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RESEARCH

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Hot water treatment in combination with silicate salts dipping for controlling apple gray mold caused by *Botrytis cinerea* Pers.:Fr.

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Abstract

Background Gray mold is the most prevalent postharvest disease of apple fruits in Egypt. In this study, five isolates of *Botrytis cinerea* were isolated from apple fruits that had postharvest decay symptoms. Investigations were made into the pathogenicity tests of these isolates as well as the molecular identification of the most virulent isolate. A study was done in vitro to see how *B. cinerea*'s mycelial growth and conidial germination would be affected by hot water treatments (HWT) at temperatures of 25, 50, 52, 54, or 56 °C for 10, 20, 30, or 40 s as well as silicate salts (SS), specifically potassium silicate and sodium silicate at 0.0, 2.0, 4.0, and 6.0%. The effectiveness of hot water treatment and silicate salts dipping (SSD), both separately and together, for preventing *B. cinerea* infection and preserving the natural qualities of apple fruits was investigated in vivo.

Results Pathogenicity tests on apples (Anna cv.) revealed that *B. cinerea* isolate (Bc-1) was found to be the most virulent. This isolate was identified as belonging to the fungus *B. cinerea* through molecular testing using internal transcribed spacer (ITS) sequencing and phylogenetic analyses, and it has since been added to Gene Bank with the accession number ON1498639.1. The lethal temperature for *B. cinerea* mycelial growth and spore germination in vitro was 54 °C/30 s and 54 °C/10 s, respectively. At a 6.0%, the SS, specifically potassium silicate and sodium silicate, completely prevented pathogen growth. When applied separately, HWT (60 °C/30 s) and SSD (6.0%/1 min) significantly reduced *B. cinerea* decay of apple fruits stored at 20 ± 2 °C for 15 days. In terms of control efficacy, the HWT (60 °C/30 s) and SSD (6.0%/1 min) combination performed better.

Conclusions When apple fruits are stored at 20 ± 2 °C for 15 days, the combination of HWT (60 °C/30 s) and SSD (6.0%/1 min) may be an efficient way to control the gray mold disease. The amount of total soluble solids (TSS) in apple fruits was unaffected by these treatments, but they significantly lessened fruit weight loss after 40 days of storage at 20 ± 2 °C.

Keywords Apple, Gray mold, HWT, Postharvest, SSD

Background

One of the most dangerous apple (*Malus domestica* Borkh.) diseases is gray mold disease, which is brought on by *Botrytis cinerea* Pers.:Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) (Hua et al. 2018; Sernaite et al. 2020). Although disease infection can happen in the field before or during harvest, disease development typically happens after harvest (Hua et al. 2018). During harvest, *B. cinerea* enters apple wounds through handling and bruising (Hua et al. 2018). By secreting a

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number of pathogenic endo-polygalacturonases, it significantly reduces the value of apple fruits (Breen et al. 2022). The resultant rot is medium firm and pale brown in color (Hua et al. 2018). In cold storage, aerial mycelium can form coarse bunches and gray conidiophore clusters. Conidiophores' branched tips produce conidia in a polyblastic manner. Because it can spread from apple to apple inside storage bins, botrytis rot is known as a nesting fungus. Because fruit can begin to rot in less than three weeks after initial contact, even small quantities of fruit that are infected can result in significant losses (Hua et al. 2018). *B. cinerea* is difficult to control because it has a wide host range, numerous attack modes, and both asexual and sexual stages to survive in favorable and unfavorable conditions (Hua et al. 2018). This pervasive fungus pathogen is also frequently present as a latent infection (Hu et al. 2019). Latency is generally defined as the time between infection and the appearance of visible symptoms, and it can be variable in the case of *B. cinerea* (Fernández et al. 2014). Currently, the primary method of controlling gray mold rot caused by *B. cinerea* remains the application of synthetic fungicides, which may make up about 8% of the global fungicide market, and annual global Botrytis control costs typically exceed €1 billion (Yan et al. 2022). The control effects of fungicides on *B. cinerea*, whose genome is flexible and prone to developing drug resistance genes, are insufficient (Shao et al. 2021). Fungicides are also harmful to the environment and people (Gikas et al. 2022). Therefore, fresh fruit gray mold rot caused by *B. cinerea* requires new preventative measures (Nyamende et al. 2021).

Significant work has been done recently to create apple fruit pre-storage HWT that is affordable and easy to use (Hosseinifarahi et al. 2020; Bhatta 2022). The commodity can be treated using hot water dips and sprays, hot vapor, dry air, infrared, or microwave radiation (Wassermann et al. 2019). After-harvest HWTs are physical, nondestructive methods with no residues left on the produce for controlling postharvest decay in fruits and vegetables (Strano et al. 2022). HWT can be applied in packinghouses to lessen infections brought on by postharvest fruit pathogens as a result. HWTs have a number of advantages, such as simple application, quick treatment times, and regular monitoring of the water and fruit temperatures. These procedures can also clean fruit skins by getting rid of surface-borne pathogens that cause fruit decay (Fallik 2004). Because HWT is now recognized as a commercial technique to preserve the postharvest quality of some fruit commodities, fruit immersion in hot water is preferred over other heat treatments (Fallik 2004). The incidence of apple fruit rot was found to be significantly reduced by incubation times of 3 min at 50–54 °C (dipping) and 20 or 25 s at 55 °C (rinsing), followed by up to 100 days of cold storage at 2 °C and 14 days at 18 °C (Maxin et al. 2012). HWT at 55 °C significantly decreased the severity of apple fruit rot infection and the decay index (Imhammed and Alhdad 2022). One method for enhancing the efficacy of HWT is integration with other approaches (Ayón-Reyna et al. 2017; Kumari et al. 2019).

Silicon (Si) is the second most abundant element in both the earth's crust and in plants, with a dry weight content of 0.1 to 10% (Katz et al. 2021). According to reports, Si is effective at preventing fungal disease (Abd-El-Kareem et al. 2019a, 2019b). Blue mold in sweet cherry fruit was reduced by mixing yeast *Cryptococcus laurentii* with Si, according to Qin and Tian (2005). They found that adding Si at 1% to yeast significantly reduced the growth of *P. expansum* on fruit. After harvest, Hami melons (*Cucumis melo* L. var *inodorus*) were treated with sodium silicate to prevent *Trichotecium roseum*'s fungal decay, according to Bi et al. (2006). According to research by Etebarian et al. (2013), silicon concentrations, particularly when combined with hot water, have an impact on how apples react to *P. expansum* and may be a key control measure for apple blue mold. Postharvest dipping of fresh produce in silicate solutions by themselves or in conjunction with HWT is another method of applying silicon to the prevention of disease in fruits and vegetables (Weerahewa and Somapala 2016). By increasing total polyphenol synthesis, maintaining higher flesh firmness, and reducing pre- and postharvest peach brown rot, Pavanello and Brackmann (2016) found that using 6 g l^{-1} sodium metasilicate may be beneficial. According to El-Mehrat et al. (2018), potassium silicate spraying combined with modified air packing preserved grape quality during storage and marketing and decreased the incidence of postharvest diseases. The effects of a 100 m mol l^{-1} sodium silicate treatment on apple weight loss and flesh firmness were discovered by Ge et al. (2019). Potassium silicate and sodium silicate have a positive effect on plant physiological and physical characteristics such as biotic and abiotic stress, element toxicity, plant growth improvement, and resistance to fungal pathogen infections (Elshahawy et al. 2021). Before harvest, peach fruit was treated with sodium metasilicate to prevent fungal decay brought on by *Monilinia fructicola*, as shown by Pavanello et al. (2022). This study set out to determine how well HWT worked on its own or in combination with SSD in potassium silicate and/or sodium silicate to control apple gray mold caused by *Botrytis cinerea*.

Methods

Treatments

In vitro and in vivo tests were conducted using a stainless steel digital water bath (Grant 40 L, with a Grant temperature controller unit (0.1 °C, 1.5 kW heater model

JSWB-22T, Korea) with continuous hot water recirculation and stirring to maintain the appropriate temperature. The silicate salts (SS) used in this study in in vitro and in vivo trials were potassium silicate solution (K_2SiO_3, 99% purity) the net weight of K_2SiO_3 per ml at 20 °C was about 1.33 g, (Lobo Chrmie Pvt. LTD. India) and sodium meta silicate pentahydrate (Na_2 $SiO_3.5H_2O$, 99% purity, Central Drug House, India).

Pathogen isolation

Apple fruits (Anna cv.) with typical postharvest apple rot symptoms were gathered from commercial markets in 6-October city, Giza governorate, Egypt. For isolation procedures, diseased fruits were collected and put in sterilized polythene bags before being transported to the Plant Pathology Laboratory at the National Research Centre in Egypt. Apples with the disease were cut into 5 mm squares, placed in 1% sodium hypochlorite solution for 30 s, 70% ethanol for 30 s, and sterilized distilled water (SDW) for 1 min in order to isolate the pathogen (Madbouly et al. 2020). The tissues were then placed on water agar (WA) and incubated for 5 days at 20 ± 2 °C. The emerge hyphae were transferred and incubated at 20 ± 2 °C on freshly prepared potato dextrose agar (PDA, Difco, Detroit, MI, USA). To induce sporulation, cultures were exposed once daily to a 10-min burst of near-UV light (BLB-30W, max = 365 nm), VL-215LC, France. Using the single spore method outlined by Williamson et al. (2007), the pathogen was purified, and the purified cultures were sub-cultured on fresh PDA plates. Five fungal isolates were found in naturally infected apple fruits. The isolates were designated Bc-1, Bc-2, Bc-3, Bc-4, and Bc-5 and identified as *Botrytis cinerea* based on morphological traits (Williamson et al. 2007). The culture of each isolate was maintained on a PDA slant and incubated for additional research at a cold temperature.

Pathogenicity tests

To support Koch's postulates, pathogenicity tests of the obtained *B. cinerea* isolates (Bc-1, Bc-2, Bc-3, Bc-4, and Bc-5) on apple fruits (Anna cv.) were carried out. These isolates were grown on PDA medium at 20 ± 2 °C for 10 days. To induce sporulation, cultures of isolates were exposed to a 10-min burst of near-UV light (BLB-20W, max = 365 nm). The conidial inoculum for each isolate was made by saturating the culture with 10 ml of sterile distilled water and filtration through two layers of sterile cheesecloth to remove hyphal fragments. The conidial concentration of each *B. cinerea* isolate was measured on a hemocytometer slide and corrected to 1×10^6 conidia ml^{-1} by adding SDW. Apple fruits (Anna cv.), which appeared to be in good health, were cleaned with 70% Ethanol for 1 min, then rinsed three times in SDW and allowed to air dry. The apple fruits were then uniformly wounded after being surface sterilized. Each side of the fruit had six randomly placed, 1 mm deep wounds made by a sterile insulin needle. *B. cinerea* conidia were sprayed onto the injured apple fruits using an atomizer at a rate of 200 ml per 100 fruits to inoculate them with the pathogen. Fruits in the control received the same quantity of SDW sprayed on them. Fruits that had been inoculated and controls were kept at 20 ± 2 °C in plastic trays that were sealed off and had a high relative humidity (RH) of about 90%. Each isolate had four replicates, and each replicate had five fruits. Fruits were checked for disease incidence after 15 days, and the percentage was calculated as follows: Disease incidence (%) = (number of decayed fruits/total fruits) × 100. The weight percentage (g) of the affected portion of each fruit was divided by the fruit's total weight to determine the disease's severity (Abd-El-Kareem et al. 2016) as follows: Disease severity (%) = weight of rotten parts/weight of fruit × 100. There were two runs of the experiment.

Molecular identification

We sequenced the entire ITS rDNA region of the fungus using the primers ITS1: (5′TCCGTAGGTGAACCTGCG G-3′) and ITS4: (5′TCCTCCGCTTATTGATATGC-3′) to confirm the identity of the most virulent *B. cinerea* isolate, Bc-1 (White et al. 1990). The primers were obtained from the Operon Technologies Company, Netherlands. A Bc-1 isolate's fresh mycelium (100 mg) was powdered using liquid nitrogen. According to the manufacturer's instructions, total DNA was extracted using the i-genomic BYF DNA extraction Mini Kit (iNtRON Biotechnology Inc., South Korea). The final 50 µl reaction mixture contained 1 unit Taq DNA polymerase (NEB, England), 1× PCR buffer (NEB, England), 1 nmol of dNTPs, 1 pmol of 2 mM $MgSO_4$, 0.25 pmol of forward and reverse primers, and 10 µl template DNA (Elshahawy et al. 2018). On a T100TM Thermal Cycler (BIO-RAD, Singapore), PCR was conducted using the following protocol: 94 °C for 2 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 70 °C for 30 s, and final extension at 72 °C for 10 min. The amplified products were separated by electrophoresis in 1.0% (w/v) agarose gels with 1X TBE (Tris–borate–EDTA) buffer. One visible DNA product was generated by the PCR amplification; this product was isolated from an EtBr-stained gel and purified in accordance with the manufacturer's instructions using a GeneJETTM PCR Purification Kit (Biotechnology, Seoul, Korea). On an ABI 3730xl DNA sequencer, the purified PCR product was sequenced using a forward primer (GATC Company, Germany). The Basic Local Alignment Search Tool (BLAST) was used to compare the DNA sequence of the

fungal isolate to sequences in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/blast/). Reference taxa located in open databases were compared to the sequence. The evolutionary distance was calculated using the parameter model, and the phylogenetic tree was created using the neighbor-joining technique (Elshahawy et al. 2018).

In vitro effect of HWT on the pathogen

(a) Agar disk assay: Agar disk assay was performed in accordance with Maxin et al. (2012) to ascertain the direct impact of hot water treatment on the viability of *B. cinerea* (isolates Bc-1). The effects of four exposure times (10, 20, 30, and 40 s) and three temperature levels (52, 54, and 56 °C) on pathogen mycelial growth were examined. Mycelial disks (5 mm diameter), from the growing edge of a 7-day-old pathogen grown in a PDA dish, were aseptically transferred into a test tube with 15-ml sterile distilled water. The test tube containing the agar disks was immersed in hot water at the temperatures and exposure times mentioned above. Following the hot water treatments, the disks were dried with sterile filter paper and then seeded centrally into a fresh PDA plate. Mycelial growth was measured seven days after incubation at a temperature of 20 ± 2 °C. A pathogen mycelial disk was subjected to hot water at 25 °C for 10, 20, 30, and 40 s served as a control. The experiment was carried out twice, with ten replicated plates given to each treatment and control. After an incubation period, the growth of agar disks on PDA plates was watched to estimate the pathogen's mycelial growth. Visual observation of the fungus's growth was made. While the symbol "●" indicates that there is no growth "lethal hot water temperature," while the symbol "○" indicates that there is growth "non-lethal hot water temperature."

(b) Spore germination assay: The direct impact of HWT on the conidial viability of *B. cinerea* (isolate Bc-1) was assessed using the spore germination assay described by Gramaje et al. (2010). Four exposure times (10, 20, 30, and 40 s) and three temperature levels (52, 54, and 56 °C) were tested. To induce sporulation, cultures of the Bc-1 isolate grown on PDA media were once daily exposed to a 10-min burst of near-UV light (BLB-20W, max = 365 nm). To create conidial suspension, 10 ml of sterile distilled water containing 0.05% Tween-20 (polyoxyethylene sorbitan monolaureate, Sigma) was poured over the 10-day-old fungal culture in PDA Petri dishes. Then, to combine the fungal spores with the water, the agar surface was gently stirred with a sterile glass rod. The spore suspension was purified through two layers of sterile cheesecloth to get rid of hyphal fragments. A 500-ml stock solution was created with spore concentration set to 10^6 ml^{-1} using a hemocytometer slide. From the stock solution, a 10-ml spore suspension was transferred to a test tube. The test tube containing the spore suspension was submerged in hot water for the aforementioned amount of time and at the aforementioned temperatures. By using a glass rod to evenly distribute 1.0 ml of spore suspension over the top of fresh PDA plates, the spores' germination was measured. A pathogen spore suspension was subjected to hot water at a temperature of 25 °C for 10, 20, 30, and 40 s served as a control. The experiment was carried out twice, with ten replicate tubes given to each treatment. Estimating spore germination was by measuring the growth of germination conidia on PDA plates. While the symbol "●" indicates that there is no spore germination "lethal hot water temperature," while the symbol "○" indicates that there is spore germination "non-lethal hot water temperature."

In vitro effect of SS on the pathogen

(a) Mycelial growth assay: The effect of potassium silicate or sodium silicate on the mycelial growth of *B. cinerea* (isolate Bc-1) was examined according to Youssef and Roberto (2014) on potato dextrose agar (PDA) at 20 ± 2 °C. PDA medium was added in 100-ml portions to 250-ml Erlenmeyer flasks and autoclaved at 121 °C for 20 min. Prior to solidification, silicate salts were added to autoclaved PDA medium containing 0.1% Tween-20 (polyoxyethylene sorbitan monolaureate, Sigma) to increase solubility. To achieve final concentrations of 0.0, 2.0, 4.0, and 6.0%, potassium silicate or sodium silicate was carefully incorporated into PDA medium. These concentrations were selected based on the earlier results (Elshahawy et al. 2021). Each flask was poured into 9-cm diameter sterilized Petri plates and inoculated with disks (5 mm diameter) from cultures of 10-day-old *B. cinerea* (isolates (Bc-1) and then incubated at 20 ± 2 °C. The average mycelial growth (mm) of the pathogen was determined once the control plates were full. Five plates were used as replicas for each treatment.

(b) Spore germination assay: Using the technique outlined by Youssef and Roberto (2014), the impact of potassium or sodium silicates on *B. cinerea* (isolates (Bc-1) spore germination was examined. A 10-ml test tube was filled with 5 ml of the potato dextrose broth (PDB, Difco, Detroit, MI, USA) medium before being sterilized at 121 °C for 20 min. PDB was gently amended with 0.1% Tween-20 after being treated with potassium or sodium silicates to achieve final concentrations of 0.0, 2.0, 4.0, and 6.0%. After adding one ml of spore suspension (10^6 spores/ml) to the test tubes, they were incubated for 24 h on a rotary shaker at 20 ± 2 °C. The percentage of spores that germinated was evaluated under a microscope. The experiment used five replicates (tubes) for each treatment.

In vivo effect of HWT and SSD separately and in combination on gray mold

Apple fruits were harvested early in the morning in a local orchard at the National Research Centre farming, El-Behera governorate, Egypt, and immediately transported to the Laboratory of Plant Pathology. Fruits that were damaged were discarded, and fruits with uniform color, shape, and size were chosen. There are four different categories for apple fruits. Each group was subjected to the subsequent experiment separately.

(a) Testing of HWT on gray mold: The apple fruits in this group were surface sterilized for one minute with 70% ethanol before being washed with sterile water. As previously mentioned in the pathogenicity test, apple fruits were punctured with sterile insulin needles. As previously mentioned, spore suspension inocula of *B. cinerea* (isolate Bc-1) were made. Apple fruits with wounds were inoculated using a *B. cinerea* spore suspension (10^6 spores/ml), which was sprayed using an atomizer at a rate of 200-ml conidial suspension per 100 fruits. Inoculated fruits were exposed to hot water at 54, 56, 58, and 60 °C for varying exposure times of 10, 20, 30, and 40 s twenty-four hours later. Apple fruits that had only been inoculated with *B. cinerea* isolate (Bc-1) were kept as controls. Ten fruits per carton box were packed into carton boxes with both treated and untreated fruits in order to store them for 15 days at 20 ± 2 °C and 90–95% relative humidity. As previously mentioned in the pathogenicity test, the prevalence and severity of the disease were determined. Five boxes (replicates) were used for each treatment.

(b) Testing of SSD on gray mold: The apple fruits in this group were sterilized, punctured, and inoculated with *B. cinerea* (Bc-1) isolates as previously mentioned. Fruits were dipped for 60 s in potassium or sodium silicates at 2.0, 4.0, and 6.0% concentrations before being air dried twenty-four hours later. Apple fruits that had only been inoculated with *B. cinerea* isolate (Bc-1) were kept as controls. Ten fruits per carton box were packed into carton boxes with both treated and untreated fruits in order to store them for 15 days at 20 ± 2 °C and 90–95% relative humidity. The pathogenicity test determined the incidence and severity of the disease, as was previously mentioned. Five boxes (replicates) were used for each treatment.

(c) Testing of HWT combined with SSD on gray mold: The previously mentioned pathogen was injected into wounded apple fruits in this group after they had been surface disinfected. Twenty-four hours later, the following treatments were used: (1) hot water treatment at 60 °C for 30 s, (2) dipping in potassium or sodium silicates at 6.0% for 60 s, (3) dipping in hot water at 60 °C for 30 s followed by dipping in 6.0% potassium or sodium silicates for 60 s, and (4) control (untreated inoculated fruits). Ten fruits per carton box were packed into carton boxes with both treated and untreated fruits in order to store them for 15 days at 20 ± 2 °C and 90–95% relative humidity. As previously stated, in the pathogenicity test, the disease incidence and severity were determined. Five boxes (replicates) were used for each treatment.

(d) Testing of HWT combined with SSD on fruits quality: The apple fruits in this group, as previously mentioned, were only superficially disinfected. The following treatments were used: (1) dipping in hot water at 60 °C for 30 s, (2) dipping in potassium or sodium silicates at 6.0% for 60 s, (3) dipping in hot water at 60 °C for 30 s followed by dipping in potassium or sodium silicates at 6.0% for 60 s, and (4) control (untreated fruits). Ten fruits per carton box were packed into carton boxes with both treated and untreated fruits in order to store them for 40 days at 20 ± 2 °C and 90 95% relative humidity. Apple fruit weight loss and total soluble solids (TSS) were calculated. Each sample box's weight loss during cold storage was measured. The weight loss as a percentage of the starting weight was used to express the results. A handheld refractometer (ATAGO-1; Atago Co. Ltd., Tokyo, Japan) was used to measure the total soluble solids (TSS) in the undiluted filtered juice obtained from 10 apple fruits from each treatment.

Statistical analysis

Every experiment was carried out at least twice. Prior to statistical analysis, the data were checked for normality and variance homogeneity. However, untransformed data were also shown. Percentage data were transformed using an arcsine square-root transformation to improve variance homogeneity. All data were put through an analysis of variance to see if there was a significant difference between the means. To compare the means at $P = 0.05$, CoStat6303 software calculated Duncan's multiple range test were used.

Results

Isolation, pathogenicity test and identification of *B. cinerea*

The casual fungus was isolated by putting small pieces of diseased apple fruit tissue on WA and then on PDA media. Five fungal isolates were obtained as a result of the isolation procedures (Fig. 1). All isolates had conidiophores and conidia that closely resembled those of *B. cinerea* in terms of morphology. All *B. cinerea* isolates (Bc-1, Bc-2, Bc-3, Bc-4, and Bc-5) were pathogenic to apple fruits according to pathogenicity tests, and they also caused gray mold symptoms that were identical to those that occur naturally (Fig. 1). These isolates were re-isolated from diseased lesions of inoculated fruits in order to confirm Koch's postulates.

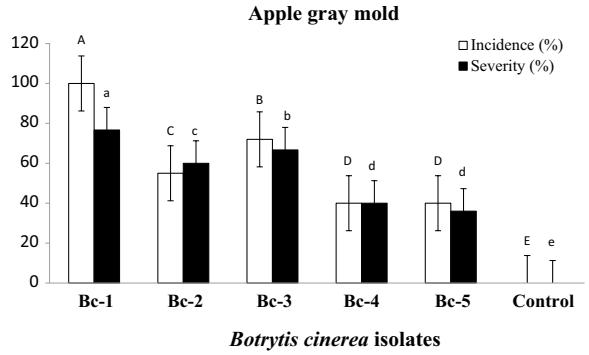

Fig. 1 Gray mold incidence and severity of apple fruits (Anna cultivar) incited by different isolates of *Botrytis cinerea* during pathogenicity test. Values are means of four replications. Disease incidence (%) = (number of decayed fruits ÷ number of total fruits) × 100. The severity of the disease was determined by calculating the weight percentage (g) of the infected part of the fruit relative to the total weight of the fruit. Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P = 0.05$, according to Duncan's multiple range tests. Percentages data were transformed into arcsine square-root transformation for analyses of variance, however, untransformed data are presented

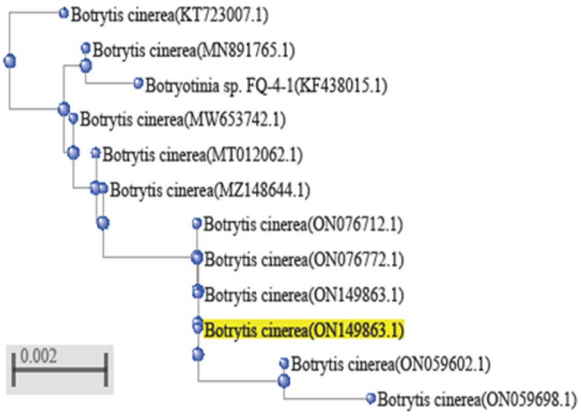

Fig. 2 The phylogenic tree showed convergence between our isolated (yellow color) and Gene bank isolates

The re-isolated isolates from inoculated fruits shared the same morphology as the original isolates. The apple fruits receiving the control treatment exhibited no symptoms (Fig. 1). Apple fruit pathogenicity varies significantly between isolates (Fig. 1). All of the *B. cinerea* isolates used in the study were pathogenic to apple fruits despite variations in disease incidence and severity on fruits. Within 15 days of inoculation, the isolate Bc-1 caused 100% disease incidence and 73.3% severity of gray mold in apples, exhibiting high pathogenicity. Additionally, compared to Bc-1 isolate, disease incidence and severity were significantly lower in Bc-2, Bc-3, Bc-4, and Bc-5 isolates. The Bc-4 and Bc-5 isolates were the least pathogenic when compared to the other isolates and the Bc-1 isolate. Following pathogenicity testing, isolate Bc-1, a representative strain of *B. cinerea*, was selected for molecular analysis, in vitro testing, and in vivo testing. To confirm the identity of the responsible fungus, we amplified and sequenced the ITS rDNA region of isolate Bc-1, the most virulent isolate in pathogenicity tests. DNA sequences were looked up using the BLAST program. The isolate Bc-1 isolated from apple rot showed 100% identity to *B. cinerea* in a BLAST search using the ITS rDNA region sequence, demonstrating that the ITS rDNA region sequence analysis was sufficient for species-level identification. A phylogenetic analysis of the ITS rDNA region sequence assigned the Bc-1 fungus (accession number

Table 1 The in vitro sensitivity of *Botrytis cinerea* to HWT using agar disk and spore germination assays

Hot water treatment	Viability of *B. cinerea*	
	Agar disk assay[a]	Spore germination assay[b]
25 °C/10 s	○	○
25 °C/20 s	○	○
25 °C/30 s	○	○
25 °C/40 s	○	○
52 °C/10 s	○	○
52 °C/20 s	○	○
52 °C/30 s	○	○
52 °C/40 s	○	○
54 °C/10 s	○	●
54 °C/20 s	○	●
54 °C/30 s	●	●
54 °C/40 s	●	●
56 °C/10 s	●	●
56 °C/20 s	●	●
56 °C/30 s	●	●
56 °C/40 s	●	●

[a] Agar disk of the pathogen was subjected to hot water at the desired concentration and exposure time then seeded on PDA medium for 7 days at 20 ± 2 °C

[b] Spore suspension of the pathogen was subjected to hot water at the desired concentration and exposure time then seeded on PDA medium for 7 days at 20 ± 2 °C. In both assays, ten replicated plates were provided for each treatment and the growth of the fungus was determined visually. The symbol "●" indicates no growth "lethal hot water temperature" and the symbol "○" indicates there is a growth "non-lethal hot water temperature"

ON1498639.1) to a clade with the *B. cinerea* group (Fig. 2).

Sensitivity of *B. cinerea* to HWT

(a) In vitro test: In vitro tests were conducted using HWT of 25, 50, 52, 54, and 56 °C and exposure times of 10, 20, 30, and 40 s. Table 1 displays how *B. cinerea* (Bc-1) mycelium growth and spore germination are affected by hot water treatment. The sensitivity of the pathogen to HWT varied with temperature and exposure duration. In general, a combination of hotter water and extended exposure prevented the growth of pathogen mycelium and the germination of spores. The findings demonstrated that at different exposure times of 10, 20, 30, and 40 s, pathogen growth could not be significantly inhibited at lower water temperatures, such as 25 and 52 °C. Agar disks are less sensitive to temperature and exposure time than spore suspension, as shown in Table 1. HWT, however, had no effect on *B. cinerea* (Bc-1)'s agar disks at 54 °C for 10 and 20 s, while it killed it at 54 °C for 30 and 40 s. *B. cinerea* (Bc-1) myclium and spores were susceptible to all exposure times of 10, 20, 30, and 40 s at 56 °C (Fig. 3). *B. cinerea* mycelium and spores had lethal exposure times of 54 °C for 30 s and 54 °C for 10 s, respectively.

(a) In vivo test: Gray mold was tested in vivo using HWT of 54, 56, 58, and 60 °C and exposure times of 10, 20, 30, and 40 s. The incidence and severity of gray mold on apple fruits were significantly reduced across all temperature ranges and exposure durations tested, as shown in Table 2. The most efficient treatments are 60 °C HWT and 30 s exposure time, which had the lowest incidence and severity of gray mold, being 20.0 and 16.0%, respectively, compared to untreated fruits, which had 100.0 and 73.3%, respectively. Other temperatures and exposure durations only had a minor impact.

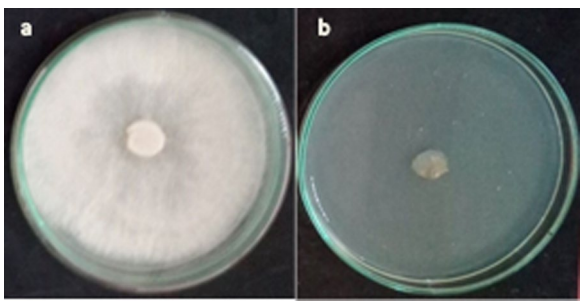

Fig. 3 The in vitro sensitivity of *Botrytis cinerea* to hot water treatment (HWT) using agar disk assay. **a** Untreated agar disk of the pathogen seeded on PDA medium and **b** hot water-treated (56.0 °C for 10 s) agar disk of the pathogen seeded on PDA medium. Inhibition of *Botrytis cinerea* mycelial growth as affected with hot water treatment was evaluated by measuring the colony diameter seven days after incubation at 20 ± 2˚C in the dark. Photographs were taken on the same day

Table 2 The in vivo effect of HWT and exposure times on the incidence and severity of gray mold disease of *Botrytis cinerea*-inoculated apple fruits

Hot water treatment	Gray mold	
	Incidence (%)	Severity (%)
54 °C/10 s	61.0 ± 1.00 b	55.0 ± 0.58 b
54 °C/20 s	58.0 ± 1.00 c	52.0 ± 1.15 b
54 °C/30 s	50.0 ± 1.00 d	44.0 ± 1.00 c
54 °C/40 s	41.0 ± 1.00 f	37.0 ± 0.58 d
56 °C/10 s	51.0 ± 0.58 d	44.0 ± 1.00 c
56 °C/20 s	45.0 ± 0.58 e	38.0 ± 1.00 d
56 °C/30 s	41.0 ± 1.00 f	33.0 ± 0.58 e
56 °C/40 s	34.0 ± 1.53 h	28.0 ± 0.58 f
58 °C/10 s	42.0 ± 1.00 f	37.0 ± 0.58 d
58 °C/20 s	37.0 ± 1.53 g	33.0 ± 1.00 e
58 °C/30 s	27.0 ± 0.58 i	24.0 ± 0.58 g
58 °C/40 s	25.7 ± 0.67 i	21.0 ± 1.00 g
60 °C/10 s	37.0 ± 0.58 g	32.0 ± 1.00 e
60 °C/20 s	32.0 ± 0.58 h	24.0 ± 1.00 g
60 °C/30 s	20.0 ± 0.58 j	16.0 ± 0.58 h
60 °C/40 s	20.0 ± 0.58 j	16.0 ± 0.58 h
Control	100.0 ± 0.0 a	73.3 ± 3.33 a

Values are mean of five replicates for each treatment as well as the control. Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$. Disease incidence (%) = (number of decayed fruits ÷ number of total fruits) × 100. The severity of the disease was determined by calculating the weight percentage (g) of the infected part of the fruit relative to the total weight of the fruit. Percentages data were transformed into arcsine square-root transformation for analyses of variance, however, untransformed data are presented

Sensitivity of *B. cinerea* to SS

(a) In vitro test: *B. cinerea*'s growth and spore germination were subjected to in vitro tests to determine the inhibitory impact of potassium or sodium silicates at concentrations of 0.0, 2.0, 4.0, and 6.0%. The findings in Fig. 4 demonstrate that *B. cinerea* (Bc-1) growth and spore germination are inhibited at all tested concentrations of both treatments. *B. cinerea* mycelial growth and spore germination were completely inhibited by potassium or sodium silicates at 6.0% (Fig. 5). The mycelial growth and spore germination were both significantly inhibited by potassium or sodium silicates at 4.0%, inhibiting them by more than 87.4 & 87.1 and 75.7 & 75.4%, respectively. Other therapies, meanwhile, had a moderate effect.

(a) In vivo test: To test the effects of potassium and sodium silicates on gray mold, apple fruits were dipped

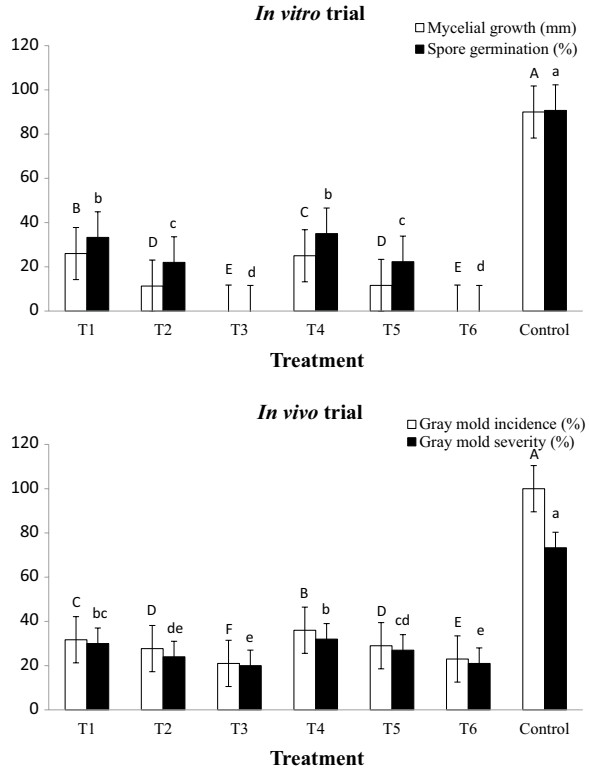

Fig. 4 In vitro and in vivo sensitivity of *B. cinerea* (Bc-1) to silicate salts. In vitro the silicate salts were amended to autoclaved PDA medium before solidification to obtain the desired concentration of 0.0, 2.0, 4.0, and 6.0%. In vivo *B. cinerea*-inoculated fruits were dipped for 60 s in the silicate salts then stored at 20 ± 2 °C for 15 days, which disease incidence and severity were calculated. Disease incidence (%) = (number of decayed fruits ÷ number of total fruits) × 100. The severity of the disease was determined by calculating the weight percentage (g) of the infected part of the fruit relative to the total weight of the fruit. Potassium silicate/2.0% (T1), potassium silicate/4.0% (T2), potassium silicate/6.0% (T3), sodium silicate/2.0% (T4), sodium silicate/4.0% (T5), sodium silicate/6.0% (T6), and untreated control. Values are means of four replications. Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P = 0.05$, according to Duncan's multiple range tests. Percentages data were transformed into arcsine square-root transformation for analyses of variance, however, untransformed data are presented

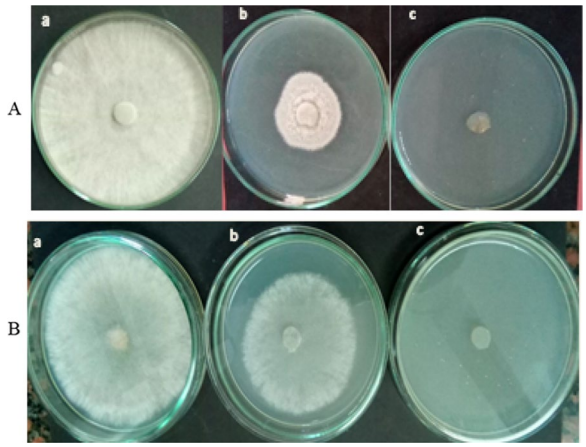

Fig. 5 The in vitro evaluation of silicate salts efficiency against the colony of *Botrytis cinerea* using mycelial growth assay. **A** The potassium silicate was evaluated at a rate of 0.0% (a), 4.0% (b), and 6.0% (c). **B** The sodium silicate was evaluated at a rate of 0.0% (a), 4.0% (b), and 6.0% (c). Inhibition of *Botrytis cinerea* mycelial growth by silicate salts was evaluated by measuring the colony diameter seven days after incubation at 20 ± 2 °C in the dark. Photographs were taken on the same day

in them for 60 s at concentrations of 2.0, 4.0, and 6.0% (Fig. 4). Results indicate that every treatment tried successfully suppressed gray mold. With potassium and sodium silicates dipping at 6.0% for 60 s, the highest suppression was attained, which decreased the incidence of gray mold by 79.0 and 77.0% as well as the severity of the disease by 80.0 and 77.0%, respectively. Potassium and sodium silicates at 4% followed this treatment statistically. The least impact was produced by silicate salts at a concentration of 2.0%.

Integration effect of HWT and SSD

(a) Effects on gray mold: To prevent gray mold, apple fruits were immersed in hot water at 60 °C for 30 s and/or potassium or sodium silicates at 6.0% for 60 s, either individually or in combination. Figure 6 findings demonstrate that every treatment tested significantly decreased the gray mold on apple fruits. The promising treatments reduced the incidence of gray mold by 90.0 and 88.0%, respectively, and the severity of the disease by 93.0 and 92.0%, using hot water at 60 °C for 30 s followed by potassium or sodium silicates at 6.0% for 60 s (Figs. 6 and 7). Single therapy, meanwhile, had a moderate effect.

(b) Effects on apple fruit quality: Apple fruits were dipped in hot water at 60 °C for 30 s and/or dipped in potassium or sodium silicates at 6.0% for 60 s to study their effect on apple fruit quality, *i.e.*, fruit weight loss percentage and total soluble solids percentage (TSS). The outcomes in Fig. 6 demonstrate that all of the tested treatments had a favorable impact on fruit quality. The promising treatments involved combine hot water at 60 °C for 30 s followed by dipping in potassium or sodium silicates at 6.0% for 60 s. This combination significantly decreased fruit weight loss percentage when compared to untreated fruits. Total soluble solids percentage (TSS) was not significantly different between treatments, however.

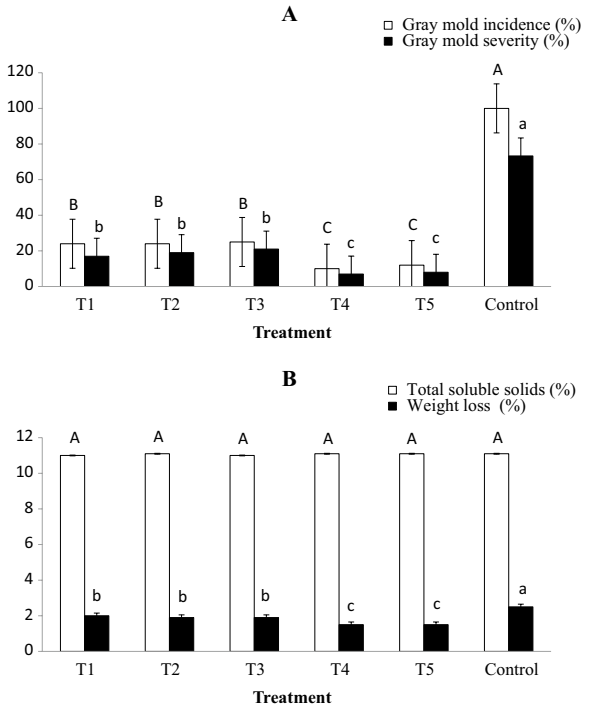

Fig. 6 In vivo, integration effect of HWT with SSD. **A** against gray mold of apple fruits inoculated with the fungus *Botrytis cinerea* and **B** on quality, i.e., total soluble solids and weight loss (%) of non-inoculated apple fruits stored for 40 days at 20 ± 2 °C. Hot water 60 °C/30 s (T1), potassium silicate/6%/60 s (T2), sodium silicate/6%/60 s (T3), hot water 60 °C/30 s/potassium silicate/6%/60 s (T4), hot water 60 °C/30 s/sodium silicate/6%/60 s (T5), and untreated control. Values are means of four replications. Bars with the same letters within each variable indicate that the means ± standard errors are not significantly different at $P = 0.05$, according to Duncan's multiple range tests. Percentages data were transformed into arcsine square-root transformation for analyses of variance, however, untransformed data are presented

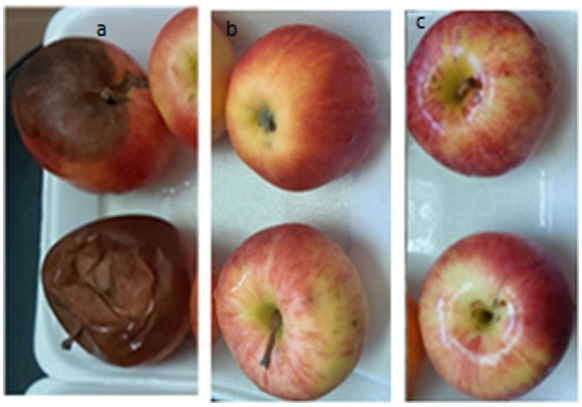

Fig. 7 In vivo, integration effect of HWT with SSD against gray mold of apple fruits inoculated with the fungus *Botrytis cinerea*. **a** Untreated fruits (control), **b** fruits treated with hot water (60 °C/30 s) followed by dipping for 60 s in potassium silicate (6.0%), and **c** fruits treated with hot water (60 °C/30 s) followed by dipping for 60 s sodium silicate (6.0%)

Discussion

Five fungi, designated Bc-1 through Bc-5, were found in apple fruits that were showing signs of gray mold in the current study. Based on morphological characteristics, all isolates belonged to *B. cinerea* (Jakobija et al. 2020). To evaluate the virulence of different *B. cinerea* isolates, pathogenicity tests on apple fruits were conducted. All isolates were able to infect apple fruits under artificial inoculation conditions, but there were variations in disease incidence and severity (Fig. 1). *B. cinerea* isolate Bc-1 showed better infection behavior overall. This may be caused by the presence or absence of genes with high virulence, such as the gene cluster responsible for the biosynthesis of the phytotoxin botcinic acid (Plesken et al. 2021). Botrydial (BOT) and botcinic acid (BOA), the two phytotoxins that *Botrytis cinerea* produces, have been shown to be necessary for full virulence (Breen et al. 2022). We chose to use the PCR molecular method to confirm the identification of the isolate because it was found to have high virulence. This isolate belonged to the genus Botrytis and shared 100% of its genetic makeup with *B. cinerea*, according to internal transcribed spacer (ITS) analysis. The most prevalent postharvest pathogen of apples is a gray mold fungus called *Botrytis cinerea*. The outcomes obtained concur with those of Sun et al. (2019). They found that one of the main pathogens causing apple gray mold is *B. cinerea*.

Potassium silicate and sodium silicate completely stopped *B. cinerea*'s growth at a dose of 6.0%. The results showed that postharvest applications of silicate salt were very effective at preventing apple gray mold disease. The best treatments involve dipping apple fruits in potassium or sodium silicates at a 6.0% concentration for 60 s (Fig. 4). According to Li et al. (2009), sodium silicate at 100 and 200 mM strongly inhibited spore germination and mycelial growth of *Fusarium sulphureum*, the cause of potato tuber dry rot. According to Khan et al. (2013), *Macrophomina phaseolina* growth could be effectively inhibited in vitro by silicon in the form of sodium silicate. Youssef and Roberto (2014) found that sodium silicate completely prevented *B. cinerea* mycelial growth and spore germination at a concentration of 0.25%. Additionally, they claimed that using this salt could greatly lower the occurrence of gray mold. According to Lopes et al. (2014), potassium silicate has a suppressive effect on the postharvest gray mold of strawberries caused by *B. cinerea*. Applying potassium silicate to apple fruit wounds significantly decreased the occurrence of gray and blue molds, according to Mbili et al. (2020a). According to Rayon-Diaz et al. (2021), sodium silicate prevented *Colletotrichum gloeosporioides* from growing.

In vitro trails conducted in the present study show the effectiveness of hot water treatment (HWT) against *B. cinerea* (Bc-1). In general, conidial germination and colony growth were inhibited by increased temperature and time combinations. Conidia were more sensitive to HWT than mycelium. The myclium and spores of *B. cinerea* (Bc-1) could be killed by all exposure times of 10, 20, 30, and 40 s at 56 °C (Fig. 3). The incidence and severity of gray mold in apple fruits inoculated with *B. cinerea* were significantly decreased in in vivo tests by HWT of 54, 56, 58, and 60 °C, as well as exposure times of 10, 20, 30, and 40 s. The best treatments used HWT at 60 °C for 30 s of exposure time. This demonstrated a close relationship between control effectiveness and HWT time. Similar findings were reported by Zhang et al. (2008), who found that HWT at 46 °C for 15 min. was more effective than HWT at 5 and 10 min. against *P. expansum* in pear fruit. According to Lichter et al. (2003), a lethal heat dose of 46.3 °C for 2 min killed *Botrytis cinerea* spores. In a related in vitro experiment, Bleach et al. (2009) discovered that HWT at 40–50 °C for 15–30 min inhibited conidial germination, whereas temperature above 52–53 °C was required to significantly reduce the mycelial growth of *Cylindrocarpon liriodendri*, *C. macrodidymum*, and *C. destructans*. For both *C. macrodidymum* and *C. liriodendri* isolates, Gramaje et al. (2010) found that temperature above 45 °C inhibited conidial germination, while temperatures above 48 °C were necessary to inhibit mycelial growth. It has long been known that *P. expansum* and *Botrytis cinerea* on apples can be successfully eradicated with hot water (Maxin et al. 2012). The in vivo findings from this study are in line with those from Alvindia (2012), who found that HWT lessened the severity of banana fruit crown rot by 50% after 7 days and by 33% after 14 days. According to Maxin et al. (2012), HWT of just-picked fruits for three minutes at 50–52 °C had high efficacies against the majority of apple storage rots. Li et al. (2013) found that HWT of papaya fruit (54 °C, 4 min) significantly decreased the carrier rate of *Colletotrichum gloeosporioides* in fruit peel and significantly decreased the incidence of anthracnose and stem-end rot.

HWT may act in at least two ways: (1) directly and fatally on the fungal inoculum inside or outside the apple; and (2) indirectly through the physiological response to stress that the fruit exposed. Spores from the fruit surface are eliminated, and fungal spores and mycelia are inactivated by hot water treatment (Hojnik et al. 2019). In addition, HWT inhibited the growth of fungal pathogens in inoculation experiments even when the fruit was inoculated soon after the heat shock (Maxin et al. 2012; Li et al. 2021). Various types of fruit have been shown to produce heat-shock or pathogenesis-related proteins in response to heat treatments, supporting the latter point (Li et al. 2021). HWT may have controlled fruit pathogens because it inhibited ethylene production and respiration rate, according to Li et al. (2013). Other researchers, on the other hand, reported that the primary factor determining the efficacy of HWTs against apple postharvest rots must be a fruit property rather than a direct heat effect on the fungus (Maxin et al. 2012). According to research on citrus fruits (Fallik 2004), HWTs trigger a physiological response that involves rapid transcription and translation of heat-shock proteins (HSPs). These may have immediate physiological functions in response to the stress factor that causes their synthesis (Li et al. 2021). Pathogenesis-related proteins (PRPs), such as chitinases or β-1,3-glucanases, are known to be present in a subset of HSPs (Li et al. 2021). It has also been demonstrated that heat shock raises the concentrations of small molecules with antifungal activity (phytoalexins) in injured citrus fruit (Lade et al. 2022). Additionally, heated apple peel has been used to extract antifungal compounds with low molecular weight, but not from untreated fruit (Lade et al. 2022).

Due to the best efficacy of HWT at 60 °C for 30 s, it was chosen to control postharvest diseases of apple fruit combined with silicate salts dipping. For controlling gray mold in artificially inoculated fruits, HWT combined with 60 s of dipping in potassium or sodium silicates at 6.0% was more effective than either HWT or silicate salts dipping alone (Figs. 6 and 7). Similar findings were obtained in the effect of treatments on fruit quality (TSS and weight loss) under natural infection, where the combination of silicate salts and HWT also demonstrated the greatest efficacy (Fig. 6). According to this, HWT might increase the silicate salts' impact on apple fruits. These results confirmed earlier research, showing that silicon and hot water treatment worked better together than they did separately to control *P. expansum* on apple fruits (Etebarian et al. 2013). The interactions between HWT, silicate salts, and fruits could all contribute to the synergistic effect. Other combinations have also shown this synergistic effect (Rayon-Diaz et al. 2021). They discovered that the additive effects of sodium silicate and chitosan reduced the growth of the *C. gloeosporioides* mycelium by 51–99%. Mbili et al. (2020b) discovered that combining potassium silicate and yeast antagonists resulted in 95 and 77.5% control of *B. cinerea* and *P. expansum*, respectively, in 'Golden Delicious' apples, compared to 62.5 and 65% control on Imazalil® fungicide treated fruits. It is unclear how silicon might prevent gray mold growth on apples. Li et al. (2009) reported that scanning electron microscopy revealed morphological changes in *Fusarium sulphureum* hyphae treated with sodium silicate, including mycelium

sparsity and asymmetry, hyphal swelling, curling, and cupped shape. They added that transmission electron microscopy revealed ultrastructural alterations such as thickening of hyphal cell walls, cell distortion, cavity, or electron-dense material in hyphal cells. According to Qin and Tian (2005), Si caused biochemical defense reactions (polyphenoloxidase, peroxidase, and phenylalanine ammonia-lyase) in the fruit of sweet cherries. According to a theory, Si treatment of host plants increases their resistance to pathogenic fungi because Si $(OH)_4$ specifically accumulates and polymerizes in cell walls, which increases host tissue resistance to pathogen penetration (Wang et al. 2017).

HWT at 60 °C for 30 s in combination with SSD significantly decreased weight loss in non-inoculated apple fruits, improving apple fruit quality. This outcome is consistent with earlier research, in which HWT found no appreciable variations in weight losses (Chiabrando et al. 2017). Inhibition of ethylene synthesis and cell wall hydrolytic enzymes like polygalacturonase and pectin methylesterase may be the cause of this (Zhao et al. 2008). Li et al. (2013) found that HWT inhibited ethylene production and respiration rate, which may be what caused the physiological change in the fruit. According to some studies, decreased activity of 1-aminocyclopropane-1-carboxylic acidsynthase (ACS) and ACC oxidase could be the cause of the reduced ethylene production in HWT-fruit (Houben and Van de Poel 2019). HWTs have been demonstrated by Elizabeth et al. (2022) to lessen ethylene production and postpone fruit ripening. Amiri and Bompeix (2011) discovered that the duration of antifungal protection after heat treatment was approximately 3–4 months against *P. expansum* infections. According to Li et al. (2013), HWT (54 °C, 4 min) of papaya fruit effectively delayed fruit softening while slightly promoting fruit coloring. According to Li et al. (2013), the water soluble pectin (WSP) content increase was postponed in HWT-fruit, which could explain why fruit softening was inhibited. Postharvest HWT on rock melon fruit increased shelf life, according to Abubakar et al. (2020), by minimizing weight loss, maintaining firmness, preventing sucrose damage, retaining rind size, maintaining fruit appearance, and lowering fungal infection.

Conclusions

Five isolates of *Botrytis cinerea* were isolated from apple fruits displaying postharvest decay symptoms in the current study. The most dangerous isolate was molecularly determined to be 100% identical to *B. cinerea* and was then added to the Gene Bank with the accession number ON1498639.1. The use of HWT and SSD alone or in combination for the control of gray mold on 'Anna cv.' apples was investigated. The results showed that using HWT (60 °C/30 s) followed by potassium silicate or sodium silicate dipping (6.0%/60 s), which are safe for consumers and the environment, the incidence of gray mold caused by *B. cinerea* on apple fruits (Anna cv) can be significantly reduced. This combination preserves the postharvest quality of apple fruits for 40 days at 20 ± 2 °C by reducing weight loss.

Abbreviations

HWT	Hot water treatment
SS	Silicate salts
SSD	Silicate salts dipping
WA	Water agar medium
PDA	Potato dextrose agar medium

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Author contributions

IE, NS, and FA participated in the planning and designing of the experiments. IE provided the molecular identification of the causal organism. IE, NS, and FA participated in writing and revising of the paper scientifically, and checking analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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