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FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

INNOVATIVE STRATEGIES TO MANAGE POSTHARVEST DISEASES OF FRESH STONE FRUIT

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ABSTRACT

Chitosan and lactoserum are among novel plant protection products to be used as alternatives to synthetic fungicides for the control of postharvest decay of fresh fruit and vegetables. Chitosan and lactoserum (whey) have been granted the status of basic substance by the European Commission. This study evaluated the *in vitro* antifungal activities of chitosan-based products and lactoserum in inhibiting mycelial growth of postharvest pathogens, mainly *Monilinia fructicola*, *Monilinia fructigena*, *Monilinia laxa*, *Alternaria alternata*, and *Botrytis cinerea*. Product with chitosan 5% formulation completely inhibited growth of *M. fructicola*, *M. fructigena*, at 1%, 0.5% and 0.1% concentration. For *M. laxa*, *A. alternaria*, and *B. cinerea* complete inhibition was achieved only at 1% and 0.5%. Chitosan formulations of 5%, 1.9%, and 2% active ingredient were the most effective in inhibiting mycelial growth of all the pathogens of this study at 1% and 0.5% concentration; there were no significant differences among the treatments. *Monilinia* spp. were most sensitive to chitosan-based products. Lactoserum formulation of 10% active ingredient was effective against all *Monilinia* spp.; inhibition of *M. fructigena*, *M. laxa*, and *M. fructicola* was 100%, 80.5%, and 80.4% respectively. Additionally, latent infection monitoring was carried out on one peach and three nectarine cultivars using the ONFIT method. Different pathogens, including *Monilinia* spp., *Cladosporium* spp., *Botrytis cinerea*, *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. were detected in the analysis. This study shows that chitosan products can be used effectively in plant disease management, and ONFIT can be a good method to predict postharvest disease incidence in fresh stone fruit production.

Keywords: Basic substances, Chitosan, Disease management, Lactoserum, Latent infection, *Monilinia* spp., Postharvest diseases

RIASSUNTO

Il chitosano ed il siero di latte fanno parte di una serie di sostanze innovative per la protezione delle piante utilizzate come alternative ai fungicidi di sintesi per il controllo delle malattie fungine di ortofrutticoli freschi in postraccolta. Sia il chitosano sia il siero di latte hanno ottenuto l'approvazione come sostanze di base dalla Commissione Europea e, nel primo studio di questa tesi, è stata valutata la loro efficacia sull'inibizione, *in vitro*, della crescita del micelio dei principali patogeni fungini postraccolta che colpiscono le drupacee. Lo studio si è concentrato sulla valutazione dell'attività antimicrobica nei confronti di *Monilinia fructicola*, *Monilinia fructigena*, *Monilinia laxa*, *Alternaria alternata* e *Botrytis cinerea*. Dai risultati si è visto che la formulazione al 5% di chitosano, alle concentrazioni dell'1%, 0,5% e 0,1%, ha inibito completamente la crescita di *M. fructicola* e *M. fructigena*. Per *M. laxa*, *A. alternaria* e *B. cinerea* l'inibizione completa è stata raggiunta solo con concentrazioni dell'1% e dello 0,5%. Le formulazioni di chitosano al 5%, 1,9% e 2% di principio attivo si sono dimostrate efficaci nell'inibire la crescita del micelio di tutti i patogeni valutati ad una concentrazione dell'1% e dello 0,5%; Non ci sono state differenze significative tra i trattamenti. La formulazione di siero di latte al 10% di principio attivo è risultata efficace contro tutte le *Monilinia* spp. *M. fructigena*, *M. laxa* e *M. fructicola* hanno mostrato un'inibizione rispettivamente del 100%, 80,5% e 80,4%. Un secondo studio è stato condotto per valutare la presenza di infezioni latenti su cultivar di pesche e nettarine utilizzando il metodo ONFIT. Diversi patogeni, tra cui *Monilinia* spp., *Cladosporium* spp., *Botrytis cinerea*, *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp. sono stati rilevati su frutti immaturi. I risultati hanno mostrato una maggiore incidenza di *Rhizopus* spp. e differenze significative tra le cultivar per quanto riguarda *Monilinia* spp., *Aspergillus* spp. e *Cladosporium* spp. Questo studio ha dimostrato che i prodotti a base di chitosano possono essere utilizzati efficacemente nella gestione dei patogeni postraccolta delle drupacee e che il metodo ONFIT può essere utile per prevedere l'incidenza delle malattie postraccolta di pesche e nettarine.

Parole chiave: Chitosano, Gestione della malattia, Infezione latente, Malattie postraccolta, *Monilinia* spp., Siero di latte, Sostanze di base

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I dedicate this study to my father IHSAN BULGAK, I know that life is going but you will always be in my deep heart. Rest in peace.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
µL	Microliter
µM	Micrometer
BCA	Biological control agents
Cv.	Cultivar
DMI	Demethylation inhibitor
EPPO	European and Mediterranean Plant Protection
EU	European Union
mL	Milliliter
PDA	Potato dextrose agar
Spp.	Species

1. INTRODUCTION

Fruits and vegetables form an important and essential part of a healthy diet because they are rich in vitamins, minerals, and fibre. In recent years there has been a shift in consumer behaviour as regards consumption of fresh and minimally processed produce; nowadays consumers demand quality fresh fruits and vegetables that are free of pesticides. Conventional pesticides are applied in the preharvest stage to prevent infection of fruits by pathogenic microorganisms that cause deterioration of fruit quality. Even with the consistent application of pesticides at preharvest, pathogens still manage to infect fruits and remain latent until conditions become conducive for their growth. The common pathogens that cause diseases of fresh produce in postharvest include *Botrytis cinerea*, *Monilinia* spp., *Alternaria* spp., *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. At postharvest use of pesticides is very limited; treatments such as hot water treatments are applied instead. Therefore, there is a need for alternative strategies to replace synthetic fungicides to prevent preharvest and postharvest losses while maintaining the quality and nutrition of the fresh produce (Dukare *et al*., 2019).

1.1. POSTHARVEST DISEASE OF FRESH FRUITS AND VEGETABLES

1.1.1. Gray mold

Gray mold, caused by *Botrytis cinerea*, is a disease of economic importance in fruit production. *B. cinerea,* [kingdom: Fungi, family: *Sclerotiniaceae,* order: *Helotiales*, class: *Leotiomycetes,* phylum: *Ascomycota,* genus: *Botrytis,* species: *Botrytis cinerea*], is a common and wide ascomycete fungus that causes gray mold (Verhoeff *et al*., 1982; Mitrev *et al*., 2020). The disease is very common on grapes and strawberries; but it also affects other fruits and vegetables such as peaches, nectarines, and cucumbers, as can be seen in **Figure 1.1** (Williamson *et al*., 2007). *B. cinerea* can infect many components of plant tissues, including stems, leaves, flower petals, and fruit. This pathogen is defined as the second critical fungal species as it causes significant crop loss in all countries each year (Dean *et al*., 2012). This necrotrophic pathogen is characterized by hyaline conidia (asexual spore). The conidium of *B. cinerea* is dispersed by wind, rainwater, and infected host tissue. *B. cinerea* is mentioned as an asexual form (anamorph), however, *Botryotinia fuckeliana* [genus: *Botryotinia*, kingdom: fungus, order: *Helotiales*, class: *Leotiomycetes*, family: Sclerotiniaceae] is known as a sexual form (teleomorph), which leads to the production of apothecia. Some *Botrytis* spp. have a narrow host range and exclusively infect the flower bulbs of plants. *B. cinerea* affects only flowers and dead plant components of bulbous flowers (Aboelghar *et al*., 2013). However, most *Botrytis* species exhibit widespread distribution or occurrence, spread to different hosts.

Although the host chosen to distinguish *Botrytis* spp. from each other is an important feature, *Botrytis* spp. are characterised according to their morphological and growing regions (Gligor *et al*., 2020). Morphological characteristics of *B. cinerea* isolated from fruits include a white to gray or dark gray colony colour, ellipsoidal to ovoid and dark brown conidial shape and colour of sizes varying between 5.1–8.5 × 5.2–9.8 µM. Sclerotia has a flat shape and black colour. The optimum temperature range of sclerotia production in *B. cinerea* is 11-13 °C. However, the fungus can sporulate at a temperature range of 12-22 °C and can form appressorium at 27-28 °C (Li *et al*., 2022).

Figure 0.1 - (a) *Botrytis cinerea* sporulation on a ripe strawberry (Bauer, 2008), and (b) gray mold on nectarine (*Prunus persica* var. nucipersica) (Suckow) (Louadfe, 2015).

1.1.2. Brown rot

Brown rot is a disease that reduces preharvest and postharvest quality, yield, and market value of stone fruits, and to a lesser extend pome fruits. Brown rot is caused by *Monilinia* species, the most common species being *Monilinia fructicola, Monilinia fructigena,* and *Monilinia laxa* (Hrustić *et al*., 2013). *Monilinia* spp. are common in countries where stone and pome fruits are grown. Infection can occur in the field and present the first symptoms at postharvest. The symptoms appear as an oil stain on the petals of the flower, over time the entire flower becomes turns brown and dry, these dried flowers usually hang on the branch for a long time. The disease spreads from the flower stalk to the branch. Brown spots appear under the bark of the diseased branch, and after a while, the branch dries up completely. The disease agent can develop on the branch and infect the leaves as well. Infected leaves turn brown with the petiole and dry together with the branch. In fruits, the first symptoms begin with the formation of brown spots at the point of infection and cover the whole fruit over time. The spots have a light brown ring around them. The rot develops into the fruit flesh, and the fruit becomes covered with fungal

spores over time, leaving the fruit mummified. Mummified fruits hang on the branches or fall to the orchard ground becoming sources of inoculum if not removed. Spores found on the mummies can be spread easily by rain, wind, insects, and/or human movement. Brown rot can be quite destructive under storage conditions as it is easy for the rot to spread between adjacent fruits compromising the quality of the whole unit if not properly maintained. Since *Monilinia* spp. can live in a wide variety of climates, they cause significant damage in almost every region where stone and pome fruit species are grown (Aiello *et al*., 2019). The *Monilinia* genus [family: Sclerotiniaceae, order: *Helotiales*, class: *Leotiomycetes*, phylum: *Ascomycota*] include fungal pathogens that affect many economically significant crops (Schoch, 2020). *M. laxa* (Aderhold and Ruhland) Honey, *M. fructigena (*Aderhold and Ruhland) Honey*,* and *M. fructicola* (G Winter) Honey are the most widespread pathogens of the *Monilinia* species around the world causing brown rot (Byrde *et al*., 1977). The host range of *Monilinia* spp. include stone and pome fruits such as peaches and nectarine (*Prunus persic*a (L.) Batsch), plum (*P. domestica* L.), apricot (*P. armeniaca* L.), sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.), apple (*Malus domestica*), and pear (*Pyrus communis*) (**Figure 1.2**) (EPPO, 2002). *M. laxa* is the most prevalent species in Europe and South Africa. *M. fructicola* was introduced to Europe in 2001 when it was first detected in peach orchards in France. It has since been reported in other countries, including Italy, Poland, Germany, Spain, and Hungary (Rungjidamai *et al*., 2014). The pathogen has been reported in several regions of Italy including Emilia Romagna, Lazio, Piemonte, Puglia, and Marche on stone and pome fruits (Landi *et al*., 2016). On the other hand, *M. fructigena* is not widespread on stone fruits. In a recent study, *M. yunnanensis*, which is very close to *M. fructigena* but a phylogenetically separate species, has been reported as the causative agent of brown rot in China (Hu *et al*., 2011). *M. azaleae, M. mali,* and *M. oxycocci* are other *Monilinia* species that have been detected in recent years. *M. polystroma* has been reported from Japan, China, and Hungary (Zhu *et al*., 2010). De Miccolis Angelini *et al*. (2022) reported that phylogenetic studies on *Monilinia* spp. is important to understand the host-plant mechanism. According to them, *M. laxa* and *M. fructigena* are close to each other, while *M. fructicola* is more distant from them.

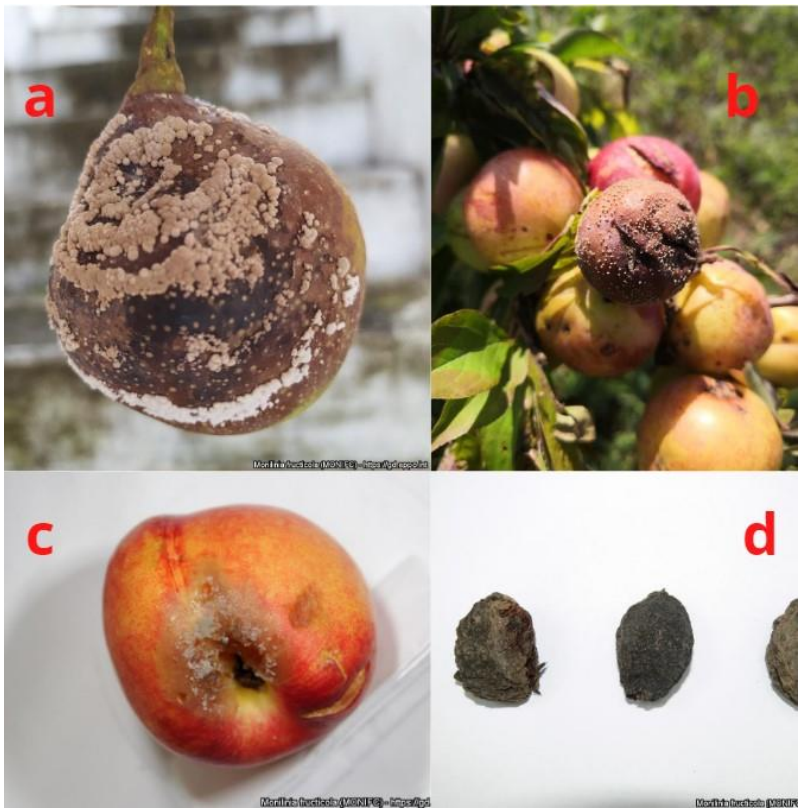

Figure 0.2 - Brown rot on symptoms on (a) pear, (b) plum, (c) nectarine, and (d) mummified peach fruit (EPPO, 2002).

1.1.2.1. Monilinia laxa

M. laxa is more common in Europe and causes flower blight and brown rot on stone fruit including apricots, apples, plums, cherries, sour cherries, nectarines, and peaches (EPPO, 2002; Hrustić *et al*., 2013). The curves in the development of mycelium of *M. laxa* look like the petals of an opened flower and form distinct layers. The species has a low growth rate compared to *M. fructicola*. The stromata of *M. laxa* are greenish brown, disc-shaped, 3-8 mM in diameter, and the stipe is 10-30 x 1-2 mM, the asci are 120-190 x 7.5-12 µM (Hu *et al*., 2011). The conidia of the agent are usually ellipsoidal, ovoid lemony-shaped and chain-like on the mycelium. Ascospores are broadly oval 7–19 x 4.5-8.5 µM in size. The dimensions of the conidia were between 8.0-16.0 x7.0-10.0 µM (Kiprovski *et al*., 2018). Genome sequence studies open a door to understand the mechanism of pathogenicity of *M. laxa* and further studies is necessary to find an effective management tools (Landi *et al*., 2019).

1.1.2.2. Monilinia fructicola

M. fructicola is known as American brown rot fungus and has increased in different fruits in continents such as Asia, Australia, Africa, North and South America and in European countries,

for example Switzerland, Czech Republic, Italy, Spain, Slovak Republic, Slovenia, Germany, and Turkey. *M. fructicola* is also listed as a quarantine fungus by the global database of plant protection organizations in Europe and the Mediterranean (EPPO, 2021).

M. fructicola colonies have green-brown colour in when grown on PDA and the conidia dimensions are at 12.5-14.5 × 8-10 µM. Each conidia develop one germ tube; and the dimensions are higher than *M. laxa* and *M. fructigena*. The length of the conidia range between 750-900 µM. Colonies shows fast develop and most destructive pathogen compared to other *Monilinia* species. The ideal temperature for conidia germination of *M. fructicola* is between 15 to 20 ºC (Holb, 2008). The complete genome sequence of *M. fructicola* is critical to understanding the mechanism of pathogen diffusion and adaptation to the environment (De Miccolis Angelini *et al*,. 2019).

1.1.2.3. Monilinia fructigena

Monilinia fructigena (phylum Ascomycota, family Sclerotiniaceae) is causes brown rot and blossom blight which decrease the quality and economic market value of the stone and pome fruit mostly in the postharvest. The pathogen is widely spread in Europe, Asia, and North Africa and some parts of South America. It is a quarantine pathogen in the USA, Canada, Australia and New Zealand (Hu *et al*., 2011).

M. fructigena colonies appear as yellow to cream on PDA, mycelia are arranged in tufts. Conidia dimensions are 17.5-20.5 × 10.5-12.5 µM. The optimum temperature for producing conidia is 15-20 °C. The sporulation of this pathogen is not common (Yangyang *et al*., 2021). Compared to *M. laxa* and *M. fructicola*, *M. fructigena* is less aggressive and virulent (Cox *et al*., 2018). Complete genome sequence of *M. fructigena* is a further step for better understanding the pathogen epidemiology and to find the most effective brown rot management systems (Landi *et al*., 2018).

1.1.3. *Alternaria* spp.

Alternaria genus is one of the *Deuteromycetes* class fungi with dark pigmentation, forming chain-like spores on conidiophores, with an unknown sexual reproduction period. This pathogen, which has about 100 species in the world, causes economic damage by infecting many cultivated plants such as cereals, ornamental plants, oilseed plants, vegetables, and fruit trees (Akimitsu *et al*., 2003). The characteristic features of this genus are the production of multicellular, dark-coloured chain-shaped spores with longitudinal and transverse divisions and tapering end cells.

This disease usually appears as blight and necrotic spots on leaves. The disease symptoms on vegetable seedlings include stem, tuber, and fruit rot. In addition, they cause fruit rot in, especially in orange, lemon, apple, pomegranate, sour cherries and strawberries, pomegranate, apple, onion and cucumber, and early blight in tomatoes and potatoes. Usually, rotten tissue and leaf spot symptoms are characteristics properties of *Alternaria* spp. It occurs in the form of target board-like concentric rings, expanding dark black and brown spots as seen in **Figure 1.3**.

Figure 0.3 - *Alternaria alternata* black spot-on strawberry (Louws, 2019).

Fungal-induced deterioration during the storage of fruits is one of the most important problems. The main reason for this is that the fungal inoculum is transmitted to the warehouse by infecting the fruit stem and flower tip during the pre-harvest period and the decay continues in the warehouse. However, the severity of fungal decay during storage increases even more if the storage temperature is above 5 °C (Almenar *et al*., 2007). At the same time, fruit infections have been detected more frequently in gardens where there is excessive nitrogen use and in-garden aeration is not good.

Alternaria alternata is generally known as a weak and opportunistic plant pathogen and can reach the internal tissues of the host by penetrating directly through the natural openings such as the cuticle layer or on the surface such as wounds, lenticels, stem tips and pedicles to enter plant tissues. After reaching the inner tissue of the immature fruit, it remains inactive until the fruit ripens. Typically, tissues weakened by stresses, aging or injury are more susceptible to these infections. Its pathogenicity is due to enzymes that directly affect the structural components of the host cells, their substances in the cell or components of the membranes, and the protoplast, thereby interfering with the vital function of the cell. In addition to enzymes, *A. alternata* produces a group of mycotoxins, host-specific toxins that have been implicated as critical determinants of pathogenicity or virulence in various plant-pathogen interactions.

These toxins suppress the host defence mechanism and increase sensitivity to the pathogen (Jiaping *et al*., 2014). *A. alternata* causes leaf blight in plants. Disease conidia are light or yellowish-brown in colour, 20-63 x 9-18 µM in size, with 3 or 5 transverse, occasional longitudinal divisions. The conidia of the tail are shorter than 1/3 of its length (Koike *et al*., 2010). Considering the morphology on the PDA; colonies of *A. alternata* were generally observed to develop in grayish, brown, olive-green colour. When examined microscopically, it was observed that the hyphae were dense, branched, segmented and close to golden yellow in colour, while the spores were pear-shaped and segmented (Berbegal *et al*., 2014).

Alternaria brassicicola causes black lesions on the leaves and stems. *Alternaria* species need nutritional requirements and a saprophytic stage outside the host. A resting stage consisting of chlamydospores has been reported for *A. brassicicola.* Conidiophores of the *A. brassicicola* is 170 µM long. The mycelia are branched and septate, and conidia are produced on conidiophores that are generally pale brown or brown and arise separately or in clusters (Köhl *et al*., 2010). The conidia are mostly in chains and arise through pores in the conidiophore wall. They are typically ovoid with transverse and longitudinal septa. *A. brassicicola* sporulate and forms a white, cottony mycelium, varying from thick, dark, heavily septate to one with characteristic chlamydospores. Under *in vitro* conditions, sporulation occurs at a temperature range of 8-24 ºC, where mature spores occur at optimum temperatures of 16 and 24 ºC with sporulation time ranging from 12 to 14 h.

1.1.4. *Penicillium* spp.

The *Penicillium* species was first described in 1809 by the fungal scientist and botanist Johann Heinrich Friedrich Link. He mentioned three different species of *Penicillium* in his studies. Namely, *P. candidum*, *P. expansum* and *P. glaucum*. The common features of these species are that they produce brush-type conidiophores (Groot *et al*., 2019). Its conidia are light and small, they can be easily transported from one environment to another by air. For this reason, the number of spores in an environment varies depending on weather, climate, and environmental factors. Its colonies with a diameter of 2.5 cm can produce an average of 400 million spores per day if the environmental conditions are suitable (Onions *et al*., 1987). Some species of *Penicillium*; *Penicillium expansum, Penicillium digitatum,* and *Penicillium italicum* cause significant losses on apple, citrus, and tomato plants.

Blue mold caused by *P. expansum* is one of the most important postharvest diseases of stone fruits. The first symptoms of blue mold are characterised by the development of light brown, soft and watery lesions on the fruit. As the lesion ages, the conidia turn blue green (Nigam *et*

al., 2018). The conidia of *P. expansum* usually enter through wounds that occur during harvest. Blue mold can occur even at temperatures below 0 °C, but in this case the infection progresses slowly (Neri *et al*., 2006). The developing colonies are 30-40 mM in diameter and radial in shape. There is white mycelium on the margins of these colonies. Conidia are dull green in colour. At 5 °C, micro-colonies usually form 2-4 mM in diameter. At 37 °C, there is no growth. The optimum temperature for this species is 25 °C. The oxygen requirement of *P. expansum* is quite low. Conidiophores are composed of hyphae at or just below the surface (Onions *et al*., 1987).

Green mold is caused by *P. digitatium*. The disease first appears as soft, watery, and slightly discoloured patches. Later, this softness expands, and a white mycelial layer of the fungus is formed, starting from the middle of the spot. Within 1-2 days following the formation of the mycelial layer, a green spore mass is seen on the mycelia mass, again starting from the center, and as a result, the fruit takes the form of a green mold ball. It has been reported that the conidia of this fungus are cylindrical, round or slightly elliptical in the form of chain of 4-7 x 6-8 µM. *P. digitatum* reproduces asexually by spores and conidia. On PDA it starts to create a white mycelia and spreads in circles. Germinated spores are yellow-greenish or olive-coloured and surrounded by a small circle of white mycelia, hence the name green mold (Zheng *et al*., 2015).

1.1.5. *Fusarium* spp.

Fusarium spp. belongs to the family Nectriaceae, order *Hypocreales*, class *Sordariomycetes* and phylum *Ascomycota*. It is one of the most important plant pathogens in the world. More than 20 species of *Fusarium* spp. have been identified. The most common species are *F.* solani, *F. oxysporum*, and *F. chlamydosporum* (Early, 2009). Aerial mycelia of *F. oxysporum* can vary from white to purple to dark pink, cultures can be cream or orange depending on the density of spores (Tadesse, 2021). *Fusarium spp.* microconidia have a kidney shape while macroconidia usually have a falcate to almost straight shape **Figure 1.4**.

Figure 0.4 - Colony morphology of *Fusarium oxysporum* on potato dextrose agar medium (PDA) (Rafique *et al*., 2015).

In some species, macroconidia and microconidia are of the same form; but in other species they are quite different from each other. Chlamydospores produced on lateral branches can be either intercalary or terminal (Early, 2009; Janssen *et al*., 2019). *Fusarium* spp. may infect the seed during the seedling period causing seedling collapse, and the disease may become widespread in the spring-summer period, which is the germination period of the seeds. Hot and humid weather is suitable for the spread of the disease (Broders *et al*., 2007). This pathogen can also cause root rot and wilt and tip blight in plants. The wrong application of the plant and cultural application; the factors leading to the formation of the disease include using previously infected seed material, host genotypes, temperature, excess rainfall, availability of inoculum, wind, and insect damage (Knights *et al*., 2016)

1.1.6. *Rhizopus* spp.

Rhizopus spp. is different from other molds; it is non-septate and produces sporangiospores rather than conidia. It has white mycelia and black sporangia with a fast growth rate; it spreads easily. It causes spoilage in fruits and vegetables commonly in sweet potato, strawberries, and berries (Bullerman, 2003). *Rhizopus stolonifer* [kingdom: fungi, division: *Mucoromycota*, order: *Mucorales*, family: *Mucoraceae*, genus: *Rhizopus*, species: *R. stolonifer*] is a mold in the genus *Rhizopus*, which is considered the most important species in terms of fruit and vegetable spoilage. It should be kept under control in foods since it is very common in the air, has many hosts, and can penetrate and colonize its host quickly. Infection on fruits usually occur during harvest and handling. The diseases caused by this mold is known as soft rot (*Rhizopus* rot) or black mold (Bhaskara *et al*., 1998).

Rhizopus species such as *R. oryzae* and *R. artocarpi* cause serious problems in fruits. Papaya, banana, tomato, peach, durian, mango, guava, grape, litchi, mango, and jackfruit are the most common fruits affected by *Rhizopus* spp. This pathogen is mesophilic and easily

affected by environmental factors such as temperature, humidity, and water content. After *R. stolonifer* infect the injured tissue, it initially covers the infected area with thin, fluffy, cottony mycelia, forming a black mass of hyphae on the surface (**Figure 1.5**). The mold mycelia then spread around the infection site (Bautista-Baños *et al*., 2014).

Figure 0.5 - *Rhizopus* spp. rot in strawberry in early infection (Clark, 2000).

1.1.7. *Aspergillus* spp.

Aspergillus spp. [kingdom: Fungi, division: *Ascomycota*, class: *Eurotiomycetes*, order: *Eurotiales*, family: *Trichocomaceae*, genus: *Aspergillus*] is consisting of a few hundred mold species. *Aspergillum* is an asexual spore-forming structure common to all *Aspergillus* species; around one-third of species are also known to have a sexual stage. *Aspergillus niger* is a fungus causes black mold disease on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food (Bennet, 2010). Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins. *A. flavus* produces cyclopiazonic acid and aflatrem mycotoxins, which lead to infecting the crops (Dyer *et al*., 2012). *A. niger* is more prevalent in warmer climates, both in field situations and stored foods. Growing temperature is between 6–47 °C with a high optimum ranging between 35–37 °C. The minimum required media water activity for growth is 0.77–0.88. *A. niger* can grow under very wide pH conditions, ranging from 1.4–9.8. It produces large colonies, minimum 60 mM in diameter, with relatively fast growth. The colony is made of plain, white mycelia, above which a layer of closely packed, black to dark-brown conidial heads roughly 2–3 mM high is formed (Hocking, 2016).

1.1.8. *Cladosporium* spp.

Cladosporium spp. [kingdom: Fungi, division: *Ascomycota*, class: *Dothideomycetes*, order: *Capnodiales*, family: *Davidiellaceae*, genus: *Cladosporium*] is a genus of fungi that produces green, brown-black colonies (Bensch *et al*., 2012). Many species of *Cladosporium* are commonly found on living and dead plant pathogens. It can be isolated from cereal grains, peanuts, and fruits. Refrigerated stored conditions are optimum for the growth of this species. *C. cladosporioides*, *C. sphaerospermum*, *C. elegant*, *C. dominicanum*, *C. musae* are most common plant pathogens. *C. musae* causes *Cladosporium* speckle on bananas and which occurs in most countries in which the fruit is cultivated. It remains latent during the harvesting stage (Crous *et al*., 2006).

The colony of this fungi on agar is very dark greenish-black or blue-black. Usually this species produces many one-celled conidia, but also two and three-celled forms are found in its isolates. The structure of the conidiophores is tree-like, a prominent feature of the genus *Cladosporium*. Usually, conidiophores have swollen nodes at the branching points. Conidia are characteristically circular to ellipsoid with a diameter of 3.4–4.0 μM. The species can grow at temperatures from as low as −5 °C to 35 °C; growth does not occur at temperatures of 37 °C and above. This fungus is xerotolerant, it can thrive in environments with low water activity. This fungus has been observed to grow in as low as 0.815 a_w *in vitro* (Virginia *et al*., 2021).

1.2. Conventional Strategies To Control Pre- And Postharvest Diseases

1.2.1. Chemical control

Brown rot and gray mold are predominant pathogens that cause fruit loss in postharvest stage both in stone and pome fruits. Latent infection observed in stone fruits mostly caused by *Monilinia* spp. in the preharvest period. Disease management is important at the preharvest period to reduce the level of disease in further steps. According to the European Union regulation, application of fungicide is allowed only at preharvest period (Luo *et al*., 2001). In the postharvest period, the use of fungicide is restricted due to consumers demanding pesticide free, quality fresh produce. Fruit loss at postharvest can be as high as 60% when fruits are not maintained properly (Mari *et al*., 2004).

Sterol demethylation-inhibitors (DMIs) fungicides are used to control blossom blight and preharvest brown rot on stone fruits that caused by *Monilinia* spp. The difenoconazole and fenbuconazole are lead DMIs in the Europe to control preharvest brown rot. Cyprodinil (4-cyclopropyl-6-methyl-pyrimidin-2-yl) is a fungicide to control disease on pome and stone

fruits like strawberry, grape, and on vegetables and field crops with low toxicity on plants. It is a single site active fungicide and used to control brown rot disease caused by *Monilinia* spp. (Emery *et al*., 2002). Fenhexamid is single site hydroxyanilide fungicide has great controlling effect on *Botrytis cinerea* and *Monilinia* spp. It can be used with the DMI maximum four times per year (Malandrakis *et al*., 2013). In addition to the fungicide, the application of insecticides can reduce the wound occurrence caused by the insects' pests, this can decrease the brown rot on the fruit.

1.2.2. Cultural control

In fruit and vegetable production the planting of certified disease-free plant material is an important first step in ensuring the health of the orchards. Plant materials such as seedlings, rootstocks, grafted plants, and others must be purchased only from certified nurseries. Additionally, thinning the fruit is necessary in the orchards, this process is started 40-60 days after first bloom. It helps to increase the quality of the fruit by leaving the more space per fruit. Open spaces between the fruits helps to improve aeration thus reducing the risk of spreading the infection through fruit contact; especially for pathogens such as *Monilinia* spp. which spreads easily between closely clustered fruits (Torres *et al*., 2021). Air circulation and good lightning of the plants, helps to keep the tree at optimum moisture (Barrett *et al*., 2004). Sanitation of the orchard must be conducted regularly; plant material such as mummified fruits that might serve as source of inoculum must be quickly removed and disposed of in appropriate manner. In the processing, good handling and management will increase the quality of the fruit product and it will reduce the risk of the disease. Regular controlling the environmental conditions helps to keep the tree healthy. Disinfection of the fruits is another aspect of disease management. Fruits and trees should be checked regularly and the wounds, and infected fruits, should be discarded from the healthy fruits. Besides, cleaning the handling apparatus, packaging, and tools used in the orchard is critical (Nierop Groot *et al*., 2019).

1.2.3. Physical control

Temperature control and interaction with oxygen are the physical controlling subtitle. In the orchard, maintaining the fast cooling after the harvesting period will reduce the heat capacity and the moisture loss of the fruits. In the cooling step, hydro cooling treatment is the most preferred method for stone fruits. This method protects the fruit from fungal infection (Kalbasi-Ashtari, 2004). Good handling of the environmental condition of the fruit reduces the latent infection. Heat treatments can also be used to prevent the development of pathogens. Hot water

treatment for stone fruits at 55-65 °C for 60 seconds reduces or prevents the development of fungal infections on the fruit. Controlling the gas level of the fruit helps to prevent latent infection in the fruit, that is, controlled atmosphere storage in which oxygen level are reduced and carbon dioxide is increased (Baker, 1987).

1.3. INNOVATIVE STRATEGIES TO CONTROL PRE- AND POSTHARVEST DISEASES

1.3.1. Basic substances

The world population is increasing rapidly and meeting the nutritional needs of this increasing population has become one of the most important problems of today. However, due to the limited agricultural lands, various production techniques have been developed to close this gap and different methods have been applied during the processing of agricultural products. The use of all modern production systems to get high efficiency from limited areas has caused a deterioration in nature that is difficult to return and caused the products to lose their naturalness (Kavlock *et al*., 2010). In addition to the danger of losing nature, which is the only source of human nourishment, both the desire to get more products from the unit area and the desire to leave a liveable environment for future generations emphasize the need to develop and expand sustainable agricultural practices. Agricultural pesticides are considered indispensable substances in terms of easy application and fast results. However, due to change in consumer behaviour and demands of pesticide free fresh produce the heavy use of pesticides in agricultural practices has been in question. Additionally, the development of resistance by fungi to these pesticides is causing major problems. Therefore, new sustainable strategies need to be developed for plant disease management without compromising the quality of fresh produce while also keeping the health of consumers and the environment (Romanazzi *et al*., 2022). Basic substances are a group of substances that are not usually used for plant protection purposes but are useful in plant protection. European Commission (EC) Regulation 1107/2009 approved those basic substances as safe and alternative methods to be used in agricultural fields (EC, 2009). There are 24 basic substances that have been approved (Romanazzi *et al.*, 2022). These products present a new avenue of plant protection that could be implemented in agriculture.

1.3.1.1. Chitosan

Chitosan poly [4-O-(2-acetamido-2-deoxy-β-D glucopyranosyl)-2-amino-2-deoxy β-D-glucopyranose] is a second biopolymer found in the world after cellulose. Chitosan is obtained from crustaceans and sometimes fungi. It one of the best known, non-toxic polymers that can be destroyed naturally. The difference of chitosan from the chitin molecule is the deacylated form of chitin. Chitosan is of commercial interest due to its high nitrogen concentration. According to the European Commission Regulation No 2022/456 chitosan is approved as a basic substance and can be used in plant protection (EC, 2022).

Although chitin, chitosan and cellulose are structurally similar to each other, the difference between them arises from the C-2 positions. Instead of the hydroxyl group in the C-2 position in cellulose, acetamide ($-NHCOCH_3$) is present in chitin and an amine ($-NH_2$) group in chitosan. This amine group of chitosan is protonated during dissolution, and thus chitosan becomes positively charged. This gives chitosan the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, protein, and macromolecules (Kumar, 2000). Chitosan has a triple action effect when used in plant protection; it has antimicrobial, film-forming and eliciting properties. For antimicrobial, chitosan alone or in combination with other substances has antifungal and antibacterial activity in that it suppresses growth of microorganisms. When applied at preharvest chitosan acts as an elicitor of plant defences. According to Romanazzi *et al*. (2018) the eliciting activity of chitosan accounts for 30 to 40% of its effectiveness in the control of postharvest decay of fresh fruit. Chitosan has been used in edible coatings applied at postharvest to improve the shelf life of fresh produce as well as maintaining quality (Romanazzi *et al*., 2018).

In previous studies, on fruits and vegetables treated with chitosan, it inhibited the spread of the disease by acting as a fungicide. The effects of chitosan plant activator and fungicide against *M. fructicola* were investigated. After harvest, chitosan plant activator was applied to peach fruits at 5 and 10 mg/L doses. Then, the fruits were inoculated with *M. fructicola* in 105 spores/ml suspension after 12 hours and incubated at 23 °C for 6 days. Chitosan plant activators 5 and 10 mg/L doses were able to inhibit brown rot at rates of 56.7% and 42.2%, respectively (Li *et al*., 2001).

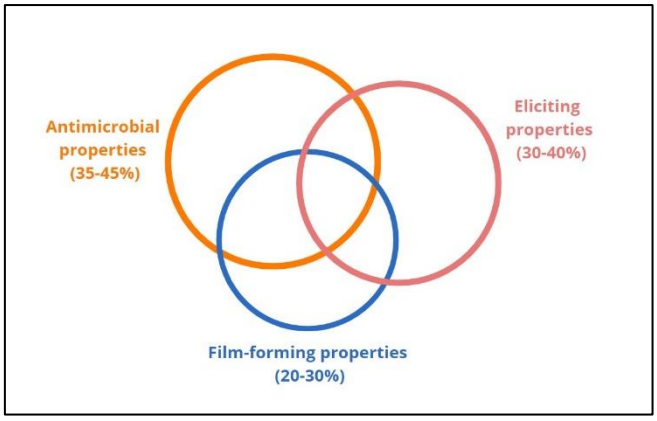

Figure 0.6 - Proportion of antimicrobial, eliciting and film-forming properties of chitosan (Romanazzi *et al*., 2018).

1.3.1.2. Lactoserum (Whey)

Whey is an important by-product containing serum proteins such as lactalbumin, lactoglobulin, lactose, fat, mineral substances, and vitamins. After coagulating the milk with yeast (rennet) or organic acid and breaking the curd, the green-yellow part remaining is called whey. About 80-90% of the milk used in cheese making is separated as whey. However, this amount varies according to the type of cheese made (Keri Marshall, 2004). Whey, which is rich in proteins with high nutritional value, is important in terms of nutrition. Although approximately 93% of the composition of whey is water, most of its dry matter consists of lactose and serum proteins with high biological value (Saudades, 2017).

Whey proteins are known as serum proteins different molecular weights and biological properties. Whey proteins can be grouped as major and minor according to their whey content. Major serum proteins include β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, and glycomacropeptides while minor serum proteins consist of transferrin, lacto-peroxidase, lactoferrin, macroglobulin and α-glycoprotein (Deeth *et al*., 2019). In addition to increasing soil and product quality in sustainable agricultural systems, which have come to the fore in recent years, research and applications are carried out to increase the conservation and effectiveness of symbionts and other similar beneficial microorganisms in the soil microflora. One of these applications is seen as spraying whey into the soil.

Considering the composition of whey, it has been revealed by various researchers that the protein nitrogen in this product is converted to inorganic nitrogen by 30-60% by microorganisms in the soil and lactose is an energy source for microorganisms (Tallapragada *et al*., 2019). In the studies, the information about the use of various whey is limited, but it has been observed that it inhibits some types of fungi. With the limited information and application

methods, there is a need to investigate the disease control in fruits and vegetables using whey (Lactoserum).

1.3.2. Biological control agents (BCA)

Biological control of plant diseases is defined as "reduction in inoculum or disease-producing activity of a pathogen through one or more organisms, including the host plant, but excluding humans" (Baker, 1987). The use of antagonistic microorganisms is one of the most preferred methods as a biological control agent (BCA). Moreover, the use of plant-derived fungicides from secondary plant metabolites and the manipulation of resistance responses in harvested crops; are among the methods used (Wilson *et al*., 1991). Antagonistic microorganisms have been used to control plant pathogens. The studies were conducted both in the laboratory and in the field. There are several antagonistic microorganisms for specific pathogens. *Trichoderma viride* has been used as an antagonistic microorganism for the biocontrol of *P. digitatum* (green mold) on lemon (Colyer *et al*., 1984). *Pseudomonas synxantha* has been used to biocontrol the *M. fructigena* and *M. fructicola* as a causal agent of brown rot on peach and nectarines (Aiello *et al*., 2019).

It is very difficult to control the fungal pathogens, which can be transported from the field to the warehouse, from time to time with latent infections, in the fight against the diseases of blue mold caused by *P. expansum* and brown rot caused by *M. fructigena*, especially cultural measures. At this stage, alternative methods to chemical control with fungicides come into play. At the beginning of the alternative control methods is the use of some biological agents that do not have residue problems and do not have harmful effects on human and environmental health. It is seen that yeasts, which are naturally isolated from the fruit surface, gain weight in research (Rivera *et al*., 2012). Methods to prevent diseases and mycotoxin production caused by *Fusarium* spp., plant rotation, fungicides, and seed resistance. Studies are also continuing the fight against bacteria and natural products (Broders *et al*., 2007).

1.3.3. Latent infection

It often happens that despite the production appearing healthy at the moment of harvesting, it hosts latent infections capable of causing considerable problems during storage. Latent infection refers to infections that occur in the field but present symptoms at postharvest when conditions become conducive for the pathogen (Gell *et al*., 2009). Preharvest contaminations represent a sort of inoculum that finds suitable conditions for growth when postharvest handling causes mechanical injury to the product and, according to Michailides *et al*. (2009)

plays a major role in both the incidence and severity of postharvest disease. If conditions are favourable, incidence and severity of latent infections will be higher and the risk for postharvest disease development will increase. The quality of fruit and vegetables begins in the field, therefore the first step to reduce postharvest qualitative decay is to arrive at the harvest with a healthy and safe product, avoiding any source of contamination. Latent infection can be used as a model to predict diseases that are most likely to develop at postharvest, BOTMON (*Botrytis* monitoring in kiwifruit sepals and/or fruit stems and in stems of grape berries) (Michailides *et al*., 2000) and ONFIT (overnight freezing incubation technique) in stone fruit, other fleshy fruit, and in nut crops (Luo *et al*., 2003). The ONFIT model is used to monitor latent infections in stone fruit for the control of *Monilinia* spp. These pathogens are the most common latent infection pathogens in stone fruit production. Infected plant tissue, especially overwintered mummified fruits, serve as primary source of inoculum in the following production season (Luo *et al*., 2001). States that there is a correlation between latent infection incidence and fruit rot incidence at harvest and postharvest on stone fruit. Therefore, frequent monitoring of latent infect ion is important in planning and implementing effective management of diseases at all production stages. Reducing the source of inoculum (sanitation) can reduce the incidence of latent infection of fruit, with the ultimate result in reducing postharvest disease. The development of efficient techniques can facilitate the detection and quantification of disease inoculum and latent infection of fruit and help predict incidence of postharvest disease (Gell *et al*., 2009). This information will help farmers to reduce and rationalize treatments only at certain opportune moments by reducing the amount of inoculum and the number of treatments in accordance with the EU Directive 128/2009 related to sustainable use of pesticides.

2.OBJECTIVES OF THE STUDY

The aim of this study was to investigate innovate strategies to control postharvest pathogens of stone fruits.

The main objectives were identified as follows:

a) Evaluate antifungal effectiveness of chitosan-based products and lactoserum on mycelial growth inhibition of *Alternaria alternata*, *Botrytis cinerea*, *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola*:
    - collect pure cultures of fungal pathogens that causes postharvest diseases on stone fruits;
    - identification of chitosan-based products and lactoserum to be studied;
    - *in vitro* assay on inhibition of mycelial growth of fungal pathogens;
    - inhibition analysis using SPSS software.

b) Evaluate latent infection incidence on several cultivars of peach and nectarine fruit using ONFIT method:
    - sampling of various cultivars of peach and nectarine fruits at pit hardening stage;
    - decontamination of fruits in chlorine solution, drying at ambient temperature and freezing overnight at -20 °C;
    - incubation of fruits at ambient temperature to assess sporulation;
    - latent infection incidence analysis with SPSS software.

3. MATERIAL AND METHODS

3.1. ANTIMICROBIAL EFFECTS OF BASIC SUBSTANCES

3.1.1. Fungal species

In this study six postharvest fungal pathogens were used to study the inhibitory effect of selected basic substances. The pathogens were collected and cultured on potato dextrose agar (PDA; 40 g L^{-1}; Liofilchem Srl, Roseto degli Abruzzi, Italy) in Petri dishes (diameter, 90 mm) to obtain pure cultures.

Table 3.1 - Fungal species details.

Fungal Species	Source/Host Species	Location
Alternaria alternata	Seed/*Cucurbita moschata*	Italy
Alternaria brassicicola	-*	-
Botrytis cinerea	-	Netherlands
Monilinia laxa	Fruit/*Prunus persica*	Italy
Monilinia fructigena	Fruit/*Prunus persica*	Italy
Monilinia fructicola	Fruit/*Prunus persica*	Italy

*Data not available

3.1.2. *In vitro* antifungal activities on mycelial growth of pathogenic fungi

In vitro antifungal effects of various chitosan-based formulations: **chitosan 5%** (C1; Prevatect, Blexia, Ascenza Agro, S.A, Italy), **chitosan 1.9%** (C3; 1.9%, Biorend, Bioplanet, Srl, Cesena, Italy), **chitosan 2%** (C4; Chitosan DC, Dal Cin, Milan, Italy), **COS-OGA** (C5; 15%, Ibisco, Gowan, Srl, Italy), and **Lactoserum** (C6; 10%,) were evaluated.

Table 3.2 - Details of the Basic Substances used in the *in vitro***.**

Basic substances	Active ingredient (%)	Brand name	Source
Chitosan	5	Prevatect	Italy
Chitosan	1.9	Biorend	Cesena, Italy
Chitosan	2	Chitosan DC	Milan, Italy
COS-OGA	15	Ibisco	Italy
Lactoserum	10	-	Italy

The antifungal activities of basic substances **Table 3.2** were determined according to the *in vitro* inhibition of mycelial growth of *A. alternata, B. cinerea, M. fructigena, M. fructicola, M. laxa.* In this experiment, chitosan-based products at concentration of 1%, 0.5% and 0.1% and lactoserum at 10% were prepared. The products were mixed in sterilized distilled water. PDA medium was autoclaved, and the temperature brought to 45°C before adding the product to PDA. The PDA and product mixture was homogenised using a rotator, poured in 90 mm petri dishes, and left to solidify. Once the medium solidified, each plate was inoculated under aseptic conditions with 8 mm plugs from 7 days old plates taken from the edges of growing cultures. The experiments were carried out as five replicates per concentration and treatment. The inoculated plates were sealed with parafilm and incubated at 23±1 °C. The control consisted of PDA only. Assessments of mycelial growth were performed two days post inoculation. The orthogonal diameters of the colonies were measured daily until the control plates were either completely covered by the mycelia or growth had stopped for 3 consecutive days. Mycelial growth inhibition was calculated based on Equation (1):

$$\text{Mycelial growth inhibition (\%)} = [(dc - dt)/dc] \times 100 \quad (1)$$

where dc and dt represent the mean diameter of the mycelial growth of the control and treated fungal strains, respectively.

3.2. LATENT INFECTION MONITORING

3.2.1. Characteristics of the orchards

This study of latent infection monitoring of postharvest fungi was carried out on peach and nectarine producing orchards located in the province of Ascoli Piceno (AP) and Fermo (FM) in Marche, Italy. The main features and geographical position of orchard are reported below **Table 3.2.3**, **Table 3.2.4**, **Figure 3.2.1** and **Figure 3.2.2**.

Table 3.0.3 - Main characteristics of orchards of Acciarri Company.

Location	Ortezzano
Province	Fermo
Proprietary	Acciarri Società Agricola SRL
Latitude	43°01'51.8"N
Longitude	13°37'19.4"E

Altitude	301 m a.s.l
Species	Nectarine
Variety	Nectadiva

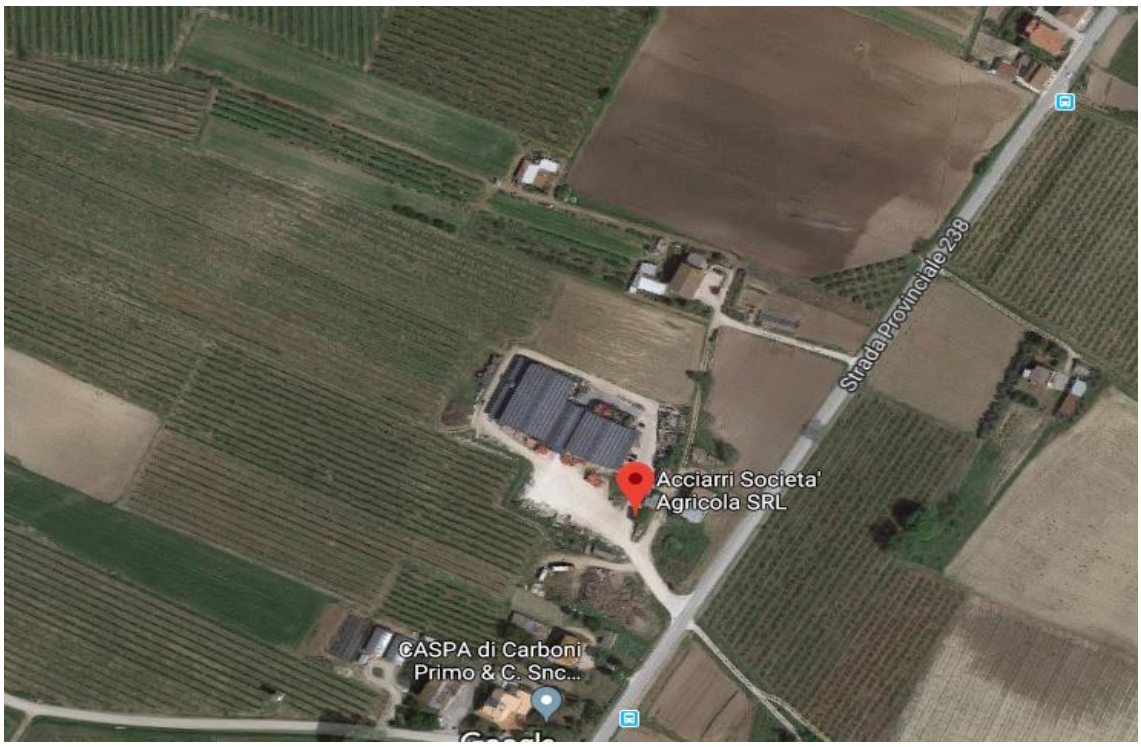

Figure 3.0.1 - Geographical location of Acciarri Company.

Table 3.0.4 - Main characteristics of orchards of Mazzoni Company.

Location	Montedinove	
Province	Ascoli Piceno	
Proprietary	Azienda Mazzoni	
Latitude	42°59'15.6"N	
Longitude	13°33'24.4"E	
Altitude	249 m a.s.l	
Species	Nectarine	Peach
Variety	Honey Royale Big Top	Lucie

Figure 3.0.2 - Geographical location of Mazzoni Company.

3.2.2. Fruit sampling

In the summer of 2022 samples were collected from Acciarri (AC) and Mazzoni (MA) Companies; the orchards had undergone standard management practices with regular spray program, irrigation, and other cultural practices carried out during the production season. Peach and nectarine fruits were collected at the pit hardening stage. Samples were collected from four different cultivars: peach cv. Lucie and nectarine cvs. Big top, Honey Royale, and Nectadiva. In each batch 48 fruits were collected into 8 replicates for Big Top, 7 replicates for Lucie, 9 replicates for Honey Royale and 4 replicates for Nectadiva.

3.2.3. Treatment and assessment

In order to determine latent infection incidence, the sampled nectarine and peach fruits were subjected to the Overnight Freezing Incubation Technique (ONFIT) treatment (Luo and Michailides, 2003). Briefly, the fruits were surface decontaminated in a chlorine solution (32 mL 0.525% sodium hypochlorite, 32 mL of 95% ethyl alcohol, and 0.01 mL of surfactant Tween-20). The fruits were submerged in the solution for 15-20 minutes, then washed in sterilized distilled water 10 times and left to dry at ambient temperature. After the fruits were dry, they were placed into plastic bags and stored to freeze at -20 °C for 24 hours. After which the fruits were placed into crates lined with packing plastics and incubated at ambient

temperature **Figure 3.3** to observe development of diseases. Assessments were performed for five consecutive days after two days of incubation. Disease incidence was calculated, and data were subjected to analysis of variance (ANOVA).

Figure 3.3 - Latent infection monitoring treatment: (a) decontamination in chlorine solution, (b) drying at ambient temperature, (c) freezing at -20 °C for 24 hours, and (d) packing.

3.3 STATISTICAL ANALYSIS

The data were subjected to analysis of variance (ANOVA) using the SPSS package (SPSS version 21.0 for windows, SPSS Inc.; Chicago, IL). The means were separated using Tukey's honestly significant difference (HSD) tests at $P \leq 0.05$. Data are expressed as means ± standard deviation (SD) and means ± standard error for the *in vitro* study and latent infection monitoring respectively.

4. RESULTS

4.1. *IN VITRO* ANTIFUNGAL ACTIVITIES ON MYCELIAL GROWTH

The antifungal effectiveness of different formulations of chitosan-based products and lactoserum at different concentrations were investigated on mycelial growth of the fungi *Alternaria alternata, Botrytis cinerea*, *Monilinia laxa, Monilinia fructigena,* and *Monilinia fructicola*.

As can be seen in **Figure 4.1**, **4.2**, and **4.3** and can be summarised in **Table 4.1**, mycelial growth of *M. fructicola* and *M. fructigena* was completely inhibited by the chitosan 5% (C1) at concentrations 1%, 0.5% and 0.1% compared to PDA control seen in **Figure 4.1**. Whereas complete inhibition of *M. laxa, B. cinerea,* and *A. alternata* mycelial growth was observed only at concentrations in 1% and 0.5% for the same product. For *M. laxa*, there were no significant differences in inhibition between 1% and 0.1% concentrations; inhibition for the latter was 96.83%. At 0.1% concentration, inhibition of *A. alternata* and *B. cinerea* was significantly reduced to 54.33% and 39.74%, respectively. Mycelial growth of *M. fructigena* was completely inhibited by chitosan 1.9% (C3) in all concentration used in the study while for *M. fructicola* and *M. laxa* complete inhibition was seen only at 1% and 0.5% concentrations. For the same product, *B. cinerea* and *A. alternata* growth was completely inhibited at 1% concentration. *B. cinerea* and *M. fructigena* had the same significant inhibition level at 0.5% and 0.1% concentration. At 0.1% concentration, *A. alternata, B. cinerea* and *M. laxa* and 0.5% *A. alternata* were 77.95%, 73.37.%, 85.72% and 77.23%, respectively.

Chitosan 2% (C4) completely inhibited the mycelial growth of *M. fructicola*, *M. fructigena*, and *A. alternata* at 1% and 0.5% concentrations seen in **Figure 4.2**. For *M. laxa* and *B. cinerea* complete inhibition was observed at 1% and 0.5% concentration respectively. At 1% inhibition for *M. laxa* was 97.59% and for *B. cinerea* at 0.5% inhibition was 83.02%. There were no significant differences between 1% and 0.5% concentration for all the pathogens. However, significant differences were observed at 0.1% concentration. At 0.1%, inhibition for *A. alternaria*, *M. fructigena*, *M. fructicola*, *B. cinerea,* and *M. laxa* was 68.19%, 65.57%, 44.05%, 43.61%, and 42.73% respectively. Chitosan 15% (C5) was not effective in inhibiting growth of the fungal pathogens used in the study. Nonetheless, inhibition of mycelial growth of the *M. fructigena*, *M. fructicola*, *M. laxa* and *A. alternata* at 1% concentration was 45.60%, 36.66%, 22.99% and 22.05%, respectively. For the pathogens and product, at 0.5% inhibition was 20.00%, 15.66%, 5.32%, and 0.48% respectively. At 0.1% inhibition ranged between 0.60% and 3.14%. This product did not inhibit growth of *B. cinerea* in any of the tested concentration.

Mycelial growth of *M. fructigena* was completely inhibited by lactoserum (LS; 10%) seen in **Figure 4.3**, whereas for *M. laxa*, *M. fructicola*, and *B. cinerea* inhibition was observed at 80.53%, 80.40%, and 66.77%, respectively. However, inhibition was significantly low for *A. alternata* at 3.73%.

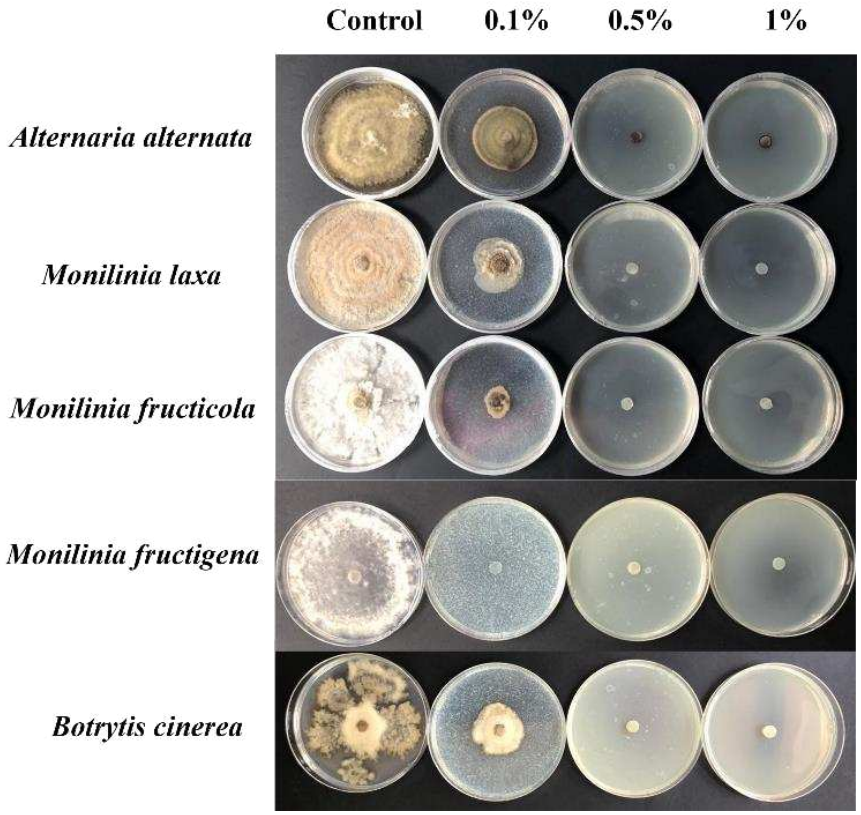

Figure 4.1 - Mycelia growth of *Alternaria alternata*, *Monilinia laxa*, *Monilinia fructicola*, *Monilinia fructigena*, and *Botrytis cinerea* on PDA amended with chitosan 5% (C1) formulation after 13 days incubation at 23 ± 1 ℃.

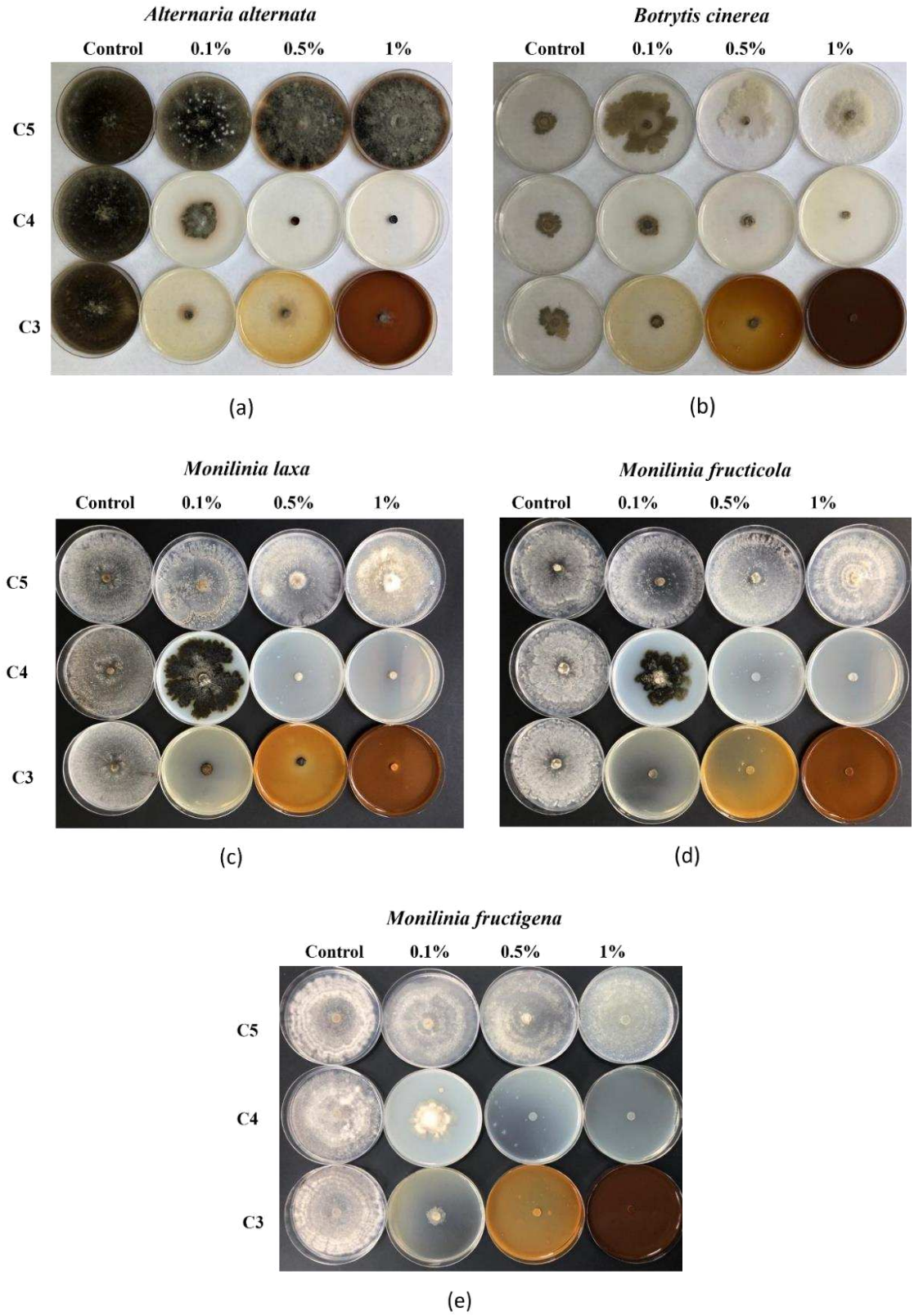

Figure 4.2 - Mycelia growth of (a) *Alternaria alternata*, (b) *Botrytis cinerea*, (c) *Monilinia laxa*, (d) *Monilinia fructicola*, and (e) *Monilinia fructigena* on PDA amended with various formulations of chitosan: chitosan 15% (C5), chitosan 2% (C4), and chitosan 1.9% (C3) at concentrations of 1%, 0.5% and 0.1% after 13 days incubation at 23 ± 1 °C.

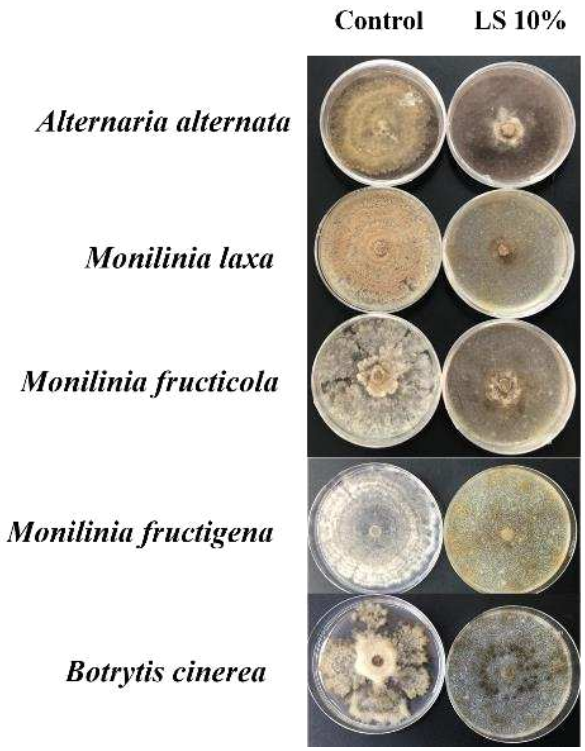

Figure 4.3 - Mycelia growth of *Alternaria alternata*, *Botrytis cinerea*, *Monilinia laxa*, *Monilinia fructicola*, and *Monilinia fructigena* on PDA amended with 10% lactoserum (LS) after 13 days incubation at 23 ± 1 °C.

Table 4.1 - Mycelia growth inhibition (%) of *Alternaria alternata*, *Alternaria brassicicola*, *Botrytis cinerea, Monilinia laxa, Monilinia fructicola*, and *Monilinia fructigena* by chitosan-based products and lactoserum after 13 days incubation at 23 ± 1 °C

Treatment*	*AA*	*BC*	*MLA*	*MFG*	*MFC*
C1-1%	100**a**	100**a**	100**a**	100**a**	100**a**
C1-0.5%	100**a**	100**a**	100**a**	100**a**	100**a**
C1-0.1%	54.33±0.66**c**	39.74±9.94**d**	96.83±7.09**a**	100**a**	100**a**
C3-1%	100**a**	100**a**	100**a**	100**a**	100**a**
C3-0.5%	77.23±3.87**b**	85.40±13.35**ab**	100**a**	100**a**	100**a**
C3-0.1%	77.95±0.69**b**	73.37±3.32**b**	85.72±1.53**b**	81.89±1.56**ab**	100**a**
C4-1%	100**a**	100**a**	97.59±5.39**a**	100**a**	100**a**
C4-0.5%	100**a**	83.02±11.62**ab**	100**a**	100**a**	100**a**
C4-0.1%	68.19±1.50**b**	43.61±24.60**c**	42.73±12.49**c**	65.57±3.14**bc**	44.05±4.22**b**
C5-1%	22.05±11.62**d**	0.00**d**	22.99±2.48**d**	45.60±50.26**c**	36.66±35.60**b**
C5-0.5%	15.66±12.07**d**	0.00**d**	5.32±3.24**e**	0.48±0.66**e**	20.00±44.72**bc**
C5-0.1%	0.60±1.04**e**	0.00**d**	2.79±2.18**e**	3.14±1.77**e**	0.84±1.01**c**
LS 10%	3.73±6.74**e**	66.77±18.62**b**	80.53±8.12**b**	100**a**	80.40±14.96**a**
Control**	0.00**e**	0.00**d**	0.00**e**	0.00**d**	0.00**c**

*C1= chitosan 5%; C3= chitosan 1.9%; C4= chitosan 2%; C5= COS (chito-oligosaccharides) - OGA (oligo-galacturonides) 15%; LS= lactoserum 10%; AA= *Alternaria alternata*; BC=*Botrytis cinerea*; MLA=*Monilinia laxa*; MFG=*Monilinia fructigena*; MFC=*Monilinia fructicola*. **PDA control. Data are means ± SD (n = 5). Data with different letters are significantly different between treatments (P ≤0.05, Tukey's HSD).

4.2. LATENT INFECTION MONITORING

From the assessment carried out on fruit samples of different cultivars taken from Mazzoni and Acciarri farms to verify the presence of latent infections caused by fruit rot agents. We have detected various pathogens including *Monilinia* spp., *Cladosporium* spp., *Botrytis* spp, *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. In the statistical analysis **Table 4.2** of the collected data, significant differences were observed between the cultivars. There was a significant incidence of latent infection caused by *Monilinia* spp. between the cultivars; Lucie and Nectadiva showed significantly lower values compared to Honey Royal and Big Top. For *Cladosporium* spp. infection was significantly high only on cv. Lucie compared to the other cultivars. Significant differences were observed for infection caused by *Aspergillus* spp.; cv. Nectadiva, Big Top, and Honey Royal had significantly higher infection incidence compared to cv. Lucie. *Rhizopus* spp. was found to be the most dominant

pathogen in all the cultivars studied; infection was significantly high on Nectadiva cv. There were no significant differences for infection caused by *Botrytis* spp., *Alternaria* spp., *Fusarium* spp., and *Penicillium* spp. on all the cultivars studied. In general, the results showed a low level of infection.

Table 4.2 - Latent infection incidence of various peach and nectarine cultivars.

Cultivar	***Monilinia*** **spp.**	***Cladosporium*** **spp.**	***Botrytis*** **spp.**	***Alternaria*** **spp.**	***Fusarium*** **spp.**	***Penicillium*** **spp.**	***Aspergillus*** **spp.**	***Rhizopus*** **spp.**
Lucie	0.30 ±0.30**b**	6.03 ±1.48**a**	2.53 ±1.64**a**	3.82 ±3.07**a**	0.74 ±0.48**a**	0.96 ±0.68**a**	0.70 ±0.70**b**	15.11 ±4.19**b**
Honey Royal	2.76 ±0.50**ab**	1.21 ±0.90**b**	0.00**a**	0.46 ±0.31**a**	1.48 ±0.73**a**	8.43 ±2.72**a**	4.60 ±1.46**ab**	7.11 ±4.90**b**
Big Top	5.18 ±2.01**a**	1.36 ±0.96**b**	6.68 ±4.51**a**	4.16 ±2.23**a**	1.09 ±0.56**a**	3.82 ±1.84a	15.15 ±5.35**ab**	30.96 ±5.85**b**
Nectadiva	0.00**b**	0.00**b**	1.09 ±1.09**a**	0.69 ±0.69**a**	0.00**a**	5.91 ±2.00**a**	15.52 ±5.31**a**	74.96 ±11.48**a**

*Data are means ± SE. Data with different letters are significantly different between cultivars (P ≤0.05, Tukey's HSD).

5. DISCUSSION AND CONCLUSIONS

Stone fruits, including peach, nectarine, plum, apricot, cherry, and pome fruits, apple and pear, have an important position in agriculture all over the world. These fruits are affected by numerous by pre- and postharvest diseases. Brown rot, which mostly occurs in fields, is one of the most important diseases on stone fruits caused by *Monilinia* spp.: *Monilinia fructigena*, *Monilinia laxa*, and *Monilinia fructicola*. The fungi infect various parts of host plants resulting in appearance of distinct symptoms, which include blight of flowers and leaves, cankers on woody tissues, and fruit rot along in the production process. At postharvest brown rot can be most destructive (Aiello *et al*., 2019). Other important postharvest diseases include gray mold caused by *Botrytis cinerea*, heart rot disease caused by *Aspergillus* spp. and *Alternaria* spp., and green and blue mold caused by *Penicillium* spp. (Jiaping *et al*., 2014). Proper and effective disease management strategies must be implemented to control these diseases. The strategies must also be environmentally friendly and sustainable. Chitosan is a natural biopolymer that has antimicrobial, film-forming, and eliciting properties (Romanazzi *et al.*, 2018). It is one of the most used polysaccharides in coating formulations due to its antimicrobial and barrier properties. However, chitosan is mostly investigated usually combined with other products like essential oils, or the application is done as an edible film barrier (Wang, 1992). Numerous studies have reported that chitosan is effective in controlling postharvest decay in several fruit. Moreover, chitosan is a good carrier of substances that help to improve the physicochemical, nutritional and microbiological quality of food, and the incorporation of essential oils may enhance these properties (Garcia *et al*., 2014). Lactoserum is a residue left from cheese-making that has a short efficacy span. Both chitosan and lactoserum have been approved by European Commission (Romanazzi *et al*., 2022).

According to the overall result, different chitosan products with different percentage are experienced and chitosan 1% and 0.5% concentrations has greatest inhibition effect in *Monilinia* spp., *A. alternata* and *B. cinerea*. Chitosan 5% (C1) showed a significant effect over the control treatment against *in vitro* mycelium growth of mostly Brown rot pathogens that are involved in postharvest diseases, assays were performed *in vitro* to evaluate antifungal activities of five substances on mycelial growth inhibition of five fungal pathogens. Chitosan 5% was the most effective product completely inhibiting mycelial growth of the *M. fructicola* and *M. fructigena* at all concentrations studied and for *A*. *alternata*, *B*. *cinerea*, and *M*. *laxa* at 1% and 0.5% concentrations. There is an obscure to understand the antifungal activity of the chitosan, however, studies support the role of chitosan in inhibition of the disease development. According to the (Li and Yu, 2001) peach treated with different concentrations of chitosan (5%

and 10) and chitosan inhibit the growth of *Monilinia* spp. on peach. Chitosan is helped to protect fruits from disease and increase the shelf life of the fruit by inhibition the ripening of the product. Further, the application of chitosan on the fruit creates a barrier that helps in maintain fruit quality and extending shelf life (Casals *et al*., 2011). Lactoserum was most effective in the inhibition of the mycelial growth of the *Monilinia* species. However, the mycelial growth of *A. alternata* was not inhibited by the treatment of lactoserum. Biancini *et al.* (2009) reported that lactoserum had an inhibitory effect on *Penicillium* species.

Latent infection monitoring can be used as a model to predict the level of postharvest disease of fresh fruits and vegetables. In this study, the ONFIT method was used to detect latent infection on nectarine, cvs. Honey Royal, Big Top and Nectadiva and peach cv. Lucie. The most common pathogens that were identified were *Monilinia* spp., *Cladosporium* spp., *Botrytis cinerea*, *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. A positive correlation between latent infection incidence and postharvest disease could be identified based on these results. In the previous studies, latent infection incidence was used for earlier disease management. Immature peach samples were harvested and treated with chemicals then left for one night in the freeze, at the end of the 14 days, the type of disease was analysed. According to the results, at the beginning of the study, no signal of fungal disease was seen. Still, at the end of the analysis *Monilinia fructicola* colonies were determined in a fruit batch (Emery *et al*., 2000).

In conclusion, the mycelial growth of tested brown rot fungi was significantly inhibited by chitosan-based products. However, it is crucial to investigate the different *Monilinia* species occurring in an area for the development of correct and effective disease management strategies. Among the tested products, COS-OGA (C5) was the least effective in inhibiting the growth of all the fungi used in this study. In further experiments, chitosan-based products need to be tested in spore assays to observe if there is any difference in inhibition, particularly for the treatments that did not inhibit mycelial growth, such as COS-OGA. Also, more studies could be carried out to elucidate mechanism of action of chitosan in the antifungal activity. The research on environmentally friendly products against disease factors is increasing. Considering the studies conducted around the world, it is predicted that the use of fungicides in postharvest agricultural products can be significantly reduced with the application of alternative control methods.

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