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*Communication*

# Effects of Commercial Natural Compounds on Postharvest Decay of Strawberry Fruit

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**Abstract:** Gray mold and Rhizopus rot, which is caused by *Botrytis cinerea* and *Rhizopus stolonifer*, respectively, are the most destructive forms of postharvest decay of the strawberry fruit. In this work, we tested the effectiveness of the control on the postharvest decay of the strawberry fruit (*Fragaria* × *ananassa* Duch cv. 'Monterey') following postharvest applications of six commercial natural compounds: chitosan-based coating compound (1% of 'ChitP', 'ChitS', 'ChitK', 'ChitO'), commercial essential oil (EOs) products based on grapefruit seed extract (0.5% of 'GraFr'), sweet orange (0.5% of 'SwOr'), a product that included eugenol, geraniol, and thymol EO, (0.4% of 'EuGeTh'), an organic compound as humic acid (0.5% *w*/*v* of 'HuAc'), and, lastly, methyl jasmonate plant growth regulator (1% *v*/*v* 'MeJA'). Strawberries were dipped in solution for 30 s and incubated at room temperature (20 ± 0.5 °C) or at cold storage conditions (4 ± 0.5 °C) following 4 days of shelf life at 20 °C. The treatments with 'ChitP', 'ChitS', and 'ChitO' provided ~30%–40% reduction of gray mold in cold storage conditions, while the 'MeJA', 'SwOr', and 'GraFr' with high activities of volatile substances were more effective at controlling gray mold at room temperature. 'HuAc', 'ChitK', and 'ChitO' were more effective at controlling Rhizopus rot in both cold storage (~50%) and room temperature conditions.

**Keywords:** basic substances; *Botrytis cinerea*; *Rhizopus stolonifer*; strawberry



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## 1. Introduction

The strawberry fruit (*Fragaria* × *ananassa* Duch.) is highly appreciated by consumers for its unique taste and flavor as well as its health benefits and exceptional nutritional value [1,2]. Indeed, strawberries are rich in bioactive compounds, such as natural antioxidants, polyphenols, anthocyanins, vitamins, and amino acids [3–7]. However, strawberries are particularly perishable, especially during postharvest storage, and they are susceptible to both mechanical damage and fungal disease, which limits their commercialization and consumption [8]. Gray mold and Rhizopus soft rot caused by *Botrytis cinerea* (Pers.) and *Rhizopus stolonifer* (Ehrenb.), respectively, are the main pathogens of the postharvest decay of the strawberry [9,10]. A primary infection of gray mold could occur at bloom time and remain quiescent in the field [10,11]. *B. cinerea* produces large numbers of spores, and it was able to survive in a dormant state in a variety of environmental conditions. [12]. Therefore, it is not surprising that *B. cinerea* ranked second in the top 10 fungal plant pathogens list based on scientific and economic importance [13]. *R. stolonifer* is a common wound pathogen of a very wide range of fruits and vegetables, causing a rapidly spreading watery soft rot. Rhizopus rot can spread at temperatures greater than 4–6 °C. Both gray mold and Rhizopus soft rot spread quickly to other fruit, and this phenomenon is known as nesting [10,14].

Despite the effectiveness of the synthetic fungicides in the management of strawberry fruit disease, natural eco-friendly alternative compounds are desirable, and they have

attracted the attention of scientists, who aim to provide growers, consumers, and the whole community with information on the strategies that are effective and, at the same time, safer for consumers.

In recent years, the antimicrobial activity of a large number of compounds similar to plant and animal extracts, such as gums, resins, etc., have been tested against both pre-and postharvest pathogens [15,16]. These compounds were non-toxic for human health and the environment, had no negative effects on the quality of the fruits, and might complement or even improve current productive practices. Natural compounds are characterized by antimicrobial activities against the main postharvest pathogens and/or are resistance inducers that activate plant defenses in order to simulate the presence of a pathogen.

Among the natural compounds, chitosan has received much attention for its application in agriculture and in the food industry. Chitosan is a natural biopolymer, derived from chitin of both marine crustaceans [17] and the cell wall of many pathogenic fungi [18–20]. This compound has been reported to stimulate plant defenses and prevent disease development [15,21]. A number of promising approaches for the postharvest application of different types of chitosan formulation have been suggested [22–24], and the effectiveness of the combination of chitosan with essential oils (EOs) has also been observed [25–27].

Essential oils from aromatic plants have been gaining interest, and their effectiveness at controlling the postharvest decay of fruit has been documented [28–32]. Other compounds, such as humic acid [33,34], an organic compound known as a promoter in sustaining plant growth [35], have been reported to have efficacy in the control of several plant diseases, inducing host resistance and direct antimicrobial activity [36]. In the same way, methyl jasmonate (MeJA) [37] is an endogenous plant growth substance that can modulate many physiological processes, including responses to environmental stress [38].

The objective of this study was to verify the effectiveness of a list of promising commercial compounds (listed in Table 1) based on chitosan, EOs, organic compounds, and plant growth regulator on the control of the postharvest decay of strawberries kept at either room temperature or cold stored and then exposed to shelf life.

**Table 1.** Commercial names and sources of the formulations containing the active ingredients used in the postharvest treatments of strawberries.

| Name | Formulation Commercial Name | Source (Country) | Active Ingredient | Application Dose (*v*/*v*); (*w*/*v*) * |
|---|---|---|---|---|
| 'ChitP' | Chito Plant powder | ChiPro GmbH; (Bremen, Germany) | Chitosan | 1% * |
| 'ChitS' | Chito Plant Solution | ChiPro GmbH; (Bremen, Germany) | Chitosan | 1% |
| 'ChitO' | OII-YS | Venture Chemicals, Inc.; (Lafayette, LA, USA) | Chitosan | 1% |
| 'ChitK' | Kaitosol | Advanced Green Nanotechnologies Sdn Bhd; (Cambridge, UK) | Chitosan | 1% |
| 'GraFr' | DF-100 Forte | Agritalia, (Rovigo, Italy) | Grapefruit seed extract | 0.5% |
| 'SwOr' | Prev-Am plus | Nufram, (Milano, Italy) | Sweet orange extract | 0.5% |
| 'EuGeTh' | 3Logy | Sipcam, (Milano, Italy) | Eugenol, geraniol, and thymol extracts | 0.4% |
| 'HuAc' | Humic acid | Sigma-Aldrich, (Saint Louis, MO, USA) | Humic acid sodium salt | 0.5% * |
| 'MeJA' | Methyl jasmonate | Sigma-Aldrich, (Saint Louis, MO, USA) | Methyl jasmonate | 1% |

* = weight by volume solution (*w*/*v*).

## 2. Materials and Methods

### *2.1. Fruit Material*

Commercial strawberries (*Fragaria* × *ananassa* Duch, cv 'Monterey') were collected from an orchard located in Montalto (AP) in the Marche region in central-eastern Italy. The strawberries were harvested at the mature stage, and were selected for the absence of defects, uniformity in size, and the degree of ripening (2/3 red on the surface) [39]. They were used for the experiments on the day that they were harvested [14].

### *2.2. Preparation of Natural Compounds Solution*

A list of chitosan-based commercial compounds available on the market together with other formulation alternatives to synthetic fungicides that could have an effect on the postharvest decay of strawberries were included in the investigation. The compounds used for the postharvest treatments are summarized in Table 1. All of the compounds were dissolved in Tween 80, 20 µL/L (Sigma Chemical Co., St. Louis, MO, USA) water solution for 1 h.

### *2.3. Postharvest Treatments*

The strawberries were immersed in the solutions ready to be tested according to Feliziani et al. [15]. In detail, the strawberries were soaked for 30 s inside each solution, air dried for 3 to 4 h, and then arranged in small plastic boxes. They were incubated in two different conditions: room temperature (20 ± 0.5 °C) and cold temperature (4 ± 0.5 °C) for 7 days, 95%–98% RH, and they were then exposed to 4 days of shelf life at 20 °C, 95%–98% RH. Each treatment consisted of 66 fruits (6 fruits in 11 plastic boxes). Three replications were performed for each treatment. The infections that subsequently developed resulted in naturally occurring inoculum for the following treatments: (i.) natural compound solution (treated strawberry fruit), and (ii.) sterile distilled water (untreated strawberry fruit).

### *2.4. Data Recording*

During storage, data were recorded based on the percentage of the incidence of decay on the strawberries. Disease severity was also measured according to an empirical scale with five degrees: 0, healthy fruit; 1, 1%–20% fruit surface infected; 2, 21%–40% fruit surface infected; 3, 41%–60% fruit surface infected; 4, 61%–80% fruit surface infected; and 5, more than 81% of the strawberry surface infected and showing sporulation [15]. The empirical scale allowed the calculation of the McKinney index, which was expressed as the weighted average of the disease as a percentage of the maximum possible level [40,41]. This parameter also included information on both disease incidence and disease severity.

### *2.5. Statistical Analysis*

Statistical analysis was performed based on the Fisher test. Differences among the means of the values were analyzed by one-way analysis of variance (ANOVA). Difference was considered as statistically significant at $p < 0.05$. Moreover, the treatments were subjected to rank analysis that allowed us to combine heterogeneous data (Excel 2007) [42,43].

## 3. Results

### *Decay Evaluation*

The postharvest treatments with commercial compounds generally reduced the development of the decay of the strawberries after 4 days of shelf life at both room temperature (20 ± 1 °C) and cold temperature (4 ± 1 °C), which was mainly gray mold followed by Rhizopus rot. However, the more significant decrease in both disease incidence and severity was observed in the cold temperature condition (data not shown). The McKinney index of decay was significantly decreased compared to the control: the compounds based on chitosan, 'ChitP', 'ChitS', 'ChiK', and 'ChiO', had decreases of 35.36%, 26.82%, 24.39, and 45.12%, respectively, whilst the compounds based on EOs, 'GraFr', and 'EuGeTh', had

decreases of 28.65% and 29.26%, respectively, and, finally, those with 'MeJA' and 'HuAc' had decreases of 31.7% and 32.92%, respectively (Figure 1).

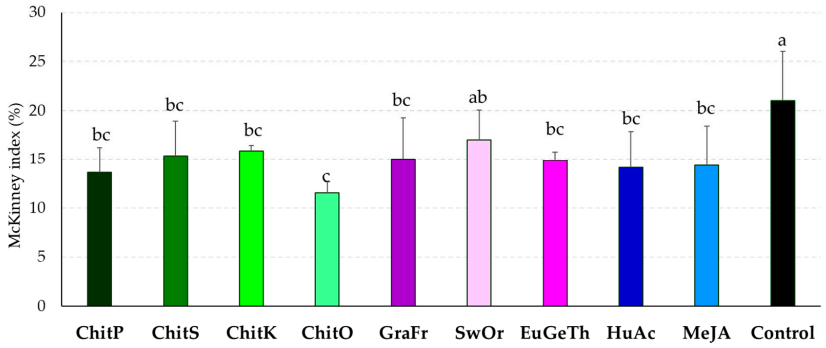


**Figure 1.** McKinney's index of gray mold of the 'Monterey' strawberry fruit. Strawberries were treated after harvest, stored for 7 days at 4 ± 0.5 °C, and then exposed to 4 days of shelf life at 20 ± 1 °C and 95% to 98% relative humidity. Values with different small letters are different at $p < 0.05$. Note: 'ChitP' = Chito Plant powder; 'ChitS' = Chito Plant solution; 'ChitK' = KaitoSol; 'ChitO' = OII-YS; 'GraFr' = DF-100 Forte; 'SwOr' = Prev-Am plus; 'EuGeTh' = 3Logy; 'HuAc' = Humic acid; 'MeJA' = methyl jasmonate.

The treatment with the 'SwOr' decreased the McKinney index of decay by 19.26%, although it did not show a significant reduction compared to the control. A more direct analysis of the degree of comparative effectiveness for the reduction of disease incidence was obtained through the application of rank analysis. At both room temperature and cold storage conditions, the untreated fruits had the highest sum of ranks, namely, 8.5 and 8.6, respectively, and, therefore, all of the treatments were more effective compared to the control (Figure 2). However, some differences occurred among the treatments at different storage temperatures. The commercial compounds 'ChitP', 'ChitS', and 'ChitO' were more effective at controlling postharvest disease in strawberries in cold storage conditions (sum of ranks 2.2, 3.1, and 5.2, respectively) compared to room temperature storage (sum of ranks 3.9, 4.1, and 7.5, respectively) (Figure 2). In contrast, the 'MeJA', 'SwOr', and 'GraFr' were more effective at controlling postharvest disease in strawberries at room temperature conditions (4.8, 2.5, and 2, respectively) compared to cold storage ones (8.4, 5.8, and 6.5, respectively). The other compounds tested showed efficiency at controlling postharvest rot in strawberries that was similar to the two storage conditions that we tested (Figure 2).

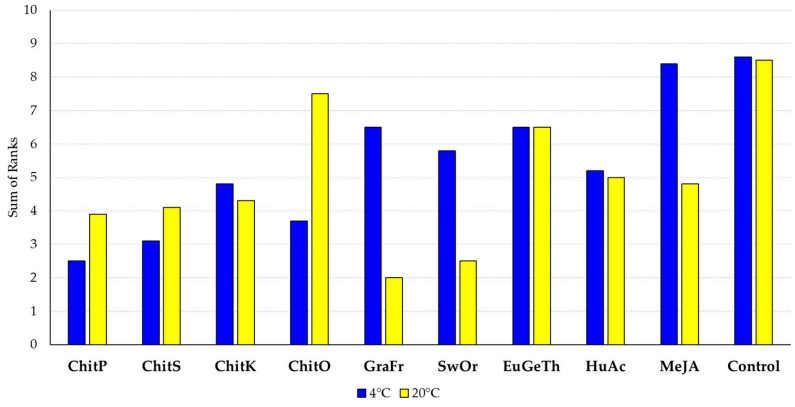


**Figure 2.** The effect of postharvest treatment with natural compounds on the reduction of gray mold on strawberries according to rank analysis. The fruit was kept at 4 °C and 20 ± 1 °C, 95%–98% RH. Note: 'ChitP' = 'Chito Plant powder; 'ChitS' = Chito Plant solution; 'ChitK' = KaitoSol; 'ChitO' = OII-YS; 'GraFr' = DF-100 Forte; 'SwOr' = Prev-Am plus; 'EuGeTh' = 3Logy; 'HuAc' = Humic acid; 'MeJA' = Methyl jasmonate.

Based on cumulative incidence, the effectiveness of different natural compounds against *R. stolonifer* on strawberries was measured. The Rhizopus rot cumulative incidence for all of the successful edible coating was about 1%–2% lower than the control at both temperatures. However, 'HuAc', 'ChitK', and 'ChitO' were the most successful compounds at reducing Rhizopus rot at cold storage conditions, and the cumulative incidence for these compounds was less than half of the control (data not shown).

## 4. Discussion

The present study shows that compounds from natural sources, such as chitosan different emulsions, commercial EOs, and organic plant growth regulator compounds with promising properties, can reduce the development of postharvest rots in strawberry fruits. All of the tested compounds significantly reduced decay on cold-stored strawberry fruits, and the best results were observed using the chitosan compounds. On strawberries kept at room temperature, the rank analysis showed that all of the tested compounds were effective at decay control compared to the control. The commercial products tested decreased the development of gray mold on strawberries, prolonging the shelf life of the fruit. Based on rank analysis, the effectiveness of the tested compounds was different according to the storage temperature of the strawberries: 'GraFr' and 'SwOr' provided the highest reduction of gray mold (76.4% and 70.5%, respectively, compared to the control) on strawberries kept at room temperature, while the compounds based on chitosan, 'ChitP' and 'ChitP', showed the best performance on cold-stored fruit (76.4% and 63.9%, respectively, compared to the control). The higher effectiveness of 'GraFr' and 'SwOr' at room temperature can be ascribed to their high activity of volatile composition. A similar result was also observed for the 'MeJa', a volatile compound that is an important cellular regulator, and which is able to reduce the gray mold and brown rot, thereby extending the shelf life of fruits [43,44]. Room temperature crucially influences the stability of EOs in several aspects. On these lipophilic compounds, which are highly volatile and plant secondary metabolites, the chemical reactions generally accelerate with increasing heat [45,46]. Consequently, the application method can affect the efficacy of postharvest treatments of EOs [29], as has been observed for the EO of oregano, red thyme, peppermint, and lemongrass incorporated in chitosan coatings on strawberry fruits [47]. Strong antifungal activity from the above EOs could be attributed to their components [48,49]. 'SwOr' and 'GraFr' consisted of sweet orange essential oils and grapefruit seed extract, respectively. The composition of 'EuGeTh' included eugenol, geraniol, and thymol, which are very well known for their bioactivity against fungal pathogens [50–52]. The activity of 'EuGeTh' as a biocontrol agent for grape vineyards against gray mold has also been observed [32]. In our work, we did not detect the same effectiveness on the postharvest strawberry treatment. Among the EO-based compounds, 'EuGeTh' was the least effective in the control of the storage decay of strawberries. Concerning the compounds based on chitosan, the refrigerated storage was effective in maintaining the postharvest quality of strawberries. The effectiveness of chitosan in disease control showed triple activity associated with antimicrobial activity, host defense activation, and film formation on the treated surface [19,53,54]. Previous works estimated that chitosan is one of the most effective alternative compounds to control the disease and prolong shelf life at cold storage conditions. It is known that chitosan coatings delay changes in weight loss, soluble solids, and total sugars, and reduce the ethylene production; these actions could be improved at low temperature conditions, leading to a lower disease incidence of fungal pathogen [53,55]. Chitosan is one of the most common resistance inducers available on the market, and elicitation of host defenses allows postharvest decay to be managed, limiting the application of synthetic pesticides and increasing the production of nutraceutical compounds [56].

## 5. Conclusions

The tested natural compounds were effective at both cold storage and room temperature at containing the postharvest decay of strawberries, and they had a variable action

according to the storage conditions. For cold-stored strawberries, all of the tested compounds, with the exception of 'SwOr', were effective at reducing gray mold infections. Overall, chitosan formulations, including 'ChitP', 'ChitO', and 'ChitS', were the most effective compounds for controlling *B. cinerea*, while the compounds based on EOs, 'SwOr' and 'GraFr', showed the highest effectiveness at room temperature. Our work emphasizes that storage temperature and the formulation of compounds are both factors that influence the effectiveness of the compounds. However, our work was run with the immersion of the strawberry fruit, and to progress to practical application, field experiments will be necessary.

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