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ANTAGONISTIC YEASTS AND ALTERNATIVE CHEMICAL COMBINATIONS AS POTENTIAL BIOCONTROL AGENTS IN POSTHARVEST APPLICATION

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Abstract
Synthetic chemical residues inflict unrepairable damage on living things and the ecology, hence the need to search for safer and more effective alternative methods for postharvest disease management. To make these alternative methods economically viable, their resilient and persistent performance must be improved. Several studies suggest that combining two or more alternative biocontrol agents give them broad-spectrum potency against postharvest diseases. In line with this, remarkable progress has been made toward development of multifaceted approaches at each stage of the disease control process. The present review highlights these alternative integrated biocontrol measures, their consequential effects on stored fruit and corresponding mechanisms of action in postharvest disease control. It also reviewed the challenges involved in the application of postharvest biocontrol agents.

Key words: ***Postharvest, Penicillium expansum, Biological Agents, Unconventional Chemicals Compounds, Antagonistic Yeasts***

Introduction
In recent decades, there has been considerable attention on antimicrobial qualities of antagonistic yeasts as effective biological control (biocontrol) agents by enhancing their consistency and persistence against postharvest diseases. Apart from the physical methods (such as microwave and hot water) (Zhang et al., 2017a), some alternative chemical control methods involving plant hormones, plant defense elicitors, and adjuvants have shown the potency to offer such improved capacity to antagonistic yeasts. Although, alternative chemical compounds used as single treatments have shown various degrees of microbial control, satisfactory results are expected from different agents combined (Zhang et al., 2017b). Limitations of using single agents may include inability to provide comprehensive protection, instability of efficacy, short-lived effect or detrimental effects on the quality attributes of produce (Mahunu et al., 2015). Over the years, minimal toxicity substances such as food additives, preservatives, or those generally recognized as safe (GRAS) substances have demonstrated compatibility with antagonistic yeasts against fungal diseases of stored fruit.

Fruits are highly vulnerable to fungal infection, especially at the stage of ripening due to modifications of pH upwards, skin softening, carbohydrates breakdown, and decline in defense mechanisms (Salas et al., 2017). All these changes make conditions favourable for fungal invasion. Fungal attack has contributed significantly to deterioration of pome fruit especially. The various genera of fungi such as *Penicillium, Alternaria and Aspergillus* have been reported to cause most important postharvest diseases (Liu et al., 2013). Therefore, this paper highlighted the alternative integrated biocontrol agents, their consequential effect on stored fruit and corresponding mode of action of disease management at storage were summarized. Lastly, the challenges encountered during the application of biological control agents were also highlighted.

Alternative Chemical Combinations for Enhancing Biocontrol Activities of Antagonistic Yeasts

Integration of two or more biocontrol agents involving unconventional chemical compounds and antagonistic yeasts to treat fruits and vegetables at postharvest have attracted special attention. This occurrence has progressed to the development of novel and nontoxic control agents to replace those produced from synthetic chemical constituents. The presence of bioactive compounds represents important components to enhance the capabilities of antagonistic yeast to acquire a broad-spectrum control effect (Wisniewski et al., 2016) and protect produce against spoilage (Tian et al., 2000). Actually, the advantage of yeast suspensions in postharvest applications is that they precipitate naturally, which tend to aid fruit protection against diseases. The food industry has benefited immensely from the use of antagonistic yeast through the various ways of action including antimicrobial activity, competition for space and nutrients, acidification of medium, capacity to resist stressful conditions (including ethanol) (Prusky et al., 2004; Spadaro & Droby, 2016). For instance, antagonistic yeast treated with alternative chemical (phytic acid or glycine betaine) have initiated increases in yeast population and blocked the germination of spore and subsequent growth of pathogen mycelia (Mahunu et al., 2016; Zhang et al., 2017a).

Some substances may also play the role of disease resistance elicitors and possibly trigger various genes of plant in different ways according to the task set by diverse pathogens. Elicitors are combination of chemicals that produce resistance in host-plants; here, the various biosynthetic paths are activated based on the compound present. Some of these bioactive compound elicitors have been studied with respect to actions of the various defense-related enzymes. Substances such as chitin has been certified by the United States (US) Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) as biodegradable hemostatic agent and food additive respectively. According to Prashanth and Tharanathan (2007), chitin is the second most abundant natural biopolymer compound after cellulose. It has been used extensively in agriculture and food industries. As natural compounds, chitin and its de-acetylated derivative chitosan have antimicrobial activity capable of stimulating defense responses in plant tissues (Fisk et al., 2008). Like other compounds, the use of chitin and its derivative to treat antagonistic yeasts has demonstrated the control of postharvest diseases and subsequent improvement of shelf life of fruits and vegetables. Similarly, the combination of three agents (*C. laurentii*+ chitosan +calcium chloride) showed even more control efficacy and steady reduction of blue mold in pome fruits than the use of any single agent (Yu et al., 2012).

Elicitors such as salicylic acid (SA), jasmonic acid (JA), brassinosteroids (BRs) and strigolactones are among plant hormones that play essential roles in the plant defense system. They have been widely accepted for their performance in stress tolerance or application in plant growth and development, aiding the regulation of resistance in plants either acting alone or in union with primary defense hormones (Torres-Vera et al., 2014). The mechanism of plant reactions to pathogen may be influenced by the relationship between pathways of the hormone present (Figure 1).

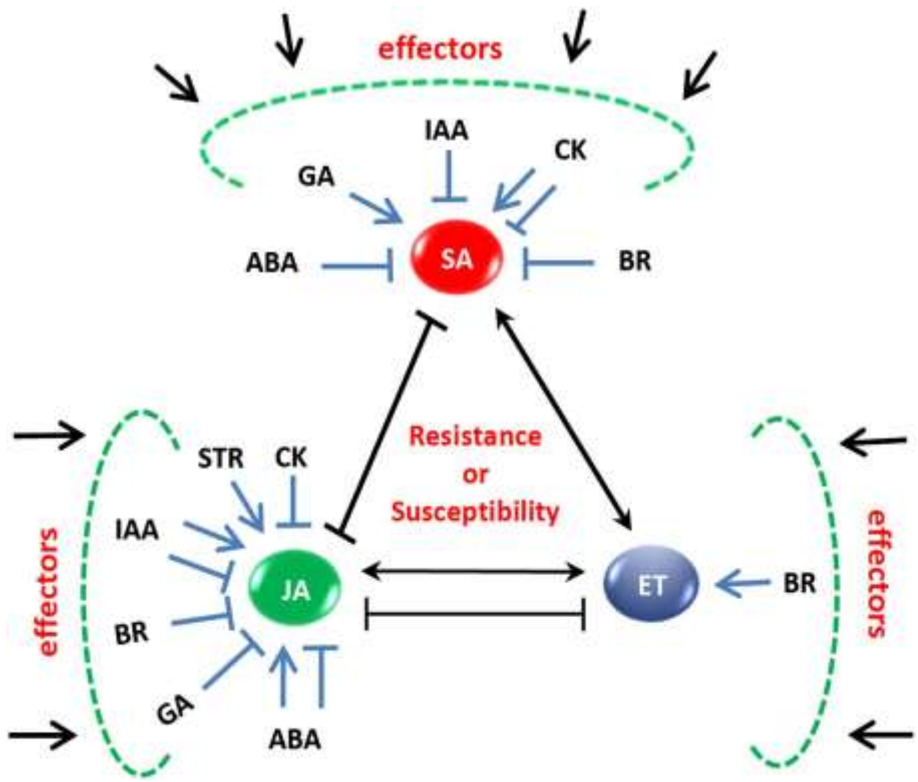

Figure 1. Complex Signaling Interactions among Phytohormone Pathways (Torres-Vera et al., 2014).

Either microbe- or elicitor-stimulated transduction pathways can result in various effects including cell wall reinforcement; phytoalexins (antimicrobial metabolites) production pathogenesis-related (PR) proteins and protection of oxidative stress enzymes; production of lignin; and then hypersensitive response (HR). All these reactive responses are methods of programmed host cell death, which restricts the development of pathogen at the infection cortex. For instance, the presence of salicylic acid has the tendency to trigger defense responses and produce a long-term systemic acquired resistance (SAR) in the whole palnt system against a large spectrum of pathogens. Wang et al. (2013) indicated that the induction of transcription of PRs genes by oligandrin increased the postharvest disease resistance of tomato fruit, which could also be linked to its systemic acquired resistance. Essentially, food additives have acted to improve the life span of stored food and stimulated growth and efficiency of antagonistic yeasts in general. Over the years, considerable interest has grown towards the use of food additives for improvement of antagonistic microbes' bio-efficacy. Table 1 shows selected exogenous bioactive compounds that have been used successfully to hasten antagonistic microbe activities but suppressed postharvest

pathogen.

Table 1: Concentration of exogenous compounds detected to enhance antagonistic yeast against fruit decay pathogens

Additives/concentration	Antagonistic yeast	Host/Produce	Pathogen	References
Salicylic acid (100 µg/ml)	*R. mucilaginosa*	Strawberries	*Rhizopus stolonifer*	(Zhang et al., 2010).
Salicylic acid (100 µg/ml)	*R. glutinis*	Strawberries	*Botrytis cinerea*	(Zhang et al., 2010)
Chitin (0.5% w/v)	*R. glutinis*	grey mold	strawberries	(Ge et al., 2010)
calcium chloride $CaCl_2$ (0.5% w/v)	*Rhodotorula paludigenum*	cherry tomatoes	*A. alternata*	(Wang et al., 2010)
Salt NaCl (6.6% w/v)	*Rhodotorula paludigenum*	pears and Chinese winter jujubes	*P. expansum* *A. alternata*	(Wang et al., 2010)
Sodium carboxymethyl cellulose (0.3% w/v)	*Rhodotorula paludigenum*	Jujubes	*A. alternata*	(Wang et al., 2011).
calcium chloride ($CaCl_2$)	*C. laurentii*	pear fruit	*P. expansum* *A. alternata*	(Yu et al., 2012)
Burdock fructooligosaccharide (0.32% w/v))	*R. mucilaginosa*	Peaches	*Rhizopus stolonifer* and *P. expansum*	(Zhang et al., 2013)
Phytic acid (4 µmol/ml)	*Rhodotorula mucilaginosa*	strawberries	*Botrytis cinerea*	(Zhang et al., 2013)
Ascorbic acid (250 µg/mL)	*P. caribbica*	Apples	*P. expansum*	(Li et al., 2014)
Phytic acid (4 mol/ml)	*Rhodotorula mucilaginosa*		*P. expansum*	(Yang et al., 2015)
Phytic acid (0.2% v/v)	*P. caribbica*	Apple	*P. expansum*	(Mahunu et al., 2016)
Phosphatidylcholine (1.5%)	*Hanseniaspora uvarum*	Citrus	*Penicillium digitatum*	(Li et al., 2016)
Glycine betaine (1mM)	*P. caribbica*	Apple	*P. expansum*	(Zhang et al., 2017b)
Bamboo leaf flavonoid (0.01% w/v)	*P. caribbica*	Apple	*P. expansum*	(Mahunu et al., 2018)
Trehalose (0.5% w/v)	*Hanseniaspora uvarum*			(Apaliya et al., 2017; Apaliya et al., 2018)
Adansonia digitata seed extract	*Sporidiobolus pararoseus*	Apple	*P. expansum*	(Abdelhai et al., 2019)

Prospects of Essential Oils as Bioactive Compounds for Possible Integration with BCAs

Recently, the combination of two essential oils (EOs) yielded more effective conditions suitable for reducing decay, extend storage of perishable produce, without compromising the quality, nutrient standards as well as consumer acceptability (Sivakumar & Bautista-Baños, 2014). Increasingly, different bioactive compounds have shown promising applications in food industries. The bioactive component of EOs obtained from plants play essential roles in the biocontrol system. The key advantage in the application of these EOs against pathogen attack is reducing the speed of antimicrobial agent diffusion rate; thus, allowing their action to persist steadily. When higher dosages of active EOs are applied directly to surfaces of intact fruits, they are able to adhere to fruit surfaces, where they persist steadily and eventually out-stage the pathogen causing decay. Wax coating amended with EO is effective in preventing the inoculum capacity of the pathogen to initiate fruit decay. The presence of coatings on fruit surfaces can also reduce weight loss during postharvest handling. Here, the coating exhibits its higher water vapour resistance properties (Sánchez-González et al., 2011), which serve as a barrier to dehydration on fruit surfaces.

Generally, the properties of EOs (therapeutic, antimicrobial and antioxidant activities) serve as important raw materials in food and other manufacturing industries (Prins et al., 2010). EOs can also be used to manufacture herbicides, pesticides and anticancer agents due to their wide biological activities. As a collection of secondary metabolites, some EOs can act in plant defense or may have ecological functions such as pollinator attraction (Prins et al., 2010). EOs extracted from plant materials like garlic, sesame, baobab, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, eucalyptus, ginger, rosemary, and peppermint have antimicrobial properties (Hyldgaard et al., 2012). Notably, they have a wide range of antibacterial properties as well as being insecticidal antiparasitic, antiviral, antifungal and antioxidants (Silva et al., 2011). EOs can also function as growth promoters in animal husbandry (Ahmadifar et al., 2011) and this application can be explored further

The volatile lipophilic components of EOs can degrade and evaporate simultaneously. Therefore, it is necessary to make suitable formulations with biodegradable compounds that can protect (encapsulate) them from degradation and evaporation and at the same time allowing for their sustained release. These qualities will support their commercial applications. There was possible encapsulation of EOs with calcium chloride or Ca-alginate microspheres, which led to effective decline in evaporation rate and increased the potential of antifungal activity for pathogen suppression (Soliman et al., 2013). Also, cassia oil applied together with magnesium sulphate ($MgSO_4$) against *A. alternata* was investigated. The results revealed that integration effect for cassia oil was detected at 500 µl/ml and 0.25% (w/v) for $MgSO_4$, which reduced rot of cherry tomatoes stored for 3 days at 20 °C (Feng et al., 2008). Similarly, Feng et al. (2008) demonstrated that thyme oil was more effective as a fumigant than through direct contact treatment of cherry tomatoes against *A. alternata*. Various studies have been conducted on EOs for their mechanism of action, which confirmed their ability to breakdown cell membrane and improve the ATP permeability through cytoplasmic membrane (Li et al., 2014). Active ingredients in EOs can restrict or terminate fungal growth in any of the two ways: temporarily as fungistatic effect or permanently as fungicidal effect. The most important attribute of cassia oil is the antifungal activity, which is proportional to its concentration and exposure time (Feng et al., 2011).

Generally, antimicrobial activities are linked to EOs and this is mainly due to the presence of phenolic and alcoholic compounds. In treatment mixtures, various elements of EOs come together to produce the antifungal effect. Besides, some phenolic compounds that possess the antimicrobial properties are also considered to be involved in plant defense reactions as low molecular weight defense chemicals**.** Similarly, biofilms properties of EOs are essential for protecting yeasts and for that matter are able to perform their important roles as biocontrol agents. It was also noticed that the monoterpene components of EOs can increase the biofilm formation in certain microorganisms (Sandasi et al., 2008). These biofilms are proffered adaptation mechanisms of natural microorganisms to unstable biocontrol environments, serving as a form protective microenvironments for the microorganism (s) involved (Mitra et al., 2012).

Some food-grade dilution-stable microemulsions are now drawing attention in food preservation and may contribute to the improvement of postharvest disease control. They have many important uses commercially. Subsequent to their introduction, the definition and concept of microemulsion have been modified in many instances (Lawrence & Rees, 2012). Microemulsions are the combinations of water and oil to constitute a sole optically isotropic and thermodynamically steady liquid solution. They are also described as a translucent or nearly transparent, quasi-homogeneous, thermodynamically constant blend of two immiscible liquids stabilized by a surfactant or mixtures. In this treatment agent, the medium-chain alcohols are often used as co-surfactants. Other names such as translucent emulsions, swollen micelles, micellar solutions and solubilized oils are often used for the description of these systems. Several of these formulations have mixed well with antagonistic microbes to control decay pathogens. Zhang et al. (2010) reported degradation of fungal cell viability caused by the application of microemulsion formulation (glycerol monolaurate, ethanol, Tween 40, sodium diacetate and water). In their investigation, there was complete viability loss of different fungal cells. It was observed that within first minute after treatment more than 99% of the fungal (*Aspergillus niger and P. expansum)* spores were damaged and within 2 min after treatment 99.9% of *C. albicans* cells were destroyed. According to the same report, 5 min after treatment the fungal cells had completely lost their viability. Again, assessment of citrus ('Satsuma' mandarin) fruit treated with thyme oil did show significant reduction of wound infections (85.9%) and intact fruit decay caused by sour rot (*Geotrichumcitri-aurantii*) also reduced by 65% after storage (Liu et al., 2009).

Investigations were conducted to determine the effect of antimicrobial microemulsions mode of action on bacterial membranes. The results revealed signifiant decrease of the bacterial cell surface hydrophobicity and the quick release of 260 nm-absorbing materials disrupted and caused dysfunction of the biological membranes and cell walls (Zhang et al., 2010). The response of microemulsion was attributed to the ability of surfactants to amend the visible hydrophobic surface of the microorganism’s cell wall, as a consequence of adsorption of surfactant on the microbial cell (Kaczorek et al., 2008).

Challenges of Biocontrol Agents Application in Postharvest Application

In order to attain a reasonable preservation efficacy with biocontrol agents, the viability of cells must become constant. The continuous cell viability can be achieved by amending their exogenous unconventional chemical products. As a matter of fact, several challenges contribute to the slow commercialization of promising candidates in the formulation of biocontrol agents/products. The following discussion will highlight some of these challenges.

First, it is often the situation that activities observed *in vitro* (in culture medium) have shown remarkable results than the actual activity *in vivo* performance. It was found that several microorganisms perform incredibly well under *in vitro* selection conditions but rather decline significantly in actual counts when introduced in targeted food samples. The observed differences in the two scenarios are joined to the complex host environment, which is characteristic of diverse factors impacting the growth and metabolism of culture solution or the biological availability and action of bioactive compounds. Interaction among population of the decay causing fungi, antagonistic microbe, the bioactive compound concentration, and the treatment time may all stimulate the biocontrol efficiency of treatments. Hence, it is extremely essential to authenticate definite biocontrol competence of the prospective bioprotective agent on its final products intended for usage.

Some biocontrol agents demonstrate strong enzymatic performance (proteolytic, amylolytic, or lipolytic); which directly affect texture and overall characteristics of products. Several investigations are still underway to establish the appropriate proportions of mixture components, which largely influence the extent of biocompatibility or incompatibility. This will, however, require optimization of the various mixture components to actualize biocontrol efficiency. Another key component is the well-being of the selected media, which is very important during selection of strains for biotechnological use. Safety evaluations where regulatory considerations need to be met were carried out by the Qualified Presumption of Safety (QPS) approach developed by the European Union. Often this approach is used to assess the safety of a broad range of biocontrol agents, which are sources of food and feed additives, enzymes, and plant protection products (Ricci et al., 2017). Generally, it can be used as premarket evaluation of microorganisms in food and feed production (Bourdichon et al., 2012) to guarantee their safe use. In all the above-mentioned cases, rigorous safety assessment procedures are needed to ensure that the intended and safe addition of biocontrol agents to fruit or food matrix lead to consumer safety while maintaining their bioprotective properties. Before microbial agents are selected for commercial application the following standards may be taken into consideration: (i) general historical knowledge and anticipated usage; (ii) definite taxonomy with precise species identification; and (iii) any likely threats to safety involving pathogenicity factors, resistance profiles of unwanted antibiotic (especially acquired resistances by means of horizontal gene transferal actions with possibility of further dissemination of trait), and adverse formation of compounds like biogenic amines, allergens, or toxins (Ricci et al., 2017), and (iv) to determine if the strain of fungi produces additional undesirable compounds specifically mycotoxins; if mycotoxin (s) is detected then it is excluded in industrial applications (Salas et al., 2017).

The manifold requirements and rigorous criteria that must be fulfilled have resulted in the release of only few commercially bioprotective products on the market. The preliminary evaluation must ensure high microbial stability, cell viability and maintenance of bioprotective activity during the storage period (Liu et al., 2013). There is the high possibility that the protective stability and/or activity of agents may be altered under commercial conditions. Therefore, careful testing under simulated commercial conditions must be undertaken to ensure the efficiency of protective cultures (formulations). Furthermore, viability of liquid cultures is difficult to maintain over an extended period. For this reason, the use of dry formulations is more advantageous since they have longer shelf life effect, easier to store under non-refrigerated conditions and easy to distribute (Li, Zhou, & Tian, 2008). More so, its ability to support satisfactory shelf life warrants efficient use of bioprotective agent. However, environmental conditions encountered in the host can influence antimicrobial activities positively or negatively. Nevertheless, it is appropriate to adhere strictly to thorough *in situ* evaluation of potential protective agents before upscaling. In some countries the regulations that define biocontrol agents can be elevated or varied frequently to suit changing trends. However, this may add to the cost of long research work and investment support.

Conclusion

The storage of perishable produce or foods is essential to make them available all-year-round. However, various fungal diseases are major contributors to significant losses during storage and for that matter threaten food safety and security. Therefore, development of practical management strategies remains essential to ensure quality, economic benefit and consumer safety to ensure that food is available all-year-round. Significant reduction of postharvest losses has been achieved by the treatment of harvested produce with antagonistic yeasts but then, their survival and biocontrol efficacy can further be augmented with unconventional chemical compounds. This paper underscored amendments with bioactive properties that influence various metabolic reactions and stress responses in biocontrol yeasts to prolong storage life of perishable produce without compromising their physicochemical parameters. Obviously, the respective treatment effects depend on the ability of bioactive compounds to boost the growth of biocontrol yeasts against produce decay.

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