ISSN- 2230-7346
*Journal of Global Trends in Pharmaceutical Sciences*

# ALTERNATIVE METHODS TO CONTROL *BOTRYTIS* INFECTION TO PLANT CROPS

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**ARTICLE INFO**

**Key words:**
*Bortytis* pathogens, fungicides, antifungal activity, Antimicrobial

Access this article onlineWebsite: https://www.jgtps.com
Quick Response Code:

**ABSTRACT**

*Botrytis* spp. are efficient pathogens, causing devastating diseases and significant crop losses in a wide variety of plant species. Moreover, changes in populations in response to selection by exposure to xenobiotics, especially fungicides, are quite common in the genus and fungicide resistance has been recorded in *Botrytis* populations throughout the history of the modern fungicide era. The application of fungicides has for years been an efficient way to control this disease. However, fungicides have negative environmental consequences that have changed popular opinion and clarified the need for more sustainable solutions. Biopesticides are products formulated based on microorganisms (bacteria or fungi) with antifungal activity through various mechanisms. The present review provides an overview of the recent primary physical, chemical, and biological control treatments adopted against the development of Botrytis in table grapes to extend shelf life. These treatments preserve product quality and safety.

## 1. Introduction

The genus *Botrytis* is highly diverse, with numerous species identified that differ in terms of their biology, ecology, morphological features and host range, Species of *Botrytis* are responsible for heavy losses in a number of economically important horticultural and floral crops (Elad *et al*., 2004 ) The plant hosts of *Botrytis* spp. include species of 170 families of cultivated plants (Walker *et al*., 2011 ; Plesken *et al*., 2015 ). The genus *Botrytis* contains around 35 species some have a very broad host range such as *B.cinerea* and *B.pseudocinerea* which cause gray mold disease for many important plants such as tomato, grape, apple , strawberry and among others (Elad *et al*., 2016). The gray mold disease characterized morphologically by a fine white or gray mycelium that occursover the infected necrotic or decayed area of the plant host (Spadaro *et al*., 2020). *Botrytis* spp. can develop on almost all plant tissues, at every developmental stage Early in the season, the presence of inoculum sources at stem emergence enables early blight infections to develop almost immediately. Infected young shoots only a few inches tall suddenly wilt and fall over due to lesions which can fully girdle the stem (Garfinkel and Chastagner 2016). *Botrytis* is considered anamorphic fungi, reproducing almost exclusively via asexual reproduction. A sexual stage of the fungal life cycle is known to exist, however this is rarely observed. The fungus favours prolonged leaf wetness (Ciliberti, Fermaud et al. 2015), and warm conditions, around 15-20°C, although it will infect at temperatures as low as 5°C (Bulger, Ellis et al. 1987). Hyphae, housed within sclerotia, present on the surface of overwintered decayed peony foliage, germinate under wet conditions in the spring. Within hours germ tubes emerge leading to the development of appressorium, specialized flattened hyphae. These contain penetration pegs which secrete enzymes, including cutinases and lipases (Schäfer 1998), as well as hydrogen peroxide to breach the cuticle and penetrate the host. Once breached, the penetration peg grows into the epidermal cells triggering an oxidative burst and cell death (Boddy 2016). These dead cells provide resources for fungal growth, where it will continue to survive as a saprotroph (or necrotroph in the case of plant death). Conidiophores, containing masses of conidia, asexual non-motile spores (the primary inoculum source for botrytis infections) are produced. Once mature, these are dispersed locally by water splash, or by plant movement, whilst further spread is facilitated by wind, insects or by crop workers and equipment/machinery. Secondary infections occur when conidia gain entry into plants via natural openings, or wounds. Once attached to the host, conidia germinate developing germ tubes for invasion of the host restarting the cycle which may repeat several times per year. Sclerotia may also form, often as a consequence of low nutrient availability or environmental conditions. These comprise a compact mass of melanised mycelium which enable long-term survival and overwintering of the fungus. In milder locations, conidia may develop on over-wintered mycelium, skipping the requirement for the sclerotial stage of the life cycle entirely (Garfinkel and Chastagner 2016). This can increase the inoculum level present during stem emergence leading to increase shoot collapse. In the future a warming climate may mean that conidia arising from overwintered mycelium may become the primary source of early inoculum. In addition to spread by conidia, infected petals may also act as a source of infection. Petal fall, and subsequent adhesion onto healthy leaves (often as a consequence of rainfall) enables close contact of mycelium from infected petals to directly infect healthy tissue (Daughtrey, Wick et al. 2000).

## 2. Control of *Botrytis* infection:

Although chemical control of *Botrytis* still largely relies on the use of chemical pesticides, suppression of diseases can significantly benefit from cultural management of crops mainly by restricting the humidity around the plant and by limiting plant surface wetness. Plant and microbial extracts, organic compounds, and biocontrol agents have become available in some countries for *Botrytis* suppression either for use at pre- or postharvest.

### 2.1. Physical Methods to Control infection

Physical technologies mainly include modification of several parameters such as temperature, absolute and relative gas pressure, UV irradiation, and sonication. Table grapes for fresh consumption often need a long period of storage for commercial purposes such as export and ready-to-eat. They are usually stored in chambers with strictly controlled temperature and humidity. To this aim, cold storage (~0 $^{0}$C) is the primary method to avoid post-harvest infections without affecting the main physicochemical features of the product (Youssef et al., 2015). However, Botrytis survives at low temperatures, and any variation of temperature can promote water condensation, thus favouring fungal growth and sporulation (Crisosto et al., 2002). In general, physical methods are often considered eco-friendly and residue-free emerging technologies, widely accepted by consumers. Although these methods have been extensively investigated in different fruit and vegetable products, only a few studies report their employment for the reduction of grey mould in table grapes. Ultraviolet irradiation (UV) (wavelengths between 10 to 400 nanometers (nm)) and sonication by ultrasound are non-thermal treatments considered simple, reliable, and eco-friendly emerging technologies for lengthening the shelf life of fresh fruits during storage. Ultraviolet irradiation C (UV-C, 10–280 nm) treatment induced a general stimulation of the phenylpropanoid pathway, associated with plant defence mechanisms, leading to an increased resistance to the diseases in artificially inoculated berries (Freitas et al., 2015). UV-C irradiation is effective, with dosages between 0.125 to 0.5 kJ/m2 at a fixed distance of 25 cm (Nigro et al**., 1998).**

### 2.2. Chemical Methods to Control infection:

At present, sulphur dioxide (SO2) remains the main method that is used to control the microbial spoilage of post-harvest fruit commodities. The employment of SO2 provides long term storage due to its antioxidant, antibacterial, antifungal and anti-browning properties (Youssef et al., 2015; Carter et al., 2015). However, excessive residue levels of SO2 in berry peels can result in quality deterioration, such as bleached berries, production of off-flavour, or hairline disorder (Gao et al., 2002; Zoffoli et al., 2008). Significant health risks to consumers are also reported due to the emergence of allergies, nausea, respiratory distress and skin rashes (Lou et al., 2017). For this reason, the United States Environmental Protection Agency (USEPA) categorized SO2 as a pesticide, with maximum tolerance in final products of 10 ppm, and, more generally, sulphur dioxide residuals on table grapes are internationally regulated, including in the European Union (European Commission, 2008). Its use is also excluded from certified “organic” grapes. Therefore, several chemical alternatives have been proposed to replace SO2 in the restraint of Botrytis in table grapes. The use of conventional synthetic fungicides is generating increasing concern among consumers due to the potential negative efects on human health (Muri et al., 2009), soil microbiota (Meena et al., 2020), and on microorganisms beneficial for food and beverage fermentations (Russo et al., 2019). Even if the use of conventional synthetic fungicides is forbidden for organic grapes (Council regulation, 2007) application is widespread to prevent spoilage mould formation in conventional agriculture (El Ghaouth et al., 2004). Despite the fact that some studies have focused on the positive action of different combinations of synthetic fungicides or bioactive compounds (Xu et al., 2018), the occurrence of resistant strains of Botrytis has been reported (Latorre et al., 2002). The most recently introduced class of synthetic fungicides belongs to the Succinate Dehydrogenase Inhibitors (SDHIs) (Avenot and Michailides, 2010). In 2012, a novel SDHI, named fluopyram, was registered against Botrytis and it was able to control grey mould infections in table grapes, with effecacy of inhibition in the range 80.1–94.4% (Vitale et al., 2016). However, high risks of rapid occurrence of resistance without appropriate management has already been underlined in other crops (Amiri et al., 2014). For this reason, alternative control methods are needed. Among these, resistance induced by elicitors, molecules able to activate defence gene expression and enhance their antimicrobial-related pathways (Ge et al., 2012), is an attractive alternative because it is associated with minor environmental risk.

### 2.3. Biological Methods to Control infection

#### 2.3.1. Microbial Resources

Several yeast species are found in association with the surface of the grapes, in particular, the genera Saccharomyces, Candida, Dekkera, Pichia, Hanseniaspora, Metschnikowia, Kluyveromyces, Saccharomycodes, Schizosaccharomyces, Torulaspora, and Zygosaccharomyces (Berbegal et al., 2017; Garofalo et al., 2016) . Highly variable in terms of relative proportion, often as a function of the sanitary condition of the grapes, these species have different significances in oenology, i.e., pro-technological, spoilage, biocontrol, production of toxic catabolites (Benito et al., 2019; Russo et al., 2016). On the other hand, it is possible to find prokaryotic organisms present on the grape surface that exert their biotechnological action in the last phases of the winemaking process (Berbegal et al., 2019). This broad microbial diversity justifies massive isolation of yeasts and bacteria to preserve and characterize strains of biotechnological interest (Zhang et al., 2019; Gómez et al., 2019). This isolation can be of microorganisms from plants, grape bunches, musts or wines and selection is made of those capable of inhibiting undesired microbe development on grapevines (Ab Rahman et al., 2018; Bleve et al., 2006) up to the final steps of wine production (Berbegal et al., 2017). This reservoir of microbial-based biocontrol solutions has also been exploited in fruits (Nally et al., 2015) in several cases offering the option to inhibit Botrytis in table grapes. Among yeast species, strains belonging to Saccharomyces are the most commonly studied because of their pivotal function in alcoholic fermentation and their role as a biological model organism (Walker and Stewart, 2016; Feyder et al., 2015). Recently, Nally et al. 2012 used a fruit decay test on wounded table grape berries to screen the activity of 65 yeasts, previously tested against Botrytis by using in vitro approaches. They found that 15 S. cerevisiae strains and one strain of Sch. pombe, isolated from grape must, were able to reduce grey mould decay . Among these, the disease incidence of grapes treated with Sch. pombe BSchp67 reached 29.9%, while 9 strains of S. cerevisiae were able to fully inhibit decay development when added at a concentration of 107 cells/mL . Regarding the non-Saccharomyces yeasts, H. uvarum is a species of enological interest, usually present on the grape surface (Tristezza et al., 2016; Capozzi et al., 2019) . In various studies, it has demonstrated an antagonistic property, mainly based on competition for living space (Liu et al., 2010). The addition of this yeast has been implicated in the reduced incidence of grey mould disease in artificially inoculated table grapes (Qin et al., 2015). Moreover, this antagonistic activity was enhanced by the addition in the formulation of salicylic acid or salts, such as sodium bicarbonate or ammonium molybdate (Cordero et al., 2017; Liu et al., 2010).Starmerella bacillaris (synonym Candida zemplinina) is another species of interest, commonly isolated from grapevines/musts (Russo et al., 2020; Russo et al., 2020).

### 2.3.2. Antimicrobial Compounds of Biological Origin:

Among the vegetal compounds, volatiles generated from cellulose soaked with garlic hydro-alcoholic extract and its derived sulfur compounds have shown anti-grey mould activity in packaged table grapes both at 4 and 25 $^{0}$C, during the 14 days of experimental trials (Campa et al., 2017). Cinnamic acid, extracted from cinnamon bark, is widely used as a food additive. Dipping the berries in a solution of 10 mM cinnamic acid can significantly decrease the incidence of decay development up to half of that in control after four days of storage at 25 $^{0}$C (Zhang et al., 2015). Hinokitiol is a natural monoterpenoid mainly extracted from the wood of Cupressaceae. In a recent study, no decay was visible after 60 h at 22 $^{0}$C in artificially wounded/inoculated table grape berries treated with a 3 g/L hinokitiol solution (Wang et al., 2020). Essential oils (EOs) from many plants, such as thymus and lemongrass, have revealed great potential in post-harvest disease control (Sivakumar and Bautista, 2014). In addition, the effect of mint EOs was recently investigated by using direct contact (e.g., dipping) and volatile methods (filter paper) (Xueuan et al., 2018). In this study, EO released by the paper was more effective than the direct contact and was capable of inhibiting Botrytis in artificially inoculated trials during nine days of shelf-life .However, the effect on product flavour and consumer acceptance was not investigated. Another research field involves the use of vegetal hormones, plant activators, and inner signaling molecules. These molecules act through a complex signalling network under the control of salicylic acid, ethylene, jasmonic acid, and phenylpropanoid pathways, which leads to the increase of specific secondary metabolites (e.g., flavonoids, soluble sugars, and phytoalexins). Methyl jasmonate is a volatile compound that mediates stress responses in plants and has shown to promote fungal resistance in various fruit crops. Recently, it was found to be effective in lessening the development of Botrytis in artificially infected table grapes (Jiang et al., 2015). In this study, the fruits were packed in the presence of a filter paper soaked with a solution of methyl jasmonate at 10 mol/L and stored at 25 $^{0}$C. The disease incidence in the treated fruits after 24, 36, and 48 h was 41.7%, 60.6%, and 86.5% of that in the control trial, respectively. Among secondary metabolites, phytoalexins are synthesized by the plants as broad-spectrum inhibitors. Stilbenoids, including pterostilbene and piceatannol, are phytoalexins commonly found in vine leaves and wine (Xu et al., 2018). "Mare's milk" table grapes treated with 50 mg/L pterostilbene did not show any sign of infection while piceatannol at the same concentration reduced grey mould disease by 75% after nine days storage at 22 $^{0}$C .These molecules seemed to be the most effective in a group of seven phenolic compounds, including resveratrol and coumarin .Edible coatings made with natural polymers like chitosan or alginate can act as a cover material able to wrap the berry. Thus, these formulations can extend the shelf-life of fruit crops and maintain quality reducing water losses (Zhou et al., 2008; Azarakhsh et al., 2014) Chitosan is a linear polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine linked by a ß-(1-4) bond obtained by treating the exoskeleton of arthropods with alkaline solutions. Recently, it was found that chitosan-silica nanocomposite polymers can reduce the incidence of decay in grape berries by 59% (Youssef et al., 2019). Alginate is another biocompatible and biodegradable polymer extracted from brown algae and used as a food additive with the code E401. It was demonstrated that the incorporation of vanillin, a phenolic compound, in a coating formulation prolongs the shelf life of table grapes until 35 days of storage, by reducing total yeasts and mould counts (Takma et al., 2017). However, the retention of soluble solids, titratable acidity, firmness, and color was also enhanced.

**Conclusion:**

More research needs to be developed to identify the bioactive components in biological extracts which may add to crops to excess their resistant against *Botrytis* infection

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