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BIOCHEMICAL CHARACTERIZATION OF WHEAT SEED LECTIN AND ITS ANTIFUNGAL ACTIVITY AGAINST SEED-BORNE *FUSARIUM GRAMINEARUM IN-VITRO* AND *IN-SITU*

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Abstract

The objective of the study was to examine the inhibitory effect of lectin as a glycoprotein isolated from wheat seeds (*Triticumaestivum*) against the fungus *Fusarium graminearum in vitro*. The purified wheat seed lectin is a glycoprotein composed of two subunits of 111 and 15 kDa with an isoelectric point of 6.0. The most abundant sugars found in lectin are galactose (160 mg) followed by galacturonic acid (80 mg) and arabinose (60 mg) per 100g sample respectively. The antioxidant activity of the wheat seed lectin investigated in the study revealed dose-dependent variations in DPPH (2,2-Diphenyl-1- picrylhydrazyl) free radical scavenging activity, with a low IC_{50} value (4.3 µg/mL). Lectin prevented the growth of mycelium at a broad concentration range (100–400 µg/mL). Maximum inhibition was observed at a concentration of 400 µg/mL, which is equivalent to 77.77% of linear growth reduction after seven days of incubation at 25ºC. The effect of the seed dressing treatment with lectin at various concentrations (1000, 200, and 400 µg/mL) on the percentage of pre- and post-emergence of damping-off grown in *Fusarium graminearum* infested soil under greenhouse conditions was also estimated. The results reveal that all the examined lectin concentrations decreased damping-off in comparison to the control. The study shows that lectin can be applied as an antifungal agent against wheat damping-off.

Key words: Lectin; Antifungal activity; Damping-off; Cereal; *Fusarium graminearum*.

Introduction

Damping-off of cereal is a common plant disease that minimizes grain evolution, and leads to poor plant stand and yield (Luz *et al.*, 1998). The damping-off disease of wheat and other cereal crops is caused by the plant pathogenic fungus *Fusarium graminearum* (Fakhrunnisa *et al.*, 2006). Damping-off may occur during the pre- and post-emergence of seedlings, culminating in the reduced development of the infected plants. Fungicides are applied widely to address abundant soil-borne diseases, but their performance is inconsistent. With growing concerns over the negative environmental impact of most synthetic fungicides, public interest has increased around alternate procedures for controlling crop diseases (Mao *et al.*, 1997; Osman *et al.*, 2016). Consequently, the desire to explore new antifungal agents as substitutions for artificial fungicides is pressing. Natural products are components with a broad structural variety that represent a significant source of novel chemical components with possibly important antimicrobial activity (Abdel-Shafi *et al.*, 2019a). Glycoproteins are a major group of such natural products that have been found to play a pivotal function in biological procedures (Abdel-Shafi *et al.*, 2019b). In particular, lectins are members of natural glycoproteins that are associated with the antifungal activity (Lannoo *et al.*, 2014). Lectins are found in different plant species and are known to be involved in various biological functions, most importantly as a cell-cell communicator (Baker *et al.*, 2009). Furthermore, lectins are found in relatively high concentrations and can be easily extracted from popular dietary staples, such as legumes and cereals (Matucci *et al.*, 2004; Cheema *et al*., 2010). The range of plant-based lectins is diverse, and already a variety of these biomolecules have been identified. Research has been directed towards categorizing their molecular structures and bioactivity (Lannoo *et al*., 2014). Novel lectins with antimicrobial activity have been isolated in numerous plant species including mistletoe (*Viscum album*) (Costa *et al*., 2010), rhizomes of stinging nettle (*Urticadioica* L.) (Broekaert *et al*., 1989) as well as jack fruit (*Artocarpus integrifolia*) (de Azevedo & Ainouz, 1981).

A significant amount of research has been directed towards wheat germ agglutinin (WGA), the principal lectin found in wheat seeds (Allen *et al*., 1973, Caruso *et al*., 1996, Tong *et al*., 2020). WGA lectin has been isolated and purified, and shown to possess enhanced antimicrobial effects, particularly in inhibiting the growth of destructive fungal species (Mirelman *et al*., 1975). The main reason for their antifungal activity is due to the selective binding properties of the lectin (Nagata & Burger, 1974). WGA lectin has shown a high affinity for binding to chitin, which is the main constituent of fungal cell walls, thus the growth and proliferation of fungal infestation is prevented in the presence of WGA lectin (Dias *et al*., 2015).

Although the antimicrobial activity of WGA lectin is well known, there is a lack of information regarding the utilization of wheat seed extracts in treating fungal infections. Furthermore, it has been established that due to factors such as the globalization of the food industry, as well as climate change, there is an increase in both the frequency and severity of food contamination through mycotoxins (Marroquín-Cardona *et al*., 2014; Marin *et al*., 2013). Therefore, low cost, non-toxic agents for the treatment of such fungal infections are sorely needed.

The present work aims to evaluate the inhibitory effect of the lectin from wheat seeds against the fungus *Fusarium graminearum In vitro*, and the possible

application of this lectin under greenhouse conditions as a seed dressing for preventing wheat seeds damping-off in *Fusarium graminearum* infested soil. This study may help in exploring a potential low cost, efficient antifungal agent for enhanced agricultural practices. Moreover, the seed dressing treatment with lectins may minimize the use of synthetic fungicides and thereby reduce environmental toxicity.

Materials and Methods

The wheat seeds (*Triticum aestivum*) cultivar Giza 168 were obtained from the local market and Ministry of Environment, Water and Agriculture, Saudi Arabia, for isolation and purification of lectin. The plant pathogen *Fusarium graminearum* was obtained from the Department of Microbiology, College of Science, Princess Nourah bint Abdulrahman University, Saudi Arabia. Molecular mass markers for SDS–PAGE were purchased from Sigma (St. Louis, MO, USA). 3-(2-amino ethyl) indole (hydrochloride) and methanesulfonic acid was procured from Eastman Organic Chemicals, Rochester, N.Y. SDS, glycerol (analytical grade), β-mercaptoethanol, Tris HCl, and bromophenol blue were obtained from Amresco (Solon, OH, USA). Calibration mixtures of amino acids were purchased from the Pierce Chemical Co. All other reagents were obtained from Sigma and were either of analytical grade or the highest quality available.

Isolation and purification of antifungal protein: One Kg of wheat seeds was ground and extracted in 1L of 0.01 M phosphate-buffered saline (pH 7.2) followed by centrifugation at 5000× g at 4°C for 15 min. The precipitate was discarded and solid ammonium sulfate was added to the supernatant up to 60% saturation level. This was followed by continuous stirring overnight at 4°C, and precipitate obtained through centrifugation (1200× g for 15 min) was dialyzed against a 10 mM sodium bicarbonate buffer (pH 8.0). The fraction obtained by dialysis was incubated at room temperature for 1 hour in a mixture of 100g colloidal chitin added to 200 mL of 10 mM sodium acetate buffer (pH 8.0) to remove chitinase and chitin-binding proteins. The chitin free fractions were further centrifuged and dialyzed against a 10 mM sodium acetate buffer (pH 6.0). The fractions collected were then evaluated for antifungal activity (Mostafa *et al*., 2019).

DPPH radical scavenging assay: The DPPH free radical-scavenging activity of crude lectin was determined, as described by Oyaizu, (1986). DPPH (2,2-Diphenyl-1- picrylhydrazyl) radical scavenging assay was used to measure the antioxidant capacity of lectin. A volume of 0.5 ml of lectin samples at different concentrations (100, 200, 300, 400, 500 µg/mL) was added to 0.375 mL of ethanol (99%) and 0.125 mL of DPPH solution (0.02% in ethanol). The mixtures were then incubated at room temperature for 60 min in the dark. Absorbance values were measured spectrophotometrically at 517 nm, the absorbance band of DPPH. The absorbance decreases with the decrease in the antiradical compound. The gradual decrease in the absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity of the tested compound. Similarly, different concentrations of ascorbic acid were used as a standard antioxidant (positive control). Scavenging activity of DPPH free radical was calculated using the equation:

$$\text{DPPH antiradical scavenging activity \%} = (A-B)/(A) \times 100$$

where A is the absorbance of the control reaction (containing all reagents except the sample) and B is the absorbance of the test sample (with DPPH solution).

IC_{50} values were estimated from the calibration curve and are the amount of test sample required to scavenge 50% of DPPH radicals.

Antifungal agent characterization

Electrophoretic analysis: 20 mg from wheat seeds lectin was dissolved in 1mL sodium dodecyl sulfate for 10 minutes. Then, it was centrifuged at 10,000× g for 15 min. 20 µL of the extract was blended with a loading buffer [SDS 4% and 3%, glycerol 20% v/v, β-mercaptoethanol 5% v/v, Tris HCl 50 mM (pH 6.8) and traces of bromophenol blue (0.1 mg/mL)]. Ten microliters were loaded per lane as per the standard method (Laemmli *et al*., 1970).

Iso-electric point estimation: The iso-electric point was measured by using protein pH solubility curves at different pH values ranging from 2 to 10, according to the protocol outlined by Sitohy & Osman (2010).

Amino acids analysis: The composition of amino acids for lectin isolated from wheat seeds was evaluated according to the method set out by Simpson *et al*., (1976), using an amino acid analyzer ("Eppendorf LC3000"), as described by Abdel-Shafi *et al*., (2016).

Carbohydrates analysis by HPLC: The carbohydrates analysis of lectin was estimated by HPLC as per the standard procedure (Wilson *et al*., 2005).

In-vitro antifungal activity

Linear growth of *Fusarium graminearum* using potato dextrose agar (PDA) media: The effect of lectin isolated from wheat seeds was tested at several concentrations (0, 100, 200, and 400 µg/mL) on the linear growth of *Fusarium graminearum* using PDA as the media, as described by Abdel-Shafi *et al*., (2019a). Linear growth was calculated as follows:

Scanning electron microscopy (SEM): *Fusarium graminearum* was treated with isolated wheat lectin (0 and 400 µg/mL) for 5 h at room temperature and was subjected to SEM analysis, as according to Sitohy *et al*., (2013).

Effect of lectin on wheat damping-off (under greenhouse conditions): *Fusarium graminearum* was applied to infest the experimental soil. To gain a higher amount of mycelia, 1 cm disc of actively grown fungi on PDA media was transported to an Erlenmeyer flask (250 mL) containing a yeast extract broth (100 mL) and held in the dark for 12 days at 25ºC. Finally, the mycelial mat from the Erlenmeyer flasks was washed with distilled water, and 1 g was mixed with 200 mL distilled water for 1 min. The mixed mycelia (100 mL) were subjected to infest the soil (5 Kg/pot) (Abdel-Monaim *et al*., 2011).

Three days before planting, wheat seeds were surface sterilized with 2% sodium hypochlorite for 1min and rinsed three times with distilled water. The disinfected wheat seeds were drenched in different concentration of lectin solution (100, 200, and 400 µg/mL) for 12 h. The seeds were dispersed in inoculated pots at a rate of six seeds/pot. Likewise, in the control treatment, wheat seeds were drenched in distilled water and dispersed in *Fusarium graminearum* inoculated soil following the above procedure. For each treatment, three pots were used. Pre- and post-emergence damping-off (%) was recorded during and after 45 days from planting, respectively.

$$\text{Linear growth reduction (\%)} = \frac{\text{[Control growth – Treatment growth]}}{\text{[Control growth] x 100}}$$

Statistical analysis: The statistical analyses were performed using SPSS (version 19.0) and data was expressed as mean ± SD. The data on the effect of different concentrations of lectin on pre- and post-emergence damping-off and plant survival were analyzed by using analysis of variance (ANOVA), and treatment means were compared according to the least significance difference (LSD) at $p<0.05$.

Results and Discussion

Lectin characterization

Electrophoretic analysis and isoelectric point estimation: The SDS-PAGE of wheat seeds lectin (Fig. 1) indicates two bands corresponding to the molecular weights (11 and 15 kDa). Similar to this, de Azevedo and Ainouz (1981) recorded two iso-lectins isolated from *Artocarpus integrifolia* L. using an ammonium sulfate precipitation method with a molecular weight of 11 and 15 KDa.

The iso-electric point (IEP) of lectin isolated from wheat seeds was estimated by pH solubility at different pH ranges from two to 10, and the results are presented in Fig. 2. The IEP (the pH at which the protein is less soluble) was presented in the form of pH- solubility curves of protein, and the minimal soluble points were gained at pH 6 (Osman *et al.*, 2018). The result corroborates with the findings of Mendoza-Blanco *et al*., (2012).

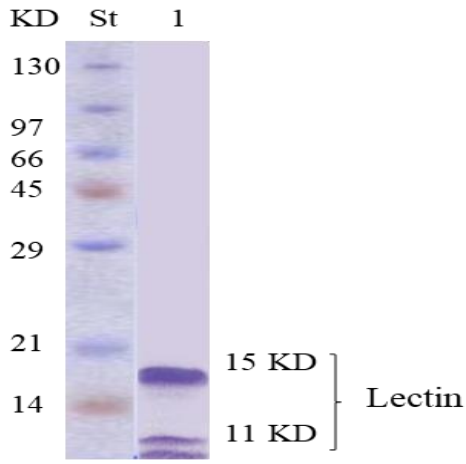

Fig. 1. SDS-PAGE of crude lectin isolated from wheat seeds (lane 1) compared to a standard protein marker (St).

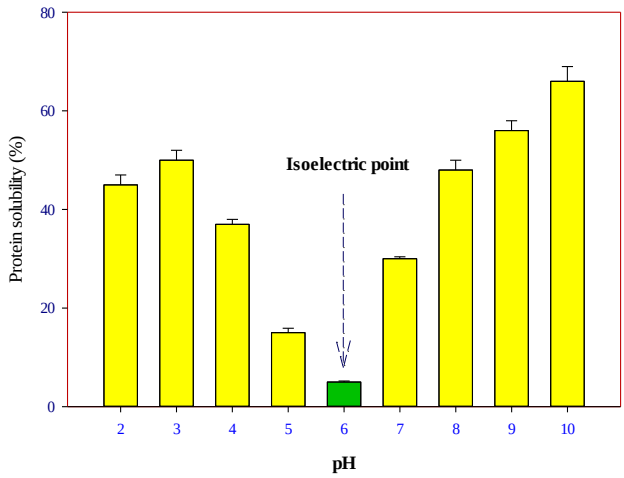

Fig. 2. The isoelectric point of lectin isolated from wheat seeds estimated by pH solubility at pH range 2 to 10. Error bars correspond to the standard deviation of these replications.

Amino acids analysis: Amino acids analysis indicated the presence of 15 amino acids in the lectin isolated from wheat seeds, and the results are presented in Fig. 3. The most abundant amino acids found in lectin are glutamic acid, aspartic acid, serine, valine, and lysine (11%, 10%, 8.9%, 6.4%, and 6.2%, respectively). The contents of the acidic amino acid residues (glutamic and aspartic acids) make up 21%, and the basic amino acids (lysine, arginine, and histidine) make up 13.8%. The contents of the essential amino acid residues (lysine, histidine, threonine, methionine, isoleucine, leucine, tyrosine, and phenylalanine) are 41.5%. Aspartic acid, glutamic acid, and serine are some of the amino acids in lectin isolated from different sources (Fornstedt & Poath, 1975).

Carbohydrate content of lectin: The carbohydrate analysis of lectin was carried out using HPLC, and the results are presented in Fig. 4. Lectin contains three saccharides, including two monosaccharides (arabinose and galactose) and one sugar acid (galacturonic). The most abundant sugars found in the lectin were galactose (160 mg) followed by galacturonic (80 mg) acid and arabinose (60 mg) per100 g sample, respectively. Similar results were obtained by Desai *et al*., (1981) in their studies on *Datura stramonium.*

DPPH radical scavenging activity: DPPH assay is one of the extensively used techniques for determining the antioxidant potential of plant-derived compounds. In the presence of an antioxidant, DPPH undergoes scavenging and gradually converts into 1,1-diphenyl-2-picrylhydrazine, with colour changes from purple to yellow. These changes indicate the scavenging potential of the tested compound. Thus, a gradual disappearance of absorption due to reduction in the antiradical compound is observed, which is used to measure the antioxidant properties. The current results have revealed a dose-dependent trend (100-500 µg/mL) in the DPPH radical scavenging activity of lectin. Maximum DPPH scavenging activity was observed at a concentration of 500 µg/ml lectin (Fig. 5). The DPPH scavenging activity obtained from the lectin of wheat seed was relatively higher compared to standard ascorbic acid. This result is supported by previous work on lectin, where the dose-dependent trend of DPPH scavenging activity was observed (Sadananda *et al*., 2014). A similar dose-dependent trend was reported by Loganayaki *et al*., (2013) where extracts from *Helicteresisora* fruits and *Ceiba pentandra* seeds were used to determine antioxidant activity. Saha *et al*., (2014), also observed significant free radical scavenging activity from crude lectin isolated from the seeds of *Lablabpurpureus.* Lower IC_{50} is the indicator of higher antioxidant activity. In the present study, lectin showed an IC_{50} value of 4.3 µg/mL compared to ascorbic acid (IC_{50} value of 2.4 µg/mL) taken as a positive control. The lower IC_{50} value of ascorbic acid is due to its strong antioxidant properties (Saha *et al*., 2014). The findings from the current study revealed lectin to be an important bioactive compound with rich antioxidant properties.

Antifungal activity *in vitro*: The antifungal activity of lectin isolated from wheat seed against *Fusarium graminearum* was estimated at several concentrations (100, 200, and 400 µg/mL) and compared to a control (Fig. 6A, B, and C**)**. Lectin prevented the growth of *Fusarium* mycelium at a concentration range of 100–400 µg/mL. In addition, lectin carried out maximum inhibition at 400 µg/mL after seven days of incubation at 25ºC and is equivalent to 77.77% of linear growth reduction. The antifungal activities of lectin isolated from different sources were reported in some of the earlier studies against different pathogenic fungal species (Chen *et al*., 2009; Maria das Graças *et al*., 2002; Kheeree *et al*., 2010; Tian *et al*., 2008; Yan *et al*., 2005).

Scanning electron microscopic images of *Fusarium graminearum* after treatment with lectin (400 µg/mL for 5 h at 25ºC) showed reduced mycelial growth (Fig. 7). The control fungus showed profuse hyphal growth with clear intact walls. After treatment with lectin, the hyphal structures were fully destabilized and malformed.

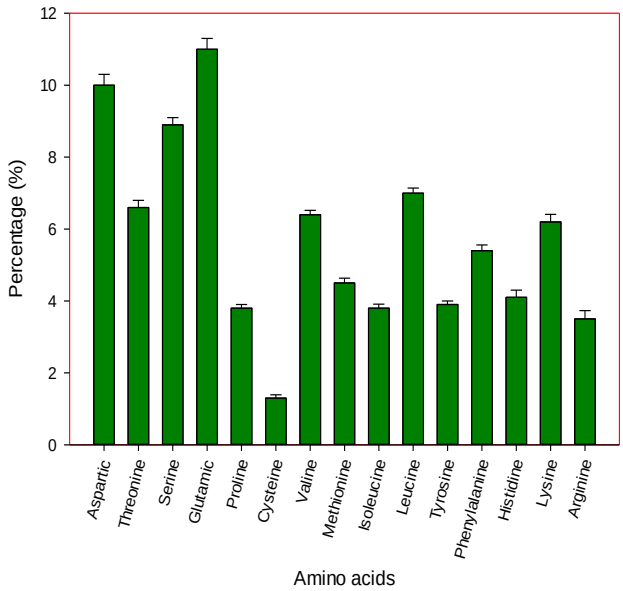

Fig. 3. Amino acids analysis of lectin (%) isolated from wheat seeds. Error bars correspond to the standard deviation of these replications.

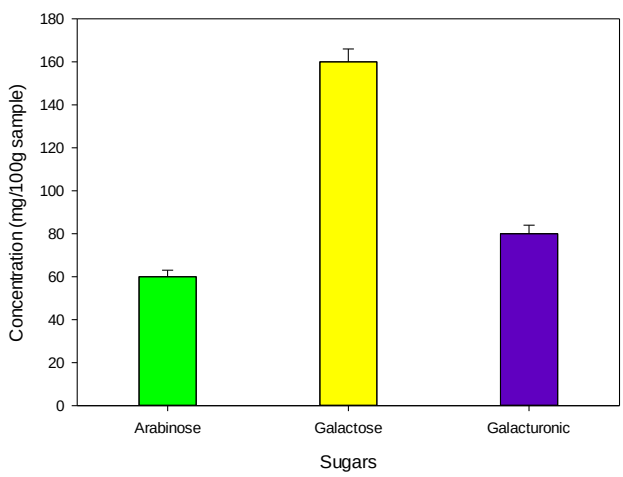

Fig. 4. Carbohydrate content of lectin (mg/100g sample) isolated from wheat seeds. Error bars correspond to the standard deviation of these replications.

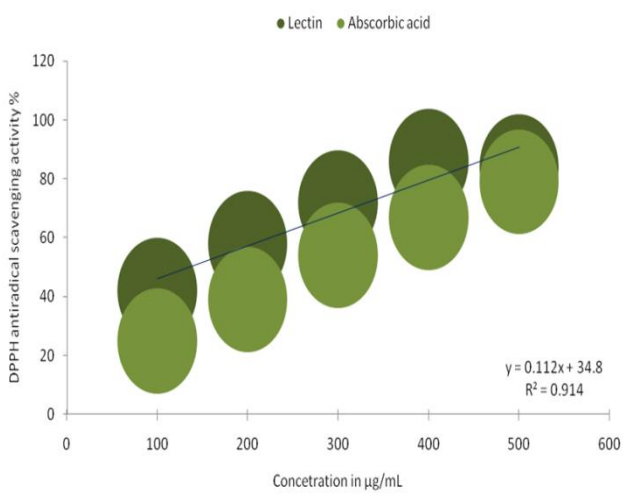

Fig. 5. Antioxidant activity of lectin at different concentrations compared to ascorbic acid as a standard.

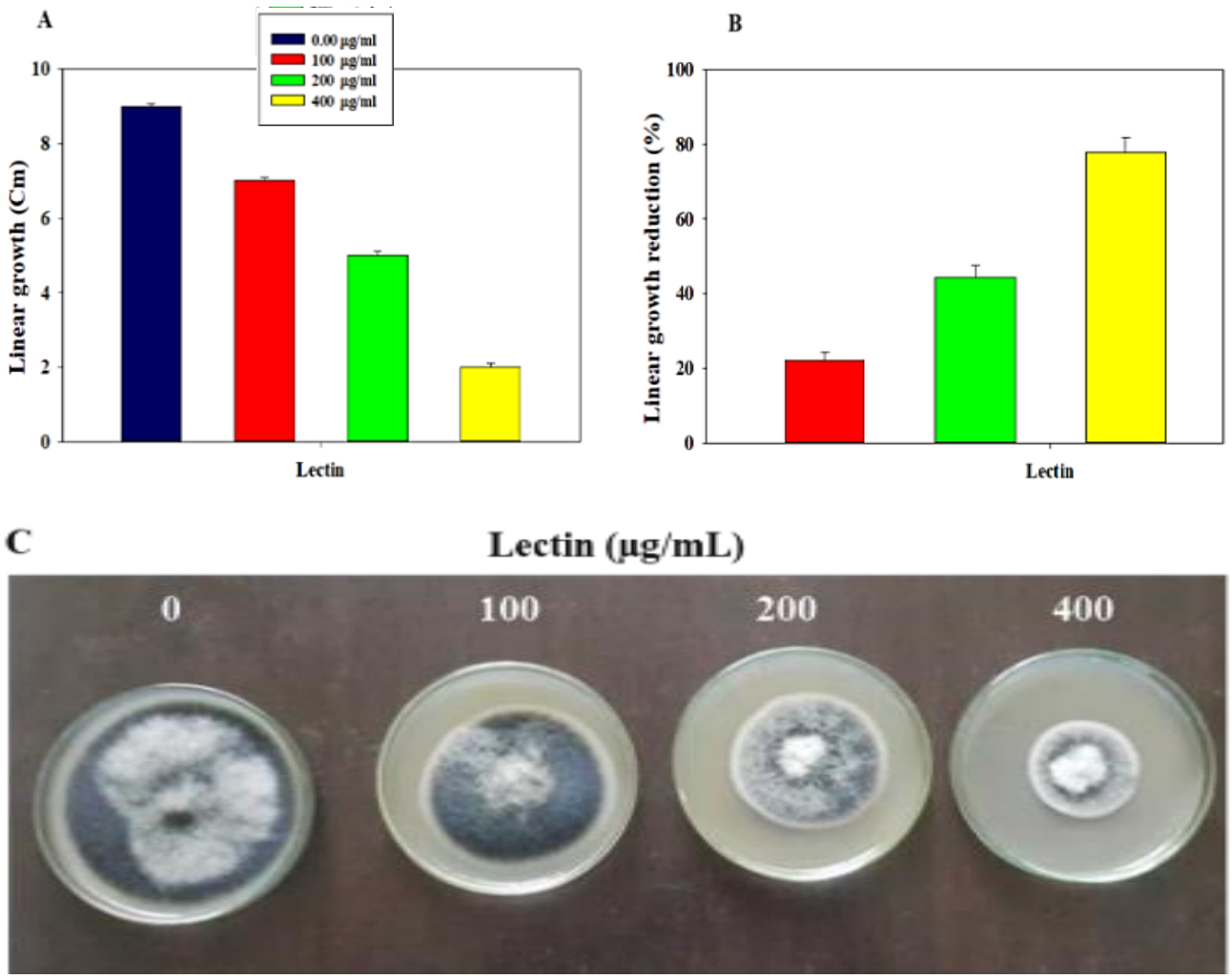

Fig. 6. Growth of *Fusarium graminearum*as expressed by (A) Linear growth of mycelium (cm), (B) Linear growth reduction (%) on potato dextrose agar medium (Error bars correspond to the standard deviation of these replications), and (C) Petri dishes showing zone of inhibition after 7 days of incubation at 25ºC, at different concentrations (0, 100, 200 and 400 µg/mL) of lectin.

Effect of lectin on wheat damping-off (under greenhouse conditions): The effect of seed dressing with lectin at different concentrations (100, 200, and 400 µg/mL) on the percentage of emergence of wheat seeds damping-off grown in *Fusarium graminearum* infested soil under greenhouse conditions was estimated. All the lectin concentrations tested showed a significant decrease in the damping-off of wheat seeds caused by *F. graminearum* under greenhouse conditions (Table 1). At 400 µg/mL concentration, lectin was most effective in controlling *F. graminearum* with seedling survivability of 83.33% (p<0.05). This was followed by a low concentration of lectin (200 µg/mL), with seedling survivability of 63.34% (p<0.05). Thus, the optimum concentration of inhibition in the present experiment was found to be 400 µg/mL. Different soil-borne fungi attack wheat during its different stages of growth, from seedling till ripening, causing damping-off. The results have established the efficacy of wheat seed extract (lectins) against *Fusarium* infestation - a species that is regarded as one of the most widespread sources of mycotoxins in the world (Nagata & Burger, 1974; Marroquín-Cardona *et al*., 2014). More importantly, with the increase in the concentration of wheat seed extract, till optimal, there is a remarkable increase in antimicrobial activity. Earlier studies demonstrate the use of eucalyptus and neem oils as topical antifungal agents, and were found to be ineffective in controlling the damping-off of seedlings (Somda *et al.*, 2007). Although lemongrass oils did show some potential for fungal control, such studies do not quantify a suitable concentration range of the extract to be used in an agricultural setting (Somda *et al*., 2007). A wide range of plant extracts (*Juglan scathayensis*, *Broussonetia papyrifera*, *Rumex dentatus*, *Glycyrrhiza uralensis*, *Sophora alopecuroides*, *Stellera chamaejasme*, and *Punica granatum*) have been studied for their antimicrobial activities, but most of them were found to be effective only at very high concentrations (1000 mg/mL) (Yu *et al*., 2001). This is more than two orders of magnitude higher than the concentrations required when using wheat seed extract as in the present study. In a more refined study, the minimum inhibitory concentrations of 22 plant extracts used in folkloric medicine were investigated (Ali-Shtayeh & Ghdeib, 1999), and there it was found that concentrations as low as 15 µg/mL can show antimicrobial effects. These are contrary to the present findings where concentrations in the range of 200-400 µg/mL were found to be effective.

Table 1. Effect of seed dressing with lectin at different concentrations (100, 200, and 400 µg/mL) on the pre- and post-emergence (%) of wheat seeds damping-off grown in *Fusarium graminearum* infested soil under greenhouse conditions.

Lectin concentration (µg/mL)	Pre-emergence (%)	Post-emergence (%)	Plant Survival (%)
100	$^{a}15.1 \pm 2.3$	$^{a}7.1 \pm 1.6$	$^{d}55.3 \pm 3.4$
200	$^{a}15.5 \pm 1.2$	$^{b}13.4 \pm 1.1$	$^{b}63.4 \pm 2.2$
400	$^{c}12.2 \pm 0.9$	$^{c}2.3 \pm 0.60$	$^{a}83.4 \pm 3.1$
Control	$^{d}16.3 \pm 2.6$	$^{d}15.9 \pm 1.9$	$^{c}56.5 \pm 3.1$

Values are mean ± standard deviation of three replicates. Values in the same column followed by different letters are significantly different at $p<0.05$

Disease monitoring measurement, which has been used to reduce infection from fungal disease, commonly implicates the utilization of fungicides (Matthiesen *et al*., 2016), breeding for disease resistance (Nagendran *et al*., 2009), chemical control (Konod *et al*., 2001), essential oils (Dhingra *et al*., 2004) and plant extracts (Niaz *et al*., 2008; Abdel-Monaim *et al*., 2011). However, research into plant-derived proteins like lectin as an antifungal agent is scarce and broader studies are required.

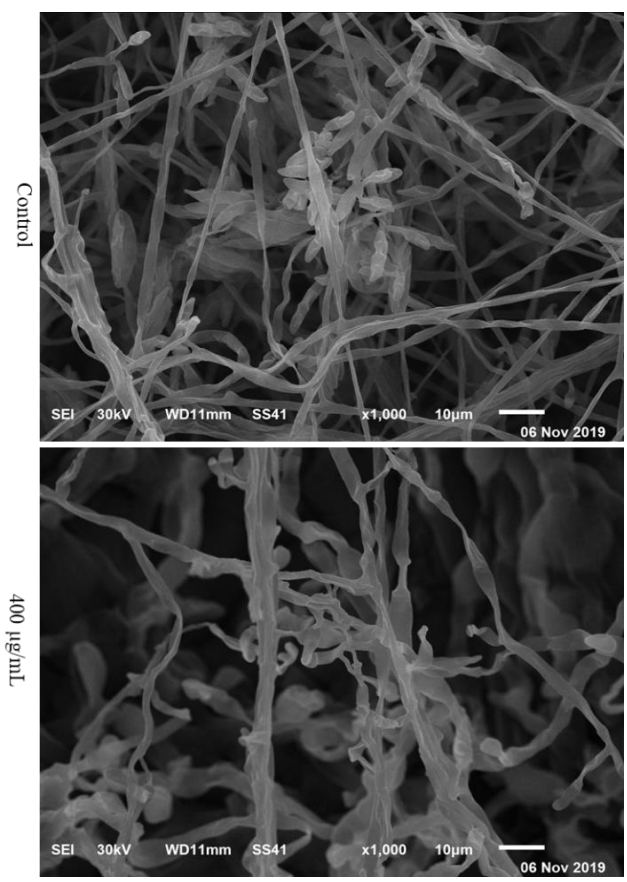

Fig. 7. Scanning electron microscope of *Fusarium graminearum* before and after treatment with lectin (at 400 µg/mL).

Conclusion

The results of the current work show that all of the lectin concentrations tested decreased damping-off and increased the survivability of plants, as compared to the control. It has been established that even at concentrations as low as 200 µg/mL, wheat seed extract can reduce seed damping-off. A concentration-dependent efficacy was observed with higher concentrations showing better results. The optimum concentration recorded in the present study is 400 µg/mL, at which, marked changes of fungal growth were observed. The present study has some significance for food security research. Moreover, it is a low-cost alternative and is an ideal candidate as a non-toxic biocontrol agent against wheat damping-off.

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References

Abdel-Monaim, M.F., K.A. M.Abo-Elyousr and K.M. Morsy. 2011. Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (*Lupinus termis* Forsik). *Crop Prot*., 30: 185-191.

Abdel-Shafi, S., A. Osman and A.R. Al-Mohammadi. 2019b. Biochemical, biological characteristics and antibacterial activity of glycoprotein extracted from the epidermal mucus of African catfish (*Clarias gariepinus*). *Int. J. Biol. Macromol.,* 138: 773-780.

Abdel-Shafi, S., A.R. Al-Mohammadi, and M. Sitohy. 2019a. Antimicrobial activity and chemical constitution of the crude, phenolic-rich extracts of *Hibiscus sabdariffa, Brassica oleracea* and *Beta vulgaris*. *Molecules*, 24: 4280.

Ali-Shtayeh, M.S. and S.I. Abu Ghdeib. 1999. Antifungal activity of plant extracts against dermatophytes. *Mycoses*., 42: 665-672.

Allen, A.K., A. Neuberger and N. Sharon. 1973. The purification, composition and specificity of wheat-germ agglutinin. *Biochem. J*., 131: 155-162.

Baker, R.L., R.L. Brown, Z.Y. Chen, T.E. Cleveland and A.M Fakhoury. 2009. A maize lectin like protein with antifungal activity against *Aspergillus flavus*. *J. Food Prod.,* 72: 120-127.

Broekaert, W.F., J.A.N. Van Parijs, F. Leyns, H. Joos and W.J. Peumans. 1989. A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science,* 245: 1100-1102.

Caruso, C., C. Caporale, G. Chilosi, F. Vacca, L. Bertini, F. Magro, E. Poerio and V. Buonocore. 1996. Structural and antifungal properties of a pathogenesis related protein from wheat kernel. *J. Prot. Chem*., 15(1): 35-44.

Cheema, N.M., M.A. Malik, G. Qadir and M.A. Rabbani. 2010. Characterization of castor bean genotypes under various environments using SDS-PAGE of total seed storage proteins. *Pak. J. Bot*., 42(3): 1797-1805.

Chen, J., B. Liu, N. Ji, J. Zhou, H. Bian, C. Li, F. Chen and J. Bao. 2009. Anovelsialic acid-specific lectin from *Phaseoluscoccineus* seeds with potent antineoplastic and antifungal activities. *Phytomed*., 16: 352-360.

Costa, R.M., A.F. Vaz, M.L. Oliva, L.C. Coelho, M.T. Correia and M.G. Carneiro-da-Cunha. 2010. A new mistletoe Phthirusapyrifolia leaf lectin with antimicrobial properties. *Proc. Biochem*., 45: 526-533.

de Azevedo Moreira, R. and I.L. Ainouz. 1981. Lectins from seeds of jack fruit (*Artocarpus integrifolia* L.): isolation and purification of two isolectins from the albumin fraction. *Biol. Plant*, 23: 186.

Desai, N.N., A.K. Allen and A. Neuberger. 1981. Some properties of the lectin from *Datura stramonium* (thorn-apple) and the nature of its glycoprotein linkages. *Biochem. J*., 197: 345-353.

Dhingra, O.D., M.L.N. Costa and G.J. Silva Jr. 2004. Essential oil of mustard to control *Rhizoctonia solani* causing seedling damping-off and seedling blight in nursery. *Fitopatol. Bras*., 29: 683-686.

Dias, R.D.O., L.D.S. Machado, L. Migliolo and O.L. Franco. 2015. Insighrs into animal and plants lectins with antimicrobial activities. *Molecules*, 20: 519-541.

Fakhrunnisa, M.H. Hasmi and A. Ghaffar. 2006. Seed-borne mycoflora of wheat, sorghum and barley. *Pak. J. Bot.*, 38(1): 185-192.

Fornstedt, N. and J. Porath. 1975. Characterization studies on a new lectin found in seeds of *Vicia ervilia*. *FEBS Lett*., 57: 187-191.

Kheeree, N., P. Sangvanich and S. Puthong. 2010. Antifungal and antiproliferative activities of lectin from the rhizomes of *Curcuma amarissima* Roscoe. *Appl. Biochem. Biotechnol.*, 162: 912-925.

Kondoh, M., M. Hirai and M. Shoda. 2001. Integrated biological and chemical control of damping-off caused by *Rhizoctonia solani* using *Bacillus subtilis* RB14-C and flutolanil. *J. Biosci. Bioeng*., 91: 173-177.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Lanoo, N. and E.J.M.V. Damme. 2014. Lectin domains at the frontiers of plant defence. *Front. Plant Sci.*, 5: 397.

Loganayaki, N., P. Siddhuraju and S. Manian. 2013. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J. Food Sci. Technol*., 50(4): 687-695.

Luz, W.C. da, G.C. Bergstrom and C.A. Stockwell. 1998. Seed applied bioprotectants for control of seedborne Pyrenophoratritici-repentis and agronomic enhancement of wheat. *Can. J. Plant Pathol*, 20: 384-386.

Mao, W., J.A. Lewis, P.K. Hebbar and R.D. Lumsden. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. *Plant Dis*., 81: 450-454.

Maria das Graças, M.F., V.M. Gomes and R.E. Corsini. 2002. Isolation and partial characterization of a novel lectin from *Talisia esculenta* seeds that interferes with fungal growth. *Plant Physiol. Biochem.*, 40: 61-68.

Marin, S., A.J. Ramos, G. Cano-Sancho and V. Sanchis. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol*.., 60: 218-237.

Marroquín-Cardona, A.G., N.M. Johnson, T.D. Phillips and A.W. Hayes. 2014. Mycotoxins in a changing global environment–a review. *Food Chem. Toxicol*., 69: 220-230.

Matthiesen, R., A. Ahmad and A. Robertson. 2016. Temperature affects aggressiveness and fungicide sensitivity of four *Pythium* spp. that cause soybean and corn damping-off in Iowa. *Plant Dis*., 100: 583-591.

Matucci, A., G. Veneri, C.D. Pellegrina and G. Zoccatelli. 2004. Temperature-dependent decay of wheat germ agglutinin activity and its implications for food processing and analysis. *Food Cont.*, 15: 391-395.

Mendoza-Blanco, W., L. Ponce-Soto and S. Marangoni. 2012. Purification and primary structure of a lectin from *Buddleja coriacea* seeds. *Sciéndo*., 15: 81-8.

Mirelman, D., E. Galun, N. Sharon and R. Lotan. 1975. Inhibition of fungal growth by wheat germ agglutinin. *Nature*, 256: 414-416.

Mostafa, S.A., H.A. Badr and A. Osman. 2019. Antimicrobial activity of lectin isolated from Egyptian wheat cultivar Giza 171. *Zagazig J. Agric. Res.*, 46: 783-792.

Nagata, Y. and M.M. Burger. 1974. Wheat germ agglutinin molecular characteristics and specificity for sugar binding. *J. Biol. Chem*., 249: 3116-3122.

Nagendran, S., R. Hammerschmidt and J.M. McGrath. 2009. Identification of sugar beet germplasm EL51 as a source of resistance to post-emergence *Rhizoctonia* damping-off. *Eur. J. Plant Pathol*., 123: 461-471.

Niaz, I., U. Sitara, S.A.R. Kazmi and S. Qadri. 2008. Comparison of antifungal properties of neem seed oil contaminated from different parts of Pakistan. *Pak. J. Bot.*, 40(1): 403-408.

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 beta-conglycinin. *J. Gen. Plant Pathol.*, 82: 293-301.

Osman, A., H.A. Goda and M. Sitohy. 2018. Storage stability of minced beef supplemented with chickpea legumin at 4℃ as a potential substitute for nisin. *LWT Food Sci Technol.*, 93: 434-441.

Oyaizu, M. 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *JPN. J. Nutrit*., 44: 307-315.

Sadananda, T.S., M. Govindappa and Y.L. Ramachandra. 2014. In vitro antioxidant activity of lectin from different endophytic fungi of *Viscum album* L. *Br. J. Pharm. Res*.., 4(5): 626-643.

Saha, R.K., S.H.M. Tuhin, N. Jahan, A. Roy and P. Roy. 2014. Antibacterial and antioxidant activities of a food lectin isolated from the seeds of *Lablab purpureous. Amer. J. Ethnomed.*, 1 (1): 008-017.

Simpson, R.J., M.R. Neuberger and T. Liu. 1976. Complete amino acid analysis of proteins from a single hydrolysate. *J. Biol. Chem.*, 251: 1936-1940.

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

Sitohy, M., S. Mahgoub, A. Osman, R. El-Masry and A. Al-Gaby. 2013. Extent and mode of action of cationic legume proteins afainst *Listeria monocytogenes* and *Salmonella* Enteritidis. *Probiotics Antimicrob. Proteins*., 5: 195-205.

Somada, I., V. Leth and P. Sereme. 2007. Evaluation of lemongrass, eucalyptus and neem aqueous extracts for controlling seed-borne fungi of sorghum grown in Burkina Faso. *World J. Agric. Sci*.,3: 218-223.

Tian, Q., W. Wang, C. Miao, H. Peng, B. Liu, F. Leng and L. Dai. 2008. Purification, characterization and molecular cloning of a novel mannose-binding lectin from rhizomes of *Ophiopogon japonicus* with antiviral and antifungal activities. *Plant Sci*., 175: 877-884.

Tong, S., M. Li, N.O. Keyhani, Y. Liu, M. Yuan, D. Lin, D. Jin, X. Li, Y. Pei and Y. Fan. 2020. Characterization of a fungal competition factor: production of a conidialcellwall associated antifungal peptide. *PLOS Pathog.*, 16(4): e1008518. https://doi.org/10.1371/journal. ppat.1008518.

Wilson, I.D., R. Plumb, J. Granger and H. Major. 2005. HPLC-MS-based methods for the study of metabonomics. *J. Chromatogr. B*., 817: 67-76.

Yan, Q., Z. Jiang, S. Yang, W. Deng and L. Han. 2005. A novel homodimeric lectin from *Astragalus mongholicus* with antifungal activity. *Arch. Biochem. Biophy*., 442: 72-81.

Yu, P., H. Shao, J. Feng, M. Zhu and X. Zhang. 2001. Preliminary study on antifungal activity of 62 plant extracts. *Acta Universitatis Agriculturalis Boreali-occidentalis*., 29: 65-69.

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