolate I an edible yeast

culture obtained from jowar flour, Probiotic isolate II an edible yeast culture obtained from bajra flour, Probiotic isolate III an edible yeast culture obtained from dosa flour, Probiotic isolate IV an *Lactobacillus* obtained from curd.

### 2.3 Commercial Probiotics

The Commercial probiotics which are easily available in the market and used in the study are Flora (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium boulandii*) Prowel (*Lactobacillus acidophilus*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*) V Bact (*Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, *Lactobacillus sporogenes*).

### 2.4 Pathogenicity

The pathogenicity test of isolated fungal pathogens were carried out by undertaking the inoculation experiments on healthy fruits of mango. These healthy fruits were washed in tap water, followed by spray of disinfectant 1:1000 sodium hypochloride (NaOCl) solution and were immediately washed with sterile water to remove the traces of disinfectant. Seven days old cultures of each isolate having good conidial and mycelial growth were suspended in sterilized water for preparation of inoculum spray suspension. The fruits were sprayed with respective fungal spore suspension with the hand sprayer under aseptic conditions and kept at ambient temperatures in the desicator for growth and infection of post-harvest pathogen. The type of symptoms developed/ fungal growth developed on the fruits were recorded eight days after inoculation.

#### 2.4.1 *In vitro* efficacy of probiotics against the post harvest pathogens

The efficacy of Probiotics on pathogens was tested *in vitro* conditions by using dual culture Agar plate technique [14] a 5 mm disc mycelium inoculated on centre of petri dish containing PDA medium. In parallel a loop full of probiotic isolates and commercial probiotics were streaked 2.5 cm away one either sides of disc in the same plate. Paired cultures were incubated at  $27-29^{\circ}\text{C}$  for 7 days. After 7days, growth diameter of the fungus (distance between the point of inoculation of fungal disc and actively growing edges of the fungus) was measured in both control



**Fig. 1. Commercial probiotics used in the experiment**

and inoculated plate. The percent growth inhibition will be calculated using the formula proposed by Vincent (1927).

$$\text{Percent inhibition} = C-T/T \times 100$$

C = Growth of pathogen in control plates,  
T = Growth of pathogen in dual culture plate

#### 2.4.2 *In vitro* effect of probiotics against diseased fruit

A 2% sugar solution prepared by mixing 20 gm of sugar in one litre of water. In it one gm of probiotic Powder from respective probiotic sachet was added. This solution was incubated at 27±2°C in BOD for 3 days for the growth of probiotic organisms. The suspension thus obtained was used for application on fruits. In case of probiotic isolates obtained the solution were prepared by taking one litre distilled water having 2 per cent sugar. A growth of individual probiotic I, II, III and IV from a single tube was added to this solution and incubated at 28 ± 2°C in BOD for the growth of probiotic organisms for 3 days. To study the antagonistic effect of probiotics on post-harvested fruits the experiments were laid out *in vitro* conditions. The harvested mango fruits were brought to laboratory. These were washed with distilled water and dried. These fruits were then spray inoculated with suspension of respective post-harvest pathogen and again air dried. The respective probiotic suspension was sprayed on these pathogen inoculated fruits were air dried and kept at room temperature. The fruits sprayed with only with pathogen but no probiotic served as control. The observation was taken up to 10 days for growth of pathogen.

### 3. RESULTS AND DISCUSSION

#### 3.1 *In vitro* Efficacy of Probiotics against the Post Harvest Pathogens

The efficacy of Probiotic isolates viz., isolate I, II, III and *Lactobacillus* and Commercial probiotics viz Flora, Prowel, V bact were evaluated against three test post-harvest pathogens viz., *Colletotrichum*, *Alternaria* and *Aspergillus* obtained from mango by employing dual culture agar plate technique.

The results depicted in (Table 1) show that both Probiotic isolates and Commercial probiotics did not form any specific inhibition zone but the Probiotic isolates were comparatively inhibiting the pathogen slowing down the growth. Among the applied probiotic sprays on mango pathogens the Probiotic isolate – IV was more effective inhibiting the growth of *colletotrichum* up to 70.99% and Probiotic isolate-II was effectively inhibiting the growth of *Alternaria* up to 67.41% and Probiotic isolate – I was effective against *Aspergillus* by inhibiting the growth up to 48.76%. Among the Commercial probiotics the Commercial probiotic-III was effective in inhibiting the growth of *Colletotrichum* and the Commercial probiotic-I was effective against *Alternaria* compared to others.

#### 3.2 Performance of Probiotics in the Management of Post-harvest Pathogens of Mango Fruits

The freshly harvested mango fruits were sprayed separately with individual inoculums of post harvest pathogens *Colletotrichum*, *Alternaria* and *Aspergillus*, which are the post harvest

pathogens associated with mango. These pathogen suspension were sprayed on mango fruits and were subsequently sprayed with the probiotic suspension of Prowel, Flora, Vbact and Probiotic isolate after one hour. The treated fruits were kept in room temperature and were observed for the infection of test pathogens. The results (Table 2) indicated that none of the probiotic was able to control the post-harvest pathogen infection of *Aspergillus* and there was a full growth of the post-harvest pathogen on fruits. The Probiotic isolates and Commercial probiotic I were able to control *Colletotrichum* and Probiotic isolates and Commercial probiotic I and II was effective

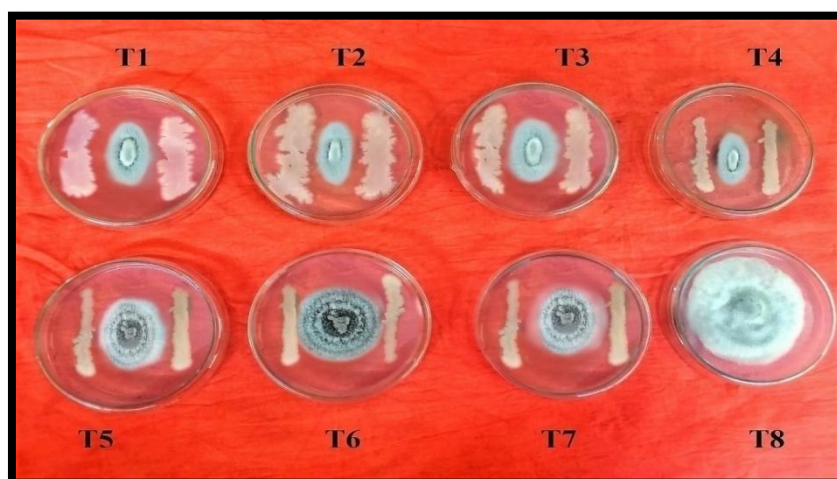
against *Alternaria*. The results (Table 3) depict that when the fruits were treated by combining all Probiotic isolates they were more effective in inhibiting the pathogen.

### 3.3 Effect of Probiotic Application on Fruit TSS (Total Soluble Sugars) Content in Mango

The effect of application of Probiotic isolates and Commercial probiotics was seen on the fruit TSS content of Mango. For this the harvested fruits of mango were obtained and were sprayed with suspension of probiotics. The sprayed fruits were kept at room temperature and the TSS

**Table 1. *In vitro* efficacy of probiotics on post harvest pathogens of mango**

Treatments	<i>Colletotrichum</i>		<i>Alternaria</i>		<i>Aspergillus</i>	
	Means	Inhibition %	Means	Inhibition%	Means	Inhibition %
T1 Probiotic isolate –I	3.70	54.71%	2.87	66.23%	4.37	48.76%
T2 Probiotic isolate –II	4.00	51.04%	2.77	67.41%	8.10	5.04%
T3 Probiotic isolate –III	3.03	62.91%	3.67	56.82%	8.40	1.52%
T4 Probiotic isolate –IV	2.37	70.99%	3.23	62.00%	8.00	6.21%
T5 Commercial probiotic-I	4.20	48.59%	3.37	60.35%	8.43	1.17%
T6 Commercial probiotic-II	6.37	22.03%	4.17	50.9%	8.50	0.35%
T7 Commercial probiotic-III	3.10	62.05%	3.73	56.11%	8.40	1.52%
T8 CONTROL	8.17		8.50		8.53	
S.E.±	0.1067		0.1917		0.1512	
C.D@5%	0.3114		0.5596		0.4414	
CV%	2.357		7.6049		6.4876	



**Fig. 2. *In vitro* dual culture agar plate technique of probiotics against mango pathogen (*Colletotrichum*)**

**Table 2. Efficacy of probiotic isolates (applied separately) and commercial probiotics in the management of post-harvest pathogens of mango**

Treatments	<i>Aspergillus</i>				<i>Colletotrichum</i>				<i>Alternaria</i>			
	3 DAI	5 DAI	7 DAI	10 DAI	3 DAI	5 DAI	7 DAI	10 DAI	3 DAI	5 DAI	7 DAI	10 DAI
Probiotic isolate-I	-	+	++	++	-	-	-	±	-	-	±	+
Probiotic isolate-II	-	+	++	++	-	-	±	±	-	-	-	±
Probiotic isolate-III	-	+	++	++	-	-	-	±	-	-	±	+
Probiotic isolate-IV	-	+	++	++	-	-	-	±	-	-	-	±
Commercial probiotic-I	-	+	++	++	-	-	-	±	-	-	-	±
Commercial probiotic-II	-	+	++	++	-	±	+	++	-	-	-	±
Commercial probiotic-III	-	+	++	++	-	-	±	+	-	-	±	+
Control	+	++	++	++	±	++	++	++	±	++	++	++

+, ++ = Fungal growth present. ± = Traces of fungal growth. - = No fungal growth

**Table 3. Efficacy of probiotic isolate (Applied in combination) and commercial probiotics in the management of post-harvest pathogens of mango**

Pathogen	Without probiotics	Growth of post harvest pathogen in probiotic sprayed mango fruits			
		Flora	Prowel	Vbact	Probiotic isolate
Post harvest pathogens					
<i>Colletotrichum</i>	+	-	±	-	-
<i>Alternaria</i>	+	-	-	-	-
<i>Aspergillus</i>	+	+	±	±	±

+= Fungal growth present. ± = Traces of fungal growth. - = No fungal growth

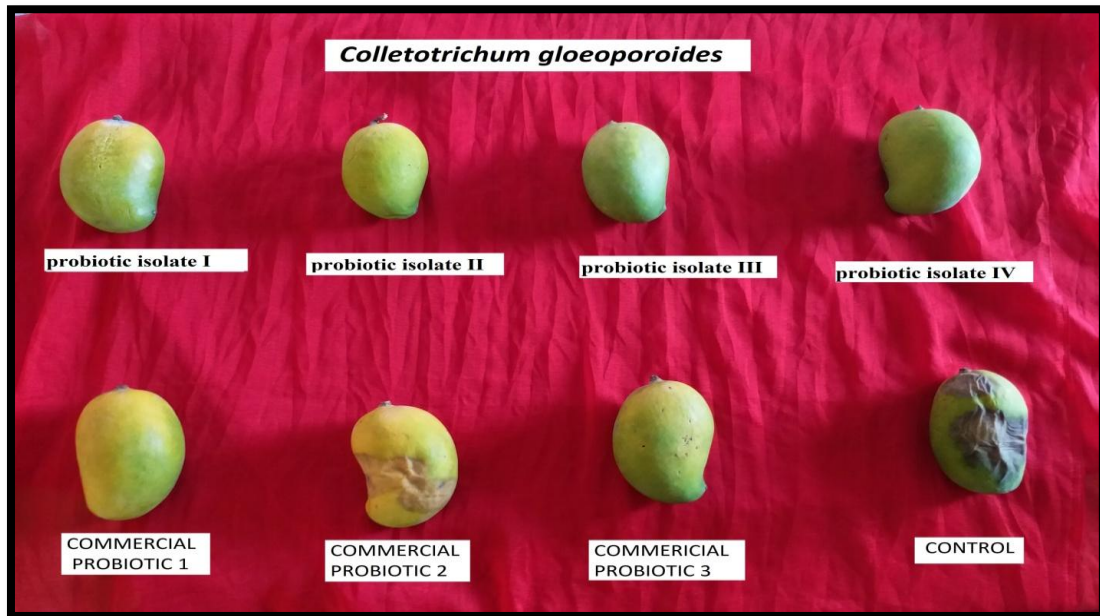
**Table 4. Effect of application of probiotics treated separately on fruit TSS content harvested mango**

Probiotic used	Increase in °Brix of mango over control
Probiotic isolates I	0.8(13.4)
Probiotic isolates II	1.4(13.4)
Probiotic isolates III	1.8(13.4)
Probiotic isolates IV	2.5 (13.4)
Flora	0.6(13.4)
Prowel	3.1 (13.4)
Vbact	2.0 (13.4)

Figures in parenthesis indicates the actual TSS (°Brix) in control (without probiotic application)

content was measured in these treated fruits after 9 days using hand refractometer. The results (Table 4) indicated that all the probiotic when treated separately increased the TSS content of mango fruits, the increase in

TSS(°Brix) was in the range of 0.8 to 3.1 over control. The maximum increase was by the probiotic Prowel, Probiotic isolate IV and followed Vbact.



**Fig. 3. Effect of probiotics on post harvest disease (*Colletotrichum*) of mango**

The Probiotic *Lactobacillus* treated fruits showed inhibition of 48% on Post harvest pathogens in Papaya and Banana and also it increased the shelflife of the fruits [15,16] and the probiotic formulations and commercial probiotics were effective in controlling post harvest diseases in grapes when the inoculum load was less and also reported that it improves the quality of Grape fruits [17]. In the same lines the present research was taken up. When the plants are treated with probiotic bacteria *Paraburkholderia fungorum*, *Bacillus amylolequefaciens* improved the antioxidant content, growth and yield in strawberry [18]. Probiotic microorganisms apply the mechanism of secreting a variety of antimicrobial compounds, including organic acids, hydrogen peroxide, bacteriocins and biosurfactants, that inhibit the growth of pathogenic bacteria; however, bacteriocins have stronger antimicrobial activity against pathogens under acidic conditions [19].

#### 4. CONCLUSION

The studies on use of probiotics in the management of post-harvest diseases of mango suggested that the post-harvest pathogens particularly *Colletotrichum*, *Alternaria*, can be checked by the sprays of probiotics under *in vitro* conditions when the load of inoculums is low. The probiotics can be used to increase the TSS content as well as the shelf life of the fruits and to keep them fresh for a longer time. The efficacy of

probiotics against post-harvested pathogens indicated that probiotics can be used in management of post harvest diseases and they can form an integral part of organic farming systems and also integrated disease management.

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The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:  
The peer review history for this paper can be accessed here:  
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