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# Management of Postharvest Decay of Strawberries by Using Natural Compounds

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## ABSTRACT

### Management of postharvest fungal decay of strawberries by using natural compounds

Postharvest losses of fruit and vegetables amounted up to half of the production through the chain from the field to the customer table. Therefore, the investigations on sustainable approaches to minimize postharvest decay and food losses would be the goal of research in the next years. In order to reduce the presence of pathogens, the application of chemical fungicides was used, however, due to potential environmental and health issues, this practice need to be evaluated carefully. Alternative approaches such as the use of edible coatings, essential oils (EOs), salts, and natural compounds (plant extracts) were emerging technologies to manage postharvest decay of fresh fruit.

The aim of this work was to investigate the effectiveness of natural compounds known as plant defense inducers on postharvest decay. In the first work, we used the meta-analyses approach to investigate the potential effect of 1% chitosan on postharvest fungal pathogens, summarizing 13 years of data related to disease incidence, mycelium growth of *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium* spp., *Colletotrichum* spp., *Alternaria* spp. and the activity of enzymes linked to host defense mechanisms. This meta-analysis confirmed chitosan antimicrobial and eliciting activity.

The other works were focused on testing, the effectiveness of the control of postharvest decay of strawberry following the application of commercial compounds, mainly chitosan-based coating and plants essential oils. The preharvest application of (‘Chito Plant powder’,’KiForce’ ‘Prev-Am Plus’ compounds) and postharvest application of (‘Chito Plant powder’, ‘Chito Plant solution’, ‘Kaitosol’, ‘OII-YS’, ‘MeJA’, ‘3 Logy’, ‘DF-100 Forte’, ‘Prev-Am Plus’, ‘Humic acid’ compounds and bergamot, rosemary, mentha EOs, combine under hypobaric environment at 50 kPa, were tested. Gray mold, caused by *B. cinerea*, and Rhizopus rot, caused by *R. stolonifer*, were monitored after incubating fruits at 20±1 °C or 7 day storage at

4±1 °C following two days shelf life at 20±1 °C. Besides, the changes of qualitative parameters in preharvest application was analyzed.

Our results showed the effectiveness of the different compounds on strawberry decay, however, among the chitosan-based coating ‘Chito Plant powder’ was the most effective compound at both pre and postharvest application at cold storage condition and in maintaining fruit quality. Rosemary and mentha EOs, combine under hypobaric environment, and sweet orange EOs with commercial formulation Prev-Am Plus following grapefruit seed extract EOs with commercial formulation DF-100Forte were the most effective compounds against gray mold infection at room temperature. Our results showed that the application of these innovative compounds was a suitable approach for strawberry postharvest decay management.

# RIASSUNTO

## Metodi innovativi di gestione delle malattie in fragola in postraccolta

Le perdite di frutta e verdura in postraccolta rappresentano fino alla metà della produzione e interessano tutte le fasi della filiera alimentare, dal campo alla tavola. Pertanto, gli studi relativi alla ricerca di nuovi approcci sostenibili utili a ridurre al minimo i marciumi postraccolta e le perdite di prodotti alimentari sono un obbiettivo primario della ricerca in campo agroalimentare. Al fine di ridurre la presenza di agenti patogeni, viene utilizzata l'applicazione di fungicidi di sintesi. Tale pratica è tuttavia sotto attenta osservazione per il potenziale negativo impatto sull'ambiente e sulla salute del consumatore. Sistemi alternativi di protezione degli ortofrutticoli freschi basati sull'uso di rivestimenti commestibili, oli essenziali, Sali e composti naturali (estratti vegetali) sono sempre più studiati.

Lo scopo di questo lavoro è stato quello di analizzare l'efficacia contro le malattie in postraccolta di composti naturali noti o studiati come capaci di elicitare una risposta di difesa nella pianta. Nel primo lavoro abbiamo utilizzato un approccio statistico basato sulla meta-analisi per la sintesi dei dati presenti in letteratura relativi all'uso del chitosano sui patogeni fungini postraccolta. Tredici anni di dati relativi a studi sull'incidenza della malattia, sull'effetto sulla crescita del micelio *in vitro* di *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium* spp., *Colletotrichum* spp., *Alternaria* spp. e sull'attività degli enzimi collegati ai meccanismi di difesa dell'ospite sono stati riassunti attraverso una meta-analisi. Questo studio ha confermato l'attività antimicrobica ed elicitante del chitosano.

I lavori sperimentali successivi si sono concentrati sull'analisi dell'efficacia di composti commerciali basati principalmente sul chitosano e di altri a base di oli essenziali contro i marciumi postraccolta della fragola. Trattamenti preraccolta ("Chito Plant powder", "KiForce", "Prev-Am Plus") e postraccolta ("Chito Plant powder", "Chito Plant solution", "Kaitosol", "OII-YS", "MeJA", "3Logy", "DF-100 Forte", "Prev-Am Plus", "acido umico", e oli essenziali di bergamotto, rosmarino, menta piperita usati in ambiente ipobarico a 50 kPa) sono stati testati per verificarne l'efficacia nel

contenimento della muffa grigia, causata da *B. cinerea*, e del marciume acquoso, causato da *R. stolonifer*, dopo 7 giorni a 20±1 °C o 7 giorni a 4±1 °C e quindi esposti a shelf life a 20±1 °C. Nei trattamenti preraccolta sono stati analizzati i principali parametri qualitativi.

Dai nostri risultati emergono diversi composti potenzialmente utili per il contenimento in postraccolta del marciume delle fragole, tuttavia tra i rivestimenti a base di chitosano il "Chito Plant powder" si è dimostrato il più efficace sia quando applicato in pre sia in postraccolta in condizioni di frigoconservazione. Inoltre, questo composto ha aumentato l'acidità titolabile, la concentrazione di fenoli e ridotto la perdita di peso dei frutti. Gli oli essenziali a base di rosmarino e di menta sono risultati i più efficaci nel ridurre l'infezione da muffa grigia a temperatura ambiente. I nostri risultati hanno dimostrato una buona efficacia delle applicazioni di questi composti innovativi per la gestione dei marciumi postraccolta della fragola.

# 1. INTRODUCTION

## 1.1 Postharvest losses

In the recent years, due to increasing world population, the demand for food had increased dramatically. According to some reports, it was projected that the production of crops for the years 2030/2050 should be 70% higher than the productions that were available previously Food and Agriculture Organization - FAO, (2012). Fruits and vegetables, with 45 to 50% of losses, had the highest wastage rate of any other food products in the world (Lipinski et al., 2013; High Level Panel of Experts on Food Security and Nutrition - Hlpe, 2014; Affognon et al., 2015). FAO (2019) estimated that, almost half of all the fruit and vegetables produced were wasted globally, with higher rates in developing countries. Therefore, decrease in postharvest losses of horticultural perishables products can provide an effective way of increasing food availability (Kuchi et al., 2019).

## 1.2 Causes of damage

Among fruit and vegetables, considering berries, raspberry, strawberry, blueberry and blackberry were considered as highly perishable. The commercial viability of these crop was continually subjected to various risks during picking, packing, storage and transportation. Susceptibility to the disorder has recently been linked to cultivar firmness (Salgado and Clark, 2016), vibration injury during transportation (Pérez-Pérez et al., 2018) and nitrogen fertilizer application rate (Edgley et al., 2016).

New studies (McCoy et al., 2016; Yin, 2017; Lawrence and Melgar, 2018; Edgley et al., 2019) have linked the time of harvested with temperature conditions during harvest which had a significant influence on the disorder, this could influence the physiology and postharvest quality of horticultural commodities.

Environmental variables such as sun exposure, humidity, and water availability have all been reported to affect firmness and bruise susceptibility across a range of fruit, including, strawberries, apples, and apricots (Paull, 1999; Sams, 1999; Hussein et al., 2018). Storage air speed, atmospheric composition (concentrations of oxygen, carbon dioxide, and ethylene), and sanitation procedures, physiopathological injury due to excessive chilling or lighting are involved the losses as well (Estrada et al., 2018). Besides, it was improved that, physical damage, such as mechanical injury could be associated with postharvest decay from many causes (Holt and Schoorl, 1982; Vergano et al., 1991; Crisosto et al., 1993; Pérez-Pérez et al., 2018). However, disease from microbial growth represented a major problem throughout pre and postharvest procedure, causing significant losses at the commercial level (Romanazzi et al., 2016).

Fruit and vegetables like berries or stone fruits were very prone to microbial spoilage because of their succulent nature. Infection by microorganisms that causes postharvest decay can occur before the harvest, at the field stage. They could remain latent until storage, when the environmental conditions would be favorable for disease development. A diversity of postharvest diseases caused by various bacterial and fungal pathogens have been estimated as one of the most serious problem, and the diseases caused by fungi are in a first level of infection. More than 50 different genera of fungi could affect fruits like berries. Many of these could develop very rapidly from rotted fruit next to the healthy fruit, causing extensive breakdown of the commodity, and sometimes spoiling entire product. Moreover, aside from direct economic considerations, disease produced a potential health risk, as some fungal genera were known to produce mycotoxins under certain conditions. Common postharvest diseases and pathogens of fruit were identified as *Botrytis*

spp., *Colletotrichum* spp., *Mucor* spp., *Alternaria* spp., *Monilinia* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizoctonia* spp. (Feliziani et al., 2015; Romanazzi et al., 2016; Janisiewicz et al., 2017).

The association between harvest conditions and the incidence of postharvest berries with fungal pathogen, required to be identified. Recognition of the factors involved in the development of berries fruit losses, should be of importance to the worldwide industry in order to develop standard management practices to reduce the incidence and severity of the disorder (Crisosto et al., 1993; Aliasgarian et al., 2015) reported that 51% of damage to strawberries including abrasions, bruises and penetrations were caused during harvest with 17% occurring during packing and 32% in delivery to market, based on this, damaged fruits were provided good substrate for fungal infection and led to heavy losses.

### 1.3 Postharvest management

Problems and prospects of the research and development of controlling fungal pathogen was concerning plant pathologist. In recent years, scientists worldwide devoted their time and energy to discover the high effect, low toxicity, safety and inexpensive plant-derived fungicides. The optimization of postharvest practices for fresh berries would be a significant interest of researchers due to their high susceptibility to postharvest loss of quality, high market value and affect the financial benefits (Sui et al., 2016; Edgley et al., 2019). Regarding this evaluation, postharvest management not only plays an important role toward fruit quality and preservation, but also, it has a strong effect on the control of fungal pathogen.

The correct choice of fruit maturity at harvest, and careful handling and use of technologies that delay fruit ripening during storage were fundamental for decay control (Mari et al., 2009). At the same time, modern

research technology efforts have led to the development of novel and alternative method for controlling postharvest disease which were recognized as natural compounds (Romanazzi et al., 2012), namely: (i) edible coating generally recognized as safe or GRAS: chitosan (Betchem et al., 2019), essential oils (Ding et al.,2019), plant extracts (Chen et al., 2019); (ii) biological control agents (BCAs) (Dukare et al., 2019); (iii) plant hormones (Xixi et al., 2019) and (iv) physical methods (Liu et al., 2018). All these treatments can be used alone or in combination. **Table 1** summarized examples of control of most common postharvest diseases and pathogens of fruit by using natural compounds. Among those, edible coatings were considered as interested compounds for further investigation. Use of edible coating would be an innovative tool to preserve the quality of fresh fruits and minimized fungal pathogen aggressiveness (Flores et al., 2018; Lopez et al., 2018). They were used for their antimicrobial properties or their induction of plant defenses and activate systemic acquired resistance (Banani et al., 2018).

**Table 1 -** Summarizes the control of most common postharvest diseases and pathogens of fruit by using natural compounds

| Natural compounds | Fruits | Causal agents | Impact | References |
|---|---|---|---|---|
| **Essential oils** | | | | |
| *Cinnamomum zeylanicum* | Banana | *Colletotricum* spp. | antifungal activity | Kamsu et al., 2019 |
| *Menta piperita* | Grape | *Botrytis cinerea* | conidial germination | Xueuan et al., 2018 |
| *Thymus vulgaris* | Apple | *Botrytis cinerea* | decay, PR proteins | Banani et al., 2018 |
| **Chitosan** | | | | |
| Submicron chitosan | Jujube | *Alternaria alternata* | cell penetration | Malerba et al., 2018 |
| Film based on chitosan | Papaya | *Rhizopus stolonifer* | mycelium growth | Guo et al., 2019 |
| Chitosan | Avocado | *Colletotricum* spp. | up regulates PAL | Obianom et al., 2019 |
| **Plant extract** | | | | |
| *Zingiber officinale* | Chilli | *Colletotricum capsici* | lesion reduction | Debjani et al., 2017 |
| *Aloe vera* | Sapodilla | *Penicillium italicum* | decay, quality | Khaliq et al., 2019 |
| *Cassia* spp. | Strawberry | *Botrytis cinerea* | reduce decay | Elmogy and Alsanius, 2012 |
| **Plant hormone** | | | | |
| MeJA | Strawberry | *Botrytis cinerea* | Slow down fungi | Saavedra et al., 2017 |
| Abscisic acid | Sweet orange | *Penicillium digitatum* | defensive role | Lafuente et al., 2019 |
| Jasmonic acid (JA) | Strawberry | *Botrytis cinerea* | defense response | Zhang et al., 2006 |
| **Biocontrol agents (BCAs)** | | | | |
| *Psedomonas synxantha* | Nectarine | *Monilinia fructicola* | reduce incidence | Aiello et al., 2019 |
| *Thrichoderma* spp. | Strawberry | *Botrytis cinerea* | decay reduction | Awad et al., 2017 |
| *Bacillus subtilis* | Pear | *Pencillium expansum* | competition | Lastochkina et al., 2019 |
| **Physical treatment** | | | | |
| Cold storage | Citrus | *Penicillium* spp. | extend shelf life | Thompson et al., 2008 |
| Hypobaric | Table grape | *Botrytis cinerea* | induce resistance | Romanazzi et al., 2016 |
| Hot water | Table grape | *Botrytis cinerea* | decay, quality | Karabulut et al., 2004 |

Edible coatings were able to alter various physical, physiological and biochemical aspects of fruit growth, to carry out its effect (Prasad et al., 2018). Regarding edible coating, the natural bio polymer chitosan (Antunes and Cavaco, 2010) and essential oils (Hua et al., 2019) have been reported to control postharvest diseases, both *in vitro*, *in vivo* and to prolong the overall quality and storage life of fresh commodities. These safe compounds facilitate the consumer benefits and acceptability to have safe fruit without any chemical treatments.

## 1.4 Chitosan

Edible coatings such as chitosan was applied in thin layers to the surface of the fresh fruit and acted as a semi-permeable barrier to respiratory gases and water vapor between the fruit and the surrounding atmosphere, thereby, establishing a modified atmosphere around the product, which slow down respiration, senescence, and enzymatic oxidation. They were water-resistant and stable during cold storage and did not cause excessive $O_2$ reduction or $CO_2$ accumulation, permeable to water vapor, improved fruit gloss and appearance and did not impart off-flavors and changes in aroma, taste, texture and appearance and have low viscosity (Dhall, 2013; Mahajan et al., 2014). Edible coatings were composed of polysaccharides, proteins, and lipids, alone or in combination, whose presence and abundance, determined the barrier properties of the material. However, none of the three constituents could provide the needed protection by themselves and so they were usually used in a combination in order to obtain the best results (Valencia Chamorro et al., 2010; Dhall, 2013; Mahaian et al., 2014). They could be applied on whole or on fresh-cut fruits and vegetables. Several scientist were improved the effect of edible coating on fruit gloss, fruit firmness, shelf life extenuation, decrease weight loss, delay changes in color,

PH, acidity, increase total soluble solid content, ascorbic acid, maintain higher concentration of total phenolics and anthocyanins and decreased decay incidence (Fisk et al., 2008; Eum et al., 2009; Gol et al., 2013; Arnon et al., 2014).

## 1.5 Essential oils

Other natural preservatives such as essential oils (EO) have been extensively used due to its biodegradable and antimicrobial properties. EO contains a variety of substances called 'phytochemicals', which belong to natural components in plants (Pratt, 1992). The phytochemical preparations with dual functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending shelf life of food products (Singh et al., 2007; Fadli et al., 2012). Generally, EO possesses high volatility. When EO was applied as a vapor, less oil was used. Further, the residues of EO on the product were minimized, and there would be less of a problem with tainting (Szczerbanik et al., 2007). It might be more appropriate to use EOs in their vapor phase for postharvest applications (Oliveria et al., 2019). However, the successful results were not obtained always, based on this, it would be necessary to applied more effective method. Positive effects of coating were also observed in fruit treated with edible coating incorporate with other alternative treatment, which presented a better external appearance, a less dehydrated surface apparently without fungal growth and lower mass loss, maintaining the storage quality and extending shelf life of fruit (Medeiros et al., 2012). In some study the combinatory treatment delayed fruit decay more than single treatments (Hussain et al., 2013; Vanoli et al., 2015; Shi et al., 2019).

The success of edible coating and EOs depended on its ability for enhancing food safety along with retaining nutritional and sensory attributes (Siddiqui et al.,

2018). In addition, to minimizing the fungal pathogen infection. Intelligent selection and use of edible coating alone or incorporated with other natural treatment help the scientist to develop fresh fruit in market, which in turn offers resistance to fruit against various quality-deteriorating factors and pathogen infection, in order to achieve the goal related to customer health benefits and positive effect on climate condition.

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# META-ANALYSIS OF CHITOSAN EFFECTIVENESS STUDIES PROVIDES INSIGHTS IN TO THE CONTROL OF POSTHARVEST DECAY


## Abstract

Although numerous studies have reported the effectiveness of chitosan, a natural polymer, to control postharvest diseases, the findings remain controversial mainly due to the mixed claims in the literature.
Here, we used the meta-analysis random effect model to investigate the potential effect of 1% chitosan on postharvest fungal pathogens *Botrytis cinerea*, *Penicillium* spp., *Rhizopus* spp., *Colletotrichum* spp. and *Alternaria* spp., and its effects on defense enzyme activity: β-1,3-glucanase, phenylalanine ammonia-lyase and chitinase at 24 hours post treatments. Overall data showed that chitosan reduced disease incidence in *in vivo* test [Mean Difference (MD) -30.22; 95% Confidence Intervals (CI) = -36.48, -23.96; $P < 0.00001$] and *in vitro* test on mycelium growth (MD -54.32; 95% CI: -64.35, -44.28; $P < 0.00001$). However, different response among pathogens, according to *in vivo* and *in vitro* test were detected. Regarding host defense response investigation (MD 74.58; 95% CI: 41.15, 108.01; $P < 0.0001$) significant up-regulation for β-1,3-glucanase and chitinase were recorded. This study demonstrated that 1% chitosan has antimicrobial, and eliciting defense activity. These are a key properties for innovative compounds, useful in the control of postharvest decay.


**Keywords:** chitosan; defense enzyme activities; fungal pathogens; meta-analysis; postharvest decay.

## 2.1 Introduction

Postharvest diseases of fruit and vegetable were mainly caused by the fungal pathogens, resulted in economic losses (Romanazzi et al., 2016). Therefore, controlling these pathogens became critical for extending the shelf life of fresh product (Prusky, 2011). Despite the efficacy of synthetic fungicides in controlling postharvest diseases, public concerns regarding chemical residues in food (Kim et al., 2019), and an increase in drug-resistant strains of pathogens have led to a need for new agents to control postharvest diseases (Zuccolo et al., 2019). Over the last decades, there was an increased interest in the study of postharvest control methods obtained from natural sources. Alternative compounds act both as a resistance inducer, activating plant defense mechanisms, or showed strong antimicrobial activities against the main postharvest fungal pathogens (Romanazzi et al., 2009; Ribes et al., 2018). However, a few fungicides have been approved for use as control agents for postharvest diseases due to strict regulatory policies for food safety. Among those, chitosan, a natural biocompatible polysaccharide derived by deacetylation of chitin, emerged as a promising eco-friendly alternative to synthetic fungicides (Betchem et al., 2019; Romanazzi et al., 2018). The chitosan was the first compound in the list of basic substances approved in the European Union for plant protection purposes (Reg. EU 66 2014/563), for both organic agriculture and integrated pest management.

From several years, chitosan met the interest of many researchers, that used it to prolong the storage of an array of fruit and vegetables worldwide, since it has shown to have a triple activities including film formation on the treated surface (Valencia-Chamorro et al., 2011; Romanazzi et al., 2018), antimicrobial activity (Kong et

al., 2010; Feliziani et al., 2015; Duan et al., 2019) and elicit host defense mechanism (Landi et al., 2014; Coqueiro et al., 2015; Landi et al., 2017; Xoca-Orozco et al., 2019; Obianom et al., 2019). For these reasons, chitosan could be used as biodegradable fungicides (Liang et al., 2017). However, the heterogeneity of chitosan, related to its effectiveness, used in a wide range of experimental conditions could lead to different interpretations, as a result, different recommendation on treatment methods were provided (Ramos-Garcia et al., 2012; Bill et al., 2014; Flores et al., 2018; Betchem et al., 2019). Besides, researchers reported that the value of disease reduction based on evaluating the similar or different fungal strains among studies was varied (Hua et al., 2019). Furthermore, due to many papers related to chitosan treatment and their influence, no single study could make all the appropriate comparisons. Thus, given the mixed claims in the literature, there was a need to understand the overall effectiveness of chitosan, to highlight useful conclusions for future investigations.

A meta-analysis could be applied as a tool for analyzing a vast collection of data from published primary studies, in which the main purpose is to integrate and interpret the findings to gain conclusions that the individual studies alone would not show clearly. This statistical procedure integrated the results of several independent studies. Combining outcome increased statistical power made it easier to detect small effects (Chen et al., 2019; Gonzalez-Dominguez et al., 2019).

The aim of this study was to use the meta-analysis approach to quantitatively review the results of studies on 1% chitosan effectiveness, the most common tested concentration for controlling postharvest fungal pathogens (Romanazzi et al., 2018). Hence, the objectives of the present meta-analysis study were: (i) analyzed the effectiveness of chitosan in reducing postharvest disease

incidence; (ii) investigated the effectiveness of chitosan on *in vitro* mycelium growth reduction related to fungal pathogens involved in postharvest decay; (iii) investigated chitosan effects of the β-1,3-glucanase, chitinase and phenylalanine ammonia-lyase (PAL) activity associated with host defense mechanisms, at 24 hours post-treatment (hpt).

## 2.2 Materials and methods

### *2.2.1 Literature search strategy and study selection*

A systematic literature search through 2019, was performed using the databases of PubMed, Scopus and Web of Science searching for the following terms: 'chitosan' and 'fruit'. The papers using chitosan mixed with other compounds were not considered. The selection of works was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Moher et al., 2009).

Article selection for the meta-analysis used the following inclusion criteria: 1% chitosan, *in vivo* disease incidence and *in vitro* mycelium growth according to specific postharvest fungi and activity of enzymes involved in the plant defense mechanism. The eligibility of the articles was performed and the articles without: (i) 1% chitosan, (ii) disease incidence, mycelium growth or defense enzymes, (iii) detailed decay-causing agent, were excluded.

In detail three categories were included and papers related to: (i) disease incidence caused by *Botrytis cinerea*, *Penicillium* spp., *Rhizopus* spp., *Colletotrichum* spp. *Alternaria* spp., published between 2010-2019 were considered as subgroups; (ii) *in vitro* test, mycelium growth of fungal pathogens: *B. cinerea*, *Penicillium* spp., *Colletotrichum* spp., and *Alternaria* spp., published between 2007-2019 were considered as subgroups; (iii)

enzyme activity variation associated with host defense mechanisms detected at 24 hpt, including PAL, chitinase, β-1,3-glucanases published between 2009-2018 were considered as subgroups. All studies included at least two treatments, chitosan and untreated control. Fruit varieties, application and time detection varied among studies. In some studies, treatment consisting of application times and rates were reported, in such cases, only the treatments applied at the same time as the standard treatment was considered in the meta-analysis.

### *2.2.2 Tests of heterogeneity*

The risk of bias and test for asymmetry of the funnel plot were used to evaluate for possible publication bias. Cochran's $I^2$ indices, $Tau^2$ and $\chi^2$ tests were calculated to estimate the statistical heterogeneity of the studies (Tufanaru et al., 2015). If heterogeneity was significant ($I^2 > 75\%$; or/and $P < 0.05$), a random effects model was applied to all subgroup included in disease incidence, mycelium growth and defense enzyme activity categories.

### *2.2.3 Data extraction*

Data were recorded from the same days of chitosan treatments in each paper. All studies related to the effect of chitosan toward disease incidence was calculated as a percentage. The studies on the effect on mycelium growth, resulted on three different units including (percentage, mm, cm), were unified to percentage. To unify the different measurement units used across the enzyme activity studies, the values were converted into percentage mean (% mean) with respect to normal control (mean of treatment/mean of normal control × 100) (Viswanatha et al., 2017). If standard deviation (SD) or standard error (SE) was not reported, the data were transformed according to the P value (Weir et al., 2018). Data from Figures would be extracted using Plot Digitizer software

(Kadic et al., 2016). The changes scores with the corresponding standard deviation were used based on the guideline of the Cochrane handbook https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD012276/epdf/full.

### 2.2.4 Data analysis

All meta-analyses were conducted in Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014 (http://tech.cochrane.org/revman). Data type was selected as a continues. The statistical method was considered as an inverse variance. Weighted mean, effect size, 95% CI includes 0 were calculated. In all analysis, P-value < 0.05 was considered statistically significant. The difference among groups was obtained when 95% CIs were overlapped with a vertical line. If 95% CIs were not overlapped, it is suggested that difference was significant (Dardiotis et al., 2018). Studies were presented in Forrest plots in order of statistical power.

## 2.3 Results

### *2.3.1 Studies selection and characteristics*

A detailed flow chart of the search criteria to include or exclude studies on chitosan effectiveness was showed in **Figure 1**. A total of 56 articles including 117 studies were involved in the meta-analysis studies, among these 49 studies were looking at *in vivo* disease incidence (total cases 8,543 for both, chitosan treatment and control) (**Figure 2**), 41 studies were looking *in vitro* mycelium growth (total cases 1,077), (**Figure 3**) and 27 studies linked to defense enzymes group (total cases 1,332), (**Figure 4**). All the papers included in the meta-analysis study were listed in **Table 1**.

### *2.3.2 Tests for heterogeneity*

All studies in selected articles were included in the risk of bias assessment. Since there were not published actual data, it was not possible to assess the domains of 'other bias', which resulted in the occurrence of blanks. Besides, in these studies, blinding of participants and personnel (performance bias) was not necessary to be considered so it is ignored to be checked for risk of bias. Domains considered for risk of bias have been chosen based on each study reporting data and scientific information. All studies had explicitly described that basic characteristics of treatment and control groups were balanced and treated in equal environmental conditions. No knowledge of the allocated intervention by outcome assessors was detected. None of these studies announced the misleading samples and all outcomes emphasizing at all time point, as a result, selection bias, detection bias, the attrition bias and reporting bias were free of bias and publications were decided at low risk of bias (**Figure 5**). The funnel plot presented from data for disease incidence, mycelium growth, and defense enzyme activity did not reveal any significant asymmetry (**Figure 6 a, b, c**).

### *2.3.3 Effect of 1% chitosan on* in vivo *disease incidence*

Based on meta-analysis, the overall data demonstrated the effectiveness of chitosan on disease incidence [(Mean Difference (MD) -28.72; 95% Confidence Intervals (CI) -35.19 -22.26; $I^2$ 90.0%; $P < 0.00001$) (**Figure 2**).

Regarding the subgroup analysis, results showed that chitosan was effective to reduce incidence of gray mold caused by *B. cinerea* (MD - 23.97; 95% CI -32.25, -15.68; $I^2$ 77.0%; $P < 0.00001$). Out of twelve studies that described the activity of chitosan on *B. cinerea*, (total cases, 1,473) (Shao et al., 2012; Feliziani et al., 2013; Gao et al., 2013; Romanazzi et al. 2013; Feliziani et al., 2015;

Kanetis et al., 2017; Zheng et al., 2017; Gramisci et al., 2018; Hajji et al., 2018), 9 studies showed high effectiveness of this compound against this pathogen (Shao et al., 2012; Gao et al., 2013; Romanazzi et al. 2013; Feliziani et al., 2015; Kanetis et al., 2017; Zheng et al., 2017; Gramisci et al., 2018; Hajji et al., 2018) (**Figure 2**). The effectiveness of chitosan was observed against *Penicillium* spp. (-30.85; 95% CI -41.91, -19.79; $I^2$ 90.0%; $P < 0.00001$). Out of 16 studies (total cases, 1,968) (Xing et al., 2011; Shao et al., 2012; Feliziani et al., 2013; Romanazzi et al. 2013; Wang et al., 2014; Lu et al., 2014; Shao et al., 2015; El Guilli et al., 2016; Zheng et al., 2017; Gramisci et al., 2018; Kharchoufi, et al., 2018; Liu et al., 2018; Shi et al., 2018), 9 studies showed high ability to reduce disease (Xing et al., 2011; Romanazzi et al. 2013; Lu et al., 2014; Shao et al., 2015; El Guilli et al., 2016; Zheng et al., 2017; Liu et al., 2018; Shi et al., 2018), (Figure 2). Chitosan reduced the incidence of Rhizopus rot (MD -28.80; 95% CI -46.13, -11.47; $I^2$ 87.0%; $P = 0.001$). Among five studies (total cases, 1,740) (Cia et al., 2010; Ramos-Garcia et al., 2012; Romanazzi et al., 2013; Xing et al., 2015) three studies (Cia et al., 2010; Ramos-Garcia et al., 2012; Romanazzi et al., 2013) showed lower disease development (**Figure 2**). All 11 studies related to the *Colletotrichum* spp. reported a positive result in decreasing disease incidence, (MD -46.64; 95% CI -61.54, -31.73; $I^2$ 92.0%; $P < 0.00001$) (total cases, 2,134) (**Figure 2**) (Maqbool et al., 2010; Zahid et al., 2012; Bill et al., 2014; Edirsinghe et al, 2014; Ali et al., 2015; Gutierrez-Martinez et al., 2017; Obianom et al., 2019). While concerning the *Alternaria* spp., meta-analysis estimated lower effectiveness (MD -8.50; 95% CI -15.75, -1.25; $I^2$ 27.0%; $P = 0.24$). Among five studies (total cases, 1,228) (Meng et al., 2010; Yan et al., 2011; Feliziani et al., 2013; López-Mora et al., 2013; Guo et al., 2017;) only one

(López-Mora et al., 2013) showed a positive effect of chitosan against incidence reduction.

### *2.3.4 Effect of 1% chitosan on* in vitro *mycelium growth*

Overall effect showed high effectiveness of chitosan against *in vitro* mycelium growth of all fungal pathogen involved in postharvest disease (MD -54.32; 95% CI; -64.35, -44.28; $I^2$ 95.0%; P < 0.00001) (**Figure 3**).

The subgroup analysis related to the effectiveness of chitosan against *B. cinerea* was medium high (MD -49.38; 95% CI, -72.98, -25.79; $I^2$ 94.0%; P < 0.0001) and all single studies showed the high activity of chitosan against this pathogen (total cases, 37) (**Figure 3**), (Xu et al., 2007; Feliziani et al., 2013; Kanetis et al., 2017; Munhuweyia et al., 2017; Flores et al., 2018). The highest effectiveness was observed against *Penicillium* spp. (MD -73.00; 95% CI -89.71 -56.30; $I^2$ 92.0%; P < 0.00001) and all nine studies exhibited the positive result (total cases, 65) (**Figure 3**), (Kader et al., 2011; Xing et al., 2011; Nisia et al., 2012; Wang et al., 2014; Shao et al., 2015; Waewthongrak et al., 2015; Munhuweyi et al., 2017; Madanipour et al., 2019). The overall data evidenced that chitosan activity against mycelium growth of *Colletotrichum* spp., had the lowest effectiveness (MD -48.18; 95% CI -62.83, -33.53; $I^2$ 96.0%; P < 0.00001). Based on point estimate, sixteen studies (Jitareerat et al., 2007 ; Rahman et al., 2008 ; Maqbool et al., 2010; Zahid et al., 2012 ; Bill et al., 2014 ; Ali et al., 2014 ; Varela et al., 2015 ; de Olivera et al., 2017 ; Ramos-Guerrero, et al., 2018 ; Xoca-Orozco et al., 2018) out of twenty-four (total cases, 955) (Jitareerat et al., 2007 ; Rahman et al., 2008 ; Munoz et al., 2009; Maqbool et al., 2010; Zahid et al., 2012 ; Mohamed et al., 2013 ; Bill et al., 2014 ; Edirsinghe et al., 2014 ; Ali et al., 2014 ; Ali et al., 2015 ; Varela et al., 2015 ; Gutiérrez-Martínez, et al., 2017 ; de Olivera et al., 2017 ; Ramos-Guerrero, et al., 2018 ; Xoca-Orozco et

al., 2018), exhibited the higher activity of chitosan on fungal reduction (**Figure 3**). Respect to *Alternaria* spp., mycelium growth of all three studies (total cases, 15) exhibited the positive activity of chitosan on fungal reduction (MD -55.20; 95% CI -80.50 -29.90; $I^2$ 90.0%; P < 0.0001) (Yan et al., 2011; Feliziani et al., 2013; López-Mora et al., 2013) (**Figure 3**).

### *2.3.5 Effect of 1% chitosan on enzymes activity associated with host defense*

Meta-analysis investigation showed that 1% chitosan at 24 hours post treatments (hpt) modified the enzyme activity associated with the host defense (MD 74.58; 95% CI 41.15, 108.01; $I^2$ 99.0%; P < 0.0001) (**Figure 4**). In detail, 1% chitosan elicited both, β-1,3-glucanase (MD 115.06; 95% CI 38.24, 191.88; $I^2$ 100.0%; P = 0.003 and chitinase enzyme (MD 75.95; 95% CI 36.18, 115.73; $I^2$ 99.0%; P = 0.0002), in treated fruits. Regarding β-1,3-glucanase, out of eight studies (total cases 266) (Hewajuliage et al., 2009; Wang et al., 2013; Ali et al., 2014; Bill et al., 2014; Landi et al., 2014; Shao et al., 2015; Jongsri et al., 2017; Shen et al., 2017), the enzyme activity was up-regulated in five studies (Hewajuliage et al., 2009; Wang et al., 2013; Ali et al., 2014; Bill et al., 2014; Landi et al., 2014).

Chitinase activity in eight studies (Hewajuliage et al., 2009; Wang et al., 2013; Feliziani et al., 2013; Ali et al., 2014; Bill et al., 2014; Landi et al., 2014; Shen et al., 2017) out of ten (Hewajuliage et al., 2009; Wang et al., 2013; Feliziani et al., 2013; Ali et al., 2014; Bill et al., 2014; Landi et al., 2014; Shao et al., 2015; Jongsri et al., 2017; Shen et al., 2017), (total cases, 491) was showed an up-regulation compared to the control. Regarding the PAL activity, chitosan did not induce a significant difference compared to the control at 24 hpt (MD 37.06; 95% CI -17.28, 91.40; $I^2$ 99.0%; P = 0.18). However, four single studies (Bill et al., 2014; Landi et al., 2014;

Waewthongrak et al., 2015; Shen et al., 2017;) among seven studies, (Bill et al., 2014; Landi et al., 2014; Shao et al., 2015; Waewthongrak et al., 2015; Song et al., 2016; Jongsri et al., 2017; Shen et al., 2017; Batista et al., 2018) showed significance difference for PAL activity (total cases, 330) (**Figure 4**).

## 2.4 Discussion

Our work summarized the results of 1% chitosan effectiveness on reducing postharvest pathogens according to both, disease incidence, and *in vitro* mycelium growth as well as the ability to induce host defense response linked to the more common analyzed enzymes associated with host defenses. According to meta-analysis, we have emphasized the primary role of this biopolymer against the pathogens associated with postharvest decay (Romanazzi et al., 2018; Betchem et al., 2019). The results from pooled estimate highlighted that chitosan was effective to decrease postharvest diseases, caused by several fungal pathogens, infecting different plant species. Based on our results, although the heterogeneity was very high, due to the low risk of bias and higher validity of each study, no substantial baseline differences between the treatment and control groups existed. Besides, the funnel plot as a method to assess the potential role of publication bias (Harbord et al., 2006), supposed that no bias detected through studies. Therefore, the values of $I^2 > 90\%$ could refer to real differences among studies, which could potentially be explained by each study level varieties.

Our study underlined the transversal effectiveness of chitosan in postharvest diseases management. However the subgroups analysis of *in vitro* mycelium growth emphasizing that the most powerful growth reduction was on *Penicillium* spp. following *Alternaria* spp. and *B. cinerea* respectively, while, lower effectiveness was

estimated toward *Colletotrichum* spp.. This results showed that fungal species in the presence of chitosan acted differently, likely since chitosan can control the fungal development and lytic enzyme activation (Geoghegan et al., 2017; Ramos-Guerrero et al., 2018). Exist a direct link between the cell wall and membrane since the synthesis of key cell wall components (glucans and chitin) are performed by the plasma membrane associated with synthase enzyme complexes (Maddi and Free, 2010). Previous studies have shown that plasma membrane of chitosan-sensitive fungi was more fluid and richer in polyunsaturated free fatty acid than in chitosan-resistant fungi (Palma et al., 2010). Although the meta-analysis highlights the different relationship between fungal specie and chitosan effectiveness, our work underlined the fundamental role of the plant species to determine the success of chitosan against the pathogen. The summarized study related to disease incidence showed the highest effectiveness against anthracnose caused by *Colletotrichum* spp., while a minor effectiveness was found toward *Penicillium* spp., *Rhizopus* spp., *B. cinerea*, and especially *Alternaria* spp.. Our work confirming that disease incidence could be influenced by the film-forming characteristic in the plants or by eliciting defense response affecting the decay incidence (Romanazzi et al., 2018). In this context, chitosan could be considered as a plant defense modulator (Lopez-Moya et al., 2019). The link between pathogenicity and synthesis enzymes of a fungal cell wall has been demonstrated in numerous studies (Oliveira-Garcia and Deising, 2013; Geoghegan et al., 2017), the depolymerization in the cell wall of plant-pathogenic fungi following the structures infection or avoiding plant immune recognition were observed (Geoghegan et al., 2017). It was detected that the strategy of some fungal pathogens to evade plant immunity, was to convert chitin into chitosan, comparatively, in poor

activator, chitin activated immune response (Lopez-Moya et al., 2019). According to systemic acquired resistance (SAR) (Pieterse et al., 1998) and/or induced systemic resistance (ISR) (Heil and Bostock, 2002), chitosan could induce resistance in plant to control postharvest fungal pathogen of fruit and vegetables. In this regard, meta-analysis results related to the ability of chitosan to elicit host defense enzymes and activating the induce resistance (Walters et al., 2013) could help to understand this aspect.

In the last years, the whole transcriptome analysis in sweet orange (Coqueiro et al., 2015) and in strawberry fruit (Landi et al., 2017) were performed and able to suggest that the activation of defense mechanisms was mainly associated with plant host. The most common approach related to the study of enzyme activity variation (Wang et al., 2012; Ali et al., 2014; Shao et al., 2015) and on the singular genes expression (Landi et al., 2014; Chun et al., 2019) both associated with reactive oxygen species (ROS), specific pathogenesis-related (PR) proteins, cell wall enzymes and secondary metabolites were investigated. Usually, these individual studies showed great variability associated with host fruit species, application methods and time from treatment. In this work, we have analyzed the most studied enzymes, PAL, associated with phenylpropanoid pathway (Dixon et al., 2002), β-1,3-glucanase and chitinase linked to the cell wall hydrolyses (Gupta et al., 2013) choosing most common analyzed time point, 24 hpt our results did not show a significant effect of chitosan to induce PAL at 24 hpt, while high induction of chitinase and β-1,3-glucanase were detected independently of host species. This funding was in agreement with the plants immunity mechanism that indicated the chitinase and β-1,3-glucanase were able to release the glucan oligomers from the chitin of fungal cell walls trigging plant immune response (Jones and Dangl, 2006; Lopez-Moya et al., 2019), although the

induction of defense mechanisms could be changed greatly based on the time from treatment. Our work suggested that the analysis 24 hpt of β-1,3-glucanase and β-1,3-chitinase activity could be a marker for verifying the plant defense induction by chitosan.

Meta-analysis provided the knowledge based on three robust findings consist of the effect of 1% chitosan on disease incidence, mycelium growth reduction and defense enzyme activity. These results suggested the potential benefit of chitosan, in plant protection as a natural fungicide and plant defense booster, which should be involved more in postharvest management strategies.

## 2.5 Conclusions

These results suggested the potential benefit of chitosan, in plant protection as a natural fungicide and plant defense booster, which should be involved more in postharvest management strategies. It met the interest of many researchers, that used it to prolong the storage of an array of fruit and vegetables worldwide and recommended to increase the role of this natural compound not only in the level of research activity, but also to be developed in the bigger scale for customer consumption in the future. However, more studies are required to better understand the possible factor included to achieve this success.

**Table 1** - General characteristics of the studies included in meta-analysis related to 1% chitosan effect on disease incidence, mycelium growth reduction, and activities of defense enzymes associated with host defense.

| First Author | Year | Fungal pathogens | Studies | |
|---|---|---|---|---|
| | | | ***In vivo* (fruits)/ *In vitro* (*)/ plant defense mechanisms (fruit[a])** | **Defense Enzyme** |
| Xu | 2007 | *B. cinerea* | * | - |
| Jitareerat | 2007 | *Colletotrichum* spp. | * | - |
| Rahman | 2008 | *Colletotrichum* spp. | * | - |
| Hewajulige | 2009 | - | Papaya[a] | β-1,3-glucanase |
| | | | | chitinase |
| Munoz | 2009 | *Colletotrichum* spp. | * | - |
| Meng | 2010 | *Alternaria* spp. | Pear | - |
| Maqbool | 2010 | *Colletotrichum* spp | Banana | - |
| Cia | 2010 | *Rhizopus* spp. | * | - |
| Yan | 2011 | *Alternaria* spp. | Jujubo/* | - |
| Kader | 2011 | *Penicillium* spp. | * | - |
| Xing | 2011 | *Penicillium* spp | Jujubo | - |
| Nisia | 2012 | *Penicillium* spp. | * | - |
| Ramos-Garcia | 2012 | *Rhizopus* spp. | Tomato | - |
| Shao | 2012 | *Penicillium* spp. | Apple | - |
| | | *B. cinerea* | | |
| Shafer | 2012 | *Penicillium* spp. | Orange | - |
| Zahid | 2012 | *Colletotrichum* spp. | Banana/* | - |
| | | | Papus/* | |
| | | | Dragon/* | |
| Feliziani | 2013 | *B. cinerea* | Table grape[a]/* | chitinase |
| | | *Alternaria* spp. | | |
| | | *Penicillium* spp. | | |
| Wang | 2013 | - | Strawberry[a] | β-1,3 glucanase |
| Mohamed | 2013 | *Colletotrichum* spp. | * | - |
| Gao | 2013 | *B. cinerea* | Table grape | - |
| López-Mora | 2013 | *Alternaria* spp. | Mango/* | - |
| Romanazzi | 2013 | *Penicillium* spp | Strawberry | - |
| | | *B. cinerea* | | |
| | | *Rhizopus* spp. | | |

| | | | | |
|---|---|---|---|---|
| Bill | 2014 | *Colletotrichum* spp. | Avocado [a/*] | PAL |
| | | | | chitinase |
| | | | | β-1,3-glucanase |
| Ali | 2014 | *Colletotrichum* spp. | Dragon [a/*] | chitinase |
| | | | | β-1,3-glucanase |
| Wang | 2014 | *Penicillium* spp. | Jujube* | - |
| Lu | 2014 | *Penicillium* spp. | Orange | - |
| Landi | 2014 | | Strawberry[a] | PAL |
| | | | | chitinase |
| | | | | β-1,3-glucanase |
| *Edirisinghe* | 2014 | *Colletotrichum* spp | Bell pepper/* | - |
| Zahid | 2015 | | Dragon[a] | PAL |
| Feliziani | 2015 | *B. cinerea* | Strawberry | - |
| Waewthongrak | 2015 | *Penicillium* spp. | Citrus [a/*] | PAL |
| Varela | 2015 | *Colletotrichum* spp. | * | - |
| Shao | 2015 | *Penicillium* spp. | Mandarin [a/*] | PAL |
| | | | | chitinase |
| | | | | β-1,3-glucanase |
| Xing | 2015 | *Rhizopus* spp. | Jujube | - |
| Ali | 2015 | *Colletotrichum* spp. | Bell pepper/* | - |
| Song | 2016 | | Loquat[a] | PAL |
| El Guilli | 2016 | *Penicillium* spp. | Citrus | - |
| Zheng | 2017 | *B. cinerea* | Kiwi | - |
| Gutiérrez-Martinez | 2017 | *Colletotrichum* spp. | Mango/* | - |
| | | | Banana/* | |
| | | | Soursop/* | |
| Guo | 2017 | *Alternaria* spp. | Jujube | - |
| Shen | 2017 | - | Table grape[a] | PAL |
| | | | | chitinase |
| | | | | β-1,3-glucanase |
| Jongsri | 2017 | - | Mango[a] | PAL |
| | | | | chitinase |
| | | | | β-1,3-glucanase |
| de Oliveria | 2017 | *Colletotrichum* spp. | * | - |
| Kanetis | 2017 | *B. cinerea* | Table grape/* | - |
| Munhuweyi | 2017 | *B. cinerea* | * | - |
| Batista | 2018 | | Guava[a] | PAL |
| Gramisci | 2018 | *B. cinerea* | Pear | - |
| | | *Penicillium* spp. | | |

| | | | | |
|---|---|---|---|---|
| Hajji | 2018 | *B. cinerea* | Strawberry | - |
| Kharchoufi | 2018 | *Penicillium* spp. | Orange | - |
| Flores | 2018 | *B. cinerea* | * | - |
| Ramos-Guerrero | 2018 | *Colletotrichum* spp. | * | - |
| Liu | 2018 | *Penicillium* spp. | Blueberry | - |
| Shi | 2018 | *Penicillium* spp. | Grapefruit | - |
| Xoca-Orozco | 2018 | *Colletotrichum* spp. | * | - |
| Obianom | 2019 | *Colletotricum* spp. | Avocado | - |
| Madanipour | 2019 | *Penicillium* spp. | * | - |

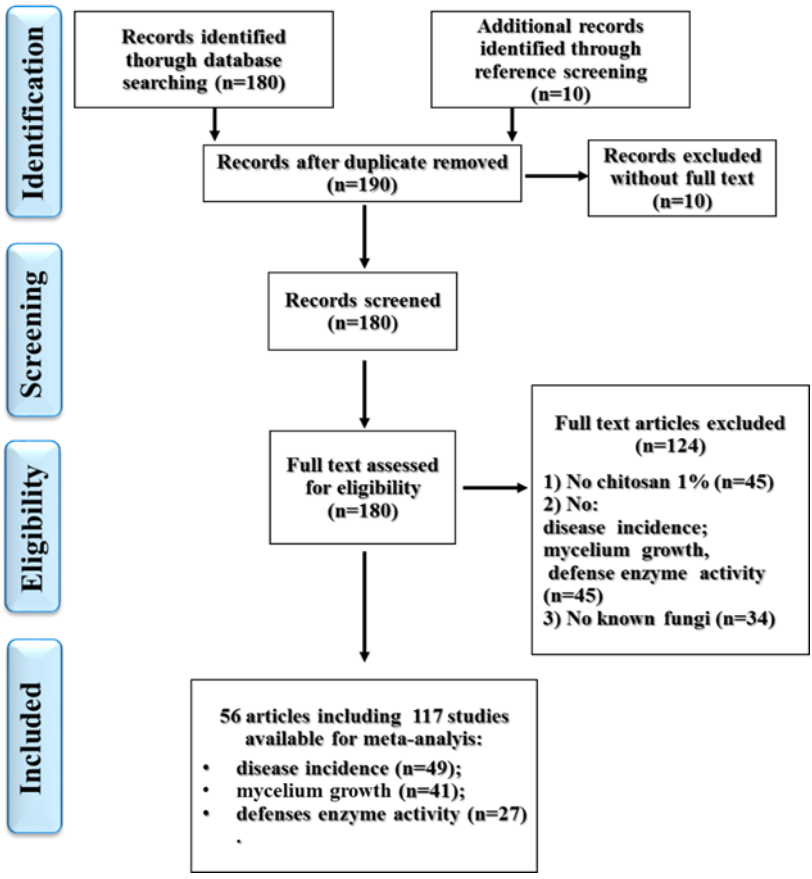


**Figure 1** - Flow chart exhibiting the selection process of eligible studies.

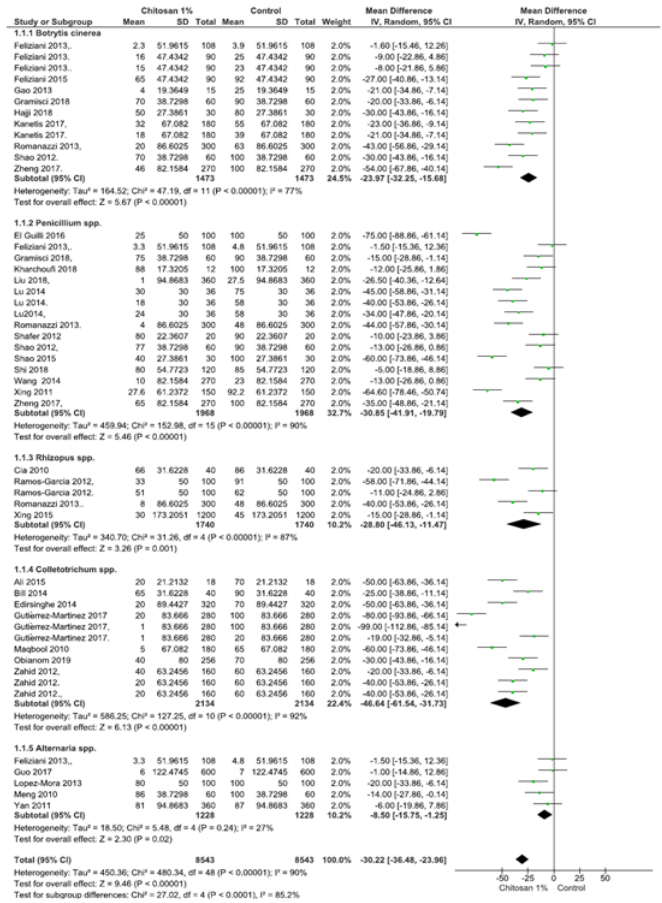


**Figure 2** - Forest plot using RavMan 5.3 software for random effect analysis related of effectiveness of 1% chitosan on *in vivo* disease incidence. *B. cinerea*, *Penicillium* spp., *Rhizopus* spp., *Colletotrichum* spp., and *Alternaria* spp. were considered as subgroups. Note: IV= Inverse Variance; CI =confidence interval.

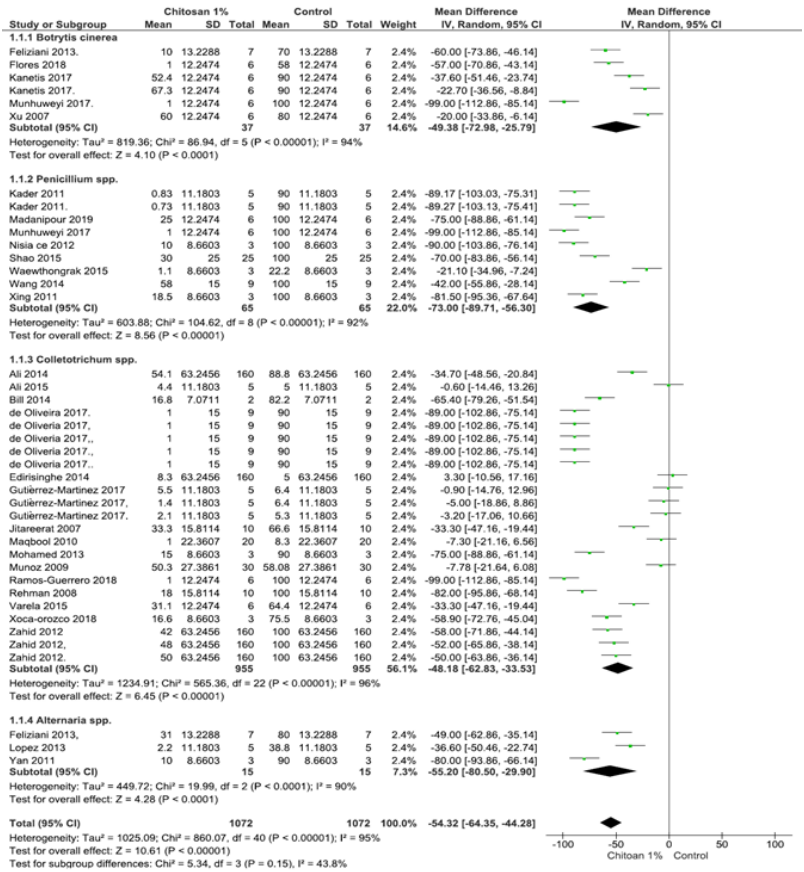


**Figure 3** - Forest plot using RavMan 5.3 software for random effect analysis related of effectiveness of 1% chitosan on *in vitro* mycelium growth. *Botrytis cinerea*, *Penicillium* spp., *Colletotrichum* spp. and *Alternaria* spp. were considered as subgroups. Note: IV= Inverse Variance; CI =confidence interval.

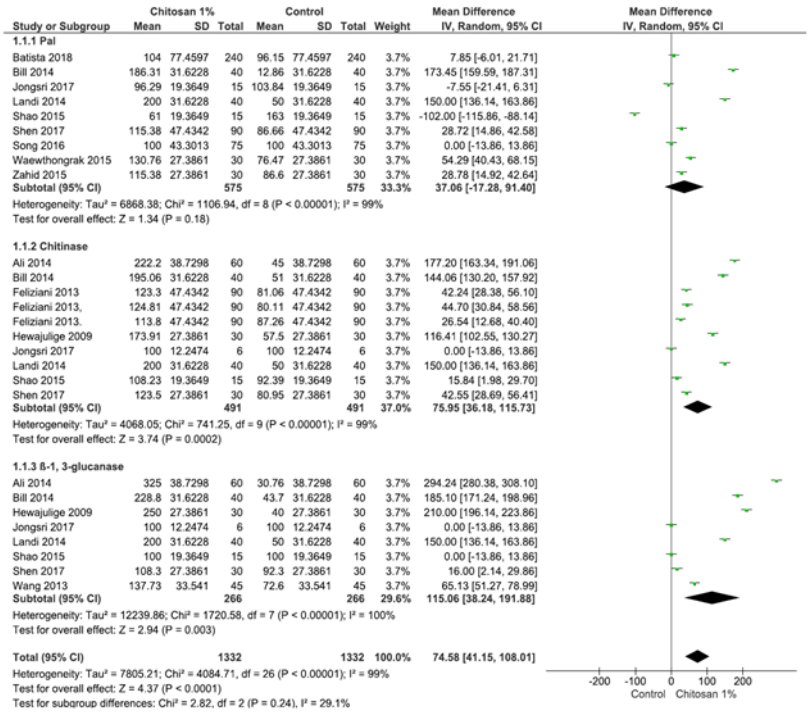


**Figure 2** - Forest plot using RavMan 5.3 software for random effect analysis related of effectiveness of 1% chitosan on plant defense mechanisms. β-1,3-glucanase, chitinase and PAL were considerd as subgroup. Related to Feliziani 2013, several studies were included from each article into subgroup. Note: IV= Inverse Variance; CI =confidence interval.

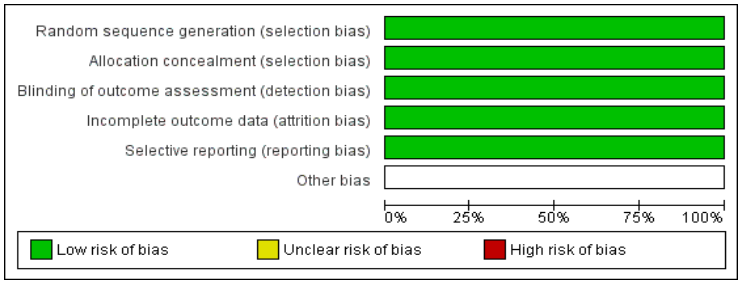


**Figure 5** - Summarized results after the use of the Cochrane risk of bias assessment tool. Review author's judgments about each risk of bias item presented as percentages across all included studies on disease incidence, mycelium growth, and defense enzyme activity.

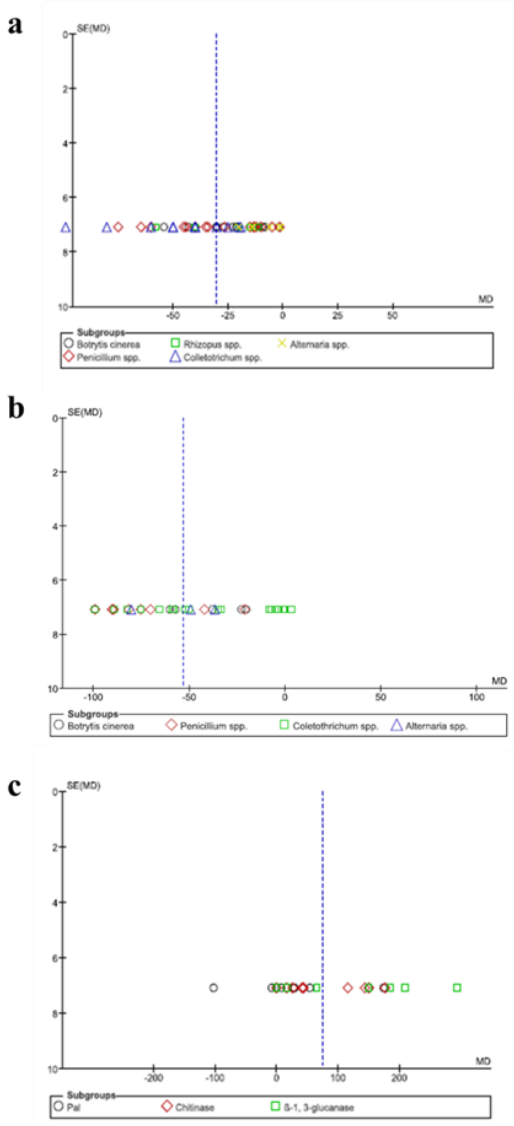


**Figure 6 -** Funnel plot to detect publication bias in the studies. Disease incidence (a), mycelium growth (b) and enzyme activity (c) detected after 1% chitosan treatment compared to control were reported.

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# POSTHARVEST TREATMENTS WITH NATURAL COMPOUNDS TO CONTROL STRAWBERRY DECAY

## Abstract

The effectiveness of commercial natural compounds on postharvest decay of strawberry fruits (*Fragaria* × *ananassa* Duch, cv. 'Monterey') by applying:1%'Chito Plant powder', 'Chito Plant solution', 'Kaitosol', 'OII-YS', and Methyl Jasmonate ('MeJA'), 0.4%'3 Logy', 0.5% 'DF-100 Forte', 'Prev-Am Plus', and 'Humic acid' has been tested. Strawberry fruits were dipped in solution for 30s and incubated at room temperature (20°C ± 1°C) and at cold storage conditions (4°C ± 0.5°C) following 2 days shelf life at 20°C. The highest gray mold reduction was obtained with 'Chito Plant powder' and 'Prev-Am Plus', while 'MeJA' and 'Kaitosol' were more effective to control Rhizopus rot at both, cold storage and room temperature conditions.

**Keywords:** *Botrytis cinerea*, natural compounds, *Rhizopus stolonifer,* strawberry.

## 3.1 Introduction

Strawberry (*Fragaria* × *ananassa* Duch) is one of the most popular fruit in the world for its delicious flavor, health benefits, and exceptional nutritional value (Mikulic-Petkovsek et al., 2013; Van de Velde et al., 2013). However, strawberry fruits were particularly perishable during postharvest storage, susceptible to mechanical damages, especially, the degradation by a fungal disease, limiting its commercialization and consumption (Ugolini et al., 2014). Gray mold and Rhizopus rot caused by *Botrytis cinerea* (Pers.) and *Rhizopus stolonifer* (Ehrenb.) respectively, were the main pathogens of strawberry postharvest decay (Maas, 1998).

Primary infection of gray mold could occur at bloom time and remained quiescent in the field (Powelson, 1960). *B. cinerea* produced large numbers of spores and able to survive in a dormant state. This pathogen was presented in a variety environmental conditions (Hahn, 2014). According to a previous review, *B. cinerea* ranked second into the world top 10 fungal plant pathogens list based on scientific and economic importance (Dean et al., 2012). This pathogen could also develop at low temperatures (even at 0 °C) with the consequent reduction of the length of the storage period. *R. stolonifer* was a common wound pathogen of a very wide range of the fruit and vegetables, causing a rapidly spreading watery rot. Rhizopus rot could spread at temperature greater than 4–6 °C. Both, of these diseases spread quickly to other fruit, a phenomenon was known as nesting.

Despite the efficiency of the synthetic fungicides in fruit disease management, natural eco-friendly alternative compounds were recommended, and attracted scientist attention.

In recent years, the antimicrobial activity of a large number of compounds similar to ‘plant and animals’

extracts as gums, resins etc. against both, pre- and postharvest pathogens were tested (Feliziani et al., 2015; Lelario et al., 2018). 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 improved current productive practices. Natural compounds were characterized by antimicrobial activities against the main postharvest pathogens, and/or they were resistance inducers that activate the plant defenses, to simulate the presence of a pathogen.

Among the natural compounds, chitosan had received much attention for application in agriculture and for food industry. Chitosan is a natural biopolymer, derived from chitin of both marine crustaceans (Dima et al., 2016) and the cell wall of many pathogenic fungi. (Benhabiles et al., 2012; Romanazzi et al., 2015). This compound has been reported to both stimulate plant defenses and prevent disease development (Feliziani et al., 2015; Landi et al., 2017). A number of promising approaches for postharvest application of different type of chitosan formulation as 'Chito Plant powder' (Gramisci et al., 2018; Liu et al., 2019) were suggested.

Essential oils (EOs) from aromatic plants gaining interest and their effectiveness for the control of postharvest decay of fruit has been documented (De Corato et al. 2010; Lopez-Reyes et al. 2013; Sivakumar and Bautista-Banos 2014; Mari et al., 2016; Rotolo et al., 2017). Others compounds as 'Humic acid' (Wei et al., 2018), an organic compound known as promoter in sustaining plant growth (Suh et al., 2016), have been reported to have efficacy in the control of several plant diseases, according to both, induce host resistance and direct antimicrobial activity (Xu et al., 2019). In the same way, the Methyl Jasmonate ('MeJA') (Glowacz a et al., 2017) was endogenous plant growth substances that modulated many physiological processes including,

responses to environmental stresses (Greelman et al., 1997).

Therefore, in the present study, we selected a list of promising commercial compounds based on chitosan, as 'Chito Plant powder', 'Chito Plant solution', 'Kaitosol' and 'OII-YS'; based on EOs as '3 Logy', 'PrevAm Plus' and 'DF-100 Forte' and organic compounds as 'Humic acid' and 'MeJA'. The aim of this study was to determine the effectiveness of the compounds in the control of postharvest decay of strawberry during the storage at room temperature (20±1°C) and at cold temperature (4 ±0.5°C).

## 3.2 Materials and Methods

### *3.2.1 Fruit materials*

Commercial strawberry (*Fragaria* × *ananassa* Duch*,* cv 'Monterey'), were harvested from orchard located in Montalto (AP) in the Marche region, central-eastern Italy, The fruit were harvested at the mature stage and selected for the absence of defects, uniformity in size and degree of ripening (2/3 red on the surface) (Rosati and Cantoni, 1993) and were used for the experiments on the day of harvested (Ugolini et al., 2019).

### *3.2.2 Preparation of natural compounds solutions*

The compounds used for postharvest treatments included chitosan-based product at different emulsions:1%w/v 'Chito Plant powder', 1% v/v 'Chito Plant solution', 1% v/v 'Kaitosol' and 1% v/v 'OII-YS' (Chito Plant, ChiPro GmbH, Bremen, Germany). Commercial EOs products at 0.5%, v/v as 'DF-100 Forte' (Agritalia, Rovigo, Italy), 0.5%, v/v 'Prev-Am plus' (Nufram, Milano, Italy), and at 0.4%, v/v '3Logy' (Sipcam, Milano, Italy).

Organic compounds at 0.5% w/v 'Humic acid'(Sigma-Aldrich, United State), and 1% v/v 'MeJA' (Sigma-Aldrich) were applied in different experiments for

postharvest treatments. All compounds were dissolved in water directly and stirred for 1 hour. Tween 80 (Sigma Chemical Co., St. Louis, MO, USA) surfactant agent, 20 µl/L was included in the solutions.

### *3.2.3* In vivo *experiment*

Deep inside solution method was used according to Feliziani et al., (2015), for following treatments: (i) natural compounds solution (treated strawberry fruit); (ii) sterile distilled water (untreated strawberry fruit). The method was performed by soaking strawberries for 30s inside each solution. After the treatments the fruits were air dried for 3 to 4 hours and individually arranged in small plastic boxes. Fruits were incubated at two different temperature conditions, room temperature (20 ± 1°C) or cold temperature (4 ± 0.5°C) for 7 days, 95–98% RH, and exposed for 2 days shelf-life at 20°C, 95–98% RH. Each treatment consisted of 66 fruits (6 fruit for 11 plastic boxes). Three replications were performed for each treatment. The naturally occurring infections were evaluated by the end of storage.

### *3.2.4 Decay evaluation*

During the storage, the percentage of strawberry decay was estimated. Disease severity was recorded 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; 5, more than 81% of the strawberry surface infected and showing sporulation (Romanazzi et al., 2000). The empirical scale allowed the calculation of the McKinney's index, expressed as the weighted average of the disease as a percentage of the maximum possible level (McKinney, 1923), this parameter included information on both disease incidence and disease severity.

### *3.2.5. Statistical analysis*

Statistical analysis was performed based on Fisher test. Differences among means of values was 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 (Conover and Iman, 1981; Romanazzi et al., 2009).

## 3.3 Results

### *3.3.1 Effect of natural compounds on strawberry gray mold*

The postharvest treatments with commercial compounds among three different experiments showed various level of inhibition on gray mold disease in strawberry fruit (data not showed). Interesting, the natural compounds were able to reduce the total gray mold infection of strawberry compared to the control on fourth day post treatment (dpt) at cold storage ($4 \pm 0.5$°C) (**Figure 1**).

The McKinney's index of gray mold in strawberry treated with 'Chito Plant powder', 'Chito Plant solution', 'Kaitosal', 'OII-YS', 'DF-100 Forte', '3Logy', 'Humic acid', 'Me JA', 'Prev-AM' Plus', was decreased compared to the control by, 35.4%, 26.8%, 24.3%, 45.1%, 28.6%, 29.3%, 32.9%, 31.7%, 19.3% respectively, (**Figure 1**). Notably, the most effective treatment to control postharvest decay of strawberry was 'OII-YS', while, 'Prev-Am Plus' did not showed any significant different compare to the control (**Figure 1**).

### *3.3.2 Rank analysis*

A more direct analysis of the degree of comparative effectiveness for the disease incidence reduction was obtained through the application of rank analysis. This analysis confirmed that the control had the highest sum of

ranks (8.5); therefore, all treatments were more effective compared to control, in particular, in 'OII-YS', '3Logy'and 'Humic acid' slightly lower values was observed (7.5, 6.5 and 5 respectively), while the lower sum of ranks for 'Prev-Am Plus' and 'DF-100 Forte' (2.5 and 2 respectively) showed, that they were largely as effective as each other at room temperature condition (**Figure 2**).

The result of this analysis at cold temperature condition confirmed, that the control had the highest sum of ranks (8.6); therefore, all treatments were more effective compared to control, however for 'MeJA, '3Logy'and 'DF-100 Forte' slightly lower values was observed (8.3, and 6.5, respectively), while the lower sum of ranks for 'Chito Plant Powder' 'Chito Plant Solution' (3.3 and 2 respectively) showed that they were largely as effective as each other (**Figure 3**).

### *3.3.3 Efficiency of natural compounds on Rhizopus rot*

Based on the cumulative incidence, the effectiveness of natural compounds against Rhizopus rot increased by the time at both temperature conditions (**Figure 4** and **5**).
'Kaitosol', 'Chito Plant powder', 'Chito Plant solution', 'DF100-Forte', 'Prev-Am Plus', and '3Logy' recorded as the most effective compounds compare to the control at 20°C (**Figure 4**), while, 'OII-YS', 'Kaitosol' and 'Humic acid' were more active to reduce the progress of Rhizopus rot on 4 dpt at 4°C (**Figure 4**). The cumulative incidence on natural compounds treatments was about 1-2% lower than the control at both temperature conditions and (**Figure 4** and **5**). The 'MeJA' showed a similar effectiveness at both temperature conditions (data not shown).

## 3.4 Discussion

In this work, compounds from natural sources such as chitosan different emulsions, commercial EOs, and organic acid as 'MeJA' and 'Humic acid' with promising properties, have shown their potential for the preservation of strawberry fruits. In particular, 'OII-YS' reduced the development of gray mold and Rhizopus rot of strawberry fruits, cv 'Monterey' at cold temperature condition.

Despite the high heterogeneity, the rank analysis among different treatments was performed and showed that all compounds tested in this work were more effective compare to control, this specify that our commercial products decreased the development of strawberry decay of gray mold, thus could be used for prolonging the shelf-life of the fruit. It is interesting to note that, results were different according to fruit storage temperature conditions. In detail 'Prev-Am Plus' and 'DF100-Forte' were the best product at room temperature, while the compounds based on chitosan as 'Chito Plant powder' and 'Chito Plant solution' showed the best performance at cold conditions. This result confirmed that the temperature affected the effectiveness of the compounds, recommending, in products based on natural compound, it is particularly important to respect the stability of the product conditions. The highest effectiveness of 'Prev-Am Plus' and 'DF100-Forte' at room temperature could be due to their high activity of volatile composition. Ambient temperature critically affected EOs stability in several aspects. Usually, on these highly volatile plant lipophilic compounds, the chemical reactions accelerate with increasing heat (Peng et al., 2013; Hosseini et al., 2018). Consequently, the application method could affect the efficacy of postharvest treatments of EOs (Lopez-Reyes, 2013). Strong antifungal activity from above EOs could be attributed to their components (Císarová et al., 2016; Tančinová et al., 2019). 'Prev-Am Plus' and 'DF-100Forte' consisted of sweet orange essential oils and grapefruit seed extract

respectively. '3Logy' was a biocontrol agent on grape vineyard. The determined EOs composition in '3Logy' showed that the most abundant constituents were geraniol (6.6%), thymol (6.6%) and eugenol (3.3%). These terpenoids compounds were able to reduce the postharvest fungal pathogen infections like gray mold and *Rhizopus* spp. (Rotolo et al. 2017; Zhou et al., 2018, 2019). Similar results from essential oils consist of such terpenoids has been reported previously (Servili et al., 2017; Oliveria et al., 2019). In addition, the inhibitory effect of citrus volatile or grapefruit seed extract on fungal pathogen, the causal agent of postharvest fruit disease, was already tested (Droby et al., 2008; Chen et al., 2019; Mahato et al., 2019).

Concerning our results related to the compounds based on chitosan, the refrigerated storage, was effective against gray mold on strawberries. The chitosan effectiveness in disease control showed triple activity associated with antimicrobial activity, host defenses activation, and film formation on the treated surface (Romanazzi et al., 2017, Romanazzi et al., 2018; Martinez et al., 2018). Previous works estimated this compound as the most qualified one to decline the disease and prolong the shelf life at cold storage condition. It is known that chitosan coatings delayed changes in weight loss, soluble solids, total sugars and reduced the ethylene production, these actions could be improved at low temperature conditions, leading to a lower disease incidence of fungal pathogen (Romanazzi et al., 2018; Thabet et al., 2019). Regarding Rhizopus rot the effectiveness of the compounds was more variable and less influenced by the temperature storage conditions. All natural compounds, except 'OII-YS' and 'Humic acid' were more powerful to reduce mycelium growth at room temperature compare to the cold storage condition, while 'MeJA' and 'Kaitosol' were the most effective compound at both temperature

conditions. (Haggag et al., 2010). 'MeJA', a major derivative of the plant hormone jasmonic acid, played a critical role in inducing resistance to fungal pathogens (Mohammad et al., 2015). 'MeJA' has been used effectively as a postharvest application in several fruits and has been reported to suppress *B. cinerea* in strawberry (Moline et al., 1997). The successful activity of chitosan (Thabet et al., 2019) and 'MeJA' (Jin et al., 2009) against gray mold or *Rhizopus rot* reduction on different fruit species was proved.

## 3.5 Conclusions

In conclusion, postharvest treatments with alternatives to conventional fungicides could reduce postharvest disease of strawberry. The comparison of the natural compound activity on strawberry pathogen at different temperature condition showed that most of the compounds during gray mold infection were more active at cold storage, while, their effectiveness toward Rhizopus rot was higher at room temperature. In addition, the storage conditions affected natural compounds effectiveness, emphasizing that the organoleptic characteristics of the compounds affected the efficiency of postharvest disease contamination. In particular, the action of volatiles molecules of 'Prev-Am Plus' following 'DF100Forte' against gray mold improved at the room temperature while the 'Chito Plant powder' based on chitosan was showed a better efficiency at cold storage condition. 'MeJA' and 'Kaitosol' were effective at both temperature conditions against Rhizopus rot.

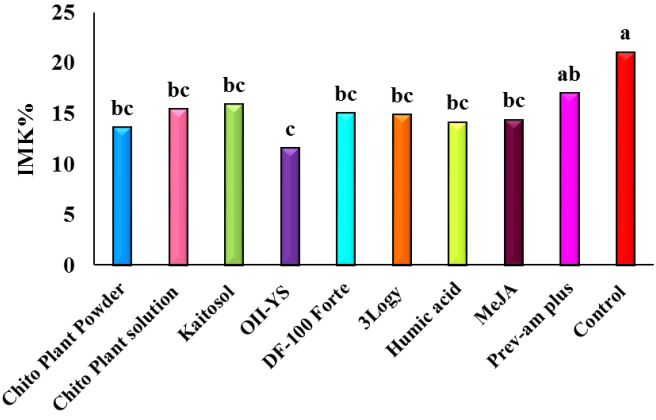


**Figure 1** - McKinney index of gray mold recorded on strawberries treated with commercial natural compounds formulation. The fruit were kept for 7days at 4 ± 0.5 °C, 95–98% RH. Different letters indicate that the values are significantly different according to Fisher test honestly significant difference, at $P \leq 0.05$.

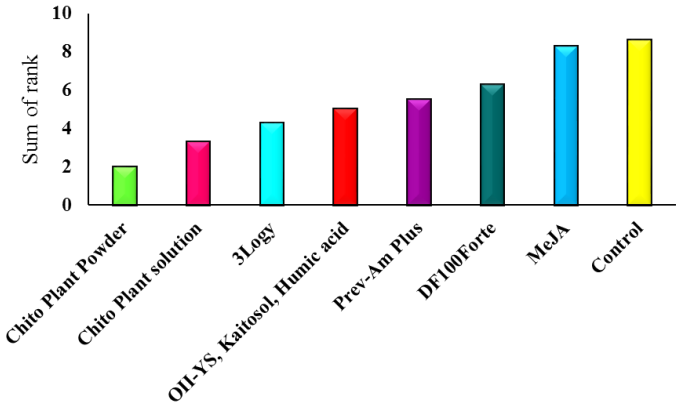


**Figure 2** - The effect of postharvest treatment with natural compounds on the reduction of gray mold on strawberry according to rank analysis. The fruit were kept at 20 ± 1 °C, 95–98% RH.

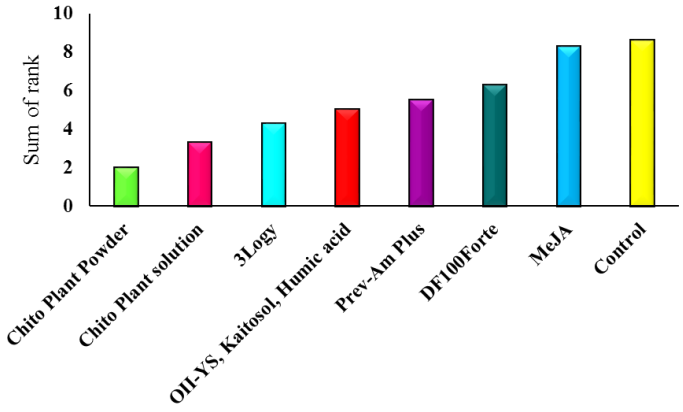


**Figure 3** - The effect of postharvest treatments with natural compounds on the reduction of gray mold on strawberry according to rank analysis. The fruit were kept for 7 days at 4 ± 0.5 °C, 95–98% RH.

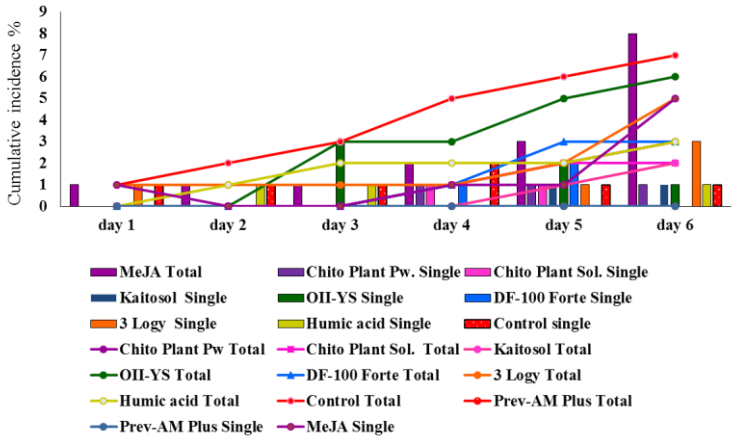


**Figure 4** - The effect of postharvest treatments with natural compounds against Rhizopus rot based on cumulative incidence. The fruits were kept at 20 ± 1 °C, 95–98% RH. Total = decay in total fruit; Single = decay in single fruit.

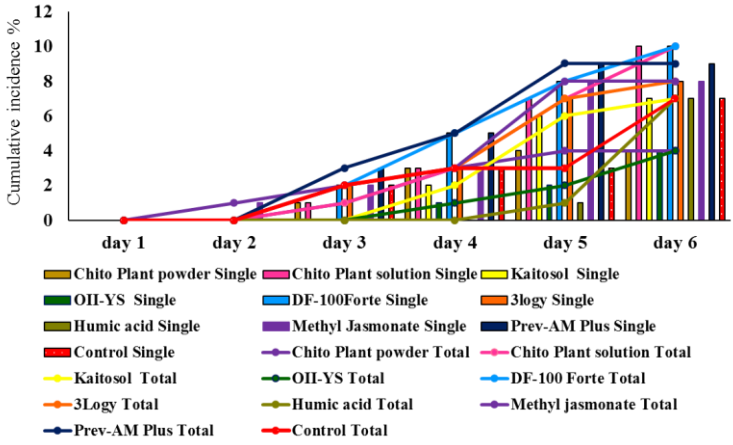


**Figure 5** - The effect of postharvest treatments with natural compounds against Rhizopus rot based on cumulative incidence. The fruits were kept for 7 days at 4 ± 0.5 °C, 95–98% RH. Total = decay in total fruit; Single = decay in single fruit.

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# EXPOSURE TO VOLATILES OF ESSENTIAL OILS UNDER HYPOBARIC TREATMENT TO CONTROL POSTHARVEST GRAY MOLD AND RHIZOPUS ROT ON STRAWBERRY

## Abstract

Strawberry fruits were highly sensitive to the infection of postharvest gray mold and Rhizopus rot, caused by *Botrytis cinerea* and *Rhizopus stolonifer*, respectively. The application of essential oils (EOs) combined with hypobaric treatments were promising alternatives to synthetic fungicides. The aim of this study was to control postharvest decay of strawberry fruits, exposure to volatiles of EOs of *Citrus bergamia* (bergamot) *Rosmarinus officinalis* (rosemary) and *Mentha piperita* (mentha) under hypobaric treatment at 50 kPa (0.5 atm) compared to hypobaric treatment alone, for 16 h. The McKinney's Index of gray mold in strawberries, exposure to volatiles of rosemary and mentha EOs under hypobaric conditions was reduced by around 43% and 48% respectively at room temperature (20°C). Bergamot and rosemary EOs controlled strawberry gray mold by around 17% and 13% respectively at cold temperature (4°C). Rosemary was the most active EOs against Rhizopus rot, the cumulative incidence was about 5-7% lower than the control during 5 days at room temperature, while, mentha was more effective and the disease was 4% lower than the control at cold storage conditions.

**Keywords:** essential oils, gray mold, hypobaric condition, strawberry decay

## 4.1 Introduction

Strawberry (*Fragaria* × *ananassa* Duch) is one of the most important fruits and famous for health- promoting effect as a source of macro and micronutrients, vitamins for human diet (Basu et al., 2014; Giampieri et al., 2015; Wang and Lin, 2000). However, these properties could be undermined since this fruit was susceptible to mechanical damages or fungal diseases infection, that limited its commercialization and consumption (Ugolini et al., 2014; Mohammadi et al., 2015). Several fungal diseases infected postharvest strawberry (Zamani-zadeh et al., 2014), among them, gray mold and Rhizopus rot, caused by species of *Botrytis cinerea* and *Rhizopus stolonifer*, have been determined as the main pathogens of this fruit (Perdones et al., 2016; Oliveira et al., 2019;), resulting in enormous economic losses in a postharvest stage (Hahn et al., 2014). Management of this pathogen required precise strategy. However, in these years the negative environmental and human health impact of pesticides had generated interest in developing effective and non-toxic approaches. At this regard plant essential oils (EOs) were gaining interest due to their apparently safe nature and their potential effectiveness as biopesticides for crop protection. Their application in fruits had advantages such as high effectiveness against several pathogens and low toxicity (no target microorganism and humans) (Pavela et al., 2016). Several investigations have been reporting the efficacy of EOs in *in vitro* and *in vivo* tests (Guerra et al., 2015; Boubaker et al., 2016; Perumal et al., 2016; Wang et al., 2019), in particular postharvest control of gray mold and Rhizopus rot with testing EOs extracted from the species belonging to *Lamiaceae* family including *Rosmarinus officinalis* (de Sousa et al., 2013), *Citrus bergamia* (Murmo et al., 2018), *Thymus vulgaris* (Camele et al., 2010), *Mentha piperita* (Servili et al., 2017) and

*Melissa officinalis* (El Ouadi et al., 2017). The potential ability of EOs to enhance the antifungal activity in various types of fruits: banana, (Madjouko et al., 2018), grape (Wang et al., 2015), and strawberry (Zhang et al., 2016; Shao et al., 2013) was strongly associated with its monoterpenic components as, thymol, limonene, a-pinene and eugenol (Barrera-Necha et al., 2008; Sellamuthu et al.,2013). In addition, their efficiency to inhibit the fungal mycelium growth and spore germination was associated with concentration and temperature condition (Sangeetha et al., 2010; Ambrico et al., 2019). The mechanism related to membrane disruption by their lipophilic compounds was suggested (Cowan, 1999). These compounds passed easily through cell membranes and caused damage to the fungal cell organization (Chao et al., 2005; Shukla et al., 2012). EOs could be applied on fruits directly (vapor phase) or incorporated with other alternative treatment at pre- or postharvest stages, which could reduce the fungal decay and upgrade the fruit shelf life (Estrada et al., 2018). However, because of the negative organoleptic effects of EOs in fruit due to their intense aroma, the novel technique for the applications of EOs incorporate with other treatment were developed. Using EOs under hypobaric conditions to control postharvest decay was highly recommended (Romanazzi et al., 2001; Hashmi et al., 2013; Servili et al., 2017), as the vaporization and distribution over the fruit surface could be improved, led to minimizing the loss of nutritional and physical attributes and reduced pathogen decay of strawberries (Harker et al., 2000; Castello et al., 2010; Inestroza-Lizardo et al., 2019).

Therefore, the objective of this study was to determine the effectiveness of exposure to volatiles of essential oils of *Rosmarinus officinalis* (rosemary), *Mentha piperita* (mentha), and *Citrus bergamia* (bergamot) to control postharvest decay of strawberry

including gray mold and Rhizopus rot under hypobaric conditions (50 kPa) at two different temperature conditions: room temperature (20 ± 1°C) and cold storage (4± 0.5°C).

## 4.2 Materials and Methods

### *4.2.1 Fruits*

The experiments were performed on strawberry (*Fragaria × ananassa*, cv. 'Monterey') which was grown in greenhouse located at Montalto, (AP) Marche region, central-eastern Italy. Fresh fruits were harvested and transferred to the laboratory and selected according to homogeneous size, shape, color, and weight, and absence of the injuries.

### *3.2.2 Treatments*

Eight strawberry were placed in small plastic trays. Twelve plastic trays were transferred in airtight boxes for 16h. In addition, the airtight boxes contained flasks with 0.5 mL water or the commercial EOs of rosemary, mentha or bergamot at concentration of 500µl/L were added. A total of 96 strawberries for each treatment were analyzed. The chemical compositions of the EOs used in the experimental trials was shown in Table 1. The hypobaric treatment of 50 kPa (0.5 atm) was created within the airtight boxes using a vacuum pump for the trails. The new pressure was recorded immediately after 16h. The plastic trays containing the strawberries were moved out from the airtight boxes and placed into the large covered plastic boxes. They were transferred at two different temperature conditions, room temperature (20 ± 1°C); cold storage (4 ± 0.5°C) for 7 days, following 2 days shelf life at room temperature 20 ± 2°C.

### *4.2.3 Decay evaluation*

Postharvest decay of strawberry was recorded daily for five days. Disease incidence was expressed as percentages of infected fruit. Disease severity was recorded according to an empirical scale with five degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5 >81% of fruit surface infected (Romanazzi et al., 2001). This empirical scale allowed the calculation of the McKinney's Index, which was expressed as the weighted average of disease as a percentage of the maximum possible level (McKinney, 1923). This was calculated according to Eq. $I = [\Sigma(d \times f)/(N \times D)] \times 100$, where d was the category of rot intensity scored on the strawberry, f was its frequency, N was the total number of strawberry examined (i.e., healthy and rotted), and D was the highest category of disease intensity that can occur on the empirical scale (Romanazzi et al., 2001). The cumulative incidence was evaluated as the number of infected fruits divided by the total number of individuals in population for a specific time period.

### *4.2.4 Statistical analysis*

Each experiment was repeated three times. Statistical analysis was performed based on Fisher test. Differences among means of values was analyzed by one-way analysis of variance (ANOVA) Difference was considered as statistically significant at $P < 0.05$.

## 4.3 Results

### *3.3.1* Effectiveness of essential oils on strawberry against *Botrytis cinerea*

The McKinney's index of gray mold in strawberry exposed to the vapors of the rosemary and mentha EOs, was significantly decreased compared to the control, by 43.6% and 48.3% respectively after 2 days at 20°C (**Figure 1**).

The McKinney's index of strawberry decay treated with bergamot and rosemary was significantly decreased compared to the control, by 17%, and 13% respectively, after 5 days from treatment at 1°C (**Figure 2**).

### *4.3.2* Effectiveness of essential oils on strawberry against *Rhizopus stolonifer*

Rosemary was evaluated as the most successful compound at room temperature condition. The cumulative incidence in rosemary treatment was 5-7% lower than the control during five days (**Figure 3**). The result from the cold storage conducted that, all EOs could reduce Rhizopus rot 1-4% less than the control at the last day two days, in particular, mentha was the most efficient compound on mycelium growth reduction (**Figure 4**).

## 4.4 Discussion

In the recent years, essential oils have gained great attention as natural preservatives that could be used to prolong the postharvest life of fruit and vegetables (Mari et al., 2016; Ambrico et al., 2019). In this work we applied EOs combined with hypobaric method testing the synergistic effect of these two treatments. Under hypobaric conditions, partial pressure of component gases, and consequently the total pressure, was reduced. In general, $O_2$ was the primary gas molecule for quality preservation of processed foods and fresh produce, therefore, reduction of $O_2$ partial pressure was the main principle of hypobaric storage (Napassawan et al., 2018). Hypobaric storage was effective in maintaining fruit quality and preserving fruits from harmful metabolites, such as ethanol and acetaldehyde. The fungal decay of strawberries under hypobaric storage, was reduced (Hashmi, et al., 2013). On the other hand, over recent decades, antifungal activities of EOs have been well

documented (Deans and Ritchie, 1987; Hyldgaard et al., 2012), and there have been several more recent studies describing the effectiveness of EOs on the control of postharvest decay of fruit (Cindi et al., 2016; Mari et al., 2016). The effectiveness of combined application of these two treatments could increase postharvest control diseases. In the hypobaric condition, the vaporization of the EOs was higher compared to the atmospheric pressure. Due to this, as the pressure in the airtight containers was lower than the atmospheric pressure (i.e., 50 kPa), there was greater vaporization which lead to upgrade the function of EOs. Previous study (Servili et al., 2017) has shown the efficiency of this method, against gray mold on grapes. This research for the first time showed that strawberry postharvest treatments of EOs combined with hypobaric method, decreased disease incidence of both, gray mold and Rhizopus rot at cold and room temperature storage conditions. This work highlights a synergistic action of EOs volatile components and hypobaric treatment. This followed the approach of the multiple hurdle's theory, according to which, the use of an individual alternative to conventional fungicides might not be effective enough to completely control a disease, while the integration of many might replace the use of conventional fungicides (Romanazzi et al., 2012).

Based on our result, the effectiveness of rosemary and mentha against gray mold and Rhizopus rot started from the first days at room temperature, while the efficacy of bergamot against above diseases was observed at 5 dpt at both room temperature and cold storage conditions. Our work emphasized that the interaction of the active components of EOs with the pathogens and the treatments environmental conditions, affected the effectiveness of postharvest strawberry decay. Usually, cold application delayed the effectiveness of EOs. This phenomenon was associated with the initial oxidant volatile organic

compounds that were temperature-dependent. In particular, our work suggested that the main bergamot active molecules as monoterpene structure of limonene (Van de vel et al., 2019) and a-pinene (Tampieri et al., 2005) affected pathogen activity. Previous works were showed that EOs molecular compositions, influenced their antimicrobial function (Chalchat et al., 2000; Tampieri et al.,2005; Alitonou et al., 2012) or their ability to trigger resistance to pathogen attack locally and systemically (Durrant et al., 2004; Walters et al., 2009; Murmu et al., 2018; Chow et al., 2019). Van de vel et al., (2019), reported that EOs mechanism could be connected to fungi toxic properties and fungal cell membranes characteristics (Guo et al., 2017; Xueuan et al., 2018).

## 4.5 Conclusions

In conclusion, rosemary and mentha showed more activity compare to bergamot under hypobaric condition against gray mold besides, rosemary was the most effective compound against gray mold and Rhizopus rot at room temperature. Based on this improvement, rosemary following mentha might be more successful for prevention ofthese fungal decay. This hypothesis need to be verified in small and later in large scale tests.

**Table 1** - The main chemical components of the essential oils (%) used in this study. (%) - Flora Srl, Pisa, Italy

| ***Citrus bergamia*** **(bergamot)** (%) | ***Mentha piperita*** **(mentha)** (%) | ***Rosmarinus officinalis*** **(rosemary)** (%) |
|---|---|---|
| limonene 38.41<br>linalil acetate 28.86<br>linalool 13.37<br>γ-terpinene 6.42<br>β-pinene 5.48 | menthol 33.39<br>menthone 26.67<br>cineol 5.13<br>menthfuran 4.67<br>menthyl acetate 4.38 | α-pinene 25.9<br>cineol 21.65<br>camphor 15.03<br>canfene 8.82<br>limonene 4.68 |

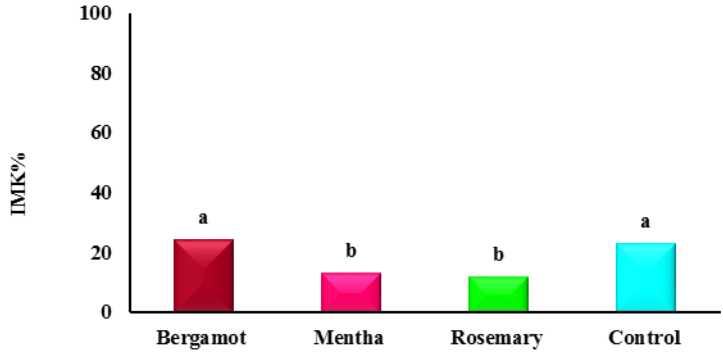


**Figure 1 -** McKinney's Index of postharvest gray mold of strawberry cv. 'Monterey' stored under hypobaric conditions (50 kPa) in airtight boxes containing flasks with water or essential oils of rosemary, mentha, or bergamot for 16 h, then, stored at 20 ± 1°C. Values within columns followed by the same letter are not significantly different (Fisher test. significant difference (HSD) tests; $P \leq 0.05$).


**Figure 2 -** McKinney's Index of postharvest gray mold of strawberry cv. 'Monterey' stored under hypobaric conditions (50 kPa) in airtight boxes containing flasks with water or essential oils of rosemary, mentha, or bergamot for 16 h, then stored at 4 ± 0.5°C for 7 days following 2 days shelf life. Values within columns followed by the same letter are not significantly different (Fisher test. significant difference (HSD) tests; $P \leq 0.05$).

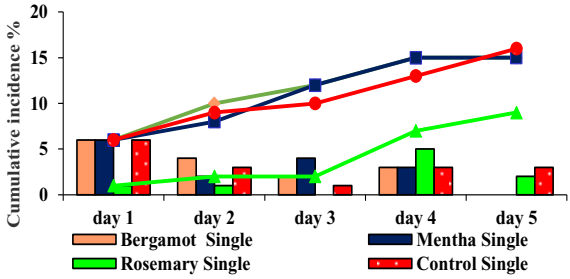


**Figure 3 -** Cumulative incidence of postharvest Rhizopus rot on strawberry cv. 'Monterey' stored under hypobaric conditions (50 kPa) in airtight boxes containing flasks with water or essential oils of rosemary, mentha, or bergamot for 16 h, then stored at 20 ± 1°C. The data were recorded for 5 days. Total = decay in total fruit; Single = decay in single fruit

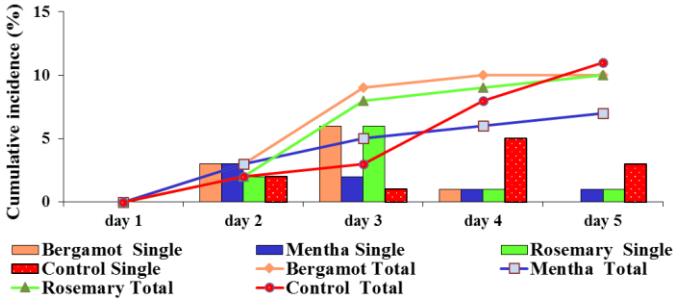


**Figure 4 -** Cumulative incidence of postharvest Rhizopus rot on strawberry cv. 'Monterey' stored under hypobaric conditions (50 kPa) in airtight boxes containing flasks with water or essential oils of rosemary, mentha, or bergamot for 16 h, then stored at 4 ± 0.5°C for 7 days following 2 days shelf life. The data were recorded for 5 days. Total = decay in total fruit; Single = decay in single fruit

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# PREHARVEST APPLICATION OF NATURAL COMPOUNDS AGAINST GRAY MOLD AND ITS EFFECT ON STRAWBERRY QUALITY DURING STORAGE TIME

## Abstract

The effectiveness of 'Chito Plant Powder', 'KiForce' based on chitosan and 'Prev-Am Plus', based on essential oils, on both, postharvest decay and quality of strawberry fruits, were analyzed. The strawberry fruits were harvested, on the third day after treatments. Gray mold after storage at room temperature (20˚C ± 1°C) and cold storage for 7 days at (4 ± 0.5°C) following two days shelf-life was evaluated. 'Chito Plant powder' formulation was effective in the control of postharvest decay at cold temperature condition. The qualitative parameters were recorded after harvested (*Time 1)* and after 3 days at room temperature condition (*Time 2-RT*) or cold storage (*Time 2-CS*). 'Prev-Am Plus', increased the FRAP and flavonoid content at *Time 1* while they decreased at *Time 2-RT*. At the same time point, the ethylene production increased, and the weight loss was increased at *Time 2-CS*. Regarding chitosan-based compounds, 'Chito Plant powder', maintained fruit quality and improved titratable acidity. Our results suggested, 'Chito Plant powder' was the most active compounds in the control of postharvest decay and quality of strawberry.

**Keywords:** **'**Chito Plant Powder', fruit quality parameters, 'KiForce', preharvest treatments, 'Prev-Am Plus'.

## 5.1 Introduction

Strawberry fruits (*Fragaria × ananassa* Duch.) was highly appreciated by consumers for its unique taste and flavor and its health benefits. Indeed, strawberries were rich in bioactive compounds, such as natural antioxidants, polyphenols, anthocyanins, vitamins and amino acids (Afrin et al., 2016). However, strawberry had a short postharvest life due to the high susceptibility to fungal pathogens. Fungal infection in strawberry, occurred in the field and more notably, during the postharvest phase infection could easily move to nearby fruits (Maas, 1998; Ugolini et al., 2019). The main pathogen that affected strawberry during storage was *Botrytis cinerea* a causal agent of gray mold. Until now, the strategy to control gray mold of strawberry using synthetic fungicides and preservation agents have been the first line of defense in conventional agriculture. But, increasing restrictive legislation on their use, together with the insurgence of pathogen resistance and consumer attention to food safety and sustainability, led research towards alternative strategies. In this context, the use of bioactive natural compounds was one of the most promising alternative options for controlling fruit pathogens and delaying senescence in harvested product (Ugolini et al., 2019; Hua et al., 2019).

The use of edible coatings with high active antimicrobial ingredients (Basak and Guha, 2017) such as chitosan (Chen et al., 2019; Grande-Tovar et al., 2018) or essential oils (EOs) with multicomponent nature (Rehman et al., 2016), was recommended for reducing the risk of pathogen growth (Falguera et al., 2011; Romanazi et al., 2016). Several studies have evaluated the antifungal efficacy of different EOs (Abbey et al., 2019) or chitosan (Romanazzi et al., 2018) on *Botrytis cinerea* through stimulating plant defenses (Shi et al., 2019) or film

formation on fruit (Feliziani and Romanazzi, 2013). Moreover, the strawberries treated with EOs (Sivakumar and Bautista-Baños 2014) and chitosan maintained fruit quality with higher levels of phenolics, anthocyanins, flavonoids (Wang and Gao, 2013; Feliziani et al., 2015).

The aims of this study was to determine the effectiveness of natural compounds to control postharvest decay of strawberry fruit using preharvest greenhouse application of 'Chito Plant powder', 'KiForce' and 'Prev-Am plus' compounds based on chitosan and EOs compounds. In addition, the fruit qualitative parameters were recorded.

## 5.2 Materials and Methods

### *5.2.1 Preharvest treatments*

Strawberry plants (*Fragaria* × *ananassa* Duch, cv. 'Festival') were selected for the experiment in February 2019, which were grown in a commercial greenhouse located at Kolossi, Limassol (Cyprus). One-month old plants were treated with fosetyl-Al using dipped inside method before planting. Common cultivation practices were applied during the study, plants were fertigated (20-20-20; 18-9-27) through irrigation system at the beginning and later of crop season. Besides, the soil application of azoxystrobin for *Sphaerotheca macularis* f. sp. *fragariae*, emamectin benzoate for worm and orange oil were sprayed. Type of the soil considered as Silt loam (6.76% clay, 39.2% sand and 54% silt), organic matter of 3.03%, pH of 7.93, Electrical Conductivity (EC) of 6.58 mS/cm, and CaCO3 of 21.67%. A completely randomized design was set-up and six-month-old strawberry plant were used for this experiment. Three different commercial products ('Chito Plant powder', 'KiForce' and 'Prev-Am Plus') were tested, resulting in four treatments, namely i) control, ii)

'Chito Plant powder' (1%), iii) 'KiForce' (1%), and iv) 'Prev-Am Plus' (0.08%). Solutions were prepared by dissolving them in distilled water and stirred for 60 min. Distilled water was used as the control. For each preharvest treatment, 90 plants were selected. Freshly prepared solution were applied by spraying 1.5 L of each compounds throughout the plants with a mechanical mist sprayer. Three days after application, fruits were harvested at commercial ripening stage based on the typical color (red skin) and shape. For each treatment, 200 strawberries were counted at the picking date. Small fruit with defects were discarded. Strawberries were transferred to the laboratory within 1 h for further experiment. Treated strawberries without visual damage and homogeneous in color and size were selected and placed into small containers (1L capacity). For each treatment 42 fruits were tested (six fruits for each of seven replications). Strawberries were incubated in two different temperature conditions. A batch of fruits were stored in a room temperature at 20°C, and a second batch of fruits were stored in a chilled temperature at 4°C for one week then exposed to a shelf life at 20±1˚ C.

### *5.2.2* Decay evaluation

Decay evaluation after the shelf-life period was performed on strawberry fruit for 7 days. Decay severity was recorded according to an empirical scale with five degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5, ≥ 81% fruit surface infected and showing sporulation (Romanazzi et al., 2000). The infection index (or McKinney index), which integrate both the incidence and severity of the decay, was expressed as a percentage of the maximum possible level (McKinney, 1923).

### *5.2.3 Quality experiment*

According to each foliar application, strawberries were randomly selected, labeled and weighted for fruit quality (three replications with six fruit/replication). Fruits were examined at harvest (*Time 1*) following three days of storage at room temperature (20 ± 1°C) (*Time 2-RT*) and chilled conditions (4 ± 0.5°C) (*Time 2-CS*) with 95% relative humidity (RH). Their physical and chemical properties were measured based on weight loss, color, firmness, respiration rate, ethylene production, total soluble solids (TSS), titratable acidity (TA), ascorbic acid (AA), anthocyanin content, total phenolics, total flavonoids, antioxidant activity of ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) at the day three.

### 5.2.4 *Weight loss, color and fruit firmness*

The weight difference between the initial day and the third day of strawberries was measured. The results were expressed as a percentage of weight loss (%) = [(m0−m3)/m0] × 100, where m0 is the initial weight and m3 is the weight after three days storage.

A konica minolta colorimeter (Chroma Meter CR 400 Konica Minolta, Japan) was used to determine the surface color of the samples on day 0 and 3 (two measurements per replicate/three replicates per treatment). Strawberries color was detected by measuring values of L* (lightness), a* (greenness [-] to redness [+]) and b* (blueness [-] to yellowness [+]). The chroma (C) value, whiteness index (WI) and the color index (CI) value were calculated based on the following equations: C = (a*2 + b*2)1/2, WI = 100−[(100 − L*)2 + a*2 + b*2]1/2 and CI= (a*1000)/(L*b) respectably (Bolin and Huxsoll, 1991). The firmness of the strawberry was determined using a texture analyzer, text plus (TA-XT

plus, UK), that, equipped with prob sms p/3 (3mm dia cylinder stain less). The penetration depth of the probe into the sample was 7 mm and the cross head speed of the texture analyzer considered as 1 mm/s for strawberry fruit. The results were reported as the peak force in Newton (N).

### *5.2.5 Total soluble solids, titratable acidity and ascorbic acid*

The Total Soluble Solids (TSS) content of storage strawberry fruit was estimated by using digital pocket refractometer (Atago, Tokyo, Japan) according to the method of Kou et al. (2019). Fruits were homogenized with a blender for 2 minutes and filtrated through layers for squeezing 5.0 mL juice. Few drops of the extract were placed on the refractometer prism glass and data was noted from direct reading. Results of TSS was expressed as °Brix.

Titratable acidity (TA) was analyzed using Mettler Toledo (DL22, Switzerland) as described by Tzortzakis et al. (2007). Homogenized strawberry juice (5 mL) added in 50 mL volumetric flask and final volume achieved 50 mL with $dH_2O$. The extract titrated with 0.1 N NaOH (pH 8.2). Results were conducted as g citric acid/ L of fresh juice.

Vitamin C content was calculated according to the 2,6-dichlorophenolindophenol titration method (Cao et al., 2010). Briefly, 5 g strawberry was extracted with 50 mL of 3% meta-phosphoric acid solution and upper aqueous phase was collected by centrifugation at 3000 rpm for 15 min at 25°C. An aliquot of 10 mL was titrated by using 0.1% 2,6-dichlorophenolindophenol to a pink color. Ascorbic acid concentration was calculated and expressed as (mg AA/100 g Fw).

### *5.2.6* Respiration rate and ethylene production

The respiration rate of the strawberry was analyzed, using the electronic Dual gas analyzer, (International Control Analyzer Ltd, UK), and expressed as volume of carbon dioxide produced per kilogram fresh weight of strawberry per hour ($CO_2$/kg/h). Strawberries were removed from cold storage (4°C) and placed in the laboratory bench for approximately one hour to warm up to ambient temperature (20°C). A 1 mm diameter hole was drilled on the lead of the container and sealed with a tape to prevent gas flow. Gaseous samples were drawn through septa with a syringe to prevent gas leakage from the packages. Strawberries of known fresh weight and volume were considered for calculation of the respiration rate. Respiration rate (mL $CO_2$/kg/h) = (V1 – V2) x%$CO_2$ *10/W x T whereas, V1 = Volume of container (L), V2 = Volume of fruits (L), W= Weight of fruit (kg), T= Time (h).

The ethylene production of the strawberry was analyzed, using the Ethylene analyzer (ICA 56, International Controlled atmosphere Ltd, UK). Following respiration measurement, gaseous samples were tested for ethylene production. Ethylene rate was expressed as mL of ethylene produced kilogram fresh weight of strawberry per hour (mL/kg/h).

### *5.2.7 Polyphenols, flavonoids, anthocyanins and antioxidant activity*

Strawberry fruit tissue (1 g) was milled with 10 mL of methanol (50% v/v) for 30 s. Extraction was transferred to the ultra-sonication for 30 min. Samples were placed in the shaker for 1h at 200rpm. The extract was centrifuged (Sigma 3-18K, Germany) at 4000 × g for 15 min at 4 ∘C. The supernatant was used for the analysis of total phenolic, flavonoid content and total antioxidant activity (FRAP, ABTS).

Polyphenols content was determined using the Folin-Ciocalteu method at 755 nm according to Marinou et al (2013). Crude extract (50 µL) was added to distilled water and achieved to final volume 1.5 mL. It mixed thoroughly with 125 µL of Folin-Ciocalteu reagent for 5 min, followed by the addition of 1.25 mL of 7% (w/v) Na2CO3.The mixture was allowed to stand further for 60 min in the dark at room temperature. The absorbance was measured at 755 nm using multiskan GO. The total phenolic content was calculated based on the calibration curve, and the results were expressed as mg GAE/g Fw.
The total flavonoid content of crude extract was determined by using aluminium chloride method (Meyers et al., 2003). The fruit extract (250 µL) was mixed with 0.75 mL of 5% $NaNO_2$ and stand for 5 min. The mixed extract was added to the 0.15 mL $Al(NO_3)_3$ 10% and incubated for 5 min. A 0.5 mL buffer of 4% NaOH was added to the mixture and total volume achieved to 2.5 mL with distilled water. The absorbance was measured at 510 nm spectrophotometrically (Multiskan GO, Thermo Fischer Scientific, United States); the result was expressed as mg Rutin/g Fw.

FRAP was determined based on the following method from (Chrysargyris et al., 2016). The FRAP reagent solution was prepared by combining 40 µL sample with 0.3M $CH_3COONa$, pH=3.6, 10 mM Tripyridil-s-triazine (TPTZ) and 20 mM $FeCl_3$. Standard procedure was performed using Trolox equivalent antioxidant capacity (TEAC) assay. The absorbance at 593 nm was measured spectrophotometrically (Multiskan GO, Thermo Fischer Scientific, United States) and result expressed as mg Trolox/g Fw.

The antioxidant capacity of strawberry fruit in the reaction with ABTS radical cation was determined according to the method, described by (Wojdyło et al., 2007). It was performed by the effect of ABTS reagent

mixed with 10 µL sample. The mixture was incubated for 6 min at room temperature. Standard procedure was performed using Trolox equivalent antioxidant capacity (TEAC). The absorbance at 734 nm was measured spectrophotometrically (Multiskan GO, Thermo Fischer Scientific, United States). Results were expressed as mg Trolox/ g Fw.

The pH-differential method was used to determine the total anthocyanin content, using two buffers systems (Lee et al., 2005). Strawberry tissue (2 g) was mixed with 15 mL of methanol: $dH_2O$:HCl with volumes 70:29:1. The homogenized extract centrifuged at 4000 rpm for 5 min at 4°C. Prepared extract was diluted with corresponding buffers (0.025 M KCl, pH=1 and 0.4 M $CH_3COONa$, pH = 4.5). After 15 minutes, the absorbance of each solution was measured at 520 and at 700 nm consequently, using multiskan GO spectrophotometric device and calculated as equivalents of (mg cyn-3-glu/ g Fw).

### *5.2.8 Statistical analysis*

Data were analyzed using analysis of variance (ANOVA) with SPSS software. Mean separation was determined by Tuckey test. Significance was defined at $P < 0.05$.

## 5.3 Results

### *5.3.1* Decay evaluation

'Chito plant powder' was the most effective compounds to reduce gray mold incidence on 5 days post treatments at cold storage condition, the McKinney's index of decay was 36.19% lower compared to the control by 55.71% (**Figure 1**). The reduction of disease with this treatment was 10-20% less than the control in the last two days (data not shown). No significant difference was observed

between the fruit treated with 'KiForce' and 'Prev-Am Plus' and the control.

### 5.3.2 *Fruit quality*

The effect of preharvest application of 'Chito Plant powder', 'KiForce' and 'Prev-Am Plus' on fruit quality parameters were tested.

At *Time1* the amount of FRAP in fruit treated with 'Prev-Am Plus' was higher than the control (5.14 mg/trolox/g/FW, and 3.53 mg/trolox/g/FW respectively) (**Table 1**). Among phenolic compounds, flavonoid content in fruit treated with 'Prev Am Plus', (1.25 mg rutin/g FW) and 'KiForce', (1.07 mg rutin/g FW), increased compared to the control (0.66±0.59 mg rutin/g FW) (**Table 1**). The treatments on the other parameters did not show any significant difference compared to the control (**Table 1**).

At *Time 2-RT*, ethylene production in strawberry fruits treated with 'Prev-Am Plus' increased by (170 ml/kg/h) compared to control (33.15 ml/kg/h) (**Figure 2a**). While, the amount of FRAP activity was lower (2.25± 0.5mg trolox/g FW) than the control (3.49±0.6 mg trolox/g Fw) (**Figure 2b**). TSS in fruits treated with 'KiForce', was (14.11±0.6 °Brix) lower than the control (15.56 ±0.3 °Brix) **(Figure 2c)**.

At *Time 2-CS* the weight loss in fruits treated with 'Prev-Am Plus' was higher (0.65%) compare to the control (0.36%) (**Figure 2d**). The production of TA in fruit treated with 'Chito Plant powder' increased compared to control (8.29 g/l and 6.47 g/l respectively) (**Figure 2d**).

The were no significance different between the other parameters in treated and untreated fruits on the third day at both temperature condition observed (data not shown).

## 5.4 Discussion

The present study confirmed that, preharvest treatment with 'Chito Plant Powder' could reduce the development of gray mold on strawberry, at cold storage condition. Our work showed that the longer storage time affected the effectiveness of 'Chito Plant Powder' on strawberry cv. 'Festival'. This compound could interfere with the life cycle of *B. cinerea*, led to slow down gray mold infection at the low-temperature condition. This could be attributed to the compound formulations affecting film-formation (Romanazzi et al., 2018; de Oliveira et al., 2020) responsible for decreasing spore germination or lysis the mycelium of *B. cinerea* in preharvest applications (Munoz and Moret, 2010; Saavedra et al., 2017). However, differently from 'Chito plant powder', 'KiForce' based on chitosan but with liquid formulation and 'Prev- Am Plus' based on EOs, were ineffective in controlling postharvest decay of strawberry in preharvest application underlined, that the pre- or postharvest application (see this thesis chapter N°3) affected the effectiveness of natural compounds (Romanazzi et al., 2013). The results related to qualitative parameters showed that, differently from 'Chito plant powder' and 'KiForce', a major physiological impact of strawberry was observed on 'Prev- Am Plus' treatment based on EOs. 'Prev-Am Plus' was a fungicide and insecticide, based on orange (*Citrus* x *aurantium* L.) EO, that acted for direct contact.

According to this compound, FRAP and flavonoids increased at *Time 1* but, decreased at *Time 2-RT*, at the same time point, the ethylene production increased. In addition to ripening, ethylene was also known to be involved in other processes such as pathogen and wounding responses, leaf senescence and abiotic and biotic stress responses (Alexander and Grierson, 2002; Jia et al., 2017). This result could be connected with molecule

characteristics of 'Prev- Am Plus' based on EOs, as vapor volatile components might affect fruit quality parameters.

Interestingly, 'Chito plant powder' and 'KiForce' maintained fruit quality. In fact, 'Chito Plant powder', did not have any negative effect on fruit quality at all tested conditions. According to 'Prev- Am Plus' treatment at *Time 2-RT*, the FRAP and flavonoid decreased, and ethylene production increased as well as weight loss at *Time 2-CS*. These results were in agreement with ineffectiveness of this compound to the control of strawberry decay. Minor impact of strawberry quality value was observed on chitosan-based compounds except for the increasing of TA value, known to influences microbiological stability of fruits and phenolic compounds (Olawuyi et al., 2018; Xu et al., 2018). The influence of 'Chito Plant powder' on the control of weight loss was a vital factor with a great impact on the appearance of strawberry fruits, attributed to the formation of an extra layer by the natural compounds coated the stomata, resulting in reduction of transpiration against oxygen, carbon dioxide, ethylene levels (Maqbool et al., 2011; Hernandez-Munoz et al., 2006) and dehydration, therefore, limited the weight loss (Guerreiro et al., 2015). These results showed that the firmness stability as a symbol of high quality related to the pectin and other cell wall components (Rico et al., 2019) was maintained, besides, the presence of anthocyanin in fruit epidermis and cortex related to the red pigment did not change after coating with all compounds (Rodrigo et al., 2007). Our work confirmed that edible coatings acted as barriers, thereby restricting water transfer, moisture loss and weight loss. This barrier also reduced oxygen uptake by the strawberry, which in turn slowed down the rate of respiration and associated weight loss from the fruit surface. This proof was in accordance with the result of the study from (Arowora et al., 2013; Yuan et al., 2019).

## 5.5 Conclusions

This research linked to the preharvest effectiveness of natural compounds based on chitosan as 'Chito Plant powder' and 'KiForce' and based on EOs as 'Prev- Am Plus' on strawberry decay and physiological qualitative parameters valuations. Our results showed the greenhouse preharvest application of 'Chito Plant powder' was effective against gray mold. At the same way, the application of this compound maintained high fruits qualitative parameters. Our research suggested that this compound as one of the most important natural compounds in the research program, could be used for further investigation at the commercial level.

**Table 1** - Effects of foliar spray of 'Chito Plant powder', 'KiForce', and 'Prev-Am Plus' on strawberry fruit quality after harvest. [Y]values (n=6 for color measurements; n = 3 for others quality attributes) in rows followed by the same letter are not significantly different, $P < 0.05$.

| Attributes | Control | Chito Plant Powder | KiForce | Prev-Am Plus |
|---|---|---|---|---|
| **Fresh weight (g)** | 8.95 ± 0.3 a | 6.33 ± 0.4 a | 4.21 ± 0.3 a | 13.20 ± 0.8 a |
| **Color L*** | 35.98 ± 1.5 a | 39.01 ± 4.9 a | 39.66 ± 3.7 a | 38.4 ± 3.4 a |
| **Color a*** | 34.45 ± 1.9 a | 34.46 ± 4.1 a | 32.00 ± 4.4 a | 31.07 ± 3.2 a |
| **Colorb*** | 19.12 ± 2.7 a | 33.70±19.4 a | 28.14 ± 17.2 a | 22.00 ± 3.1 a |
| **Chroma** | 39.48 ± 2.6 a | 50.65 ± 17.1 a | 44.24 ± 14.8 a | 38.15 ± 3.9 a |
| **Hue** | 0.50 ± 0.5 a | 0.67 ± 0.2 a | 0.65 ± 0.2 a | 0.61 ± 0.6 a |
| **WI** | 24.72 ± 1.6 a | 19.15 ± 13.4 a | 24.02 ± 10.0 a | 27.23 ± 1.4 a |
| **Color index** | 51.76 ± 7.9 a | 38.12 ± 14.9 a | 36.19 ± 12.0 a | 38.23 ± 7.1 a |
| **Respiration ($CO_2$ ml/kg/h)** | 26.75 ± 8.2 a | 32.84 ± 6.8 a | 29.31 ± 10.9 a | 35.69 ± 15.8 a |
| **Ethylene (ml/kg/h)** | 19.40 ± 11.9 a | 26.79 ± 16.6 a | 37.55 ± 24.5 a | 23.10 ± 19.2 a |
| **Texture(N)** | 1.74 ± 0.6 a | 2.11 ± 1.1 a | 2.74 ± 0.7 a | 2.88 ± 1.2a |
| **TSS (Brix)** | 8.06 ± 0.9 a | 6.26 ± 2.0 a | 7.90 ± 1.3 a | 9.03 ± 1.6 a |
| **TA (g/L)** | 9.27 ± 1.2 a | 6.42 ± 0.9 b | 8.05 ± 0.2 ab | 9.26 ± 0.7 a |
| **Anthoc (mgcyn-3-glu/100 g/FW)** | 3.15 ± 0.96 a | 3.41 ± 2.20 a | 2.34 ± 0.72 a | 2.87 ± 0.34 a |
| **Phenol (mg GAE/g FW)** | 2.56 ± 0.47 a | 2.66 ± 0.12 b | 2.92 ± 0.21 ab | 3.37 ± 0.48 a |
| **FRAP (mg trolox/g FW)** | 3.53 ± 0.79 b | 3.89 ± 0.97 ab | 3.78 ± 0.50 ab | 5.14 ± 0.65 a |
| **ABTS (mg trolox/g FW)** | 10.34 ± 1.67 a | 10.51 ± 1.03 a | 7.18 ± 0.46 b | 7.46 ± 0.66 b |
| **Flavonoid (mg rutin/g FW)** | 0.66 ± 0.59 c | 0.80 ± 0.20 bc | 1.07 ± 0.69 ab | 1.25 ± 0.21 a |

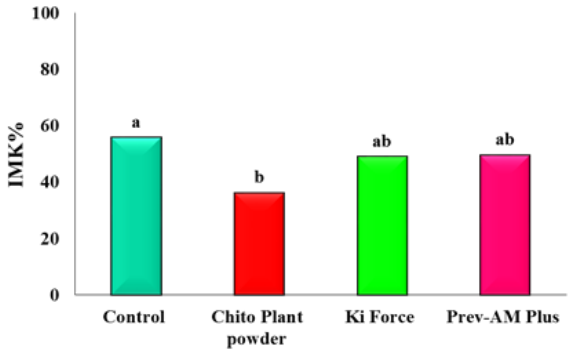


**Figure 1 -** McKinney index of gray mold recorded on strawberries treated with commercial natural compounds formulation. The fruit were kept for 7 days at 4 ± 1 °C, 95–98% RH.-Different letters indicate that the values are significantly different according to Tukey's honestly significant difference, at $P \leq 0.05$. Data were recorded on fifth day of treatment.

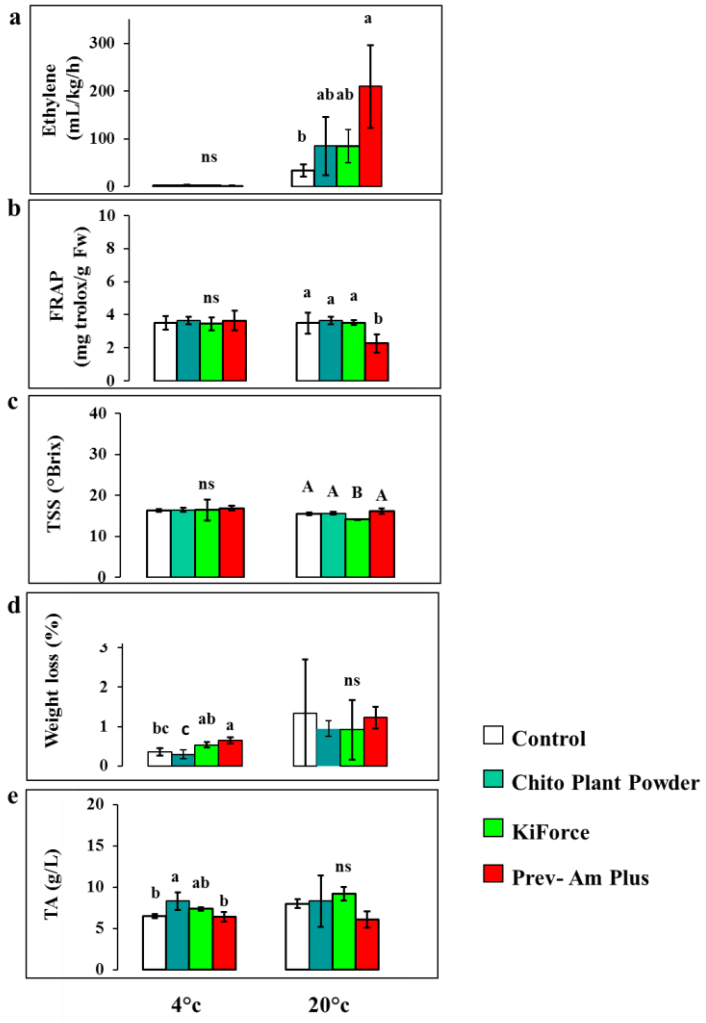


**Figure 2 -** Effect of 'Chito Plant', 'KiForce' and 'Prev-Am Plus' treatment on FRAP, weight loss, TA, TSS and ethylene production of strawberry fruit at *Time 2-RT* (20°C) and *Time 2-CS* (4°C) (see materials and methods section). Error bars represent the standard deviation of the mean of (three/six fruit) replicates. Tukey's honestly significant difference, at $P \leq 0.05$.

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# 6. OVERALL CONCLUSIONS

Considering the new tendencies in fruit industry and marketing, the use of alternative methods represented a suitable approach for several agriculture commodities not only for controlling postharvest diseases but also for maintaining fruit quality. In this PhD work, extensive research was carried out with the aim of evaluating several commercial formulations based on natural compounds against postharvest strawberry decay. We focused on the chitosan-based coating and essential oils derived from plants containing a variety of active volatile compounds. All tested natural compounds showed promising results suggesting they could be as effective for strawberry postharvest decay control. However, their effectiveness was affecting by pathogens, treatments and storage conditions. In postharvest treatments, 'Prev-Am Plus' following 'DF-100 Forte' were the best compounds against gray mold at room temperature, while 'Chito Plant powder' was the most effective compound at cold storage condition. 'MeJA' and 'Kaitosol' were the best compounds against Rhizopus rot at both, cold storage and room temperature. Application of EOs under hypobaric conditions was affected by temperature, in this regard, rosemary and mentha were more effective against gray mold and Rhizopus rot at room temperature, while their effectiveness was reduced with cold storage. This result underlined that volatile aromatic compounds of essential oils were more activated at room temperature. Among all tested compounds 'Chito plant powder' were more effective to control postharvest decay of strawberry when applied at both, pre- and postharvest conditions. Interestingly, 'Chito plant powder' did not adversely affected fruit quality, as the organoleptic characteristics of the fruit were more maintained than other compounds. Our work highlighted that chitosan was the most promising compound in the control of postharvest diseases, which

led to using this compound in meta-analysis study. Meta-analysis investigation related to chitosan on postharvest decay, summarized last 13 years of investigations, involving more than 130 studies, concerning the effectiveness of chitosan on postharvest disease control.

Overall PhD research provided valuable information related to practical aspects, associated with the most effective formulations, some of those, were already commercially available, and their application alone or in combination with other alternative treatment was feasible.

## 7. CONTRIBUTIONS TO CONFERENCES

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