Production and characterization of xylanase from pomegranate peel by *Chaetomium globosum* and its application on bean under greenhouse condition

Sherien M. M. Atalla 1* and Nadia G. El Gamal 2

**Abstract**

**Background and objective:** The main objective of the present study was production of xylanase from different agricultural wastes using *Chaetomium globosum* on pomegranate waste, isolation of fungi associated with some legumes seeds, and studied the effect of safe product which obtained from xylanse under laboratory and greenhouse conditions on bean seeds.

**Results and discussion:** Different agricultural wastes were tested for xylanase production by *Ch. globosum*; from them, pomegranate peel was most effective at concentration 40 g/l using 2 disks 6 mm in diameter after 7-day incubation period produces 1398.34 U/ml. Addition of calcium chloride increases xylanase activity to 1469.40 U/ml. The crude xylanase activity was active after 20 min of starting of reaction at 6.6 pH, and 40 °C of the reaction mixture produces 1587.27 U/ml. Xylanse is still active at 40 °C for 30 min. Seeds of some legumes were examined for seed borne mycoflora by the agar plate method. The most common fungi were *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*.

Testing of crude xylanase as biocide was more effective in reduction of linear growth of some pathogenic fungi also applied the biocide as seed treatment of bean. Seed coating with biocide gave significant protection to emerge bean seeds against invasion of pathogenic fungi at the pre-emergence stage. Seed coating recorded more than 50% protection compared with the untreated control.

**Conclusion:** Production of xylanase from pomegranate peel wastes using *Chaetomium globosum* under optimum conditions. Testing crude xylanase after optimization as an effective biocide.

**Keywords:** Xylanase, *Chaetomium globosum*, Pomegranate peel, Soil born fungi

**Introduction**

Xylan (E.C.2.8.1.8) is one of the major hemicellulose components of plant cell wall consist of backbone of β-14-linked xylopyranose residues with branches containing arabino-furanosyl, acetyl, and glucuronosyl residues. In the plant cell wall, hemicellulose is highly branched and tightly associated with other biopolymers (Jae et al. 2009).

Xylanase from different microorganisms such as fungi, bacteria, marine algae, and yeast gained interest due to their potential applications in many industrial processes as well as using agro industrial residues are good source of nutrition for the growth of the microorganisms and cheap natural carbon sources. The production of the enzyme xylanase can be used for industrial applications, commercial purposes including chlorine free bleaching of the wood pulp prior to papermaking, food additives in
poultry and in wheat flour for increasing the quality of baked products and improving the handling of dough, and for the extraction of coffee, extraction of starch and plant oils and considered important as they contain large amount hemi celluloses and serve as inducers for the production of enzymes such as xylanases which led to the development of bioprocesses for the effective use of agro wastes (Shabeena et al. 2017; Nitin et al. 2017; El-Mohamedy et al. 2018; Atalla et al. 2019).

Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productive. Some of these fungi produce aflatoxins which damage the liver and induce carcinogenic, mutagenic. Thus, control of seed borne fungi is extremely important, and the damaging effects can be relieved through integrated approaches (Agarwal et al. 2011).

The seeds are locally produced or imported and stored by the farmers under poor quarantine. Therefore, seed contamination can occur by seed borne fungi which adversely affect the production and productivity of these crops.

Seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant, a necessary condition for good yields (Diaz et al. 1998). Hence, this study has been undertaken to investigate the percentage incidence of seed borne fungi associated with some varieties of legumes.

During the industrial processing of pomegranate, large volume of wastes is produced, which have a wide range of nutritional values. Therefore, in the recent years, scientists have focused on the industrial byproducts of pomegranate that have a high potential of antioxidant and antifungal properties (Dahham et al. 2010).

The main objective of the present study was production of xylanase from different agricultural wastes using Chaetomium globosum on pomegranate waste, isolation of fungi associated with some legumes seeds, and studied the effect of safe product which obtained from xylanase under laboratory and greenhouse conditions on bean seeds.

Materials and methods

Microorganisms

Chaetomium globosum is a local fungal strain isolated from Egyptian soil. It was identified in Plant Pathology Department of National Research Centre, Giza, Egypt, according to Barnett and Hunter (1972) and Nelson et al. (1983). The strain was kept on a potato dextrose agar medium and stored at 4 °C.

Three fungal pathogens F. solani, M. phaseolina, and R. solani were selected from preliminary test of isolated from legume seeds. The fungi in pure culture were identified after pathogenicity test according to the keys given by Barnett and Hunter (1972) and Nelson et al. (1983).

Common legume seed samples were collected in polythene envelopes from different market from Egypt and taken to the laboratory for isolation of fungal pathogens.

Substrates

Different agricultural residues (banana peel, pea peel sawdust, peanuts, pomegranate peel (Pp), potato peel, wheat straw, sugar cane bagasse, soy bean) were used as sole carbon source and tested for xylanase production. All wastes were washed, dried at 70 °C in oven, and cut into small pieces (1 cm × 1 cm) before used.

Fermentation condition

The ability of the microorganism for xylanase production was examined in medium containing (g/l) NaNO₃ 2.0, K₂HPO₄ 0.5, KCl 0.5, MgSO₄ 0.7, H₂O 0.5, and different agricultural wastes (20.0). Erlenmeyer flasks 250 ml each containing 50 ml fermentation medium were inoculated with two disks 6 mm in diameter of the selected microorganism and incubated at 28 ± 30 °C for 7 days in incubator shaker at 200 rpm.

Xylanase assay

Determination of enzyme activity was carried out according to the method of Monreal and Reese (1969).

One milliliter of 1% birch wood xylan (Sigma) in acetate buffer (pH 4.6) in test tubes was added to 1 ml of the culture filtrate and mixed by shaking. The mixture was incubated in a water bath at 50 °C for 30 min and then cooled and centrifuged before assaying. The amount of reducing sugar was determined with 1 ml of 3,5-dinitrosalicylic acid (DNS). One unit of enzyme activity was taken of the catalyst converts one μmol of substrate in 1 min under specific condition.

Effect of different agricultural residue

In order to induce xylanase synthsis from microbial sources from cheap and most economic carbon source, 20 g of different agricultural residues (banana peel, pea peel, sawdust, peanuts, pomegranate peel, potato peel wheat straw, sugar cane bagasse, and soy bean) in comparison with control was added to fermentation medium.

Optimization of culture conditions

Optimization conditions were carried out depends on addition of different (Pp) concentrations ranging from 5 to 120 g/l. Different inoculum size 2 disks (2, 4, 6, and 8 mm) in diameter, different incubation period (5, 7, and 10 days), and addition of different salts (sodium bicarbonate, calcium carbonate, potassium sorbate, calcium
chloride) were obtained from Sigma Aldrich Spruce Street, St. Louis, USA.

**Characterization of crude xylanase activity**
Optimization of crude xylanase activity was carried out using crude filtrate of *C. globosum*. Effect of different reaction time was carried out in the reaction mixture ranging from 10 to 60 min, followed by changing pH values from 3.6 to 7.0. The temperature of the reaction was analyzed by incubated reaction for 10–60 °C.

**Thermal stability**
Thermal stability was analyzed by incubating the crude filtrate at different temperature with different time intervals, and then, residual activity was measured.

**Collection of the seed samples**
Twenty seed samples are from 5 leguminous crop, namely, *Pisum sativum* (pea), *Vicia faba* (faba bean), *Cicer arietinum* (chickpea), *Phaseolus vulgaris* (white bean), and *Lens culinaris* (lentil).

**Seed Borne fungi analysis**
Detection of seed borne fungi from selected legume seeds was done by agar plate method as recommended by ISTA (1976).

**Frequency of occurrence of isolated fungi**
Frequency of fungal isolates was calculated according to Al-Abdalall (2008).

\[
\% \text{Frequency} = \frac{\text{total number of fungal isolates/crop}}{\text{number of fungal isolates}} \times 100
\]

**Evaluation of the efficacies of crude xylanase produced against different fungal isolates**
Agar well diffusion method crude xylanase from *C. globosum* was screened for antifungal activity using sterile cork borer of size 6.0 mm in diameter according to Bobbarala et al. (2009). Five hundred microliters of crude xylanase solution homogenized added to 0.02 ml of inoculums filled in deep blocks. Incubation period of 48–72 h at 25 °C was maintained for antifungal activity. Radial inhibition was calculated when growth of mycelia in the control plate reached the edge of the petri dish. The toxicity of the extracts to growth of fungi was calculated by using the formula percentage (%) inhibition = dc – dt/dc × 100, where dc = average increase in mycelial growth in control and dt = average increase in mycelial growth in treatment (Singh and Tripathi 1999).

**Greenhouse experiment**
Three fungal pathogens *F. solani*, *M. phaseolina*, and *R. solani* were selected from preliminary test of isolated from legume seeds which showed the highly percentage of occurrence and evaluate the biocide prepare from *C. globosum* grown on Pp waste under greenhouse conditions for bean seed *P. vulgaris*. Sandy clay soil was transferred in pots. Inoculum from each cultures was colonized separately and infested at the rate of 3 g/100 g soil. The disinfected bean seeds were coated with a biocide at the rate of 4 ml/kg seeds. Seed dressing was carried out by applying the biocide to the gum moistened seeds in polyethylene bags and shaking well to ensure even distribution of the added materials then left to air dried. Five replicates/treatments, pathogen free seeds were surface sterilized and planted (5 seeds/pot) in inoculated and non-inoculated soils. All pots maintained in greenhouse under natural condition, for 15 days after sowing. Disease ratios were determined by recording the number of non-emerged seeds (pre-emergence damping-off) while post-emergence damping off was recording from 30 to 45 days after sowing. The equations described by Khalifa (1987) were as follows:

- Pre-emergence (%) damping off
  \[= \frac{\text{no. of non-emerged seeds}}{\text{no. of sown seeds}} \times 100\]

- Post-emergence (%) damping off
  \[= \frac{\text{no. of killed seedling}}{\text{no. of sown seeds}} \times 100\]

**Statistical analysis**
Tukey test for multiple comparisons among means was utilized (Neler et al. 1985).

**Results**
**Production of xylanase from different agricultural residues**
The results in Fig. 1 showed that pomegranate peel (Pp) produces maximum xylanase activity 1046.20 U/ml, followed by wheat bran produces 663.87 U/ml, then pea peel produces 618.13 U/ml; other wastes showed moderate to low activities in comparison with control.

**Different pomegranate peels (Pp) concentrations**
The results in Fig. 2 showed that xylanase activity increases by increasing Pp concentrations till reached 40 g/l which was the most suitable concentration producing 1398.34 U/ml followed by 50 g/l producing 1286.01 U/ml then 60 g/l producing 1280.01 U/ml.

**Effect of different inoculum size**
The results in Fig. 3 showed that activity on the xylanase increases till reaching 1398.34 U/ml after inoculation with 2 disks 6 mm in diameter; then, the activity decreased.
Different incubation periods (days)
The results recorded in Fig. 4 showed that 7-day incubation period was the most suitable for xylanase production producing 1401.13 U/ml followed by 5-day incubation period producing 1398.34 U/ml.

Addition of different salts
The data in Fig. 5 showed that the addition of calcium chloride increases xylanase activity producing 1469.40 U/ml, followed by potassium sorbate producing 975.60 U/ml while addition of other salts reduces xylanase activity.

Characterization of crude xylanase activity
Effect of different reaction time
The results in Fig. 6 revealed that time of reaction increases till reached 20 min after the start of the reaction produces maximum xylanase activity of 1473.39 U/ml; then, activity of reaction begins to decrease.

Fig. 1 Effect of different agricultural wastes on xylanase production by C. globosum

Fig. 2 Effect of different concentrations of pomegranate peel on xylanase activity
Different pH values

Different pH values were analyzed using crude filtrate of xylanase after 20 min of reaction. The results in Fig. 7 presented that xylanase activity increased with increasing pH values till reaching pH 6.6 producing 1708.74 U/ml followed by pH 6.0 producing 1726.05 U/ml; then, the activity decreased. This means that addition of pomegranate peel as a carbon source in the fermentation medium increases the ability of the crude filtrate to resist increase in different pH values.

Different temperature of the reaction mixture

The results in Fig. 8 showed that xylanase activity increases till reached 40 °C which was the most promising temperature for xylanase activity producing 1587.27 U/ml followed by 30 °C producing 1473.39 U/ml then 20 °C producing 1405.27 U/ml.

Thermal stability

The data present in Fig. 9 showed that increasing the exposure time of crude xylanase up to 40 °C for 30 min decreases xylanase activity up to 45%, while at a 60 °C enzyme lost 85% of its activity. Thermal stability is an interesting enzymes property due to the great industrial importance. Crude xylanase from *P. sclerotiorum* was stable at 50 °C; increase in thermal stability would be interesting and could be achieved with directed site mutagenesis.

Seed borne fungi analysis:

The fungi which appeared on seed were isolated in pure culture for identification and for further study. In agar plate method, pre-sterilized petri plates were poured with 15 ml of autoclaved (PDA). On cooling the medium, three replicates of ten seeds/plate of the sample were equidistantly placed aseptically. The plates were
incubated at 25 ± 2 °C under diurnal conditions. On sev-
enth day of incubation, seeds were first examined under
stereoscopic microscope for determining the various fun-
gal growths. Seed borne fungal pathogens were isolated
from seeds on potato dextrose agar (PDA) using agar plate
method as described by Anonymous (1993). The isolated
fungi were identified using cultural appearance and micro-
scopic characteristics (Ogbulie et al. 2001).

Frequency of fungal isolates
Many fungal species were isolated and identified from the
collected seed samples using the agar plate method. Fungi
were frequently isolated from all seeds (Table 1); *R. solani* is
the most frequently isolate (27.3 to 43.5% in Faba bean) from
all samples and also *F. solani* (26.0 to 33.3%) and *M. phaseo-
lina* (32.0%) in white bean, followed by *A. niger* (8.0 to
17.4%) and *P. spp.* (13 to 13.3%). Differences in isolation and
frequency may be due to variation in the moisture content of
seeds, varietal differences, and seed susceptibility to infection
besides specific environmental conditions in each seed store.

A seed borne pathogen presents externally, internally,
or associated with the seed as contaminant may cause
seed abortion, seed rot, seed necrosis, reduction, or elim-
ination of germination capacity as well as seedling dam-
age resulting in the development of disease at later
stages of plant growth by systemic or local infection.
In vitro antifungal activity of crude xylanase (biocide)

Effect of crude xylanase (biocide) on mycelia growth and zone of inhibition of some pathogenic fungi isolated from legume seed. Results showed that biocide showed higher antifungal activity than control in (Table 2) highest inhibition against R. solani, F. solani, and M. phaseolina shown by the zone of inhibition 13.0, 13.0, and 7.0, respectively, as shown by the reduction in linear growth 78.8, 73.3, and 60.0 for all organisms.

Fig. 7 Effect of different pH values on crude xylanase activity

![Graph showing xylanase activity at different pH values](image)

Fig. 8 Effect of different temperature °C on crude xylanase activity

![Graph showing xylanase activity at different temperature](image)
Greenhouse experiments
The data in Table 3 show that basal could highly significantly reduce disease incidence of beans which have been artificially infested with root rot pathogens, compared to untreated control treatments. Seed coating with biocide gave significant protection to emerge bean seeds against invasion of pathogenic fungi at the pre-emergence stage. Seed coating recorded more than 50% protection compared with the untreated control. At the post-emergence stage, data also showed that treatment with biocide could reduce the percentage of root-rot incidence more than the untreated control (treatment with pathogens alone). Treatment with biocide caused reduction in the percentage of root-rot incidence recorded at 59.5%, 59.2%, and 42.2% in soil infested with R. solani, F. solani, and M. phaseolina, respectively.

Discussion
Utilization of pomegranate peel (Pp) produces maximum xylanase activity. This result was coincided with Shabeena et al. (2017) who found that production of xylanase by Aspergillus japonicas was most promising using wheat bran as carbon source; use of alternative agricultural residues as carbon sources reduces the cost of final products. Also, Betini et al. (2009) found that the use of agricultural wastes as carbon source reduces the cost of xylanase production by studying the effect of orange

| Fungal sp.                  | % Occurrence Seed/sample | Chick pea | Faba bean | Lentil | Pea | White bean |
|-----------------------------|--------------------------|-----------|-----------|--------|-----|------------|
| Alternaria alternate        | –                        | –         | –         | –      | 21.7| –          |
| Aspergillus flavus          | –                        | –         | 9.1       | 21.4   | 4.3 | –          |
| Aspergillus niger           | 13.3                     | 22.7      | –         | –      | 17.4| 8.0        |
| Fusarium spp.               | 33.3                     | –         | 35.7      | –      | –   | –          |
| Fusarium solani             | –                        | 27.3      | –         | –      | 43.5| 24.0       |
| Macrophomina phaseolina     | –                        | –         | –         | –      | –   | 32.0       |
| Rhizoctonia solani          | 40.0                     | 27.3      | –         | –      | –   | 36.0       |
| Penicillium spp.            | 13.3                     | 13.6      | 26.0      | 13.0   | –   | –          |

Table 1 Frequency of seed borne fungi recorded from five different legume seeds
peel, orange pomace, lemon peel, lemon pomace, apple peel, banana peel, pear peel, hazelnut shell, and melon peel on the production of xylanase from *Trichoderma harzianum* 1073-D3, as well as Seyis and Aksoz (2005) who discovered that the addition of molasses as carbon source increases xylanase production followed by melon peel. Production of xylanase by *Penicillium janthinellum* uses different agricultural wastes (Oliveira et al. 2006), in addition to Dobrev et al. (2007) who use corn cob for xylanase production in shaking media inoculated with *Aspergillus niger* B03. Also, this results were similar to Venkatesh and Tallapragada (2009) and Nitin et al. (2017) where *A. niger* produces xylanase using wheat bran and pea peel as sole carbon source.

Comparison between different agricultural wastes on xylanase production by *C. globosum*, Darshna et al. (2017) found that 10 g/l of Pp was most suitable for xylanase production from *A. niger* and Venkatesh and Tallapragada (2009) who illustrated that higher inoculum size for xylanase from *A. Niger* was most promising after 6-day incubation period, and Su et al. (2011) found that maximum xylanase *Thermomyces lanuginosus* SKYKY-1 was after 3-day incubation period produce 38.7 g/l from corncobs and 17.5 g/l from soybean meal. The maximum production of xylanase for optimum fermentation depends on the organism, the nature of the substrate, additive nutrients, and many other fermentable conditions (Dekker 1983; Mishra et al. 1985).

Addition of molasses as illustrated by Carlos et al. (2014) increases xylanase production after 7-day incubation period while with Antoine et al. (2010) found that *Penicillium canescens* produce xylanase after 7 days of incubation. While these results were not coincided with Venkatesh and Tallapragada (2009) who discussed that production of xylanase from *A. Niger* was most promising after 6-day incubation period, and Su et al. (2011) found that maximum xylanase *Thermomyces lanuginosus* SKYKY-1 was after 3-day incubation period produce 38.7 g/l from corncobs and 17.5 g/l from soybean meal. The maximum production of xylanase for optimum fermentation depends on the organism, the nature of the substrate, additive nutrients, and many other fermentable conditions (Dekker 1983; Mishra et al. 1985).

The same results obtained by DOS Reis et al. (2015) who found that higher concentration of xylanase from *Penicillium echinulatum* SIM29 was obtained by the addition of calcium chloride in fermentation media. Addition of other salts illustrated by Maciel et al. (2009) who noticed that *Aspergillus niger* LPB 326 produced in fermentation media containing sugarcane bagasse in addition of the mineral salt solution. Also, Nayyar et al. (2017) discuss that production of xylanase from *A. niger* increases in presence of salts in fermentation media and Radhika and Monika (2014) who illustrate that *Gordonia* sp. increase xylanase production in presence of sodium chloride.

### Table 2

| Pathogenic fungi | Biocide from pomegranate | Linear growth (mm) | Reduction (%) | Zone of inhibition (mm) |
|-----------------|--------------------------|--------------------|--------------|------------------------|
| A. alternate    |                          | 51.0               | 43.3         | 4.0                    |
| A. solani       |                          | 50.0               | 44.4         | 3.0                    |
| F. solani       |                          | 24.0               | 73.3         | 13.0                   |
| F. oxysporum    |                          | 39.0               | 56.7         | 5.0                    |
| M. phaseolina   |                          | 36.0               | 60.0         | 7.0                    |
| R. solani       |                          | 19.0               | 78.8         | 13.0                   |
| Control         |                          | 90.0               | –            | 0.0                    |

### Table 3

| Treatments | Percentage (%) pre-emergency | Percentage (%) reduction | Percentage (%) post-emergency | Percentage (%) reduction |
|------------|------------------------------|--------------------------|------------------------------|--------------------------|
| F. solani  | 28.0a                        | –                        | 33.3a                        | –                        |
| F. solani + Biocide | 12.0b                        | 57.1                    | 13.6b                        | 59.2                     |
| M. phaseolina | 32.0a                       | –                        | 41.2a                        | –                        |
| M. phaseolina + Biocide | 16.0b                       | 50.0                    | 23.8b                        | 42.2                     |
| R. solani  | 32.0a                        | –                        | 35.3a                        | –                        |
| R. solani + Biocide | 16.0b                       | 50.0                    | 14.3b                        | 59.5                     |

Figures with the same letter are not significantly different (p = 0.05)
Simone et al. (2009) found that the maximum xylanase activity obtained from the culture filtrate of A. fumigatus and A. niveus was after 1 h of the beginning of the reaction. As well as Taneja et al. (2002) who used A. nidulans KK-99 to report that xylanase was active in after 1 h and retained almost 80% of its activity 60 min after the start of the reaction was most suitable for xylanase production from Aspergillus niger DFR-5 (Ajay and Farhath 2010).

This result of different pH values was not the same with Cai et al. (2004) who found that the optimum pH value of xylanase activity from the culture filtrate of Pleurotus ostreatus SYJ042 was 6.0. Simone et al. (2009) found that pH 5.0–5.5 increase xylanase activity from crude filtrates of A. fumigatus while optimum pH of A. niveus was 4.5–5.0. Pill (2000) illustrated that xylanase activity from Penicillium sp. was at acid range.

This result of temperature was coincided with Ryan et al. (2003) who found that 40 °C was the optimum for xylanase production from Penicillium sp., while not coinciding with Hsin-Yu et al. (2007) who found that the optimum temperature for crude xylanase activity from Aspergillus carneo M34 was 60 °C. As well as Adriana and Eleonora (2008) found that 50 °C was the optimum temperature for xylanase activity from P. sclerotiorum.

Thermal stability is an interesting enzymes property due to the great industrial importance as discussed by Eijink et al. (2005) due to the stability of enzyme at different temperatures and times (Adriana and Eleonora 2008). Simone et al. (2009) xylanase enzymes from both A. fumigatus and A. niveus were stable at 60 °C for 30 min while after 1 h activity retained up to 85%.

Khanzada et al. (2002) and Al-Abdalall (2008) found that seed borne pathogen presents externally, internally, or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction, or elimination of germination capacity as well as seedling damage resulting in the development of disease at later stages of plant growth by systemic or local infection.

The antifungal activity of xylanase extract against some phytopathogenic fungi has been previously reported; the different pomegranate extracts on linear growth of different fungi; the highest antifungal activity was reported; the different pomegranate extracts on linear growth of A. alternata, F. oxysporum, Phoma destructiae, R. solani, and Sclerotium rolfsii with different degrees of activity against the tested fungi (Al-Askar 2012; Mangang and Chhetry 2012).

Greenhouse experiments obtained results are in agreement with those reported by Satish et al. (2007), the antifungal activity against seed borne pathogens of Aspergillus spp. as the result of application pomegranate peel extract. Also, pronounced decrease was found in citrus green mold disease P. digitatum as a result of application of pomegranate peel extract (Tayel et al. 2009). In another study, soil treatment with a pomegranate leaf extract before sowing effectively reduced damping off disease of French bean caused by Rhizoctonia solani, under greenhouse and field conditions. Xylanase is good biocontrol for green and blue mold of orange and lime fruit (El Shamy et al. 2016).

Conclusion
Different agricultural wastes were tested for xylanase production from them; pomegranate peel was most effective at concentration 40 g/l using 2 disk 6 mm in diameter after 7-day incubation period in addition of calcium chloride increases xylanase activity. The crude xylanase activity was active after 20 min of starting of reaction at 6.6 pH and 40 °C of the reaction mixture. Xylanase retained 85% of activity at 60 °C.

Seeds of some legumes were examined for seed borne mycoflora by the agar plate method. The most common fungi were Fusarium solani, Rhizoctonia solani, and Macrophomina phaseolina.

Testing of crude xylanase as biocide was more effective in reduction of linear growth of some pathogenic fungi. Seed coating with biocide gave significant protection to emerge bean seeds against invasion of pathogenic fungi at the pre-emergence stage. Seed coating recorded more than 50% protection compared with the untreated control.

Acknowledgements
The authors acknowledge National Research Centre especially Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, Plant Pathology Department, and Agricultural and Biological Research Division for their helpful and encouragement.

Availability of supporting data
Not applicable

Authors’ contributions
All authors read and approved the final manuscript. Sherien Mohamed Mabrouk Atalla (SH), selection of microorganism, enzymes assay, and optimization conditions. Nadia G. El-Gamal (NG), selection of microorganism and field experiment.

Authors’ information
Not applicable

Funding
Not applicable

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
Not applicable

Author details
1Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (NRC), Dokki, Giza 12622, Egypt. 2Plant Pathology Department, Agricultural and Biological Research Division, National Research Centre (NRC), Dokki, Giza 12622, Egypt.
Received: 14 January 2020 Accepted: 9 June 2020
Published online: 23 June 2020

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Page 11 of 11

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