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RESEARCH ARTICLE

PROBIOTIC *LACTOBACILLUS* AS BIO-CONTROL AGENT OF POST-HARVEST DISEASES OF BANANA AND PAPAYA FRUITS

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ABSTRACT

In the present study, isolation of post-harvest diseases of fruit banana and papaya was carried out such as *Fusarium* sp., *Colletotrichum* sp. *and Alternaria* sp. based on the cultural and morphological test, the diseased pathogens were identified as *Fusarium moniliforme* and *Colletotrichum musae* from banana and *Alternaria alternata* from papaya. Commercially used *Lactobacillus* probiotic bacteria was found most effective against *Fusarium moniliforme, Colletotrichum musae and Alternaria alternata. Lactobacillus* was showed highest percent inhibition 37%, 42% and 48% against *Fusarium moniliforme, Colletotrichum musae* and *Alternaria alternata* respectively. In the *in vivo* study, the healthy Fruits of banana were infected with *Fusarium moniliforme* and *Colletotrichum musae* were remain uninfected and healthy up to 10 days when treated with *Lactobacillus* and in case of *Alternaria alternata* was infected to healthy papaya, remain in healthy condition up to 8 days when treated with *Lactobacillus*. A Probiotic bacterium *Lactobacillus* increases the shelf life of fruit such as Papaya and Banana.

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INTRODUCTION

The fruit Crops like Banana (*Musa paradisica* L) and Papaya (*Carica papaya* L) are the most important horticultural crops in Maharashtra as well as in India. However, these crops are well acclimatized to tropical and subtropical climatic regions. Fruit plays vital role in nutrition. World health organization (WHO) recommend, daily per capita consumption on of fruit is total 400g for healthy person (Chu *et al*., 2010). China's share has been highest with 21.2% in world's fruit production followed by India and Brazil. India is the second largest food producer in the world, after China and one of the centers of origin of F&V with the total production of 88.977 million metric tonnes of fruits till the year end 2014 (NHB, 2015). Anthracnose, crown rot and blossom end are common and serious postharvest diseases of banana. Crown rot affects export of bananas in all banana-producing countries and is considered to be one of the main export banana post-harvest diseases (Krauss and Johanson, 2000). Anthracnose, caused by the fungus *Colletotrichum musae*, it is necessary to improve the post-harvest behavior of banana attempting extends shelf life and quality of banana. Fruit infection and subsequent losses of banana were caused essentially by anthracnose due to *colletotrichum musae* and *fusarium spp*., in frequent association and various fungal species like *Aspergillus flavus, Aspergillus nigar*, high temperature as well as lack of sanitation in strong room play an important role on shelf life of banana (Diedhiou *et al.*, 2014). One of the major reasons of post-harvest losses of papaya production is fungal diseases. Post-harvest disease organisms of papaya are Stem end root like, *Rhizopus stolonifer, Alternaria alternata* (Robert, 1997).

Probiotics are defined as live microorganisms that beneficially affect the host by improving its intestinal microbial balance. Probiotic bacteria in foods have been used to promote health benefits for 20 years (Lynne, 2009). *Lactobacillus* is a type of bacteria. These are "friendly" bacteria that normally live in our digestive. Probiotics are the living micro-organisms that can be formulated into many different types of products including food, drugs and dietary supplement (Robin, 2014). Probiotic organisms are *Lactobacillus, Bifidobacteria, Enterococcus, and Streptococcus.* The efficiency of lactic acid bacteria (LAB) isolated from fresh fruits as bio-control agents against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris* and *penicillum expansum* (Rosalia *et al.,* 2008). Post harvest losses are reported up to 35 to 40% with reduction in quality and quantity of marketable fruits. Various Chemicals are used for controlling post harvest diseases, but Chemical control is risky due to possibility of residual effect and awareness of people about health, and also results in considerable financial loss to the sellers as well as

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the consumers. Biological control is one of the safest approaches for controlling post harvest diseases. In the view of the post harvest spoilage due to various fungal pathogens indicated above, it was thought these probiotic organisms can be useful for control of post harvest diseases of different fruit crops. Taking in the consideration, need of development of alternative strategies which are economically safe and risk free to human being for controlling post harvest diseases. In the view of post harvest diseases control in major fruit crops (Banana and Papaya), it was thought to undertake use of probiotics for study.

MATERIALS AND METHODS

Isolation of pathogens associated with post-harvest diseases of fruits

The isolation of post harvest diseases of fruit banana and papaya were done by using potato dextrose agar (PDA) by employing tissue isolation method, which were collected from different fruit markets of Aurangabad, Maharashtra, India. These diseased parts were disinfected by surface sterilization with 1:1000 sodium hypochlorite solution for one followed by three of corrosive sublimate. These small pieces of infected fruits were transferred aseptically to sterilized petri plates containing PDA medium (3 to 4 pieces /plates). These plates were incubated at room temperature (27± 1^0c) for six days.

Identification of isolates

Pathogens isolated from diseased samples of fruit Banana and papaya was identified on the basis of morphological characters observed under microscope and on the basis of pathogenicity (Barnett and Barry, 1972).

Pathogenicity

The pathogenicity test of the isolated fungi was conducted by undertaking the inoculation experiments on healthy fruits. (Jimenez *et al.,* 1993 and Douglas *et al.,* 1996).

Antagonist

Commercially used different probiotics were used as antagonist. Suspensions of probiotic bacteria were prepared by growing cultures in nutrient yeast dextrose broth (NYDB) (nutrient broth 8 g l^{-1}, yeast extract 5 g l^{-1}, dextrose 10 g l^{-1}) for 24 h at 25 ± 1°C.

In vitro effect of probiotics against post-harvest pathogens

Paper disc method was used to evaluate efficacy of probiotics against post harvest pathogens. The seven days old cultures of each isolate having good growth were used separately for preparation of spore suspension in sterile water. The pathogen spore seeded plates were allowed to solidify. The sterilized Whatman's filter paper discs (5mm dia) loaded with probiotics bacterial suspension were kept on to the surface of the Petri-plates containing spore seeded PDA medium. The paper disc loaded with sterile distilled water served as respective check. The plates were incubated at 28±2^0c for 7 days. The inhibition zone around the paper disc was measured with millimeter scale and percent inhibition was calculated by using 1-TG/CG×100.

In vivo effect of probiotics against post-harvest pathogens

In vivo effect of probiotics against post harvest pathogens were laid out by using wound inoculation method. Small wounds (3mm wide x 5 mm deep) were made by pinching sterile paper pins on to healthy fruits, banana and papaya washed in 1:1000 mercuric chlorides for one minute followed by rinsing twice in sterile distilled water. The seven days old culture of pathogens was used for spore suspension. Inoculation was made by dipping the wounded fruits in spore suspension of pathogenic fungi. After drying for 1 hour, fruits were sprayed with probiotic *Lactobacillus* bacterial suspension and kept in moist chamber. Control was also maintained by inoculating spore suspension of post harvest pathogens on fruits. The fruits were observed for expression of symptoms.

Effect of temperature on sporulation of post-harvest pathogens

The study was conducted on potato dextrose Ager medium. The plates were inoculated at centre with different fungal post harvest plant pathogens disc of 5mm diameter and incubated at 0, 5, 20, 27, 35 and 45^0c.

RESULTS AND DISCUSSION

Isolation of pathogens associated with post-harvest diseases of fruits

Three isolates of fungal pathogens *Fusarium* sp., *Colletotrichum* sp., from the diseased fruits Banana (*M. paradisica*) and *Alternaria* sp. from Papaya (*C. papaya*) were isolated from banana and papaya at MGM's Institute of Bioscience and Technology, Aurangabad, Maharashtra, India. Several workers have reported the pathogens associated with the post harvest diseases of fruits. Sangeetha and Rawal (2008) had pointed out association of fungal pathogens like *Penicillium, Aspergillus, Fusarium, Colletotrichum* and *Alternaria are* responsible for spoilage of fruits.

Identification of cultures

The pathogenic fungi which are isolated from the rotted fruits banana and papaya were identified on the basis of morphological characteristics as *Fusarium moniliforme, Colletotrichum musae* and *Alternaria alternate,* respectively. Colony colour of *Fusarium moniliforme* was pinkish white mycelium, septate and profusely branched mycelia. Macro conidia were curved, sickle shaped and septate. Micro conidia were usually cylindrical, mostly non septate. The findings are similar to those reported by Bagi *et al*. (1999). Mycelium of *Colletotrichum musae* is septate, acervuli develop profusely. Conidia are straight cylindrical or sickle shaped with rounded ends, aseptate, single celled. The conidia in mass are pinkish but hyaline individually. Mycellium turned brown when old. Conidiophores were cylindrical and unbranched. The present studies are similar to those reported by Kumar *et al.* (2010). The mycelium of *Alternaria alternata* was non septate, irregularly branched, brown to deep grey in colour. Conidia

formed in long often branched chain, having conical or cylindrical beak with cross vertical as well as oblique septa. Conidia were muriform in shape and light brown in colour. Conidiophores arose singly or branched. The present observations are similar to those reported by Ramjegathesh and Ebeneear (2012).

Pathogenicity and Symptoms of banana and papaya

In this experiment, fruits of banana and papaya were artificially inoculated with the pathogen isolated earlier from diseased fruits. All the pathogens gave positive results and symptoms appeared on fruits within 2 to 4 days after inoculation. Re-isolation was made from artificially inoculated fruits of banana and papaya showed typical symptoms of lesions and patches which yielded fungi (Pathogens) similar to those of original ones.

Fusarium moniliforme

Banana fruit inoculated with *Fusarium moniliforme* showed small, olive brown spots on the surface of fruit, mainly at tip. Infection become deep seated, leaking of juice of foul odour and fungal growth on surface. In advance stage large clove brown to mummy brown patches observed on the fruit. The present observations are similar to the symptoms observed by Prasad *et al*. (2000) and Jimenez *et al*. (1993).

Colletotrichum musae

Banana fruits inoculated with *Colletotrichum musae* showed small black circular sunken spots coalesce to form large spots. At advanced stage, severely infected fruits become dark due to blemishes. Finally fruits rotten in 4-5 days.

Alternaria alternata

Papaya fruits inoculated with *Alternaria alternata* showed symptoms in the form of grey brown, circular to semi circular spots and patches on the fruit surface covered with mycelium and conidiophores. At advanced stage spots become dark brown and fruits rotten within 4-5 days.

***In vitro* efficacy of probiotics on the post-harvest fungal pathogens**

Commercially available different probiotics were used for *in vitro* efficacy. In which *Lactobacillus* probiotic was showed the inhibition zone against *Fusarium moniliforme, Colletotrichum musae and Alternaria alternata.* Percent inhibition of *Lactobacillus* against *Fusarium moniliforme, Colletotrichum musae and Alternaria alternata* were 37%, 42% and 48%, respectively. (Table 1) Shrishikar (2002) reported that the antifungal activity of garlic extract against *C. gloeosporioides*. Antibacterial activity against *Xanthomonas* sp. and *Erwinia* sp. and spoilage molds, *Leuconostoc* isolates could effectively inhibit bacteria than molds. Culture broth of LM 15 recorded the highest zone of inhibition (13.59 cm2) followed by LM 30 (11.20 cm2) against *Xanthomonas* sp. (Hitendra *et al.* 2015).

***In vivo* efficacy of probiotics on the post-harvest fungal pathogens of banana and papaya**

Banana Fruits treated with 1ml (10^7cfu/ml) *Lactobacillus* against *Fusarium moniliforme* and *Colletotrichum musae* were remain uninfected and healthy up to 10 days. Fruits in control get rotted within 3 days. Papaya fruits treated with 1ml (10^7cfu/ml) *Lactobacillus* against *Alternaria alternata* were remain in healthy condition up to 8 days only. Fruits in control get rotted within 4 days. (Table 2) Only few probiotic strains including the commercial *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12 have been investigated to enrich fresh-cut apples and papaya (Tapia *et al.,* 2007).

Table 1. *In vitro* efficacy of *Lactobacillus* probiotic on fugal pathogens of fruit banana and papaya

Sr. No.	Post harvest pathogen	% Inhibition
1.	*Fusarium moniliforme*	37.00 %
2.	*Colletotrichum musae*	42.00 %
3.	*Alternaria alternata*	48.00 %

Table 2. *In vivo* efficacy of *Lactobacillus* Probiotic on post harvest pathogens of Banana and papaya

Sr. No.	Treatments	*Fusarium moniliforme*				*Colletotrichum musae*				*Alternaria alternata*		
		2 D	4 D	8D	10D	2 D	4 D	8D	10D	2 D	4 D	8D
1.	*Lactobacillus* probiotic	+	+	+	+	+	+	+	+	+	+	+
2.	Control	+	-	-	-	+	-	-	-	+	+	-

Where, + = No Infection - = Infection started D= Days

Table 3. Effect of temperature on sporulation of fungal diseases of banana and papaya

Temperature	*Fusarium moniliforme*	*Colletotrichum musae*	*Alternaria alternata*
0^0C	-	-	-
5^0C	-	-	-
20^0C	++	-	++
27^0C	++++	++	+++
35^0C	++	+++	+
45^0C	-	-	-

- = No sporulation, + = Poor sporulation,
++ = Moderate sporulation, +++ = Good sporulation,
++++ = Abundant sporulation.

Probiotics can play their beneficial role if they reach the gut lumen in an enough number to provide health gain to the host. Bio-preservation is based on the antagonistic effect of some microorganisms, including LAB that can play a protective role in the product itself during storage (Alegre, 2011)

Effect of temperature on sporulation of isolated post-harvest pathogens

It is observed that the fungus *Fusarium moniliforme* could grow at temperature range 20-35^{0}C. Maximum growth and abundant sporulation observed at 27^{0}C temperature while moderate sporulation at 20^{0}C and 35^{0}C temperature. There was no growth at temperature level 0^{0}C, 5^{0}C and 45^{0}C. It is observed that the fungus *Colletotrichum musae* could grow at temperature range 27 to 35^{0}C. Highest growth was noted at 35^{0}C temperature. There was no growth at temperature level 0^{0}C, 5^{0}C and 45^{0}C. It is observed that the fungus *Alternaria alternate* could grow at temperature range 20-35^{0}C. Highest growth was noted at 27^{0}C temperature. There was no growth at temperature level 0^{0}C, 5^{0}C and 45^{0}C. Good growth and sporulation occur at 27^{0}C temperature while moderate at 20^{0}C, poor sporulation at 35^{0}C temperature. (Table 3) These results are similar to the observations made by Nagaraja *et al.* (2011). They have reported that temperature 25^0 to 27^{0}C is optimum for *Fusarium* growth. Hudge and Datar (2009) observed that 25^0 to 27^{0}C temperature are good for sporulation and growth of *Alternaria alternata*. (Smilanick and Mansour, 2007).

Conclusion

In conclusion, The probiotic *Lactobacillus* was found as potential bio-control agent against post harvest diseases of banana and papaya *viz. Fusarium moniliforme, Colletotrichum musae* and *Alternaria alternata* both *in vitro* and *in vivo* study. *Lactobacillus* is safe to consume. A Probiotic bacterium *Lactobacillus* increases the shelf life of fruit such as Papaya and Banana. This strain may be developed as new bio-control agent for post harvest disease control of banana and papaya. But further study is necessary to understand the mode of action mechanism of this bio-agent.

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