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REGULATION OF *EUROTIUM REPENS* REPRODUCTION AND SECONDARY METABOLITE PRODUCTION

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ABSTRACT

Eurotium repens (Anamorph *Aspergillus repens*) was isolated from spoiled fruit. It reproduced sexually at different sucrose concentrations up to 50% (w/v); water activity, 0.79. It reproduced asexually at high sucrose concentration 60% (w/v) water activity, 0.75. The concentrations of all detected amino acids were higher in the teleomorph than the anamorph stage except that of glycine, while α-aminoadipic acid and alanine were detected in teleomorph only. The extracellular secondary metabolites produced by the teleomorph and anamorph stages were variable and different except epoxysuccinic acid and 2-pyruvylamino benzamide which were produced by both stages. Glycine, arginine and calcium chloride unlike glutamic acid, aspartic acid and alanine, are important in the induction of teleomorph stage formation at high sucrose concentration 60 % (w/v).

Keywords: *Aspergillus repen*s, reproduction, fungi, amino acids, sucrose.

INTRODUCTION

Eurotium species often dominate the fungal population in stored grain and are responsible for spoilage of jams, dried foods, dried salted fish and sponge cake (Abellana *et al*., 1999; Bluham *et al*., 2005). *Eurotium repens* sexually reproduces as an ascomycete (telomorph) whereas asexual conidial reproduction of the same fungus (Anamorph) is classified as *Aspergillus repens*.

Water activity (a_w) measurements estimate the proportion of the available water in a system, i.e. the water available for biological (biochemical) and chemical reaction. Water activity can be controlled through water removal or solute addition; solutes that can be used for this purpose are polyols, salts and sugars (Rose, 1983). Xerophilic fungi are characterized as being capable of growing below a_w of 0.85, and are most commonly associated with intermediate moisture foods, including cereals, nuts species and several dried food stuffs (Hocking, 1988). The majority of xerotolerant fungi belongs to the genera *Aspergillus* and *Penicillium* or are perfect forms of *Aspergillus* such as *Eurotium* and *Emericella.* One of the principal factors controlling the growth of these organisms in food is a_w,: the effective growth range can be as low as 0.61 (Corry, 1987; Jay, 1992).

Low a_w significantly reduced spores germination of *Aspergillus spp* (Nesci *et al*., 2003; Ni and Streett, 2005). The spores only germinated on a medium with high a_w values; 0.982 and 0.937, while the spores did not germinate with a_w values 0.747 and 0.809.

Fungi reproduce asexually under favorable condition and sexually under stress conditions (Griffin, 1994). Bluhm *et al*. (2005) reported that *Aspergillus nidulans* and *Aspergillus flavus* strains grew only at 0.98 a_w. At 0.86 a_w. No growth of *Aspergillus nidulans* or *Aspergillus flavus* was visible after 8 days. At 0.83 a_w, *Aspergillus* nidulans was not observed, nor were sclerotia produced by *Aspergillus flavus.*

Secondary metabolites are low-molecular-weight natural products generated by filamentous fungi, plants, algae, bacteria, and animals in response to environmental abiotic and biotic stimuli. Secondary metabolites have a strong impact on humankind via their application in health, medicine, agriculture, and industry; they include useful (e.g. antibiotics) and detrimental compounds (e.g. mycotoxins). These metabolites are frequently associated with asexual and sexual development (Chang *et al*., 2001; Wilkinson, *et al*., 2004). Adams *et al*. (1998) and Pena *et al*. (1998) found a positive correlation between cleistothecial formation and secondary metabolites production in wild type and mutant strains of *Emericella nidulans.*

Aspergillus spp. produce an array of secondary metabolites including aflatoxin, cyclopiazonic acid, aflatrem, patulin, penicillin, kojic acid, lovastatin, carotenoids, and spore pigments; novel secondary metabolites have also been discovered that are synthesized from so called silent gene clusters in *A. nidulans*, such as terrequinone.

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A, monodictyphenone, emodins, and polyketides (Bok *et al.*, 2009).

Some ascomycetes may require exogenous vitamins, minerals, or other natural materials for ascocarp production that are often not duplicated in synthetic media (Moore – Landecker, 1992). *Venturia inaequlis* produced large number of ascocarps with glycine but no ascocarps were produced with ammonium tartrate (Roos and Bremner, 1971). Engelkes *et al*. (1997) found that the tyrosine was one of the better nitrogen sources for production of *Taloromyces flavus* ascospores. Also, fatty acids or related lipids are important for sexual development of filamentous fungi (Nukina *et al*., 1981; Goodrich – Tanrikulu *et al*., 1998). The objectives of this study was to assess the metabolic regulation through stress conditions on growth, reproduction and secondary metabolites biosynthesis of *Eurotium repens* which cause spoilage of fruits.

MATERIALS AND METHODS

Fungal strain

The fungal isolate was isolated from spoiled fruit and identified as *Eurotium repens* according to Rapper and Fennel (1965).

Media

Dox's agar medium (sucrose, 20g; $NaNO_3$, 2g; KH_2PO_4, 1g; KCl, 0.5g; $MgSO_4$. $7H_2O$, 0.5g; Fe SO_4. $7H_2O$, 0.001 g; agar 20 g and distilled water, 1L) and Malt extract agar medium (malt extract, 20g; peptone, 1g; dextrose, 20g; agar, 20g and distilled water, 1L) were used for isolation, cultivation and identification of the fungal isolate.

Growth and culture conditions

Dox's agar medium was supplemented with different sucrose concentrations; 2, 30, 40, 50, 60, 70 and 80% (w/v) to adjust the water activity (a_w) 0.99 , 0.86, 0.82, 0.79 , 0.75 , 0.72 and 0.70, respectively according to Hefnawy, 1993. A plug of inoculum from the loading edge of a colony growing on an agar plate was either inoculated in the center of another plate contain the above medium (for growth and detection of the anamorph and teleomorph stages) or transferred to 500 ml conical flask (s) for detection of amino acids, secondary metabolites, metals and antimicrobial activity. Dox's agar medium supplemented with different sucrose concentrations and pHs, were inoculated and incubated were at different temperatures for 8 days to study their effects on anamorph and teleomorph stages formation.

Nitrogen free Dox's agar medium supplemented with different sucrose concentrations was amended with selected amino acids in equivalent weigh to N of $NaNO_3$ and certain metals; calcium chloride and aluminum chloride, (0.01mg/100ml medium) for metabolic regulation of anamorph and teleomorph stages formation. The percentage of teleomorph and anamorph forms, as represented by the presence of cleistothecia and conidial heads, respectively was calculated by using a hemacytometer.

Secondary metabolites detection

Secondary metabolites were determined by the method described by Paterson and Bridge (1994) as follows the fungal mat of *Eurotium repens* was harvested and the fungal growth medium was filtered and extracted with equal volume of chloroform : methanol (2 :1, v/v), left to evaporate till dryness and then dissolved in 1 ml of extraction solvent.

The extraction concentrates were spotted on a pre-coated thin layer chromatography (TLC) plate (20 × 20 cm aluminum sheet silica gel 60, layer thickness 0.2 mm) along with griseofulvin as a standard reference. The metabolites were eluted using toluene: ethyl acetate: 90% formic acid (5:4: l, v/v/v). The developed secondary metabolites spots were visualized for their colour and R_f under white, UV (365 nm), UV (254 nm) and back under UV (365 nm) light respectively. The plate was then sprayed with 0.5 % (w/v) ρ -anisaldehyde in methanol: acetic acid: concentrated sulphuric acid (17:2:1, v/v/v) and visualized under white light. The plat was heated for 8 minutes at 105°C and reexamined under white, UV (365 nm) and UV (254 nm) light respectively.

Amino acids analysis

Cell free extracts was prepared by grinding the fresh fungal mycelium (5gm) in sterile mortar with 70% ethanol (v/v). The slurry was centrifuged at 600 rpm for 10 minutes, and the supernatant was concentrated using a vacuum desiccators. The concentrated cell free extract was analyzed for amino acids qualitatively and quantitatively with a full automated Amino Acid analyzer: Model Lc 3000 (Eppendorf-Biotronik, Germany) at the Regional Center for Mycology and Biotechnology Al-Azhar University.

Metals analysis

Dry fungal mycelium (0.5gm) was ground and analyzed for metals with a Fei QUANTA 200 Environmental scanning electron microscope with Edex Unit Micro-analysis.

Antimicrobial activity

The antimicrobial activity of extra-and intracellular secondary metabolites were determined by the filter paper disc method (Nester *et al*., 1983). The filter paper discs, 6 mm in diameter were separately soaked in the extracts and transferred onto the surface of the growth medium seeded with the test organism after the incubation period, the diameter of the inhibited growth area around the disc (s) was measured.

RESULTS

As showing in table 1, growth of *Eurotium repens* increased with increasing sucrose concentration up to 40% reflect a decreasing water activity (a_w, 0.82), but then decreased slightly, and failed to grow at 80% sucrose concentration (a_w 0.70). The percentage of teleomorph and anamorph stages formation detected as shown in figure 1a and 1b, decreased and increased respectively, with increasing sucrose concentration up to 50%. At 60 and 70% sucrose concentration, the fungus failed to reproduce sexually (Table 1).

The percentage of teleomorph and anamorph stages formation at stress temperatures (20 & 40ºC) and pHs (4 & 8) was relatively similar to those of control (30ºC & pH6) at the same sucrose concentrations (Tables 2, 3) but among the conditions compared, the optimum-growth temperature and pH were 30°C and pH 6.

Secondary metabolites

The extracellular secondary metabolites produced by teleomorph and anamorph stages of *Eurotium repens* were different except for two metabolites; epoxy succinic acid and 2-pyruovylaminobenzamid (Table 4). The number of extracellular secondary metabolites produced by teleomorph stage was more than that produced by anamorph stage.

Antimicrobial activity

The intra- and extracellular secondary metabolites of the teleomorph stage exhibited antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, while the intracellular secondary metabolites of the anamorph stage exhibited antimicrobial activity against *E. coli* and *B. subtilis* (Table 5a).

Amino acids

The free amino acids in teleomorph and anamorph stages were varied (Table 5b). Although, the level of all detected free amino acids except glycine was higher in teleomorph than anamorph stage. Alanine and the secondary amino acid ∝-amino adipic acid were detected only in the teleomorph stage. The concentration of glutamic acid, alanine, phosphoethanol amine and aspartic acid were considerable higher in teleomorph stage (253. 93, 61.88, 61.79 and 40. 50µg/ml, respectively) than other detected amino acids in the same stage. On the other hand, glutamic acid and glycine concentrations (82.79 and 31.97µg/ml, respectively) were higher than other detected amino acids in anamorph stage.

Metals analysis

There was considerable variation among the teleomorph and anamorph stages in their elemental analysis (Table 6). Most of the detected elements in anamorph stage were

Table 1. Teleomorph and anamorph stages formation at different sucrose concentrations.

Sucrose concentration % (w/v)	Colony radius (cm)	Percentage (%) of the formation of	
		Teleomorph	Anamorph
2	3.2	95	5
30	3.5	90	10
40	4.3	87	13
50	3.9	80	20
60	2.5	0.0	100
70	1.6	0.0	100
80	0.0	0.0	0.0

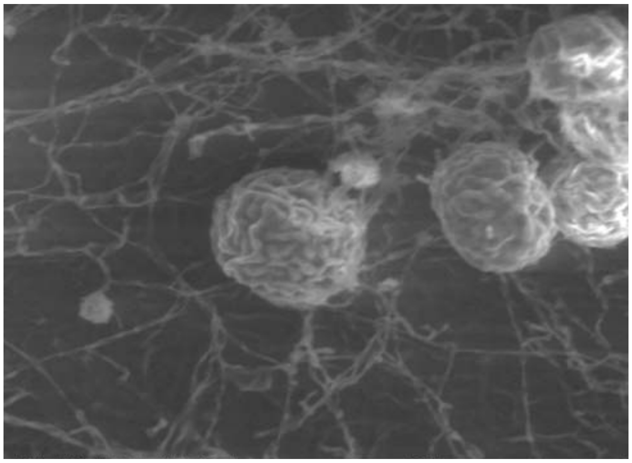

Fig. 1a. Teleomorph stage represented by cleistohecia.

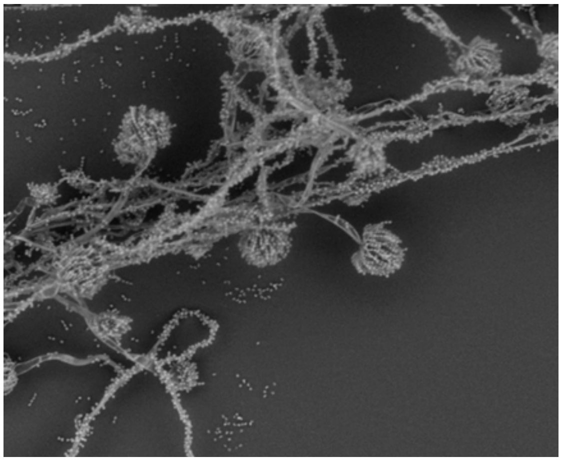

Fig. 1b. Anamorph stage represented by conidial heads.

Table 2. Effect of temperature on growth, teleomorph and anamorph stages formation of *Eurotium repens* at different sucrose concentrations % (w/v).

Sucrose % (w/v)	Temperature (ºC)														
	10			20			30			40			45		
	Cr (cm)	T (%)	A (%)	Cr (cm)	T (%)	A (%)	Cr (cm)	T (%)	A (%)	Cr(cm)	T (%)	A (%)	Cr (cm)	T (%)	A (%)
2	0.0	0.0	0.0	2.2	95	5	3.1	96	4	1.1	96	4	0.0	0.0	0.0
30	0.0	0.0	0.0	2.8	88	12	3.5	90	10	2.4	87	13	0.0	0.0	0.0
40	0.0	0.0	0.0	2.9	84	16	4.2	85	15	2.6	82	18	0.0	0.0	0.0
50	0.0	0.0	0.0	0.0	0.0	0.0	3.7	81	19	1.1	81	19	0.0	0.0	0.0
60	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0
70	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	10	0.0	0.0	0.0	0.0	0.0	0.0
80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0

Cr, colony radius; T, teleomorph stage; A, anamorph stage

Table 3. Effect of pH on growth, teleomorph and anamorph stages formation of *Eurotium repens* at different sucrose concentrations % (w/v).

Sucrose concentration % (w/v)	pH														
	4			5			6			7			8		
	Cr(cm)	T (%)	A (%)	Cr(cm)	T (%)	A (%)	Cr(cm)	T (%)	A (%)	Cr(cm)	T (%)	A (%)	Cr(cm)	T (%)	A (%)
2	2.1	90	10	2.3	93	7	2.9	94	6	2	88	12	0.9	89	11
30	2.5	88	12	2.9	88	12	3.2	89	11	2.2	90	10	2.1	92	8
40	2.6	85	15	2.8	84	16	4.0	86	14	2.6	83	17	2.4	90	10
50	2.0	79	21	3	80	20	3.5	80	20	3.1	79	21	1.9	80	20
60	1.6	0.0	10.0	1.7	0.0	100	2.1	0.0	100	1.9	0.0	100	1.5	0.0	100
70	0.0	0.0	0.0	0.0	00	0.0	1.1	0.	10	0.9	0.0	10	0.0	0.0	0.0
80	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Cr, colony radius; T, teleomorph stage; A, anamorph stage

Table 4. Extracellular secondary metabolites production by teleomorph and anamorph stages.

Secondary metabolites produced by	
Teleomorph	Anamorph
* Epoxysuccinic acid	* Epoxysuccinic acid
* 2-pyruovylaminobenzamide	* 2-pyruovyl aminobenzamide
* Lapiosin	* Kojic acid
* Wartmannin	*2-carboxy–3,5, dihydroxyphenyl acetyl- carbinol
* Gentisyl alcohol	
* (-) Flavoskyrin	* Unknown (2)
* Compactin	* Unknown (3)
* Unknown (1)	

present in higher concentration than teleomorph stage except for potassium. Silicon and copper were not detected in teleomorph stage. On the other hand iron and calcium were not detected in anamorph stage.

Regulation of reproduction by amino acids and metals

Alanine and arginine unlike aspartic acid, glutamic acid and glycine, exhibited stimulatory effect on growth of *Eurotium repens* at low sucrose concentration (2% w/v), while glycine and arginine exhibited stimulatory effect on growth and teleomorph stage formation at high sucrose concentrations (Table 7). At high sucrose concentration (70 % w/v), the fungus failed to grow on medium amended with alanine and aluminum chloride.

DISCUSSION

In this study the high sucrose concentration 80% (w/v), (a_w 0.70) inhibit the growth of *Eurotium repens*. Pitt (1975) showed that the lower a_w limit for growth of *Eurotium* species is approximately 0.70. Fungi reproduce asexually under favorable condition and sexually under stress condition (Griffin, 1994). However *Eurotium repens* did not reproduce sexually under stress of low water activity 0.75 and 0.72 adjusted by sucrose

Table 5a. Antimicrobial activity of *Eurotium repens*.

Test organism	Intracellular secondary metabolites inhibition zone (mm)		Extracellular secondary Metabolites inhibition zone (mm)	
	Anamorph	Teleomorph	Anamorph	Teleomorph
Fusarium oxysporium	0	0	0	0
Aspergillus terreus	0	0	0	0
Candida albicans	0	0	0	0
Cunninghamella sp	0	0	0	0
Escherichia coli	2.2	22	0	24
Bacillus subtilis	27	23	18	25
Pseudomonas aeruginosa	0	17	0	20
Salmonella typhi	0	0	0	0

0, Inhibition zone not detected.

Table 5b. Amino acids pool analysis of teleomorph and anamorph stages.

Amino acids	Concentration (μg/ml) of amino acids in	
	Teleomorph	Anamorph
Phosphoserine	23.42	5.22
Taurine	14.50	7.72
Phosphoethanol amine	61.79	20.72
Aspartic acid	40.50	15.21
Threonine	14.60	6.83
Serine	30.32	18.09
Glutamic acid	253.93	82.79
∝-Aminoadipic acid	14.54	0.0
Glycine	9.76	31.97
Alanine	61.88	0.0
∝-Aminobutyric acid	10.25	7.56
Methionine	8.69	5.58
Isoleucine	9.80	8.04
Leucine	6.72	3.20
Tyrosine	11.39	7.43
Phenylalanine	7.53	2.01
3-Methylhistidine	6.64	5.38
Carnosine	29.48	27.75
Ornithine	7.36	4.84
Lysine	34.99	11.19
Arginine	18.91	1.89

0.0, Amino acid not detected.

concentration 60% (w/v) and 70% (w/v) respectively. Recently, Bluhm *et al*. (2005) found that the conidial heads of *Eurotium rubrum* were visible after 6 days at 0.98 a_w. Cleistothecia were produced only at 0.98 a_w, however mature ascospres were not detected unitl 10 days.

From the current study there is indirect relationship between the low a_w and reproduction in *Eurotium repens* where at low a_w certain amino acids were produced while others not produced. Generally the free amino acids are known to play an important role in the regulation of synthesis of some enzymes, on secondary metabolites production and osmoregulation. The unusual amino acid ∝ amino adipic acid and alanine were not detected when the *Eurotium repens* reproduce asexually (anamorph). On the other hand, glycine was only detected in higher concentration in teleomorph than in anamorph. These amino acids may be involved in the regulation of *Eurotium* repens reproduction. Mc Alpin and Wicklow (2005) stated that high nitrate (0.3% - 0.6% $NaNO_3$) and high sucrose (10 – 20 %) concentrations were optimal for stromata development. No stromata were produced by *Petromyces alliaceus* (Anamorph *Aspergillus alliaceus)* on media in which cystine or ammonium sulphate represented the only source of nitrogen, while the percentage of stromata containing ascocarps was the greatest with ammonium tartrate, glutamic acid, glycine or serine substituted for $NaNO_3$.

Table 6. Metals analysis of teleomorph and anamorph.

Metal	Metal weight (%) of	
	Teleomorph	Anamorph
Sodium	4.11	7.49
Magnisum	2.69	4.70
Alumnium	8.08	16.81
Silicon	0.0	4.43
Phosphrus	19.88	20.99
Sulfur	7.80	10.70
Chloride	1.54	11.34
Pottasium	24.91	17.63
Calcium	2.44	0.0
Copper	0.0	5.92
Iron	28.53	0.0

0.0, metal not detected.

Table 7. Effect of certain amino acids and metals on growth, teleomorph and anamorph stage formation, at different sucrose concentrations.

Amino acids of Elements	Sucrose concentration % (W/V)								
	2			60			70		
	$Cr_{(cm)}$	$T_{(\%)}$	$A_{(\%)}$	$Cr_{(cm)}$	$T_{(\%)}$	$A_{(\%)}$	$Cr_{(cm)}$	$T_{(\%)}$	$A_{(\%)}$
Control	3.1	92	8	2.5	0	100	1.5	0.0	100
Glycine	3.0	80	20	2.9	40	60	1.9	35	65
Alanine	3.3	95	5	1.9	0.0	100	0.0	0.0	0.0
Aspartic acid	2.9	90	10	2	0.0	100	1.2	0.0	100
Glutamic acid	3.0	91	9	1.8	0.0	100	1.4	0.0	100
Arginine	3.5	83	17	2.3	45	55	0.0	0.0	0.0
Aluminum	2.1	90	10	1.0	0.0	100	0.0	0.0	0.0
Calcium	2.7	88	12	1.5	48	52	1.2	0.0	100

Cr, Colony radius; T, Teleomorph; A, Anamorph

There is a direct relationship between osmotic stress and polyols, phospholipids and lipid composition in filamentous fungi (Hefnawy, 1993). The growth of *Eurotium repens* at low water activity (high osmotic stress) may induce synthesis of compounds which may then regulate their reproduction. This information is consistent with previous studies, where fatty acids or related lipids (Nukina *et al*., 1981; Goodrich-Ta-nrikulu *et al*., 1998) and polyols (Feofilova *et al*., 2000) affected sexual development in filamentous fungi.

The secondary metabolites detected in teleomorph and anamorph stages of *Eurotium repens* were generally different; this may be due to differentiation or may be related to other physiological changes. Many previous studies revealed that the production of fungal secondary metabolites is associated with differentiation (sexual and asexual development) and environmental stress (Cotty *et al*., 1994; Trail *et al*., 1995; Adams and Yu, 1998; Pena *et al*., 1998; Chang *et al*., 2001; Michael *et al*., 2001).

From the elemental analysis, calcium was detected only in teleomorph stage, and therefore, when added to the growth medium it stimulates the sexual reproduction at 60% (w/v) sucrose concentration in *Eurotium repens.* Changes in microcellular Ca^{+2} concentration are known to play an important role in the regulation of all physiological processes occurring in the cell such as growth, division, secretion and development of microbial resting forms (Jackson and Heath, 1993; Berridge *et al*., 2000). On the other hand, aluminum suppresses the growth and sexual reproduction in the *Eurotium repens*, the reduction of spore germination by aluminum was documented by Dursun *et al*., 2002.

REFERENCES

Abellana, M., Magri, X., Sanchis, V. and Ramos, AJ. 1999. Water activity and Temperature effects on Growth of *Eurotium amstelodami; E. chevalierie* and *E. herbariorum* on a sponge cake analogue. Inter J. Food Microbiol. 52:97-103.

Adams, TH., Wieser, JK, and Yu, JH. 1998. Asexual sporulation in *Aspergillus nidulans.* Microbial Mol. Biol. Rev. 62(1):35-54.

Adams, TH. and Yu, JH. 1998. Coordinate Control of Secondary metabolite Production and Asexual Sporulation in *Aspergillus nidulans.* Curr. Opin. Microbiol. 1(6):674-677.

Berridge, MJ., Lipp, P. and Bootman, MD. 2000. The Versatility and Universality of Calcium Signaling. Nature Rev. Mol. Cell boil. 1:11-12.

Bluham HB., Reuhs, BL. and Woloshuk, CP. 2005. Glass – Fiber Disks provide suitable medium to study Polyol Production and Gene Expression in *Eurotium rubrum.* Mycologia 97(4):743-750.

Bok, JW., Chiang, YM., Szewczyk, E., Reyes-Dominguez, Y., Davidson, AD., Sanchez JF., Lo, HC., Watanabe, K., Strauss, J., Oakley, BR., *et al*. 2009. Chromatin-level regulation of Biosynthetic Gene Clusters. Nat. Chem Biol. 25(7):462-464.

Chang, P., Bennett, JW. and Cotty, PJ. 2001. Assocation of Aflatoxin Biosynthesis and Sclerotial development in *Aspergillus parasiticus* Mycopathologia. 153:41-48.

Corry, JEL. 1987. Relationship of Water activity to Fungal growth. In: food and Beverage Mycology. Ed. Beuchat, LR. York, Van Nostr and Rienhold. 51-88.

Cotty, PJ., Bayman, P., Egel, DS. and Elias, DS. 1994. Agriculture, Aflatoxins and *Aspergillus.* In the genus *Aspergillus.* New York: Plenum Press. 1-27.

Dursun, S., Boddy, L. and Franklaand, J. 2002. Effects of pH and Aluminum ion concentration on Spore germination and Growth of some Soil Fungi. Turk J. Biol. 26:99-107.

Engelkes, CA., Nuclo, RL. and Fravel, DR. 1997. Effect of Carbon, Nitrogen and C: N ratio on Growth, Conidiation, and Biocontrol Efficacy of *Taloromyces flavus*. Phytopathology. 87:500-505.

Feofilova, EP., Tereshina, VM., Khokhlova, NS. and Memorskaya, AS. 2000. Different Mechanisms of the Biochemical adaptation of Mycelial Fungi to Temperature Stress: Changes in the cytosol carbohydrate composition. Microbiology. 69(5): 504-508.

Goodrich–Tanrikulu, M., Howe, K., Stafford, A. and Nelson, M. 1998. Changes in Fatty acid composition of *Nerospora crassa* accompany Sexual Developmentad Ascospore Germination. Microbiology. 114:1713-1720.

Griffin, DH. 1994. Fungal Physiology (2nd ed.). New York John Wiley and Sons. pp458.

Hefnawy, MA. 1993. Influence of Certain Stress Condition on a Metabolic Disorders of Some Fungi. Ph.D. Thesis, Faculty of Science, Minoufiya University, Egypt.

Hocking, AD. 1988. Moulds and Yeasts associated with Foods of reduced water activity: Ecological Interactions. In: Food Preservation by Moisture Control. Ed. Seow, CC. London. Elsevier Applied Science. 57-72.

Jackson, SL. and Heath, IB. 1993. Roles of Calcium Ions in Hyphal tip Growth. Microbiological Reviews. 57:367-382.

Jay, JM. 1992. Intrinsic Parameters of Foods that Affect Microbial Growth. In: Modern Food Microbiology. Ed. Jay, JM. Chapman and Hall, New York. 38-62.

Mc Aplin, CE. and Wicklow, DT. 2005. Culture Media and Sources of Nitrogen Promoting the Formation of Stromata and Ascocarps in *Petromyces alliaceus (Aspergillus* section Flavi). Can J. Microbiol. 51:765-771.

Michael, JC., Sarah, CW. and Graham, WG. 2001. The Fungi. (2nd ed.). London Syney. Tokyo.

Moore-Landecker, E. 1992. Physiology and Biochemistry of Ascocarp Induction and Development. Mycol . Res. 96:705-716.

Nesci, A., Rodriguez, M. and Etcheverry, M. 2003. Control of *Aspergillus* Growth and Different Conditions of Water Activity and pH. J. Appli. Microbiol. 95:279-287.

Nester, EW., Pearsal, NN., Roberts, CE., Nester, MT. and Lidstrom, MF. 1983. Microbiology. (3rd ed.). CBS College Publishing, New York. 10:273.

Ni, X. and Streett, DA. 2005. Modulation of Water Activity on Fungicide Effect on *Aspergillus niger* Growth in Sabouraud Dextrose Agar Medium. Letters in Applied Microbiology. 41:428-433.

Nukina, M., Sassa, T., Ikeda, M., Takahasi, K. and Toyota, S. 1981. Linoleic acid Enhances Perithecial Production in *Neurospora crassa*. Agric Biol. Chem. 45:2371-2373.

Paterson, RR. and Bridge, PD. 1994. Biochemical Techniques for Filamentous Fungi. CAB International, Wallingford, UK.

Pena, D., Aguirre, J. and Ruiz-Herrera, J. 1998. Correlation between the Regulation of Sterigmatocystin Biosynthesis and Asexual and Sexual Sporulation in *Emericella nidulans*. Antonie van Leeuwen hoek. 73:199-205.

Pitt, JI. 1975. Xerophilic Fungi and the Spoilage of Foods of Plant Origin. In Water Relations of Foods. Plant Origin. In: water Relations of Foods. Ed. Duckworth, RB. Academic press, London.

Rapper, K.B. and Fennel, DI. 1965. The Genus *Aspergillus.* The Williams and Wilkins Company, Baltimore, USA.

Rose, AH. 1983. Food Microbiology. Academic Press. London, New York. Toronto, Sydney, San Francisco. 174-198.

Ross, RG. and Bremner, FDJ. 1971. Effect of Ammonium Nitrogen and Amino acids on Perithecial formation of *Venturia inaequalis.* Can. J. Plant. Sci. 51:29-33.

Trail, F., Mahanti, N. and Linz, J. 1995. Molecular Biology of Aflatoxin Biosynthesis. Microbiology. 141:755-765.

Wilkinson, HH., Sim, SC. and Keller, NP. 2004. Increased Conditions Associated with Progression along the Sterigmatocystin Biosynthetic Pathway. Mycologia. 96 (6):1190-1198.

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