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Research Article

Enhancing quality characteristics and controlling gray mold disease caused by *Botrytis cinerea* in strawberries fruits using various edible abiotic coatings

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Abstract

The study aimed to evaluate the efficiency of chitosan, potassium silicate, and calcium chloride as edible abiotic coatings in controlling the postharvest gray mold disease of strawberries caused by *Botrytis cinerea*, reducing the use of chemical fungicides and managing fruit decay. Two pure isolates of *B. cinerea* were extracted from strawberry fruits of cv. 'Festival', identified based on morphological features, and their rDNA sequences were sequenced using BLAST and phylogenetic analysis, showing 98.9-100% equivalence. The ITS sequences have been deposited in Gene Bank and assigned accession numbers MT708074 and MT704983. *In vitro*, all treatments inhibited linear growth of both isolates, with chitosan and potassium silicate were the most effective against the two isolates. *In vivo* test showed a significant decrease in gray mold incidence and severity. The study revealed that potassium silicate significantly reduced disease incidence in strawberry fruit cultivars 'Fortuna' and 'Festival' from Qalyubia governorate, while chitosan achieved the greatest reduction in disease severity in samples from Beheira governorate. Both treatments increased the total phenolic and peroxidase activity. The study found that application of potassium silicate and chitosan to strawberry fruit in 'Fortuna' and 'Festival', resulted in higher sugar and ascorbic acid content, increased fruit firmness, and decreased respiration rate, suggesting that these treatments could potentially reduce postharvest decay and enhance fresh strawberry fruit quality.

Keywords: calcium chloride; chitosan; gray mold; potassium silicate; strawberry

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Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an economical imperative fruit crop that is grown worldwide due to their attractive taste (Bai *et al*., 2021). Also, it is one of the most vital crops in Egypt for domestic consumption and exportation. However, strawberries have a short shelf life after harvesting due to their susceptibility to fungal diseases which can negatively impact and reduce the fruits' quality throughout storage (Feliziani and Romanazzi, 2016; Azam *et al*., 2019). In the field strawberry fruits are impacted by fungal infections, which cause significant pre- and post-harvest losses. Gray mold caused by *Botrytis cinerea* Pers (teleomorph: *Botryotinia fuckeliana*) is the most common postharvest disease affecting strawberries. Due to its genetic flexibility, numerous types of fungicides have been ineffective in controlling it, even though they exist. (Williamson *et al*., 2007; Huma *et al*., 2023). *B. cinerea* is a significant necrotrophic plant pathogen that damages ended 500 plant species, particularly fresh fruits, and causes major global economic losses (Hua *et al*., 2018; El-fawy *et al.,* 2020).

There is a growing interest in finding secure and efficient non-fungicide alternatives of managing post-harvest infections due to public concerns about synthetic pesticides in food and the environment. This forces operators to work hard to avoid these toxic chemicals, replace them with safe products, and search for alternative means of controlling the disease. Nowadays, spraying, coating, or dipping strawberry fruits with fungicide alternatives is extensively used to increase their shelf life (Kahramano *et al*., 2022; Lin *et al*., 2023). Certain strategies, such as pre- or postharvest treatment of potassium silicate, chitosan, and calcium chloride, were suggested for decreasing the risk of pathogen development (Hassan and Chang, 2017; Abd-El-Kareem *et al.*, 2019; Thabet, 2019; Hussein *et al*., 2021; Romanazzi and Moumni, 2022).

Chitosan is used as a plant protector owing to its antimicrobial capacity, to induces resistance responses in host tissues, and to create films on treated surfaces (Elsabee and Abdou, 2013; Romanazzi *et al*., 2019; Shreen *et al*., 2019; Rajestary *et al*., 2021; Omar *et al.* 2021). For different types of fruits chitosan-based coatings are thought to be the most edible and safe preservation coatings due to their functional benefits, which include decreased microbial development, longer storage times, slower respiration rates, and firmness retention (Romanazzi *et al*., 2015). Instead, Toivonen and Stan (2001) reported only minor effects of calcium sprays on strawberries and retention of quality during short-term storage. However, Wojcik and Lewandowski (2003) found that calcium spray increased firmness and reduced fungal decay caused by gray mold (*B. cinerea*). Calcium addition showed positive effects on fruit density and fruit nutrition value growth (Niazi *et al*., 2021; Liu *et al*., 2023). Under various biotic and abiotic stress conditions the positive effects of silicon (Si) are frequently stated more obviously in Si-accumulating plants. Silicon is contributed to stimulating growth and increases plant strength and stress resistance (Xiao *et al*., 2022). It acts efficiently for managing a wide range of bacterial and fungal diseases in various plant species. (Wang *et al*., 2017; Nikagolla *et al*., 2019). Furthermore, Moscoso-Ramírez and Palou (2014) showed that treatment with potassium silicate decreased the severity of green mold and extended the shelf life of orange fruits. In general, potassium silicate postharvest treatments demonstrated promise as non-polluting methods to manage penicillium decay in citrus fruit. Additionally, Elshahawy *et al*. (2023) revealed that the dipping with silicate salt prevented *B. cinerea* infection and preserved the normal qualities of apple fruits.

The purpose of the current investigation was to estimate the effect of postharvest treatments, i.e., chitosan, potassium silicate, and calcium chloride, on controlling fruit mold and maintaining the quality of strawberry fruits.

Materials and Methods

Source of plant material

Strawberry fruits of cvs. ‘Fortuna’ and ‘Festival’ were collected from Qalyubia and Beheira governorates, Egypt. The collected fruits were transferred to the laboratory and kept in the refrigerator at 5 °C. The separating of the fruits was based on their uniformity and the lack of physical injuries or decay. Then, before treatment, a random distribution of the chosen fruits was made into four groups.

Isolation and morphological identification of the causal pathogen

According to Abdel Wahab (2015) and Wagih *et al.* (2019) the fungal pathogens were isolated from naturally infected strawberry fruits cv. ‘Festival’ viewing numerous types of gray mold symptoms collected from Qalyubia and Beheira governorates, Egypt, and cultured on potato dextrose agar (PDA) at 20 ± 2 °C for 2 weeks using the hyphal tip and/or single spore technique under alternate light and dark conditions (Vinodkumar and Nakkeeran, 2017). The purified fungal isolates were recognized based on their cultural properties and morphological features, as defined by Ellis (1971) and Nielsen *et al.* (2002).

Molecular identification

Morphological identification was verified by matching of the ITS sequences of nuclear ribosomal DNA. The two tested fungi's genomic DNA was extracted for molecular identification, and the ITS region was used for PCR amplification in accordance with the DNeasy Plant Mini Kit (Qiagen). Using the Nano Drop technique (ND 1000, Thermo Scientific, Waltham, MA), the concentration of DNA was determined. Samples were kept in storage at -20°C. Universal primers ITS-1: 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS-4: 5′-TCC TCC GCT TAT TGA TAT GC-3′ were used to amplify the whole ITS region using PCR (Glass and Donaldson, 1995). Using an ABI 3730xl DNA sequencer, the purified DNA samples were sequenced at GATC Company (GATC Biotech Ltd., The London Bioscience Innovation Centre, London, United Kingdom). Using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nih.gov), the acquired sequences were compared to sequences in the public database to assess similarity to sequences in the Gene Bank database (Shayne *et al.*, 2003). A phylogenetic analysis was conducted using MEGA6 software (Tamura *et al.*, 2013) and the neighbor-joining technique (Saitou and Nei, 1987).

Treatments

Chitosan, potassium silicate, and calcium chloride were used to determine their effects in controlling strawberry gray mold infection. A chitosan crab shell was obtained from Roth Co., Germany, and was used at a concentration of 1% (W/V). Potassium silicate (12% Si) and calcium chloride (15% Ca) were produced by Central Lab of Organic Agriculture and used at a rate of 0.5 mL / 1 L water.

Effect of the treatments on the linear growth of the pathogens in vitro

Selected concentrations were incorporated into the PDA and poured into glass petri dishes (9 cm) to determine the effect of treatments on the linear growth of *Botrytis cinerea*. As a source of inoculums, plugs of 0.5 cm agar covered with mycelia and fungal pathogen spores were cut from the growing edge of colonies that had been growing on PDA for two weeks. These agar discs were set in the middle of petri plates containing PDA with the corresponding compounds at various concentrations then plates were incubated at 20 ± 2 °C (Guo *et al.*, 2006). As a control, non-treated plugs for pathogens were inserted in the middle of PDA plates and incubated in the same conditions. Four replicates were constructed for each treatment and until the control plates were completely covered with mycelia, the colony diameter was measured every day. The percentage of mycelial growth inhibition (MGI) % was calculated as the following formula proposed by Ong *et al.* (2013).

$$\text{Percentage of mycelia growth inhibition (MGI) \%} = \frac{\text{DC - DT} \times 100}{\text{DC}}$$

Where: DC = average diameter of the mycelia growth in the control.

DT = average diameter of the mycelia growth in the treatment.

Disease assessment of strawberry fruit postharvest decay in vivo

Strawberry fruits were dipped in chitosan, potassium silicate, and calcium chloride at the above-mentioned concentrations to assess their impact on the incidence and severity of decay. The fruits were dipped individually in each treatment for two minutes, then raised and left to air dry. Three punnets (250 g) were used for each treatment. Strawberry fruits dipped in distilled water only act as a control. Then, all treated strawberry fruits were kept at room temperature at 20 °C for 7 days. Every day, until gray mold symptoms appeared in the control treatment, all the punnets were inspected. The percentage of disease incidence was calculated as the percentage of decayed or infected fruits according to Ali *et al.* (2015). Also, to assess the effectiveness of the above treatments on decay development, the disease severity was measured using the disease index as described by Eccleston *et al.,* (2010): 1: No visible disease on fruit; 2: No greater than ¼ of fruit infected; 3: No greater than ½ of fruit infected; 4: No greater than ¾ of fruit infected; 5: Whole fruit surface infected

% Disease severity = (sum of n × v) × 100/ 5N

Where n = number of fruits in each category; v = numerical value of each category; N = total number of fruits in sample.

Total phenolic content and peroxidase enzyme activity

The total phenolic content was determined using the Folin-Ciocalteu reagent and gallic acid as standards (Slinkard and Singleton, 1977).

Peroxidase enzyme extract was obtained by grinding fruits tissues (2 ml / g fruits tissue) in 0.1 M sodium phosphate buffer at pH (7.1). The extracted tissues were centrifuged at 3000 rpm for 20 min. at 6 °C. The collected supernatants were considered as crude enzyme extract. Peroxidase activity was expressed as changes in absorbance/min at 425 nm using the method of Chen *et al.* (2000)

Determination of quality properties of strawberry fruits

Measurements of respiration rate were determined at the California-Egypt project for Agricultural System Development, Faculty of Agriculture., Cairo University, using 250 g of strawberry fruits consistent with the method of Fonseca *et al.* (2002), which measure carbon dioxide production as mL CO_2/kg/hr.

Firmness was determined according to Bourne (2003) at the Horticulture Processing Research Department, Food Technology Institute.

Titratable acidity was determined along with the glass electrode technique defined by A.O.A.C. (2005). The obtained results were represented as grams of citric acid per 100 g sample.

Ascorbic acid content was determined using 2,6-dichlorophenol indophenol according to the method described by Askar and Treptow (1993). The results were shown as mg of ascorbic acid per 100 g of sample.

Sugar contents were extracted from samples with 70% ethyl alcohol and clarified with lead acetate. Sodium oxalate precipitated the excess lead acetate. As outlined in A.O.A.C. (2005) total sugars were determined in the clarified solution. The results were represented as grams of glucose per 100 g of sample.

Statistical analysis

The obtained data were statistically analyzed using SAS software with a complete randomized design, version 2004, in accordance with the methods described by Snedecor and Cochran (1980). Duncan's test was used to compare the treatment means at the 0.05 probability level.

Results

Morphological identification and molecular classification of the pathogen

Two isolates of *B. cinerea* were isolated from strawberry fruits of cv. 'Festival'. The first isolate (B1) was isolated from Qalyubia governorate, while the second isolate (B2) was isolated from Beheira governorate, Egypt. Botrytis species were identified by sequencing and phylogenetic analysis

PCR using genomic DNA of two fungal isolates as template and universal ITS primers for 5.8s rDNA, produced one fragment of 550 bp (Figure 1). The two fungal isolates' amplified fragments were exposed to nucleotide analysis using the identical primers and the sequences compared with the NCBI GenBank database. The nucleotide sequence data for (B1) and (B2) have been deposited in the NCBI GenBank with accession numbers MT708074 and MT704983, respectively. Using the nuclear ribosomal internal transcribed spacer (ITS rDNA), a phylogenetic tree of the 16 isolates of *B. cinerea* that are distributed globally as well as those from Egypt has been created. The result showed that between the Egyptian isolates, the nucleotide sequence of the ITS rDNA gene was closely related to some worldwide isolates and ranged from 98.9 to 100% (Table 1 and Figure 2).

Table 1. Accession numbers of both isolated *Botrytis cinerea*, identity and origon compared to other registered *Botrytis* and *Sclerotinia* isolates at the NCBI gen bank database

Origin	Isolate	Host	Identity (B1)	Identity (B2)	GenBank Accession No.
China	*Botrytis cinerea*	Grape	100 %	100%	KP737304
The Netherlands	*Botrytis fabae*	Broad bean	99.8 %	99.8%	AJ716303
Pakistan	*Botrytis cinerea*	Bell pepper	100 %	100%	MF521932
Egypt (B2)*	***Botrytis cinerea***	**Strawberry**	**100%**	-----	**MT704983**
Bangladesh	*Botrytis cinerea*	Guava	100 %	100%	MN756674
USA	*B. caroliniana*	Blackberry	99.7 %	99.7%	NR111839
China	*B. fabiopsis*	Broad bean	99.7 %	99.7%	EU519204
Egypt (B1)*	***Botrytis cinerea***	**Strawberry**	----	**100%**	**MT708074**
China	*Botrytis cinerea*	Blueberry	100 %	100%	KT343755
India	*Botrytis sp.*	Lilies	99.9 %	99.9%	MN783427
China	*Botrytis cinerea*	Plum	99.9 %	99.9%	KP234034
China	*Botrytis cinerea*	Grapes	100 %	100%	JX840480
China	*Botrytis fabae*	Faba Bean	100 %	100%	MN589852
USA	*B. californica*	Blueberries	99.7 %	99.7%	NR151843
China	*Botrytis fabae*	Faba Bean	99.8 %	99.8%	MT877057
The Netherlands	*Botrytis cinerea*	Grapes	100 %	100%	AJ716294
China	*Botryti cinerea*	Blueberry	99.5 %	99.5%	MT954024
The Netherlands	*Botrytis porri*	Onion	98.9 %	98.9%	NR_147419
The Netherlands	*Sclerotinia sclerotiorum*	Bean	42.0%	42.0%	AJ745716

*The new isolates obtained in this study

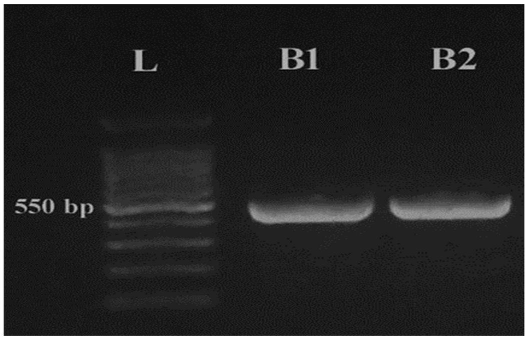

Figure 1. Electrophoresis patterns of 5.8S rDNA gene of *Botrytis cinerea*
L: 100 bp Ladder marker and lanes 1 and 2 refer to the two isolated fungi.

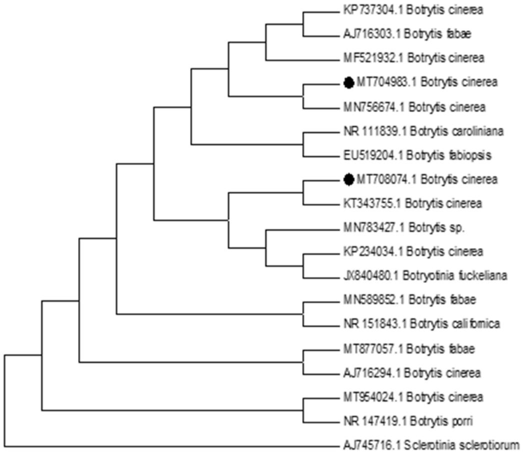

Figure 2. Phylogenetic analysis of *B. cinerea* isolates MT708074 and MT704983 constructed using the neighbor-joining method and based on combined ITS-5.8S rDNA data
Sclerotinia sclerotiorum strain was used as the out group. The isolates obtained in this study are shown with asterisk.

Impact of some inorganic compounds on B. cinerea growth in vitro

To investigate the inhibitory effects of potassium silicate, chitosan, and calcium chloride on the linear growth of *B. cinerea* isolates B1 and B2, these treatments were applied *in vitro*. Data in Figure 3 indicated that when compared to the control, all treatments decreased the linear growth of both isolates. Treatment with chitosan and potassium silicate was the most effective against the two isolates compared with the other treatments. A potassium silicate followed by chitosan clearly reduced the liner growth of B1 and B2 by 73.2%-65.3% and 80.4%-60.2%, respectively, when compared with calcium chloride and control.

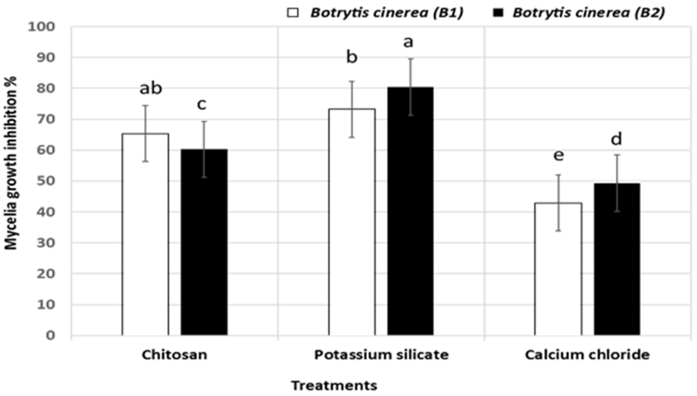

Figure 3. Effect of different inorganic compounds on the liner growth of *B. cinerea* after 2 weeks at 20 ± 2 °C.

Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P = 0.05$.

Impact of some inorganic compounds on strawberry fruits infection with gray mold in vivo

Chitosan, potassium silicate, and calcium chloride were used as postharvest treatments in an *in vivo* test to examine their impact on the incidence and severity of gray mold disease in strawberries that were kept at 20 °C for seven days. Data in Table 2 and Figure 4 indicated that all treatments generally reduced the development of gray mold disease compared with control. The findings also showed that, when compared to other treatments, potassium silicate and chitosan were the most successful in reducing decay infection in the two strawberry cultivars (cvs. 'Fortuna' and 'Festival') that were gathered from the Qalyubia governorate. They exhibited a significant decrease in disease incidence that could reach to 50.6%-46.2% and 44.1%-40.2%, respectively. A similar trend was also observed with disease severity, in which potassium silicate followed by chitosan caused a clear reduction that could reach to 8.3%-10.1% and 10.3%-12.5%, respectively. In Beheira governorate, chitosan was the most effective treatment in reducing the incidence of gray mold in both strawberry cultivars by 57.7% and 49.8%, respectively, followed by potassium silicate, which recorded 47.8% and 44.9%, as compared to other treatments. However, when strawberry fruits cv. 'Fortuna' were treated with chitosan (11% DS) or potassium silicate (11.5% DS), no significant differences in disease severity (DS) were observed. In the meantime, potassium silicate proved to be the most effective treatment in reducing the severity of the disease in strawberry fruits (cv. 'Festival'). Moreover, the least effect on disease development was produced by the treatment with calcium chloride in fruits collected from the two tested governorates.

Figure 4. Effect of dipping strawberry fruits in some inorganic compounds on gray mold decay development on cv. 'Fortuna' after 7 days of storage

A: calcium chloride; B: potassium silicate; C: chitosan; D: control

Table 2. Effect of dipping strawberry fruits in some inorganic compounds on gray mold incidence, severity and phenol content *in vivo*

Treatment	**Qalyubia**			**Beheira**		
	%Reduction in disease incidence	**% Disease severity**	**Phenols content mg/100 g**	**%Reduction in disease incidence**	**% Disease severity**	**Phenols content mg/100 g**
'Fortuna' cv.						
Control	00.0 ^{f}	42.4 ^{b}	172.8 ^{d}	00.0 ^{f}	46.3 ^{b}	175.9 ^{de}
Chitosan	44.1 ^{c}	10.3 ^{e}	200.2 ^{a}	57.7 ^{a}	11.0 ^{g}	209.4 ^{a}
Potassium silicate	50.6 ^{a}	8.30 ^{f}	205.3 ^{a}	47.8 ^{b}	11.5 ^{g}	195.8 ^{b}
Calcium chloride	31.5 ^{e}	12.7 ^{d}	188.0 ^{bc}	34.7 ^{d}	18.3 ^{d}	185.6 ^{c}
'Festival' cv.						
Control	00.0 ^{f}	44.8 ^{a}	161.5 ^{e}	00.0 ^{f}	48.5 ^{a}	162.4 ^{f}
Chitosan	40.2 ^{d}	12.5 ^{d}	182.8 ^{c}	49.8 ^{b}	15.8 ^{e}	184.6 ^{cd}
Potassium silicate	46.2 ^{b}	10.1 ^{e}	190.9 ^{b}	44.9 ^{c}	13.4 ^{f}	190.0 ^{bc}
Calcium chloride	31.3 ^{e}	18.3 ^{c}	166.8 ^{de}	29.0 ^{e}	21.3 ^{c}	170.0 ^{ef}

* For each column, means followed by the same letter are not significantly different according to Least Significant Difference (LSD) test at ($P \leq 0.05$).

Impact of treatments on the total phenol content of strawberry fruits

Table 2 illustrates changes in the total phenol content of strawberry fruits following a 7-day treatment with abiotic agents. The results clearly showed that, in comparison to the control treatment, treating strawberry fruits with all abiotic agents significantly increased their total phenolic content. Furthermore, it is evident that increased phenol content is positively correlated with a decline in disease incidence. According to the findings in Table 2. The link was quite significant when strawberry fruit cv. 'Fortuna', harvested from Beheira, was dipped in chitosan, which recorded the highest phenol content (209.4 mg/100 g) compared with the least effective treatment calcium chloride (185.6 mg/100 g). Likewise, potassium silicate was the highest active treatment for reducing gray mold disease in strawberry fruit cv. 'Fortuna' collected from Qalyubia by 50.6%, which recorded 205.3 mg/100 g phenol content.

Impact of treatments on peroxidase enzyme activity of strawberry fruits

In the present study, the activity of peroxidase was determined in treated strawberry fruits (cvs. 'Fortuna' and 'Festival') with abiotic treatments, which were then stored for 7 days. Data presented in Figure 5 revealed that all treatments increased enzyme activity as compared to those of control. When compared to other treatments and the control, treatment with potassium silicate and chitosan considerably ($P<0.05$) increased the amount of enzyme activity. Meanwhile, the least activity was cleared with calcium chloride treatment. It could be easily concluded that chitosan was the most effective treatment for increasing the activity of peroxidase in strawberry fruit cv. 'Fortuna' collected from Beheira. Also, potassium silicate, was the most effective treatments for increasing the activity of peroxidase in strawberry fruit cv. 'Fortuna' collected from Qalyubia.

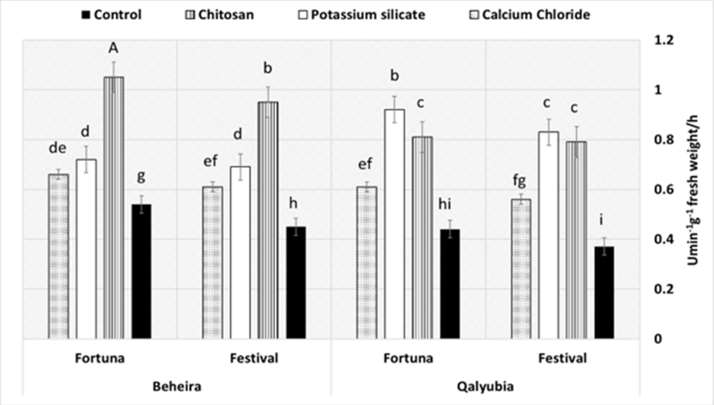

Figure 5. Effect of some inorganic treatments on peroxidase activity of strawberry fruits in different cultivars after storage at 7 days

Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at *P≤ 0.05.*

Impact of treatments on total sugars, acidity and ascorbic acid content

All abiotic treatments significantly increased total sugar content compared with the control in both strawberry fruit cultivars collected from Qalyubia or Beheira governorates after 7 days of packing (Table 3). The data also disclosed that treatment with potassium silicate showed the maximum content of total sugar in 'Fortuna' and 'Festival' fruits obtained from Qalyubia (8.05% and 7.20%) and Beheira governorate (7.87% and 6.92%), respectively, followed by chitosan treatment, compared with other treatments. Similarly, data elucidated that treatment with potassium silicate recorded the highest total acidity value in fruits of cv. 'Festival' collected from Qalyubia and Beheira governorates to be 0.94% and 0.91%, respectively. Alternatively, 'Fortuna' fruits treated with chitosan recorded the highest total acidity value, reaching 0.90% and 0.85% in fruits obtained from Qalyubia and Beheira governorates, respectively. Likewise, results in Table 3. demonstrated that as compared to the control all treatments reduced the deterioration in ascorbic acid of strawberry fruits. The greatest increase in ascorbic acid content was detected in strawberry fruits coated with potassium silicate to be 56.02 and 62.55 mg/100 g for 'Fortuna' and 'Festival' cultivars, respectively, obtained from Qalyubia governorate, followed by chitosan-treated fruits reaching 55.95 and 60.62 mg/100 g for 'Fortuna' and 'Festival' cultivars, respectively. Meanwhile, calcium chloride treatment showed the lowest ascorbic acid value in treated fruits, with 48.61 and 50.39 mg/100 g for 'Fortuna' and 'Festival' cultivars, respectively.

Table 3. Effect of dipping strawberry fruits in some inorganic compounds on total sugars, acidity and ascorbic acid content

Treatment	**Qalyubia**			**Beheira**		
	Total sugars %	**Total acidity %**	**Ascorbic acid mg/100 g**	**Total sugars %**	**Total acidity %**	**Ascorbic acid mg/100 g**
'Festival'						
Control	4.71 ^{g}	0.90 ^{ab}	50.03 ^{dc}	4.53 ^{h}	0.86 ^{ab}	50.22 ^{dc}
Chitosan	6.47 ^{d}	0.92 ^{a}	60.11 ^{ab}	6.21 ^{d}	0.89 ^{ab}	60. 62 ^{ab}
Potassium silicate	7.20 ^{c}	0.94 ^{a}	62.55 ^{a}	6.92 ^{c}	0.91 ^{a}	62.10 ^{a}
Calcium chloride	4.83 ^{g}	0.91^{ab}	50. 39 ^{dc}	4.68 ^{g}	0.86 ^{ab}	50.50^{dc}
'Fortuna'						
Control	5.52 ^{f}	0.83 ^{c}	45.04 ^{d}	5.39 ^{f}	0.78 ^{d}	44.79 ^{d}
Chitosan	7.49 ^{b}	0.90 ^{ab}	55.38 ^{bc}	7.35 ^{b}	0.85 ^{bc}	55.95 ^{bc}
Potassium silicate	8.05 ^{a}	0.88 ^{bc}	56.02 ^{b}	7.87 ^{a}	0.83 ^{cd}	56.33 ^{ab}
Calcium chloride	5.94 ^{e}	0.85 ^{bc}	48.61 ^{d}	5.81 ^{e}	0.79 ^{cd}	48.55 ^{d}

* For each column, means followed by the same letter are not significantly different according to Least Significant Difference (LSD) test at (*P*≤ 0.05).

Impact of treatments on respiration rates and firmness

It appears from the results in Figure 6A that all tested treatments were successful in lowering the treated fruits' respiration rates when compared to the control treatment. As well, treatment with chitosan showed to be the most effective treatment of cv. 'Festival' fruits, harvested from Qalyubia and Beheira governorates, scored 18.50 and 18.34 mg CO_2 kg^{-1} h^{-1}, respectively, followed by potassium silicate treatment, which recorded 20.83 and 20.94 mg CO_2 kg^{-1} h^{-1}, respectively. Alternatively, the treated fruits of cv. 'Fortuna' from Qalyubia and Beheira governorates with chitosan recorded the lowest respiration rate values (20.67 and 21.10 CO_2 kg^{-1} h^{-1}, respectively), followed by potassium silicate treatment, which recorded 21.27 and 21.17 mg CO_2 kg^{-1} h^{-1}, respectively. Correspondingly, data from Figure 6B makes it clearly apparent that, in comparison to the control, all treatments considerably decreased the loss of fruit firmness. Furthermore, chitosan treatment showed a higher fruit firmness (2.45 and 2.37 N°), followed by potassium silicate, which recorded 2.14 and 2.25 N° within the cv. 'Festival' obtained from Qalyubia and Beheira governorates, respectively. Moreover, it could be observed that a similar trend of findings was demonstrated in the treated 'Fortuna' cultivar with chitosan recorded height firmness values (2.26 and 2.30 N°), followed by treatment with potassium silicate (2.20 and 2.21 N°), respectively.

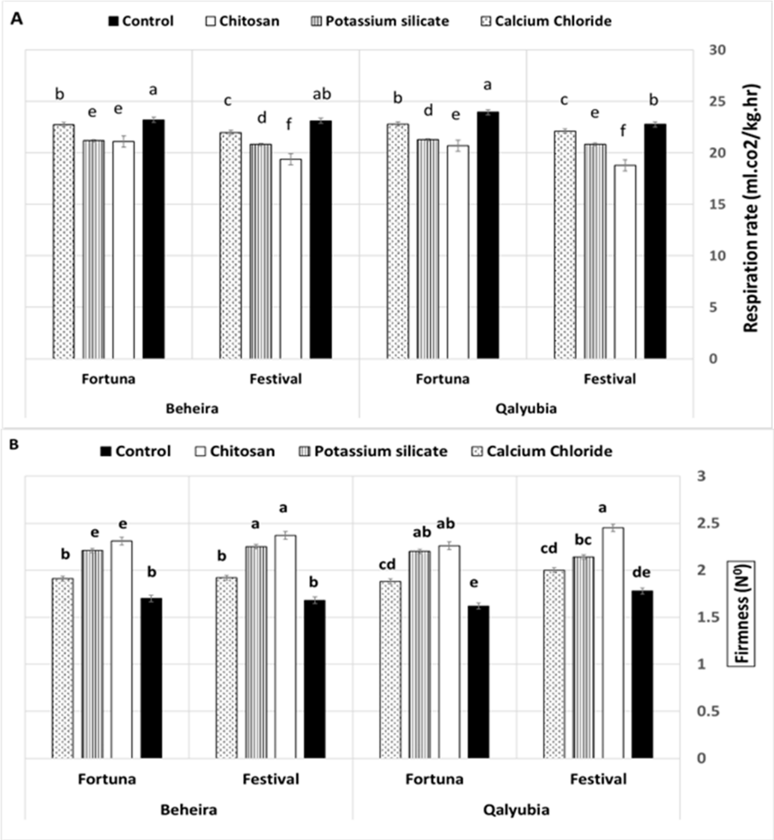

Figure 6. Effect of some inorganic treatments on respiration rate (A) and firmness (B) of strawberry fruits in different cultivars after storage at 7 days

Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at *P≤ 0.05.*

Discussion

Strawberries (*Fragaria x ananassa* Duch.) are the most widely distributed berries due to their distinct taste and diverse phytochemical combinations (Lombardi *et al*., 2020). Because of their rapid respiration rate and morphological characteristics, strawberry fruits are extremely sensitive and succulent for postharvest storage (Kahramano *et al*., 2022). They are also highly sensitive to phytopathogens; *B. cinerea* is the primary cause of these infections, which result in gray mold, a serious disease that can affect strawberries, particularly when they are being stored. In order to reduce the need for chemical fungicides and manage the emergence of postharvest diseases, the use of natural compounds as postharvest treatments has been examined (Shreen *et al.,* 2019; Thabet, 2019; Rajestary *et al.*, 2021; 2022; Romanazzi and Moumni, 2022). The current study was started to investigate the impact of treating strawberry fruits with chitosan, potassium silicate, and calcium chloride on the gray mold disease caused by *Botrytis cinerea* during storage.

Two pure strains of *B. cinerea* were isolated from strawberry fruits of cv. 'Festival' collected from Qalyubia and Beheira governorates, Egypt. Based on morphological features, all isolates related to *B. cinerea* (Jakobija *et al*., 2020). Botrytis species were identified by sequencing and phylogenetic analysis. The nucleotide sequence data for (B1) and (B2) have been deposited in the NCBI GenBank with accession numbers MT708074 and MT704983, respectively. By using BLAST and phylogenetic analysis, the internal transcribed spacer (ITS) sequences were shown to be 98.9-100% identical to the equivalent sequences of other *B. cinerea* strains worldwide. It's related to the results by Wagih *et al.* (2019); Gaber *et al.* (2020) and Hussein *et al.* (2021), who studied the pathological variety of *B. cinerea* isolates in infected strawberries in Egypt.

To investigate the inhibitory effects of chitosan, potassium silicate, and calcium chloride on the linear growth of *B. cinerea* isolates B1 and B2, these treatments were applied *in vitro*. According to our findings, as compared to the control, all treatments reduced the linear growth of both isolates. Treatment with chitosan and potassium silicate was the most effective against the two isolates compared with the other treatments. similar trend of results was also obtained by Ayon-Reyna *et al.* (2017); Ehab and Abeer (2018); Thabet, (2019) and De Oliveira *et al.* (2020) Their suggestion was that chitosan could cause deformity in the spores and hyphae of pathogenic fungus such *B. cinerea*, *R. stolonifera*, and *P. expansum* because it has been shown to inhibit spore germination, germ tube extension, and radial growth. Because chitosan's molecule contains positively charged amine groups that bind to the negatively charged surfaces of microorganisms including walls and plasma membranes, the molecule has an antifungal effect. This involves modifying cellular permeability, which results in ions and proteins being excluded from cells. Furthermore, chitosan can bind to nucleic acids and inhibit the normal functioning of the cellular molecular apparatus (Perinelli *et al*., 2018). Moreover, Youssef and Roberto (2014) demonstrated that spore germination and *B. cinerea* mycelial growth were totally inhibited by sodium silicate. Scanning and transmission electron microscopy were used in another investigation by Li *et al.* (2009) to detect deformation in *Fusarium sulphureum* hyphae treated with sodium silicate. This deformation included hyphal swelling, thickening of the hyphal cell walls, and cell distortion.

Chitosan, potassium silicate, and calcium chloride were used as postharvest treatments in an *in vivo* test to examine their impact on the incidence and severity of gray mold disease in strawberries that were kept at 20 °C for seven days. The results indicated that all treatments generally reduced the development of gray mold disease compared with control. The findings also showed that, when compared to other treatments, potassium silicate and chitosan were the most successful in reducing the incidence and severity of disease in two strawberry cultivars (cvs. 'Fortuna' and 'Festival') that were collected from the governorates of Qalyubia and Beheira. These outcomes are consistent with the findings published by Jayawardana *et al.* (2014) and Sak (2016), who suggested that silicon (Si) applied has the ability to delay the disease process and is effective in controlling many fungal and bacterial diseases. Similar, Lopes *et al.* (2014); Mbili *et al.* (2020), and Elshahawy *et al.* (2023) demonstrated the effectiveness of potassium silicate in decreasing *B. cinerea* caused gray mold infections.

Additionally, Dallagno *et al.* (2021) confirmed that treatment with potassium silicate reduces postharvest nectarine decay caused by *Rhizopus stolonifer*. According to Wang *et al.* (2017), because Si (OH)4 preferentially accumulates and polymerizes in cell walls, increasing host tissue resistance to pathogen penetration, treating plants with Si boosts their resistance to pathogenic fungus. Correspondingly, Awad (2017), Thabet, (2019), Melo *et al.* (2020) and Rajestary *et al.* (2022) concluded that, coating strawberry fruits with chitosan has a great effect on reducing the progression of the disease and its spread to fruits during the storage period. Besides, Youssef *et al.* (2019) claimed that *B. cinerea* caused gray mold infections in table grapes may be defeated by the combined action of chitosan and silica nanocomposites. Consistent with Romero *et al.* (2022), chitosan's antibacterial and antioxidative qualities are thought to have an impact; Strawberries become less susceptible to postharvest degradation when a thin, semipermeable polymer coating forms on their surface, reducing respiration and slowing down fruit ripening. Also, Wang *et al.* (2021) indicate that chitosan interacts with negatively charged phospholipids in host cell membranes through its polycationic molecule, activates defense responses in plant tissue, and plays a role in the immune signaling networks of plants through hormone pathways (jasmonic acid and abscisic acid pathways). These processes also control fruit's resistance to disease.

Changes in the total phenol content in strawberry fruits after 7 days of treatments with abiotic compounds were determined. Results clearly showed that as compared to the control treatment all abiotic compounds significantly enhanced total phenolic content in treated strawberry fruits. Furthermore, it is evident that increased phenol content is positively correlated with a decline in disease incidence. When strawberry fruit cultivars from Beheira or Qalyubia were dipped in chitosan and potassium silicate, our findings showed a very strong association when compared to the least efficient treatment, calcium chloride. Our findings are consistent with those acquired by Romanazzi *et al.* (2015); Ventura-Aguilar *et al.* (2018) and Rico *et al.* (2019) They reported that chitosan coatings enhanced the amount of phenolic compounds in strawberries and stimulated the activities of polyphenol oxidase and phenylalanine ammonia lyase (PAL). Additionally, Tesfay *et al.* (2011) and Hussein *et al.* (2021) revealed that the potassium silicate and potassium phosphate applications, respectively, had great effects on fruit firmness, increasing total phenolic concentrations as well as catalase activities, which responded positively to control gray mold disease in strawberry fruit.

Enzyme activity and the induction of resistance in plants are closely connected. Numerous investigations have verified that potassium silicate and chitosan are exogenous inducers of host defense reactions, such as the accumulation of β-1,3-glucanase and peroxidase (Maxin *et al.*, 2012; Weerahewa and Somapala, 2016). In the current study, the activity of peroxidase was determined in treated strawberry fruits (cvs. 'Fortuna' and 'Festival') with abiotic treatments, which were then stored for 7 days. Results revealed that all treatments increased enzyme activity as compared to control. Treatment with potassium silicate and chitosan significantly induced an increase in enzyme activity compared with other treatments and controls. These results follow the same trend as those gained by Jin Peng *et al.* (2016); Ehab and Abeer (2018) and Thabet, (2019) They revealed that, chitosan enhanced peroxidase, β-1,3-glucanase, and phenylalanine ammonia-lyase activities in strawberry fruits as compared with controls. Additionally, Petriccione *et al.* (2015) discovered that applying chitosan as a coating treatment increased the activity of certain antioxidant enzymes, limiting browning of the flesh and minimizing damage to the membranes. According to Wang *et al.* (2017) because Si $(OH)_4$ preferentially accumulates and polymerizes in cell walls, increasing host tissue resistance to pathogen penetration, treating host plants with Si enhances their resistance to pathogenic fungus. Also, Qin and Tian (2005) showed that the application of Si to the fruit of sweet cherries enhanced the biochemical defensive processes (polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase).

In the present study, all abiotic treatments significantly increased total sugar content compared with the control in both strawberry fruit cultivars collected from Qalyubia or Beheira governorates. This finding aligns with the outcomes attained by Bahri and Rashidi (2009) and Nie *et al.* (2020). They revealed that the increase in total sugar was probably because the treatment might decrease respiration rates. Consequently, when compared with untreated fruits it could delay the use of total sugar in the enzymatic reactions of respiration.

Also, our data disclosed that treatment with potassium silicate showed the highest content of total sugar, followed by chitosan treatment in 'Fortuna' and 'Festival' fruits obtained from Qalyubia and Beheira governorates, compared with other treatments. Parallel to this, data showed that in both cultivars gathered from the governorates of Qalyubia and Beheira, treatment with potassium silicate followed by chitosan produced the highest overall acidity value. In this regard, studies by Maftoonazad *et al.* (2008) and Sogvar *et al.* (2016) demonstrated a direct correlation between the fruit's total acidity and its concentration of organic acid. The fruit's changed metabolism or the respiratory process's usage of organic acids may be responsible for the fruit's decreased acidity. Additionally, as compared to the control our data revealed that all treatments reduced the deterioration in ascorbic acid in strawberry fruits. Also, strawberry fruits coated with potassium silicate followed by chitosan showed the greatest increase in ascorbic acid content. Meanwhile, calcium chloride treatment showed the lowest ascorbic acid value in treated fruits. Moreover, Khodaei *et al.* (2021) showed that coating treatments acted as a barrier and altered the fruit's internal environment by regulating the permeability of carbon dioxide and oxygen, which slowing the metabolic processes and consequently retarded fruit senescence. Thus, delayed the deteriorative oxidation reaction of ascorbic acid. These findings match with the results that were obtained by Gol *et al.* (2015); Wang *et al.* (2021); Bal and Bahtiyar (2021) and Rajestary *et al.* (2022) observed that coating harvested strawberries with chitosan substantially maintained high levels of soluble sugars and total acidity.

The rate of respiration is an important key to fruit and vegetable quality. It has frequently been noted that fruits with rapid respiration rates had a short postharvest life (Aked, 2002). In the current study, results showed that in comparison with the control all estimated treatments succeeded in reducing the respiration rates of treated fruits. As well, treatment with chitosan turned out to be the most efficient treatment of cv. 'Festival' fruits, harvested from Qalyubia and Beheira governorates, followed by potassium silicate treatment. Alternatively, the treated fruits of cv. 'Fortuna' from Qalyubia and Beheira governorates with chitosan recorded the lowest respiration rate values, followed by potassium silicate treatment, These results could be discussed in light of the findings of Bal (2013); Yuan *et al.* (2019), and Rajestary *et al.* (2022), They showed that applying chitosan on strawberry fruits inhibited the flow of gases between the fruit and its surroundings due to the fruits have varying permeabilities to gases like CO_2 and O_2. This resulted in coated strawberries having a lower respiration rate than untreated ones.

A common way to assess the maturity and ripeness of horticultural products is to utilize mechanical measures to quantify their firmness. Furthermore, texture plays a significant role in handling practices and is one of the factors influencing consumers' sensory perception of fruit, as they view texture as a positive quality trait that indicates the freshness of produce and enhances the enjoyment of eating (Konopacka and Plocharski, 2004). Our data clearly indicated that as compared to the control all treatments significantly reduced the loss in fruit firmness. Furthermore, chitosan treatment showed higher fruit firmness, followed by potassium silicate, in both strawberry fruit cultivars collected from Qalyubia or Beheira governorates. According to other research, chitosan and silicon edible coating modify gas diffusion, which in turn modifies the exchange of CO_2 and O_2 between fruit tissue and the external atmosphere. This preserves fruit moisture and lowers the rate of respiration, which delays textural changes and slows down the ripening process. (Wang *et al.*, 2007; Zhu *et al.,* 2008; Nguyen *et al.*, 2020).

Conclusions

Strawberry is an extremely perishable fruit with a short postharvest life, mostly due to fungal decay. In this study, we tried to shed light on the possibility of using alternatives to fungicides. Overall, the study's findings demonstrated that applying potassium silicate and chitosan as postharvest treatments decreased fruit deterioration without adversely affecting the fruits' quality criteria. Moreover, it enhances fruit quality, especially when disease pressure is low.

Authors' Contributions

Conceptualization: AMA, MT, MAZ and AJ: Conceived and designed the experiments. AMA. MT, MAZ, AJ and DA: Performed the experiments. AMA. MT, MAZ, AJ and DA: Analysed the data. DA: Genetic analysis and Molecular identification. AMA. MT, MAZ, AJ and DA: Wrote or proofread the paper. AJ, TMA. EAA and SAA: Provided funding. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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