Journal of Environmental Studies [JES] 2017. 16: 21-36
Original Paper

Screening and optimization of extracellular cellulase and pectinase enzymes produced from post-harvest fungi of apple (*Pyrus malus* L.) and tomato (*Solanum lycopersicum* L.)

A.H.M. El-said[1], M.A. Hussein[2], Thanaa A. Maghraby[3] and S.M. Meghezel[4]

[1]Biology Department, Faculty of Science, Taif University, Saudi
[1,2,3]Botany Department, Faculty of Science, South Valley University, 83523 Qena, Egypt
[4]Botany Department, Faculty of Science, Sohag University, Sohag, Egypt

Rec. 14 May, 2016 Accept. 29 June, 2016

Abstract
Fourty-eight species and two species varieties belonging to 19 genera were collected from 50 samples of apple and tomato fruits (25 from each) from Sohag governorate in Egypt on dichloran rose-Benegal chloramphenicol agar medium at 28^{O}C. The most common genera were *Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium.* Form the above genera the most prevalent species were *Alternaria alternata, A. tenuissima, Aspergillus niger, A. flavus, A. fumigatus, Cladosporiumcladosporioides, C.herbarum* and *Penicillium oxalicum.* Fourty-eight species and two species varieties fungal species screened for their abilities to produce cellulase (C_1 enzyme**)** and pectinase enzymes. Six and seven species showed high cellulolytic and pectinolytic activity, while 23 and 17 species were moderately activity for the two enzymes, respectively. The remaining species were low activity in both enzymes. The highest cellulase and pectinase activities were recorded by *Aspergillus chevalieri.* Maximum production of cellulase enzyme by *A.chevalieri* was obtained after 6 days of incubation at 30^{O}C with initial pH 6 in culture medium containing sucrose and peptone as carbon and nitrogen sources, respectively. Regarding to pectinase enzyme the highest pectinase production by *A. chevalieri* was recorded after 6 days of incubation at 30^{O}C with initial pH 8 in culture medium containing pectin and ammonium sulphate as carbon and nitrogen sources, respectively.

Key words: mycology, postharvest diseases, extracellular enzymes, cellulase, pectinase, apple, tomato, *Aspergillus chevalieri.*

Introduction

Fruits are widely distributed in nature, this keeps the body in a good and healthy condition and help in human daily diet (Ewekeye *et al*., 2013). The fruit is often attacked by microorganisms especially fungi after harvest (Udoh *et al.,* 2005). Mebratie *et al.* (2015). estimated that the total postharvest loss of banana was to be 26.5% in Ethiopia. The common postharvest and storage fungi of fruits are *Alternaria, Aspergillus, Fusarium* and *Penicillium* sp. (Bhale, 2011).

Apples (*Pyrusmalus* L.) are members of family Rosaceae in the most common genus *Malus* (Smock and Neubert, 1950). Ilyas *et al*. (2007) reported that the fungi isolated from rotten on Potato Dextrose Agar (PDA) medium were *Aspergillus niger, A. fumigatus, Alternaria tenuis, A. tenuissima, Cladosporium herbarum, Penicillium expansum, P. italicum* and *Rhizopus nigricans.* Fatima *et al*. (2009). showed that *Alternaria alternata* and *Geotrichum candidum* were the most common fungi caused diseases of apple fruits. Juhneviča *et al*. (2011). stated that microorganisms found on apple fruit surface before and after storage were related to the genera *Penicillium, Alternaria, Aspergillus, Cladosporium, Candida* on Potato Dextrose Agar (PDA) and Malt Extract Agar

* Corresponding author:
Dr. S.M. Meghezel
✉ safa_meghezel@yahoo.com

(MEA). Ammar and El-Naggar (2014) could isolate *Alternaria alternata* (1.38±0.02), *Penicillium* sp. (2.08±0.05), *Fusarium equisti* (1.04±0.02) and *A. niger* (0.69±0.05) from rotted apple samples in France.

Tomato (*Solanum lycopersicum* L.) is a berry plant in the Solanales order, Solanaceae family and genus *Solanum* (ACMSF, 2005). Etebu *et al*. (2013). confirmed that different species of fungi such as *Alternaria* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp. and *Geotrichum* caused postharvest diseases of tomato fruits. Oyemaechi *et al.* (2014) studied the microbial agents of tomato spoilage in Nigeria and noticed that *Candida tropicalis, Penicillium notatum, Aspergillus niger, Fusarium oxysporum, Absidia corynbifera* and *Rhizopus stonolifer* were the most causal agents of tomato. Samuel and Orji (2015) indicated that the fungi associated with the spoilage of tomato fruits were *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum* and *Geotrichum candidum. Aspergillus niger* had the highest percentage occurrence (47.27%) in the fruits examined.

The plant cell wall of fruit contains cellulose, hemicellulose and pectin (McNeill *et al*., 1984; Nathalie, 2006). Cellulose is a α-1,4 linked linear polymer of 8000~12000 glucose units. Cellulose is commonly degraded by an enzyme called cellulase. El-said *et al*. (2014) reported that the most prevalent species isolated from broad bean plant were *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium cladosporioides, Fusarium merismoides* and *Penicillium chrysogenum.*They screened these species for their abilities to produce exo-β-1,4-glucanase enzymes (C_1) and noticed that the highest cellulase activity was recorded by *Alternaria citri* and *Cochliobolus spicifer*. Maximum production of C_1 enzyme by *A. citri* and *C. spicifer* was obtained after 6 days of incubation at 30°C with initial pH 6 in culture medium containing lactose and calcium nitrate as carbon and nitrogen sources, respectively. Pectinases are the first enzymes to be secreted by fungal pathogens when they attack plant cell walls (Idnurm and Holett, 2001). Saleem *et al.* (2012). indicated that maximum production of pectinase produced by *A. citri* and *A. raphani* was recorded after 8 days at 30°C and pH 6 in the liquid medium supplemented with *Citrus* pectin and ammonium sulphate as carbon and nitrogen sources, respectively. Sethi *et al*. (2016). tested *Aspergillus terreus* NCFT4269.10 for biosynthesis of pectinase. Among various substrates, banana peel was most suitable for pectinase. 96 h of incubation at 30^{O}C and pH of 5.0 with urea and ammonium persulfate have positive influence on pectinase production.

This article aimed to isolation and identification of the Mycobiota associated with rotted post-harvest fruits. Also, study the potential of fungal species isolated from fruits for cellulase and pectinase enzymes production. The effect of different environmental and nutritional factors affecting secretion of the enzymes was assessed.

Materials and Methods

Collection of fruit samples:

Fifty infected samples of apple (*Pyrus malus L.*) and tomato (*Solanumlycopersicum L.*) (25 from each) were collected from different Markets/ shops in Sohag governorate, in Upper Egypt. Each sample was put in a sterile polyethylene bag and transferred to Mycological laboratory for fungal analysis.

A. Mycological analysis of fruit samples:

The dilution-plate method was used for the estimation of fungal flora associated with spoilage fruits described by Christensen (1963) and employed by Moubasher *et al*. (1972,1980). The developing colonies were counted, examined and identified. Dichloran rose- Bengal chloramphenicol agar (DRBC) medium used for isolation of various groups of fungi (King *et al*., 1979).

Cellulase and pectinase activity of fruit spoilage fungi

Fourty-eight and two species varieties related to 19 genera isolated from spoilage fruits, were screened for their abilities to produce cellulase (C_1 enzyme) and pectinase.

Screening of fungal isolates for cellulase production:

Fungal species were cultured on Eggins and Pugh medium (1962). Cultures were

incubated at 28±1°C for 7 days. Using a sterile cork borer, 10 mm diameter discs were cut to inoculate 50 ml sterile liquid medium (in 100 ml Erlenmeyer conical flasks) of Eggins and Pugh medium (1962) for C_1cellulase production. The cultures were filtered and the filterates were used to detect the activity of the enzyme. Using a sterile cork borer 3 cavities (10 mm diameter) were made in plates containing solid Eggins and Pugh medium (1962). Using a sterile cork borer 3 cavities (10 mm diameter) were made in plates containing solid Eggins and Pugh medium (1962). A 0.2 ml of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 h, then the plates were flooded with chloroiodide of zinc solution and the clear zones around cavities were measured.

Screening of fungal isolates for pectin lyase production:

All fungal species were screened for their abilities to produce pectinase enzyme as described by Osman (2005). Fungal species were cultured on Czapek's agar medium. Cultures were incubated at 28°C for 5 days. Using a sterile cork borer, 10 mm diameter discs were obtained. For each fungal isolate, two sterile 250 ml Erlenmeyer flasks containing 50 ml of the liquid Hankin *et al*. (1971) medium. Cultures were incubated at 28°C without shaking for 7 days after which the mycelium was harvested by filtration. Filtrates were used to detect pectin lyase activity of fungi according to Ammar *et al*. (1995). Aliquots of 0.1 ml of a culture filtrate were pipetted into 10 mm cavities which were made in plates containing solid medium of Hankin *et al*. (1971). After 24 h incubation at 28°C, plates were flooded with iodine solution. Uncoloured zone indicated the production of pectin lyase, the average diameter of clear zones (in mm) of the triplicates for each isolates was recorded.). Using a sterile cork borer 3 cavities (10 mm diameter) were made in plates containing solid Hankin *et al.* (1971) medium. A 0.2 ml of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 h, then the plates were flooded with iodine solution and the clear zones around cavities were measured.

Factors affecting cellulase and pectinase production:

The effect of different ecological and nutritional factors on production of exo-β-1,4glucanase (C1 enzyme) and pectinase by *Aspergillus chevalieri* was studied, whereas it was found to be highly active producer for the two enzymes.

For cellulase activity: The test isolate grown on Deacon (1985) medium. Fifty ml of the medium were dispensed into each 100 ml Erlenmeyer flask and each was inoculated with an agar mycelial disc (10 mm diameter) of the mould obtained from 7 day old cultures growing on the solid basal medium.

For pectinase activity: The previous isolate was grown on liquid Hankin *et al*. (1971). Fifty ml of the medium were dispensed into each 100 ml Erlenmeyer flask and each was inoculated with an agar mycelial disc (10 mm diameter) of the mould obtained from 5 day old cultures growing on the solid basal medium.

1- Effect of temperature and incubation period:

Aspergillus chevalieri was grown on the basal medium of Deacon (1985) for cellulase C_1 enzyme and liquid Hankin *et al*. (1971) medium for pectinase enzyme. The inoculated flasks were incubated at 20, 30 and 40°C for 14 days and harvested at 48 h intervals. Cultures were filtered and centifugated at 5000 r. p. m for 10 min. The clear supernatents were assayed for enzyme activity.

2- Effect of pH values:

The initial pH of the medium was adjusted with 0.1 N NaOH or 0.1 N HCl to different values ranging from 2,4, 6, 8, 10and12. After inoculation with *A. chevalieri* for C1 enzyme, cultures were incubated at 30°C for 6 days. At the end of the incubation period cultures were filtered and centrifugated. The clear supernatents were assayed for cellulase activity.

3- Effect of different carbon sources:

The basal medium (Deacon, 1985) with pH 6 (the best pH for exo-β-1,4glucanase production) and the basal medium (Hankin *et al*., 1971) with pH 8 (the best pH for pectinase enzyme)were supplemented with 1% of one of the following carbon sources: fructose,

glucose, CMC (Carboxymethylcellulose), maltose, starch and sucrose, in addition to cellulose and pectinase control. After inoculation cultures were incubated at 30°C (the best temperature of C_1 enzymes production) for 6 days (the best incubation periods) followed by filtration and centrifugation. Clear filtrates were used to detect the cellulase and pectinase activity.

4- Effect of various nitrogen sources:

The sodium nitrate (2g/ L) in the basal medium was replaced by the same amount of various nitrogen compounds such as; ammonium nitrate, ammonium sulphate, calcium nitrate, potassium nitrate, peptone, urea in addition to sodium nitrate as control. Cultures in flasks were incubated at 30°C for 6 days (for C_1 enzymes) and the cultures were filtrated, centrifuged and the filtrate was used for the detection of cellulase and pectinase activity.

Assay of cellulase activity (C_1 enzyme):

The method described by Nelson (1944) and modified by Naguib (1964) was employed. The amount of reducing sugars produced was estimated by determining the optical density (absorption spectrum) at 700 nm wave length with a spectrophotometer model (Bausch and Lomb Spectronic 2000 colorimeter). A standard curve was plotted using aqueous solutions of D-glucose with concentrations from 0.1-0.00001 g/ l.

Assay of pectinase activity:

The method described by Osman (2005) was employed. Pectin lyase (PL enzyme) was assayed spectrophotometrically by determining the optical density (absorption spectrum) at 235 nm wavelength with a spectrophotometer model (Bausch and Lomb Spectronic 2000 colorimeter). A wavelength at which unsaturated uronide product of pectin degradation absorb test (Sherwood, 1966).One unit of pectin lyase activity was defined as that amount of enzyme causing an increase in absorbance of 0.01 in 30 minutes.

Results and Discussion

1- Mycobiota associated with apple fruits:

Thirty-five species and 1 species variety belonging to 14 genera were collected from apple species on agar (DRBC) medium. The most common genera were *Aspergillus*, *Alternaria* and *Penicillium*. From the above genera the most common species were *Aspergillus niger, A. flavus, A. fumigatus*, *Alternaria alternata* and *A. tenuissima*. They were recorvered from 28-96% of the samples and 1.7-15.9%of total fungi (Table 1).Our result is in agreement with Hasan (2000) and Karaibrahimoglu *et al*. (2004). who reported that *Alternaria alternata* followed by *A. niger* was found in rotten apple fruits. Juhneviča *et al*. (2011) mentioned that *Alternaria* sp. and *Aspergillus* sp. were isolated on potato dextrose agar (PDA) and malt extract agar (MEA) media from apple in France. Our study didn't match with Ammar and El-Naggar (2014) who evaluated that *Aspergillus niger* was the lowest rate of occurrence and *Alternaria alternata* ranked the second rate of occurrence in his study in France. Ewekeye *et al*. (2016) showed that *Aspergillus niger* are causative agent in the deterioration of apple fruit from different areas in Nigeria. In this work, *Penicillium* sp. was isolated in high frequency of occurrence. These results are in agreement with other researchers like Ammar and El-Naggar (2014) who reported that *Penicillium* sp. was one of the most genera associated with apple storage in France.

2- Mycobiota associated with tomato fruits:

Thirty-two species and two species varieties belonging to 15 genera were collected from tomato fruits on DRBC medium. The most prevalent genera were *Aspergillus* and *Penicillium* which were recovered in high frequency of occurrence; emerging in 76% and 80% of the samples and 9.2% and 11.5% of total fungi, respectively. From the above genera the most common species were *Aspergillus niger, A. flavus, A. fumigatus* and *Penicillium oxalicum.* They were collectively encountered from 36- 72% of the samples and from 2.08- 7.76 % of total fungi (Table 1). Results of our study corroborate with previous workers reported by Samuel and Orji (2015) who found that *Aspergillus niger* had the highest percentage occurrence (47.27%) and Yeast (*Saccharomyces cerevisiae* and *Geotrichum candidum*) having the lowest percentage occurrence (3.64%) in Nigeria. Udoh *et al*. (2015) and Samuel and Orji (2015) reported that *Rhizopus stolonifer* and

Aspergillus niger responsible for the soft rot of tomato. This result is in agreement with Picos-Munoz *et al*. (2011) and Khokhar and Bajwa (2014) reported that *Penicillium oxalicum* was the most prevalent species caused blue mold rot on tomato fruit in Mexico and Pakistan. In addition, Wogu and Ofuase (2014) detected that *Penicillium* sp. ranked the second fungal genera isolated from spoilt tomato fruits in Benin city in Nigeria. But Samuel and Orji (2015) showed that *Penicillium digitatum* was the commonest species associated with the spoilage of tomato fruits.

Cellulolytic activity:

Total of fourty-eight fungal species and 2 species varieties screened for their abilities to produce C_1 enzyme (exo-β-1,4 glucanase) on solid media proved to be active to utilize cellulose, but with different degrees. Six species (12% of total isolates) showed high cellulolytic activity. On the other hand, 23 species (46% of total isolates) found to be of moderate cellulolytic activity of exo-β-1,4-glucanase enzyme. The remaining 21 isolates (42% of total isolates) could be regarded as weak producers of C_1 enzyme. Our study indicated that *A. chevalieri* was the highest cellulase producer strain (Table 2). In this respect, Abdel-Hafez *et al.* (2010) and Massoud (2013) revealed that *Aspergillus flavus* was the highest fungal isolates in cellulase production. There are revious results contrast with our ones supported by khokhar *et al.* (2011). who revealed that *P. waskmanii* showed the highest growth stimulation in the cellulose and starch medium. Adejuwon *et al*. (2009). demonstrated that *Penicillium funiculosum* exhibited cellulase activity which might be responsible enzyme in pathogenicity of tomato.

Optimization of cultural and nutritional condition for C1 cellulase production by *Aspergillus chevalieri*:

1-Effect of temperature and incubation period:

Our results showed that the maximum dry fungal growth was achieved on 8 days of incubation at 30^{O}C as 0.65 g from *A. chevalieri* (Fig.1, A). Maximum production of exo-β-1,4-glucanase by *A. chevalieri* was achieved on 6 days after incubation at 30^{O}C (Fig. 2, A). Several researchers approved the present study where Abd El-Zaher and Fadel (2010) reported that the high cellulase activity was obtained by *Trichoderma reesei* for 5 days incubation at 28±2˚C. Sakthi *et al*. (2011) reported that maximum production of exo-β-1,4-glucanases by *Aspergillus niger* which was isolated from the spoiled coconut after 6 days of incubation at 30℃. However, other researchers contrast with our study like Utharalakshmi (2015) reported that *Aspergillus flavus* SB4 has higher productivity of cellulase when temperature and incubation time were 35^{O}C and 84 hours, respectively.

2- Effect of pH values:

Our results showed the best mycelial growth of *A. chevalieri* was recorded at pH 6 as 0.5 g (Fig. 1, B) and the optimum pH for cellulase production was 6 (Fig. 2, B). These result approved with Ahmed *et al*. (2009) and Sakthi *et al*. (2011). They found that optimum pH for maximum production of cellulase was 6.0 and 5.5 by *Aspergillus niger* and *Trichoderma harzianum*. Recently, Yadav *et al*. (2016) reported that the optimum pH of cellulase production was 6.0 exhibited by P1 isolate which was isolated from different soil samples. Our results don't match with Utharalakshmi (2015) who reported that the higher productivity of cellulase by *Aspergillus flavus* SB4 was shown at pH 1.5.

3- Effect of carbon source:

Among 7 carbon sources incorporated separately in the culture medium, sucrose yielded the maximum production of C_1enzyme and mycelial growth produced by *Aspergillus chevalieri* (Fig. 1 and 2, C). These results are also in agreement with the ones obtained by other workers like Gautam *et al.*(2011) and Kaur and Joshi (2015). They reported that sucrose was the best carbon source for cellulase production by *Trichoderma* sp. In addition, Deep *et al*. (2014) confirmed that among synthetic carbon sources, sucrose produced maximum exo-β-1,4-glucanse activity which exhibited from *Alternaria brassicicola*. Our results are disagreement with other researchers such as El-Said and Saleem (2008) who reported that maltose was the most suitable carbon source for cellulase

production by *Chaetomiumglobosum*. But Bagga *et al*. (1989) and Yadav *et al*. (2016) showed that lactose was the best inducer for *Aspergillus* sp.

4- Effect of nitrogen source:

The highest yields of mycelial growth and the maximum amounts of C_1 enzyme by *Aspergillus chevalieri* was produced in the presence of peptone (Fig. 1 and 2, D). Our investigations correlate with the result of Bamigboye (2013) who found that the highest cellulase activity for *A. niger, A. oryzae* and *P. expansum* occurred in peptone. Yadav *et al*. (2016) reported that peptone was the best nitrogen source for enhancing cellulase production using solid state fermentation by P1 isolate. Our results contrast with Afifi (2003) who reported that urea was the best biosynthetic abilities of *Mucor fuscus* (MS22) for cellulase production. In addition, Dutt and kumar (2014) observed that the best nitrogen source for inducing cellulase production was $(NH_4)_2SO_4$ from *A. flavus* AT-2 and *A. niger* AT-3 strains.

Pectinase activity:

The ability of fourty-eight species and two species variety belonging to 19 genera were screened for their abilities to produce pectin lyase using cup-plate method. All isolates were pectin lyase producers, but with variable degrees. Seven species (14% of total isolates) exhibited high pectinolytic activity. 17 fungal species (34% of total isolates) were found to be moderate pectinolytic activity. The remaining species (52% of total isolates) were low producers of the enzyme. Our results assessed that *A. chevalieri* was the most fungal species with maximum production of pectin lyase (Table 2). Gummadi and Panda (2003) confirmed that *A. niger* is the most commonly used fungal species for the industrial production of pectinases. In contrast, Martins *et al*. (2002) and Silva *et al*. (2002) showed that *Trichoderma* sp. and *Penicillium* sp. to be the most common pectinase producers. Ibrahim (2013) found that *Penicillium citrinum* has been found to be the best producer of pectinolytic enzymes.

Optimization of cultural and nutritional condition for pectinase production by *A. chevalieri*:

with culture medium containing pectin as carbon source and ammonium sulphate as nitrogen source and initially adjusted to pH 8 (Fig.4).

1. Effect of temperature and incubation period:

Our results showed the highest yield of mycelial growth and maximum production of pectinase by *A. chevalieri* were achieved on 6 days after incubation at 30˚C (Fig. 3 and 4, A). Our results are similar to those obtained by Akhter *et al*., (2011). demonstrated that the maximum pectinase production by *A. niger* was peaked on the seventh day of incubation. Sethi *et al*. (2016). tested that *Aspergillus terreus* NCFT4269.10 have positive influence on pectinase production at 30 °C. On the other hand, Bhardwaj and Garg (2012) revealed that the optimum temperature was found to be 45°C for 24 h of incubation period and further increase in temperature reduces the pectinase production. Khatri *et al.* (2015) demonstrated that a maximum pectinase production by *Aspergillus niger* MCAS2 was observed at 48 h of fermentation at 50°C.

2. Effect of pH on pectinase activity:

Our results indicated that the maximum production was achieved at pH 8 and mycelial growth *A.chevalieri* was increased with the increasing of pH values giving maximum at pH 8 (Fig. 3 and 4 B). These results agree with Yadav *et al*. (2007) and Sandhya and Kurup (2013). They found that the optimum pH for pectinase production in culture filtrate of *Aspergillus flavus* and *Penicillium citrinum* were 8.0. In spite of this, other studies different with our results such as Khatri *et al.* (2015) and Sethi *et al*. (2016) who noticed that a maximum pectinase production by *Aspergillus niger* MCAS2 and *Aspergillus terreus* NCFT4269.10 were observed at pH 8.2 and pH of 5.0, respectively.

3. Effect of carbon source on enzyme activity:

Our results assessed that pectin as a carbon source is the most indicible carbon sources for

pectin lyase production and maximum dry weight of pectinase by *A. chevalieri* (Fig. 3 and 4, C). These results approved with Saleem *et al.* (2012) reported that maximum production of pectinase produced by *Alternaria citri* and *A. raphani* was supplemented with *Citrus* pectin in liquid media. Bhardwaj and Garg (2012) disagreed with our results as the supplementation of production medium with 1% sucrose as carbon source resulted in maximum production of pectinase.

4. Effect of nitrogen source on enzyme activity:

Ammonium sulphate supported highest microbial enzymes production and dry growth in this study (Fig. 3 and 4, D). Saleem *et al.* (2012) reported that the highest yields of pectinase produced by *Alternaria citri* and *A. raphani* were achieved in the presence of ammonium sulphate as nitrogen source followed by peptone. On the contrary, Alcântara *et al.* (2010) reported that the concentration of ammonium sulphate had a negative effect on enzyme activities.

Conclusion:

This article revealed that different fungi from different taxonomic genera were responsible for post-harvest rot of apple and tomato fruits. The gross total count often reflects the outbreaks in the counts of some heavily sporulating fungi such as *Aspergillus* and *Penicillium* species. All fungal species exhibited cellulase (C_1 enzyme) and pectinase activity with variable degrees. The nutritional and environmental conditions of the two enzymes were studied by *Aspergillus chevalieri*.

Genera and species	Apple			Tomato		
	ATC	NCI	OR	ATC	NCI	OR
Acremonium	5500	9	M			
A. buytri	4150	8	M			
A. rutilum	1350	1	R			
Alternaria	13050	16	H	22400	12	M
A. alternata	10200	16	H	22000	11	M
A. chlamydospora	100	1	R			
A. raphani	400	2	R			
A. tenuissima	2350	7	M	400	1	R
Aspergillus	32150	25	H	22200	19	H
A. aegyptiacus				200	1	R
A. flavus	6550	12	M	5000	12	M
A. flavus var. *columnaris*	350	2	R	200	1	R
A. fumigatus	2050	12	M	5900	9	M
A. niger	21800	24	H	10900	18	H
A. ochraceus	1400	2	R			
Bahusakala olivaceonigra				1500	1	R
Cladosporium	5350	11	M	20500	12	M
C. cladosporioides	2500	6	L	17300	10	M
C. herbarum	2100	7	M	1500	3	R
C. sphaerospermum	750	3	R	1700	4	L
Drechslera halodes	250	1	R			
Emericella				3600	2	R
E.nidulans var. *echinulatus*				3600	2	R
Epicoccum purpurascens				3000	2	R
Eurotium chevalieri	100	1	R	200	1	R
Fusarium	750	2	R	14900	8	M
F. dimerum				3600	2	R
F. equistei	100	1	R			
F. heterosporum				1500	1	R
F. moniliforme	650	1	R	2200	3	R
F. oxysporum				4900	3	R
F. subglutinans				1700	1	R
F. tabacinum				1000	2	R

Table (1): Average total counts (ATC), number of cases of isolation (NCI, out of 25 samples) and occurrence remarks (OR) of fungal genera and species recovered from50 samples of apple and tomato on dichloran rose-Benegal chloramphicol agar.

Genera and species	Apple			Tomato		
	ATC	NCI	OR	ATC	NCI	OR
Myrothecium roridum	1300	1	R			
Penicillium	8850	13	H	27600	20	H
P. camembertii	1050	2	R	1100	4	L
P. chrysogenum				4000	2	R
P. citrinum	1400	2	R			
P. dendriticum	50	1	R	400	2	R
P. expansum	100	1	R			
P. funiculosum	500	1	R	500	3	R
P. griseofulum	400	1	R			
P. olivicolor	200	2	R			
P. oxalicum	2400	6	L	18600	14	H
P. purpurogenum	1300	5	L	1600	3	R
P. rubrum	500	2	R			
P. viridicatum	650	3	R	800	3	R
P. waksmanii	300	3	R	600	4	L
Rhizopusnigricans				2100	1	R
Scytalidium lignicola	550	1	R			
Stemphylium	100	1	R	1800	3	R
S. botryosum	100	1	R			
tate of *pleospora herbarum*				400	1	R
S. vesicarium				1400	3	R
Sterile mycelia				500	1	R
Trichoderma viride				1100	2	R
Ulocladium	850	3	R	800	2	R
U. alternariae	250	2	R			
U. botrytis	600	1	R	800	2	R
Wiesneriomyces javanicus				1500	1	R
Yeast sp.				1900	5	L
Average total counts	137100			239400		
No. of genera 20	14			15		
No. of species 65	35+1			32+2		

Table (1): cont.

*Occurrence remarks: OR (out of 25 samples), H= high occurrence from 13-25 cases, M= moderate occurrence from 7-12 cases, L= low occurrrence from 4-6 cases and R= rare occurrence from 1-3 cases.

Fungal isolates	Exo-1,4-β cellulase	Pectinase Activity
Acremoniumbuytri	12 W	16 W
A. rutilum	14 W	21 M
Alternariaalternata	15 W	18 W
A. chlamydospora	25 H	15 W
A. raphani	14 W	13 W
A. tenuissima	18 M	19 M
Aspergillus aegyptiacus	20 M	20 M
A. flavus	25 H	30 H
*A. flavus*var. *columnaris*	26 H	33 H
A. fumigatus	21 M	26 M
A. niger	18 M	23 M
A. ochraceus	12 W	19 M
Bahusakalaolivaceonigra	16 W	11 W
Cladosporiumcladosporioides	23 M	28 H
C. herbarum	16 M	12 W
C. sphaerospermum	18 M	25 M
Drechslerahalodes	15 W	12 W
Emericellanidulans var.*echinulatus*	22 M	21 M
Epicoccumpurpurascens	13 W	13 W
Eurotiumchevalieri	30 H	35 H
Fusarium dimerum	19 M	18 W
F. equistei	22 M	28 H
F. heterosporum	15 W	15 W
F. moniliforme	19 M	15 W
F. oxysporum	25 H	13 W
F. subglutinans	21 M	24 M
F. tabacinum	20 M	19 M
Myrothecium roridum	22 M	23 M
Penicilliumcamembertii	11 W	14 W
P. chrysogenum	14 W	17 W
P. citrinum	15 W	18 W
P. dendriticum	14 W	21 M
P. expansum	22 M	28 H
P. funiculosum	15 W	20 M
P. griseofulvum	19 M	16 W
P. olivicolor	11 W	23 M

Table (2): Screening of fungal isolates for their abilities to produce cellulase and pectinase lyase enzymes.

Fungal isolates	Exo-β-1,4 cellulase	Pectinase Activity
P. oxalicum	20 M	29 H
P. purpurogenum	17 M	18 M
P. rubrum	13 W	17 W
P. viridicatum	12 W	13 W
P. waksmanii	13 W	10 W
Rhizopusnigricans	25 H	14 W
Scytalidiumlignicola	13 W	25 M
Stemphyliumbotryosum	13 W	13 W
*S.*state of*pleosporaherbarum*	18 M	14 W
S.vesicarium	17 M	16 W
Sterile mycelia	19 M	12 W
Trichoderma viride	20 M	18 M
Ulocladiumalternariae	18 M	12 W
U. botrytis	20 M	14 W
Wiesneriomycesjavanicus	10 W	12 W

Table 2: cont.

Activity remarks for cellulase: High activity, H= from 30-23 mm; Moderate activity, M= 22-16mm; and Weak activity, W= less than 16 mm. Activity remarks for pectinase enzyme: high activity, H=from 35-27 mm; Moderate activity, M=26-19 mm; and weak activity, W=less than 18 mm.

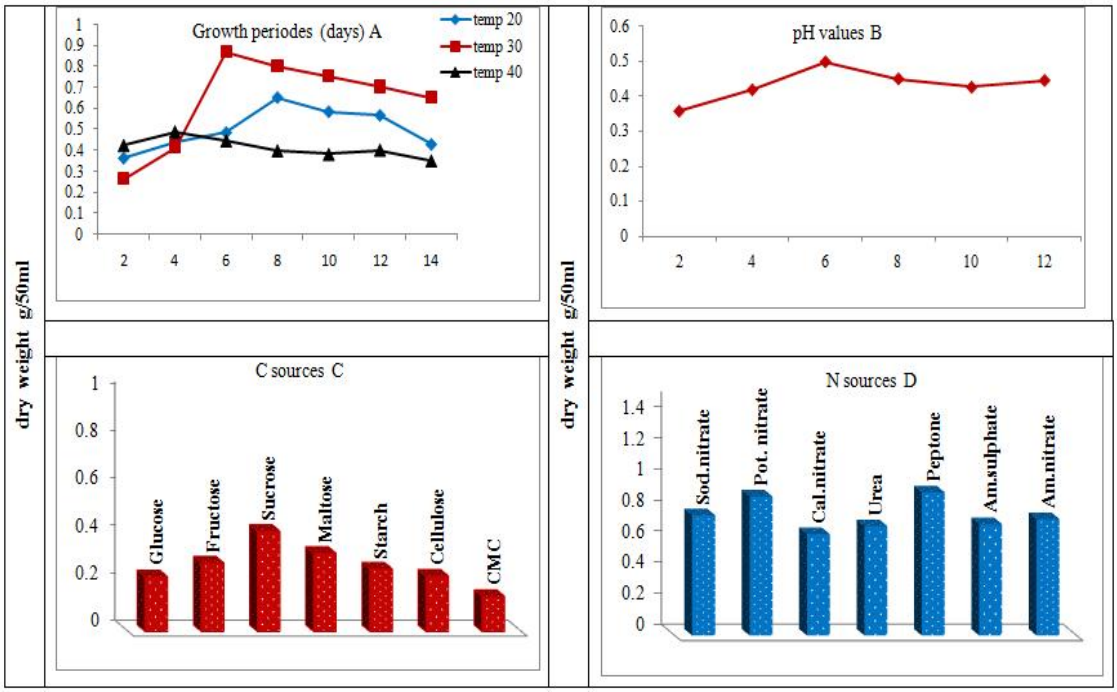

Fig. (1): Effect of temperature and incubation period (A), effect of pH values (B), effect of carbon source (C) and effect of nitrogen sources (D) on exo-β-1,4-glucanases production by dry weight of *Aspergillus chevalieri*.

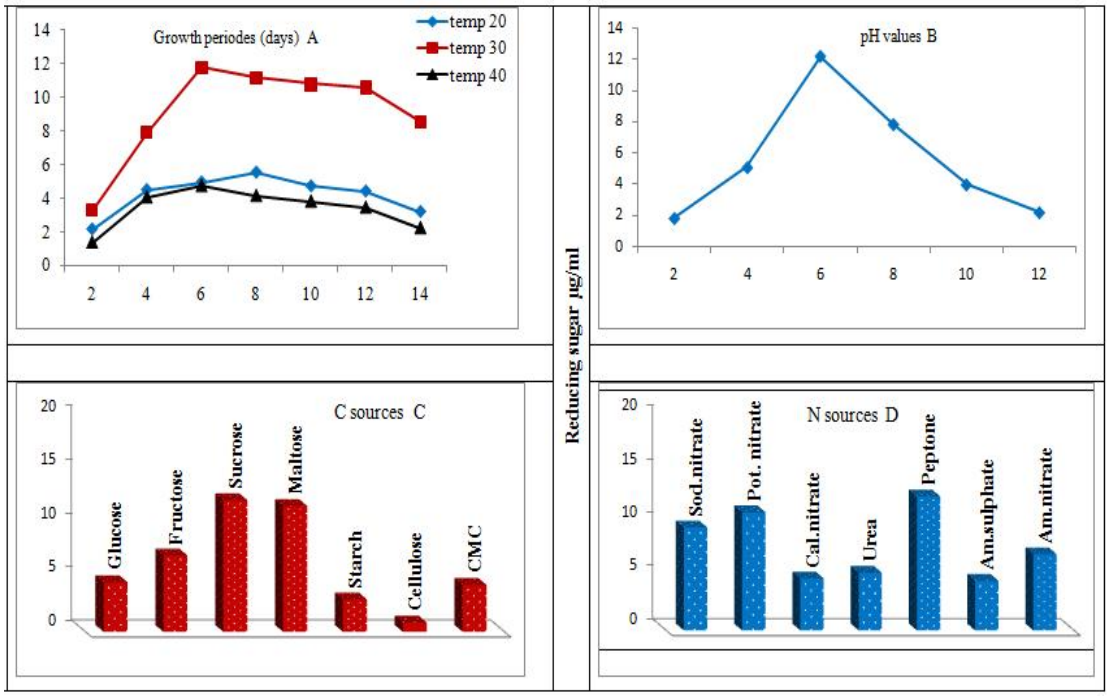

Fig.(2): Effect of temperature and incubation period (A), effect of pH values (B), effect of carbon source (C) and effect of nitrogen sources(D) on exo-β-1,4-glucanases production by *Aspergillus chevalieri*.

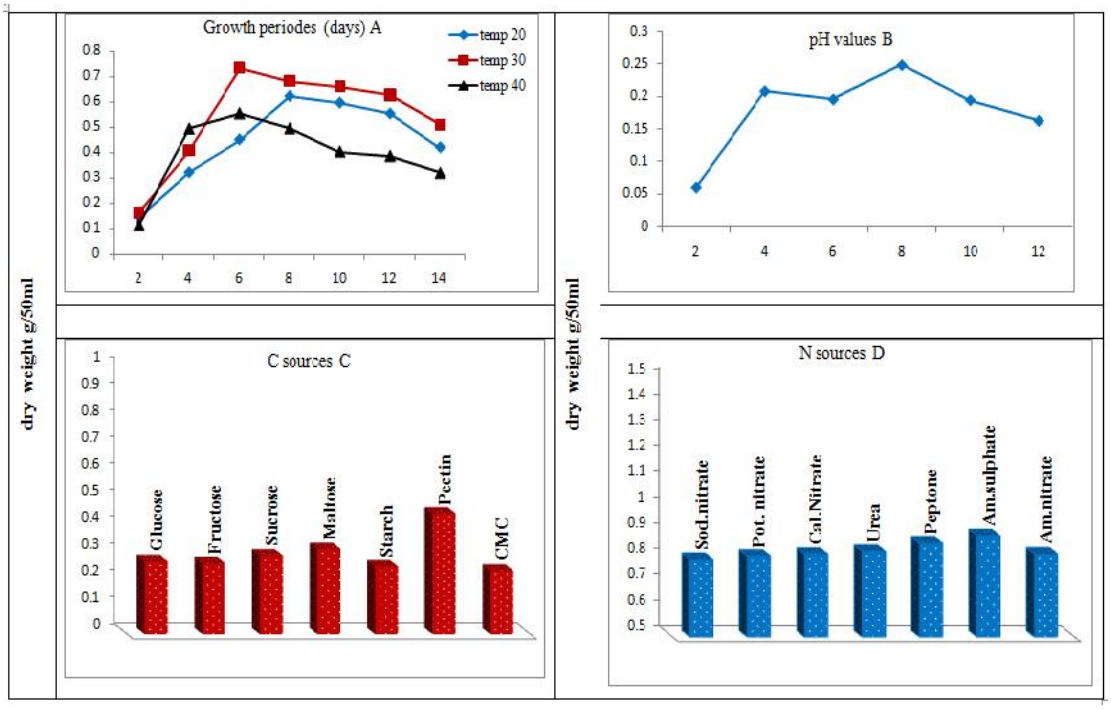

Fig. (3): Effect of temperature and incubation period (A), effect of pH values (B),effect of carbon source (C) and effect of nitrogen sources(D) on pectinase production by dry weight of *Aspergilluschevalieri.*

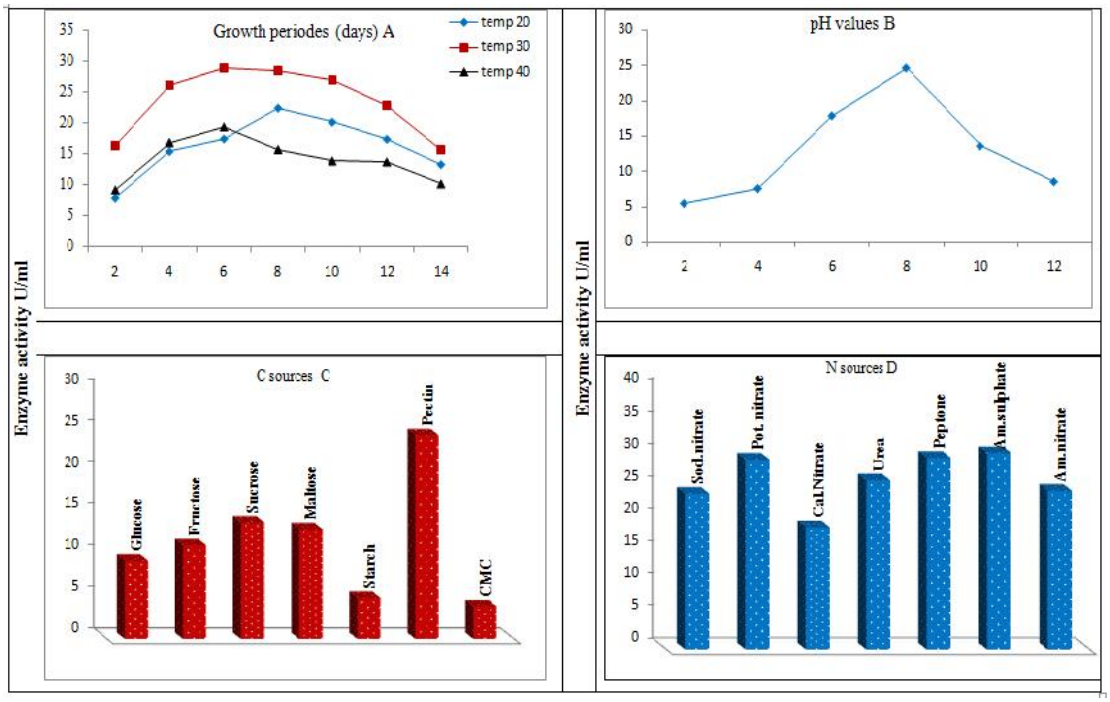

Fig. (4): Effect of time course and temperature(A), effect of pH values (B), effect of carbon source (C) and effect of nitrogen sources (D) of pectinase by *A. chevalieri*.

References:

Abd El-Zaher, F.H. and Fadel, M. (2010). Production of bioethanol via enzymatic saccharification of rice straw by cellulase produced by Trichoderma reesei under solid state fermentation. New York Science Journal, 3 (4): 72-78.

Abdel-Hafez, S.I.I., El-Said, A.H.M., Moharram, A.M. and Saleem A. (2010). Effect of two insecticides, Sparkill (25% Cypermethrin) and Tafaban (48% Chorpyrifos) on mycobiota of maize plants in Upper Egypt. Archives of Phytopathology and Plant Protection, 43 (7-9): 783-800.

ACMSF, (Advisory Committee on the Microbiological Safety of Food) (2005). Microbiological status of ready to eat fruit and vegetables. Retrieved February 1, 2012, from http://www.food.gov.uk/multimedia.

Adejuwon, A.O., Oni, A.O., Ajayi, A.A. and Olutiola, P.O. (2009). Cellulase activity in tomato fruit infected with *PenicilliumFuniculosum* Thom. African Journal of Plant Science 3:113-116.

Afifi, M.M. (2003). Biotechnological applications of cellulose and pectinase enzymes produced by some isolated from soil in upper Egypt. The Asssiut University Bulletin for Environmental Research. 6 (2): 53-63.

Ahmed, S., Bashir A., Saleem, H., Saadia, M. and Jamil, A. (2009). Production and Purification of Cellulose-Degrading Enzymes from A Filamentous Fungus Trichoderma harzianum. Pakistan Journal of Botany, 41(3): 1411-1419.

Akhter, N., Morshed M.A., Uddin, A., Begum, F., Tipu Sultan and Azad A.K. (2011). Production of pectinase by*Aspergillus niger* Cultured in Solid State Media. International Journal of Biosciences.1 (1):33-42.

Alcântara, S.R., Almeida, F.A.C., Silva F.L.H. (2010). Pectinases production by solid state fermentation with apple bagasse: water activity and influence of

nitrogen source. Chemical Engineering Transactions 20 :121-126.

Ammar, M.I. and El-Naggar, M.A. (2014). Screening and Characterization of Fungi and their associated Mycotoxins in some Fruit Crops, International Journal of Advanced Research 2 (4): 1216-1227.

Ammar, M.S., Louboudy, S.S., Azab M.S. and Afifi M.M. (1995) A new method for the estimation of fungal pectinase (s) using the pectin clearing zone (P.C.Z.) technique and its application in food industries. Al-Azhar Bulletin of Science, 6: 325-339.

Bagga, P.S., Sandhu, D.K. and Sharma, S. (1989). Catabolite repression of cellulose production in *Aspergillus nidulans*. Process Biochemistry, 24: 41-45.

Bamigboye, O.O. (2013). IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 6 (5): 15-19.

Bhale, U.N. (2011). Survey of market storage diseases of some important fruits of Osmannabad District (M. S.) India. Science Research Reporter. 1: 88 - 91.

Bhardwaj, V., Garg, N. (2012). Production, Purification of Pectinase from Bacillus sp. MBRL576 Isolate and its Application in Extraction of Juice.International Journal of Science and Research (IJSR): 3.358 pages 648-652.

Christensen, C.M. (1963). Influence of small differences in moisture content upon the invasion of hard red winter wheat by *Aspergillusrestrictus* and *A. repens*. Cereal Chemistry 40: 385-395.

Deacon, J.W. (1985). Decomposition of filter paper cellulose by thermophilic fungi acting in combination and in sequence Trans. Br. Mycol. Soc., 85: 663-669.

Deep, S., Sharma, P. and Behera, N. (2014). Optimization of extracellular cellulase enzyme production from *Alternariabrassicicola*. International Journal of Current Microbiology and Applied Sciences. 3(9) 127-139.

Dutt, D. and Kumar, A. (2014). Optimization of cellulose production undersolid-state fermentation by *Aspergillus flavus* (AT-2) and *Aspergillus niger* (AT-3) and its impact on stickies and INK particle size of sorted office paper. Cellulose Chemistry Technology. 48 (3-4),285-298.

Eggins, H.O.W. and Pugh, G.J.F. (1962). Isolation of cellulose decomposing fungi from the soil Nature, 193: 94-94.

El-Said, A.H.M. and Saleem, A. (2008). Ecological and physiological studies on soil fungi at western region, Libya.Mycobiology, 36 1: 1-9.

El-Said, A.H.M., Saleem, A., Maghraby, T.A. and Hussein, M.A. (2014).Cellulase activity of some phytopathogenic fungi isolated from diseased leaves of broad bean. International Journal of Current Microbiology and Applied Sciences, 3(2): 883-900.

Etebu, E., Nwauzoma, A.B. and Bawo, D.D.S. (2013). Postharvest Spoilage of Tomato (*Lycopersiconesculentum*Mill.) and Control Strategies in Nigeria, Journal of Biology, Agriculture and Healthcar.3 (10): 51-61.

Ewekeye, T.S., Oke O.A. and Esan O.O. (2016). Studies on post harvest rot of apple (*Malus domestica Borkh*). Indian Journal of Plant Sciences5 (1): 36-41.

Ewekeye, T.S., Oke O.A., Quadri, A.I., Isikalu, A.O., Umenwaniri M.O. and Durosinmi, M.L. (2013). Studies on Post Harvest Deterioration of Some Fruits and Vegetables in Selected Markets in Lagos State, Nigeria.American Journal of Research Communication.**1:** 209-222.

Fatima, N., Batool, H., Sultana, V., Ara, J. and Ehteshamul-Haque, S. (2009). Prevalence of Post-Harvest rot of vegetables and fruits in karachi, Pakistan, Pakistan Journal of Botany, 41(6): 3185-3190.

Gautam, S.P., Bundela P.S., Pandey, A.K., Jamaluddin, L., Awasthi, M.K. and Sarsaiya, S. (2010). Optimization of the medium for the production of cellulase by the Trichoderma viride using submerged fermentation. International Journal of Environmental Science and Technology.14: 656-665.

Gummadi, S.N. and Panda, T. (2003). Purification and biochemical properties of microbial Pectinases a review Process Biochemistry 38 : 987-996.

Hankin, L., Zucker, M. and Sands, D.C. (1971). Improved Solid Medium for the Detection and Enumeration of Pectolytic Bacteria.Appl Microbiology 22(2): 205–209.

Hasan, H.A.H. (2000). Patulin and aflatoxin in brown rot lesion of apple fruits and their regulation, World Journal of Microbiology & Biotechnology16: 607-612.

Idnurm, A., Howlett, B.J. (2001). Pathogenicity genes of phyto-pathogenic fungi.Mol. Plant Pathol. 2: 241-255.

Ilyas, M.B., Ghazanfar, M.U., Khan, M.A., Khan, C.A. and Bhatti, M.A.R., (2007). post harvest losses in apple and banana during transport and storage, Pakistan Journal of Agriculture Science 44(3):534-539.

Juhneviča, K., Skudra, G., Skudra, L. (2011). Evaluation of microbiological contamination of apple fruit stored in a modified atmosphere. Environmental and Experimental Biology. 9: 53–59.

Karaibrahimoglu, Y., Fan, X., Sapers, G.M. and Sokoraj, K. (2004). Effect of pH on the survival of Listeria innocuain calcium ascorbate solutions and on quality of fresh cut apples. Journal of Food Protection., 67(4): 751-757.

Kaur, H.P. and Joshi, D. (2015). Optimization of cellulose produced by fungus isolated from water, World Journal of pharmacy and pharmaceutical sciences. 4 (2): 521-534.

Khatri, B.P., Bhattarai, T., Shrestha, S. and Maharjan, J. (2015). Alkaline thermostable pectinase enzyme from *Aspergillus niger* strain MCAS2 isolatedfrom Manaslu Conservation Area, Gorkha, Nepal. Springer Plus 4:488.

Khokhar, I, Mukhtar, I, Mushtaq, S. (2011). Comparative Studies on the Amylase and Cellulase Production of *Aspergillus* and*Penicillium*.Journal of Applied Sciences and Environmental Management 15 (4): 657- 661.

Khokhar, I. and Bajwa, R. (2014). Prevalence of post-harvest rot of fruits and vegetables by Penicillium species, International Journal of Advanced Research in Biological Sciences. 1(9): (2014): 14–19.

King, D.A.Jr., Hocking, A.D. and Pitt, J.I. (1979). Journal of Applied and Environmental Microbiology. 37: 959-964.

Martins, E.S., Silva, D., Da Silva, R. and Gomes, E. (2002). "Solid state production of thermostable pectinases from thermophilic *Thermoascusaurantiacus*," Process Biochemistry, 37 (9); 949–954.

Massoud, M.S. (2013). Survey of Fungal Diseases of Some Vegetables and Fruits in Aswan. Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 6 (3): 39-42.

McNeill, M., Darvill, A.G., Fry, S.C., Albersheim, P. (1984). Structure and function of the primary cell walls of plants.Annual Review of Biochemistry. 53: 625-663.

Mebratie, M.A., Haji, J., Woldetsadik, K., Ayalew, A. (2015). Determinants of Postharvest Banana Loss in the Marketing Chain of Central Ethiopia, Science and Quality Management, 37: 52-63.

Moubasher, A.H., El-naghy, M.A. and Abdel-Hafez, S.I.I. (1972). Studies on the fungus flora of three grains in Egypt. MycopathologiaetMycololgiaApplicata. 47:261-274.

Moubasher, A.H., Abdel-Hafez, S.I.I., El-Hissy F.T. and Hassan S.K.M. (1980). Effect of temperature and moisture content on Egyptian Peanut seed-borne fungi.Mycopathologia. 70:149-154.

Naguib, M.I. (1964). Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds, Indian journal of experimental biology. 2: 149-152.

Nathalie, J. (2006). Plant protein inhibitors of cell wall degrading enzymes. Trends Plant Science, 11: 359-367.

Nelson, N. (1944). Aphotometric adaptation of the somogyi method for determination of glucose. Journal of Biological Chemistry, 153: 375-380.

Osman, N.F.A. (2005). Ecological and physiological studies on fungi associated with post-harvested rot of some fruits.

Oyemaechi, C.U., Chukwuezi, O. and Ozougwu, E.O. (2014). Microbial Agents of Tomato Spoilage in Onitsha Metropolis .Advances in Biological Research 8 (2): 87-93.

Picos-Munoz, P.A., Garcia-Estrada, R.S., Carrillo-Fasio, J.A., Leon-Felix, J., and Allende-Molar, R. (2011). First Report of Blue Mold Caused by *Penicilliumoxalicum* in Tomato (*Solanum lycopersicum*) in Mexico, The American Phytopathological Society, 95 (9),

Sakthi, S.S., Saranraj, P. and Rajasekar, M. (2011). Optimization for cellulose production by *Aspergillus niger* using paddy straw as substrate.International journal of Advanced Scientific and Technical Research1 (1): 69-85.

Saleem, A., El-Said, A.H.M., Maghraby, T.A., Hussein, M.A. (2012). Pathogenicity and pectinase activity of some facultative Mycoparasitesisolated from *Viciafaba*diseased leaves in relation to photosynthetic pigments of plant. Journal of Plant Pathology and Microbiology3 (6): 1-7.

Samuel, O., Orji, M.U. (2015). Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria.Universal Journal of Microbiology Research 3(2): 11-16.

Sandhya, R. and Kurup, G. (2013). Screening and Isolation of Pectinase from Fruit and Vegetable Wastes and the Use of Orange Waste as a Substrate for Pectinase Production International Research Journal of Biological Sciences 2(9): 34-39.

Sethi, B. K., Nanda, P. K., Sahoo, S. (2016). Enhanced production of pectinase by *Aspergillus terreus* NCFT4269.10 using banana peels as substrate. 3 Biotechnology, 6:36.

Ibramim, A. Shima (2013). Enhancement of fungal Pectinolyticenzymes production using gamma radiation under solid state fermentation. MSC Thesis in Botany department, faculty of science, Assiutuniversity.

Sherwood, R.T. (1966). Pectin lyase and polygalacturonase production by Rhizoctoniasolani and other fungi. Phytopathology 56: 279-286.

Silva, D., Martins, E. da Silva, Silva, R. da, and Gomes, E. (2002). "Pectinase production by *Penicilliumviridicatum*RFC3 by solid state fermentation using agricultural wastes and agro-industrial by-products", Brazilian Journal of Microbiology, 33 (4): 318–324.

Smock, R.M. and Neubert, A.M. (1950). Apples and apple products.Wiley Interscience, New York, NY, USA.Page 486.

Udoh, D.J., Ndon, B.A., Asuquo, P.E. and Ndaeyo, N.U. (2005). Crop Production Techniques for the Tropics. Concept publications limited, Lagos, Nigeria. Pp238-399.

Udoh, I.P., Eleazar, C.I., Ogeneh, B.O., Ohanu, M.E. (2015). Studies on Fungi Responsible for the Spoilage/Deterioration of Some Edible Fruits and Vegetables, Advances in Microbiology, 5: 285-290.

Utharalakshmi, N., Kumar, A.G. and Narendrakumar, G. (2015). Optimization of cellulaseproducing *Aspergillusflavus* SB4 by solid state fermentation using response surface methodology (rsm)-ccd. Research Journal of Pharmacy and Technology: 8(4): 349-354.

Wogu, M.D. and Ofuase, O. (2014). Microorganisms responsible for the spoilage of tomato fruits, lycopersicumesculentum, sold in markets in Benin City, Southern Nigeria.

Scholar's Academic Journal of Bioscience, 2(7): 459-466.

Yadav, S.,Yadav, P.K., Yadav, K.D.S. (2007). Pectin lyases of a few indigenous fungal strains. Journal of science & Industrial Research 66:601-604.

Yadav, P.R., Chauhan, P.B., Gahlout, M. and Prajapati, H. (2016). Isolation, Screening and Optimization of process parameters for enhanced production of cellulase by solid state fermentation.International Journal of Advanced Research in Biological Sciences. 3(5): 21-27.

الملخص العربى

تم فى هذا البحث دراسة المحتوى الفطرى لخمسين عينة من الفاكهة (طماطم وتفاح) فى محافظة سوهاج بمصرعلى وسط غذائى واحد وهو داى كلوران روز بنجال كلورام فينيكول اجار والتحضين عند ٢٨ م . كشفت الدراسة عن عزل ثمانى واربعون نوعا فطريا وصنفان تنتمى الى ١٩ جنسا .وجد ان اكثر الاجناس انتشارا على فاكهة الطماطم والتفاح قيد الدراسة هما الاسبريجلس والبنسيلوم والكلادوسبويم والالترناريا والفيوزاريم ومن اكثر الانواع الفطرية شيوعا هى الالترنايا الترناتا والالترناريا تونيسيما والاسبيرجلس نيجر والاسبيرجلس فلافس والاسبيرجلس فيوميجاتس وكلادوسبويم كلادوسبرويدس وكلادوسبويم هيربارم وبنيسليوم اوكزليكم. و تم دراسة مقدرة خمسين عزلة فطرية (٤٨ نوع وصنفان) على انتاج انزيمى السيليلوزC1 والبكتينيز.واظهرت النتائج ان ٦ و ٧عزلات فطرية لها قدرة عالية على انتاج انزيمى السيليلوز والبكتينيز على التوالى . وبينما ٢٣ و١٧ عزلات فطرية لها قدرة متوسطة على انتاجالأنزيمين على التوالى. أما باقى العزلات الفطرية لها قدرة منخفضة على انتاج الانزيمين. واظهرت الدراسة ان اقوى العزلات الفطرية انتاجا لانزيمى السيليلوز والبكتينيز هو الاسبرجلس شيفاليرى أظهراكبر انتاج لانزيم السيليلوز بواسطة هذه العزلة عند ٦ ايام من التحضين و ٣٠ درجة مئوية ودرجة الاس الهيدروجينى ٦ فى الوسط غذائى محتوى على السكروز والبيبتون كمصدر كربونى ونيتروجينى. وبالنسبة لانزيم البكتينيز سجل الاسبرجلس شيفاليرى اعلى انتاج لانزيم البكتينيز بعد ٦ ايام من التحضين و ٣٠ درجة مئوية ودرجة الاس الهيدرجينى للوسط الغذائى ٨ ومحتوى على البكتين وكبريتات الامونيوم كمصدر كربونى ونيتروجينى على التوالى.