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Use of Probiotics for the Management of Post Harvest Diseases in Papaya

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ABSTRACT: The diseases caused by pathogenic microorganisms pose a potential threat to agriculture. Owing to the limitations of conventional methods, an effective alternative is needed. Probiotics are live micro organisms provide a number of health benefits to the host when consumed. The use of probiotics in crop production and crop protection programme is gaining a momentum in recent years. Probiotics usage for the management of post-harvest diseases of papaya has opened a new avenue in the disease management replacing toxic chemical fungicides. Along with the challenges in use of probiotics such as screening for efficient strain and identification of appropriate carrier for their inoculation. The present investigation was conducted at MPKV, Rahuri using different probiotic isolates, which are isolated and cultured from curd, fermented dosa material, fermented jower and bajra flour and the commercial probiotics (Prowel, Flora and Vibact) were tested for their efficacy in controlling major post harvest diseases of papaya. The study suggests that the growth of post-harvest pathogens particularly *Colletotrichum gloeosporoides, Alternaria alternate* were inhibited up to 71.8 % and 73.3 % respectively and the probiotic treated fruits showed a reduced disease development under *in-vitro* conditions and resulted in increase in the TSS brix (1.0- 4.0 over control) in the fruits and also showed an increased shelf life over the control.

Keywords: Papaya, Post-harvest disease, Probiotics, Management, Bio control

INTRODUCTION

Papaya (*Carica papaya*) also known as "the Fruits of the Angels" according to Christopher Columbus, grows mainly in tropical regions is rich source of vitamins A, E and C. The fruit also contains K, Cu, P, Fe, Mg, carotenes, flavonoids, folate, pantothenic acid and fiber and has antimicrobial properties (Alara *et al.*, 2020). In India, papaya is cultivated over an area of 142 thousand m.ha. with a total production of 6011 thousand million tonnes (Horticulture statistics division, 2019-2020). The state Maharashtra with suitable tropical climatic conditions for the growth of fruit crop occupies 11.45 thousand ha area and with production of 424.22 thousand m.t ranks fourth with about 50 per cent of countries total production.

The fruit of papaya is highly perishable, the softening of fruit accompanied with ripening makes this fruit susceptible to various biotic and abiotic stresses. Especially a wide range of diseases, caused by fungi which include *Colletotrichum, Phytophthora, Alternaria, Aspergillus, Fusarium, Stemphylium* resulting for major post harvest losses (Gabrekiristos *et al.*, 2020) of which anthracnose showed highest incidence 46% (Hamim *et al.*, 2014). The losses caused due to the post harvest micro organisms accounts for millions of dollars of perishable goods every year (Narayana Swamy, 2005). To overcome these losses caused by micro organisms, it is necessary to control these post-harvest diseases by various biological, chemical and physical treatments (Qadri *et al.*, 2020)

The use of toxic chemical fungicides as pre-harvest treatment to control the pathogen poses a serious threat on the consumer health as a result, their usage on edible products is being restricted worldwide (Igbedioh *et al.*, 1991). Therefore, there is a need to identify a novel alternate to control these post-harvest diseases and save our edible produce. The probiotics use in the management of various human, animal and aquaculture diseases has taken a great improvement in recent years (Zubrod *et al.*, 2019). However, their use in plant disease management has taken a momentum over the last few years with increase in the health consciousness of people.

Probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit to the host (FAO/WHO, 2002). Most of the probiotics includes bacterial genera such as *Bifidobacterium, Lactococcus, Carnobacterium, Enterococcus, Streptococcus, Pediococcus, Propionibacterium, Leuconostoc*, and *Bacillus* species, of which lactic acid bacteria is the most common type. (Oyetayo & Oyetayo, 2005; Mulaw *et al.*, 2019). Few molds (*Aspergillus*) and yeasts (*Saccharomyces*) are also being used as probiotics (Amara and Shibl, 2015).

A good probiotic must be a micro organism that has potential to exert a beneficial effect on the hosts, such as increased growth and disease resistance, the probiotic must be nonpathogenic and non-toxic to host consumed exhibit high acid tolerance (Kim *et al*., 2019) and must show continued growth and metabolism in the gut environment and exert a stable performance under storage and field conditions (Bharti *et al*., 2017).

Along with commercially available probiotic formulations they also occur naturally in fermented food products such as cabbage kimchee, curd, yoghurt, kefir, sauerkraut (sour cabbage), Soybeans fermented miso and natto. As the probiotics are consumed orally and have health benefits to human (Kanmani, 2013). Their presence as biocontrol agent on the edible fruits will not have any detrimental effect on the human health and are safe to consume. Hence, Their use to control pathogenic micro organism infection in post-harvest fruit produce is interesting to study and hence forth the present investigation is taken up.

MATERIALS AND METHODS

A. Pathogen isolation from diseased samples

The post harvest pathogens were isolated from the surface sterilized diseased samples on potato dextrose media plates. The fungal growth obtained is sub cultured by mycelial tip isolation method and identified up to genus level based on their morphological and conidial characteristics.

Probiotic isolates. Probiotic isolates used for the treatment were isolated from the fermented samples of jowar, bajra, dosa flour and curd by streaking on Malt extract agar and Nutrient agar media respectively, and are named as Probiotic isolate- I for the edible yeast culture obtained from jowar flour, Probiotic isolate –II for the edible yeast culture obtained from bajra flour, Probiotic isolate III for the edible yeast culture obtained from dosa flour and Probiotic isolate IV for the *Lactobacillus* bacteria obtained from curd.

Commercial probiotics. The commercial probiotics sachets which are consumed and easily available in the market are used they are Prowel (*Bifidobacterium longum*, *Lactobacillius acidophilus, Bifidobacterium lactis, Bifidobacterium bifidum,*) Flora (*Lactobacillius rhamnossus, Bifidobacterium longum, Lactobacillius acidophilus, Bifidobacterium boulardii)* ViBact (*Bacillus mesentericus, Clostridium butyricum, Streptococcus feacails*, *Lactobacillus sporogenes).*

Pathogenicity. To prove the Koch postulates, the pathogenicity test was carried out by pathogen inoculation on healthy papaya fruits. The healthy fruits were sprayed with fungal spore suspension under aseptic conditions and were incubated in the desiccator for growth and infection of pathogen. The type of symptoms were recorded seven to eight days after inoculation.

B. Efficacy of probiotics against post harvest pathogen under in vitro conditions

The Probiotics efficacy on post harvest pathogens was evaluated under *in vitro* conditions by dual culture agar plate method. A 5mm disc of fungal mycelium was inoculated on centre of petri dish containing PDA medium. In parallel a loop full of probiotic isolates cultures and commercial probiotics were streaked one either sides of disc in the same plate respectively. The Paired cultures inoculated were incubated at 26-29°C for 6-7 days. The plate with no probiotic served as control. After 7 days, growth diameter of the fungus was measured both in control and probiotic inoculated plate. The percent growth inhibition will be calculated using the formula.

Inhibition Percent (%) = G_C-G_T/ G_T ×100

G_C = Pathogen growth in control plates,

G_T = Pathogen growth in probiotic inocultated plates

C. Effect of probiotic application against diseased fruit in in vitro conditions

Probiotic powder from commercial probiotic sachet and individual probiotic isolates were cultured in 2% sucrose solution. This inoculated solution was incubated at 25 ± 2ºC for 3 days for the efficient growth of probiotic organisms. The probiotic suspension thus obtained was further used for spray inoculation on harvested fruits under *in vitro* conditions. The surface sterilized harvested papaya fruits were inoculated with suspension of respective post-harvest pathogen and air dried. The respective probiotic suspension was sprayed on these pathogen inoculated fruits and are kept at room temperature. The observations were taken from 3 days up to 10 days until the appearance of pathogenic symptoms.

D. Effect of probiotic application on fruit quality

The outcome of probiotics application on fruit quality parameters was tested on the fruit TSS content. The harvested papaya fruits were sprayed with probiotics suspension and were kept at room temperature. The change in TSS content was measured after 9 days using hand refractometer.

RESULTS AND DISCUSSION

A. Efficacy of probiotics against the post harvest pathogen under in vitro conditions

The efficacy of both Probiotic isolates and the commercial probiotics were assessed against the isolated papaya post-harvest pathogens, *viz.,Colletotrichum, Alternaria* and *Aspergillus*.

The results depicted in (Table 1), showed that the probiotic isolates were effective in controlling the post harvest pathogens compared to commercial probiotics.

Among the applied tested probiotics the Probiotic isolate-II was effective against *Colletotrichum* showing an inhibition percentage of 71.80% and Probiotic isolate-I was effective against *Alternaria* showing an inhibition percentage of 73.3%. *Apergillus* was effectively inhibited by Probiotic isolate-I showing an inhibition of 38%. Among the Commercial probiotics tested Vi bact was successful in inhibiting the growth of pathogens *Colletotrichum* and *Alternaria* compared to others.

Table 1: Efficacy of probiotics against post harvest pathogens of papaya under *in vitro* conditions.

Treatments		*Colletotrichum*		*Alternaria*		*Aspergillus*	
		Mean	Percent of inhibition %	Mean	Percent of inhibition %	Mean	Percent of inhibition %
T1	Probiotic isolate –I	2.57	67.5 %	1.92	73.3 %	5.27	38 %
T2	Probiotic isolate –II	2.23	71.80 %	3.10	56.9 %	7.27	14.4 %
T3	Probiotic isolate –III	2.43	69.2 %	2.43	63.4 %	7.60	10.5 %
T4	Probiotic isolate –IV	3.33	57.90 %	3.30	54.16 %	7.70	9.4 %
T5	Flora	4.03	49.05 %	3.47	54.58 %	8.17	3.8 %
T6	Prowel	4 .30	45.63 %	3.83	46.80 %	7.90	7.90%
T7	Vibact	3.37	57.39 %	2.53	64.86 %	8.10	4.70 %
T8	CONTROL	7.91		7.50		8.50	
	S.E.±	0.1936		0.1317		0.1308	
	C.D@5%	0.565		0.3845		0.3820	
	CV%	4.435		6.051		6.526	

B. Efficacy of probiotic application on post-harvested papaya fruits

The efficacy of Probiotics on the post-harvested papaya fruits was evaluated under *in vitro* conditions. The results in (Table 2) indicates that the Probiotic isolates were effective to inhibit the growth of isolated post-harvest pathogens *Colletotrichum* and *Alternaria* up to 10 days. But were not effective against the pathogen *Aspergillus*. Commercial probiotics Prowel and Vibact were effective to inhibit *Alternaria* but not *Colletotrichum.* The results (Table 3) show that the Probiotic isolates in combination were able to inhibit the pathogen more compared to when sprayed separately.

C. Effect of application of probiotics on quality parameters (TSS content) of papaya

The effect of probiotic application was seen on the quality parameters-TSS content in papaya fruits. The results in (Table 4) shows that increase in TSS (^{0}Brix) in papaya fruits was in the scale of 1.0 to 4.0 over the control. The maximum increase was observed in the probiotic Prowel treated fruit followed by Probiotic isolate III and IV.

The results shown in (Table 5) indicates that probiotics when sprayed in combination the increase in TSS (Brix) in papaya was in range of 4.2 and 2.7 over the control. The maximum increase observed in case of prowel followed by probiotic isolates.

The results from the study suggest that the probiotics can be used as an effective alternative to the conventional disease management strategies with no impact on human health and the environment. The probiotics employ various direct and indirect mechanisms to enhance the plant growth and protection. Each of these probiotic microorganisms have a specific task including bacterial antagonism by secreting various antimicrobial compounds, enzymes, bacteriocins, hydrogen peroxide, etc. (di Francesco, *et al*., 2017 Zhang *et al.*, 2016). Rydlo *et al*., (2006), studied that probiotic lactic acid bacteria could protect food products against pathogenic bacteria during storage using several mechanisms, such as inhibiting the growth by production of antimicrobial compounds, including enzymes, volatile compounds and competiting for nutritional elements with pathogens. Hernandez-Montiel *et al.*, (2018) reported that the *Debaryomyces hansenii* uses various antagonistic mechanisms against *Collectotrichum* causing anthracanose in papaya. In addition, these microorganisms enhance each other, i.e. they act synergically. They eliminate the pathogenic micro-organisms by occupying the niche that a pathogen would occupy or by producing substances that kill or damage pathogen cells and break down toxic substances (Ewa, 2013) thus, improving the quality and nutrition (de Paiva *et al.*, 2017). Neelima *et al.*, (2017) studied that the probiotic *Lactobacillus* has shown a inhibition of 48% agains the post-harvest pathogens in Papaya and Banana and also the treated fruits showed an increased shelf life. Srilatha and Borkar (2017) studied that probiotic yeast species controlled post harvest diseases in grapes and it also improves the quality of grape fruits. Greeshma *et al.*, (2020) studied on that various probiotic yeast and bacteria controlled the post harvest disease anthraconose in mango resulted in increase TSS in fruits keeping them fresh for longer period.

Table 2: Efficacy of probiotic isolates (applied separately) and commercial probiotics in the management of post-harvest pathogen in Papaya.

	Aspergillus				***Colletotrichum***				***Alternaria***			
Treatments	**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	-	-	±	+	-	-	-	±	-	-	-	±
Flora	-	±	±	+	-	-	-	±	-	-	-	±
Prowel	-	±	±	+	-	-	+	±	-	-	-	±
Vibact	-	±	±	+	-	-	+	±	-	-	-	±
Control	+	++	++	++	±	++	++	++	±	++	++	++

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

Table 3: Efficacy of Probiotic isolates (applied in combination) and commercial probiotics in the management of post-harvest pathogen in Papaya.

Post Harvest Pathogens	**Growth of post harvest Pathogen (without probiotics)**	**Growth of post harvest pathogen in probiotic sprayed papaya fruits**			
		Flora	**Prowel**	**Vibact**	**Probiotic isolates**
Colletotrichum	+	-	-	+	-
Alternaria	+	-	-	+	-
Aspergillus	+	±	±	±	±

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

Table 4: Effect of probiotics application separately on fruit TSS content in papaya harvested fruits.

Probiotic treated	**Increase in ^{0}Brix of papaya over control**
Probiotic isolates I	1.0(10.0)
Probiotic isolates II	2.2(10.0)
Probiotic isolates III	3.1(10.0)
Probiotic isolates IV	3.0 (10.0)
Flora	2.1(10.0)
Prowel	4.0(10.0)
Vibact	1.3(10.0)

Values in parenthesis shows the TSS (^{0}Brix) in control (with no probiotic application)

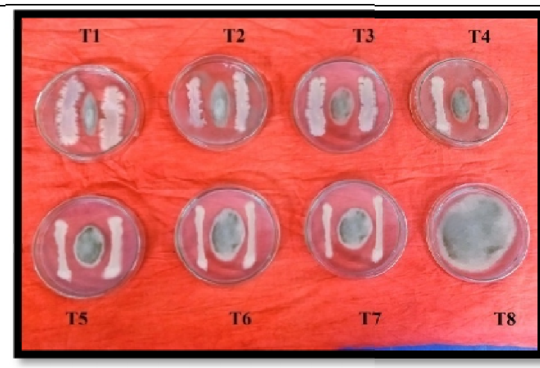
Table 5: Effect of probiotics application in combination on fruit TSS content in papaya harvested fruits.

Probiotic treated	**Increase in ^{0}Brix of papaya over Control**
Flora	2.3(11.5)
Prowel	4.2(11.5)
Vibact	1.7(11.5)
Probiotic isolates	2.7(11.5)

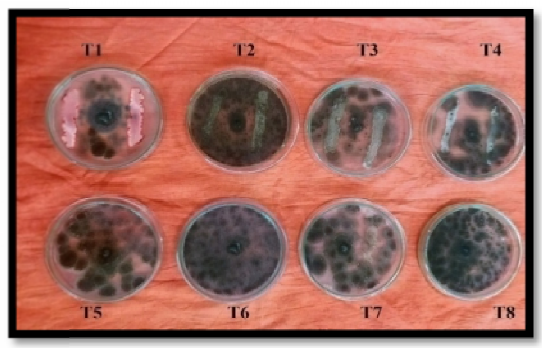
Values in parenthesis shows the TSS (^{0}Brix) in control (with no probiotic application)

Rahman *et al.*, (2018) evaluated that the plants treated with probiotic bacteria *Paraburkholderia fungorum, Bacillus amylolequefaciens* improved the antioxidants content, growth and yield in strawberry. García-Seco *et al., (*2013), showed that the inoculation of blackberry with the strain *Pseudomonas fluorescens* N21.4 improves flavonoid content as compared to the control treatment also the root treatment with *Pseudomonas fluorescens* N21.4 not only increased the expression of flavonoids biosynthetic genes, but also flavonoid concentration by 22%, in fruits (García-Seco *et al.*, 2015). The production of several organic acids, which are strong antimicrobial agents, has been reported to be efficient at fighting against /pathogens in vegetables and fruits due to a reduction in pH and anion accumulation (Rico *et al.*, 2007).

(a) *Colletotrichum*.



(b) *Alternaria*



(C) *Aspergillus*.

Fig. 1. *in vitro* dual cuture agar plate technique of probiotics against papaya post harvest pathogens.

CONCLUSION

The studies on use of probiotics as a alternative in the management of post-harvest pathogens of papaya fruits suggests that the pathogens particularly *Colletotrichum, Alternaria* can be controlled as a post-harvest pathogen by spraying of probiotics under *in vitro* conditions when the inoculum load of these pathogens is less on the fruits. The probiotics treatment also increases the shelf life and the quality of the fruits keeping them fresh for longer periods. Hence, they can be used as an effective alternate in plant disease management and form essential part of bio control and also in integrated disease management. The future scope includes Molecular identification and characterization of efficient strains and development of stable formulations with efficient carriers that enhance the shelf life along with developing standardized protocols for quality assurance.

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