INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

Review Article

APPLICATION OF ANTAGONISTIC MICROORGANISMS FOR THE CONTROL OF POSTHARVEST DECAYS IN FRUITS AND VEGETABLES

[a,b]Ernest Ekow **ABANO** & [b]Livingston Kobina **SAM-AMOAH**
[a]Agricultural Engineering Department, University of Cape Coast, Cape Coast, Ghana
[b]School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China

ABSTRACT

Over the years, synthetic fungicides are primarily used to control postharvest decays of fruits and vegetables because of their effectiveness and low cost. Nevertheless, the health and safety of synthetic fungicides as a biological control agent in delicate foods like fruits and vegetables as well its impact on the natural environment has created legal and consumer concerns. This however, has called for a safer and more environmentally friendly alternative for the control of postharvest decays in fruits and vegetables. The application of antagonistic microorganisms as an alternative to solve the challenges in the control of postharvest diseases in fruits and vegetables is becoming increasingly popular worldwide. The mechanism(s) by which microbial antagonists suppress the postharvest diseases is still unknown, but competition for nutrients and space, production of antibodies, direct parasitism, and induced resistance are most widely accepted mechanisms of their action. Microbial antagonists are applied either before or after harvest, but postharvest applications are more effective than pre-harvest applications. The new paradigm shift proposed in the biocontrol research includes the integration of low risk chemical fungicides, natural anti-microbial substances, and physical means such as hot water treatment, irradiation with ultraviolet light, microwave, and infrared treatment in the bio-control process; the enhancement in the expression of crucial biocontrol genes and/or combining genes from different biocontrol agents in the mass production, formulation and storage, or in response to exposure and contact with host plant tissue after application; the use of genetically modified organisms as biocontrol agents; and the research towards discovering new antagonists instead of the ones currently used in practice. This review critically assesses the use of wide array of antagonistic microorganisms to control postharvest decays of fruits and vegetables. Particularly, the efficacy of the technology, the effect of the method on the quality of the fruits and vegetables, and the new paradigm shift proposed after 20 years of biocontrol research. The major obstacles that have influenced the commercialization of biocontrol products have also been highlighted.

KEYWORDS: antagonistic microorganisms, fruits and vegetables, postharvest decays.

INTRODUCTION

A Postharvest decay of fruits and vegetables is a major challenge throughout the world. The degree of postharvest loss through decay is well documented. In the industrialized countries, it is estimated that about 20– 25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling (Sharma *et al*, 2009; Singh and Sharma, 2007, Droby, 2006; Zhu, 2006; El-Ghaouth *et al*., 2004). The situation is far more exasperating in the developing countries, where postharvest decays are often times over 35% , due to inadequate storage, processing and transportation facilities (Abano and Sam-Amoah, 2011). The use of synthetic fungicides such as benomyl and iprodione to control postharvest diseases of fruits and vegetables are well known in scientific literature (Zhang *et al.*, 2007; Singh and Sharma, 2007; Korsten, 2006; Zhu, 2006; El-Ghaouth *et al*., 2004; Fan *et al*., 2000). However, the health and environmental concerns associated with the continuous use of synthetic fungicides have alarmed legal enforcers and consumers to demand green technology and quality products from the food industry as well as the scientific community. This called for a new paradigm shift from the use of synthetic fungicides to a safer and environmentally friendly alternative for reducing the postharvest decay in fruits and vegetables (Mari *et al*., 2007; Ragsdale & Sisler, 1994). Among the replete of bio-control approaches, the use of the microbial antagonists like yeasts, fungi, and bacteria is quite promising and gaining popularity (Sharma *et al.*, 2009; Zhang *et al.*, 2007; Droby, 2006; Korsten, 2006; Zhang *et al*, 2005; Janisiewicz & Korsten, 2002; Roberts, 1990; Droby *et al*., 1991; Wisniewski and Wilson, 1992). The objective of this review was to critically assesses the use of microbial antagonists for controlling postharvest diseases of fruits and vegetables with particular focus on their efficacy, the influence of it application on the quality of fruits and vegetables, and the new paradigm shift proposed after 20 years of biocontrol research.

Basic approaches for using the microbial antagonists

Two basic approaches have been reported for using the microbial antagonists against the postharvest diseases of fruits and vegetables (Sharma *et al.*, 2009). These are the use of microorganisms which already exist on the produce itself, which can be promoted and managed, and those that can be artificially introduced against postharvest pathogens.

Natural microbial antagonists

The antagonistic microorganisms that are naturally present on the surfaces of fruits and vegetables, which when after been isolated are used for the control of postharvest diseases are called naturally occurring antagonists (Janisiewicz, 1987; Sobiczewski *et al*., 1996). Investigation by Chalutz and Wilson (1990) revealed that when the concentrated washings from the surface of citrus fruit were plated out on agar medium, only bacteria and yeast appeared while after dilution of these washings, several rot fungi appeared on the agar, indicating that yeast and bacteria may be suppressing fungal growth. They therefore concluded that washed fruits and vegetables are more predisposed to postharvest decays than the unwashed ones.

Recent microbial antagonist product development

A review by Droby *et al* (2009) has well documented commercial antagonistic microorganisms available in the global market for postharvest control of decays in fruits and vegetable. These are Biosave (*Pseudomonas syringae* Van Hall), which are registered in the USA and used mostly for the control of sweet potato and potato diseases (Stockwell and Stack, 2007), and "Shemer" (*Metschnikowia fructicola* Kurtzman & Droby) registered in Israel and used commercially for the control of sweet potato and carrot storage diseases (Kurtzman and Droby, 2001; Blachinsky *et al*., 2007). The two yeast-based products, AspireTM (Ecogen, US) and Yield Plus (Anchor Yeast, South Africa) developed in the USA and South Africa is no longer available (Droby *et al*, 2009). Currently, BioNext (Belgium) and Leasaffre International (France) have developed a commercial product, based on the same yeast used in AspireTM, *Candida oleophila*. A similar yeast-based product, *Candida saitoana* was developed by Neova Technologies (Abbotsford, British Columbia, Canada). Additionally, Spain has also developed a commercial formulation of *Candida sake* for use on pome fruit under the name "Candifruit".

Characteristic traits of an Ideal microbial antagonist

Several reviews have provided the good characteristic traits desired in microbial antagonist in the disease controlling process (Droby *et al*., 2009; Sharma *et al*., 2009). Wilson and Wisniewski (1989) recommended a guideline to select an ideal antagonist, which are as follows:

1. Must be stable
2. Should be effective at low concentrations
3. Must not be demanding in terms of required nutrients
4. Must be able to survive under adverse environmental conditions
5. Should be effective against a wide spectrum of commodities and pathogens under different conditions
6. Should be amenable to production on inexpensive growth media
7. Should be amendable to formulations with a long shelf life
8. Should be easy to dispense without being hazardous to human health
9. Must be resistant to chemical used in the postharvest environment
10. Must be environmentally friendly
11. Must be compatible with commercial processing practices.
12. Should not be detrimental to the quality of the fruits and vegetables it preserves.

Isolation and selection of antagonistic microorganisms

Isolation of the antagonistic microorganisms is an important step in the bio-control processes of postharvest decays in fruits and vegetables. Since antagonistic microbes are limited in commercial supplies in many part of the world, and the fact that various attempts to develop commercial products produced limited success (Droby *et al*., 2009), isolation prior to its bio-control activity is very critical. Typically, microorganisms are isolated from the leaves and the natural environment of the fruits and vegetables grown under organic conditions. In some instances antagonistic microorganisms are isolated from soil samples beneath the immediate vicinity of the fruits and vegetables. Zhang *et al* (2007) isolated antagonistic yeast *Cryptococus. Laurenti* (Kufferath) Skinner with nutrient yeast dextrose agar (NYDA) from the surfaces of pears harvested in an unsprayed orchard. In the isolation of the target yeast, 8g nutrient broth, 5g yeast extract, 10g glucose and 20g agar in a litre of distilled water was used and incubated at 28 ^{o}C for 20hrs.

In a similar experiment by Zhang *et al* (2008) the antagonistic *Rhodotorula glutinis* was isolated from the surfaces of strawberries harvested in an unsprayed orchard and identified by the VITEK 32 Automicrobeic system by a France company bioMérieux for the control of blue mold in pears. In another research, Manso and Nunes (2011) isolated epiphytic microorganisms from the surfaces of leaves from pome and citrus fruit picked from different orchards. They also used NYDA in same amount except the agar (15 g) and incubated at 25 ^{o}C for 24 hrs. The isolates were purified and maintained at 4±1 ^{o}C. In addition, *R. glutinis* and *C. laurentii* were isolated from the surface of apple fruit whereas *P. membranaefaciens* was isolated from the surface of peach (Tian *et al*., 2004; Xu, Qin and Tian, 2008). In many research, selection of the target microbial antagonist was based on the isolate with greater antagonism in the controlling process at a minimum effective concentration against the target pathogens. To select and develop a successful biocontrol agent, it is essential to evaluate its effectiveness under wide spectrum of pathogens and under different conditions typically used in practice.(Manso and Nunes, 2011). The relatively long time used and the expensiveness of the isolation, development, and commercialization of bio-control agents still remain a challenge (Droby *et al*., 2009, Blachinsky *et al*., 2007).

Pre-harvest and postharvest application of antagonistic microorganisms

Following the selection of the potential microbial antagonist, the next step is look for an application method effective enough to control or suppress the disease causing pathogens (Sharma *et al*., 2009). Till date, antagonists are applied either before harvest or after harvest. Many investigators have provided strong evidence that several pathogens infest fruits and vegetables in the field, and these infestations become critical factors for decays during transportation or storage of the commodities and hence argued that preharvest application (s) of microbial antagonistic culture are often effective to controlling postharvest decays in fruits and vegetables ((Ippolito and Nigro, 2000; Janisiewicz and Korsten, 2002; Ippolito *et al*., 2004; Irtwange, 2006). Typically, preharvest application is done to pre-colonize the fruit surface with the antagonistic microbes so that bruises inflicted during harvesting can be colonized by the antagonists before colonization by the pathogens (Ippolito and Nigro, 2000). Reports indicate that this application method could not become commercially viable, because of the poor survival of microbial antagonists in the field conditions. (Sharma *et al*, 2009). However, successes were chopped in certain cases. For example, (Benbow and Sugar, 1999) reported that a 3-week preharvest application of the antagonists *Cryptococcus infirmo-miniatus* (Okanuki) Phaff & Fell, *Cryptococcus laurentii*, and *Rhodopholus glutinis* (Fresenius) Harrison, to 'd Anjou' and 'Bosc' pears was found to reduce gray mold from 7% to nearly 1% And 13% to 4% respectively. In another experiment, Teixido *et al*. (1999) found *Candida sake* CPA-1 to reduce blue mold in wounded apples by nearly 50% when they were inoculated with the antagonist 2 days before harvest and inoculation with *Penicillium expansum* and cold storage for 4 months.

Preharvest applications with various microbial antagonists like *Gliocladium roseum* Bainier (Sutton *et al*., 1997), *Trichoderma harzianum* (Tronsmo and Denis, 1977; Kovach *et al*., 2000) and *Epicoccum nigrum* Link (Larena *et al*. (2005) have achieved successes in postharvest control of strawberries where synthetic fungicides proved ineffective. The application of secondary metabolite such as *pyrrolnitrin* produced by *Pseudomonas cepacia* was reported to achieve the highest levels of postharvest control of diseases (Janisiewicz and Roitman, 1988; Janisiewicz and Korsten, 2002). Additionally, studies by (Karabulut *et al*., 2003) found that applications close to harvest with *Metschnikowia fructicola* Kurtzman & Droby alone or in combination with ethanol and sodium bicarbonate controlled postharvest decays of grapes significantly over the control. Also, preharvest spray of *Metschnikowia fructicola* Kurtzman & Droby was found to be effective in controlling preharvest and postharvest fruit rots in strawberry (Karabulut *et al*., 2004). In a different study, the preharvest application of *Aureobasidium pullulans* reduced significantly the storage rots in strawberry (Lima *et al*., 1997), grapes (Schena *et al*., 1999, 2003), cherries (Wittig *et al*., 1997; Schena *et al*., 2003), and apples (Leibinger *et al*., 1997). There was a related reducing trend of incidence of green mold (Penicillium digitatum) on grapefruit by preharvest spray of *Pichia guilliermondii* (Droby *et al*., 1992).

In pear, field application(s) of *Cryptococcus laurentii* and *Candida oleophila* was reported to reduced storage rots (Benbow and Sugar, 1999) whereas prehavest applications of *Pantoea agglomerans* CPA-2 and *Epicoccum nigrum* were reported to be effective against citrus rots and peach brown rot under laboratory and field conditions respectively (Sharma *et al*, 2009). Similarly, Canamas *et al*. (2008) reported that preharvest application of different concentrations of Pantoea agglomerans was effective against *Penicillium digitatum* during storage of oranges [(Citrus sinensis (L.) Obseck.] . However, this method was seen to have many limitations and commercially applied to avocado (Sharma *et al.*, 2009).

In recent times, a postharvest application of antagonistic microorganisms are common and appears to be better for controlling post harvest diseases of fruits and vegetables. In the application process, the antagonists are sprayed directly onto the surfaces of the fruits and vegetables or are applied by dipping (Sharma *et al.*, 2009; Irtwange, 2006; Barkai-Golan, 2001). Investigations by many authors show that the postharvest application of microbial antagonists for controlling diseases in fruits and vegetables are more effective than the preharvest approach for citrus (Long *et al*, 2007), apples (Morales *et al*., 2008; Zhang *et al*., 2009; Mikani *et al*. , 2008), peach (Mandal *et al*., 2007), banana (Lassois *et al*., 2008), mango (Kefialew and Ayalew, 2008), tomato (Zhao *et al*., 2008), and cabbage (Adeline and Sijam, 1999). In strawberries and lemons for example, Pratella and Mari (1993) found that postharvest application of *Trichoderma harzianum, Trichoderma viride, Gliocladium roseum* and *Paecilomyces variotii* Bainier resulted in a better control of *Botrytis* and *Alternaria rots* respectively than preharvest application(s).

In a related development, postharvest applications of *Pseudomonas variotii* and *Trichoderma harzianum* were more effective in controlling Aspergillus and Fusarium rots in lemons and potatoes than their respective dips in iprodion and benomyl. A significant reduction in storage decay was reported for microbial antagonist yeast species in direct contact with wounds in the peel of harvested fruits against pathogens such as *P. digitatum, P. italicum* in citrus (Chalutz and Wilson, 1990); *B. cinerea* in apples (Gullino *et al*., 1992; Mercier and Wilson, 1995; Roberts, 1990; Wisniewski *et al*., 1988), *B. cinerea* and *P. expansum* in pears (Chand-Goyal and Spotts, 1996, 1997; Sugar and Spotts, 1999), and *B. cinerea, Rhizopus stolonifer and Alternaria alternata* in tomatoes (Chalutz *et al*., 1988). Nevertheless, there were different pathogenic reactions to a given antagonist (Sharma *et al*, 2009).

Major obstacles in the commercialization of microbial antagonistic products

1. Small number of companies in the development of biocontrol products
2. Lack of financial resources and established market networks
3. Small size of postharvest market and the fact that the microorganism need to control a wide spectrum of pathogens on different fruits and vegetables.
4. The expensiveness and too much time required to register a bioctrol agent
5. The robustness of the registration package in that it must clean safety records for humans and the environment, basic toxicological tests on the formulated products on the eyes, skin and ingestion
6. The complexities surrounding registration of biocontrol agent in somes countries. For example Europe.
7. The existing health and safety concerns regarding the introduction of antagonists into our diet

8. The dilemma of cell physiology and metabolism after rehydration
9. Lack of packaging technology that is capable of preventing contamination of the biocontrol agents with high water retention.
10. The lack of standard quality assurance guidelines for determining the acceptability of the products
11. The inability of the biocontrol agents to achieve 95-98% control efficiency as a standalone agent.
12. The skeptism with which agriculturalists view the use of microorganisms to biologically control postharvest diseases in fruits and vegetables.

Efficacy of antagonistic yeast against disease incidence under storage conditions

Many authors have reported the efficacy results against disease incidence in fruit and vegetables. In a study by Zhang *et al* (2010a), the application of antagonistic *Aureobasidium pullulans* PL5 at 10^8 cells /mL on the postharvest pathogens of peach, apple, and plun reported a 45% disease incidence on *M. laxa* in plums, 63% on *M. laxa* in peaches, and 56% and 46% on *B. cinerea* and *P. expansum* in apple respectively, under storage conditions of 1.2 ^oC and 95% relative humidity (RH) for 28 days. A combination of *R. glutinis* and Salicyclic acid was also found to prevent fungal spoilage in strawberries, which reduced the incidence of fungal spoilage of the fruit to 6.3% and 6.3% respectively compared to 37% and 46.7% for the untreated controls for fruit stored at 20 °C for 3 days or 4 °C for 7 d followed by 20 °C for 2 days (Zhang *et al*, 2010b)

In a similar experiment, Zhang *et al* (2006) found that at storage condition of 25 ^oC for 6 days for apple, a microwave treatment for 2-3 min combined with yeast antagonistic was able to reduce the disease incidence of blue mold in pear from 100% to 20.2% and lesion diameter from 2.81mm to 1.1 mm. Manso and Nunes (2011) used *Metschnikowia andauensis* strain NCYC 3728 (PBC-2) to control blue mold *Rhizopus stolonifer*, *P. expansum* and *B. cinerea*, on pears and on different apple cultivars and against *Penicillium digitatum* and *Penicilliuem italicum* on mandarins and oranges observed inhibitory activity of over 75% reduction of disease incidence and severity when the fruits were stored at 20±1 ^oC and 80±5% relative humidity for 7 days. In a related study by Zhang *et al* (2009), the application of R. glutinis resulted in low average decay incidence of 28.3% in apples compared with 75% in the control when stored at 20 ^oC for 5 days. In the same experiment, the *R. glutinis* treated apple fruits stored at 4 ^oC for 30 days followed by 20 ^oC for 4 days recorded a decay incidence of 6.67%, while the control fruit had 58.3% decay incidence. A combination of 46 ^oC hot water treatment and *Rhodotorula glutinis* was found to *control* completely the blue mould decay of pears (Hongyin *et al* , 2008). This combination achieved reduction in decay from 66.7% in the control fruit to 13.3% after 15 days at 20 ^oC, and from 46.7 to 6.7% after 4 ^oC for 60 days followed by 20 ^oC for 15 days on naturally infected and intact fruit respectively. Similarly, a combination of microwave (0.45kW) and *C. laurentii* (10^8 cells/mL) treatment, was capable of reducing percentage of decayed pear fruits to 12.1% (Zhang, Zheng, and Su, 2006)

Antagonistic microorganism's mechanism in the control of diseases

The mechanism under which antagonistic microorganism control pathogens in fruits and vegetables is still been researched. Over the years, seven main modes through which the antagonistic microbes fights the disease incidence caused by fungi have been reported (Sharma *et al*, 2009). The bio-control mechanisms are achieved through space and nutrient competition, production of antibiotics, attachments, population of the microbial antagonists, direct parasitism, and induced resistance. In the case of space, the microbial antagonistic grow more rapidly and survive under unfavorable conditions than the disease causing pathogens. It is reported that biocontrol activity of microbial antagonists increases with antagonistic concentrations, which consequently decrease the concentration of the pathogens (McLaughlin *et al*., 1990; Zhang *et al*, 2009). For example, antagonistic *Cryptococus. Laurenti* was effective at a concentration of 10^8 CFU/ml for controlling *Penicillium. expansum* in peach, and in apple storage for 5 days at 20 ^oC or for 30 days 4 ^oC followed by 20 ^oC for 4 days, *Rhodotorula glutinis* (10^8 / CFU/ml) was the most effective at controlling the gray mold and blue mold decays in apples (Zhang *et al*, 2007; 2009). Similarly, Zhang *et al* (2010) reported that for *Aureobasidium pullulans, a concentration of* 10^8 CFU/ml was better in controlling *Monilinia laxa* on plums and peaches, *Botrytis cinerea* and *Penicillium expansum* on apples.

The second most important mechanism by which antagonistic microbes inhibit the pathogens of fruits and vegetables is through production of antibodies (Sharma *et al*, 2009). For example, (Gueldner *et al*., 1988; Pusey, 1989) found the bacterial antagonists such as *Bacillussubtilis* and *Pseudomonas c epacia Burkh* to kill pathogens by producing the antibiotic iturin. The antagonism produced by *Bacillus Subtilis* was reported to be effective against fungal rot in citrus (Singh and Deverall, 1984) and *Monilinia fructicola* (Winter) Honey in peaches and cherries (Pusey and Wilson, 1984; Utkhede and Sholberg, 1986). Additionally, in apples rot, *Pseudomonas cepacia* inhibited the growth of postharvest pathogens like *B. cinerea* and P. expansum in the fruis by producing an antibiotic, pyrrolnitrin (Janisiewicz and Roitman, 1988; Janisiewicz *et al*., 1991). *Pseudomonas cepacia* was also effective in controlling green mold (Penicillium digitatum) in lemon (Citrus limon L.). In the case of attachment mechanism, there are different research findings relating to their bio-control activity. One group of authors reported that the antagonistic microorganism attaches itself directly to the disease causing pathogens for competition for nutrients. Interaction studies done using components of the *Enterobactor cloacae* (Jordon) Hormaeche and Edwards, and *Rhizopus stolonifer* (Ehrenberg: Fries) Lind (Wisniewski *et al*, 1989), and *Pichia guilliermondii* Wickerham, and *Penicillium italicum* Wehmer (Arras *et al*, 1998) revealed that direct attachment caused antagonistic yeasts and bacteria to consume more nutrients rapidly than the target pathogens and as a result prevented spore germination and growth of the pathogens (Droby *et al*, 1989, 1998; Wisniewski *et al*, 198). Another group of researchers reported that direct physical attachment does not affect antagonistic activity of o*Aureobasidium pullulans* (de Bary) Arnaud against Botrytis cinerea Pers.: Fries, *Penicillium expansum* Link, Rhizopus stolonifer, and Aspergillus niger van Tieghem\ infecting table grapes (Vitis vinifera L.) and *Botrytis cinerea* and *Penicillium expansum* on apple (*Malus domestica Borkh*.) fruit (Castoria *et al*., 2001). However, they attributed the microbial antagonism to antibiosis.

Studies that support the competition for nutrient as one of the bio-control mechanism in microbial antagonistic is well documented (Hongyin *et al*, 2007; Grebenisan *et al*., 2008; Droby *et al*., 1998). In a postharvest decay study using apple through ion depletion, (Saravanakumar *et al*., 2008) revealed that antagonistic yeast *Metschnikowia pulcherrima* consumed more nutrients than the disease causing microorganisms like *B. cinerea* and *P. expansum*. As a result of its effectiveness to inhibit postharvest decays, recommendations have been made regarding its potential usage for controlling fruit rots (Kurtzman and Droby, 2001; Grebenisan *et al*., 2008). When several nutrients were added the biocontrol efficacy of *M. pulcherrima* on gray mold (Botrytis cinerea) on apple increased indicating that competition for nutrients plays a significant role in the biocontrol capability of *M. pulcherrima* against Botrytis cinerea (Piano *et al*., 1997). A similar antagonism was observed for non-pathogenic species of Erwinia, such as, E. cypripedii (Hori) Bergey, against various isolates of *Erwinia caratovora* sub sp. Caratovora (Jones) Bergey, the pathogens of soft rot of many vegetables like carrot, tomatoes (Lycopersicon esculentum L.) and pepper (*Capsicum annuum L*.) (Moline, 1991; Moline *et al*., 1999). In vitro studies by several authors have confirmed the antagonistic microorganisms' biocontrol mechanism through rapid nutrients uptake more rapidly than their disease causing counterpart. This enables them to get established quickly and inhibit spore germination of the pathogens at the wound site (Hongyin *et al*, 2007; Wisniewski *et al*., 1989; Droby and Chalutz, 1994, Droby *et al*., 1998). Microbial antagonists was reported to induce disease resistance in harvested avocado(Persea americana Mill) fruit by the production of antifungal compounds (Prusky *et al*., 1994; Yakoby *et al*., 2001), and accumulation of phytoalexins, like scoparone and scopoletin in citrus fruit (Rodov *et al*., 1994; Arras, 1996).

Application of microbial antagonists on the quality of fruits and vegetables

As indicated early on, the quality of the fruits and vegetables subjected to antagonistic microbial treatments should be preserved during the process. A Review by Vadivambal and Jayas (2007) indicated that quality includes three principal areas: nutritional value, acceptability, and safety. Good quality is judged by freshness, firmness, expected appearance, colour, flavour, and texture.etc. Zhang *et al* (2009) investigated the effects of *R. glutinis* on postharvest quality parameters of apples and reported no significant differences in mass loss, firmness, total soluble solids (TSS), ascorbic acid (AA), and titratable acidity (TA) when the apple fruits were stored at 20 ^{o}C for 5 days or at 4 ^{o}C for 30 days followed by 20 ^{o}C for 4 days. In pears, no significant differences in quality was observed by Zhang *et al* (2008) when they studied the effect of combined hot water treatment and antagonistic *R. glutinis* on pears mass loss, fruits firmness, TSS, AA and TA, whether the pears were stored at 20 ^{o}C for 15 days or at 4^{o}C for 60 days followed by 20 ^{o}C for 15 days. These results were similar to another study by Zhang *et al* (2008) on peach using antagonistic yeast with salicylic acid on the quality of stored products. They reported no significant reduction in weight loss, fruit firmness, TSS, AA or TA when the peach was stored at 20 ^{o}C for 7 days. These quality results are in agreement with a similar study with antagonistic *C.laurentii* (10^{8} cells/mL) under microwave (0.45 kW) treatments for 2-3 min when pears were stored for two months at 2 ^{o}C and additional 15 days at 20 ^{o}C (Zhang, Zheng, and Su, 2006). Additionally, no significant reduction in the above quality parameters was observed when pears were treated with antagonistic *C. laurentii* after 60 days storage at 2 ^{o}C followed by 15 days at 20 ^{o}C. This research revealed that not many researchers concern themselves with intrinsic quality attributes after microbial antagonistic treatment. Perhaps, many authors use physical quality indicators to assume the general quality of fruits and vegetables that have undergone bio-antagonism. A lot more research is needed in this direction to re-affirm the existing believe that microbial antagonists do not influence negatively the natural quality of fruits and vegetables.

A new paradigm shift in the science of microbial antagonistic bio-control

A review by Droby *et al.* (2009) provides the biocontrol scientific community with insight regarding the new paradigm shift necessary to effectively control decays in fruits and vegetables. A number of ideas proposed includes: (1) the need to view biological control as a process rather than the control of one organism (2) the need to integrate low risk chemical fungicides, natural antimicrobial substances, and physical means such as hot water, microwave, infrared, and ultrasonic treatment in the bio-control process (3) the use of more efficient DNA-based methods to monitor bio-control agent fate and activity after application (4) the application of proteomics and functional genomics to determine and follow changes in the physiological status of biocontrol agents and the effect of environmental stress, (5) Enhancement in the expression of crucial biocontrol genes and/or combining genes from different biocontrol agents in the mass production, formulation and storage, or in response to exposure and contact with host plant tissue after application (6) the use of genetically modified organisms as biocontrol agents (7) the research towards discovering new antagonists instead of the ones currently used in practices in that only a small portion of the earth micro flora has been identified and characterized.

CONCLUSION

The applications of antagonistic microorganisms for the control of preharvest and postharvest control of diseases in fruits and vegetables have been reviewed. It was clear in this review that the use of antagonistic microbes is a promising alternative to synthetic chemicals fungicides. However, the approach has not achieved desired successes because of the dilemma and the complexities surrounding its products development and application in delicate foods like the fruits and vegetables. The efficacy levels typically achieved is not as high as the synthetic chemical fungicides and requires a new paradigm shift to enhance its biocontrol efficiency, efficacy and consistency. The review revealed that not much research is done on the intrinsic quality attributes of fruits and vegetables after microbial antagonistic treatment. Rather, many authors do not make further attempt to determine the intrinsic quality characteristics but only assume the general quality of fruits and vegetables that have undergone bio-antagonism. A lot more research is needed to make certain the current notion that microbial antagonists do not influence negatively the natural quality of fruits and vegetables.

AKNOWLEDGEMENT
The authors acknowledge the financial support of the Priority Academic Program Development (PAPD) for Jiangsu Higher Education Institutions.

REFERENCES
Abano, E.E., and Sam-Amoah, L.K. (2011) Effects of different pretreatments on drying characteristics of banana slices. *Journal of Engineering and Applied Science. Vol 6*, Article 3.

Adeline, T.S.Y., and Sijam, K.. (1999) Biological control of bacterial soft rot of cabbage. In: Hong, L.W., Sastroutomo, S.S., Caunter, I.G., Ali, J., Yeang, L.K., Vijaysegaran, S., Sen, Y.H. (Eds.), Biological Control in the Tropics: Towards Efficient Biodiversity and Bioresource Management for Effective Biological Control: Proceedings of the Symposium on Biological Control in the Tropics. CABI Publishing, Wallingford, UK, pp. 133–134.

Barkai-Golan, R. (2001) Postharvest Diseases of Fruit and Vegetables: Development and Control. Elsevier Sciences, Amasterdam, The Netherlands.

Benbow, J.M., and Sugar, D. (1999) Fruit surface colonization and biological control of postharvest diseases of pear by preharvest yeast applications. Plant Disease 83, 839–844.

Canamas, T.P., Vinas, I., Usall, J., Torres, R., Anguera, M., Teixido, N., (2008). Control of postharvest diseases on citrus fruit by preharvest applications of biocontrol agent Pantoea agglomerans CPA-2: Part II. Effectiveness of different cell formulations. Postharvest Biology and Technology 49 (1), 96–106.

Chalutz, E., Ben-Arie, R., Droby, S., Cohen, L., Weiss, B., Wilson, C.L. (1988) Yeasts as biocontrol agents of postharvest diseases of fruit. Phytoparasitica 16, 69–75.

Chalutz, E., Wilson, C.L. (1990) Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by Debaryomyces hansenii. Plant Disease 74, 134–137.

Chand-Goyal, T., Spotts, R.A. (1996) Postharvest biological control of blue mold of apple and brown rot of cherry by natural saprophytic yeasts alone or in combination with low doses of fungicides. Biological Control 6, 253–259.

Chand-Goyal, T., Spotts, R.A. (1997) Biological control of postharvest diseases of apple and pear under semi-commercial and commercial conditions using three saprophytic yeasts. Biological Control 10 (3), 199–206.

Droby, S., (2006) Improving quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticulturae 709, 45–51.

Droby, S., Chalutz, E., Wilson, C.L., Wisniewski, M.E. (1992) Biological control of postharvest diseases: a promising alternative to the use of synthetic fungicides. Phytoparasitica 20, 1495–1503.

Droby, S., Wisniewski, M., Macarisinb, D., Wilson, C.(2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm? Postharvest Biology and Technology 52, 137–145

El-Ghaouth, A., Wilson, C.L., Wisniewski, M.E. (2004) Biologically based alternatives to synthetic fungicides for the postharvest diseases of fruit and vegetables. In: Naqvi, S.A.M.H. (Ed.), Diseases of Fruit and Vegetables, vol. 2. Kluwer Academic Publishers, The Netherlands, pp. 511–535.

Fan, Q., Tian, S.P., (2001) Postharvest biological control of grey mold and blue mold on apple by Cryptococcus albidus (Saito) Skinner. Postharvest Biology and Technology 21 (3), 341–350.

Gullino, M.L., Benzi, D., Aloi, C., Testoni, A., Garibaldi, A. (1992) Biological control of Botrytis rot of apple. In: Williamson, B., Verhoeff, K., Malatrakis, N.E. (Eds), Recent advances in Botrytis research. Proceedings of the 10th International Botrytis Symposium, Heraklion, Crete, pp. 197-200.

Ippolito, A., and Nigro, F., (2000) Impact of preharvest application of biological control agents on postharvest diseases of fresh fruit and vegetables. Crop Protection 19 (8/10), 715–723.

Ippolito, A., Nigro, F., Schena, L., (2004) Control of postharvest diseases of fresh fruit and vegetables by preharvest application of antagonistic microorganisms. In: Niskanen, D.R., Jain, R. (Eds.), Crop management and postharvest handling of horticultural products Volume: IV: Diseases and disorders of fruit and vegetables. SM Science Publishers, Inc. Enfield, USA, pp. 1–30.

Irtwange, S., (2006) Application of Biological Control Agents in Pre- and Post-harvest Operations. Agri. Eng. Intl. 8, Invited Overview 3, A & M University Press, Texas.

Janisiewicz, W.J., Korsten, L. (2002) Biological control of postharvest diseases of fruit. Annual Review of Phytopathology 40, 411–441.

Janisiewicz, W.J., Roitman, J., (1988) Biological control of blue mold and gray mold on apple and pear with Pseudomonas cepacia. Phytopathology 78, 1697 – 1700.

Karabulut, O.A., Smilanick, J.L., Gabler, F.M., Mansour, M., Droby, S., (2003) Nearharvest applications of Metschnikowia fructicola, ethanol, and sodium bicarbonate to control postharvest diseases of grape in central California. Plant Disease 87 (11), 1384–1389.

Karabulut, O.A., Tezean, H., Daus, A., Cohen, L., Wiess, B., Droby, S., (2004) Control of preharvest and postharvest fruit rot in strawberry by Metschnikowia fructicola. Biocontrol Science and Technology 14, 513–521.

Kefialew, Y., Ayalew, A. (2008) Postharvest biological control of anthracnose (Colletotrichum gloeosporioides) on mango (Mangifera indica). Postharvest Biology and Technology 50 (1), 8–11.

Korsten, L. (2006) Advances in control of postharvest diseases in tropical fresh produce. International Journal of Postharvest Technology and Innovation 1 (1), 48–61.

Kovach, J., Petzoldi, R., Harman, G.E. (2000) Use of honey bees and bumble bees to disseminate Trichoderma harzianum to strawberries for Botrytis control. Biological Control 18, 235–242.

Larena, I., Torres, R., de Cal, A., Linan, M., Melgarejo, P., Domenichini, P., Bellini, A., Mandrin, J.F., Lichou, J., Ochoa de Eribe, X., Usall, J. (2005) Biological control of postharvest brown rot (Monilinia spp.) of peaches by field applications of Epicoccum nigrum. Biological Control 32 (2), 305–310.

Lassois, L., de Bellaire, L., Jijakli, M.H. (2008) Biological control of crown rot of bananas with Pichia anomala strain K and Candida oleophila strain O. Biological Control 45 (3), 410–418.

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, 1103–1110.

Lima, G., Ippolito, A., Nigro, F., Salerno, M., (1997) Effectiveness of Aureobasidium pullulans and Candida oleophila against postharvest strawberry rots. Postharvest Biology and Technology 10, 169–178.

Long, C.A., Deng, B.X., Deng, X.X. (2007) Commercial testing of Kloeckera apiculata, isolate 34–9, for biological control of postharvest diseases of citrus fruit. Annals of Microbiology 57 (2), 203–207.

Mandal, G., Singh, D., Sharma, R.R. (2007) Effect of hot water treatment and bio-control agent (Debaryomyces hansenii) on shelf life of peach. Indian Journal of Horticulture 64 (1), 25–28.

Mercier, J., Wilson, C.L. (1995) Effect of wound moisture on the biocontrol by Candida oleophila of gray mold rot (Botrytis cinerea) of apple. Postharvest Biology and Technology 6, 9–15.

Mikani, A., Etebarian, H.R., Sholberg, P.L., Gorman, D.T., Stokes, S., Alizadeh, A. (2008) Biological control of apple gray mold caused by Botrytis mali with Pseudomonas fluorescens strains. Postharvest Biology and Technology 48 (1), 107–112.

Morales, H., Sanchis, V., Usall, J., Ramos, A.J., Marín, S. (2008) Effect of biocontrol agents Candida sake and Pantoea agglomerans on Penicillium expansum growth and patulin accumulation in apples. International Journal of Food Microbiology 122 (1–2), 61–67.

Pratella, G.C., Mari, M. (1993) Effectiveness of Trichoderma, Gliocladium and Paecilomyces in postharvest fruit Protection. Postharvest Biology and Technology 3, 49–56.

Roberts, R.G., (1990) Postharvest biological control of gray mold of apple by Cryptococcus laurentii. Phytopathology 80, 526–530.

Schena, L., Ippolito, A., Zehavi, T., Cohen, L., Nigro, F., Droby, S. (1999) Genetic diversity and biocontrol activity of Aureobasidium pullulans isolates against postharvest rots. Postharvest Biology and Technology 17, 189–199.

Schena, L., Nigro, F., Pentimone, I.A., Ippolito, A. (2003) Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of Aureobasidium pullulans. Postharvest Biology and Technology 30 (3), 209–220.

Sharma, R.R., Singh D., Singh R. (2009) Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review, Biological Control , 50 (2009) 205–221.

Singh, D., Sharma, R.R. (2007) Postharvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), Sustainable Pest Management. Daya Publishing House, New Delhi, India.

Sugar, D., Spotts, R.A. (1999) Control of postharvest decay of pear by four laboratory grown yeasts and two registered biocontrol products. Plant Disease 83, 155–158.

Sutton, J.C., Li, D., Peng, G., Yu, H., Zhang, P., Valdebenito-Sanhueza, R.M. (1997) Gliocladium roseum, a versatile adversary of Botrytis cinerea in crops. Plant Disease 31, 316–328.

Teixido, N., Usall, J., Vinas, I. (1999) Efficacy of preharvest and postharvest Candida sake biocontrol treatments to prevent blue mold on apples during storage. International Journal of Food Microbiology 50, 203–210.

Tronsmo, A., Denis, C. (1977) The use of Trichoderma species to control strawberry fruit rots. Netherlands Journal of Plant Pathology 83, 449–455.

Wisniewski, M., Wilson, C.L., Chalutz, E., Hershberger, W.(1988). Biological control of postharvest diseases of fruit: inhibition of Botrytis rots on apples by an antagonistic yeast. Proceedings of the Electron Microscopic Society of America 46, 290–291.

Wittig, H.P.P., Johnson, K.B., Pscheidt, J.W. (1997). Effect of epiphytic fungi on brown rot, blossom blight and latent infections in sweet cherry. Plant Disease 81, 383–387.

Zhang , H., Zheng , X., Yu, T., (2007). Biological control of postharvest diseases of peach with *Cryptococcus laurentii*. Food Control 18, 287–291.

Zhang, H,, Wang S., Huanga, X.,, Donga,Y., Zheng, X., (2008). Integrated control of postharvest blue mold decay of pears with hot water treatment and *Rhodotorula glutinis* Postharvest Biology and Technology, 49, 308–313

Zhang, H., Wang, L., Ma, L., Dong, Y., Jiang, S., Xu, B., Zheng, X. (2009). Biocontrol of major postharvest pathogens on apple using Rhodotorula glutinis and its effects on postharvest quality parameters. Biological Control 48 (1), 79–83.

Zhang, H., Zheng, X., , Su, D. (2006) Postharvest control of blue mold rot of pear by microwave treatment and *Cryptococcus laurentii*. Journal of Food Engineering 77, 539–544.

Zhang, H., Zheng, X., Fu, C., Xi, Y. (2005) Postharvest biological control of gray mold rot of pear with Cryptococcus laurentii. Postharvest Biology and Technology 35 (1), 79–86.

Zhang, H.,, Ma, L., , Jiang, S., Lin, H.,, Zhang X., Ge, L., Xu, Z. (2010b) Enhancement of biocontrol efficacy of Rhodotorula glutinis by salicyclic acid against gray mold spoilage of strawberries. International Journal of Food Microbiology 141, 122–125

Zhao, Y., Tu, K., Shao, X., Jing, W., Su, Z.(2008) Effects of the yeast Pichia guilliermondii against Rhizopus nigricans on tomato fruit. Postharvest Biology and Technology 49 (1), 113–120.

Zhu, S.J. (2006)Non-chemical approaches to decay control in postharvest fruit. In: Noureddine, B., Norio, S. (Eds.), Advances in Postharvest Technologies for Horticultural Crops. Research Signpost, Trivandrum, India, pp. 297–313.