El-Beltagi HS *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 2, Article number 13783
DOI:10.15835/nbha52213783
Research Article

# Controlling *Botrytis* gray mold in strawberry fruit by bioactive protein isolated from kidney bean

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## Abstract

Due to the risks they pose to human and environmental health, there has been a strong push in recent times to reduce the use of chemical fungicides in phytopathogen treatment. In the present study, vicilin was isolated from kidney bean seeds and characterized by SDS-PAGE, zeta potential, and pH solubility curve. The $\alpha'$ (85 KDa), α (70 KDa), and β (60 KDa) subunits were represented by three distinct protein bands in vicilin. The linear growth of *B. cinerea* was clearly reduced by 12.96%, 14.81%, 25.92%, 35.18%, and 40.73% in response to vicilin application at 50, 100, 200, 300, and 400 µg/ml, respectively. The scanning electron microscopy (SEM) of vicilin-exposed *B. cinerea* revealed hyphae enlargement and conidia distortion. Addition of vicilin from kidney bean seeds clearly reduced the disease incidence in a concentration-dependent manner (100, 200, and 400 µg/mL). The higher doses (400 µg/mL) of vicilin provided higher activity in decreasing the disease severity of the strawberry fruits. As a sustainable glycoprotein, vicilin, found in kidney bean seeds, can be used to combat postharvest fungal infections.

***Keywords:*** *Botrytis cinerea*; gray mold; postharvest; strawberry; vicilin

*Received: 15 Mar 2024. Received in revised form: 30 Apr 2024. Accepted: 21 May 2024. Published online: 22 May 2024.*
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

## Introduction

Concerns about food loss and food security are paramount on a global scale. There is either contamination or missing ingredients in over a third of the world's food supply (Themen, 2014). The main reason behind the considerable loss of food is postharvest diseases (Godana *et al.,* 2020). Post-harvest diseases cause dangerous damage to foodstuffs and perishables during transport and storage. Perishable foods with high water content, nutrient composition, and pH provide suitable environments for various microorganisms to thrive. Due to their acidic pH levels, fungi are the primary cause of fruit spoilage. Fungi can cause the rotting and contamination of fruits by producing mycotoxins (Moss, 2002). Worldwide post-harvest loss of perishables due to fungi is between 10% and 50% (Singh *et al.,* 2021). There are several different fungal species that can cause postharvest infections, with Botrytis being the most well-known and dangerous one (Sardella *et al.,* 2016). Botrytis species, especially *Botrytis cinerea*, which can live in harsh environments, can severely reduce the output of commercially important horticultural plants because it can attack a wide range of hosts and use a variety of attack methods (Fillinger and Elad, 2016). The widespread disease *Botrytis cinerea* has an impact on more than 200 plant hosts, including strawberries (Bui *et al.,* 2019). The most popular berries are strawberries (*Fragaria* × *ananassa* Duch.), which are known for their distinct flavour and rich concentration of phytochemicals (Oszmiański and Wojdyło, 2009). Due to their high rate of respiration and physical attributes, strawberry fruits are extremely vulnerable to postharvest storage. Additionally, they are extremely vulnerable to phytopathogens, with *B. cinerea* being the primary infection-causing agent (Caleb *et al.,* 2016; El-Beltagi *et al.,* 2022).

Preharvest chemical management of early inoculation density is used to protect horticulture plants after harvest. Botrytis spp. diseases that occur post-harvest remain a common issue during storage (Droby and Lichter, 2007; Maryam 2022). To control post-harvest infections and stop the accompanying rots, artificial chemical fungicides are still widely used. However, the establishment of precise regulatory legislation for their use is a direct result of the increased focus on food safety and human health (Afify *et al.,* 2012; Liu *et al.,* 2013). Additionally, many of these manmade poisons evolve into ineffectual forms as new physiological races of pathogens emerge (Spotts and Cervantes, 1986; Afify *et al.,* 2011) requiring the search for new antifungal substances derived from natural sources. Within this pattern, isolation of soy seed glycoproteins led to the discovery and confirmation of 7S globulin as a potent fungal inhibitor (Osman *et al.,* 2016a). This compound effectively halted the formation of green mold on orange fruit after harvest and maintained the lowest levels of fungal infection, disease incidence, and severity throughout a 21-day treatment period (Osman *et al.,* 2016a). After harvesting figs, conducting *in-situ* research with chickpea 7S globulin (vicilin) as a postharvest treatment can potentially reduce the severity of *Alternaria tenuissima* infection by about 73% compared to the positive control, thus increasing the fruit's shelf life (Abbas *et al.,* 2020a). Because their protein compositions are so similar, it stands to reason those soybeans and other legume, such as chickpeas, cowpeas, and peas, serve similar purposes ( Sitohy *et al.,* 2012; Osman *et al.,* 2014b; Abdel-Shafi *et al.,* 2019a). The abundance of kidney bean protein, along with its minimal allergenicity, high nutritional value, and affordable production costs, make it a popular ingredient in the development of novel food products. Composition-wide, the majority of kidney bean protein is made up of water- and salt-soluble albumins (2S, 10-20%), as well as the vicilin (7S) and legumin (11S) globulins. The two main fractions of kidney bean protein isolates, 11S and 7S, are determined by their sedimentation coefficients (Hirano, 2021). Legumin and vicilin have been structurally described as important storage proteins ( Tzitzikas *et al.,* 2006; Klassen and Nickerson, 2012). Taking all these things into account, an experiment was made to establish a new safety strategy to counteract fungal post-harvest infections through infesting and testing the potentiality of 7S globulin of kidney bean as an environmentally friendly bioproduct in extending the shelf life of strawberries by preventing *Botrytis cinerea* fungal rotting. When these efficient, environmentally friendly antifungal agents succeed in reaching a technology for preserving the strawberry that

was identified by the current study, this can extend to many other fruits. This could motivate researchers to produce natural compounds with similar technological outputs.

## Materials and Methods

*Vicilin isolation and characterization*

The seeds of kidney beans ('Cream 7') were bought at a nearby marketplace. A laboratory mill was used to dehull the kidney bean seeds and grind them into flour. The flour was defatted for 8 hours with Chloroform: methanol 3: 1. The solvent was evaporated using a rotary evaporator, while the defatted, desiccated meal was stored in hermetically sealed plastic containers at an approximate temperature of 5 °C until analysis. Vicilin was extracted using the methodology outlined by Abdel-Shafi *et al*., (2019a) from the defatted, desiccated meal. The defatted dried meal (5%, w/v) was blended with buffer (0.03 mol/L tris hydrochloric acid, 0.4M sodium chloride, 10 mM β--mercaptoethanol, 1mM EDTA, 0.02% NaN3) at pH 8.5, stirred for 1h at 25 °C, and centrifuged at 10000 × g for 15 min. At 45 °C, the supernatant was agitated for one hour. Then, using ammonium sulphate (50-65%) vicilin was precipitated. The precipitate was dispersed in a small amount of the same buffer, dialyzed overnight, and lyophilized.

SDS-PAGE for vicilin isolated from kidney bean seeds was applied according to (Laemmli, 1970). Twenty mg of vicilin was dissolved in 1 ml of SDS 10% for 10 min and centrifuged for 15 min at 10,000 × g. Twenty µl of the supernatant was combined with 60 µl of loading sample buffer, and 10 µl was loaded per lane. By using the procedure provided by Sitohy and Osman (2010), Using protein pH solubility curves at various pH ranges between 2 and 10, the isoelectric point was calculated.

Zeta potential of vicilin (0.2%, w/w; pH 7; room temperature) was measured as described in (Elimelech *et al.,* 1994) by using a Delsa Nano C Instrument (Malvern Instruments, Westborough, MA).

*The causal organism isolation and identification*

*Botrytis cinerea* was isolated from a strawberry fruit that exhibited grey mold symptoms. After analyzing its cultural and microscopic characteristics, the fungus was identified as *B. cinerea*.

*In vitro antifungal bioassay*

The impact of different concentrations (0, 50, 100, 200, 300, and 400 µg/ml) of vicilin, extracted from kidney beans, on the linear growth of *Botrytis cinerea* was examined using a potato dextrose agar (PDA) medium. The Petri plates were incubated at 25 °C until fungal growth covered the control plates, and colony diameters were measured every day. The following equation was used to calculate linear growth.

$$Linear\ growth\ reduction\ (\%) = ((Control\ growth - Treatment\ growth))/(Control\ growth\ )\ x\ 100$$

*Scanning electron microscopy (SEM)*

SEM examination was conducted on pathogenic fungi treated with vicilin at 400 µg/mL and compared to the control based on the method described by Sitohy et al. (2013).

*Tests conducted in the fruit after harvest*

*In-situ* antifungal activity

The strawberry ('Camarosa') fruit from a local market in Zagazig City, Egypt. The postharvest changes were evaluated by visually selecting strawberries that were uniform in size, shape, color, firmness, absence of mechanical damage, and fungal infection. Strawberries were cleaned for one min with sodium hypochlorite at 1%. The fruit was then rinsed three times with sterile distilled water and dried for 20 to 25 min on sterile drying

papers. Before being used to inoculate the fresh strawberry fruit, fresh cultures on PDA plates were made using *Botrytis cinerea* and stored on PDA at 4 °C (four groups of ten fruit each, in three replicates). With the use of a sterile pipet tip (10 L), each fruit was given a single, deep (1 mm) wound used a sterile pipette tip (10 µL). Each strawberry fruit wound was filled with 20 microliters of vicillin solutions (100, 200, or 400 g/mL) or sterilised distilled water. Following that, each wound was then injected with 10 µl of a conidial suspension of *B. cinerea* isolates at a concentration of $1.0 \times 10^6$ conidia/ml. Following inoculation, the fruit (four groups of 15 fruits each, in three replicates) were sealed in 5-1 plastic boxes with polyethylene linings and incubated for 10 days at 25 °C. On the positive control (PC), the identical process was used, but distilled water was used in place of vicillin. The fruit in the negative control (NC) was similarly treated, but neither the fungus nor vicillin were present. To determine the disease incidence, the shape and width of any lesions were noted after 5, and 10 days of immunisation.

$$Disease\ incidence\ (\%) = (No.of\ fruits\ with\ lesions\ /\ Total\ no.of\ treated\ fruits)\ x\ 100$$

Based on the location of the lesion on each fruit, the severity of the disease was determined using the formula below:

$$Disease\ severity\ (\%) = (Infected\ tissue\ area/\ Total\ tissue\ area)\ x\ 100$$

*Analysis of fruit quality*

In addition to measuring disease severity and incidence, we monitored weight loss (%), pH, anthocyanin, firmness, total soluble solids, and citric acid content every five days for ten days.

At 0, 5, and 10 days after storage, the treated and untreated strawberries were weighed using an analytical balance that had a precision of 0.001 g. There were ten strawberries utilised for each condition. The weight loss percentage of the fruit sample was calculated based on its initial weight on day 0.

$$Weight\ loss\ (\%) = \frac{(Weight\ of\ fruit\ sample\ at\ day\ 0 - Weight\ of\ fruit\ sample\ at\ day\ t)}{Weight\ of\ fruit\ sample\ at\ day\ 0} x100$$

The juice's total soluble solids (TSS) were measured at 20 °C using a handheld refractometer (Atago, Tokyo, Japan). The pH of the fruit juice was then determined using a pH metre (pH 211 Hanna Instruments Inc., Nusfalau, Romania). In order to investigate the firmness of strawberry fruit a puncture test was performed with a penetrometer machine (Prob diameter, 3.5 mm). The pH differential method was used to quantify the anthocyanin levels of strawberries in mg / 100 gm of fresh weight (Kırca *et al.,* 2007). Expression of titratable acidity (TA) as grammes of citric acid Each treatment's 100 $g^{-1}$ (fresh weight) was calculated by titrating 10 g of the pulp with 10 mL of water and 0.1 mol $L^{-1}$ of sodium hydroxide.

*Statistical analysis*

The experimental design consisted of a factorial layout in a completely randomized design (CRD) with three replicates. All the data underwent ANOVA analysis for a randomized design. Tukey's range test was used to detect differences among cultivars, concentrations, and their interactions ($P \leq 0.05$).

## Results

*Vicilin chemical characterization*

Vicilin isolated from kidney bean was subjected to SDS-PAGE electrophoresis, which revealed three protein bands corresponding to α/ (85 KDa), α (70 KDa), and β (60 KDa) subunits (Figure 1A). The pH solubility curve of vicilin showed the least soluble point at pH 5.8, which was used to estimate its isoelectric

point (Figure 1B). The ζ-potential of vicilin is presented in Figure 1C. The surface charge is a crucial molecular characteristic. The ζ-potential of vicilin displayed a net negative value (-17.4 mV).

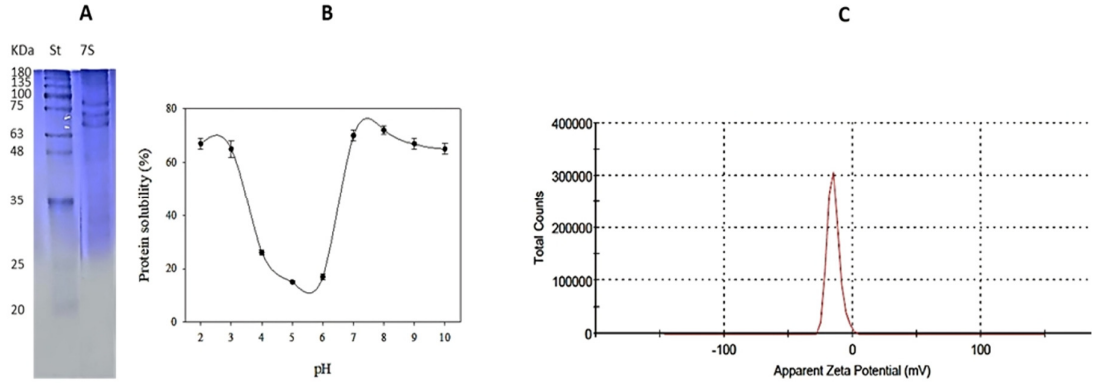


**Figure 1.** SDS-PAGE (A) and pH- solubility curve (B), and zeta potential (C) of vicilin (7S globulin) isolated from kidney bean

*Antifungal activity against B. cinerea*

The study evaluated the antifungal activity of kidney bean vicilin against *B. cinerea* at different concentrations, and the results are presented in Table 1 and Figure 2. The presence of vicilin reduced the mycelial growth of *B. cinerea* in a concentration-dependent manner. The *B. cinerea* fungal growth reduced by 12.96%, 14.81%, 25.92%, 35.18 and 40.73%, respectively, responding to 7S globulin at 50, 100, 200, 300 and 400 µg/mL.

**Table 1.** Linear growth (Cm) and growth reduction (%) of *Botrytis cinerea* grown on solid agar medium after 7 days at 25 °C in the presence of vicilin at various doses (0, 50, 100, 200, 300, and 400 µg/mL)

| Treatment | Linear growth (Cm) | Growth reduction (%) |
|---|---|---|
| 0 | 9.00±0.23 [a] | 0.00±0.00 [d] |
| 50 | 7.83±0.18 [b] | 12.96±0.32 [c] |
| 100 | 7.67±0.14 [b] | 14.81±0.37 [c] |
| 200 | 6.67±0.11 [c] | 25.92±0.45 [b] |
| 300 | 5.83±0.013 [d] | 35.18±0.48 [a] |
| 400 | 5.33±0.09 [d] | 40.73±0.76 [a] |

Means followed different letters are significantly different according to Tukey's HSD test (P ≤ 0.001).

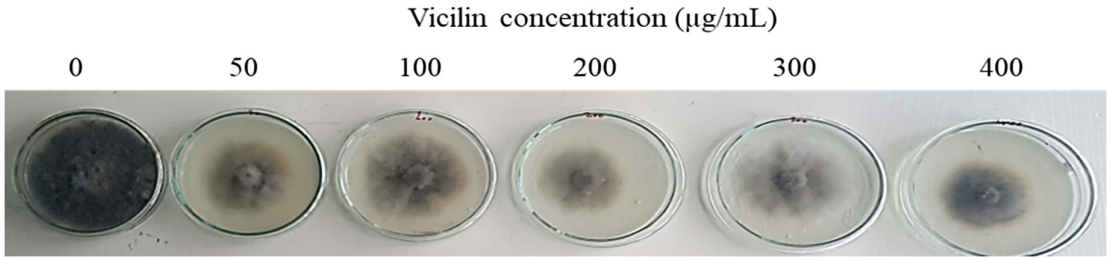


**Figure 2.** *Botrytis cinerea* fungal growth on solid agar medium after 7 days of incubation at 25 °C with vicilin present at various concentrations (0, 50, 100, 200, 300, and 400 µg/mL)

SEM images of *Botrytis cinerea* after exposure to kidney bean vicilin (400 µg/mL) are displayed in Figure 3. The normal untreated fungal exhibited typical hyphae with seemingly unbroken walls. The untreated fungal conidia of *B. cinerea* appeared regular and pear-shaped in the SEM image. Treatment with vicilin has significantly impacted the anatomical features of both fungal hyphae and conidia, fully destabilizing and deforming this shape at (400 µg/mL).

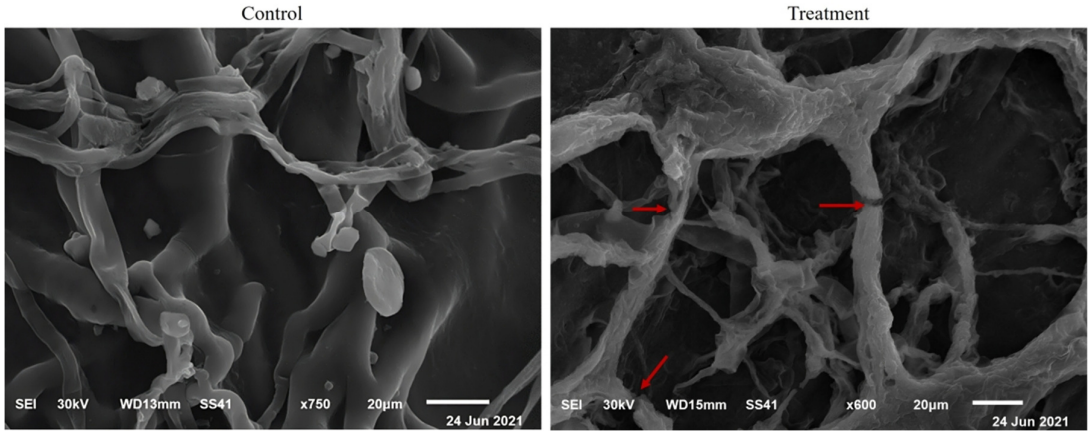


**Figure 3.** Scanning electron microscopy (SEM) showing the effect of vicilin (400 µg/mL) on *Botrytis cinerea* compared to control (untreated sample). Red arrows refer to cell wall deformation and broken

*In situ antifungal activity*

The effects of vicilin on grey mold incidence and disease severity in strawberry fruits

Table 2 and Figure 4 shows how vicilin affects the prevalence of disease in strawberries that have *B. cinerea* infection. The fruits with artificial infection (positive control) had the highest rates of grey mold rot over time, followed by the fruits with natural infection (negative control). However, the presence of vicilin clearly decreased the prevalence of the disease in a concentration-dependent manner. After 10 day of storage, incidence rates in the positive control were 100 %, compared with 74.33 % on fruit treated with vicilin (400 µg/mL).

**Table 2.** Effect of vicilin at different concentrations (100, 200, and 400 µg/mL) on gray mold incidence (%) and disease severity (%) on strawberry fruits before inoculation with *Botrytis cinerea* (10 µL of 1×106 conidia/mL) as compared with negative and positive controls (NC and PC, respectively)

| Treatment | Storage time (day) | Disease incidence (%) | Disease severity (%) |
|---|---|---|---|
| Negative control | 0 | 0.00±0.00 $^{j}$ | 0.00±0.00 $^{g}$ |
| | 5 | 26.67±0.56 $^{h}$ | 25.92±0.43 $^{f}$ |
| | 10 | 93.33±0.89 $^{b}$ | 75.33±0.95 $^{b}$ |
| Positive control | 0 | 0.00±0.00 $^{j}$ | 0.00±0.00 $^{g}$ |
| | 5 | 41.33±0.48 $^{f}$ | 51.71±0.53 $^{c}$ |
| | 10 | 100.00±0.98 $^{a}$ | 100.00±0.88 $^{a}$ |
| Vicilin (100 µg/mL) | 0 | 0.00±0.00 $^{j}$ | 0.00±0.00 $^{g}$ |
| | 5 | 36.33±0.32 $^{g}$ | 46.68±0.37 $^{cd}$ |
| | 10 | 86.33±0.78 $^{c}$ | 53.38±0.67 $^{c}$ |
| Vicilin (200 µg/mL) | 0 | 0.00±0.00 $^{j}$ | 0.00±0.00 $^{g}$ |
| | 5 | 22.67±0.21 $^{h}$ | 39.28±0.41 $^{de}$ |
| | 10 | 81.67±0.64 $^{d}$ | 45.31±0.44 $^{cd}$ |
| Vicilin (400 µg/mL) | 0 | 0.00±0.00 $^{j}$ | 0.00±0.00 $^{g}$ |
| | 5 | 17.67±0.17 $^{i}$ | 32.67±0.43 $^{ef}$ |
| | 10 | 74.33±0.63 $^{e}$ | 41.00±0.64 $^{de}$ |

Means followed different letters are significantly different according to Tukey's HSD test (P ≤ 0.05).


**Figure 4.** Effect of vicilin treatment on *B. cinerea*-infected strawberries

The strawberries were treated with vicilin at different dosages (100, 200, and 400 µg/mL) and kept at room temperature (temperature of 25 ± 5 °C; relative humidity 50-70%) for 5 and 10 days. The study included a control group that received no treatment (0 µg/mL)

**Table 3.** Effect of vicilin at different concentrations (100, 200, and 400 µg/mL) on the citric acid (%), pH, total soluble solids (%TSS), firmness (N), anthocyanin content (mg/ 100g FW), and weight loss (%) in strawberry fruits inoculated with *Botrytis cinerea* (10 µL of 1×106 conidia/mL) as compared with negative and positive controls (NC and PC, respectively)

| Treatment | Storage time (day) | Citric acid (%) | pH | TSS (%) | Firmness (N) | Anthocyanin (mg/100 g FW) | Weight loss (%) |
|---|---|---|---|---|---|---|---|
| Negative control | 0 | 1.23±0.023 $^{a}$ | 3.43±0.084 $^{a}$ | 8.00±0.21 $^{a}$ | 116.67±2.24 $^{a}$ | 244.33±3.33 $^{a}$ | 0.00±0.00 $^{h}$ |
| | 5 | 0.83±0.012 $^{bc}$ | 3.33±0.091 $^{ab}$ | 5.00±0.12 $^{f}$ | 36.67±0.56 $^{bcd}$ | 195.67±2.43 $^{b}$ | 2.43±0.043 $^{fg}$ |
| | 10 | 0.63±0.011 $^{ef}$ | 3.00±0.082 $^{c}$ | 5.20±0.10 $^{ef}$ | 0.00±0.00 $^{d}$ | 165.00±1.78 $^{cd}$ | 5.57±0.11 $^{c}$ |
| Positive control | 0 | 1.23±0.018 $^{a}$ | 3.43±0.067 $^{a}$ | 8.00±0.32 $^{a}$ | 116.67±3.21 $^{a}$ | 244.33±4.65 $^{a}$ | 0.00±0.00 $^{h}$ |
| | 5 | 0.60±0.013 $^{fg}$ | 3.10±0.083 $^{c}$ | 5.00±0.095 $^{f}$ | 8.67± 0.67$^{cd}$ | 155.00±1.89 $^{def}$ | 3.27±0.16 $^{e}$ |
| | 10 | 0.42±0.010 $^{h}$ | 3.00±0.11 $^{c}$ | 5.00±0.089 $^{f}$ | 10.00±0.87 $^{cd}$ | 137.67±1.76 $^{g}$ | 7.53±0.35 $^{a}$ |
| Vicilin (100 µg/mL) | 0 | 1.23±0.010 $^{a}$ | 3.43±0.090 $^{a}$ | 8.00±0.22 $^{a}$ | 116.67±3.76 $^{a}$ | 244.33±2.63 $^{a}$ | 0.00±0.00 $^{h}$ |
| | 5 | 0.72±0.022 $^{de}$ | 3.10±0.084 $^{c}$ | 5.20±0.087 $^{ef}$ | 40.00±0.98 $^{bcd}$ | 159.67±2.88 $^{cde}$ | 2.83±0.87 $^{ef}$ |
| | 10 | 0.61±0.019 $^{f}$ | 3.20±0.054 $^{bc}$ | 5.00±0.13 $^{f}$ | 26.67±0.76 $^{bcd}$ | 144.00±1.79 $^{fg}$ | 6.40±0.67 $^{b}$ |
| Vicilin (200 µg/mL) | 0 | 1.23±0.027 $^{a}$ | 3.43±0.073 $^{a}$ | 8.00±0.26 $^{a}$ | 116.67±2.32 $^{a}$ | 244.33±3.83 $^{a}$ | 0.00±0.00 $^{h}$ |
| | 5 | 0.81±0.013 $^{cd}$ | 3.40±0.099 $^{ab}$ | 6.40±0.23 $^{c}$ | 80.00±1.11 $^{ab}$ | 164.67±2.78 $^{cd}$ | 2.17±0.21 $^{fg}$ |
| | 10 | 0.51±0.021 $^{gh}$ | 3.10±0.13 $^{c}$ | 5.37±0.13 $^{c}$ | 43.33±1.21 $^{bcd}$ | 149.33±1.21 $^{efg}$ | 5.47±0.33 $^{c}$ |
| Vicilin (400 µg/mL) | 0 | 1.23±0.022 $^{a}$ | 3.43±0.15 $^{a}$ | 8.00±0.33 $^{a}$ | 116.67±4.12 $^{a}$ | 244.33± 3.12$^{a}$ | 0.00±0.00 $^{h}$ |
| | 5 | 0.92±0.032 $^{b}$ | 3.40±0.17 $^{ab}$ | 7.23±0.26 $^{b}$ | 116.67±2.87 $^{a}$ | 168.33±1.32 $^{c}$ | 2.03±0.14 $^{g}$ |
| | 10 | 0.74±0.026 $^{cd}$ | 3.10±0.095 $^{c}$ | 6.00±0.31 $^{d}$ | 68.33±0.89 $^{abc}$ | 153.33±1.89 $^{def}$ | 4.07±0.17 $^{d}$ |

Means followed different letters are significantly different according to Tukey's HSD test (P ≤ 0.05)

Table 3 shows how vicilin affects the severity of the *B. cinerea*-infected strawberry fruits' sickness. The fruits with natural infection had the most severe illness, followed by those with artificial infection (positive control) (negative control). Vicilin has a strong track record of preventing illness severity, as a result. The presence of vicilin clearly lowered the severity of the illness in a concentration-dependent way. The higher doses of vicilin (400 µg/mL) resulted in a greater reduction in disease severity in strawberry fruits. After 10 days of storage, the positive control showed 100% disease severity, while the fruit treated with vicilin (400 µg/mL) had 41% severity.

*Impacts of vicilin on strawberry fruit quality*

Impacts of vicilin on citric acid (%), pH, total soluble solids (%TSS), firmness (N), anthocyanin content (mg/ 100 g FW), and weight loss (%) in strawberry fruits inoculated with *B. cinerea* are presented in Table 3. Vicilin at several concentrations (100, 200, and 400 µg/mL) diminished weight loss (%) of the strawberry fruits in a concentration-dependent manner. Vicilin exhibited a more significant impact on the prevention of weight loss at larger application doses. After 10 days of storage, the positive control showed 7.53% weight loss, compared to 4.07% weight loss on fruit treated with vicilin (400 µg/mL). Throughout storage, the strawberry fruits' total soluble solids (TSS) content decreased. The deterioration of the strawberry fruits was partially inhibited by the application of vicilin in a manner that depended on its concentration. The strawberry fruits' pH decreased during storage; however, no significant difference was observed in the pH values among all vicilin treatments. One key factor contributing to the post-harvest and market quality of strawberries is their firmness. All treatments containing vicilin showed a significant increase in firmness as compared to the control group. This increase was observed in a concentration-dependent manner. The total anthocyanin content of strawberry fruit was affected by both vicilin treatment and storage time. The results showed that anthocyanin content decreased in a concentration-dependent manner in the positive control compared to the vicilin groups.

Throughout storage, the strawberry fruits' citric acid (%) decreased. The decline in strawberry fruits was partially prevented when treated with vicilin, and the prevention effect was dependent on the concentration used.

## Discussion

Because of their strong nutritional value and positive health effects, strawberries are very popular among customers. Overall, strawberries are an important agricultural product for the global economy. However, because of its sensitivity to pathogen infection, its shelf life is quite short after harvest (Singh *et al.,* 2021; Kahramanoğlu 2017; Darwish *et al.* 2021). Post-harvest losses are mainly caused by *Botrytis cinerea*, commonly known as gray mold, which leads to post-harvest deterioration (Kahramanoğlu *et al.,* 2022). Presently, controlling gray mold in strawberries depends most on the continued usage of artificial fungicides, which leads to a series of passive effects (Sun *et al.,* 2021a). Therefore, the need for safe antifungal medicines to prevent postharvest grey mould and extend the shelf life of strawberry fruit is critical. Alternative natural food preservatives could be chosen from a variety of sources, like glycoproteins (Abbas *et al.,* 2020a). After 21 days of postharvest therapy, 7S globulin, a glycoprotein derived from soy seeds, was found to be a powerful fungal inhibitor that prevented the growth of green mold on orange fruit and kept the incidence and severity of the disease at their lowest possible levels (Osman *et al.,* 2016a). The results obtained by treating fig fruit with chickpea 7S globulin (vicilin) after harvest suggest that it could be a potential solution to reduce the severity of *Alternaria tenuissima* infection on figs and extend their shelf life. The treatment resulted in a significant reduction of disease severity by approximately 73% compared to the positive control. These findings support the *in vitro* results and indicate that the treatment could be a viable way to extend the shelf life of figs (Abbas *et al.* 2020a; Ramadan *et al.,* 2022; El-Beltagi *et al.,* 2023). The protein components of soy, chickpea, pea, and kidney bean are similar, suggesting comparable uses (Osman *et al.,* 2014a). In the present investigation, 7S globulin (vicilin), an antifungal agent against *B. cinerea* obtained from strawberry fruit with grey mold disease, was extracted from pea seeds, identified, and tested *in vivo*.

Vicilin's molecular weight revealed three bands corresponding to the α, α′, and β subunits. This demonstrates that this globulin protein is similar to those of other legumes, including soybean and chickpea (Atallah *et al.,* 2021). The vicilin glycoprotein (7S globulin) obtained from peas has 5.8% carbohydrate. (Osman *et al.,* 2016a) reported a content of 6% carbohydrate in 7S from soybean. According to (Deepak *et al.,* 2003) glycoproteins have antifungal activity. According to (Kimura *et al.,* 1997) the 5-6% carbohydrate level, primarily high-mannose moieties, is responsible for this antifungal activity. Evidencing the glycoprotein nature of vicilin ( Osman *et al.,* 2016a; Abbas *et al.,* 2020a; Atallah *et al.,* 2021) was realized by its carbohydrate content of vicilin (5.8%), which will also partially explain its antifungal effect.

The concentration-dependent presence of vicilin inhibited the mycelial growth of *B. cinerea*. Previous studies have reported antifungal proteins from various sources, including soybean (Osman *et al.,* 2016a), chickpea (Abbas *et al.,* 2020a), lentil (Sitohy *et al.,* 2007), and African catfish (Abdel-Shafi *et al.,* 2019b). SEM revealed deformation of the fungal hyphae and they appeared shrivelled up after exposure to the vicilin in PDA. This trait was evident in *B. cinerea* treated with vicilin, and it may be linked to protein-protein interactions that affect membrane permeability (Osman *et al.,* 2016b; Abdel-Hamid *et al.,* 2016).

Vicilin was applied as a postharvest treatment on strawberries, and the *in-situ* results support the potential use indicated by the *in vitro* findings. Our study reported that the presence of vicilin from kidney bean seeds reduced disease incidence and severity in preserved strawberry fruit in a concentration-dependent manner. This refers to the effectiveness of vicilin as an antifungal agent in reducing disease incidence and severity.

The use of vicilin treatment has been found to have a positive impact on the quality of fruits. This treatment is effective in preventing weight loss, maintaining firmness, and improving TSS, pH, citric acid, and anthocyanin content. This improvement can be attributed to the antifungal properties of vicilin. Previous research suggests that the 7S globulin may inhibit the growth of bacteria and fungi on strawberry surfaces by interacting with other proteins to preserve the fruit's cuticle integrity and stop water and weight loss, which would preserve the fruit's firmness and turbulence (Sun *et al.,* 2021b).

## Conclusions

Current investigations have found that vicilin derived from kidney bean seeds exhibits a significant antifungal effect against *B. cinerea* mycelial development. They may also have an impact on the hyphal shape and impair the integrity of the plasma membrane. Additionally, the vicilin shielded strawberry fruits from postharvest grey mould. Similarly, the vicilin exposure delayed the strawberry's natural deterioration and preserved fruit quality. As a sustainable glycoprotein, vicilin, found in kidney bean seeds, can be used to combat postharvest fungal infections.

## Authors' Contributions

Conceptualization: AO, MS; Methodology: EA, AO; Software: HHA, TAS, SME-G; Validation: AO, HSE-B; Investigation: AO, MS, EA; Resources: HHA, TAS, SME-G; Data curation: AO, HSE-B; Writing—original draft preparation: AO, EA; Writing—review and editing: MS; Funding acquisition: HSE-B.

All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

## Acknowledgements

We thank to Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (GRANT 5663), for support this research work.

## Conflict of Interests

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

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