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Management of post-harvest diseases of fruits and vegetables with microbial antagonists

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ABSTRACT

Fresh fruits and vegetables encounter diseases between harvest and consumption, resulting in significant food waste and economic losses. According to the Food and Agriculture Organization about 45% of harvested fruits, vegetables, roots and tubers are lost. Most of this loss occurs during storage due to microbial infestation. Traditionally, chemical fungicides and food preservatives are being used to control postharvest decay. However, exposure to these chemicals is, in many cases, hazardous to the environment including humans, animals birds and fishes. Due to the toxicological chemicals residual effects in food products, their application in the postharvest has been limited to a few registered chemicals and is completely prohibited in some advanced and European countries. Several postharvest diseases can now be controlled by microbial antagonists. Although, the mechanism by which microbial antagonists suppress the postharvest diseases is still not clear. However, competition for nutrients and space is the most widely accepted mechanism of their action. Production of antibiotics, direct parasitism and induced resistance in the harvested commodity are the other modes of action by which biocontrol agents suppress the activity of postharvest pathogens in fruits and vegetables. Microbial antagonists are applied either before or after harvest, but post-harvest applications are more effective than pre-harvest applications. Different microbial antagonists like Cryptococcus laurentii Kufferath & Skinner, Bacillus subtilis (Ehrenberg) Cohn, Pseudomonas syringae and Trichoderma harzianum Rifai are being used. Efficacy of single use of microbial antagonists are one of the main constrains to manage post-harvest diseases. To improve the efficacy of microbial antagonists, mixture of two or more compatible antagonists, antagonists with low dose chemicals, heat treatments with antagonists are being used. Microbial biocontrol products like Aspire, BioSave, Shemer etc., have also been developed and registered for post-harvest application in fruits and vegetables.

***Keywords**: Biological control, post-harvest diseases, Pseudomonas spp., Trichoderma spp. chemicals hazards.*

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INTRODUCTION

The term "Biological Control" was first used by H. S. Smith in 1919 [36]. As defined by Cook et al [6], 'biological control is the use of natural or modified organism, genes or gene-products to reduce the effect of pests'. The general strategy of biological control is the technique of using one living organism for controlling the other. The biological-control agents may be antagonistic microorganisms or even natural plant and animal-derived compounds.

A wide range of microbial antagonists have been reported to control several different pathogens on various fruits and vegetables. Earlier, chemical fungicides and food preservatives were used to control postharvest decay. Fresh fruits and vegetables are exposed to diseases between harvest and consumption; consequently there are significant food wastes and economic losses. According to the Food and Agriculture Organization,

about 45% of harvested fruits, vegetables, roots, and tubers are lost due to various gaps in handling and storage. It is estimated that about 20-25% of the harvested fruits and vegetables are decayed and destroyed by pathogens during postharvest handling even in the developed countries of the world [34]. In developing countries, however, the post-harvest losses are often more severe due to insufficient storage and transportation facilities. Among all antagonists, yeast is one of the most accessible and efficient micro-organisms that can be used for biological control of postharvest pathogens. They have simple nutritional requirements and are capable of colonizing dry surfaces for longer durations. There are many such instances where successful results in disease control have been accomplished. Brown rot of peach was controlled by the application of *Bacillus subtilis an*d *Pseudomonas cepacia* [35]. *B. Subtilis* has also controlled postharvest brown rot and *Alternaria* rot of sweet cherry [37]. *Trichoderma atroviride*, *Trichoderma viride*, and *Rhodotorula* spp. can be used against brown rot diseases of peach and plum (Hong et *al.* 1998). The global trend appears to be shifting towards reduced use of fungicide on produce and hence, there is a strong public and scientific desire to seek safer and eco-friendly alternatives for reducing the decay loss in the harvested products [25]. Among different biological approaches, use of the microbial antagonists like yeasts, fungi, and bacteria is quite promising and gaining popularity (Table 1).
P. guilliermondii strain R13 were found that inhibited *C. capsici* growth with biocontrol efficacies of 93.3% and incidence of infected chilli fruits by *C. capsici* is 6.5% [4]. The two low-fermenting (*Candida intermedia* 235 and *Lachancea thermotolerans* 751) yeast strains inhibit ochratoxin A- producer (OTA) *Aspergillus carbonarius*, and remove their ability to OTA from grape juice.

Table 1.Some efficient microbial antagonists against fruits and vegetables diseases.

S.no	Antagonists	Disease (pathogen)	Fruits	References
		FUNGAL AND YEAST ANTAGONISTS		
1.	*Cryptococcus laurentii*	Bitter rot (*Glomerella cingulata*)	Apple	[2]
2.	*Trichoderma harzianum*	Anthracnose (*Colletotrichu musae*)	Banana	[8]
3.	*Rhodotorula glutinis*	Blue rot *(Penicilliu mexpansum)*	Pear	[43]
4.	*Trichoderma* sp.	Fruit rots *(Phomopsis psidi* & *Rhizopus* spp.)	Guava	[21]
5.	*Candida oleophila*	Anthracnose *(C. gloeosporioides)*	Papaya	[12]
6.	*Pestalotiopsis neglecta*	Anthracnose *(C. gloeosporioides)*	Apricot	[24]
7.	*Cryptococcus laurentii*	Rhizopus rot (*Rhizopus stolonifer*)& Gray mold (*Botrytis cinerea*)	Peach	[42]
8.	*Saccharomyces cerevisiae*	Postbloom Fruit Dro (*Colletotrichum acutatum)*	Citrus	[36]
9.	*Pantoea agglomerans*	*Penicillium*rot *(Penicillium expansum*)	Apple	[27]
10.	*Pichia anomala* (Hansen) Kurtzman	Crown rot *(Colletotrichum musae))*	Banana	[21]
11.	*Tricho sporonpullulans* (Lindner) Didlens & Lodder	Alternaria rot (*Alteria alternata*) Graymold (*Botrytis cinerea*)	Cherry	[32]
12.	*Kloeckera apiculata(*Rees) Janke	Botrytis rot *(Botrytis cinerea)*	Cherry	[19]
13.	*Aureobasidium pullulans*	*Monilinia* rot *(Monilinia laxa)*	*Banana*	[39]

14.	*Aureobasidium pullulans*	Botrytis rot *(Botrytis cinerea)*	Grape	[33]
15.	*Aureobasidium pullulans*	Soft rot *(Monilinia laxa)*	Grape	[1]
16.	*Kloeckera apiculata (*Rees) Janke	Green *(Penicillium digitatum) and* blue mold *(Penicillium italicum)*	Citrus	[23],[24]
BACTERIAL ANTAGONISTS				
17.	*Bacillus subtilis*	Green mold (*Penicillium digitatum)*	Citrus	[34]
18.	*Bacillus licheniformis*	Anthracnose *(C. gloeosporioides)*& stem end rot *(Dothiorella gregaria)*	Mango	[36]
19.	*Pseudomonas syringae*	Blue mold (*P. expansum)*	Apple	[17]
20.	*Bacillus amyloliquefaciens* CPA-8 (10^7 CFU mL^{-1})	*Monilinia laxa* and *Monilinia fructicola*	stone fruit	[41]
21.	*Pseudomonas fluorescens* Migula	Gray mold (*Botrytis mali* Ruehle)	Apple	[26]
22.	*Bacillus subtilis*	Brown rot *(Lasiodiplodia theobromae)*	Apricot	[26]
23.	*Bacillus subtilis*	Alternaria rot (*Alternaria alternata* (Fr.) Keissler)	Litchi	[18]
24.	*Bacillus subtilis*	Graymold (*Botrytis cinerea*)	Strawberry	[45]
25.	Bacillus subtilis	Alternaria rot (*Alternaria alternata*)	Litchi	[18]
26.	*Brevundimonas diminuta*	Anthracnose (*Colletotrichum gloeosporioide*s)	Mango	[20]
27.	*Burkholderia cepacia*	Anthracnose (*Colletotrichum musae*)	Banana	[7]
28.	*Pseudomonas syringae,* MA-4,	blue mold [*Penicillium expansum*] and graymold [*Botrytis cinerea*]	apples	[42]

Some microbial antagonist in vegetables

S. no.	Antagonists	Disease (pathogen)	vegetables	References
FUNGAL AND YEAST ANTAGONISTS				
29.	*Pichia guilliermondii*	Anthracnose *(Colletotrichum capsici)*	Chillies	[9]
30.	*Cryptococcus laurentii*	Gray mold *(Botrytis cinerea)*	Tomato	[40]
31.	*Pichia guilliermondii*	*Rhizopus* rot (*Rhizopusnigricans)*	Tomato	[44]
BACTERIAL ANTAGONISTS				
32.	*Pseudomonas putida*	Soft rot (*Erwinia carotovora carotovora)*	Potato	[5]
33.	*Pseudomonas aeruginosa*	Bacterial soft rot (*Erwinia carotovora* sub sp. *carotovora*	Cabbage	[5]
34.	*Bacillus subtilis*	Alternaria rot (*Alternaria alternata)*	Muskmelon	[41]

Importance of biological control

It reduces the use of chemical pesticides and their undesirable effects. It acts selectively on different pathogens. It is eco-friendly in nature and leaves no residue of poison in the soils and rivers. It does not lead to development or increase in disease intensity. Also, play a key role in integrated diseases resistance by the pathogen. The sustainability & durability of the effects makes it appealing. It is economic to use by the farmers, non-toxic to human, animals, birds and environment.

Limitations of biological control

It is specific control and usually not broad spectrum. It is difficult to maintain, requires expert supervision and occasional deleterious effects on non-target micro-organisms. It has more susceptible to environmental conditions, seasonal/ weather phenomena can make bio control agents less effective and also short shelf-life of formulation.

Mode of action of microbial antagonists

There are several modes of action that drive the mechanism of biocontrol activity of microbial antagonist. Competition for nutrient and space between the pathogen and the antagonist is considered as the major modes of action by which microbial agents control pathogens causing postharvest decay. In addition, production of antibiotics (antibiosis), direct parasitism, and possibly induced resistance are other modes of action of the microbial antagonists by which they suppress the activity of postharvest pathogens on fruits and vegetables [10].

Competition

Competition within and between species results in decrease in growth and activities of interacting organisms. Competition for nutrients and space between the pathogen and the antagonist is considered as the major mode of action by which microbial agents control pathogens causing postharvest decay in harvested fruits and vegetables.To compete successfully with pathogen at the wound site, the microbial antagonist should be better adapted to various environmental and nutritional conditions than the pathogen. (Barkai and Golan 2001). Competition for nutrients and space as the main mechanism of antagonistic action of *Candida tropicalis* YZ27 against anthracnose disease of banana (*Colletotrichum musae*). *C. tropicalis* YZ27 inhibit the germination and survival of *C. musae* spores. [46].

Competition for Space

Rapid colonization of fruit wound by the antagonist is critical for decay control, and manipulations leading to improved colonization enhance biocontrol. Thus, microbial antagonists should have the ability to grow more rapidly than the pathogen. Similarly, it should have the ability to survive even under conditions that are unfavourable to the pathogen [9]. The biocontrol activity of microbial antagonists with most harvested commodities increased with the increasing concentrations of antagonists and decreasing concentrations of pathogen (Table 2).

Table 2. Microbial antagonists with different concentrations against pathogens

S.no	Microbial antagonists (MA)	Concentration of MA	Diseases	References
1.	*Candida saitona*	10^7 CFU/ml	*Penicillium expansum* of apple	[32]
2.	*Pichia guilliermondii*	10^{10} CFU/ml	*Penicillium digitatum* of grapefruit & *Botrytis cinerea* of apples	[9]

Competition for nutrient

In vitro studies have demonstrated that microbial antagonists take up nutrients more rapidly than pathogens, get established and inhibit spore germination of the pathogens at the wound site (Table 3) [9]. Sour rot disease in citrus (*Geotrichum citri-aurantii*) was control by *Aureobasidium pullulans* strain ACBL-77. The addition of ammonium sulfate (1%) in the yeast culture stimulated biofilm production and increased the competition for nutrients between microorganisms against the disease (Klein *et al* .2017).

Table3. Microbial antagonists competing with pathogens for nutrients

S.no.	Microbial antagonists	Diseases	Nutrient depletion	References
1.	*Enterobacter cloacae*	*Rhizopus stolonifer* on peach	Iron	[38]
2.	*Cryptococcus laurentii*	*Botrytis cinerea* on apple	Iron	[38]
3.	*Pichia guilliermondii*	*Penicillium digitatum* on citrus	Iron	[9]
4.	*Metschnikowia pulcherrima* Pitt & Miller	*Botrytis cinerea* and *Penicillium expansum* on apple	Iron	[9]

Production of antibiotics

Antibiotics are secondary metabolites produced by microorganisms which inhibit the growth of other microorganisms called antibiosis. Production of antibiotics is the second important mechanism by which microbial antagonists suppress the pathogens of harvested fruits and vegetables (Table 4). Production of antifungal metabolites produced and including chitosanase by *Bacillus subtilis* V26 which suppress the fruit rot disease of tomato is caused by *Botrytis cinerea.* (Kilani-Feki *et. al.*, 2016).

Table 4. Some antibiotic compounds produced by different microbial antagonists to control pathogens.

S.no	Microbial Antagonists	Antibiotic	Pathogens	References
1.	*Pseudomonas cepacia*	Pyrrolnitrin	*Botrytis cinerea* & *Penicillium expansum* in apple	[16]
2.	*Bacillus subtilis, Pseudomonas cepacia*	Iturin	fungal rot in citrus	[11]
3.	*Pseudomonas syringae*	Syringomycin	green mold of citrus and gray mold of apple,	[9]

Direct parasitism

Parasitism occurs when the antagonist feeds on the pathogen, resulting in a direct destruction or lysis of fungal propagules and structures [38]. Lytic enzymes such as gluconase, chitinase, and proteinases were produced by Microbial antagonists that help in the cell wall degradation of the pathogenic fungi [3].

Induced resistance

Some biological agents induce a sustained change in the host plant, increase its tolerance to infection by a pathogen & this phenomenon is called Induced Systemic Resistance. *Cryptococcus saitona* induces chitinase activity and forms structural barrier (papillae) on host cell walls in apple against *Penicillium expansum* [10]; *Aureobasidium pullulans* caused an increase in the activity of 1,3-gluconase, peroxidase and chitinase enzymes in apple wounds which stimulated wound healing processes and induced defense mechanisms against *Penicillium expansum* [15]. Oligosaccharide fragments of yeast cell wall are known to be active elicitors of host defense responses (Base et al. 1992) (Table 6). The gray and blue mold disease of apple fruit caused by *Botrytis cinerea* and *Penicillium expansum* were controred by *Aureobasidium pullulans* and its ability to induce biochemical defense responses in apple tissue by increase activities of b-1,3-glucanase, chitinase, and peroxidase. In apple wounds, *A. pullulans* multiplied rapidly and controlled decay fruits [15]. Disease incidences of *Botrytis cinerea* and *Alternaria alternate* in cherry tomato were reduced by *C. laurentii* which can induce resistance by activating the expression of important defense-related genes, such as genes involved in salicylic acid (SA) and jasmonic acid (JA) signaling pathways and genes encoding pathogenesis related proteins, thus activating comprehensive defense reaction against pathogen invasion.

Table 6. Some microbial antagonists induce systemic resistance against pathogens.

S.no.	**Microbial Antagonists**	**Antibiotic**	**Pathogens**	**References**
1.	*Pseudomonas cepacia*	Pyrrolnitrin	*Botrytis cinerea & Penicillium expansum* in apple	[16]
2.	*Bacillus subtilis, Pseudomonas cepacia*	Iturin	fungal rot in citrus	[12]
3.	*Pseudomonas syringae*	Syringomycin	green mold of citrus and gray mold of apple,	[10]

Criteria for an ideal antagonist

It is non-pathogenic to the host and genetically stable and is generally effective at low concentrations. An ideal antagonist is capable of surviving under adverse environmental conditions. Moreover, it is effective against a wide range of pathogens and different harvested commodities.An ideal antagonist should be resistant to pesticides and must be able to be prepared in a form that can be effectively stored and dispensedand also should be compatible with other chemical and physical treatments. Some ideal antagonists have been commercially developed to manage postharvest diseases of fruits and vegetables (Table 7).

Table 7 Some commercially developed antagonists for postharvest diseases control of fruits and vegetables:

S.no.	Product	Microbial agent	Fruit/vegetables	Target disease(s)	Manufacturer /distributor
1.	Aspire	*Candida oleophila*strain 1–182	Apple, pear and citrus	Blue, gray, & green molds	Ecogen, Inc., USA
2.	Biosave 10LP, 110	*Pseudomonas syringae(*strain 10 LP, 110	Apple, pear, citrus, cherries &Potatoes	Blue & gray mold,*mucor*, & sour rot	Eco Science Corporation,USA
3.	Yield plus Botrytis,	*Cryptococcus albidus*	Pome fruit	*Penicillium, Mucor*	South Africa
4.	Serenade	*Bacillus subtilis*	Apple, pear, grapes and vegetables	Powdery mildew, late blight, brown rot and fire blight	Agro Quess Inc., USA
5.	Rhio-plus	*Bacillus subtilis*FZB 24	Potatoes and other vegetables	Powdery mildew and root rots	KFZB Biotechnick, Germany
6.	Messenger	*Erwinia amylovora* (Burrill) Winslow et al.	Vegetables	Fire blight	EDEN Bioscience Corporation, USA
7.	Blight Ban A 506	*Pseudomonas fluorescence* A 506	Apple, pear, strawberries and potatoes	Fire blight and soft rots	Nu Farm, Inc., USA

How to enhance bio efficacy of microbial antagonists

Microbial antagonists when applied alone usually do not bring about 100% controls of postharvest diseases of fruits and vegetables. To increase their effectiveness, and to enhance their bio-efficacy, following approaches have been useful:

1. manipulations in the physical and or chemical environment during storage
2. use of mixed cultures
3. addition of low doses of fungicides in microbial cultures
4. addition of salt additives in microbial cultures
5. addition of nutrients in microbial cultures
6. microbial cultures in association with physical treatments

Manipulations in the physical and or chemical environment during storage

Fruits and vegetables are usually stored at pre-determined temperature relative humidity and in gas combinations for varying periods with the primary objective of maintaining the quality to meet the market demands. Fruits and vegetables are often treated and/or handled in water before, during, and after the storage which provide an excellent opportunity to modify the environment (Table 8).

Table 8. Microbial antagonists and manipulation in the physical and or chemical environment during storage

S.no.	Combination	Diseases controlled	References
1.	*Candida sake*(2 x 10^6 CFU/ml) + ammonium molybdate (5 mM/l) at 20 °C for 7 days and at 1 °C for 60 days	*Penicillium expansum, Botrytis cinerea* & *Rhizopus stolonifer* in apples	[28]
2.	*Pseudomonas syringae*+ low doses of thiobendazole or imazalil (250 µg/ml),	crown rot and anthracnose of banana	[39]
3.	*Candida saitona* + 2-deoxy-D-glucose	blue mold of apple and green mold of oranges	[10]

Use of mixed cultures

It is difficult to select an individual microbial strain with a broad spectrum of activity against major postharvest pathogens. Hence, compatible strains are needed to provide the necessary spectrum of activity for effective control of postharvest diseases (Table 9)([34].

Application of mixtures of microbial antagonists has certain advantages:

1. Widening the spectrum of microbial activity results in the control of two or more postharvest diseases.
2. Enhancing the efficiency and reliability of biocontrol as the components of the mixtures act through different mechanisms like antagonism, parasitism, and induction of resistance in the host.
3. Combination of different biocontrol traits without the transfer of alien genes through genetic transformation

Table9. Application of mixtures of microbial antagonists with enhanced antagonistic character to suppress postharvest pathogens.

S.no.	Mixed culture	Pathogen	References
1.	*Pseudomonas syringae* + *Sporobolomyces roseus* (yeast)	*Penicillium expansum* in apple,	[17]
2.	*Aureobasidium pullulans* (10^6 CFU/ml) + *Bacillus subtilis*(10^8 CFU/ml),	*Penicillium expansum* and *Botrytis cinerea citrus*	[22]
3.	*Candida sake*CPA-1(2×10^7 CFU/ml) + *Pantoeaagglomerans* (2×10^7 CFU/ml)	blue mold rot on 'Golden Delicious'apples	[29]
4.	*Metschnikowia pulcherrima* + *Cryptococcus laurentii*	blue mold (*P. expansum*) on citrus	[17]

Addition of low doses of fungicides in microbial cultures

Compatibility between a microbial antagonist and a synthetic fungicide offers the option of using the antagonists in combination with reduced level of the fungicide (Table 10). This approach can be successfully added.

Table10. Application of low dose of chemicals with microbial antagonists to manage different postharvest pathogens.

S.No.	Biological agents and fungicide	Pathogens	References
1.	Cypronidil (20 ppm) and *Pseudomonas syringae* (3×10^7 CFU/ml)	blue and gray mold rots on apples	[42]
2.	*Candida oleophila* + thiobendazole	fruit decay in citrus	[9]
3.	*Cryptococcus laurentii* + imazalil (25 ppm)	storage rots of jujube	[32]
4.	*Pseudomonas syringae* + cypronidil	*Penicillium expansum* on apples, and pear	[11], [37]

Addition of salt additives in microbial cultures

The effectiveness of microbial antagonists depends upon the concentration of the antagonist, concentration of salt additive(s), their mutual compatibility and duration and time at which they are applied. Usually, the cultures should be applied well before the initiation of infection process (Barkai-Golan, 2001) (Table 11).

Table 11. Addition of salt additives with microbial antagonists to enhance the efficacy and manage different postharvest pathogens.

S. no.	Bio agents	Salt	Diseases	References
1.	*Cryptococcus laurentii*	Sodium carbonate	Blue mold of apple	[17]
2.	*Pseudomonas syringae*	Calcium chloride	Blue mold of oranges	[17]
3.	*Cryptococcus laurentii*	Sodium bicarbonate	Botrytis rot of tomato	[40]
4.	*Bacillus subtilis*Strain B34	Sodium bicarbonate +Aloe verage	Anthracnose Disease of papaya	[15]
5.	*Pichiamembranaefaciens*	Ammonium molybdate	Brown rot of Cherry	[29]

Addition of nutrients in microbial cultures

The efficacy of the microbial antagonists can also be enhanced considerably by the addition of some nutritious compounds or natural plant products. For example, additions of nitrogenous compounds like L-aspargine and L-proline, and 2-deoxy-D-glucose, a sugar analog helped in enhancing the bio-efficacy of microbial antagonists in controlling the postharvest decay rots in some fruits and vegetables [17]. The combination of *Candida saitona* and 2-deoxy-D-glucose (0.2%) controlled fruit decay on apples, oranges and lemons caused by *Botrytis cinerea*, *Penicillium expansum*, and *Penicillium digitatum* [10]. Nutrients that promoted the growth of the yeasts were applied with the antagonist to wounded fruits in cold storage. The most effective mixtures of the $CaCl_2$ with the two antagonist *Vishniacozyma victoriae* and *Pichiamembrani faciens* yeasts inhibited the growth of *Penicillium expansum* and *Botrytis cinerea*, the causal agents of blue and grey mold of pear fruits.

Microbial cultures in association with physical treatments

Integration of microbial antagonists with physical methods such as curing or heat treatments could enhance the bio-efficacy of microbial antagonists. Anthracnose disease of mango caused by *Colletotrichum gloeosporioides* is control by HW + UV-C + ES-MCO treatments which showed to suppress anthracnose disease and senescence of mango fruit was delayed during cold storage.

Table 12. Microbial cultures in association with physical treatment to manage different postharvest pathogens.

S. No.	Microbial antagonist	Physical treatment	Disease controlled	Reference
1.	*Candida oleophila*	Hot water at 55 °C for 10 seconds	Post-harvest diseases of peaches	[19]
2.	*Pseudomonas syringae*	Heat treatment	Green mould of apple	[17]
3.	*Bacillus subtilis*	Hot water treatment	Green (*P. digitatum*) and blue mold (*P. italicum*) of citrus	[28]
4.	Yeast	Fruit curing	*Botrytis cinerea* of kiwifruit	[6]

Application methods for microbial antagonists

In general, microbial antagonists are applied by two different ways i.e., preharvest application, and postharvest application.

Pre-harvest application

Pre-harvest application is to pre-colonize the fruit surface with an antagonist immediately before harvest so that wounds inflicted during harvesting can be colonized by the antagonist before colonization by a pathogen [15]. For example, pre-harvest application of *B. subtilis* under field conditions results in colonization of the apple fruit surface by the microbial antagonist, controls *Penicillium expansum* and *Botrytis cinerea* of apples [22].

Post-harvest application

This approach has been more effective than preharvest application of microbial antagonists, and has several successes. For instance, Direct contact of microbial antagonist and infested fruit peel has been quite useful for the suppression of pathogens like *Penicillium digitatum, Penicillium italicum* in citrus, *Botrytis cinerea*in apples [13]

CASE STUDIES

Antagonistic Yeasts for Biocontrol of the Banana Postharvest Anthracnose Pathogen *Colletotrichum musae* [46]

Postharvest anthracnose, caused by *Colletotrichum musae* (Berk. and M.A. Curtis) Arx, is the most important disease of harvested banana. This study was aimed at elaborating the effect of antagonistic strains of yeast, *Candida tropicalis* on the control of *C. musae*, the anthracnose-causing phytopathogen on banana cv. Martaman.

The lesion diameter of *C. musae*-inoculated wound site on banana fruits treated with *C. tropicalis* YZ27 at all tested concentrations except 1 $\times 10^5$ and 1 $\times 10^6$ CFU/ml was significantly reduced compared with those of the control fruits. The results show that better disease control was obtained at higher concentrations of the antagonist yeast. The lesion diameter progressively reduced upon increasing the concentration of *C. tropicalis* from 1 $\times 10^5$ to 1 $\times 10^{10}$ CFU/ml. (Fig.1

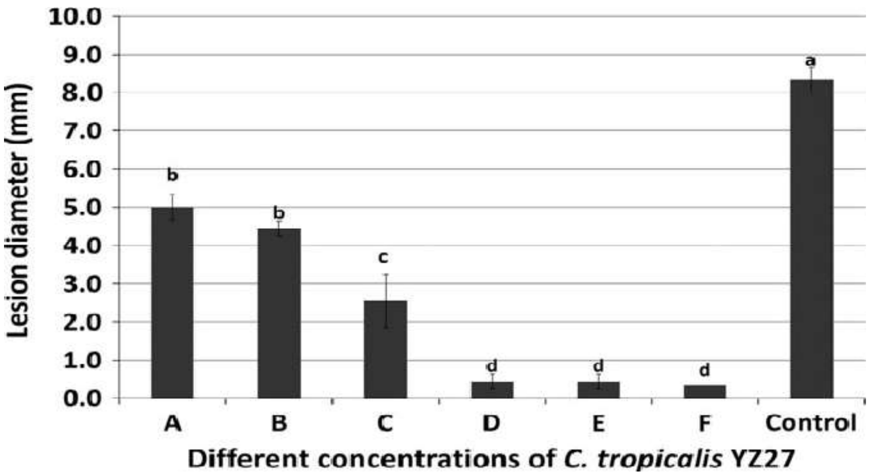

Fig. 1.Anthracnose lesion diameter on inoculated banana fruits at 4 days after application of *Candida tropicalis* strain YZ27 at different concentrations.In *in vivo*study, Anthracnose lesion diameter on artificially pathogen inoculated banana fruits at 4 days after application of *Candida tropicalis*strainYZ27 at different concentrations at 28 ± 1°C. The letters A, B, C, D, E and F represent the concentrations of *C. tropicalis*YZ 27 at 1 $\times 10^5$,1 $\times 10^6$, 1 $\times 10^7$, 1 $\times 10^8$, 1 $\times 10^9$, and 1 $\times 10^{10}$, CFU/ml [46].

Enhanced control of postharvest citrus fruit decay by means of the combined use of compatible biocontrol agents [31]

Postharvest green mold of citrus caused by *Penicillium digitatum*(Pers.) Sacc. is responsible for serious economic losses during harvest, transportation and storage.This study was aimedto determine the ability of mixtures of *Pseudomonas syringae* and *Trichoderma* spp. to improve their biocontrol activity against *Penicillium digitatum* on orange and lemon.

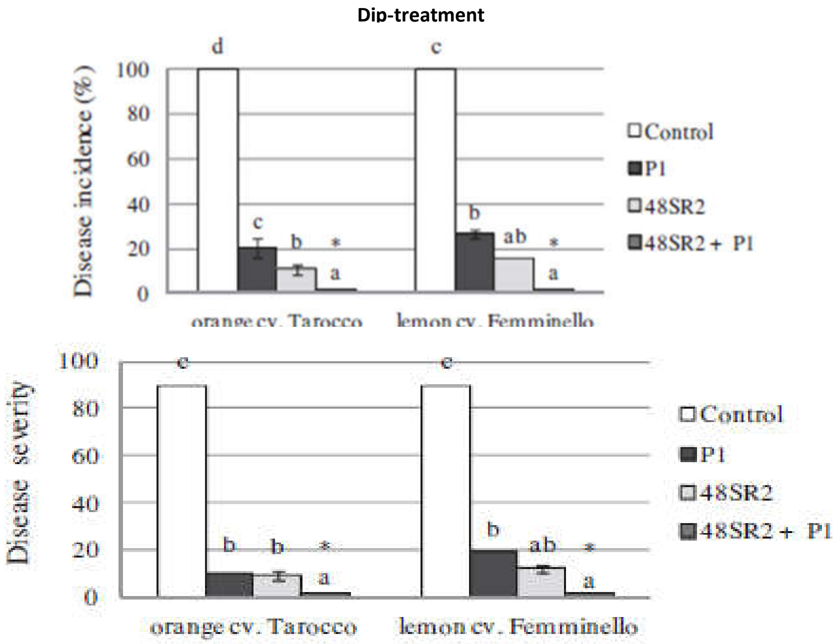

Fig. 2.Disease incidence and severity on 'Tarocco' orange and 'Femminello' lemon fruits treated with *P. syringae* 48SR2 and *T. atroviride* P1, alone and in mixture. Fruits were submerged for 2 min. in aqueous suspensions of each biological control agent and inoculated with *P. digitatum*(Green mold) 72 h later. Values of disease incidence and severity were determined with respect to controls after 5 days of incubation at 20 °C. For each host, mean data (±SEM) followed by the same letter in column indicate no difference among the treatments according to Fisher's least significant difference (LSD) test at *P*=0.01. Asterisk indicates synergistic activity was present according to Limpel's formula [31].

P. syringae 48SR2 cells and *T. atroviride* P1 conidia applied alone by dip-treatments significantly reduced disease incidence and severity on orange and lemon fruits compared to the control in addition, their mixture significantly and strongly reduced green mould incidence and severity on orange and lemon, synergistically improving the level of disease suppression compared to *Pseudomonas* and *Trichoderma* strains applied alone. Bacterial and fungal antagonistic strains controlled the disease development on citrus fruits more effectively in dip-treatments in comparison to wound inoculation, whereas the efficacy of the mixture was not significantly different as shown in fig. 2.

Effects of the yeast *Pichia guilliermondii* against *Rhizopus nigricans* on tomato fruit [44]

Antagonists have been isolated and found to have biocontrol efficacy against postharvest fruit diseases. At present, there are numerous suggestions regarding the change in defensive enzymes that correlate with the process of plant resistance. This suggests that the activation of host defence may be a mechanism in disease control [38]. Ippolito *et al.* [15] used postharvest apples as material, and examined peroxidase (POD), chitinase (CHI) andβ1, 3-glucanase (EC) activities induced by the antagonist *Aureobasidium pullulans* during storage found that CHI and β1, 3-glucanase activities of postharvest grapes were increased when spraying the suspension of the antagonistic yeast *Candida oleophila* onto the surface of grapes. In China, induced host resistance in response to the yeast *Cryptococcus laurentii* has been observed. Significant changes in PAL, CHI and β1,3-glucanase activities were found to be involved in the action of plant resistance (Tian *et. al.*, 2007).The objectives of this study were to examine:

(i)The inhibitory effect of *Pichia guilliermondii* against *Rhizopus nigricans* on tomato fruit

(ii) The influence of *Pichia guilliermondii* inoculation on Phenylalanine ammonia lyase (PALs), chitinase (CHI) and β-1,3-glucanase activity on tomato fruit.

Efficacy of *P. guilliermondii* in controlling Rhizopus rot

The experiment showed that different *P. guilliermondii* treatments had different influences on lesion development in tomato fruit. After 2 days of incubation at 20 °C, A and B both showed an inhibitory effect on the development of lesions, and the lesion diameters of tomato fruit treated with A and B were approximately 6 mm, significantly smaller than the 10.67mm of CK . (Fig. 3)

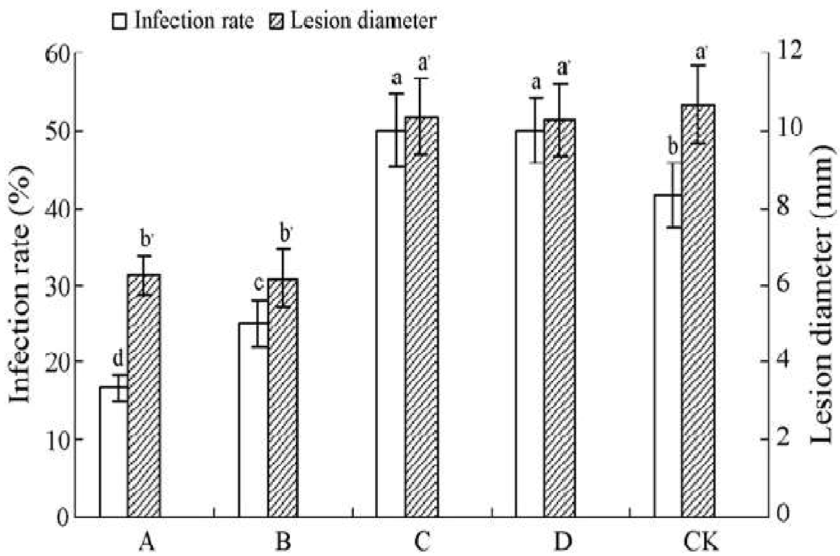

Fig. 3.Effects of different treatments of *P. guilliermondii* on the infection rate and lesion development of *Rhizopus* rot on tomato fruit (A: 1×10^{8} CFU mL^{-1} washed cell suspension; B: 1×10^{8}CFU mL^{-1} unwashed cell culture mixture; C: autoclaved culture; D: culture filtrate and CK: sterile distilled water). Vertical bars represent standard deviations of the means. Means followed by different letters are significantly different according to Duncan's multiple range test *P*=0.05 [44].

Effect of *P. guilliermondii* on enzyme activities

Tomato fruit were capable of responding to the yeast *P. guilliermondii*, which induced strong disease resistance. The activities of PAL, CHI and -1,3- glucanase were all correlated with the onset of induced resistance. (Fig 4)

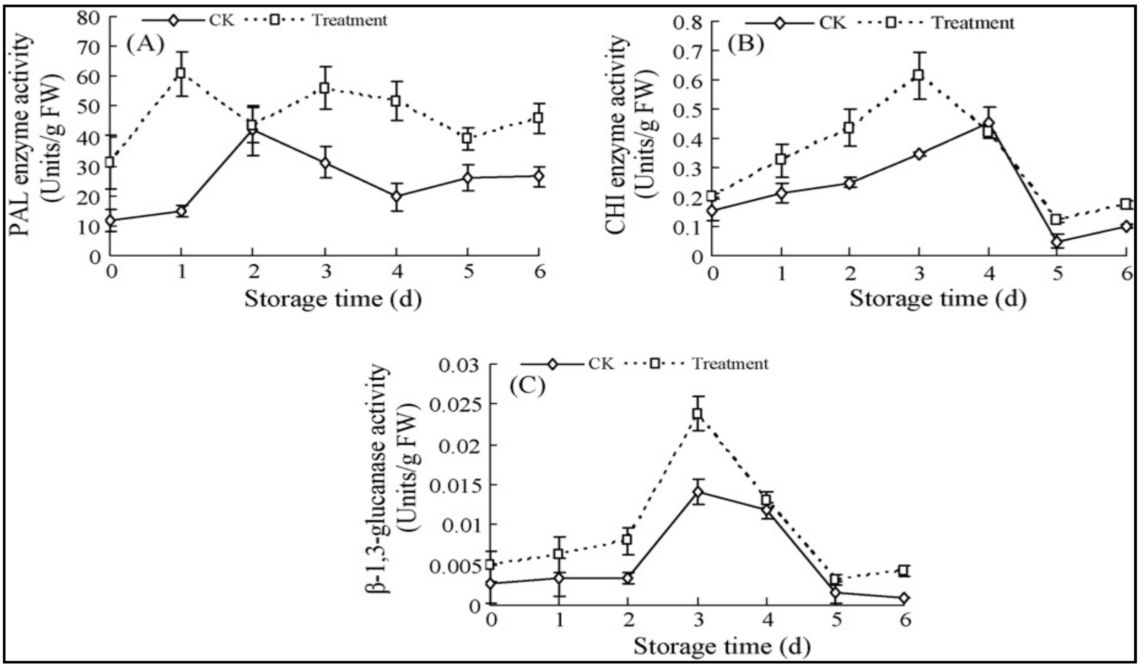

Fig. 4.Effects of the antagonistic yeast *P. guilliermondii*on Phenylalanine ammonia lyase (PALs) (A) chitinase (B) and β-1,3-glucanase (C) activities in tomato fruit at 20 °C. Vertical bars indicate standard deviations of the means [44].

Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose *(Colletotrichum capsici)* [4].

Anthracnose caused by *Colletotrichum capsici* is a major disease of tropical vegetables such as chilli (*Capsicum annuum* L. var. *Acuminatum* Fingerh.) [14],[30]. Ripe fruit-rot is more conspicuous as it causes severe damage to mature fruits in the field as well as during transit and storage. Under favourable conditions, the disease damaged up to 50% of the fruits [36]. In this study the antagonistic capabilities of epiphytic yeast strains from fruits and vegetables were isolated and identified and capabilities against anthracnose disease of chilli caused by *Colletotrichum capsici* were investigated for postharvest preservation.

In order of their efficacy *P. guilliermondii* strain R13 showed effectiveness in reducing disease incidence on C. *capsici* infected chilli fruits to as low as 6.5% in comparison to control. The application of *P. guilliermondii* is more active for preserving chilli than conventional preservation with chlorinated water (Table:13).

Table:13 The efficiency of the four antagonistic yeast strains in reduction of disease(anthracnose) incidence in *Colletotrichum capsici* infected chilli fruites.

Yeast isolate	Disease incidence [a] (%)	Biocontorl efficacy[a] (%)	Lesion diameter[b](mm)
*Pichiaguilliermondii*R13	6.7 ± 0.40a	93.3 ± 0.04a	6.7 ± 0.23a
Candida musae R6	16.9 ± 0.87b	83.1 ± 0.87b	7.8 ± 0.17b
*Issatchenkiaorientalis*ER1	23.4 ± 0.92c	76.6 ± 0.92c	9.1 ± 0.28c
*Candida quercitrusa*L2	33.6 ± 0.52d	66.4 ± 0.52d	10.3 ± 0.35d
Control	100 ± 0.00e	0.0 ± 0.00e	15.4 ± 0.40e

[a] % Disease incidence = (A/T)x100 and % Biocontrol efficacy = [(T- A)/T]x100, where T is the number of infected wounds of chilli fruits inoculated with *Colletotrichum capsici* only (control), and A is the number of infected wounds of chilli fruits inoculated with both yeast

antagonists and *C. capsici*, The results are presented as mean of three independent experiments ± standard error. Values of each column followed by a different letter indicates significant difference (P< 0.05) according to LSD test.[b] Lesion diameter is the average length of lesion in x-axis and y-axis.

SUMMARY

Biological control involves the use of microbial antagonists such as bacteria, yeast or fungi to suppress postharvest disease of fruits and vegetables. They have several importance and advantages over chemical control methods and their mode of action includes antibiosis, competition, parasitism and induced systemic resistance. There are, however, some limitations to the general use of biological control agents are variability in effectiveness, low spectrum action, short shelf life of products etc.Bio-agents including microbial antagonists bring disease suppression with no environmental hazards and seem to be the best alternative of chemicals to disease suppression specially postharvest diseases of fruits and vegetables.

FUTURE PROSPECTS

The research in the area of biocontrol is confined to the laboratory and very little attention has been paid to produce the commercial formulations of bio agents. Whatever has been commercially produced, has not been used efficiently by the farmers owing to the lack of information regarding its use. So, it is need to popularize the concept of biocontrol agent. Most of the biocontrol agents perform well in the laboratory but its fullest potential is not exploited in the field due to physiological and ecological constraints that limit the efficacy of the biocontrol agent. Genetic engineering can be effectively used to overcome this type of problem.

REFERENCES

1. Barkai-Golan R (2001). *Postharvest diseases of fruits and vegetables: development and control*. Elsevier. Biological Control 42 (3), 326–335.
2. Blum LE, Amarante CV, Valdebenito-Sanhueza, RM., Guimarães, LS, Dezanet A, & Hack-Neto P (2004). Cryptococcus laurentii aplicadoempós-colheitareduz podridõesemmaçãs. *Fitopatologia Brasileira*, *29*(4), 433-436.
3. Bonaterra, A., Mari, M., Casalini, L., &Montesinos, E. (2003).Biological control of Monilinialaxa and Rhizopusstolonifer in postharvest of stone fruit by Pantoeaagglomerans EPS125 and putative mechanisms of antagonism. International journal of food microbiology, 84(1), 93-104
4. Chanchaichaovivat A, Ruenwongsa P, Panijpan, B, 2007. Screening and identification of yeast strains from fruit and vegetables: potential for
5. Colyer PD (1983).Bacterization of potatoes with Pseudomonas putida and its influence on postharvest soft rot diseases.
6. Cook DWM, Long PG, & Ganesh S, (1999).The combined effect of delayed application of yeast biocontrol agents and fruit curing for the inhibition of the postharvest pathogen Botrytis cinerea in kiwifruit. *Postharvest Biology and Technology*, *16*(3), 233-243.
7. De Costa, DM.,&Erabadupitiya, HRUT(2005). An integrated method to control postharvest diseases of banana using a member of the Burkholderiacepacia complex. Postharvest Biology and Technology, 36(1), 31-39.
8. Devi AN, &Arumugam T, (2005).Studies on the shelf life and quality of Rasthali banana as affected by postharvest treatments. *Orissa J. Hortic*, *33*(2), 3-6.
9. Droby S, Chalutz E, & Wilson CL, (1991).Antagonistic microorganisms as biological control agents of postharvest diseases of fruits and vegetables. *Postharvest News and Information*, *2*(3), 169-173.
10. El Ghaouth A, Wilson C, & Wisniewski M, (2004).Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases of fruit and vegetables. In *Diseases of Fruits and Vegetables: Volume II* (pp. 511-535). Springer, Dordrecht.
11. Errampalli, D., &Brubacher, N R. (2006).Biological and integrated control of postharvest blue mold (Penicilliumexpansum) of apples by Pseudomonas syringae and cyprodinil. Biological Control, 36(1), 49-56.
12. Gamagae SU, Sivakumar D, Wijeratnam RW, &Wijesundera RLC, (2003).Use of sodium bicarbonate and Candida oleophila to control anthracnose in papaya during storage. *Crop Protection*, *22*(5), 775-779
13. Gullino ML, Benzi DAMC, Testoni A, & Garibaldi A, (1992).Biological control ofBotrytisrot of apple. *Recent advances inBotrytisresearch*, 197.

14. Hadden, J.F., Black, L.L., 1989. Anthracnose of pepper caused by *Colletotrichum*spp.. In: Green, S.K., Griggs, T.D., Mclean, B.T. (Eds.), Tomato and Pepper Production in the Tropics. AVRDC.Shauhua, Taiwan, pp. 189–191.
15. Ippolito A, &Nigro F, (2000).Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop protection*, *19*(8-10), 715-723.
16. Janisiewicz W, Yourman L, Roitman J, & Mahoney N, (1991).Postharvest control of blue mold and graymold of apples and pears by dip treatment with pyrrolnitrin, a metabolite of Pseudomonas cepacia. *Plant disease (USA)*.
17. Janisiewicz, WJ.,Saftner, RA., Conway, WS., & Yoder, KS. (2008). Control of blue mold decay of apple during commercial controlled atmosphere storage with yeast antagonists and sodium bicarbonate. Postharvest Biology and Technology, 49(3), 374-378.
18. Jiang, Y. M., Zhu, X. R., & Li, Y. B. (2001).Postharvest control of litchi fruit rot by Bacillus subtilis. LWT-Food Science and Technology, 34(7), 430-436.
19. Karabulut, OA.,Arslan, U., Ilhan, K., &Kuruoglu, G. (2005). Integrated control of postharvest diseases of sweet cherry with yeast antagonists and sodium bicarbonate applications within a hydrocooler.Postharvest biology and technology, 37(2), 135-141.
20. Kefialew, Y., &Ayalew, A. (2008).Postharvest biological control of anthracnose (Colletotrichum gloeosporioides) on mango (Mangiferaindica). Postharvest Biology and Technology, 50(1), 8-11.
21. Lassois, L., de Bellaire, LDL.,&Jijakli, MH. (2008). Biological control of crown rot of bananas with Pichiaanomala strain K and Candida oleophila strain O. Biological Control, 45(3), 410-418.
22. Leibinger W, Breuker B, Hahn M, &Mendgen K, (1997).Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. *Phytopathology*, *87*(11), 1103-1110.
23. Long, CA., Deng, BX., Deng, XX., (2006). Pilot testing of Kloeckeraapiculata for thebiological control of postharvest diseases of citrus. Annals of Microbiology 56(1), 13–17.
24. Long, CA., Deng, BX., Deng, XX., (2007). Commercial testing of Kloeckeraapiculata,isolate 34–9, for biological control of postharvest diseases of citrus fruit. Annalsof Microbiology 57 (2), 203–207.
25. Mari M, Neri F, &Bertolini P, (2007). Novel approaches to prevent and control postharvest diseases of fruits. *Stewart Postharvest Review*, *3*(6), 1-7.
26. Mikani, A., Etebarian, H.R., Sholberg, P.L., Gorman, D.T., Stokes, S., Alizadeh, A., 2008.Biological control of apple graymold caused by Botrytis mali with Pseudomonas fluorescens strains. Postharvest Biology and Technology 48 (1), 107–112.
27. Morales, H., Sanchis, V., Usall, J., Ramos, A.J., Marín, S., 2008.Effect of biocontrolagents Candida sake and Pantoeaagglomerans on Penicilliumexpansum growthand patulin accumulation in apples. International Journal of Food Microbiology, 122 (1–2), 61–67.
28. Nunes C, Usall J, Teixidó N, &Vinas I, (2002).Improvement of Candida sake biocontrol activity against post-harvest decay by the addition of ammonium molybdate. *Journal of applied microbiology*, *92*(5), 927-935.
29. Nunes, C., Usall, J., Teixidó, N., Fons, E., &Viñas, I. (2002).Post-harvest biological control by Pantoea agglomerans (CPA-2) on Golden Delicious apples. Journal of Applied Microbiology, 92(2), 247-255.
30. Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J., Mongkolporn, O., 2005.Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in Capsicum.Plant Breed. 124, 206–208.
31. Panebianco S, Vitale A, Polizzi G, Scala F, &Cirvilleri G, (2015). Enhanced control of postharvest citrus fruit decay by means of the combined use of compatible biocontrol agents. *Biological control*, *84*, 19-27.
32. Qin, GZ.,Tian, SP., Xu, Y., Chan, ZL., & Li, BQ. (2006). Combination of antagonistic yeasts with two food additives for control of brown rot caused by Moniliniafructicola on sweet cherry fruit. Journal of applied microbiology, 100(3), 508-515.
33. Schena, L., Nigro, F., Pentimone, I., Ligorio, A., &Ippolito, A. (2003).Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of Aureobasidiumpullulans. Postharvest Biology and Technology, 30(3), 209-220.
34. Sharma RR, Singh D, & Singh R, (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological control*, *50*(3), 205-221.
35. Smilanick JL, & Denis-Arrue R, (1992).Control of green mold of lemons with Pseudomonas species. *Plant disease*, *76*(5), 481-485.
36. Smith, K.W., Crasson, D.F., 1959. The taxonomy, etiology and control of *Colletotrichum piperatum* (E and E) and *C. capsici* (Synd) (BB).Plant Dis. Rep. 42, 1099.
37. Utkhede RS, &Sholberg PL, (1986).In vitro inhibition of plant pathogens by Bacillus subtilis and Enterobacteraerogenes and in vivo control of two postharvest cherry diseases. *Canadian journal of microbiology*, *32*(12), 963-967.

38. Wisniewski M, Wilson C, & Hershberger W, (1989).Characterization of inhibition of Rhizopusstolonifer germination and growth by Enterobacter cloacae. *Canadian Journal of Botany*, *67*(8), 2317-2323.
39. Wittig, HPP., Johnson, KB&Pscheidt, JW.(1997). Effect of epiphytic fungi on brown rot blossom blight and latent infections in sweet cherry. Plant Disease, 81(4), 383-387.
40. Xi L,&Tian S (2005). Control of postharvest diseases of tomato fruit by combining antagonistic yeast with sodium bicarbonate. *Zhongguonongyekexue*, *38*(5), 950-955.
41. Yang, DMBi, Y., Chen, XR.,Ge, YH., & Zhao, J. (2006, August). Biological control of postharvest diseases with Bacillus subtilis (B1 strain) on muskmelons (Cucumismelo L. cv. Yindi).In IV International Conference on Managing Quality in Chains-The Integrated View on Fruits and Vegetables Quality 712 (pp. 735-740).
42. Zhang H MaL, Wang L, Jiang S, Dong Y, & Zheng X, (2008). Biocontrol of graymold decay in peach fruit by integration of antagonistic yeast with salicylic acid and their effects on postharvest quality parameters. *Biological control*, *47*(1), 60-65.
43. Zhang, H., Zheng, X., & Yu, T. (2007).Biological control of postharvest diseases of peach with Cryptococcus laurentii. Food Control, 18(4), 287-291.
44. Zhao YTuK, Shao X, Jing W, & Su Z, (2008).Effects of the yeast Pichia guilliermondii against Rhizopusnigricans on tomato fruit. *Postharvest Biology and Technology*, *49*(1), 113-120.
45. Zhao, Y., Shao, X. F., Tu, K., & Chen, J. K. (2007).Inhibitory effect of Bacillus subtilis B10 on the diseases of postharvest strawberry. Journal of Fruit Science, 24(3), 339-343.
46. Zhimo VY, Dilip D, Sten J, Ravat VK, Bhutia DD, Panja B, &Saha J, (2017). Antagonistic Yeasts for Biocontrol of the banana postharvest anthracnose pathogen Colletotrichum musae. *Journal of Phytopathology*, *165*(1), 35-43.