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Biological control strategy for postharvest diseases of citrus, apples, grapes and strawberries fruits and application in Indonesia

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Abstract

Background: In Indonesia, the postharvest fruit loss is 25%, so the economic loss from the export of various fruits is estimated at US\$ 58,966,861. One of the causes for the loss is postharvest pathogens. Postharvest fruit rot is caused mainly by fungi, some of which produce mycotoxins harmful to human health. Therefore, in meeting the global food safety requirements, Indonesia should develop a biological control strategy for postharvest fruit diseases. This paper is a review based on observations, a literature review of postharvest biological control of citrus and other subtropical fruits, and an overview of strategies and prospects for their application in Indonesia.

Main body: The pathogens that cause diseases on citrus fruits, apples, grapes, and strawberries in Indonesia produce mycotoxins, namely *Fusarium* sp., *Aspergillus terreus*, *Aspergillus* sp., *Penicillium* sp., and *Alternaria* sp. The potential biological agents are from the yeast group, such as the *Candida* genera, the bacterial group, such as the *Bacillus* and *Pseudomonas* genera, and the fungal group, such as the *Muscodor* and *Trichoderma* genera.

Conclusion: Through mutually additive and synergistic multiple reduction methods in cooperation with the vanguards, postharvest disease control emphasizes disease prevention using several methods. Each method reduces the percentage of damage by a certain amount to produce highly effective controls.

Keywords: Citrus, Postharvest, Apples, Grapes, Strawberries, Mycotoxin, Microorganism agents, Botanical agents

Background

Indonesia is one of the tropical countries with the potential to develop fruits as a commodity, including citrus and other subtropical fruits (apples, grapes, and strawberries). The problem of fresh product quality and safety still needs attention. The low quality of fruits is mainly caused by a high microbial contamination, which pollutes various fruits in Indonesia because they are supported by tropical climatic conditions with high humidity levels. This contamination is economically detrimental and

harmful to health, especially for diseases caused by fungi that produce mycotoxins. Mycotoxins are toxic organic compounds produced by the secondary metabolism of fungi that can interfere with human health clinically and pathologically (Ismail and Papenbrock 2015).

Pathogens that infect fruit products, including citrus, grapes, apples, and strawberries, may come from the field, while some emerge during postharvest. In addition, inadequate postharvest handling of fruits can result in physical damage, for example, bruising because of falling during transportation. Bruises or other physical damages on fruits make them easily infected with fungi. Postharvest and food losses are around 30% of production in

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developing countries, especially those under humid climates (Bradford et al. 2018).

Some fungi that cause postharvest damage to citrus and other subtropical fruit crops (apples, grapes, and strawberries) include *Botrytis*, *Penicillium*, *Mucor*, *Alternaria*, *Colletotrichum*, *Lasiodiplodia*, *Rhizopus*, *Fusarium*, and *Aspergillus* (Pétriacq et al. 2018). In Indonesia, fungal contamination and low-safety assurance of various fruits are due to poor postharvest handling, including: (1) improper harvesting time, (2) over-packing and improper packing, (3) physical damages due to collisions and rough handling, and (4) chemical changes during the storage process. These factors result in the low-quality and low-safety status of various fruits in Indonesia due to a high level of contamination (Miskiyah and Broto 2010).

Research on postharvest diseases and control of various fruits has not been widely carried out in Indonesia. However, many similar studies have been carried out abroad, as indicated by a large number of published research results (Wallace et al. 2018). Currently, the most effective control method is the use of chemical pesticides, but this method can create a single chemical resistance to fungal pathogens, if used continuously (Hawkins et al. 2019). The use of chemical pesticides demands more attention because of their potential toxicity to humans and the environment. In Indonesia, pesticide residues are found not only in fresh agricultural products but also in soil, water bodies, animal feed, and honey. Although the residue is still below the maximum residual limit, the use of agricultural chemicals needs to be monitored as they are toxic, persistent, and accumulative (Hendriadi et al. 2021). The food safety requirements of the global market encourage Indonesia to develop biological control strategies for postharvest diseases of fresh fruits. Therefore, this review was based on observations, biological control of postharvest diseases of citrus and other subtropical fruits, and an overview of strategies and prospects for their application in Indonesia.

Main body

Postharvest diseases on citrus, apples, grapes, and strawberries in Indonesia

Fungi, bacteria, and viruses can cause postharvest diseases of citrus, apples, grapes, and strawberries. Postharvest diseases can occur when the commodity has been harvested, but infections may also happen before harvesting when the pathogen is in a dormant state. Pathogens then develop and cause symptoms when conditions are favorable. Inventory-specific research showing postharvest diseases of citrus, apples, grapes, and strawberries has not been widely carried out in Indonesia. However, from the results of literature reviews, field and market observations (Dwiastuti and Fajrin 2014;

Widiastuti et al. 2015; Dwiastuti et al. 2016), and during the authors' visit to production centers of citrus, apples, grapes, and strawberries at different times, infections of *Penicillium italicum*, *P. digitatum*, *Lasiodiplodia theobromae*, *Alternaria citri*, *Phomopsis citri*, *Geotrichum candidum*, *Pezicula malicorticis* (*Gloeosporium malicorticis*), *Glomerella cingulata*, *Physalospora abtusa*, *Podosphaera leucotricha*, *Mucor piriformis*, *P. expansum*, *Botrytis cinerea*, *Erysiphe necator*, *Colletotrichum gloesporioides*, *Rhizopus stolonifer*, *Aspergillus* sp., *Penicillium* sp., *Phytophthora cactorum*, *Fusarium* sp., *Alternaria* sp. were found in traditional markets and supermarkets (Table 1).

The types of fruits observed varied, from local to imported fruit varieties. More fungal contaminations were found in traditional markets than supermarkets. Lower fungal contaminations of fruits in supermarkets happen since supermarkets have air-conditioned storage areas, and fruits are sorted, cleaned, and covered with wrappers, reducing contact among fruits. In traditional markets, fruits are sold or stored as a reserve, placed in bamboo baskets without cooling space, while the temperature in the market tends to be high. In addition, fruits in traditional markets are not individually wrapped, so contact among fruit is possibly very high.

Some fungal diseases that contaminate citrus, apples, grapes, and strawberries in Indonesia including *Fusarium* sp., *A. terreus*, *Aspergillus* sp., *Penicillium* sp., and *Alternaria* sp. The types of mycotoxins found were patulin, ochratoxin, aflatoxin, deoxynivalenol, and others (Table 2). Each of these mycotoxins can cause quite dangerous effects on humans and animals. Table 2 shows that mycotoxins can cause mild to severe symptoms, including moderate-acute nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness, fever, respiratory and circulatory failure, paralysis, seizures and death, to carcinogenic and mutagenic effects, plasma membrane disruption, protein synthesis inhibition, and inhibition on the anti-tumor, antiviral, antibacterial activities. However, in-depth knowledge about mycotoxins, especially in fruits in Indonesia, is still limited.

Control management

Similar to pre-harvest disease management in the field, postharvest disease control must also consider an integrated pest management strategy. Postharvest handling alone is insufficient for the product after harvest, but control measures during pre-harvest will significantly determine the health quality of the harvested fruits. Postharvest disease control is highly dependent on the initial condition of the commodity, meaning that plant health, planting area cleanliness, and several other treatments in the field determine the presence or absence of postharvest diseases. In addition, after entering the postharvest

Table 1 Postharvest diseases of citrus and subtropical fruits in Indonesia. *Source:* Dwiastuti unpublished (UP): Field and market observations during visits to production centers of citrus, apples, grapes, and strawberries at different times

Diseases	Pathogen	Location	Found on		References
			TM	SM	
<i>Citrus</i>					
Green mold	<i>Penicillium italicum</i>	Midlands and highlands in East Java, West Java, Bali, East Nusa Tenggara	F	F	Soesanto (2006), Dwiastuti UP
Blue mold	<i>P. digitatum</i>	Midlands and highlands in East Java, West Java, Bali, East Nusa Tenggara	F	F	Soesanto (2006), Dwiastuti UP
Stem end rot	<i>Lasiodiplodia theobromae</i>	All altitudes of East Java, Central Java, West Java, Bali, East Kalimantan, West Kalimantan, East Sulawesi, East Nusa Tenggara	F	NF	Soesanto (2006), Dwiastuti and Aji (2021)
Circular rot	<i>Alternaria citri</i>	East Java	F	NF	Soesanto (2006), Dwiastuti UP
Stem end rot	<i>Phomopsis citri</i>	Market, storage	F	F	Soesanto (2006)
Sour mold	<i>Geotrichum candidum</i>	Market, storage	F	F	Soesanto (2006)
<i>Apples</i>					
Apple anthracnose	<i>Pezizula malicorticis</i> (<i>Gloeosporium malicorticis</i>)	East Java, West Java, East Nusa Tenggara	F	NF	Dwiastuti UP
Bitter rot	<i>Glomerella cingulata</i>	East Java, Central Java	F	F	Soesanto (2006)
Black rot	<i>Physalospora abtusa</i>	East Java, Central Java	F	F	Soesanto (2006)
Dry rot	<i>Alternaria</i> sp.	Yogyakarta	F	NF	Widiastuti et al. (2015)
Powdery mildew	<i>Podosphaera leucotricha</i>	East Java, West Java, East Nusa Tenggara	F	NF	Dwiastuti UP
Mucor rot	<i>Mucor piriformis</i>	East Java	F	NF	Dwiastuti UP
Blue mold	<i>P. expansum</i>	East Java	F	F	Soesanto (2006), Dwiastuti UP
<i>Grapes</i>					
Gray mold	<i>Botrytis cinerea</i>	East Java, Bali	F	NF	Soesanto (2006), Dwiastuti UP
Powdery mildew	<i>Erysiphe necator</i>	East Java, Bali	F	NF	Dwiastuti UP
Anthracnose	<i>Colletotrichum gloesporioides</i>	East Java, Bali	F	NF	Dwiastuti UP
Rhizopus rot	<i>Rhizopus stolonifer</i>	East Java, Bali	F	NF	Soesanto (2006), Dwiastuti UP
Dry rot	<i>Aspergillus</i> sp.	Central Java	NF	F	Widiastuti et al. (2015), Dwiastuti UP
Blue mold	<i>Penicillium</i> sp.	Market, storage	F	F	Soesanto (2006)
<i>Strawberries</i>					
Gray mold	<i>B. cinerea</i>	East Java, Central Java, West Java, Bali, North Sumatera	F	F	Dwiastuti UP
Rhizopus rot	<i>R. stolonifer</i>	East Java	F	NF	Dwiastuti UP
Phytophthora rot	<i>Phytophthora cactorum</i>	East Java	F	NF	Dwiastuti and Fajrin (2014)
Anthracnose	<i>C. gloesporioides</i>	East Java, Central Java, West Java, Bali, North Sumatera,	F	F	Dwiastuti and Fajrin (2014)
Fusarium rot	<i>Fusarium</i> sp.	East Java	F	NF	Dwiastuti and Fajrin (2014), Dwiastuti et al. (2016)
Alternaria rot	<i>Alternaria</i> sp.	East Java	F	NF	Dwiastuti UP

TM = Traditional markets, SM = supermarkets, F = found, NF = not found

period, disease progression is highly dependent on management techniques from harvesting to transportation or storage at the consumer level.

Disease control for citrus, apples, grapes, and strawberries is usually carried out from generative growth and ends one month before harvest to avoid residue on the fruits (Sumiati and Julianto 2017). Generally, fruit orchards in Indonesia are located far from consumers. Therefore, fruits are transported from the packaging

station in the production center to various destinations using various vehicles. They can even be transported several times to reach the final destination (Prabawati 2009). This causes farmers to sell on a wholesale basis. As such, sorting is not carried out by farmers but by collectors, traditional market traders, and retail traders by removing rotten fruits in simple to modern ways.

However, not all farmers or traditional traders in Indonesia carry out postharvest handling, such as washing,

Table 2 Mycotoxins produced by fungi that cause postharvest diseases on citrus and subtropical fruits and their effects

Pathogen	Mycotoxin	References	Effects	
	Mycotoxin		Effects on humans	References
<i>Fusarium</i> sp.	Deoxynivalenol , T2 toxin, zearalenone, moniliformin, Fumonisin	Rahayu (2006)	Acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever	Sobrova et al. (2010)
<i>Aspergillus terreus</i>	Citreoviridin , terreic acid, patulin	Rahayu (2006)	Aspergillosis, respiratory and circulatory failure, paralysis, seizures and death	Lass-Florl et al. (2005)
<i>Aspergillus</i> sp.	Aflatoxins , kojic acid, aspergillilic acid, cyclopi-azonic acid, patulin, stereoviridin, fumigatin, ochratoxins, sterigmatocystin, viriditoxin, citrinin, palmotoxin, rubratoxin	Rahayu (2006)	Carcinogenic, mutagenic, teratogenic, and immunosuppressive for humans	Lanier et al. (2020)
<i>Penicillium</i> sp.	Patulin , ochratoxin, citrinin, luteoskirin, tremorgenic, rubratoxin, mycophenolate, griseofulvin, phenytoin, colonic acid	Rahayu (2006)	Plasma membrane disruption; protein synthesis inhibition; Na ⁺ -coupled inhibition; amino acid transport, transcription and translation disorders; DNA synthesis inhibition; and inhibition in the production of interferon γ type 1 T-helper cells	Pal et al. (2017), Ramalingam et al. (2019)
<i>Alternaria</i> sp.	Tenuazonic acid	Rahayu (2006)	Causing <i>Onyali</i> disease, causing chronic and acute effects by inhibiting anti-tumor, antiviral, antibacterial activities, and causing mutagenic activities	Zain (2011)

sorting, and pre-cooling (Siswadi 2007). As a result, many of them are not aware of whether the fruit carries spores or parts of microbes that can develop and become new sources of infection. In addition, most of the local market traders (traditional markets) in Indonesia only select fruits based on their respective grades and put them in the same container; there is no separation among fruits. The exception is only for packaging for modern markets (supermarkets), which require more stringent terms and standards for the safety of fruit to be marketed (Siswadi 2007).

Postharvest disease control using physical method has been recommended in Indonesia, but not all can apply it to maintain fruits quality. The method used includes low-temperature management and modification of the storage room atmosphere using reduced oxygen, elevated carbon dioxide, heat treatment (hot air or immersion), or radiation (Sukasih and Setyadjit 2019). Utilizing chemicals to control the postharvest disease is considered the most effective. However, its application depends on the type of marketing and infection that occurs. For example, in citrus with a long shelf life, there should be additional safe chemical treatment to prevent the germination of fungal spores. Conversely, for strawberries that have a very short shelf life, it seems that postharvest disease control treatment is prioritized on controlling just before harvest in the field (Sudjatha and Wisaniyasa 2017).

Alternative modern techniques used to minimize losses due to postharvest diseases in developed countries are physical, biochemical, biological (Qadri et al. 2020), and breeding for resistance (Pétriaccq et al. 2018). These

modern techniques can be used as a reference for controlling postharvest diseases in Indonesia. Until now, there is no information on citrus, apple, grape, and strawberry varieties that are resistant to postharvest diseases.

Biochemical techniques for modern postharvest disease control are aimed at suppressing the use of pesticides and minimizing the side effects of fungicide resistance in major fungal pathogens such as *Penicillium*, *Monilinia*, *Botrytis*, and other common pathogens (Qadri et al. 2020). The technique being tried out involves genetic transformation with the application of fungicidal dsRNA. de Oliveira Filho et al. (2021) reviewed the results of research on the external application of synthetic fungicide dsRNA on fruits, which is safer for controlling postharvest fruit diseases, as a new generation of sustainable and environmentally friendly products. Several studies have been carried out by spraying or soaking fruit surfaces with RNA interference (RNAi) to control postharvest fungi of fruits, particularly *B. cinerea* (Wang et al. 2016) and *Fusarium* spp. (Gu et al. 2019) which causes postharvest losses on strawberries, grapes, and other fruits. This new fungicide with RNAi technology has greater specificity and efficiency. External application of double-stranded RNA (dsRNA), small interference RNA (siRNA), or hairpin RNA (hpRNA), resulting in post-transcriptional gene silencing, has emerged as a promising strategy for controlling phytopathogens (Islam and Sherif 2020). Nanomaterials have also been used as a new generation in postharvest technology in general and disease management in particular (Roberto et al. 2019). Exogenous application of calcium-based chemicals helps

stabilize plant cell walls, maintaining fruit quality (Rodriguez et al. 2019). Chitosan solution (0.5 and 1%) was effective for maintaining high quality and preventing fungal pathogens in oranges during cold storage with good chemical and physical quality (Khalil Bagy et al. 2021). In addition, Youssef et al. (2020) reported that potassium bicarbonate (PB), sodium silicate solution (SSi) and calcium chelate (CCh) (1%) were effective in inducing resistance to gray mold disease in table grapes and reduced disease incidence by 43, 50, and 41%, respectively. The salt solution acts as an antimicrobial agent against the gray mold of table grapes so that it can increase the natural host resistance associated with an increase in the antioxidant system. From this study, it was proved that the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD) increased with the above salt treatment.

Various physical techniques with refrigeration, cold atmosphere storage, low pressure storage, and modified atmosphere storage are used to control postharvest disease. The physical technique of heat treatment is considered the most effective, especially to treat fungal diseases and chilling injury. In addition, UV treatments (UV-C, UV-B, and UV-A) are used to sterilize commodities, reduce spoilage due to microorganisms, extend shelf life, and maintain fruit quality. UV treatment exposes fruit and vegetable commodities for a period of time under a UV lamp bank, with maximum emission at 254 nm. UV-C treatment has been shown to be effective for controlling the most common postharvest pathogens (Civello et al. 2006). Several studies have also proven that UV-C radiation is effective not only because of its disinfecting effect, but also because it can stimulate plant defenses. Forges et al. (2020) stated that UV-C radiation was efficient in reducing disease progression in many species, including strawberries (*Fragaria × ananassa*). Infection on leaf against powdery mildew and on fruit against *Rhizopus* spp. were greatly decreased due to the UV-C effect. Besides that, it also has an impact on the initiation of flowering and fruit development, increasing the physical integrity of strawberries in storage, although it does not increase the shelf life of strawberries.

Postharvest disease management using biological agents has been studied extensively. By involving natural enemies of the pathogen to be targeted, the population of the pathogen can be suppressed through restriction of normal growth or activity. In addition, volatile compounds are usually applied on a commercial scale for flavoring and seasoning in foods, which greatly reduces the incidence of microbial pathogens. This volatile compound has various properties such as antiprotectant, antimicrobial, less harmful to mammals, environmentally friendly, and can be used as an alternative to chemical

fungicides (Abo-Elyousr et al. 2021). Plants are a large reservoir of natural compounds that harbor fungicidal activity with the potential to replace synthetic fungicides. Many species produce volatile substances and essential oils that can serve as antifungal or antimicrobial preservatives for fruits and other harvested commodities. Thus, combining multiple treatment options can offer stakeholders and producers a more consistent, durable, practical, and sustainable solution for postharvest disease control.

The breeding techniques involved in increasing fruit resistance and postharvest disease control is priming. This is a novel adaptive strategy by inducing plant immune systems to have protection against disease (Pétriaccq et al. 2018). Priming can be long lasting and can be maintained throughout the plant life cycle (Luna et al. 2016) and can even be transmitted to the next generation (Rasmann et al. 2012), therefore representing a type of plant immunological memory. This phenomenon is characterized by increased activation of induced defense mechanisms. Stimuli can be from pathogens, beneficial microbes, or arthropods, as well as chemical and abiotic cues, thus triggering priming formation that acts as a warning signal. After perception of a stimulus, changes can occur in plants at physiological, transcriptional, metabolic, and epigenetic levels. This phase is called the priming of defense phase and is understood as the adaptive part of induced resistance (IR) (Mauch-Mani et al. 2017). After subsequent challenges, plants effectively mount a faster and/or stronger defense response that defines a postchallenge prime and results in increased resistance and/or stress tolerance. The potential for future use is very high and inexpensive; thus, detailed research support is needed.

Alternative biological control

Antagonistic microorganisms have been found to suppress the development of disease on the surface of the fruits. Controlling postharvest diseases of citrus fruit using antagonistic microorganisms is carried out in two ways: (a) using natural epiphytic antagonists that are already on the surface of the fruit; and (b) artificial introduction of selective microbial antagonists that control postharvest diseases. There have been many studies showing that antagonistic microorganisms are effective in controlling postharvest disease of citrus, apples, grapes, and strawberries, with various application methods (Table 3). In addition, some antagonistic microbial products have been developed from actively infected fruits. Until now, biological control has not completely replaced the use of fungicides or other chemicals in postharvest disease control. In the future, it is hoped that biological control can become more established as part of an

Table 3 Microorganism agents used for the successful control of postharvest diseases of citrus, apples, grapes, and strawberries

Microorganism agents	Pathogen	Mechanism of action	References
<i>Yeast</i>			
<i>Candida saitoana</i>	<i>Penicillium italicum</i>	Competition for nutrient and space, direct parasitism	El Ghaouth et al. (2002)
<i>C. oleophila</i>	<i>P. digitatum</i> <i>Botrytis cinerea</i> <i>Colletotrichum gloeosporioides</i> <i>P. expansum</i> <i>P. italicum</i> <i>Rhizopus stolonifer</i>	Induction of resistance	Droby et al. (2002), Liu et al. (2007), Spadaro (2012)
<i>C. sake</i>	<i>P. digitatum</i> <i>B. cinerea</i> <i>P. expansum</i>	ND	Spadaro (2012)
<i>C. ernobii</i>	<i>Lasiodiplodia theobromae</i>	Induction of resistance	Spadaro (2012)
<i>Kloeckera apiculata</i>	<i>P. italicum</i>	Competition for nutrient and space	Long et al. (2006)
<i>Wickerhamomyces anomalus</i>	<i>P. digitatum</i>	Antibiosis	Platania et al. (2012)
<i>Cryptococcus laurentii</i>	<i>Alternaria alternata</i> <i>B. cinerea</i> <i>R. stolonifer</i>	Antagonist	Wei et al. (2014)
<i>Pichia guilliermondii</i>	<i>A. alternata</i> <i>B. cinerea</i> <i>P. digitatum</i> <i>P. expansum</i> <i>P. italicum</i> <i>R. stolonifer</i>	Antagonist	Sui et al. (2015)
<i>Schwanniomyces vanrijiae</i> combination and ethanolic extracts of propolis (EEP)	<i>P. digitatum</i> <i>Mucor fructigena</i>	Antibiosis, antioxidant	Abo-Elyousr et al. (2021)
<i>Galactomyces geotrichum</i>	<i>M. fructigena</i>	Antagonist	Madbouly et al. (2020)
<i>Pichia kudriavzevii</i>	<i>M. fructigena</i>	Antagonist	Madbouly et al. (2020)
<i>Meyerozyma caribbica</i>	<i>C. gloeosporioides</i>	Antagonist	Navarro-Herrera and Ortíz-Moreno (2020)
<i>Rhodosporidium paludigenum</i>	<i>P. digitatum</i>	ND	Zhang et al. (2021)
<i>Bacteria</i>			
<i>Bacillus subtilis</i>	<i>P. digitatum</i> <i>A. citri</i> <i>B. cinerea</i> <i>C. gloeosporioides</i> <i>L. theobromae</i> <i>P. expansum</i> <i>P. italicum</i> <i>Phomopsis citri</i>	Antibiosis	Mohammadi et al. (2017)
<i>B. amyloliquefaciens</i>	<i>B. cinerea</i> <i>C. gloeosporioides</i> <i>L. theobromae</i> <i>P. digitatum</i> <i>P. expansum</i> <i>P. italicum</i> <i>R. stolonifer</i>	Antibiosis	Calvo et al. (2017)
<i>Streptomyces</i> sp. CACIS-1.5CA	<i>Colletotrichum</i> sp. <i>Alternaria</i> sp. <i>Aspergillus</i> sp. <i>Botrytis</i> sp. <i>Rhizoctonia</i> sp. <i>Rhizopus</i> sp.	Antagonist, antibiosis	Evangelista-Martínez et al. (2020)
<i>B. thuringiensis</i>	<i>Guignardia citricarpa</i>	Antibiosis, induction of resistance	Lucon et al. (2010)
<i>B. licheniformis</i>	<i>C. gloeosporioides</i> <i>Dothiorella gregaria</i>	Antibiosis, induction of resistance	Spadaro (2012)
<i>B. pumilus</i>	<i>B. cinerea</i>	Antibiosis, induction of resistance	Abd-El-Kareem et al. (2021)
<i>Burkholderia cepacia</i>	<i>C. musae</i>	Antibiosis	Spadaro (2012)

Table 3 (continued)

Microorganism agents	Pathogen	Mechanism of action	References
<i>B. gladioli</i>	<i>P. digitatum</i> <i>P. expansum</i>	Antibiosis	Spadaro (2012)
<i>B. glathei</i>	<i>P. digitatum</i>	Antibiosis	Spadaro (2012)
<i>Brevundimunas diminuta</i>	<i>C. gloeosporioides</i>	Antibiosis	Spadaro (2012)
<i>Enterobacter aerogenes</i>	<i>A. alternata</i>	Antibiosis	Nabi et al. (2017)
<i>E. cloacae</i>	<i>R. stolonifer</i>	Antibiosis	Spadaro (2012)
<i>Pantoea agglomerans</i>	<i>Penicillium</i> spp. <i>P. digitatum</i> <i>P. expansum</i> <i>P. italicum</i> <i>R. stolonifer</i>	Antibiosis, triggers H ₂ O ₂ production and enzymatic activities	Spadaro (2012)
<i>Pseudomonas aeruginosa</i>	<i>Erwinia carotovora</i>	Antibiosis	Spadaro (2012)
<i>P. cepacia</i>	<i>B. cinerea</i> <i>P. digitatum</i> <i>P. expansum</i>	Antibiosis	Spadaro (2012)
<i>P. fluorescens</i>	<i>B. cinerea</i> <i>P. expansum</i>	Antibiosis	Wallace et al. (2018)
<i>P. corrugata</i>	<i>Monilinia fructicola</i>	Induction of resistance	Benhamou (2004)
<i>P. putida</i>	<i>E. carotovora</i>	Antibiosis	Spadaro (2012)
<i>P. syringae</i>	<i>B. cinerea</i> <i>G. candidum</i> <i>M. fructicola</i> <i>P. digitatum</i> <i>P. expansum</i> <i>P. italicum</i>	Antibiosis	Spadaro (2012)
<i>Serratia plymuthica</i>	<i>Penicillium</i> spp.	Antibiosis and competition for nutrient	Meziane et al. (2006)
Fungi			
<i>Verticillium lecanii</i>	<i>P. digitatum</i>	Induction of resistance	Benhamou (2004)
<i>Aureobasidium pullulans</i>	<i>P. digitatum</i> <i>B. cinerea</i> <i>P. expansum</i> <i>R. stolonifer</i>	Antibiosis	Spadaro (2012)
<i>Muscodor albus</i>	<i>P. digitatum</i> <i>P. expansum</i> <i>B. cinerea</i> <i>Geotrichum citri-aurantii</i>	Production of volatile compounds	Mercier and Smilanick (2005)
<i>Acremonium breve</i>	<i>B. cinerea</i>	ND	Spadaro (2012)
<i>T. harzianum</i>	<i>B. cinerea</i> <i>R. stolonifer</i> <i>P. expansum</i>	Antagonist	Sharma et al. (2009)
<i>T. viride</i>	<i>P. digitatum</i> <i>B. cinerea</i>	Antagonist	Sharma et al. (2009)
<i>Trichoderma</i> spp.	<i>L. theobromae</i>	Antagonist	Sharma et al. (2009)

integrated program of controlling major postharvest diseases caused by fungi.

Yeast as a biocontrol agent

Yeast is one of the best and safe alternative ingredients for the biological control of citrus, apples, grapes, and strawberries. The mechanism of action of yeast in postharvest disease inhibition includes competition for nutrients and the ability to form biofilms and colonize wounds (Parafati

et al. 2016). Furthermore, yeast can produce extracellular polysaccharides, increasing its resistance and limiting the growth of pathogenic propagules. In addition, yeast can use nutrients quickly and reproduce at high speed (Sharma et al. 2009). Yeast can also tolerate common pesticides such as imazalil, tiabendazole, pyrimethanil, or fludioxonil (Perez et al. 2017). Several types of yeast were successful in controlling citrus fruit rot disease, namely *C. saitoana*, *C. famata*, *C. oleophila*, *C. sake*, *C.*

ernobii, *Kloeckera apiculata*, *Wickerhamomyces anomalus*, *Cryptococcus laurentii*, and *Pichia guilliermondii* (Platania et al. 2012). Results of the latest invention showed that several yeasts, including *Meyerozyma caribbica*, have the potential as a promising biocontrol gene for the prevention of anthracnose (*C. gloeosporioides*) in passion fruit (Navarro-Herrera and Ortíz-Moreno 2020). Abo-Elyousr et al. (2021) reported that *S. vanrijae* was also effective to control *P. digitatum* on lemon. Inhibition of the conidia of *M. fructigena* from apples in vitro was demonstrated by *S. vanrijae*, *G. geotrichum*, and *Pichia kudriavzevii* from 67.6 to 89.2% and significantly reduced the incidence of disease, respectively, up to 84.02–89.5, 80.1–86.9, 56.3–86.9% than the control (Madbouly et al. 2020). However, despite many reports regarding yeast as a biological control agent, the number of commercial products available is still limited in Indonesia.

Bacteria as a biocontrol agent

The use of bacteria for postharvest disease control of fruits is one step ahead. Its products are available as bio-fungicides. It is easy to obtain bacteria as control material because they are available abundantly in nature. In most plant species, rhizosphere bacteria can be isolated from the surface of plant tissues, soil, roots, and various plants of the rhizosphere. In addition, there are endophytic bacteria in demand as biocontrol agents because they can colonize plant tissues to form internal defenses against pathogens (Liu et al. 2013).

Antagonistic bacteria are well known for producing substances with antifungal and antibacterial properties (Yáñez-Mendizábal et al. 2011). *Bacillus* and *Pseudomonas* genera are effective antagonists in controlling pathogens because they contain the chitinase enzyme that can hydrolyze the β -1,4 bonds among N-acetylglucosamine (NacGlc) in chitin; this polysaccharide polymer makes up the cell walls of several pathogenic fungi and the exoskeleton of invertebrates. Therefore, chitinase is well known for its antifungal or microbial properties (Yu et al. 2008). Mohammadi et al. (2017) reported the isolation of extracellular enzymatic activity of potential bacteria (*B. subtilis*, *B. pumilus*, *B. megaterium*, and *Agrobacterium radiobacter*) to control blue mold diseases in citrus fruit (*P. digitatum*). Abd-El-Kareem et al. (2021) also reported the effectiveness of *B. pumilus* on strawberry black rot disease (*Rhizoctonia solani*, *F. solani*, and *Phytophthora* sp.). The results showed that the chitinase, glucanase, and protease enzyme reacted positively to control pathogenic fungi, except for the bacteria *A. radiobacter*. The inhibitory mechanism of bacteria is classified as antibiosis. Some of the antibiotics produced by antagonistic bacteria are iturin, an antifungal produced by *B. subtilis*, and pyrrolnitrin produced by *P. cepacia* (Layton et al.

2011). The process of antibiosis can also occur through the production of low molecular weight compounds produced by antagonistic bacteria, called volatile organic compounds (VOCs). This compound is active in low concentrations in several chemicals, such as alcohols, aldehydes, ketones, esters, lactones, terpenes, and sulfur compounds. Due to their volatility, these compounds can safely be transported long distances in heterogeneous environments in the form of solid, liquid, or gaseous compounds. He et al. (2020) reported that VOCs produced by *B. methylotrophicus* BCN2 and *B. thuringiensis* BCN10 were effective in controlling and suppressing the mycelium of five postharvest pathogens isolated from loquat fruits, namely *F. oxysporum*, *Botryosphaeria* sp., *T. atroviride*, *C. oryzae*, and *P. expansum* based on in vitro and in vivo experiments. The final results showed that the loquats remained fresh for ten days significantly. Significant progress was also found in the control of postharvest citrus diseases with the use of antagonistic bacteria such as *B. subtilis*, *B. amyloliquefaciens*, *P. fluorescens*, *B. thuringiensis*, *B. licheniformis*, *B. pumilus*, *B. cepacia*, *B. gladioli*, *B. glathei*, *B. diminuta*, *E. aerogenes*, *E. cloacae*, *P. agglomerans*, *P. aeruginosa*, *P. corrugata*, *P. putid*, *P. syringae*, and *S. plymuthica* (Table 2). *P. fluorescens* produces secondary metabolites products include: phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol, polysaccharides, and many others (Soesanto 2009).

Currently, there are 3 commercial *Pseudomonas* formulations are listed in the USA: Bio-Save 10 LP, Bio-Save 11 LP, and BlightBan A506 (Stockwell and Stack 2007). Bio-Save 10 LP and Bio-Save 11 LP contain the *P. syringae* strains ESC-10 and ESC-11, respectively. This product is applied in packaging stations to prevent postharvest fungal diseases on oranges, pome, stone fruit, and potatoes during storage. BlightBan A506 contains *P. fluorescens* strain A506 and is mainly applied to pear and apple trees during bloom to suppress bacterial blight. This biocontrol product can inhibit fungal diseases with moderate to very good criteria. In addition, the procedure is relatively easy to implement and can be integrated with conventional products for disease control. In 2020, it was reported that *Streptomyces* sp. CACIS-1.5CA was effective against the pathogenic fungi *Colletotrichum* sp., *Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Rhizoctonia* sp., and *Rhizopus* sp. with percentages ranging from 30 to 63% (Evangelista-Martínez et al. 2020).

Fungi as a biocontrol agent

Utilization of antagonistic fungi to control postharvest diseases is less developed than those of yeast and bacteria. Antagonistic fungi show a broad spectrum of disease control but have volatile antimicrobial compounds (Mercier et al. 2007). Six antagonistic fungi (*V. lecanii*,

A. pullulans, *M. albus*, *A. breve*, *T. harzianum*, *T. viride*, and *Trichoderma* spp.) were reported to control citrus and subtropical fruit diseases (Table 2). The fungi biofumigant *M. albus*, which produces specific low molecular weight volatiles, has been used to fumigate lemons in storehouses and has successfully controlled green fungal pathogens and acid rot (Mercier and Smilanick 2005). This fungus produces 28 volatile organic compounds that exhibit some inhibitory effects against fungal and bacterial pathogens (Strobel et al. 2001). This antagonism occurs due to antimicrobial metabolites (Verma et al. 2007). The potential as fungal antagonists can be increased continuously with improved isolation, formulation, and application methods, particularly in the post-harvest environment.

Strategy of implementing biological control for postharvest diseases in Indonesia

The application of biological control in postharvest diseases of citrus, apples, grapes, and strawberries has not been studied on a large scale. In traditional Indonesian markets, biological control is rarely implemented. Biolog-

ical control alone is not quite popular and less desirable in Indonesia because their effectiveness as inhibitors are lower than synthetic pesticides. The biological control is also biodegradable and must be applied repeatedly.

The main objective of developing a postharvest strategy is to ensure fruit products are safe and present the lowest possible risk. Therefore, a multi-method was

applied in the supply chain from harvest to market to prevent cross-contamination and the spread of pathogenic contamination to the downstream process. This method was based on intervention using technology at critical points during the harvesting, handling, storage, shipping, and marketing processes, by integrating a field-to-supermarket approach. The concept of post-harvest disease control was about using several methods, each of which reduces the percentage of damage by a certain amount, mutually additive and synergistic to produce control with 97–99% effectiveness (Fig. 1).

The following is a strategy for implementing postharvest disease biological control in Indonesia. Through mutually additive and synergistic multiple reduction methods in cooperation with the vanguards, post-harvest disease control emphasizes disease prevention using several methods. Each method reduces the percentage of damage by a certain amount to produce highly effective controls carried out sequentially or together (Fig. 2). This multi-step method is divided into preventive and curative controls. Preventive control strategies are as follows:

Action 1 = reducing the inoculum level through sanitation and/or careful harvesting and handling

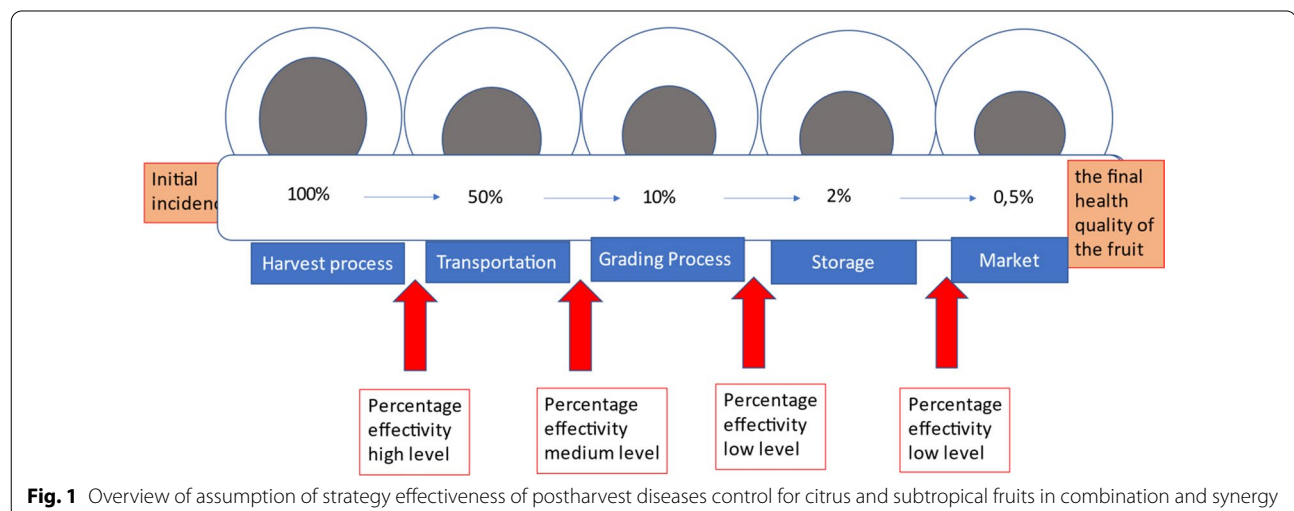
Action 2 = disinfectant treatment

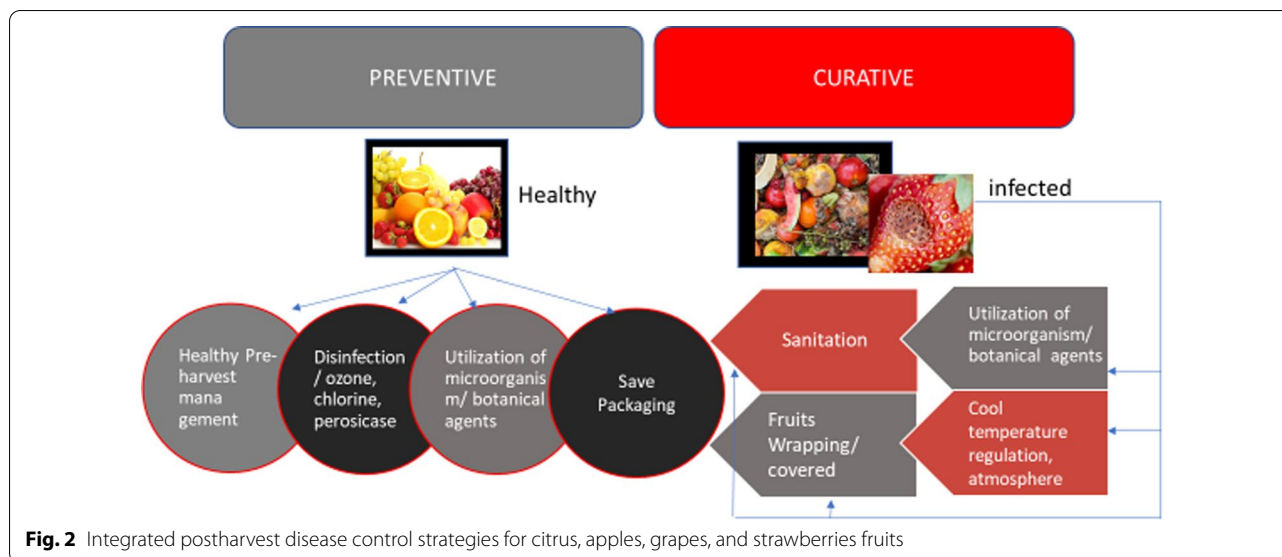
Action 3 = application of microbial antagonists

Action 4 = cold storage, modified or controlled atmosphere storage or packaging

ical control alone is not quite popular and less desirable in Indonesia because their effectiveness as inhibitors are lower than synthetic pesticides. The biological control is also biodegradable and must be applied repeatedly.

Some of the problems in the supply chain include the long distance between orchards and customers and the hilly and muddy, especially when it rains. Apart from being muddy, steep roads are also a problem to reach locations. Therefore, collaboration with local governments or ministries such as the Ministry of Agriculture,





of Industry, of Trade and Cooperatives, and of Tourism as market owners is highly recommended. In addition, fruit-picking tourism needs to be increased by promotion, especially before the main harvest season. The strategy that can be taken to keep strawberries fresh is to handle them immediately without delay after they are harvested.

Conclusions

Among postharvest diseases of citrus and subtropical fruit (apples, grapes, strawberries) found in Indonesia, some contain mycotoxins that are harmful to human health, including *Fusarium*, *Aspergillus* sp., *A. terreus*, *Penicillium* sp., and *Alternaria* sp. The biological agents in the yeast group (*Candida* spp.) have the opportunity to be a powerful postharvest disease controller. It has been developed abroad and can be synergized with the fungal groups (*Trichoderma* spp.) and antagonists bacteria (*Bacillus* spp. and *P. fluorescens*) found in Indonesia that are stable and have a broad spectrum. The potential botanical agents found in Indonesia are easy to make, have a broad spectrum, and can be combined with certain biological agents and chemicals. Through mutually additive and synergistic multiple reduction methods in cooperation with the vanguards, postharvest disease control emphasizes disease prevention using several methods. Each method reduces the percentage of damage by a certain amount to produce highly effective controls.

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Competing interests

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