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GREEN BIOCHEMICAL PROTECTION OF POSTHARVEST TABLE GRAPES AGAINST GRAY MOLD (*BOTRYTIS CINEREA*) USING 7S PROTEINS

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SUMMARY

The 7S globulins (seed storage glycoprotein) isolated from soybean and chickpea seeds have the antifungal potential against the pathogenic fungus (*Botrytis cinerea)* causing gray mold in grapes assessing table grapes in vitro or postharvest by comparing with synthetic fungicide, Switch 62.5 WG. Conventional microbiological procedures estimated the in-vitro antifungal potential of the 7S globulins, such as linear growth curves and scanning electron microscopy (SEM). Soybean-7S significantly inhibited the in-vitro growth of *Botrytis cinerea* by about 64.44%, 66.64%, and 76.67% when applied at 50, 100, and 200 µg/mL, respectively, followed by chickpea 7S with growth reduction of 52.22%, 54.44%, and 66.67%, respectively. The synthetic fungicide (Switch 62.5 WG) induced higher growth inhibition extents (83.33% and 86.66%) when applied at 50 and 200 µg/mL, respectively. The 7S-exposed *B. cinerea* displayed swollen hyphae compared with the control under scanning electron microscope examination. The 7S derived from soybean and chickpea inhibited gray mold development in table grapes when applied at 200 and 400 µg/mL for 30 days after infection with *B. cinerea*. The maintained disease severity was also minimal (40% and 25% for soybean-7S and chickpea-7S, respectively). An increased level of treatment (400 µg/mL) highly reduced the disease severity to only 7.5% after 30 days of storage at cold conditions for both proteins. The 7S globulin from legume seeds can be an alternative to synthetic fungicides for controlling *B. cinerea* as a postharvest treatment. Developing these legume proteins as natural fungicides could also progress for the safe control of various plant pathogens, causing drastic crop losses.

Keywords: Grape (*Vitis vinifera* L.), gray mold (*Botrytis cinerea*), 7S globulin, postharvest, linear growth curves, scanning electron microscopy (SEM)

Key findings: Soybean-7S significantly inhibited the in vitro growth of *Botrytis cinerea* by about 64.44%, 66.64%, and 76.67% when applied at 50, 100, and 200 µg/mL, respectively, compared with 52.22%, 54.44%, and 66.67% growth reduction by chickpea-7S, respectively. The

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synthetic fungicide (Switch 62.5 WG) induced higher growth inhibition, amounting to 83.33% and 86.66% with 50 and 200 µg/mL, respectively. The 7S-exposed *B. cinerea* displayed swollen hyphae compared with the control under scanning electron microscope examination.

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INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most precious fruits worldwide, rich in nutrients with beneficial effects on promoting human health. Grape cultivation occurs almost everywhere, with a worldwide consumer preference for their delicious taste and nutritional value. Moreover, grapes are the brewing industry's prime ingredient for wines and liquor. Therefore, as a multipurpose fruit, its production serves mainly wine, juice, and several other derivatives processing in the North. The table grape is a non-climacteric fruit with a relatively low rate of physiological activities (Chen *et al.,* 2018). The grape's long-time cultivation produced diverse genotypes and landraces with varied and improved fruit qualities. The grape's growing market is fascinating in enhancing innovative processing, storage, and marketing technologies for a longer postharvest shelf-life (Elsayed *et al.,* 2022).

Botrytis cinerea - gray mold is an omnipresent fungal disease infecting various postharvest life of fruits and vegetables (Wang *et al.,* 2020; Suirta *et al*., 2021).). The causative agent, *Botrytis cinerea* Pers. ex Fr., is the second most economically important fungus among the global top 10 pathogens and has a thoughtful impact on several horticultural crops based on its wide host range (Youssef *et al.,* 2019). It is a necrotrophic pathogen, secreting various metabolites and enzymes during infection (Nakajima and Akutsu, 2014; Rao *et al*., 2016). Moreover, it can tolerate low temperatures and maintain a high germination rate even at −0.5 °C. Particularly, gray mold may reach various parts of plants at successive developmental stages, leading to tremendous annual losses (Pande, 2009).

The infection severity depends on the pathogen's existence during harvest and postharvest stages, including handling and storing grapes in cold chambers. At the beginning of infection, dark and soft circular areas become visible on the tissues, resulting in abundant sporification from white to gray, developing from the infection site as ordinary openings and mechanical wounds. Usually, *B. cinerea* fungus spreads from a decayed fruit to uninfected ones, promoting infection and establishing a nest of rotted berries, resulting in huge losses. In addition, the fungus can infect fresh fruit by entering damaged tissues at the stem end, which is rich in nutrient exudates, damaging the whole fruit. Therefore, precise gray mold control is crucial to lessen the fruit yield and quality losses.

Chemical fungicides, like procymidone, iprodione, and pyrimethanil, are the primary means to manage and control the postharvest gray mold (Rodríguez *et al.,* 2014). However, the long-term and extensive use of these chemicals resulted in myriad problems, such as, increased pathogen resistance, soil contamination, polluted environment, unsafe for health, and deteriorating ecological structures (Kautsky *et al.,* 2000). Furthermore, the growing consumers' interest in organic fruits and their processed products is urging the search for safer alternatives to synthetic fungicides while reducing costs and protecting the environment (Wu *et al.,* 2023).

Increasing attention has targeted the use of microorganisms (de-Moura *et al.,* 2021), UV light (Abbey *et al.,* 2019), organic and inorganic salts and acids (Zaker, 2014), and natural compounds (Sitohy *et al.,* 2007; Abbas *et al.,* 2020; Atallah *et al.,* 2021) to control fungal infections. Although synthetic fungicides are still the most effective agents against postharvest fungal diseases in various fruits, they have significant drawbacks, including high cost and handling hazards, pathogens'

resurgence, biodiversity loss, increased toxicity, high residues, threatening human health, and environment (Mari *et al.,* 2014; Sajeena *et al.,* 2019). Fungi can also rapidly increase their resistance to one fungicide.

Therefore, developing safe, biodegradable, and natural fungicides is crucial. Controlling gray mold is highly challenging, particularly when most countries have banned postharvest treatments with synthetic fungicides. Within the scope of exploring safe alternatives to control postharvest gray mold of table grapes, the potential antifungal activities of 7S globulin (seed storage glycoprotein) isolated from soybean and chickpea seeds against pathogenic fungus *Botrytis cinerea* attained extensive investigation in the presented research through in vitro in postharvest table grapes. Furthermore, a comparison of results with the potency of synthetic fungicides (Switch 62.5 WG) ensued.

MATERIALS AND METHODS

Plant materials

Legume (soybean and chickpea) seeds and table grapes, purchased from the local market, transpired in Zagazig City, Sharkia, Egypt.

Chemicals and reagents

Reagents for electrophoresis came from Bio-Rad laboratories (Richmond, CA, USA). All other chemicals used in experiments were of analytical grade.

Preparation of 7S globulin

Legume (soybean and chickpea) seeds, ground and dispersed in chloroform, included methanol (3:1 v/v) for eight hours to eliminate fat. Initially dispersing 5% (w/v) defatted seed flour in water extracted the seed protein isolate (Johnson, 2005). The 7S globulin isolation from defatted dried flour used an ammonium sulfate (55%–80%) precipitation procedure according to Nagano (1992), as modified by Sitohy *et al.* (2012).

Characterization of 7S globulin

SDS-PAGE

Twenty milligrams of 7S globulin dissolved in 1 ml sodium dodecyl sulfate for 10 min continued to centrifugation at 10,000 *xg* for 15 min. Twenty microliter extracts gained blending with the loading buffer (SDS 4%, 3%, glycerol 20%, β-mercaptoethanol, Tris HCl 50mM pH 6.8, and bromophenol blue traces), with 10 mcL of the last solution loaded per lane according to Laemmli (1970).

Isoelectric point estimation

Determining the isoelectric point used protein pH solubility curves at several pHs from two to 10 according to the protocol outlined by Sitohy and Osman (2010).

Amino acids analysis

The composition of amino acids for 7S globulin (isolated from legume seeds) underwent evaluation according to Simpson *et al.* (1976) using the amino acid analyzer instrument model "Eppendorf LC3000" following Abdel-Shafi *et al.* (2016).

Carbohydrates estimation

The carbohydrate analysis of 7S globulin was estimated by the dehydration of carbohydrates into furfural derivatives and reacting them with phenol to develop a correlated color measurable at 490 nm as described by Wilson *et al.* (1981).

Fourier transform infrared (FT-IR) spectroscopy

Protein samples' preparation and handling employed the potassium bromide (KBr) pellet method (Souillac *et al.,* 2002). Generating the infrared spectra used an FT-IR spectrometer (Nicolet Nexus 470, DTGS, Thermo Scientific, Waltham, MS, USA) at 25 °C, with the 256 interferograms collected for each spectrum with a resolution of 4 cm^{-1} and 64 scans and 2 cm^{-1} intervals from 4000 to 400 cm^{-1} regions.

Working out the relative amounts of the various secondary structures of 7S globulin from the infrared second derivative amide spectra proceeded through manual computation of the areas under the bands.

Isolation and identification of the gray mold

Infected grapes showing typical gray mold symptoms were the source of the pathogen used in this study after purification and morphological-molecular identification. These isolates were on potato dextrose agar (PDA) slants for maintenance and storage at 4 °C for further use. Acquiring the molecular confirmation of the fungus was through the Internal Transcribed Sequence (ITS)-PCR identification. The DNA extraction was according to Tuna-Gülören *et al.* (2021), and ITS-PCR amplified the region ITS-5.8S rDNA according to Taylor *et al.* (2003). Sequencing proceeded at the Human Genome Center-USP, with the sequences obtained handled via the software BioEdit Sequence Alignment Editor (1997-2005), searching for corresponding successions using Blastn at NCBI.

In vitro antifungal assay

The antifungal activity of 7S globulin estimates against *B. cinerea* in vitro was as follows: Mycelia plugs (5 mm) separated from the growing border of one-week-old fungal cultures continued placing in the middle of Petri dishes, including PDA, and the tested 7S globulin at different concentrations (50, 100, and 200 µg/mL). The synthetic fungicide (Switch 62.5 WG) became a standard positive control at 50 and 200 µg/mL. Following the poisoned food technique (Osman *et al.,* 2016), the inoculated plates were incubated at 23 °C ± 1 °C, measuring the radial fungal growth (mm) of *B. cinerea* on the fifth day of gestation. The reduction percentage in colony diameter (CD) calculation was as follows:

$$CD\ (\%) = \frac{dc - dt}{dc}\ x\ 100$$

Where:
dc = the control's average diameter of linear fungal growth,
dt = treatment's average diameter of linear fungal growth.

Each treatment consisted of three Petri dish replicates, with the experiment repeated twice.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of *B. cinerea*, treated with 7S globulin from soybean and chickpea at 200 µg/mL for 4 h at room temperature, attained estimation and comparison with the control treatment (Sitohy *et al.,* 2013; Abbas *et al.,* 2020).

In vivo experiments and antifungal activity assay

Fungal conidia collection from two-week-old PDA cultures of *B. cinerea* maintained at 23 °C ± 1 °C in a Petri plate comprised adding 5 mL of distilled water containing 0.05% (v/v) Tween 80. Using a sterilized glass rod, gently dislodge the conidia from the surface, with the resulting suspensions filtered through three layers of cheesecloth to remove any adhering mycelia. Next, the filtrates' dilution with sterile water began before determining conidial content with a hemacytometer until diluting to 106 conidia mL^{-1}, the level utilized for grape inoculation. Grape clusters (100 g) immersed in *B. cinerea* spore suspension at 106 spores per L for 3 min continued to air drying at 25 °C for 30 min and then submerged in 7S globulin from different sources at various concentrations (0, 200, and 400 µg/mL) for 3 min, and air dried again for 30 min at 25 °C. Every treatment included three replicates. Placing the treated fruits in plastic trays and enclosing them with a polyethylene bag ensured keeping 95% humidity, then stored at 4 °C for 30 days. Calculating the disease incidence utilized the following formula:

$$\text{Disease incidence (\%)} = (\text{Number of infected bunches} / \text{Total number of bunches}) x100$$

Assessment of fruit quality parameters

Measuring the weight of the fruits employed a digital balance at the beginning of the experiment and after 30 days of storage. The weight loss percentage calculation had the following equation:

$$Weight\ loss\ (\%) = [Intial\ weight - weight\ at\ the\ examined\ time]/\ Intial\ weight\ x100$$

A digital refractometer (Pocket Refractometer PAL 3, ATAGO, Japan) helped assess the content of the total soluble solids in the fruit juice following Simonne *et al.* (2007). Selecting five fruits from each replicate provided for determining firmness in Newtons (N), using a fruit Push-Pull Effegi penetrometer system (Model FD 101) supplemented with a plunger penetrator 2 mm in diameter. Using a hook instead of the plunger allowed the measurement of the berry separation force, expressed in Newtons (N).

Statistical analysis

Running statistical analysis utilized the Microsoft Excel software (2010). All results' values had expressions as mean ± SD.

RESULTS

Characterization of 7S globulin from different sources

The results of the SDS-PAGE electrophoretic pattern of 7S globulin from soybean and chickpea seed proteins appear in Figure 1A. The bands corresponding to 7S globulin refer to about 70, 50, and 40 kDa in 7S globulin from soybean and 72, 60, and 50 kDa for 7S globulin from chickpea, which indicates the bands of these protein fractions, i.e., α/, α, and β subunits. The infrared spectra of 7S globulins in Figure 1B showed remarkable

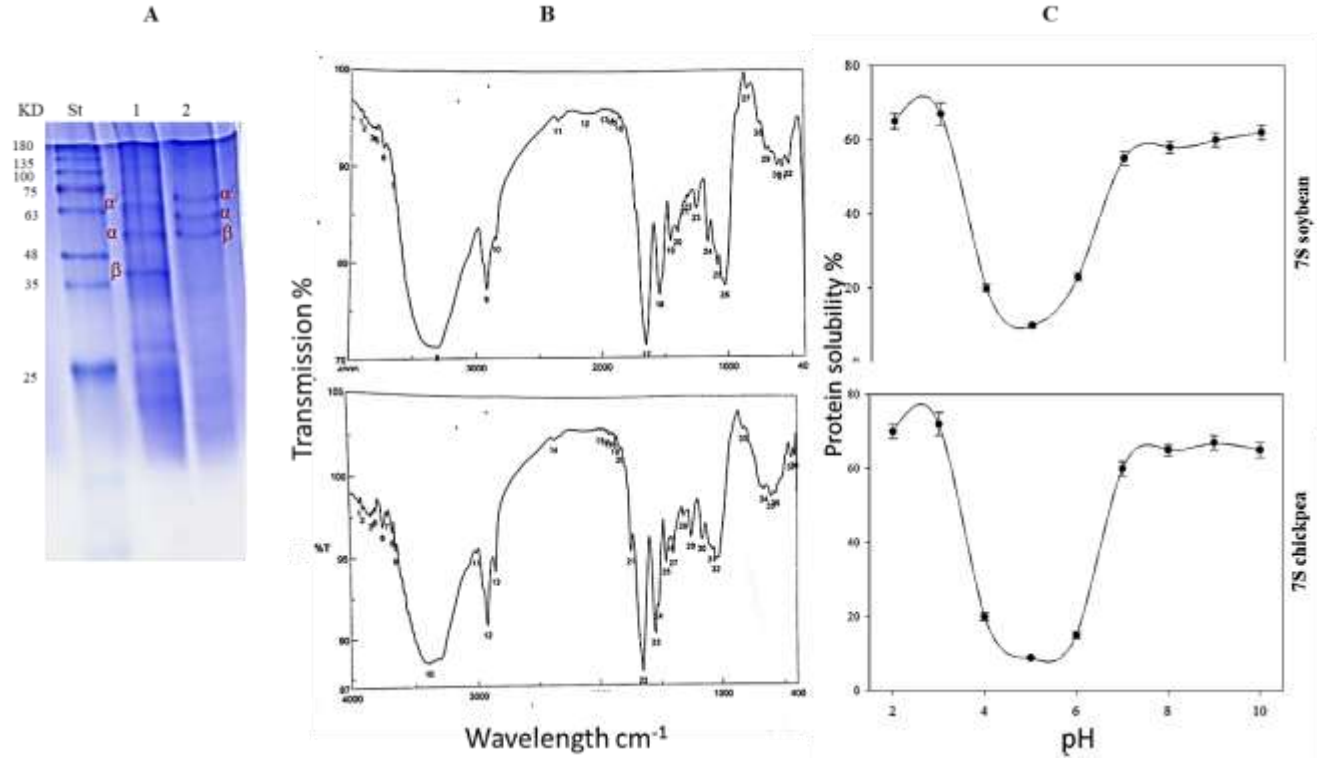

Figure 1. Biochemical information on 7S globulin isolated from soybean (1) and chickpea (2): SDS-PAGE (A), IR (B), and pH solubility curves (C).

Table 1. Amino acids composition of 7S globulin from different sources.

Amino acids	Concentration (%)	
	Soybean	Chickpea
Essential amino acids		
Methionine*	0.4	0.2
Isoleucine*	4.0	4.5
Leucine*	6.5	6.7
Valine*	3.0	3.0
Phenylalanine*	4.4	4.3
Threonine	1.3	1.5
Lysine	5.0	5.8
Total essential amino acids	24.6	26.0
Non-essential amino acids		
Aspartic acid	9.2	7.0
Glutamic acid	18.0	17.5
Serine	5.0	3.6
Glycine	2.4	2.3
Alanine*	2.4	3.0
Cysteine	2.0	1.2
Tyrosine	1.9	1.7
Arginine	6.3	6.5
Proline*	5.4	4.2
Histidine	2.4	3.2
Total non-essential amino acids	55.0	50.2
Total amino acids	79.6	76.2

*Hydrophobic amino acids

similarity between 7S globulin from the two legume crops. In addition, the protein solubility curves of both proteins were remarkably similar, indicating almost the same isoelectric point at pH 5 (Figure 1C). This apparent similarity may indicate nearly the same functionality of the soybean and chickpea proteins, extending the opportunity to have different antifungal agents

The estimated carbohydrate content of each protein indicates the presence of carbohydrate components in each protein, proving the glycoprotein nature of each (data not shown). However, 7S globulin from soybean obtained a relatively higher content of carbohydrates (6%) than chickpea-7S globulin (5.6%). Table 1 presents the amino acid composition of 7S globulins from the soybean and chickpea. Essential, nonessential, hydrophobic, acidic, and basic amino acids recorded 24.6%, 55%, 26.1%, 27.2%, and 13.7% for 7S globulin from soybean against 26%, 50.2%, 25.9%, 24.5%, and 15.5% for 7S globulin from chickpea, respectively.

In vitro antifungal activity

The synthetic fungicide (Switch 62.5 WG) effectively inhibited the mycelial growth of *B. cinerea* on solid agar medium Petri dishes at various concentrations (0, 50, and 200 µg/mL) after seven days of incubation at 25 °C in a concentration-based manner (Figure 2). However, the maximum fungal growth reduction extent reached 86.66% in response to 200 µg/mL Switch 62.5 WG compared with 83.33% in response to the low fungicidal dose (50 µg/mL). The soybean-7S globulin and chickpea-7S globulin at 0, 50, 100, and 200 mg/mL also inhibited mycelial growth of *B. cinerea;* however, to lower extents than the synthetic fungicide (Figure 2). The first agent isolated from soybean exerted its maximum reducing action (76.67%) in response to the high agent dose (200 µg/ml), which is higher than the corresponding one achieved by chickpea-7S globulin (66.67%) at the same agent dose.

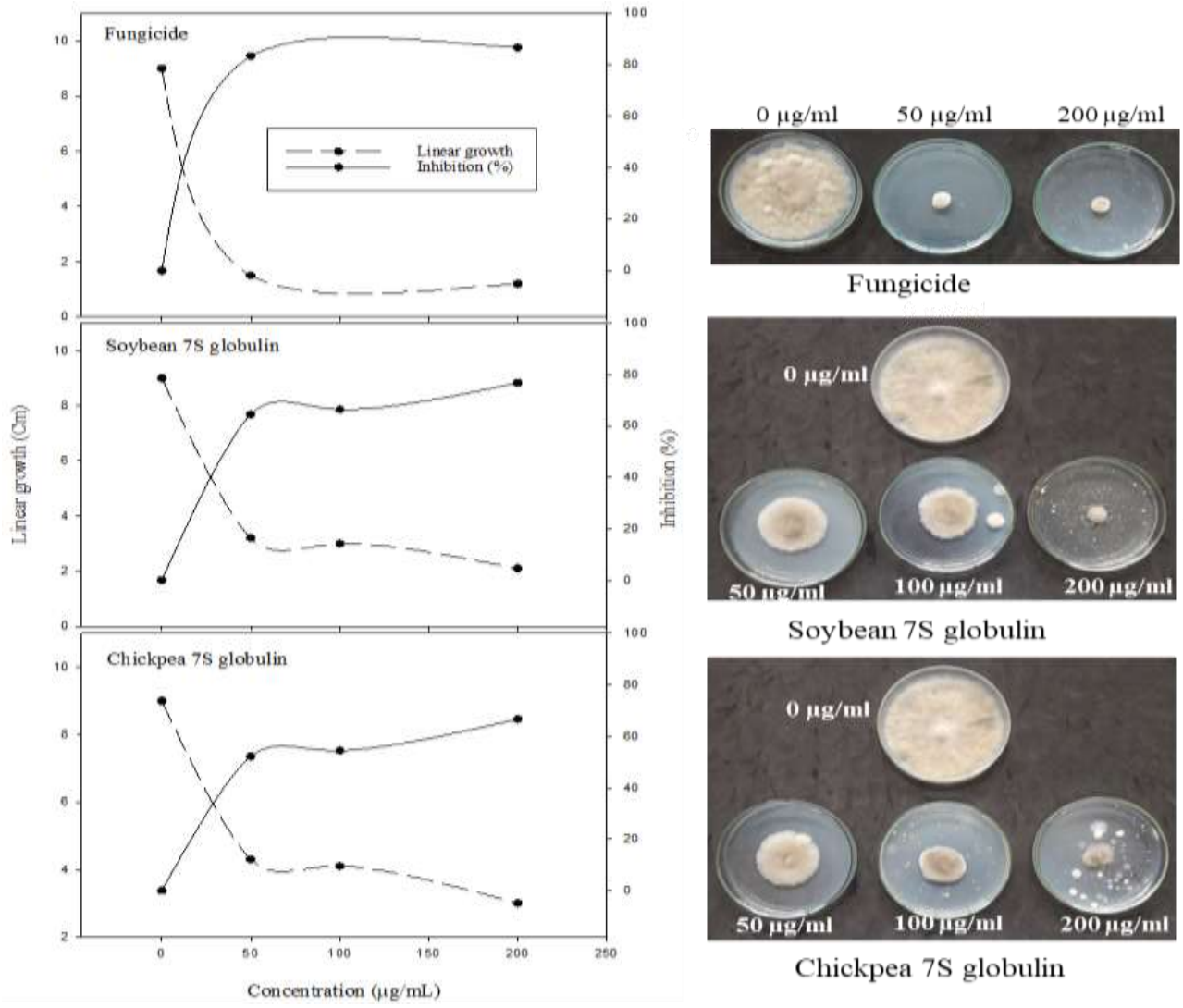

Figure 2. Fungal growth of *B. cinerea* on solid agar medium Petri dishes, as expressed in fungal linear growth (cm) and growth inhibition (%) after seven days of incubation at 25 °C in the presence of 7S globulin obtained from soybean and chickpea at various concentrations (0, 50, 100, and 200 µg/mL) compared with the synthetic fungicide (0, 50, and 200 µg/mL).

Comparing the inhibitory action of both 7S globulins with the synthetic fungicide revealed relative inhibitory potency amounting to approximately 88% and 77% of the antifungal strength of the synthetic fungicide. Therefore, considering the safety of natural products, these two organic antifungal agents (soybean-7S globulin and chickpea-7S globulin) represent safe antifungal potency. These in vitro results may be promising, encouraging the use of antifungal agents in vivo. However, doses around 200 µg/mL may be more appropriate for effectual application.

Scanning electron microscopy

Electron microscopic view indicated the effects of 7S globulin obtained from soybean and chickpea on the morphology and microstructure of gray mold germs and hypha (Figure 3). In the control treatment (without 7S globulin), the mycelium showed classic

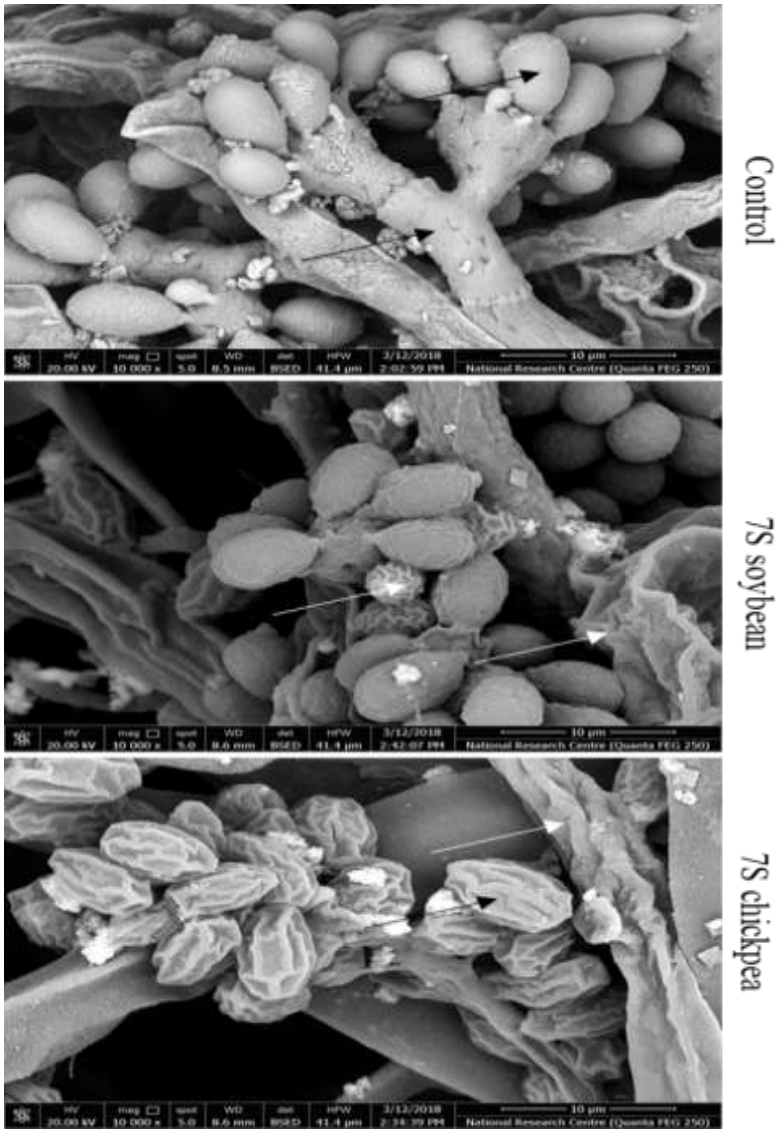

Figure 3. Scanning electron microscopy of *B. cinerea* after seven days of incubation at 25 °C in the presence of 7S globulin obtained from soybean and chickpea (200 µg/mL).

morphology (linear and homogeneous) and uniform, complete, thicker cell walls along with tapered and smooth hyphae apices. However, these typical morphological structures changed when applied with 7S globulin from soybean and chickpea seeds. Evident craters appeared with the treatments of both antifungal agents on the hyphal cell wall. The 7S-globulin treatments caused cell wall rupture, finally resulting in cell death. Overall, a conclusion can state that soybean-7S and chickpea-7S globulins reduced the fungal pathogenicity by affecting spore germination and the microstructure of *B. cinerea*. The inhibition impacts had extreme influences from the chemical composition of 7S globulin obtained from soybeans and chickpeas.

In vivo experiments and antifungal activity assay

The in vivo experimental approach showed that soybean-7S and chickpea-7S globulins negatively affect the incidence of postharvest *B. cinerea* fungal rots on grapes. Both antifungal agents inhibited the postharvest

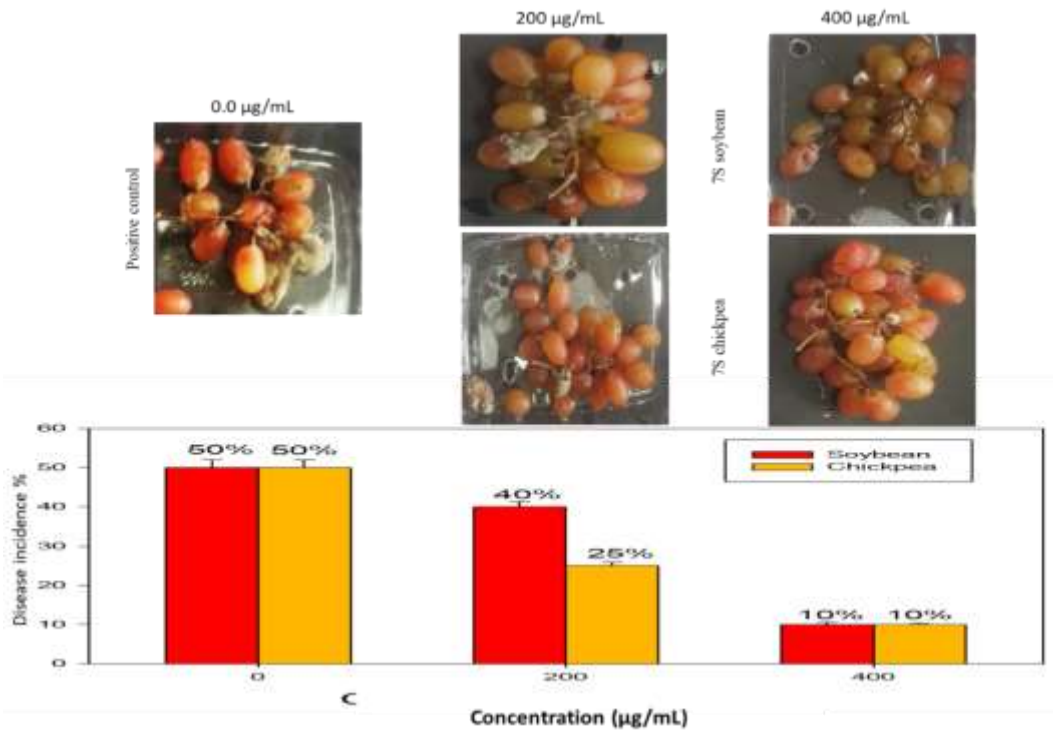

Figure 4. Disease incidence (%) in table grapes was inoculated with *B. cinerea* and treated with 7S globulins obtained from soybean and chickpea at different concentrations (200 and 400 µg/mL) compared with the control (0 µg/mL).

pathogens mainly via their direct control of reducing their mycelial growth and conidia germination, potentially affecting the pathogen's cellular metabolism. Results further showed that the antifungal activities of 7S globulin from both legumes varied in vitro and in vivo; however, they were higher under in vitro conditions.

The in vivo results utilizing 7S globulin from soybean and chickpea as postharvest treatments on grapes translate the previously stated in vitro results into potential application, minimizing the incidence of *B. cinerea* infection and extending fruit shelf life. After 30 days of cold storage, the tested substance remained highly effective even at lower concentrations (200 µg/mL), reducing the disease incidence to 40% and 25% for 7S globulin obtained from soybean and chickpea, respectively (Figure 4). However, more positive results were visible with the higher concentration (400 µg/mL) of the soybean-7S globulin and chickpea-7S globulin, reducing the disease incidence to the minimum level (10%) which was much lower than the control treatment (50%). It is realizable that this treatment (400 µg/mL) saves about 80% of postharvest fruits from disease incidence. The 400 µg/mL application gave no difference in the antifungal potency between both legumes. Therefore, protection of grapes against *B. cinerea* infection using 400 µg/mL of soybean-7S globulin or chickpea-7S globulin and even the same protein fraction from other similar vegetable sources is promising.

Assessment of fruit quality parameters

The effect of 7S globulin obtained from soybean and chickpea at different concentrations (200 and 400 µg/mL) on the quality of grapes inoculated with *B. cinerea* is available in Figure 5A. Compared with the control treatment, weight loss rates were significantly lower across all treatments. After 30 days of storage, the fruit treated with 7S

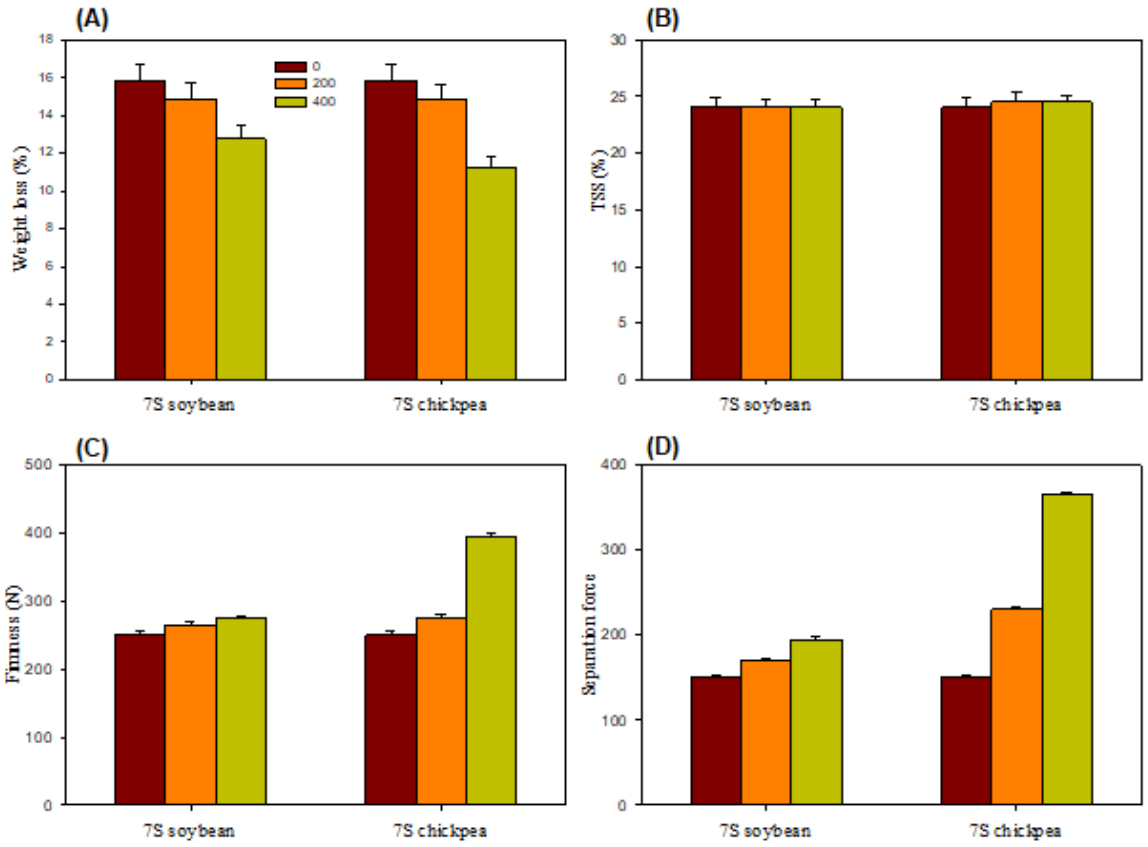

Figure 5. The effect of 7S globulin obtained from different sources (soybean and chickpea) on weight loss (A), total soluble solids (B), firmness (C), and separation force (D) in fruits of grape inoculated with *Botrytis cinerea* compared with the control treatment.

globulin from soybean and chickpea (at 400 µg/mL) lost only 12.7% and 11.23% weight, respectively, compared with 15.83% weight loss in the positive control. The effect of 7S globulin from soybean and chickpea at different concentrations (200 and 400 µg/mL) on TSS (%) in fruits of grapes infected with *B. cinerea* showed no significant differences among all the treatments compared with the control (Figure 5B).

However, 7S-globulin application with two concentrations (200 and 400 µg/mL) on grapes inoculated with *B. cinerea* improved their firmness quality during 30 days of cold storage (Figure 5C). The high-dose treatment (400 mg/ml) of 7S globulin from soybean and chickpea was the most efficient in maintaining firmness (275 N and 395 N, respectively) compared with the control treatment (250 N). Likewise, applying 7Sglobulin from soybean and chickpea at 200 and 400 µg/mL on grapes inoculated with *B. cinerea* also improved their separation force compared with the positive control (Figure 5D). Chickpea-7S globulin provided the highest separation force (365 N), followed by soybean-7S globulin (195 N), and topping in the value associated with the positive control (150 N).

DISCUSSION

The basis for traditional control and management of fungal diseases mainly applies chemical fungicides. However, the continuous use of fungicides may lead to the emergence of fungicide-resistant strains, a polluted environment, and harmful effects on human health (Baibakova *et al.,* 2019). Therefore, in current agricultural policies, eco-friendly crop

management methods, including exploring natural antifungal products, gain encouragement to control postharvest fruit diseases (Xu *et al.,* 2022). Numerous studies have reported the suitability of 7S globulin from different sources as a safe alternative to synthetic fungicides for controlling postharvest fruit diseases. So far, leguminous 7S globulin is recommendable to serve as a natural alternative to fungicides to decrease postharvest losses and increase the shelf-life of various fruits (Osman *et al.,* 2016).

The fungi *Botrytis* spp. kill the host cells by producing reactive oxygen species and toxins, triggering an oxidative plant burst (Choquer *et al.,* 2007). Two nonspecific phytotoxins identified included botcinic acid and its derivatives, sesquiterpene botryoidal, and related compounds. Botryoidal, produced during plant infection, can induce chlorosis and cell collapse (Adam *et al.,* 2015). However, the antifungal 7S globulin mode of action may proceed to affect cell viability, perturbing plasma membrane integrity, reducing lipid peroxidation, and lowering the expression of essential genes related to pathogenesis (Abbas *et al.,* 2020).

The 7S-glycoprotein components can also disrupt fungal cell membranes, probably through cross-linkage reactions, incurring leakage of electrolytes and depletion of amino acids and sugars. Other 7S constituents can selectively intervene in the lipid-rich portion of the cell membrane, disrupting membrane integrity and function (Osman *et al.,* 2016a, b; Abbas *et al.,* 2020). As an alternative to synthetic fungicides in the control of postharvest diseases, several elicitors have also undergone extensive studies for their possible use in managing various postharvest infections, including gray mold in grapes (Xu *et al.,* 2019). Plants synthesize the phenylpropanoid pathway secondary metabolites, such as, phenolic compounds and flavonoids, for essential biological functions as structural and signaling molecules in plant development and defense systems (Bartwal *et al.,* 2013).

The faster reduction in firmness in the control treatment is attributable to enhanced ripening processes occurring via storage, arising from the degradation of the middle lamella of the cell wall (Johnston *et al.,* 2002). Natural antifungal agents are also vital to function as a protective layer against fungi to prevent fruit damage (Palou *et al.,* 2015). Biological antifungal agents also aid in maintaining the cell wall carbohydrate metabolism, which is associated with decreased susceptibility to infection by fungal pathogens during storage. Fruit firmness probably decreases because of fungal infection, which may hydrolyze pectin, leading to cell wall breakdown (Sañudo-Barajas *et al.,* 2009). In the presented research, the alterations in the morphology of fungus hyphae grown on media with 7S globulin can refer to the effect of the 7S globulin on enzymatic reactions regulating cell wall synthesis (Fakoor *et al.,* 2013).

The close similarity between soybean-7S and chickpea-7S globulins for biochemical characters and composition may explain the similar results regarding their impact on fungal incidence. Thus, both 7S globulins can replace each other as antifungal agents against *B. cinerea* gray mold infection during the postharvest storage of grapes under cold conditions. Based on the SEM images of the 7S-globulin-treated fungus, the observed reduced pathogenicity of fungi came about by affecting the spore germination and microstructure of *B. cinerea,* mainly rupturing cell wall, and eventually, cell death. The deformed images of the treated *B. cinerea* fungus spores indicate the direct effect of the 7S globulins. This action was probably due to the specific chemical composition of the 7S globulin, which is a typical glycoprotein capable of interacting with specific receptors on the fungus cell membranes.

Both in vitro and in vivo experiments indicated the capabilities of 7S globulin to inhibit *B. cinerea* growth by about 67%–77% when applied at the rate of 200 µg/mL. However, 7S globulin at 400 µg/mL protects

around 80% for 30 days under cold storage conditions. Therefore, the said treatment prevents many possible losses from fungal infection. Furthermore, this protective action of 7S globulin against the fungal infection manifested in the overall quality of the fruits of treated grapes, thus raising the economic value of the grape.

CONCLUSIONS

The protein fraction 7S globulin isolated from soybean and chickpea can be appropriate as an antifungal treatment and substitute the synthetic fungicides for controlling *B. cinerea* gray mold infection in postharvest grapes during cold storage. Similar products from different legumes can proceed to screen to develop a library of novel types of selective and natural fungicides applicable within safe norms and conditions to control various plant pathogenic fungi causing drastic crop losses. These compounds responsible for antimicrobial activity will open a new and exciting research trend dealing with 7S globulin from different plant sources. Based on the observed inhibition of mycelial growth and germination of conidia of *B. cinerea* in vitro and reduced incidence of disease symptoms on 7S-globulin-treated grapes, their recommendation is necessary as a potentially effective natural fungicide. However, more studies need execution before this 7S globulin can move for commercial recommendation as a natural antifungal agent.

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