Zagazig J. Agric. Res., Vol. 50 No. (5) 2023 651-663

Plant Protection and Pathology Research
Available online at http://zjar.journals.ekb.eg
http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

ANTIFUNGAL ACTIVITY OF SOME LEGUME GLYCOPROTEINS AGAINST CUCUMBER FRUIT ROT CAUSED BY *Pythium* sp.

Entsar Abbas[1], A. Osman[2]*, F.S. Mohsen[3] and M. Sitohy[2]

1.Plant Pathol. Dept., Fac. Agric., Zagazig Univ., Egypt
2.Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt
3. Hort. Dept., Fac. Agric., Zagazig Univ., Egypt

Received: 13/08/2023; Accepted: 10/09/2023

ABSTRACT: The potential antifungal activity of seed storage glycoprotein (7S globulin) isolated from Cowpea and Lupin seeds against the pathogenic fungus *Pythium* sp. that cause fruit rot in cucumber was tested *in vitro* and *in vivo* on cucumber fruits after the harvesting compared to control. The 7S globulin obtained from Cowpea had a high content of total amino acid (78.2%) against 77.27% for 7S globulin obtained from Lupin. Both protein fractions have similar electrophoretic patterns despite different band locations on the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The pathogen was identified in the Plant Pathology Department Lab., Faculty of Agriculture, Zagazig University, as *Pythium* sp. The mycelial growth of *Pythium* sp. in liquid media was evidently reduced in the presence of 7S globulin from Cowpea and Lupin in a concentration-dependent manner. The application of 500 µg/mL 7S globulin entirely destabilized and deformed this shape in both compounds (Lupin seed 7S and Cowpea seed 7S). Scanning electron microscopy (SEM) images of the mycelium of *Pythium*. sp. in inhibition zones induced by either Lupin 7S or Cowpea 7S revealed significant morphological changes in the hyphae, including deformation and contraction. The *in vivo* tests showed that both Cowpea 7S globulin and Lupine 7S globulin effectively reduced the incidence of postharvest *Pythium.* sp. infection on cucumber fruits. It can be concluded that there are indeed alternatives to replace the synthetic fungicides for the management of this notorious soil as well as seed-borne fungi (*Pythium*), which causes big agricultural losses.

Key words: Fruit rot, legume glycoprotein, *Pythium* sp., cucumber.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated vegetables in open fields, tunnels, and greenhouses. Severe seed-borne and soil-borne pathogens, such as *Fusarium* spp., *Rhizoctonia* spp., *Macrophomina phaseolina*, *Seclrotia sclerotiorum*, and many species of *Pythium*, exist in the rhizosphere and caused seed rot and damping off disease. Cucumber seedlings are susceptible to most of these pathogens, resulting in significant economic losses in Cucumber yield quality and quantity **(Yang *et al.*, 2002)**.

Cucumbers are susceptible to several diseases, such as root rot **(Chatterton and Punja, 2009)**, *Fusarium* wilt **(Zhao *et al.*, 2012)**, Pythium damping-off, *Rhizoctonia* damping-off, downy mildew and powdery mildew **(Wang *et al.*, 2008)**. The most prevalent diseases influencing Cucumber cultivation in the field are damping-off and root rot. *Pythium* is the principal causative agent of their disease, particularly in conditions of high soil field capacity and low temperature **(McCarty and Miller, 2002)**, Some species are restricted to certain members of a family, resulting in a decrease in crop yield. *Pythium* causes pre- and post-emergence damping-off disease by infecting embryo, hypocotyl, and emergent radicle. Infected roots of mature plants inhibit plant growth and sometimes result in plant mortality **(Schroeder *et al.*, 2013).** Shoot and

*** Corresponding author: Tel. :**+201271262256
E-mail address: aokhalil@zu.edu.eg

root dry weights of Cucumber plants were significantly decreased by *Pythium ultimum* infection **(Ravnskov *et al.*, 2020)**. Several *Pythium* species can infect Cucumber seedlings, including *Pythium aphanidermatum, P. ultimum, P. deliense, P. myriotylum,* and *P. spinosum* **(Al-Balushi *et al.*, 2018)**. Based on the growth rate and morphological characteristics, such as the morphology of sporangia, oogonia, and antheridia, *Pythium* species are identified **(Uzuhashi *et al.*, 2015).** This requires extensive knowledge, and a new method has been developed to facilitate more precise identification. Species-specific molecular primers, Polymerase Chain Reaction (PCR), and the sequence of the ras-related protein gene have been developed in recent years **(Khan *et al.*, 2017).**

The *Pythium* species are fungi-like organisms that are commonly referred to as water moulds **(Verma *et al.*, 2020a)**. They are associated with a variety of habitats, including terrestrial or aquatic environments, cultivated or uncultivated soils, plants or animals, and salty or fresh water **(Chakravarthula, 2021)**. The genus *Pythium* is one of the largest Oomycete genera, with over 130 recognised species found in various regions of the globe **(Arafa *et al.*, 2020)**. Although it is widely acknowledged that *Pythium* species are not host-specific, most of them are known to parasitize and infect crop plants, ultimately causing pre- and post-emergence damping-off disease. Rapid germination of *Pythium sporangia* following exposure to exudates or volatiles from seeds or roots, followed by immediate infection, makes its control challenging **(Patil *et al.*, 2012)**.

Pythium sp. symptoms manifest first on fruit in contact with the soil, manifesting as small, water-soaked spots that spread swiftly and turn the fruits necrotic and soft. At later phases, abundant white fungal growth, resembling cotton tufts, is visible on rotted areas under conditions of high humidity **(Kolte, 2019)**. Cottony leak (*Pythium* Rot) infects nearly all cucurbits but is more prevalent on Cucumbers and squash. Typically, this disease appears first on fruit parts that come into contact with the field soil **(Babadoost, 2016)**. It typically appears first on fruit portions in contact with the soil, causing small, water-soaked areas to rapidly expand until large portions of the fruits are necrotic and soft **(Sen, 2006)**. Glycoproteins have emerged as a novel strategy for combating fungal infections. The antifungal protein isolated from *Urginea indica* bulbs was identified as a glycoprotein, with the glycan portion of the molecule exhibiting antifungal activity **(Verma *et al.*, 2020b)**. Many studies have confirmed this conclusion **(Alsohaimy *et al.*, 2007; Sitohy *et al.*, 2007)**. These glycoproteins were proved efficient as antifungal agent against plant fungi during post-harvest and pre-harvest **(Osman *et al.*, 2016; Abbas *et al.*, 2020; Atallah *et al.*, 2021)**.

The initial objective of this investigation was to isolate and identify *Pythium* sp. associated with Cucumber fruit rot. The potential antifungal activity of seed storage glycoprotein (7S globulin) isolated from Cowpea and Lupin seeds will be evaluated *in vitro* against a pathogenic fungus (*Pythium* sp.). The potential curative effect of these glycoproteins on Cucumber fruits artificially infected with *Pythium* sp. will be investigated to identify safe alternatives for controlling Cucumber postharvest fruit rot.

MATERIALS AND METHODS

Isolation of Pathogen

The isolation technique varied slightly depending on the stage of disease development in fruits. A small portion of the cottony mycelial growth was transferred directly onto plain agar medium (PA) from diseased fruits. The fruits exhibiting the earliest phases of symptom development were kept in a humid growth chamber for one to two days until mycelium grew. Fruit tissue samples were surface sterilized by immersing them in 1% NaOCl for two minutes and then rinsing them three times in sterile water before placing them on a PDA medium containing 100 ppm streptomycin. At 27°C, the plates were incubated.

Morphological Identification

On the basis of the characteristics of the isolates grown on the PDA medium and the microscopic characteristics, the isolates were identified morphologically in the Plant Pathology Department Lab., Faculty of Agriculture, Zagazig University, and recorded as *Pythium* sp.

Colony characteristics including colour, mycelial development form (e.g., aerial, flat), and growth rate were investigated under a light microscope (Olympus CX31, Japan), the vegetative and reproductive structures of the isolates were observed **(Jiang *et al.*, 2012).**

Preparation of Legume Glycoprotein (7S Glycoprotein)

Legume seeds (Cowpea and Lupin) were obtained from local market ground and dispersed in chloroform: methanol (3:1 V/V) for eight hours to eliminate fat. Seed protein isolate was extracted according to **Johnson and Brekke (1983)** by initially dispersing 5% (W/V) defatted seed flour in water. The obtained seed protein isolate was used to isolate 7S globulin according to **Nagano (1992)** as modified by **Sitohy *et al.* (2012)**.

Characterization of 7S Globulin

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Twenty mg of 7S globulin was dissolved in one ml of SDS 10% for 10 min, followed by 15 min of centrifugation at 10,000 xg. According to **(Laemmli 1970)**, 20 µL of the extract was mixed with the loading buffer (SDS 4%, 3%, glycerol 20%, β-mercaptoethanol, Tris HCl 50mM pH 6.8, and bromophenol blue traces), and ten microliters of the final solution was loaded per lane.

Iso electric point estimation

Using protein pH solubility profiles at various pHs between 2 and 10, the isoelectric point was determined according to the protocol outlined by **Sitohy and Osman (2010)**.

Amino acids analysis

The amino acid composition of 7S globulin isolated from legume seeds was analysed using the amino acid analyser instrument model "Eppendorf LC3000" according to **Simpson *et al.* (1976)** and **Abdel-Shafi *et al.* (2016)**.

Fourier transform infrared (FT-IR) spectroscopy

Using the potassium bromide (KBr) pellet method, protein samples were processed and prepared **(Souillac *et al.*, 2002)**. A FT-IR spectrometer (Nicolet Nexus 470, DTGS, Thermo Scientific, Waltham, MS, USA) was used to generate infrared spectra at 25°C. Several 256 interferograms were collected for each spectrum, with a resolution of 4 cm^{-1} with 64 scans and a 2 cm^{-1} interval from the 4000 to 400 cm^{-1} regions. From the infrared second derivative amide spectra, the relative quantities of the various secondary structures of 7S globulin were manually calculated.

In vitro Evaluation of Antifungal Activity

The effect of Cowpea and Lupin glycoprotein (0, 250, 500, and 1000 µg/mL) on the mycelial proliferation of *Pythium* sp. was examined using potato dextrose broth (PDB) according to **Teoh *et al.* (2011)** with slight modification. The medium was autoclaved at 121°C for 15 minutes. To prepare the mycelial suspension, 5 mm diameter disc was cut from plate of 7d old *Pythium* sp. mycelia agar and transferred to a sampling bottle containing sterilised distilled water 0.15 L and 0.1% (V/V) Tween 80. The mycelial suspensions were then vortexed for 5 minutes to homogenise them. In 90 ml of medium containing legume glycoprotein from Cowpea and lupin (0, 250, 500, and 1000 µg/ mL) in 250 ml Erlenmeyer flasks (3 replicates per treatment) and incubated at 25 °C, an aliquot of the mycelia suspension (10 ml, 10% V/V) was added. The fungal biomass was filtered through a Whatman No. 1 filter, and the fresh weight was measured. The biomass was desiccated in an oven at 60 °C for twenty-four hours until constant weight. The weight of the control's biomass of 7 days old cultures considered as 100% fungal growth. The weight of the fungal biomass in the treatments was compared to that of the control as a percentage, representing relative growth at fresh or dried weight.

Scanning Electron Microscopy (SEM.)

Comparing *Pythium* sp. treated with 7S globulin from Cowpea and Lupin at 500 µg/mL for 4 h at room temperature to untreated controls, SEM was performed using a JEOL scanning electron microscope JXA840A (Japan) in National Research Centre (NRC) according to **Abbas *et al.* (2020)**.

Fungal Control in Post Harvesting

The healthy cucumber fruits were surface-disinfected with 70% ethanol-dampened cotton wool. A sterilized inoculating needle was used

to create the wound. Mycelium of *Pythium* sp. from a 5-day-old culture was transferred to the wound aseptically. The portion of tissue that had been removed from the fruit was replaced, and the wound was covered. Instead of mycelium, sterile distilled water was used to treat the controls. Four hours later, fruits were sprayed with 500 µg/mL of 7S globulin from two sources (Cowpea and Lupin) versus the untreated control. The treated fruits were preserved at 4°C in plastic box and polyethylene bags to maintain high humidity (approximately 95%). Each treatment had three replicates with a minimum of three fruits per replicate, and the experiment was conducted three times. After treatment, disease incidence and lesion diameter were measured at 15, and 21 days. Using the following equation, the total number of Cucumber fruits and the number of symptomatic fruits in each treatment were enumerated to determine the disease incidence.

$$\text{Disease incidence (\%)} = \text{Number of infected fruit} / \text{total number of fruit observed} \times 100$$

Disease severity of fruit rot was determined 15, and 21 days post inoculation by measuring the area of the fruit showing the rot symptoms.

The digital balance was used to measure the weight of the fruits at the start and after 21 days after storage. To determine the hardness in Newtons (N), three fruits from each replicate were tested using a fruit Push-Pull Effegi penetrometer system (Model FD 101) with a 2 mm diameter plunger penetrator. By using a hook instead of the plunger, the force required to separate the berries could be measured and expressed in Newtons (N).

RESULTS AND DISCUSSION

Characterization of Legume Glycoprotein (7S Globulin)

The amino acid composition results are shown in Table 1. It is apparently seen that tryptophan, asparagine and glutamine were damaged during the acid hydrolysis process, and only 17 types of amino acids were identified. The 7S globulin obtained from Cowpea had a high content of total amino acid (78.2%) against 77.27% for 7S globulin obtained from lupin. It can be observed that the differences among the individual amino between the two studied proteins (Lupine 7S and Cowpea 7S) are generally minimal revealing their genetic relevance. Essential, nonessential, hydrophobic, acidic, and basic amino acids recorded 25.1, 53.1, 26.3, 25.85 and 14.6% for Cowpea-7S against 25.22, 52.05, 25.77, 25.5, and 14.85% for Lupin-7S (Table 1). The relatively higher contents of acidic amino acids (25.85% and 25.5% (7S) then the basic amino acid contents (14.6% and 14.85% for Lupine and Cowpea, respectively) indicate the more acidic nature of this protein fraction and explains its low Isoelectric point (pH 4.5) for each (Fig. 1). It is evident in the same Figure that both protein fractions have similar electrophoretic patterns despite different band locations on the SDS-PAGE. Although the amino acid composition was similar between the two-protein fraction, their IR spectra were somewhat different. This may refer to difference in the secondary structures of the two protein fractions.

The amino acid composition analysis showed similar patterns of Lupine 7S globulin and Cowpea 7S globulin revealing their genetic relevance. Similar SDS-PAGE electrophoretic patterns of the two proteins consolidated this conclusion. The relatively higher contents of acidic amino acids than the basic amino acid contents for the two fractions from Lupine and Cowpea, indicate the more acidic nature of this protein fraction and explains its low Isoelectric point (pH 4.5).

Fungai Strain Isolation and Identification

The pathogen was identified as *Pythium* sp in the Plant Pathology Research Lab., Plant Pathology Department, Faculty of Agriculture, Zagazig University.

Pythium species are a worldwide threat to vegetable production, causing damping-off, poor growth, and low yields of surviving plants. Among these fungus-like microorganisms, *Pythium* sp. appears as the most severe pathogen, capable of causing catastrophic yield losses (**Jiang *et al.*, 2012**). This organism is known to have a high optimum temperature of 35–40°C, which significantly influences infection and the subsequent damage it causes to the host plant. *Pythium* spp. are responsible for root and yield reduction in several soilless crops such as tomato and Cucumber (**Schwarz *et al.*, 2010**).

Table 1. Amino acids composition of legume glycoprotein (7S globulin) from Cowpea and Lupin seeds

Amino acids	Concentration (%)	
	Cowpea	Lupin
Essential amino acids		
Met*	0.3	0.27
Ile*	4.25	4.35
Leu*	6.6	6.4
Val*	2.8	2.95
Phe*	4.35	4.33
Thr	1.4	1.42
Lys	5.4	5.5
Total essential amino acids	25.1	25.22
Non-essential amino acids		
Asp	8.1	7.82
Glu	17.75	17.68
Ser	4.3	4.12
Gly	2.35	2.34
Ala*	2.7	2.77
Cys	1.6	1.5
Tyr	1.8	1.77
Arg	6.4	6.45
Pro*	5.3	4.7
His	2.8	2.9
Total non-essential amino acids	53.1	52.05
Total amino acids	78.2	77.27

*Hydrophobic amino acids

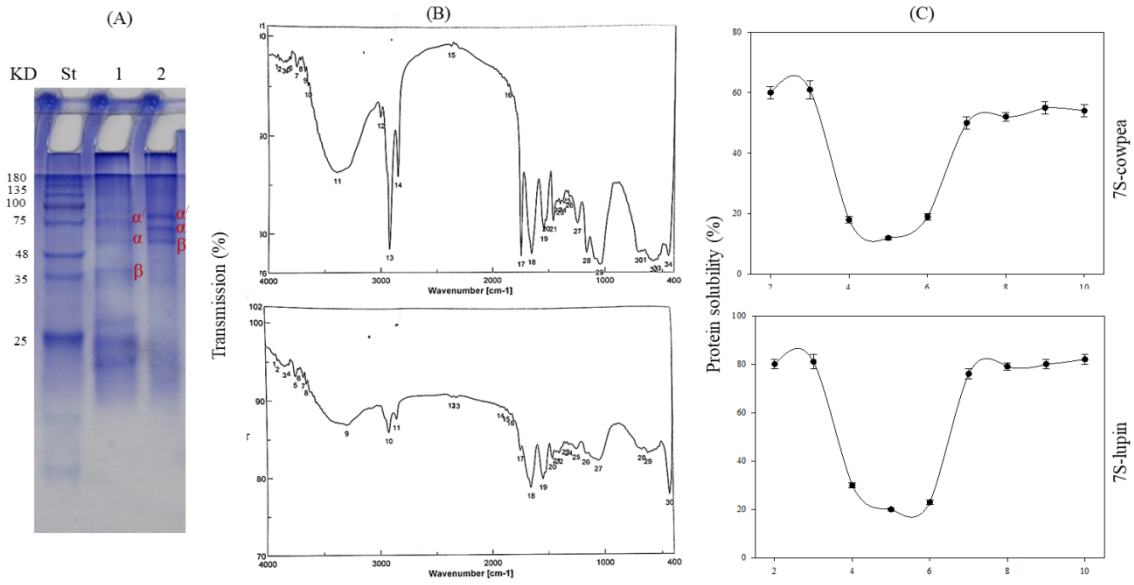

Fig. 1. Biochemical information on 7S globulin isolated from Cowpea and Lupin: SDS-PAGE (A), IR (B), and protein solubility (C)

Antifungal Activity on *Pythium* sp. Mycelial Growth

The mycelial growth of *Pythium* sp. in liquid media was evidently reduced in the presence of 7S globulin from Cowpea and Lupin in a concentration-dependent manner (Fig. 2). The weight reduction was more evident in terms of dry weight than in terms of fresh weight. The impact on fungal growth was higher in the case of 7S globulin from cowpea than 7S globulin from Lupin at substance concentration in the range of 250-500 µg/ml. However, at the higher concentration (1000 µg/ml), Lupine 7S achieved the maximum relative growth reduction, i.e., 95.17%. So, at high substance concentrations, Lupine seed 7S may be recommended for counteracting *Pythium* sp. infection.

The evident reduction of the mycelial growth of *Pythium* sp. in liquid media by 7S globulin from Cowpea and Lupin in a concentration-dependent manner evidenced the antifungal activity of these proteins. The magnitude of reduction indicates high antifungal power. The weight reduction was more evident in terms of dry weight than in terms of fresh weight. The antifungal capacity of 7S globulin was generally considerable in the substance range of 250-500 µg/ml, achieving an excellent practical result, although the maximum growth reduction reaching 95% was achieved by the higher concentration (1000 µg/ml). Growers commonly use fungistatic chemicals to control *Pythium* diseases, but there have been reports of *Pythium* strains that are resistant to fungicides **(Carmona *et al.*, 2018)**. Antifungal proteins potentially active *in vivo* are proposed to be in peripheral cell layers. Most antimicrobial proteins have this pattern of expression **(Deepak *et al.*, 2003)**. It is noteworthy that PR proteins generally exist in many isoforms, some stored in the vacuole, while others in the apoplast. Several studies suggest that antimicrobial activity *in vivo* especially the enhanced resistance to microbial pathogen is conferred to transgenic plants overexpressing thionins, defensin or lipid transfer proteins **(Sels *et al.*, 2008).** In addition, their constitutive expression and external localization in some plant cells has been described, suggesting a potential contribution to innate immunity. In fact, unlike phytoalexins, which can have adverse effects, the plant antifungal proteins do not seem to be phototropic and can attain high levels in seeds and tubers, where they are believed to play a role as protectants. Our results showed that a substantial amount of antifungal protein is located at an external position. Thus, it would be accessible to potential pathogens and is a good candidate to be in the first line of defence, limiting the infection in early contact with fungi. Currently, there is no direct proof for the linkage between the growth inhibition effect shown *in vitro* and the activity of protein in plants.

Scanning Electron Microscopy

Fig. 3 depicts the SEM images of *Pythium* sp. exposed to 7S globulin from Cowpea and Lupin seeds (500 µg/ ml) as compared to the control. Containing sporangia of *Pythium* sp., the SEM image of untreated fungal sporangia (control) revealed quite regular morphologies. Application of 500 µg/mL 7S globulin entirely destabilised and deformed this shape in both compounds (Lupin seed 7S and Cowpea seed 7S). SEM images of the mycelium of *Pythium* sp. in inhibition induced by either Lupin 7S or Cowpea 7S revealed significant morphological changes in the hyphae, including deformation and contraction. However, Cowpea 7S globulin exhibited more pronounced morphological modifications than Lupin 7S. Therefore, it can be concluded that 7S globulin from Cowpea inhibits *Pythium* sp. more effectively than 7S globulin from Lupin, although both proteins inhibited the fungus-like microorganism. The SEM analysis of *Pythium* sp. revealed morphological alterations in the hyphae and sporangia, indicating that the fungus-like microorganism was killed by 7S globulin. This may mean that treating the infected Cucumber fruits with these proteins directly affects the microorganism causing the plant disease. There are different types of antifungal proteins that can stop the production of the fungal cell wall or alter its structure and function, causing the fungal cells to break down. Some antifungal proteins affect the membrane of the fungi, leading to cell lysis **(Selitrennikoff, 2001)**.

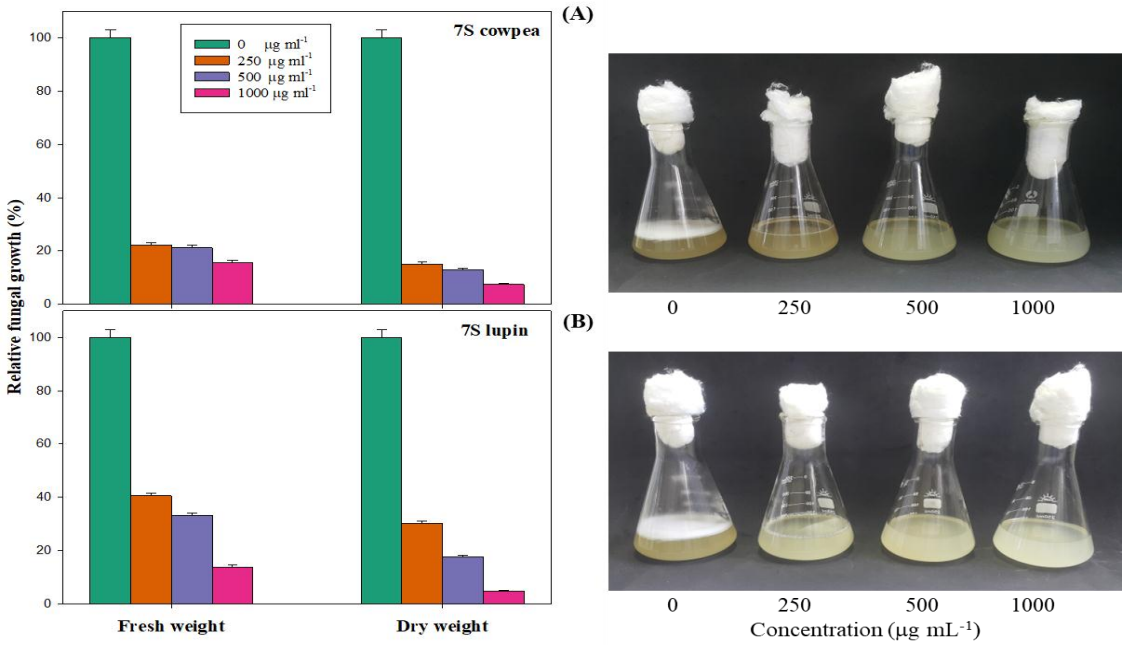

Fig. 2. Fungal fresh and dry weight in Liquid media 7 days' post incubation at 25 °C in the presence of 7S globulin from Cowpea (A), and Lupin (B)

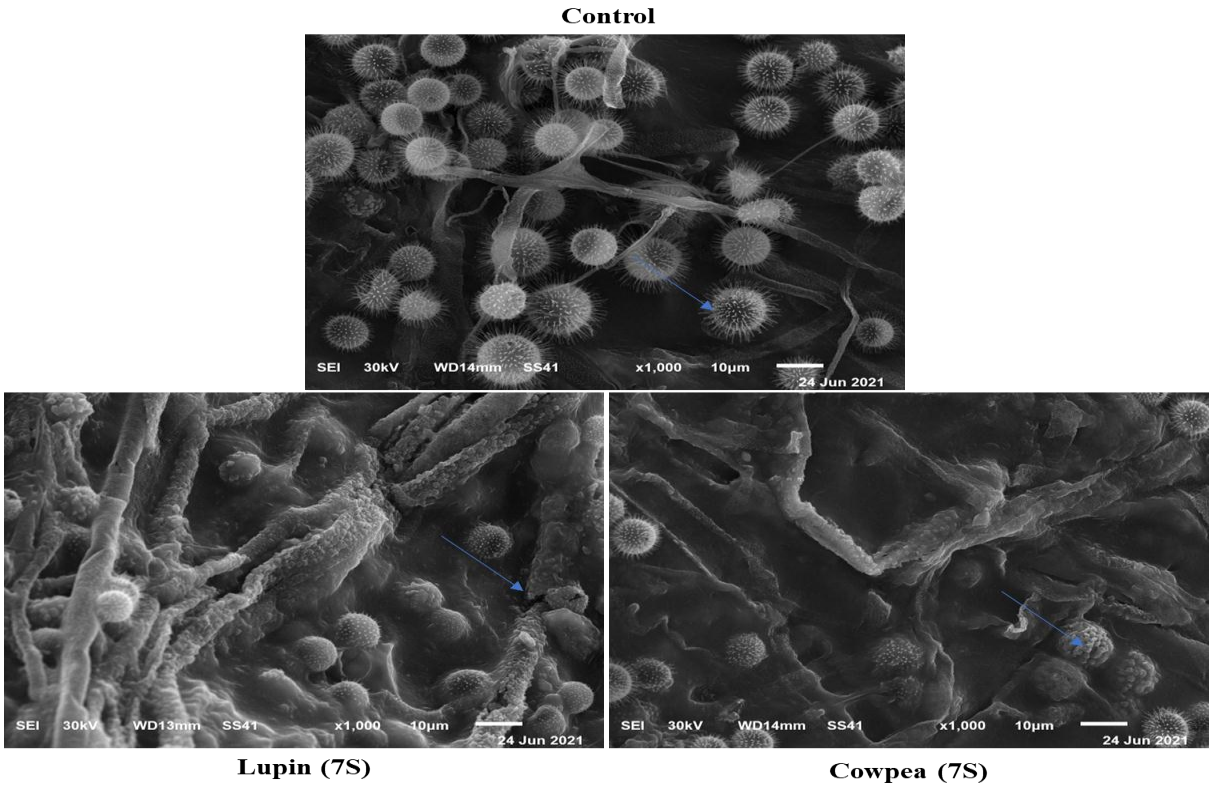

Fig. 3. Scanning Electronic Microscopic (SEM) of images *Pythium* sp. exhibiting the morphological characteristics of the fungus-like microorganism in its normal status and after being subjected to 500 µg/mL of Lupin seed 7S and Cowpea seed 7S.

The assays for antifungal activity include microtiter broth assays, agar diffusion assays, broth microdilution assays (**Hadacek *et al.*, 2000**), and in plant assays, the determination of resistance of transgenic plants overexpressing a protein of interest. Most of the antifungal proteins described below are quite potent, with MICs in the micromolar or µg/mL range, equivalent to MICs of many agricultural antifungal compounds.

Fungal Control in Post Harvesting

The *in vivo* tests showed that both Cowpea 7S globulin and Lupine 7S globulin effectively reduced the incidence of postharvest infection on Cucumber fruits caused by *Pythium* sp. (Fig. 4 and Table 2). Both antifungal agents inhibited the postharvest pathogen mainly via their direct effect on the mycelial growth and sporangia, potentially affecting the pathogen's cellular metabolism. The results of the antifungal activities of 7S globulin from the two plant sources were higher *in vitro* than under *in vivo* conditions, due probably to the interference of different constituents in the complex bio-environmental system. However, the *in vivo* results utilizing 7S globulin from cowpea and Lupin as a postharvest treatment on Cucumber fruits nearly simulate the *in vitro* results, but understandably with a lesser magnitude. The *in vivo* results generally minimized the incidence of *Pythium* sp. Infection and extended the fruit shelf life. A medium concentration of 7S globulin treatment (500 µg/mL) from the two sources (Lupine and Cowpea) could protect 100% of the postharvest fruits from disease incidence without distinction between the two sources. So, to prolong the shelf life of the postharvest Cucumber fruits, it may be advisable to have one spray treatment in a solution of 7S globulin treatment (500 µg/mL) from either Lupine seed or Cowpea seed. Studies on the subcellular level revealed that antifungal protein accumulated in the cell wall. Most plant fungal pathogens have an extracellular location during the colonization process. Following the *in vitro* results, the *in vivo* results proved high potency of 7S globulins from Cowpea and Lupine seeds to minimize the incidence of *Pythium* sp. infection and extend the fruit shelf life of the postharvest Cucumber fruits. A low concentration of 7S (500 µg/mL) from the two sources was sufficient to protect (100%) the postharvest fruits from disease incidence. The antimicrobial peptides and proteins are part of the innate immune system, acting as the first line of defence against microbial attack by limiting infections in the early hours **(Fusco *et al.*, 2021)**.

Assessment of Fruit Quality Parameters

The data in Fig. 6A indicated considerable reductions in the hardness of the control Cucumber fruits infected with *Pythium* sp. and kept under cold conditions for 21 days in a time-dependent manner. Most pronounced reduction in Cucumber fruit hardness appeared in the case of the untreated Cucumber infected with *Pythium* sp. after 21 days of cold storage. Applying 7S globulin from Cowpea and Lupin at 500 µg/mL on Cucumber fruits inoculated with *Pythium* sp. improved their hardness quality during 21 days of cold storage. The keeping effect of 7S globulin from Cowpea seemed better than 7S from Lupine cucmber fruit inoculated with *Pythium* sp.

In parallel, the effect of 7S globulin from Cowpea and Lupin at 500 µg/mL on the weight of Cucumber fruits inoculated with *Pythium* sp., is presented in Fig. 5B. Compared to the control, weight loss rates were significantly lower across all treatments compared to the untreated control. After 21 days of storage, the fruit treated with 7S globulin from Cowpea and Lupin (500 µg/mL) lost 6.04% and 7.14% weight, respectively, compared to 19% weight loss in the untreated control.

Conclusions

The status of research suggests that there are indeed alternatives to replace the synthetic fungicides for management of this notorious soil as well as seed borne fungi: *Pythium*, which causes big agricultural losses. However, the use of synthetic fungicides can have harmful effects as well as many of the commonly used synthetic fungicides are unable to control *Pythium* species as it has got resistant against these synthetic fungicides. So, the use of bio-fungicides proved to be economical alternative that can be implemented at the farm level.

Chemical analysis proved the structural similarity between 7S from Cowpea seed and Lupine seeds and an acid nature protein of an isoelectric point at 4.5. *In vitro* studies revealed evident reduction of the mycelial growth of

Fig. 4. ***In vivo*** **antifungal assay indicated no rot observed in cucumber fruit treated with 7S Cowpea and 7S Lupin until 21-day storage while fruit rot observed after 15, and 21 days in control treatment and more developed in 21 day**

Table 2. Disease incidence (%), and disease severity (%)

Treatment	Time (days)	Disease incidence (%)	Disease severity (%)
Control	15	66.66	65
	21	66.66	75
7S Cowpea	15	0	0
	21	0	0
7S Lupin	15	0	0
	21	0	0

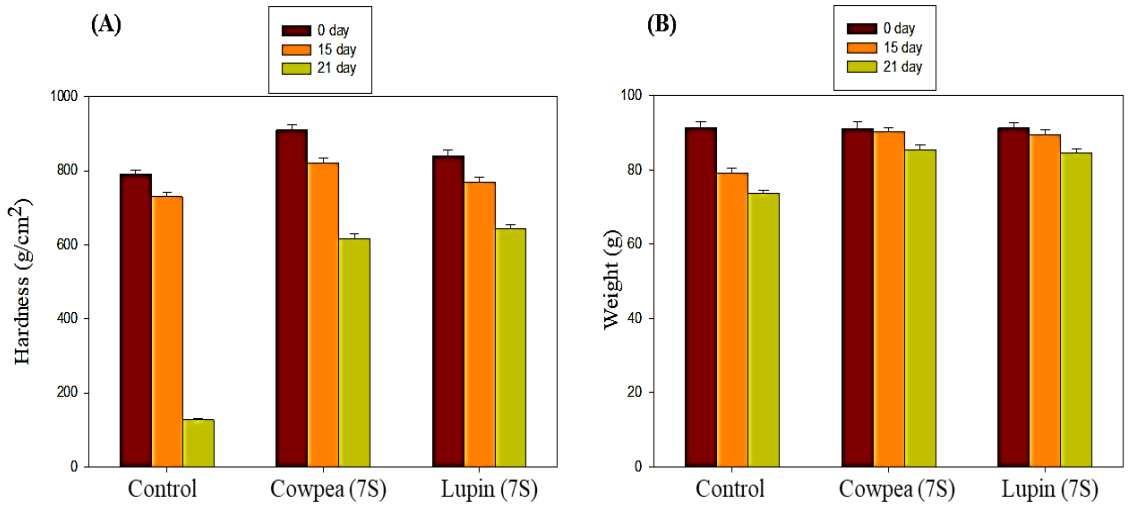

Fig. 5. The effect of 7S globulin from Cowpea and Lupin on hardness (A), and weight (B) in Cucumber fruits inoculated with *Pythium* sp. after 21 days of cold storage, compared to control

Pythium sp. in liquid media by 7S globulin from Cowpea and Lupin in a concentration-dependent manner evidencing the antifungal activity of these proteins. The SEM analysis of *Pythium* sp. manifesting the morphological changes in the hyphae and sporangia when subjected to 7S evidenced the killing action of protein. This may mean that treating the infected Cucumber fruits with these proteins directly affects the microorganism causing the plant disease. The *in vivo* results proved high potency of 7S globulins from Cowpea and Lupine seeds to minimize the incidence of *Pythium* sp. infection and extend the postharvest shelf life of Cucumber fruits.

Acknowledgments

Authors are thankful to Science and Technology Development Fund (STDF), Egypt, for funding Project ID: 43673 entitled "Fighting Plant Fungi Post-Harvest Using Environmentally Friendly Bio-Products' to support this research work.

REFERENCES

Abbas, E., A. Osman and M. Sitohy (2020). Biochemical control of *Alternaria tenuissima* infecting post-harvest fig fruit by chickpea vicilin. J. Sci. Food and Agric., 100 (7): 2889 - 2897.

Abdel-Shafi, S., A. Osman, G. Enan, M. El-Nemer and M. Sitohy (2016). Antibacterial activity of methylated egg white proteins against pathogenic G+ and G− bacteria matching antibiotics. Springer Plus, 5: 1-13.

Al-Balushi, Z.M., H. Agrama, I.H. Al-Mahmooli, S.S. Maharachchikumbura and A.M. Al-Sadi (2018). Development of resistance to hymexazol among *Pythium* species in Cucumber greenhouses in Oman. Plant Disease, 102 (1): 202-208.

Alsohaimy, S.A., M.Z. Sitohy and R.A. El-Masry (2007). Isolation and partial characterization of chickpea, Lupine and lentil seed proteins. World J. Agric. Sci., 3(1): 123-129.

Arafa, R.A., S.M. Kamel and K.A. Abd-Elsalam (2020). The genus *Pythium*: genomics and breeding for resistance. *Pythium*: Diagnosis, Dis. and Manag., 270-286.

Atallah, O.O., A. Osman, M.A. Ali and M. Sitohy (2021). Soybean β-conglycinin and catfish cutaneous mucous p22 glycoproteins deteriorate sporangial cell walls of *Pseudoperonospora cubensis* and suppress cucumber downy mildew. Pest Manag. Sci., 77(7): 3313-3324.

Babadoost, M. (2016). Oomycete diseases of cucurbits: History, significance, and management. Hort. Rev., 44: 279-314.

Carmona, M.A., F.J. Sautua, P.E. Grijalba, M. Cassina and O. Pérez-Hernández (2018). Effect of potassium and manganese phosphites in the control of *Pythium* damping-off in soybean: a feasible alternative to fungicide seed treatments. Pest Manag. Sci., 74 (2): 366 - 374.

Chakravarthula, M. (2021). Taxonomy and Ecology of Soil Fungi in India: Aspects and Prospects. Progress in Mycology: An Indian Perspective, 439-490.

Chatterton, S. and Z.K. Punja (2009). Interactions between *Clonostachys rosea*, *F. catenulata*, *Fusarium oxysporum* and cucumber roots leading to biological control of fusarium root and stem rot. Recent Dev. Manag. Plant Dis., 93-106.

Dhakar, K., A. Sharma and A. Pandey (2014). Cold, pH and salt tolerant *Penicillium* spp. inhabit the high-altitude soils in Himalaya, India. World J. Microbiol. and Biotechnol., 30: 1315-1324.

Deepak, A.V., G. Thippeswamy, M.N. Shivakameshwari and B.P. Salimath (2003). Isolation and characterization of a 29-kDa glycoprotein with antifungal activity from bulbs of *Urginea indica*. Biochem. and Biophysical Res. Commun., 311(3): 735-742.

Fusco, A., V. Savio, M. Donniacuo, B. Perfetto and G. Donnarumma (2021). Antimicrobial peptides human beta-defensin-2 and-3 protect the gut during candida albicans infections enhancing the intestinal barrier integrity: in vitro study. Frontiers in Cellular and Infection Microbiol., 11: https://doi.org/10.3389/fcimb.2021.666900

Hadacek, F. and H. Greger (2000). Testing of antifungal natural products: methodologies, comparability of results and assay choice. Phytochem. Anal.s: An Int. J. Plant Chem. and Biochem. Techniques, 11(3): 137-147.

Jiang, Y. N., Haudenshield, J. S., & Hartman, G. L. (2012). Characterization of Pythium spp. from soil samples in Illinois. Canadian J. Plant Pathol., 34(3): 448-454.

Johnson, E.A. and C.J. Brekke (1983). Functional properties of acylated pea protein isolates. J. Food Sci., 48 (3): 722-725.

Khan, M., B. Li, Y. Jiang, Q. Weng and Q. Chen (2017). Evaluation of different PCR-based assays and LAMP method for rapid detection of *Phytophthora infestans* by targeting the Ypt1 gene. Frontiers in Microbiol., 8: 1920.

Kolte, S.J. (2019). Diseases of Annual Edible Oilseed Crops: Volume I: Peanut Diseases. CRC press.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat., 227(5259): 680-685.

McCarty, L.B. and G. Miller (2002). Managing bermudagrass turf: Selection, construction, cultural practices, and pest management strategies. John Wiley and Sons.

Nagano, T. (1992). Dynamic viscoelastic study on the gelation properties of β-conglycinin. J. Agric. Food Chem., 40: 941-944.

Osman, A., E. Abbas, S. Mahgoub and M. Sitohy (2016). Inhibition of *Penicillium digitatum in vitro* and in postharvest orange fruit by a soy protein fraction containing mainly β-conglycinin. J. General Plant Pathol., 82: 293-301.

Patil, A., A. Laddha, A. Lunge, H. Paikrao and S. Mahure (2012). *In vitro* antagonistic properties of selected *Trichoderma* species against tomato root rot causing *Pythium* species. Int. J. Sci., Environ. and Technol., 1 (4): 302-315.

Ravnskov, S., C. Cabral and J. Larsen (2020). Mycorrhiza induced tolerance in *Cucumis sativus* against root rot caused by *Pythium ultimum* depends on fungal species in the arbuscular mycorrhizal symbiosis. Biol. Control, 141: https://doi.org/10.1016/j.biocontrol.2019.104133.

Schroeder, K.L., F.N. Martin, A.W. de Cock, C.A. Lévesque, C.F. Spies, P.A. Okubara and T.C. Paulitz (2013). Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. Plant Dis., 97 (1): 4-20.

Schwarz, D., U. Beuch, M. Bandte, A. Fakhro, C. Büttner and C. Obermeier (2010). Spread and interaction of Pepino mosaic virus (PepMV) and *Pythium aphanidermatum* in a

closed nutrient solution recirculation system: effects on tomato growth and yield. Plant Pathol., 59 (3): 443-452.

Sels, J., J. Mathys, B.M. De Coninck, B.P. Cammue and M.F. De Bolle (2008). Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol. and Biochem., 46 (11): 941-950.

Selitrennikoff, C.P. (2001). Antifungal proteins. Appl. and Environ. Microbiol., 67 (7): 2883-2894.

Sen, S. (2006). Studies on Cymbidium rot and its management by biocontrol agents (Doctoral Dissertation, North Bengal Univ.). https://ir.nbu.ac.in/bitstream/123456789/921/14/202020.pdf

Simpson, R.J., M.R. Shafique, H.A., V. Sultana, S. Ehteshamul-Haque and M. Athar (2016). Management of soil-borne diseases of organic vegetables. J. Plant Prot. Res., 56: (3): 221-230.

Neuberger and T.Y. Liu (1976). Complete amino acid analysis of proteins from a single hydrolysate. J. Biol. Chem., 251 (7): 1936-1940.

Sitohy, M., M. Doheim and H. Badr (2007). Isolation and characterization of a lectin with antifungal activity from Egyptian *Pisum sativum* seeds. Food Chem., 104 (3): 971-979.

Sitohy, M. and A. Osman (2010). Antimicrobial activity of native and esterified legume proteins against Gram-negative and Gram-positive bacteria. Food Chem., 120 (1): 66-73.

Sitohy, M.Z., S.A. Mahgoub and A.O. Osman (2012). *In vitro* and *in situ* antimicrobial action and mechanism of glycinin and its basic subunit. Int. J. Food Microbiol., 154 (1-2): 19-29.

Souillac, P.O., C.R. Middaugh and J.H. Rytting (2002). Investigation of protein/carbohydrate interactions in the dried state. 2. Diffuse reflectance FTIR studies. Int. J. Pharm., 235 (1-2): 207-218.

Teoh, Y.P., M.M. Don and S. Ujang (2011). Media selection for mycelia growth, antifungal activity against wood-degrading fungi, and GC-MS study by *Pycnoporus sanguineus*. Bio-Res., 6 (3): 2719-2731.

Uzuhashi, S., G. Okada and M. Ohkuma (2015). Four new *Pythium* species from aquatic environments in Japan. Antonie Van Leeuwenhoek, 107: 375-391.

Verma, C., B.K. Gupta, A. Singh, A. Kujur, R. Sahu and P. Prajapati (2020a). Biological control agents in the management of bell pepper nursery diseases: A review. J. Pharm. and Phytochem., 9 (5S): 253-259.

Verma, G., M. Shaquiquzzaman and M.M. Alam (2020b). Heterocyclic Drug Design and Development. In Promising Drug Molec. Nat. Origin. Appl. Acad., Press, 271-333.

Wang, N., Y. Ma, C. Yang, G. Dai and Z. Wang (2008). rDNA-ITS sequence analysis of pathogens of cucumber downy mildew and cucumber powdery mildew. Frontiers of Agric. China, 2: 317-320.

Yang, J., P.D. Kharbanda and M. Mirza (2002, August). Evaluation of Paenibacillus polymyxa pkb1 for biocontrol of Pythium disease of cucumber in a hydroponic system. In *XXVI* Int. Hort. Congress: Managing Soil-Borne Pathogens: A Sound Rhizosphere to Improve Productivity in 635: 59-66.

Zhao, S., C.M. Du and C.Y. Tian (2012). Suppression of *Fusarium oxysporum* and induced resistance of plants involved in the biocontrol of Cucumber Fusarium Wilt by *Streptomyces bikiniensis* HD-087. World J. Microbiol. and Biotechnol., 28: 2919-2927.

النشاط المضاد للفطريات لبعض الجليكوبروتينات البقوليه ضد عفن ثمار الخيار المتسبب عن فطر *Pythium* sp.

انتصار عباس[1] – على عثمان[2] – فريد محسن[3] – محمود سطوحى[2]

1- قسم أمراض النبات – كلية الزراعة – جامعه الزقازيق – مصر
2- قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعه الزقازيق – مصر
3- قسم البساتين – كلية الزراعة – جامعه الزقازيق – مصر

تهدف هذه الدراسة الى تقييم النشاط المضاد للفطريات لبعض الجليكوبروتينات البقولي المفصولة من اللوبيا والترمس ضد فطر *Pythium* sp. معمليًا وضد عفن ثمار الخيار المتسبب عن هذا الفطر. تم تعريف الفطر المختبر في معمل بحوث أمراض النبات بقسم أمراض النبات بكلية الزراعة جامعة الزقازيق، وتم تعريفه على أنه *Pythium* sp. يحتوى الجليكوبروتين المسمى 7S جلوبيولين الذي تم فصله من اللوبيا على نسبة عالية من إجمالي الأحماض الأمينية (78.2%) مقابل 77.27% للجليكوبروتين المفصول من الترمس. بالتفريد الكهربى لكلا البروتينين باستخدام التفريد فى مجال كهربى تبين أن لهم نفس الوحدات البنائية. يتأثر النمو الفطري لـ *Pythium* sp. فى وجود 7S جلوبيولين من الترمس واللوبيا فى البيئة السائلة حيث ينخفض النمو بشكل واضح مع زيادة التركيز. بفحص الفطر تحت الميكروسكوب الالكترونى الماسح (SEM) فى وجود 7S جلوبيولين من الترمس واللوبيا (500 ميكروجرام/ ملل) تبين وجود تشوهات وتغيرات مورفولوجية فى الفطر المعامل بالبروتينات مقارنه بالكنترول. أظهرت الاختبارات التى أجريت على ثمار الخيار بعد الحصاد تأثير البروتينات بشكل فعال على جودة الثمار وتقليل شدة الاصابة بالفطر *Pythium* مقارنة بالكنترول. ويمكن الاستنتاج أنه من الممكن استخدام بعض البروتينات الطبيعية كبدائل للمبيدات الفطريه المخلقة.

المحكمـــــون:
1- أ.د. حسان محمد محمد مصطفى الذهبي أستاذ أمراض النبات المتفرغ - قسم النبات الزراعي - كلية الزراعة - جامعة طنطا.
2- أ.د. محمـــد رضا أحمـــد تهـــــامي أستاذ أمراض النبات - قسم أمراض النبات- -كلية الزراعة- جامعة الزقازيق.