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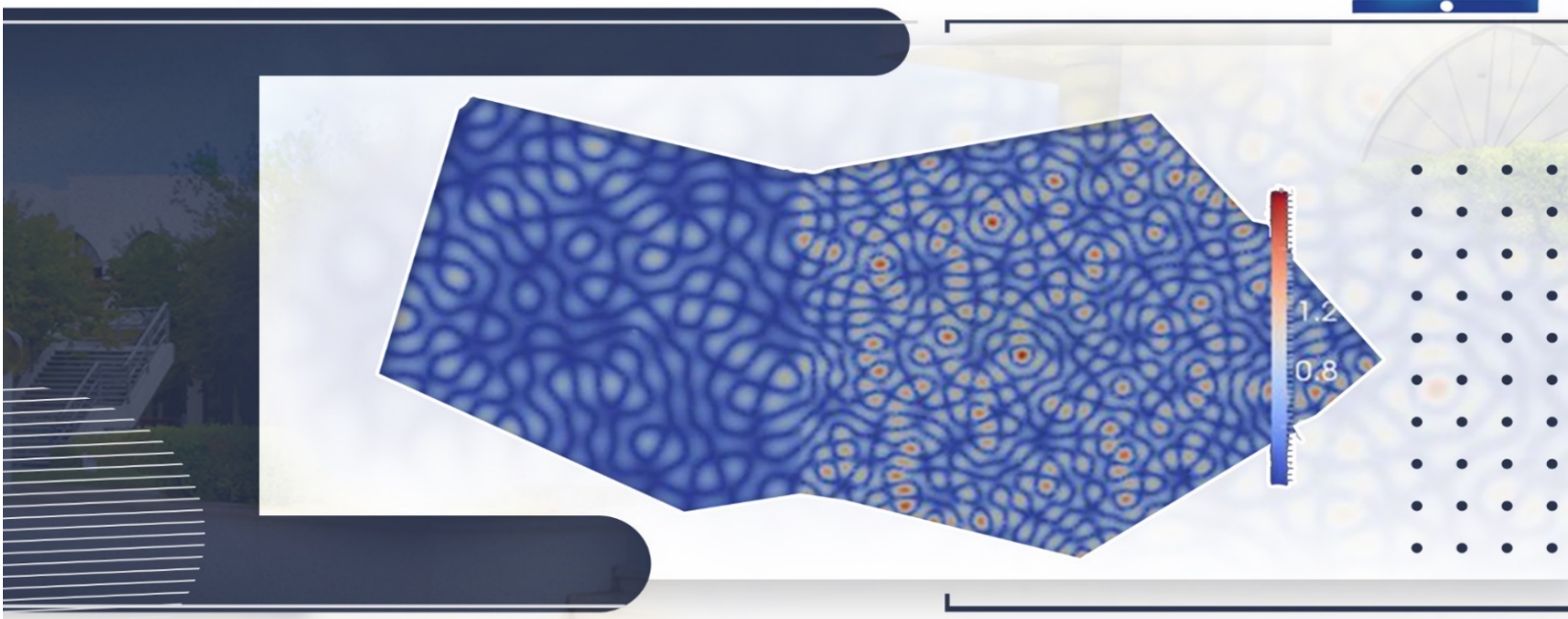

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Control of Apple Fruit Rot Caused by *Alternaria porri* and *Alternaria mali* by Using Hot Water Treatment and Some Inorganic Salts

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A B S T R A C T

Apple rot is one of the most important apple diseases worldwide. The disease causes significant losses in both the quantity and quality of apple fruits. In this study, the antifungal activity of hot water treatment and salts were investigated against apple fruit rots caused by *Alternaria porri* and *Alternaria mali*. Linear growth of tested fungi was inhibited at 5ºC, however, growth was increased by increasing storage temperature degree from 15ºC to 25ºC and decreased at 35ºC. On the other hand, hot water treatment at 55ºC significantly reduced the decay index and severity of infection. When salts such as potassium bicarbonate (KCO_3), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$) and ammonium bicarbonate (NH_4CO_3) was applied, a significant reduction in linear growth and fruit rot incidence was noticed using potassium bicarbonate. Calcium chloride, ammonium bicarbonate and sodium bicarbonate greatly inhibited growth of *A. porri* and *A. mali*. The most effective inhibitor of fruit decay was potassium bicarbonate and calcium chloride.

1 Introduction

Apple fruit (*Malus domestica*) is considered an important fruit worldwide. It is a fruit belonging to the family Rosaceae. The apple fruit has the ability to store long-term and due to this characteristic it exists in markets all year round (Petres, *et al.* 2020), furthermore it is transported from localities of production to far off places for marketing and consumption. Succulent apple fruits can be damaged and degraded with improper handling during harvest, marketing, storage, transportation and consumption. (Rotondo *et al.* 2012, Llyas et *al*. 2007 and Harteveid et al. 2014). During the transfer process and storage, apple fruit quality can be affected by several factors including plant pathogenic fungi, which can cause major postharvest losses during storage. The most common fungal species that cause storage losses apple are *Botrytis cinerea* Pers., *Monilinia fructigena* Honey, *Penicillium expansum* Link, as well as *Alternaria* spp., *Mucor* spp., *Rhizopus* spp. and *Botryosphaeria* spp., etc. (Grahovac *et al*. 2011, 2012; Petres *et al*. 2017).

Therefore, monitoring of these species in stored apple fruits is of high importance. Keeping in mind that postharvest use of synthetic pesticides are not allowed in many countries, there is a need for finding alternative strategies for apple fruit rot control.

The fungal infestation of fruit and vegetables in post-harvest storage severely limits their economic value due to degradation. Although fungicide treatments have been the primary method of monitoring post-harvest diseases, public concerns about fungicide residues in food and the developing fungicide resistance by pathogens has rising the search for suitable means of disease control agents (Tian *et al.* 2001). Non-fungicidal treatments has become a most desirable strategy for disease control (Lieperuma *et al*. 2000, Yacoub 2005, Abd-Allah ,2007

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and Nikolov *et al*. 2013). Hot water treatments might be a very successful option process to control rot, especially in organic production (Maxin *et al*. 2006; *Gasser et al.* 2015).

Heat treatment has been extensively studied as an effective method of disinfesting fruit with microorganisms (Couey 1989). Water is a more efficient medium than air, and the cost of treating hot water is much cheaper than that of treating hot air, hot water treatment is gaining commercial acceptance (Fallik 2004). According to Maxin et al. (2012) and Maxim (2012), there are three modes of action of hot water treatments: washing off the inoculum from the fruit surface, heat inactivation of spores and activation of the defense response in fruits (stress-induced transcription of heat shock proteins HSP). It is necessary to find a suitable combination of temperature and exposure time that will successfully suppress fruit rot while not damaging the fruit. Some inorganic salts used in the food industry as antimicrobial agents and preservatives have proven to be viable alternatives to synthetic fungicides in controlling plant diseases (Russell and Gould, 1991).

These compounds have demonstrated broad antimicrobial activity with little mammalian toxicity (Olivier et al., 1999), are biocompatible (Horst et al., 1992) and have been shown to be safe. Furthermore, they are less sensitive to ecological conditions than other options, for example biological control agents, which may make them suitable for controlling plant infections or suppressing mycotoxin production (Roinestad *et al*. 1993; Singh and Chand 1993).

Bicarbonates are regularly used in the food industry (Lindsay 1985) and have been found to suppress several fungal diseases in cucumber plants (Ziv and Zitter 1992). Kuepper *et al*. (2001) reviewed several research papers on the benefits of sodium bicarbonate as a safe fungicide to treat various plant diseases. Palmer et al. (1997) found that ammonium, potassium, and sodium bicarbonates can inhibit colony growth of *Botrytis cinerea* at concentrations as low as 20 mM.

The aim of the study aims to identify the best degrees of temperature, hot water treatments and salts compounds that would improve the control of pathogens caused by apple fruit rots.

2 Materials and Methods

2.1 Collection of Disease Samples

Diseased golden apple fruits were collected from the market, kept in sterilized polythene bags and transported to the Botany laboratory, Sirte University, Libya for the isolation of the pathogen.

2.2 Isolation, Purification and Identification of Pathogens

For isolation and purification of the pathogen, diseased portions from fruits were cut with a sterilized blade into small pieces (4-6 mm). The pieces were surface sterilized with sodium hypochlorite NaClO (1%) for approximately 2 min. The surface sterilized pieces of apple fruit were washed with sterilized distilled water, then placed in Petri dishes containing 15 ml Potato Dextrose Agar (PDA) media and incubated at 30ºC ± 2ºC. Petri dishes were monitored and observed for growth of fungi daily. After five days, the young growing hyphal tips were transferred to freshly prepared PDA media plates (Figures 2 and 3). The purified fungal isolates were identified according to the procedures described by Ellis (1971) and O`Donnell (1979).

2.3 Effect of Temperature Degree on Linear Growth of The Tested Pathogenic Fungi

The effect of temperature on the growth of *A. porri* and *A. mali* was carried out in accordance with the methods of Malik and Singh (2004) The influence of temperature on the growth of *A. porri* and *A. mali* was carried out according to the methods of Malik and Singh (2004).. The PDA medium was used to investigate the effect of different degrees of temperature on linear growth of the tested pathogenic fungi. Inoculated plates were incubated at **5**˚C, 15˚C, 2**5**˚C, **35**˚C for **7** days and results were recorded at the 7th day. There were three replicate panels per temperature treatment

2.4 Hot Water Treatment

For hot water treatment, healthy golden apple fruits, of uniform size, were divided into two groups. (Tohamy et al. 2004). First group was sterilized by dipping in 70% ethanol for one minute, air dried and inoculated with mycelial discs (4mm. in diameter) by tested pathogenic fungi through scratch in the surface of each fruit and stored at room temperature and served as control. The second group of fruits were inoculated with mycelial discs (4mm. in diameter) from tested pathogenic fungi through a scratch in the surface of each fruit, then stored at room temperature for 72 hrs. After that inoculated fruits, were dipped in hot water at 35ºC, 45ºC and 55ºC for 2, 4 and 6 min. per each degree and stored at room temperature for 2 weeks.

2.5 Salts

Four inorganic salts namely calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), potassium bicarbonate (KCO_3) and ammonium bicarbonate (NH_4CO_3) were used for their antifungal activity against mycelial growth and control of apple fruit decay caused by *A.porri* and *A. mali* (Zaker 2014), Moreover, they are less sensitive to

environmental conditions. Several documents are available proving this fact. (Hervieux *et al*. 2002, Jamar *et al*. 2007, Nahal and Mokhtar 2009, Turkkan 2013.).

2.5.1 Effect of Salts on Mycelial Growth

Methods used in evaluating control of mycelial growth properties of the selected salts: calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate followed the protocol detailed by Schmitz (1930). Pure isolates of selected fungi were grown in petri dishes on PDA with different salt concentrations 2%, 3% and 4% at 28 ± 2ºC. PDA discs (4mm. in diameter) of actively growing mycelia of tested fungi were used to inoculate the plates. For each plate, diameter of colony was determined after 7 days of the inoculation period. Inhibition of mycelial growth was calculated as follows:

[(control radial growth - salt amended radial growth) / control radial growth] × 100.

2.5.2 Effect of Salts on Apple Fruits Decay

Surface of apple fruits were disinfected with 2.5% sodium hypochlorite for 3 minutes, rinsed with sterilized water and air-dried, then wounded using 1 mm in diameter needle at one marked point and dipped for 3 minutes, into the solution of calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate at 4% concentration of each of the salts, then picked up and left to air dry on filter paper. After 1 hr all treated fruits were inoculated by fungi (2 mm in diameter). Control treatments consisted of apple fruits inoculated with sterilized distilled water. Thereafter, all treated apple fruits were air-dried, placed into nylon bags with 3 fruits capacity and stored in a cold room at 10ºC ± 2ºC for four weeks. Each treatment had replicates.

3 Statistical Analysis

All the experiments were repeated at least twice. The results are averages from treatments within each experiment. Data were analysed by ANOVA using SPSS v 20. Different letters above the bars on graphs or after figures in tables indicate a significant difference in means from post hoc Tukey test.

4 Results

4.1 Effect of Storage Temperature on Linear Growth

The results from Figure 1 indicate that linear growth incited by *A. porri* and *A. mali* was significantly affected by storage temperature ($P > 0.05$), with the largest linear growth at 25°C for both species at 96 mm and 85 mm in *A. porri and A. mali* respectively. The lowest linear growth was at 5 °C for both species compared to other temperatures. In general, the line of growth in *A. mali* was higher than in *A. porri*, except at 5 °C where linear growth was 19 mm for *A. mali* and 24 mm for *A. porri*. On the other hand, mycelial growth increased with increase in storage temperature degree from 5ºC to 25ºC then decreased at 35ºC.

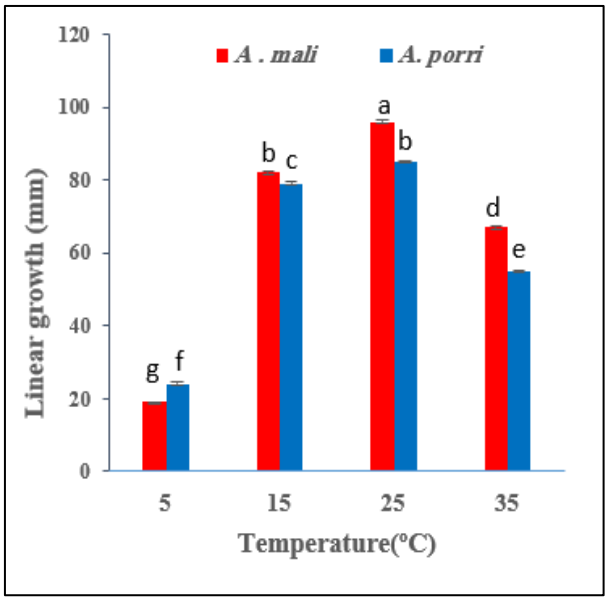

Figure (1). The effect of different degrees of temperature (5ºC, 15ºC, 25ºC and 35°C) on mycelial growth (linear growth) of *A. mail and A. porri* grown on potato dextrose agar (PDA) after 7 days. Letters above error bars (n = 3) indicate significant difference in means from post hoc Tukey tests ($P < 0.05$).

4.2 Effect of Storage Temperature on Decay Index and Severity of Infection

According to data illustrated in Figure 2A, decay index was significantly affected by storage temperatures (P > 0.001). Results showed that *A. porri* had a low decay index of 0.8% at 5ºC, followed by 1.02% by *A. mali*. It was clear that disease peak was at 25ºC and decreased at storage temperature of 35ºC.

The present investigation showed that the optimum storage temperature suitable for the development of the tested fungi was 25ºC. Severity of infection showed a similar pattern to decay index in response to different degrees of storage temperatures (Figure 2B).
Furthermore, severity of infection was lower at 5ºC than other treatments in both species.

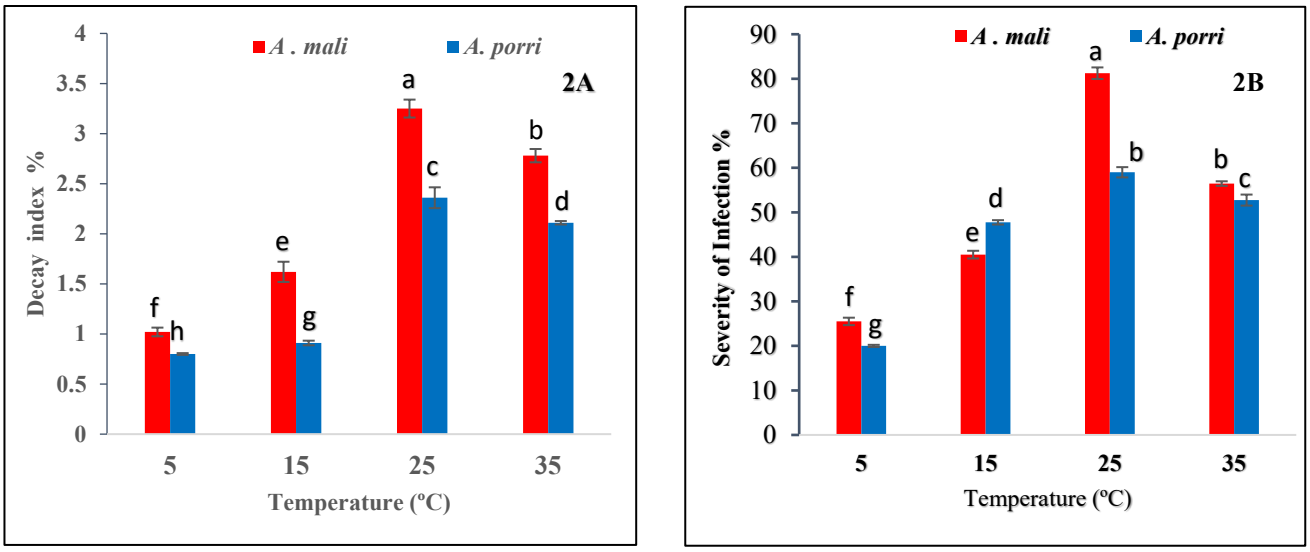

Figure (2). The effect of different degrees of temperatures (5°C, 15°C, 25°C and 35°C) on (2A) decay index and (2B) severity of infection of *A. mail and A. porri* grown on potato dextrose agar (PDA) after 7 days. Letters above error bars (n = 3) indicate significant difference in means from post hoc Tukey tests ($P < 0.05$).

4.3 Effect of Hot Water on Apple Fruit Rot Diseases

The results from (Table. 1) indicated that the three (35○C, 45○C and 55○C) temperature degrees of hot water at three different dipping times (2, 4 and 6 minutes) had significant effect on fruit rot disease development (Severity of Infection and Decay Index (SIDI)) ($P \leq 0.01$). The SIDI was significantly reduced by increasing hot water treatments compared with the control. However, the SIDI was not stable with increasing dipping time at 35○C and 45○C, while at 55○C it decreased with increasing dipping time. Fruit rot disease was completely inhibited when apple fruits were exposed to hot water at 55○C for 4 and 6 minutes in *A. porri* and 55 ○C for 6 minutes in *A. mali*. Decay index was totally inhibited at 55 degrees at 6 minutes in *A. porri* and at 4 and 6 minutes for *A. mali*.

Table (1). Effects of different hot water treatments and durations on apple fruits infected by tested pathogenic fungi. *A. porri* and *A mali* stored at room temperature for 2 weeks.

Treatment	Severity of Infection %		Decay index %	
	A. porri	*A. mali*	*A. porri*	*A. mali*
Control- not treated	89 ± 1.5^{a}	95 ± 1.7^{a}	4.2 ± 0.2^{a}	3.4 ± 0.4^{a}
35 °C, 2 min	68 ± 1.3^{b}	58 ± 2.9^{c}	2.7 ± 0.3^{b}	2.3 ± 0.3^{b}
35 °C, 4 min	37.5 ± 0.6^{c}	16.8 ± 0.6^{g}	1.5 ± 0.06^{c}	1.2 ± 0.1^{d}
35 °C, 6 min	25 ± 0.7^{h}	60 ± 1.2^{b}	1.01 ± 0.06^{ef}	2.4 ± 0.2^{b}
45 °C, 2 min	28.3 ± 1.3^{f}	$55.8\pm0.4_{d}$	1.13 ± 0.1^{e}	2.32 ± 0.2^{b}
45 °C, 4 min	31.3 ± 0.7^{e}	31.5 ± 0.6^{f}	1.25 ± 0.09^{d}	1.26 ± 0.1^{d}
45 °C, 6 min	33 ± 1.2^{d}	33.5 ± 1.6^{e}	1.32 ± 0.05^{d}	1.34 ± 0.05^{c}
55 °C, 2 min	22.5 ± 0.9^{i}	17.5 ± 0.9^{g}	0.90 ± 0.06^{f}	$0.70\pm.1^{e}$
55 °C, 4 min	0.00 ± 0.0^{j}	2.5 ± 0.3^{h}	0.10 ± 0.01^{g}	0.0 ± 0.0^{f}
55 °C, 6 min	0.0 ± 0.0^{j}	0.0 ± 0.0^{i}	0.0 ± 0.0^{h}	0.0 ± 0.0^{f}

Superscripts within results indicate significant difference in means (n = 3) from post hoc Tukey tests ($P < 0.05$).

4.4 Effect of Salts on Disease Incidence.

4.4.1 Effect of Salts on Mycelial Growth.

Data from Table 2 shows that, salt concentration had a significant effect on linear growth ($P \leq 0.05$) of mycelial fungi. Linear growth decreased with increasing salt concentrations in all treatments for both species. Potassium bicarbonate had the highest antifungal activity against tested pathogenic fungi, followed by calcium chloride, ammonium bicarbonate and sodium bicarbonate. Compared with *A. mali*, ammonium bicarbonate had the highest significant effect on antifungal activity on linear growth of *A. porri* however,

linear growth for both species for sodium bicarbonate at 2 % was 88 mm and 86 mm respectively, while potassium bicarbonate and calcium chloride had highest effect on linear growth of *A. mali* compared with *A. porri.*

Table (2). Effects of different salt concentrations on the mycelial growth (mm) of tested pathogenic fungi.

Salts	Concentration %	Linear growth of fungi (mm.) ±SE	
		A. porri	*A. mali*
Potassium bicarbonate	2	76±1.2^{c}	65±2.32^{d}
	3	54±0.3^{f}	42±1.2^{h}
	4	33±0.9^{g}	23±1.6^{i}
Calcium chloride	2	82±1.8^{b}	75±1.1^{c}
	3	64±2.3^{e}	56±1.7^{f}
	4	34±0.5^{g}	22±0.8^{i}
Ammonium bicarbonate	2	81±2^{b}	89±3^{a}
	3	52±1.2^{f}	65±2^{d}
	4	35±1^{g}	47±0.6^{g}
Sodium bicarbonate	2	88±0.6^{a}	86±1^{b}
	3	67±1^{d}	72±2^{c}
	4	31±1^{h}	59±1^{e}

Values marked with different letters (n = 3) indicate significant different in means from post hoc Tukey tests ($P < 0.05$).

4.4.2 Effect of Salts on Fruit Rot Development

Data in Table (3) revealed that potassium bicarbonate was the most effective inorganic salt for controlling the causal organisms of apple fruit rots followed by calcium chloride for both species, while ammonium bicarbonate and sodium bicarbonate had the least effect on controlling mycelial growth within both species. It was also noticed that *A. mali* was more sensitive to potassium bicarbonate and calcium chloride salts than *A. porri*. The best inhibitory effect of fruit rot was potassium bicarbonate followed by calcium chloride, while the least inhibitory effect in this experiment was observed by ammonium bicarbonate. Furthermore, the same pattern was recorded in Severity of Infection.

Table (3). Efficacy of inorganic salts on apple fruit rots development stored in cold room at 10±2ºc for four weeks

Salts	Concentration %	Decay Index %		Severity of Infection %	
		A. porri	*A. mali*	*A. porri*	*A. mali*
Potassium bicarbonate	4	0.91±0.01^{c}	0.67±0.01^{d}	25.5±.4^{c}	16.7±0.4^{d}
Calcium chloride	4	0.98±0.01^{c}	0.86±0.01^{c}	24.5±0.3^{c}	21.5±0.5^{c}
Sodium bicarbonate	4	1.14±0.03^{b}	1.52±0.05^{b}	28.5±0.5^{b}	38±0.6^{b}
Ammonium bicarbonate	4	1.23±0.07^{a}	2.42±0.06^{a}	30.7±0.8^{a}	60.5±1.4^{a}

Values marked with different letters (n = 3) indicate significant different in means from post hoc Tukey tests ($P < 0.05$).

5 Discussion

Effect of Storage Temperature on Linear Growth and Decay Index and Severity of Infection

One of the most important environmental factors affecting mycelial growth and growth is temperature, which occurs over a diverse, varying temperature range. In order to evaluate the effect of storage temperature on the linear growth of *A. porri* and *A. mali* fungi, it was necessary to expose both species to different degrees of temperature (5ºC, 15ºC, 25ºC and 35ºC). Results in Figures 1, 2A and 2B showed the highest liner growth for (2A) Decay Index and (2B) Severity of Infection was recorded at 25 ºC and the lowest was at 5 ºC. These results were similar to Neelam *et al*. (2013) who reported optimum temperature for growth of *Pleurotus ostreatus* in a variety of 25°C to 30°C. Also, Farooq *et al.* (2005) observed that growth of *Fusarium oxysporium* achieved its maximum after 7 days of incubation at 30°C and growth was drastically decreased at temperatures under 15°C and above 35°C. Similar results were obtained by Ibrahim *et al* (2011) who observed that maximum growth of *Helminthosporium fulvum* was obtained at 25˚C and 30˚C temperatures. Furthermore, Mishra and Thawani (2016) discussed poor growth of *Alternaria*

alternate at temperatures under 20°C compared to its great growth at 27°C. The same authors noted temperature of 5°C as a growth limit for *A. alternate*, which is in agreement with the results obtained for our study on apple fruits stored at 5°C. Grzegorzewska *et al* (2022) also reported that a temperature of up to 5◦C markedly reduced fungal development.

Effect of Hot Water on Apple Fruit Rot Disease

The recent research has provided evidence of a fundamental efficacy of hot water treatment against *A. porri* and *A. mali* fungus in apples that have been infected artificially. Apples inoculated with *A. porri* and *A. mali* were subjected to hot water treatment at different temperatures (35◦C, 45◦C and 55◦C) and duration (2, 4 and 6 minutes), followed by ambient conditions for 2 weeks. Hot water at 55ºC was the best temperature, which gave the lowest fruit rot disease and completely prevented the development of fruit rot at 4 and 6 minutes for *A. porri* and for 6 minutes in *A. mali* (Table 1). This result was similar to that of Petres *et al* (2020) who reported that *Fusarium avenaceum* and *Fusarium graminearum* were exposed to hot water treatments ranging from 45°C to 90°C at different durations ranging from 30 to 20 minutes, their results showed that the treatments that significantly inhibited mycelial growth were temperatures of 53°C and 57°C for 3 and 5 minutes. Also, Di Francesco et al. (2018) found that defense response in apple fruit against pathogens can be stimulated by hot water treatments. Grzegorzewska *et al* (2022) reported that heat water treatment at temperatures of 53 °C for 3 seconds and 55 °C for 3 seconds substantially inhibited deterioration during short-term storage. According to Loayza *et al.* (2012), hot water treatment at 52°C for 5 min improved the sensory profiles of intact tomato fruits of two varieties. Trierweiler *et al*. (2003) observed that stored apple fruits previously treated with 53°C for 2 minutes in hot water significantly reduced the occurrence of *Gloeosporium* fruit rot compared to untreated fruits. Many authors such as (Fallik *et al.*1995, Lieperuma *et al.* 2000, Fallik 2004 and Tohamy *et al.* 2004 reported similar results. However, Maxin *et al.* (2014) stated that a temperature of 53◦C or higher increased the incidence of fruit rot was noticed probably since this is the point where physiological damages occurs in apple fruit. These claims are opposite to results achieved in this study. In our study no visible damage was discovered on treated fruits, this is likely because each variety reacts differently to the same water temperature. Treatment 55°C for 5 minutes showed the strongest necrosis inhibition without detrimental effects on fruits stored at at ambient temperature. Our results indicated that the most promising hot water treatment was 55 °C with an exposure time of 4 to 6 minutes. This can be explained by the antifungal influence of the applied temperature, as well as by the activation of the defense reaction in apple fruits. Maxine *et al*. (2012) concluded that the main effects of hot water immersion against this fruit rot is mediated through heat-induced acquired resistance of the fruit and not heat-induced spore mortality. Amongst different solutions, hot water treatment seems to be an encouraging means to decline the physiological aging process, prevent the development of physiological disorders and minimize microbial growth in freshly cut products (Fallik and Ilic, 2017). Koukounaras *et al*. (2008), Lurie (2006) and Siddiq et al. (2013) demonstrated that hot water treatment has been illustrated to have profound special effects on tissue metabolism and maintaining the quality of fresh-cut products.

Effect of Inorganic Salts on Disease Incidence

Potassium bicarbonate, Calcium chloride, sodium bicarbonate, and ammonium bicarbonate have been shown to prevent fungal pathogens in vegetables, fruit, field crops, and ornamental plants (Ziv and Zitter 1992; Palmer *et al*. 1997). Four inorganic salts namely calcium chloride, sodium bicarbonate, potassium bicarbonate and ammonium bicarbonate, have been used for their antifungal activity against mycelial growth and control of apple fruit decay caused by *A.porri* and *A. mali*. In all salt treatments, linear growth of fungi decreased with increasing salt concentrations (Table 2) and these results agree with Nahal *et al* (2009) who stated that the application of calcium chloride or sodium bicarbonate considerably reduced early blight and its severity by increasing their concentrations. Previous research showed that raising sodium bicarbonate concentrations caused in a constant improvement in efficacy (Mlikota and Smilanick 1998, 2001). The inhibitory impact of bicarbonate salts on microorganisms may be due to a decrease in cell turgor, which causes hyphae and spores to collapse and shrink, resulting in fungistasis (Fallik *et al*. 1997).

This finding supports the results of Wisniewski *et al.* (1995) who discovered that calcium chloride can minimize fungal infections by directly inhibiting growth and spore germination. Maouni *et al*. (2007) recorded that calcium chloride considerably decreased pear fruit decay induced by *Penicillium expansum* and *A. alternata* and when used at 4% and 6%. The exact mechanism by which calcium reduces fungal infection is unknown, but it may work by interfering with the activity of pectolytic enzymes (Conway et al. 1992) and may be due in part to a decrease in cell wall maceration by polygalacturonase (PG) due to improved structural integrity caused by increased calcium content (Conway *et al*. 1998). Calcium applications have been shown to improve

membrane functionality and maintaining integrity,which could explain why calcium treated fruits possess lower linear growth(Shirzadeh *et al.* 2011).

Bicarbonate salts have an inhibitory effect on fungi due to a decrease in fungal cell turgor pressure, which caused hyphae and spores to breakdown and shrink of preventing fungi from sporulating (Fallik et al. 1997).

In addition to controlling nutritional disorders, increasing the calcium content of fruits and vegetables increases their shelf life. It is believed that this effect is mainly due to the role of calcium in alleviating physiological disorders and thereby indirectly reducing pathogen activity (Bateman and Lumsden 1965, Conway et al. 1992). Much of the research with apples to improve storage quality and reduce decay with calcium supplementation has been done in the post-harvest environment. Alan R. Biggs (1999) reported that the results of this study show that calcium salts directly suppress the bitter rot pathogens *Colletotrichium gloeosporioides* and *Colletotrichium acutatum* Suppressive effects include reduced germ tube growth, reduced in-vitro mycelial growth, and reduced severity of infection of calcium pretreated host tissues. Zaker (2014) reported that among the four inorganic salts, potassium bicarbonate achieved greatest antifungal activity against *Fusarium oxysporum*, *Alternaria alternata* and *B. cinerea*. Zaker (2014) reported that potassium bicarbonate had the highest antifungal activity against *Fusarium oxysporum*, *Alternaria alternata* and *B. cinerea* among the four inorganic salts

Calcium as a component of the cell wall plays an essential role in Cross-bridge formation that impact cell wall strength and is considered regarded the last barrier before cell separation (Fry, 2004) Exogenously supplied calcium stabilizes the plant cell wall and protects it from cell wall-degrading enzymes (White and Broadley, 2003). Post-harvest calcium treatment considerably reduced decay in peaches by *Monilinia fructicola* (Conway *et al*., 1987 a,b) and in apples by *Botrytis cinerea* (Klein *et al*., 1997). Tian *et al*. 2001, found that the biocontrol efficacy of yeast (*Trichosporon* spp.) used to control gray and blue mold apple fruit was enhanced in the presence of 2% calcium chloride. Tian *et al* (2001) reported that calcium chloride (2% w/v) significantly inhibited the growth of the pathogen *Rhizopus stolonifer.*

6 Conclusions

Different degrees of temperature, hot water treatment and inorganic salts were investigated for their efficacy in reducing the incidence and severity of infection of apple fruits by *A. porri* and *A. mali*. It is apparent from the study that a low temperature of 5°C and exposure of apple fruits to hot water at 55°C for 4 to 6 minutes using potassium bicarbonate and calcium chloride improved the sensory profiles of intact apple fruits against two cultivars of fungi.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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