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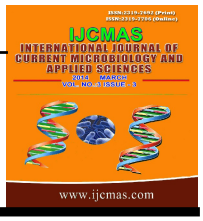

Original Research Article

Effect of moisture contents on the biodiversity of fungi contaminating *Cuminum cyminum* and *Pimpinella anisum* seeds under storage periods and Amylolytic Activity of fungal isolates

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Keywords

Cumin and anise seeds; moisture contents; storage periods; fungal populations;

A B S T R A C T

The present research work was conducted to evaluate fungal populations in storage cumin and anise seeds under various moisture contents and the relationship between fungal populations and various moisture contents and storage periods. The moisture contents and storage periods used for cumin and anise seeds 10, 15 and 20% m.c. and for four months. Seeds were taken every month for mycological analysis. The total fungal population often showed irregular monthly fluctuations with some peaks mainly due to the outbreaks in the counts of *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii* and *Mucor hiemalis*. *Trichoderma viride* was in the top of fungi in producing extracellular α-amylase among the 90 isolates recovered from anise and cumin seeds .The highest yield of the enzyme could be achieved 6 days after incubation at 30˚C with the incorporation of dextrose as carbon source and yeast extract as nitrogen source in the culture medium which is initially

Introduction

Seeds by nature are hygroscopic and tend to be influenced by the surrounding atmosphere. At high moisture content they heat up rapidly in storage. The respiration of the sample is accelerated. Coupled with this, associated micro-organisms also play an important role in the high respiratory processes. Therefore, good storage conditions are required to prevent seed deterioration thus increasing the storage life of the seed and maintaining good seed viability (Zainun Nik and Mamat, 1982).

However, spices as plants may be harmed even in the field and contaminated by bacteria and moulds before the beginning of drying and treatment (Stankovic *et al*., 2006). Later, due to the bad conditions of storage and ventilation and high percent of humidity, contamination of the stored amounts by pathogenic microorganisms frequently occurs (Omafuvbe and Kolawole, 2004).

Several studies have been made on the storage of seeds (Misra *et al*., 1995; El-

Bazza *et al*., 1996; DiGrak and Ozcelik, 2001; Marchisio and Airaudi, 2001; Bhattacharya and Raha, 2002; Bankole *et al*., 2004; Taligoola *et al*., 2004; Abramson *et al*., 2005; Park *et al*., 2005; Adebayo-Tayo *et al*., 2006; Oh *et al*., 2007; Genkawa *et al*. 2008; Oh *et al*. 2008; Weinberg *et al*., 2008).

Moharram *et al*. (1989); Regina and Roman (1992) reported that cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), anise (*Pimpinella anisum*), caraway (*Carum carvi*) and fennel (*Foeniculum vulgare*) are the most important medicinal and aromatic seeds in Egypt and in the world. Following harvest and during storage, they are subjected to attack and damage by numerous fungi, *i.e. Aspergillus* spp., *Penicillium* spp. *Rhizopus* spp. and *Fusarium* spp.

Adebayo-Tayo *et al*. (2006) showed that *Aspergillus carbonarius, Aspergillus terreus, Aspergillus flavus, Aspergillus candidus, Penicillium expansum, Aspergillus niger, Candida tropicalis* and *Aspergillus glaucus* were found to be associated with marketed bush mango seeds. Of the eight species, *A. flavus and A. tereus* were most dominant. The moulds probably infected the product during the process of cracking (shelled) to extract the cotyledons (kernels), drying, storing and transportation. The moisture content obtained from bush mango seeds ranged from 2.0-21.0%.

Oh *et al*. (2008) studied fungal and bacterial populations in unhulled and brown rice under indoor storage conditions and examined the relationship between microbial populations and environmental conditions such as temperature and relative humidity. The temperature and relative humidity of the storage room temperature ranged from 22.6˚C to 27.0˚C and 23.3% to 44.2%, respectively. Total fungal and bacterial populations remained relatively stable over the storage period. Predominant fungi included *Aspergillus candidus*, *A. flavus*, *A. fumigatus* and *Penicillium* spp.

Weinberg *et al*. (2008) studied maize at 14, 16, 18, 20 and 22% m.c. was initially conditioned for 28 days in tightly wrapped plastic bags and then stored in sealed containers at 30˚C for up to 75 days. Carbon dioxide produced within the containers replaced the oxygen. As the m.c. increased the time for O2 depletion shortened, from 600 h at 14% m.c. to 12 h at 22%. The maize at 20 and 22% m.c. exhibited the highest dry matter (DM) losses, the lowest germination rates and the highest yeast and bacteria counts.

Amylolytic activities of some fungi

Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan *et al*., 1999; Pederson and Nielsen, 2000) by degrading 1-4 linkage of starch. This enzyme is extensively used in starch liquefaction, paper industries, food, pharmaceutical and sugar industries (Nigam and Singh, 1995). Three different enzymes catalyze most steps in the degradation of starch to glucose, although still other enzymes are needed to complete the process. The first three enzymes include α-amylase, β-amylase and starch phosphorylase (Kumar and Satyanarayana, 2004). On the basis of their mode of action, these amylolytic enzymes are divided into endo-amylases and exo-amylases (Calik and Ozdamar, 2001), (Koetje *et al.,* 2003). Amylases are a groupe of enzymes that have been found

in several microorganisms including fungi (Fadel, 2000).

α-amylase is known to be produced by some representatives of the genera *Aspergillus*, *Rhizopus*, *Penicillium*, *Paecilomyces*, *Alternaria* etc. (Sadhukhan *et al*., 1990; El-Abyad *et al*., 1992; Gupta and Grautam, 1995; Goto *et al*., 1998; Pandey *et al*., 1999; Fadel, 2000; Buzzini and Martini, 2002; Ikram-ul-Haq *et al*., 2006; Shafique *et al*., 2009, Abdel-Hafez *et al*. 2010 , Moharram *et al*. , 2010 and Amany *et al.* , 2011).

Materials and Methods

Source of seeds

Anise and cumin seeds used in this part of study were obtained from the field during harvest.

Determination of moisture content

The moisture content of the seeds was determined by oven drying at 105°C for 16 h (Anonymous, 1966).

Seeds moisture content test

The fresh weight of seeds completed with a sterile distillated water for the moisture content becoming 10%, 15% and 20% m.c, these seeds put in a sterile polyethylene bags in a refrigerator for 24 h and then incubated at 28°C for 4 months (Wexler and Bornbaker, 1951). Samples were taken every month for mycological analysis.

Mycological analysis

The dilution - plate and seed – plate methods were used for the estimation of fungal flora associated with anise and cumin seeds as described by Christensen (1963) and employed by Abdel- Hafez *et al*. (1987, 1993) and modified Czapek's agar medium was used .

Screening of fungal isolates for amylase production

Eighty-seven species and 3 species varieties belonging to 32 genera were screened for their abilities to produce extracellular α-amylase. Isolates were cultured on solid starch yeast extract agar (SYE) medium with a composition (in g /L) of soluble starch, 5.0; Bacto-yeast extract, 2.0; KH_2PO_4, 1.0; $MgSO_4$. 7 H_2O, 0.5 and agar-agar, 15 (Barnett and Fergus, 1971). Cultures were incubated at 28°C for 6 days. Using a sterile cork borer (10 mm diameter), the inoculum (agar disc bearing mycelium from the agar culture) was obtained. For each fungal isolate, one sterile 100 ml Erlenmeyer flask containing 50 ml of the liquid SYE was prepared. Cultures were incubated at 28°C without shaking for 7 days after which the mycelium was harvested by filtration. Filtrates were used to detect the amylolytic activity of fungi according to the method of the society of American Bacteriologists (1957). Aliquots of 0.1 ml of a culture filtrate were pipetted into 10 mm cavities which were made in SYE plates. After 24 h incubation at 28°C, plates were flooded with iodine solution (KI, 15 g; I_2, 3 g per liter of distilled water). A zone void of blue indicates the production of amylase. In case of positive strains, the average diameter of clear zones (in mm) of the triplicates for each isolate was recorded.

Factors affecting α-amylase production

The effects of different ecological and nutritional factors on α-amylase production by *Trichoderma viride* were

studied. This isolate was found to be the most active amylase producer.

Effect of temperature and time course

Flasks, each containing 50 ml SYE medium with pH 6, were inoculated with the test organism and incubated at 20°, 30° and 40°C for 14 days and harvested at 48 h intervals. Filtrates from samples were combined and the resulting solution was assayed for α-amylase activity.

Effect of pH values

Flasks with 50 ml aliquots of SYE medium adjusted using 0.1 N HCL or 0.1 N Na OH to different pH levels ranging from 2 to 12 were inoculated and incubated at 30°C for 6 days (the optimum temperature and incubation period for α-amylase production). One flask for each pH level was prepared. At the end of incubation period, cultures were filtered, and the amylase activity was determined.

Effect of different carbon sources

The test organism was cultured on SYE medium free of starch and adjusted to pH 6 (the best pH for α-amylase production). The following carbon sources were incorporated separately in the basal medium at a concentration of 0.5% w/v: cellulose, dextrose, fructose, glucose, sucrose, in addition to starch in the basal medium as control. After 6 days of incubation at 30°C (the best period and temperature for amylase production), cultures were filtered and the α-amylase activity was determined by Nelson (1944) and modified by Naguib (1964).

Effect of different nitrogen sources

The test organism was cultured on SYE medium free of yeast extract and adjusted to pH 6 (the best pH for α-amylase production). The following nitrogen sources were incorporated separately in the basal medium at a concentration of 0.2% w/v: ammonium chloride, ammonium nitrate, ammonium sulphate and potassium nitrate, in addition to yeast extract as control. After 6 days of incubation at 30°C (the best period and temperature for amylase production), cultures were filtered and the α-amylase activity was determined by Nelson (1944) and modified by Naguib (1964).

Results and Discussion

Fungi of Stored Anise and Cumin Seeds

The effect of various moisture contents and storage periods on the mycoflora of anise and cumin seeds was studied. Over a period of four months the most common fungi isolated at various moisture contents are *Aspergillus*, *Emericella* and sterile mycelia.

During the period of storage, the fungal content of anise and cumin seeds showed great fluctuations depending on the decrease or increase of individual fungal species. In most cases control samples (with 5.3% - 6.0% moisture content) were contaminated with a reasonable number of fungal colonies which was mainly due to the high counts of *A. niger* and *R. stolonifer*.

Generally, the fungal population markedly declined during storage either in the control samples or in samples containing 10%, 15% or 20% moisture content. Unexpected outbreaks in the counts of some fungal species such as *A. flavus, A. fumigatus*, *A. niger* and *A. sydowii*. A plausible explanation of the reduction in fungal population during storage is the

release of volatile oils (essential oils) from anise and cumin seeds causing inhibition of some or many fungal spores contaminating these seeds.

Numerous investigations have been carried out on the mycoflora associated with various types of seeds stored under moisture content conditions in many parts of the world.

As suggested by Wallace (1973) cereals are preserved by reduction of moisture content to less than 13.5% and oil seeds to less than 7.8%, because storage fungi such as *Aspergillus* spp. or *Penicillium* spp. cannot grow at these low moisture content. Unfortunately low energy methods of drying seeds are not always practical as mould fungi can develop before the seed dried, especially in areas where the relative humidity is high after harvest (Sholberg *et al*., 1996).

Prasad *et al.* (1988) and Regina and Roman (1992) reported that the incidence of infection and severity of damage by storage fungi depend on storage temperature, seed moisture content, relative humidity, fungal species and their counts present at preharvest and mechanical damage of flowers.

Moharram *et al*. (1989) reported that cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), anise (*Pimpinella anisum*), caraway (*Carum carvi*) and fennel (*Foeniculum vulgare*) are subjected to attack and damage by numerous fungi, *i.e. Aspergillus* spp., *Penicillium* spp. *Rhizopus* spp. and *Fusarium* spp.

Working with wheat grains Abramson *et al*. (2005) found that *Eurotium* and *Penicillium* species colonized the durum wheat at 16% m.c. In the absence of any moisture increase, the *Eurotium* species predominated. The higher moisture (20% m.c) favored the multiplication of *Penicillium*.

Oh *et al*. (2008) studied fungal and bacterial populations in unhulled and brown rice under indoor storage conditions and examined the relationship between microbial populations and environmental conditions such as temperature and relative humidity. The temperature and relative humidity of the storage room temperature ranged from 22.6˚C to 27.0˚C and 23.3% to 44.2%, respectively. Total fungal and bacterial populations remained relatively stable over the storage period. Predominant fungi included *Aspergillus candidus, A. flavus, A. fumigatus* and *Penicillium* spp.

Amylolytic Activities of Fungal Isolates

Ninety isolates recovered from anise and cumin seeds were screened for their abilities to produce extracellular α-amylase. It was noticed that Forty-five isolates (50% of total isolates) exhibited high amylolytic activities and these were: *Acremonium kiliense, A. rutilum, Alternaria brassicicola, Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. sulphureus, A. sydowii, A. tamarii, Botryotrichum piluliferum, Chaetomium crispatum, Circinella muscae, Cochliobolus lunatus, Curvularia pallescens, Drechslera euphorbiae, Fusarium dimerum, F. oxysporum, Gibberella fujikuroi, G. tricincta, Memnoniella subsimplex, Mucor circinelloides, M. hiemalis, M. racemosus, Myrothecium verrucaria, Nectria haematococca, Paecilomyces carneus, Penicillium aurantiogriseum, P. brevicompactum, P. chrysogenum,*

Table.1 Count (per g dry weight) of common fungal genera and species after different storage periods of *Cuminum cyminum* seeds treatment with various moisture contents on glucose-Czapek's agar, using dilution-plate method.

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Acremonium kiliense	50															
Alternaria alternata	25															
Aspergillus	175	125		25	225	50	25	550	250	175	500	650	150		175	100
A. flavus					75			50			125	50				
A. fumigatus						25		475	125	50	375	525	25		175	75
A. niger	175	100		25	150	25		25	75	100		50				25
A. sydowii		25					25		25	25		25				
A. terreus var. *aureus*									25							
A. versicolor													125			
Emericella nidulans var. *lata*													25			
Eurotium chevalieri	50											25				
Mucor hiemalis																
Penicillium	25	25		100			25									
P. brevicompactum				75												
P. chrysogenum	25	25					25									
P. funiculosum				25												
Rhizopus stolonifer	250															
Stachybotrys state of *Melanopsamma pomiformis*					25					25				25		
Sterile mycelia (dark and white colour)	100		50		25		225			75		50		25	25	25
Gross total count	675	150	50	125	275	50	275	550	250	275	500	725	175	50	200	125

M.C= Moisture content, C= Control (5.3% m.c), Treatments= (10, 15, 20% m.c).

Table.2 Count (per g dry weight) of common fungal genera and species after different storage periods of *Cuminum cyminum* seeds treatment with various moisture contents on cellulose-Czapek's agar, using dilution-plate method.

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Aspergillus	275	375			450	25		25	500	475	650	150	350	500	275	
A. clavatus	25															
A. flavus	175	375			150						50		125	125	250	
A. fumigatus	75				50			25	250	300	450	150	125	125	25	
A. niger					250				250	100	75			75		
A. ochraceus														25		
A. sydowii						25					25		100			
A. terreus var. *aureus*										75	50					
Botryotrichum piluliferum														125		
Chaetomium				25		25										
C. crispatum						25										
C. madrasense				25												
Cochliobolus					25											
C. spicifer					25											
Mucor hiemalis		750														
Penicillium																
P. chrysogenum												25				
Setosphaeria rostrata	125															
Stachybotrys state of *Melanopsamma pomiformis*		25			25	25							25			
Sterile mycelia (dark and white colour)	25		375	50	25		25									50
Ulocladium					25											
U. botrytis					25											
Gross total count	425	1150	375	75	550	75	25	25	500	475	650	175	375	475	275	50

M.C= Moisture content, C= Control (5.3% m.c), Treatments= (10, 15, 20% m.c).

Table.3 Counts (per 20 seeds) of common fungal genera and species after different storage periods of *C. cyminum* seeds treatment with various moisture contents on glucose-Czapek's agar, using the seed-plate method

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Alternaria alternata													1			
Aspergillus	12	4	4	3	14	11	5	5	9	7	4	7	9	4	6	7
A. flavus	1			1		2						1			3	2
A. fumigatus					3	7	4	4	4	2	4	3		3	3	3
A. niger	11	3	4	2	10	2	1	1	5	4		2	9	1		2
A. ochraceus																
A. sydowii		1			1					1		1				
Cochliobolus	2												1			
C. lunatus	2															
C. spicifer													1			
Emericella nidulans var. *lata*											1		1			
Eurotium chevalieri		1		1												
Mucor hiemalis	5				2									1		
Penicillium	3															
P. chrysogenum	3															
Rhizopus stolonifer	2		2	2	3											
Setosphaeria rostrata		2			1								1			
Stachybotrys state of *Melanopsamma pomiformis*		3	1	2	2	1				1			2			
Sterile mycelia (dark and white colour)	8	7	6	5	5	5	4	3	2	3	2	2	5	2	3	
Gross total count	32	17	13	13	27	17	9	8	11	11	7	9	20	7	9	7

M.C= Moisture content, C= Control (5.3% m.c), Treatments= (10, 15, 20% m.c).

Table.4 Counts (per 20 seeds) of common fungal genera and species after different storage periods of Cuminum cyminum seeds treatment with various moisture contents on cellulose-Czapek's agar, using the seed-plate method

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Alternaria alternata				1												
Aspergillus	7	3	7		15	9	9	4	17	8	9	13	14	1	6	
A. flavus	1	1	2		3	1			1				3		5	
A. fumigatus					1	3	1	1	7	3	9	12			1	
A. niger	6	2	5		11	5	8	3	9	5			11	1		
A. ochraceus												1				
Chaetomium	2	1	2	3						1				1		1
C. atrobrunneum	1															
C. barilochense		1														
C. crispatum	1		2	1						1				1		
C. funicola																1
C. globosum				2												
Cochliobolus	1		2													
C. lunatus	1		1													
C. spicifer			1													
Cunninghamella echinulata									1							
Emericella nidulans var. *lata*					2											
Eurotium chevalieri													2			
Mucor hiemalis					5											
Penicillium							1									
P. aurantiogriseum							1									
Rhizopus stolonifer											8					
Setosphaeria rostrata		2														
Stachybotrys state of *Melanopsamma pomiformis*	6	5	1			2										
Sterile mycelia (dark and white colour)				3		1		6	2	2	4	2	1	2	2	2
Ulocladium	2			1												
U. chartarum	2			1												
Gross total count	18	11	12	8	22	12	18	10	20	11	13	15	17	4	8	3

M.C= Moisture content, C= Control (5.3% m.c), Treatments= (10, 15, 20% m.c).

Table.5 Count (per g dry weight) of common fungal genera and species after different storage periods of *Pimpinella anisum* seeds treatment with various moisture contents on glucose-Czapek's agar, using dilution-plate method

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Acremonium		20								10				10		
A. furcatum														10		
A. kiliense		20														
A. strictum										10						
Alternaria alternata	270	100			80											
Aspergillus	310	280	100		600	320	520	10	220	270	80	600	280	150		60
A. clavatus	20		20													
A. flavus	30	10			80	10			30				20	10		
A. fumigatus	30						490			160	70					
A. niger	200	270	80		520	280	30	10	190	100			260	140		
A. ochraceus						20										
A. sydowii	20					10					10	600				60
A. tamarii	10															
A. terreus var. *aureus*										10						
Botryotrichum piluliferum														10		
Circinella muscae						90										
Cladosporium		10											30			
C. cladosporioides													30			
C. sphaerospermum		10														
Cunninghamella echinulata									10							
Drechslera erythrospila		10														
Emericella nidulans var. lata	10		10		40	40			40	10	10		40			
Eurotium chevalieri								10						10	20	130
Nectria haematococca	10															

Table.5 Continued

Fungal Species Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Penicillium		10	10		30	10	10	30					30			
P. aurantiogriseum					20								20			
P. brevicompactum			10													
P. chrysogenum		10			10	10							10			
P. citrinum								30								
P. corylophilum							10									
Rhizopus stolonifer		50	50		10	10			70							
Setosphaeria rostrata	10															
Stachybotrys state of *Melanopsamma pomiformis*		10				30										
Sterile mycelia (dark and white colour)	20	30	10	20	30	20	20	70	20		20		200	50	40	
Ulocladium		10			40											
U. botrytis		10			40											
Gross total count	630	530	180	20	830	520	550	120	360	290	110	600	580	230	60	190

M.C= Moisture content, C= Control (6% m.c), Treatments= (10, 15, 20% m.c).

Table.6 Count (per g dry weight) of common fungal genera and species after different storage periods of *Pimpinella anisum* seeds treatment with various moisture contents on cellulose-Czapek's agar, using dilution-plate method

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Acremonium				10	20				10						20	
A. furcatum				10											20	
A. kiliense					20				10							
A. strictum																
Alternaria alternata	280	210		10	60	10	10									
Aspergillus	160	180	170	30	420	340	30	50	390	120	80	600	280	90	70	410
A. clavatus				10												
A. flavus	10	30	20		70	10		10	50				20			
A. fumigatus			50			30	20	20	30	10	70		10	10	20	10
A. niger	150	140	100	20	350	280		20	240	80	10		240	80	50	
A. ochraceus						10										
A. sydowii		10				10			60	30		600				400
A. terreus var. *aureus*							10		10				10			
Chaetomium	60	20				10										
C. atrobrunneum						10										
C. globosum	60	20														
Cladosporium		30							10							
C. cladosporioides		30														
C. sphaerospermum									10							
Cochliobolus spicifer													20			
Curvularia lunata var. *aeria*					10					20						
Emericella nidulans var. *lata*					50	10				60				60		

Table.6 Continued

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Fusarium		10			60											
F. merismoides		10														
F. oxysporum					60											
Mucor hiemalis						110			50							
Penicillium					20				40							
P. aurantiogriseum									40							
P. funiculosum					20											
Phoma eupyrena								10								
Scopulariopsis shaerospora	10															
Stachybotrys state of *Melanopsamma pomiformis*	20	100	130	10	30				20				20			
Sterile mycelia (dark and white colour)			110	130	10	20	20	60	20		20		130		40	
Ulocladium					10											
U. alternariae					10											
Gross total count	530	550	410	190	690	500	60	120	540	200	100	600	450	150	130	410

M.C= Moisture content, C= Control (6% m.c), Treatments= (10, 15, 20% m.c).

Table.7 Counts (per 20 seeds) of common fungal genera and species after different storage periods of *Pimpinella anisum* seeds treatment with various moisture contents on glucose-Czapek's agar, using the seed-plate method

Fungal Species / Periods & M.C	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
														1		
Alternaria alternata	7	3							1							
Aspergillus	18	17	14	1	21	22	14	10	23	30	7	34	19	7		2
A. flavus	4	2	2		3	1	2				1	3	4	1		
A. fumigatus					4	11		10	7	20	6	9				2
A. niger	12	14	12	1	14	10	12		16	9		2	15	6		
A. ochraceus	2	1														
A. sydowii												20				
A. terreus var. *aureus*										1						
Cladosporium		1	1	1												
C. sphaerospermum		1	1	1												
Cochliobolus		1	2			1							2	1		
C. lunatus													1			
C. spicifer		1	2			1							1	1		
Emericella nidulans var. *lata*	2		1	2	7		1		3	3				1		
Eurotium chevalieri															2	1
Mucor hiemalis						2										
Penicillium		1				2										
P. corylophilum						2										
P. funiculosum		1														
Rhizopus stolonifer	5	11	3		1	5	3						2			
Stachybotrys state of *Melanopsamma pomiformis*			2	1	1									1		
Sterile mycelia (dark and white colour)	2	3	2	4	2	2		3		2	2		1	4	4	
Trichoderma hamatum											1					
Ulocladium	1	2	1	1												
U. alternariae				1												
U. botrytis	1	2	1													
Gross total count	35	39	26	10	32	34	18	13	27	35	10	34	24	15	6	3

M.C= Moisture content, C= Control (6% m.c), Treatments= (10, 15, 20% m.c).

Table.8 Counts (per 20 seeds) of common fungal genera and species after different storage periods of *Pimpinella anisum* seeds treatment with various moisture contents on cellulose-Czapek's agar, using the seed-plate method

Fungal Species / Periods & M.	1 Month				2 Month				3 Month				4 Month			
	C	10	15	20	C	10	15	20	C	10	15	20	C	10	15	20
Alternaria alternata	5	9	2													
Aspergillus	22	16	13	1	36	30	30	19	33	30	15	40	24	6	2	2
A .flavus	6	4	1			18	18						3	1	2	2
A. fumigatus					19			19	11	20	15	20	1			
A. niger	16	12	12	1	17	12	12		19	9			16	5		
A. sydowii												20	3			
A. terreus var. *aureus*									3	1			1			
Chaetomium											1		1			
C. crispatum											1					
C. funicola													1			
Cladosporium sphaerospermum	3	3		2												
Cochliobolus spicifer	1				1	1										
Emericella nidulans var. *lata*		1				3		1	1	3	1					
Eurotium chevalieri				1												
Penicillium corylophilum	1															
Stachybotrys state of *Melanopsamma pomiformis*	4	2		1									2			
Stemphylium solani		1														
Sterile mycelia (dark and white colour)			1	1			1			2	2		5			
Ulocladium		1	1	1			1						1			
U. alternariae		1														
U. botrytis													1			
U. chartarum			1	1			1									
Gross total count	36	33	17	7	37	34	32	20	34	35	19	40	33	6	2	2

M.C= Moisture content, C= Control (6% m.c), Treatments= (10, 15, 20% m.c).

Table.9 Degree of cellulolytic and amylolytic activities (calculated as. average diameter of clear zone in mm) of the fungal isolates tested

Fungal isolates	Amylase
Acremonium furcatum	14 W
A. kiliense	29 H
A. rutilum	23 H
A. strictum	17 M
Alternaria alternata	17 M
A. brassicicola	22 H
A. chlamydospora	17 M
A. raphani	11 W
Aspergillus candidus	35 H
A. clavatus	30 H
A. flavus	22 H
A. fumigatus	20 H
A. niger	28 H
A. ochraceus	20 H
A. sulphureus	23 H
A. sydowii	20 H
A. tamarii	35 H
A. terreus var. *aureus*	17 M
A. ustus	28 M
A. versicolor	19 M
Botryotrichum piluliferum	29 H
Chaetomium anguipilium	10 W
C. atrobrunneum	10 W
C. citrinum	12 W
C. crispatum	23 H
C. dreyfussii	10 W
C. funicola	18 M
C. globosporum	10 W
C. globosum	10 W
C. jabalpurense	17 M
C. spiralotrichum	10 W
C. oblatum	10 W
C. subspirilliferum	10 W
C. uniporum	10 W

Table.9 Continued

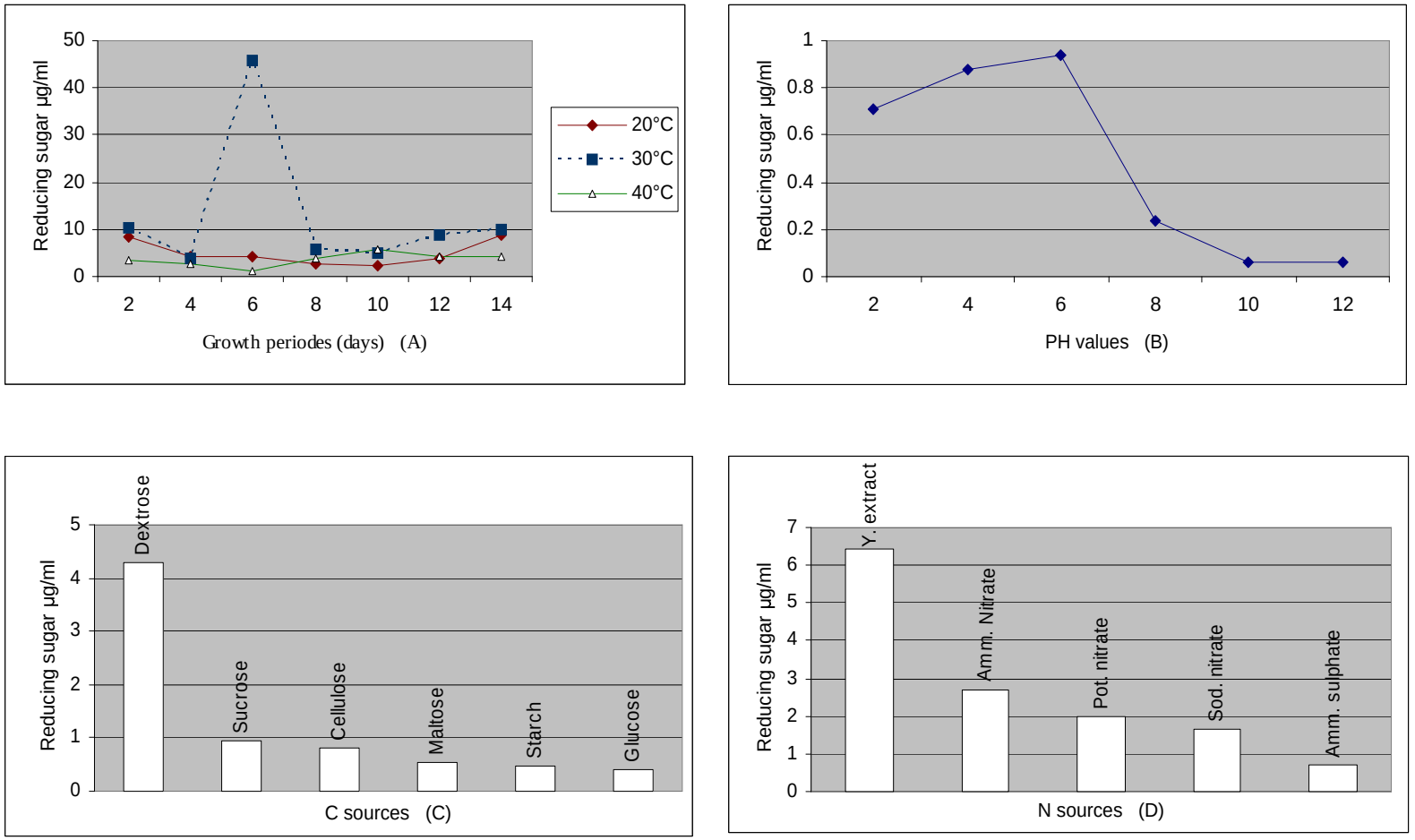
Fungal isolates	Amylase
Circinella muscae	22 H
Cladosporium cladosporioides	10 W
C. musae	19 M
C. sphaerospermum	14 W
C. variabile	15 W
Cochliobolus lunatus	27 H
C. spicifer	16 M
C. tuberculatus	18 M
Cunninghamella echinulata	10 W
Curvularia brachyspora	19 M
C. lunata var. *aeria*	16 M
C. pallescens	22 H
Drechslera australiensis	19 M
D. erythrospila	18 M
D. euphorbiae	25 H
D. state of *Trichometasphaeria pedicellata*	13 W
Emericella nidulans var. *lata*	16 M
Epicoccum purpurascens	12 W
Eurotium chevalieri	10 W
Fusarium dimerum	21 H
F. merismoides	18 M
F. oxysporum	20 H
F. semitectum	10 W
F. sulphureum	18 M
Gibberella fujikuroi	23 H
G. tricincta	31 H
Memnoniella subsimplex	23 H
Monographella nivalis	17 M
Mucor circinelloides	38 H
M. hiemalis	21 H
M. racemosus	24 H
Myrothecium verrucaria	25 H
Nectria haematococca	25 H
Paecilomyces carneus	25 H
Penicillium aurantiogriseum	22 H
P. brevicompactum	32 H

Table.9 Continued

Fungal isolates	Amylase
P. chrysogenum	34 H
P. citrinum	28 H
P. corylophilum	23 H
P. duclauxii	27 H
P. funiculosum	24 H
P. purpurogenum	22 H
P. spinulosum	17 M
Phoma eupyrena	23 H
Rhizopus stolonifer	20 H
Scopulariopsis brevicaulis	19 M
S. sphaerospora	10 W
Setosphaeria rostrata	16 M
Stachybotrys state of *Melanopsamma pomiformis*	22 H
Stemphylium solani	26 H
Syncephalastrum racemosum	23 H
Trichoderma hamatum	20 H
T. viride	41 H
Ulocladium alternariae	21 H
U. botrytis	14 W
U. chartarum	13 W

Activity Remarks: High activity, H= from 20-40 mm; Moderate activity, M= 16-19 mm; and Weak activity, W= less than 15 mm.

Fig.1 Effect of time course and temperature, pH values, different carbon and nitrogen sources (A, B, C and D) on the production of α-amylase enzyme by *Trichoderma viride.*



P. citrinum, P. corylophilum, P. duclauxii, P. funiculosum, P. purprogenum, Phoma eupyrena, Rhizopus stolonifer, Stachybotrys state of *Melanopsamma pomiformis, Stemphylium solani, Syncephalastrum racemosum, Trichoderma hamatum, T. viride* and *Ulocladium alternariae*. The remaining fungal isolates showed moderate or low amylolytic activities (Table 9).

There is evidence in the literature that the different fungal isolates exhibit variable capabilities in the production of α-amylase by several workers.

Abdel-Hafez *et al*. (2010) tested the ability of 50 fungal isolates to produce extracellular hydrolytic enzymes in solid media revealed that the most active amylase producers were *Aspergillus flavus, Cunninghamella echinulata, Fusarium oxysporum, Mucor hiemalis* and *Penicillium chrysogenum*.

Moharram *et al*. (2010) screened Forty four fungal isolates representing 35 species and 2 varieties for amylase production. All isolates were recovered from different parts of faba bean plant. All fungal isolates tested had the ability to produce amylase enzyme, but with variable degrees. Nineteen isolates (43.2% of total isolates) showed high amylase activity. Sixteen isolates (36.4% of total isolates) were found to be moderate amylase activity. The remaining isolates (9 isolates, 20.4% of total isolates) were low producers of amylase.

Chimata *et al*. (2010) found that the maximum production of amylase by *Aspergillus* sp. MK07 was achieved after 120 h. of incubation period at 30˚C with culture medium containing starch as carbon source and peptone as nitrogen source and initially adjusted to pH 5, 70% moisture content and 5% inoculum level. The highest amount of amylase production obtained under all optimized conditions was 164 U/g.

When the effect of different environmental and nutritional factors on α-amylase production by *Trichoderma viride* was studied, it was found that the highest yield of the enzyme could be achieved 6 days after incubation at 30˚C with the incorporation of dextrose as carbon source and yeast extract as nitrogen source in the culture medium which is initially adjusted to pH 6 (Fig.1).

These finding are almost in agreement with those reported by Abdel-Hafez *et al*. (1995) observed that maximum amylase production by *Penicillium chrysogenum* could be achieved after 4 days of incubation at 30˚C especially when the culture medium was initially adjusted to pH 4 and contained yeast extract as nitrogen source.

Erdal and Taskin (2010) found that production of α-amylase by *P. expansum* was 6 days after incubation at 30˚C with culture medium of loquat kernel flour (LKF) containing starch as carbon source and peptone as nitrogen source and initially adjusted to pH 6 with moisture content of 70%, particle size of 1 mm and 1 ml methanol as supplement alcohol.

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