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# METABOLIC REGULATION OF FUNGAL REPRODUCTION AND THEIR SECONDARY METABOLITES

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## Abstract

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

## Introduction

Ascomycetes may produce an ascosporic state (Teleomorph) and conidial state (anamorph) for example, *Eurotium. Eurotium* species often dominate the fungal population in stored grains and are responsible for spoilage of jams, dried foods, dried salted fish and sponge cake (Abellana *et al*., 1999; Bluham *et al*., 2005).

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, nut species and several dried food stuffs (Hocking, 1988) . The majority of xerotolerant fungi belong to the gnera *Aspergillus* and *Penicillium* or the perfect forms of *Aspergillus* such as *Eurotium* and *Emericella*. One of the principle factors controlling the growth of these organisms in food is $a_w$, since the effective growth range can be as low as 0.61 (Corry, 1987; Jay, 1992).

Low $a_w$ significantly reduced spore germination (*Aspergillus* spp). The spores only germinated on the medium with high $a_w$ values (i.e. 0.982 and 0.937) . While the spores did not germinate when $a_w$ values were 0.809 and 0.747 (Nesci *et al*., 2003; Ni and Streett, 2005).

Fungi reproduce asexually under favorable condition and sexually under stress condition (Griffin, 1994). Bluham *et al*. (2005) reported that the *Aspergillus nidulans* and *Aspergillus flavus* strains grew only at 0.98 $a_w$. At 0.86 $a_w$, no growth of *Aspergillus nidulans* and *Aspergillus flavus* could be detected after 8 days. At 0.83 $a_w$, growth of *Aspergillus nidulans* could not be observed and the sclerotia were not produced by *Aspergillus flavus.*

Filmentous fungi are unique organisms–rivaled only by actionomyctes and plants – in producing a wide range of natural products called secondary metabolites. These compounds are very diverse in structure and perform functions that are not always known. These metabolites are frequently associated with a sexual 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.*

Some ascomycetes may require exogenous vitamins, minerals, or other natural materials for ascocarp production, often not duplicated in synthetic media (Moore – Landecker 1992). *Venturia inaequlis* produced large number of ascocarps with glycine but no ascocarps formation with ammonium tartrate (Roos and Bremner 1971) . Engelkes *et al*., (1997) found that the tyrosine was one of the better nitrogen source for production of *Taloromyces flavus* ascospores. Also, fatty acids or related lipids are important to sexual development of filamentous fungi (Nukina *et al*., 1981; Goodrich – Tanrikulu *et al*., 1998). The objective of this study were 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 spoild fruit (Fig) , and identified as *Eurotium repens* according to Rapper and Fennel (1965) .

**Media:**

(1) Dox's agar medium which consists of sucrose, 20.0 g; $NaNO_3$, 2.0 g; $KH_2PO_4$, 1.0g; KCl, 0.5 g, $MgSO_4$. $7H_2O$ , 0.5 g ; Fe $SO_4$. $7H_2O$, 0.001 g; agar 20.0 g and distilled water, 1.0 L. (2) Malt extract agar medium which consists of malt extract, 20.0 g; peptone, 1g; dextrose, 20.0 g; agar, 20.0 g and distilled water, 1.0 L. This media were used for isolation, cultivation and identification of 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 plates containg the above medium (for growth and detection the anamorph and teleomorph stages formation or transferred to 500 ml conical fealsks for detection of the amino acids, secondary metabolites, metals and antimicrobial activity.

Dox's agar medium supplemented with different sucrose concentrations (adjusted at different pH, inoculation and incubation at 28ºC for 8 days) inoculated and incubated at different temperatures for studying the effect of it on anamorph and teleomorph stages formation.

Nitrogen free Dox's agar medium supplemented with different sucrose concentrations was amended with certain amino acids in equivalent weight to N of $NaNO_3$ in Dox medium and certiain metals, Calcium chloride and Alumnium chloride, (0.01 mg / 100 ml medium) for metabolic regulation of anamorph and teleomorph stages formation. The percentage of teleomorph and anamorph which represented by the presence of Cleistothecia and Conidial heads respectively was calculated by using a hemacytometer.

**Secondary metabolites detection :**

The secondary metabolites were determined according to the method described by Paterson and Bridge (1994) as follows: the fungal mat of *Eurotium repens* was harvested and the supernatant was filtrated and extracted with equal volume of chloroform:methanol (2 : 1, v/v), left to evaporate till dryness and then dissolved in 1.0 ml of extracted solvent .

The extracts were spotted on a pre-coated thin layer chromatography (TLC) plate (20 × 20 cm aluminum sheet silica gel 60 , layer thickening 0.2 mm) along with griseofulvin as a reference standard. 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) $\rho$ -anisaldehyde in methanol: acetic acid : concentrated sulphuric acid (17:2:1, v/v/v) and visualized under white light. The plate 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 (5 gm) in sterile mortar using 70% (v/v) of ethanol. The slurry was centrifuged at 600 r.p.m. for 10 minutes, and the supernatant was concentrated using a vacuum desiccator. The concentrated cell free extract was analyzed for amino acids qualitatively and quanitatively with a full automated Amino Acid analyzer : Model Lc 3000 (Eppendro – Biotro Nik, Germany) at the Regional Center for Mycology and Biotechnology Al-Azhar Unv.)

**Metals analysis:**

Dry fungal mycelium (0.5 gm) was grinded and analyzed for metals with Scaning Electron Microscope (Environmental) Fei QUANTA 200 provided 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 exracts and transferred onto the surface of a nutrient medium seeded with the test organism, the diameter of the inhibited growth area around the disc (s) was measured after the incubation period .

**Result**

The growth of *Eurotium repens* (Fig. 1 a & b) increased with increasing sucrose concentration up to 40% these concentrations 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 teleomorhp and anamorph stages formation 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) .

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

| Sucrose concentration % (w/v) | Colony radius (cm) | Percentage (%) of the formation of | |
|---|---|---|---|
| | | Teleomorph (Cleistothecium) | Anamorph ( Conidial head) |
| 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 |

On the other hand, the percentage of teleomorph and anamorph stages formation at stress temperature (20 & 40ºC) and pH (4 & 8) was relatively similar to those at control (30ºC & pH6) and at the same sucrose concentrations (Table 2 & 3) but the obtained results revealed that the optimum-growth temperature and pH were 30°C and 6 respectively .

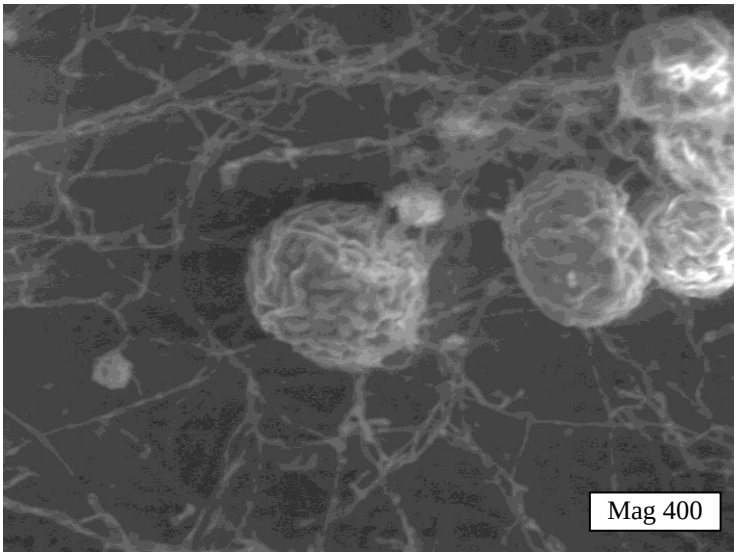


**Fig (1 a): Teleomorph stage represented by cleistothecia .**

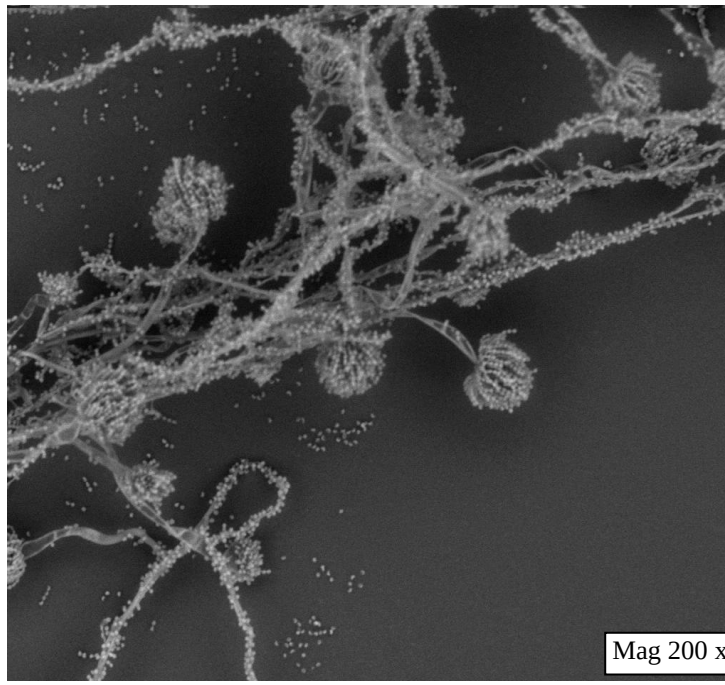


**Fig (1 b): Anamorph stage represented by conidial heads.**

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

| Sucrose concentration % (w/v) | Temperature (ºC) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | | | 20 | | | 30 | | | 40 | | | 45 | | |
| | $R.g._{(cm)}$ | $T_{(\%)}$ | $A_{(\%)}$ | $Rc_{(cm)}$ | $T_{(\%)}$ | $A_{(\%)}$ | $R.g._{(cm)}$ | $T_{(\%)}$ | $A_{(\%)}$ | $R.g._{(cm)}$ | $T_{(\%)}$ | $A_{(\%)}$ | $R.g._{(cm)}$ | $T_{(\%)}$ | $A_{(\%)}$ |
| 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 | 100 | 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 | 0.0 |

R.g., radial growth ; T, teleomorph stage; A, anamorph stage

**Table (3): Influence of temperature 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 | | |
| | R.g. (cm) | $T_{(\%)}$ | $A_{(\%)}$ | R.g. .(cm) | $T_{(\%)}$ | $A_{(\%)}$ | R.g. (cm) | $T_{(\%)}$ | $A_{(\%)}$ | R.g. (cm) | $T_{(\%)}$ | $A_{(\%)}$ | R.g. (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 | 100 | 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 | 0.0 | 0.0 | 1.1 | 0.0 | 100 | 0.9 | 0.0 | 100 | 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 |

R.g., radial growth ; T, teleomorph stage; A, anamorph stage

**Secondary metaboites:**

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

**Table (4) : Extracellular secondary metabolites production by teleomorph and anamorph stages:**

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

**Antimicrobial activity:**

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

**Table (5) Antimicrobial activity of *Eurotium repens*.**

| Test organism | Intracellular secondary metabolites | | Extracellular secondary metabolites | |
|---|---|---|---|---|
| | Anamorph | Teleomorph | Anamorph | Teleomorph |
| *Fusarium oxysporium* | -ve | -ve | -ve | -ve |
| *Aspergillus terreus* | -ve | -ve | -ve | -ve |
| *Candida albicans* | -ve | -ve | -ve | -ve |
| *Cunninghamella sp* | -ve | -ve | -ve | -ve |
| *Escherichia coli* | 22 | 22 | -ve | 24 |
| *Bacillus subtilis* | 27 | 23 | 18 | 25 |
| *Pseudomonas aeruginosa* | -ve | 17 | -ve | 20 |
| *Salmonella typhi* | -ve | -ve | -ve | -ve |

-ve, Inhibition zone not detected .

**Amino acids :**

The free amino acids in teleomorph and anamorph stages were varied (Table 5 and Fig 2 a & b). Although, the level of all the 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 considerably higher in teleomorph stage (253. 93 µg/ml, 61.88 µg/ml, 61.79 µg/ ml and 40. 50 µg/ml respectively) than other detected amino acids in the same stage. On the other hand, glutamic acid and glycine concentrations (82.79 µg/ml and 31.97 µg/ml respectively) were higher than other detected amino acids in anamorph stage.

**Table (5) Amino acids pool analysis of teleomorph and anamorph stages.**

| Amino acids | Concentration (μg/m) 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 | -ve |
| Glycine | 9.76 | 31.97 |
| Alanine | 61.88 | -ve |
| ∝-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 |

-ve, Amino acid not detected.

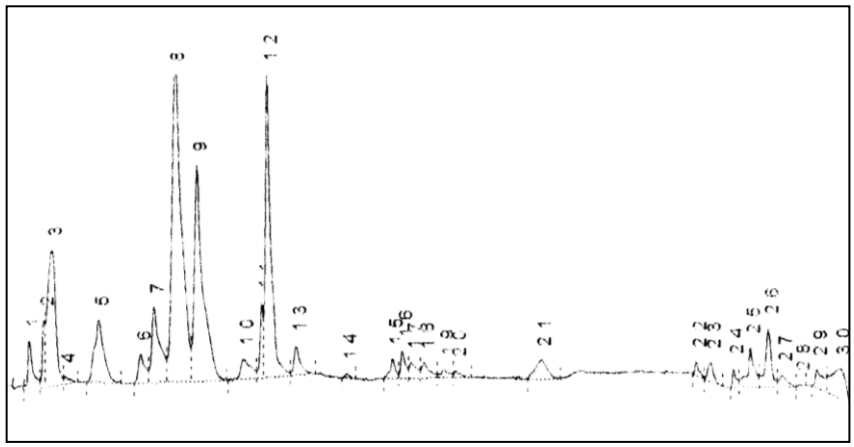

**Fig (2 a): Amino acids chromatogram of teleomorph stage.**

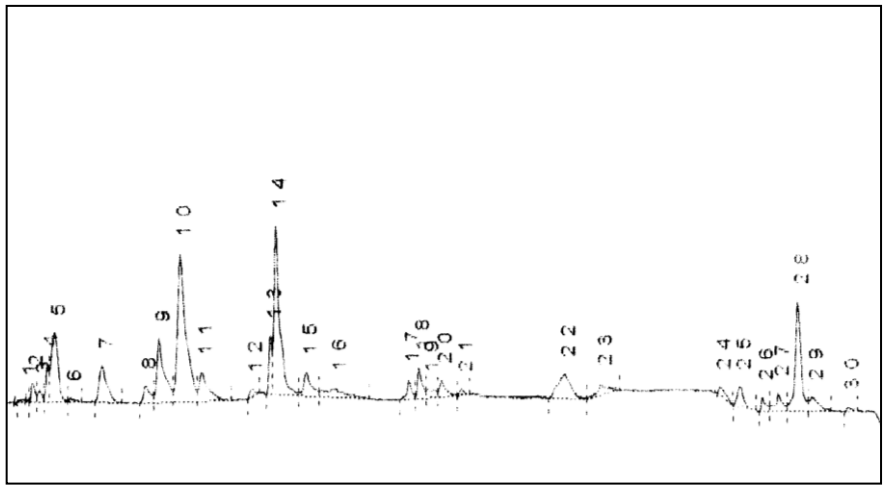

**Fig (2 b): Amino acids chromatogram of Anamorph stage.**

**Metals analysis:**

There was considerable variation among the teleomorph and amamorph stages in their elemental analysis (Table 6 and Fig. 3 a & b). Most of the detected elements in anamorph stage were present in higher concentration than teleomorph stage except potassium. Silicon and copper could not be detected in teleomorph stage. On the other hand iron and calcium could not be detected in anamorph stage.

**Table (6) Metals analysis of teleomorph and anamorph .**

| Metal | Metal concentration (%) of | |
|---|---|---|
| | Teleomorph | Anamorph |
| Sodium | 4.11 | 7.49 |
| Magnesium | 2.69 | 4.70 |
| Alumnium | 8.08 | 16.81 |
| Silicon | -ve | 4.43 |
| Phosphorus | 19.88 | 20.99 |
| Sulfur | 7.80 | 10.70 |
| Chloride | 1.54 | 11.34 |
| Potassium | 24.91 | 17.63 |
| Calcium | 2.44 | -ve |
| Copper | -ve | 5.92 |
| Iron | 28.53 | -ve |

-ve , metal not detected.

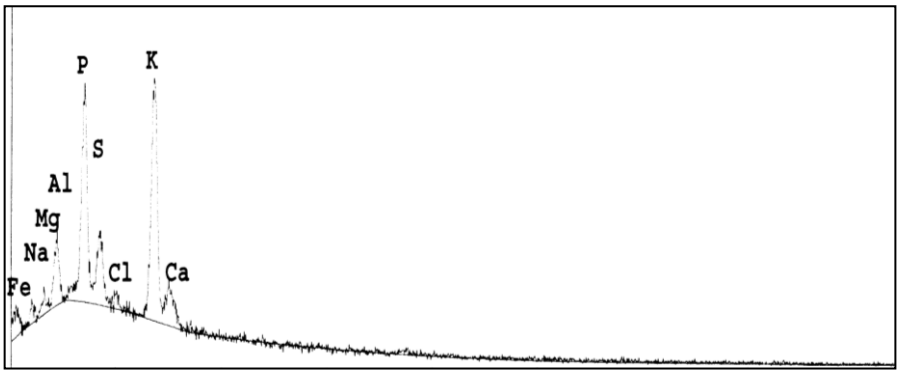


**Fig. (3 a) : Metals chromatogram of teleomorph stage..**

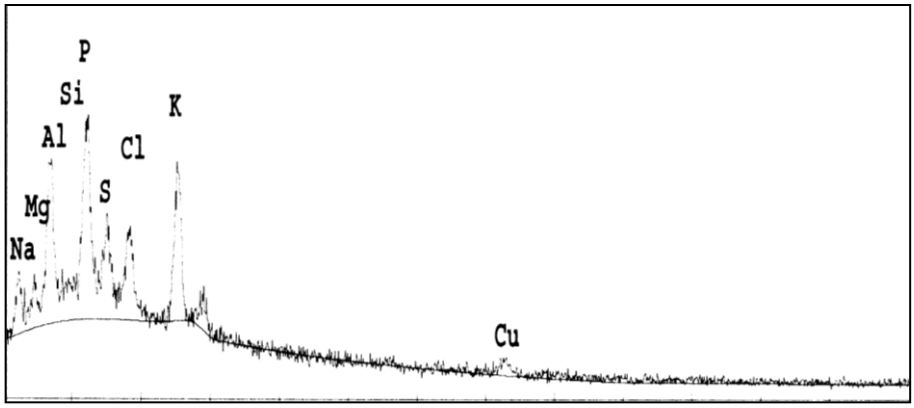


**Fig. (3 b) : Metals chromatogram of anamorph stage.**

**Regulation of reproduction by amino acids and metals:**

Alanine and arginine unlike aspartic acid, glutanic 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 concenatration (Table 7). At high sucrose concentration (70 % w/v), the fungus failed to grow on medium amended with alanine and aluminum chloride.

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

| Amino acids and Elements | Sucrose concentration % (W/V) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | 60 | | | 70 | | |
| | R.g.(cm) | T. (%) | A. (%) | R.g. (cm) | T. (%) | A. (%) | R.g. (cm) | T. (%) | A. (%) |
| Control | 3.1 | 92 | 8 | 2.5 | -ve 40 | 100 | 1.5 | -ve | 100 |
| Glycine | 3.0 | 80 | 20 | 2.9 | -ve | 60 | 1.9 | 35 | 65 |
| Alanine | 3.3 | 95 | 5 | 1.9 | -ve | 100 | -ve | -ve | -ve |
| Aspartic acid | 2.9 | 90 | 10 | 2 | -ve | 100 | 1.2 | -ve | 100 |
| Glutamic acid | 3.0 | 91 | 9 | 1.8 | 45 | 100 | 1.4 | -ve | 100 |
| Arginine | 3.5 | 83 | 17 | 2.3 | -ve | 55 | -ve | -ve | -ve |
| Aluminum | 2.1 | 90 | 10 | 1.0 | 48 | 100 | -ve | -ve | -ve |
| Calcium | 2.7 | 88 | 12 | 1.5 | | 52 | 1.2 | -ve | 100 |

R.g., radial growth
T., teleomorph
A., anamorph

## Discussion

In this study the high sucrose concentration 80% (w/v), ($a_w$ 0.70) inhibite the growth of *Eurotium repens*. Pitt (1975) showed that the lower $a_w$ limit the growth of *Eurotium* species to approximately 0.70. Fungi reproduce asexually under favorable condition and sexually under stress condition (Griffin, 1994). However *Eurotium repens* failed to reproduce sexually under stress of low water activity 0.75 and 0.72 adjusted by sucrose 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 could not be detected unitl after 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 other not produced. Generally the free amino acids are known to play an important role in the regulation of enzymes synthesis, secondary metabolites production and osmoregulation. The unusual amino acid ∝ amino adipic acid and alanine could not be detected when the *Eurotium repens* reproduce asexually (anamorph) . On the other hand, glycine could be detected in higher concentration in teleomorph than in anamorph. These amino acids may be contribute 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 could be detected by *Petromyces alliaceus* (Anamorph *Aspergillus alliaceus)* on medium in which cystine or ammonium sulphate represented the only source of nitrogen, while the percentage of stromata containing ascocarps was the greatest with either ammonium tartrate, glutamic acid, glycine or serine substituted for $NaNO_3$.

There is a direct relationship between the 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 produced such compounds which may regulate their reproduction . This information was confirmed by previous studies, where fatty acids or related lipids (Nukina *et al*., 1981; Goodrich-Ta-nrikulu *et al*., 1998) and polyols (Feofilova *et al*., 2000) possibly regulate the sexual development in filamentous fungi .

The detected secondary metabolites in teleomorph and anamorph stages of *Eurotium repens* were different except few metabolites, this may be due to differentiation or may be related to other physiological changes. There are many previous studies which revealed that the production of fungal secondary metabolites are associated with the differentiation (sexual and asexual development) and the 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 etemental analysis, calcium was detected only in teleomorph stage, and therefore when added to the growth medium it stimulate 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, enzyme secretion and development of microbial resting forms (Jackson and Heath, 1993; Berridge *et al*., 2000). On the other hand, Alumnium suppress the growth and sexual reproduction in the *Eurotium repens* the reduction of spore germination by aluminum was documented by Dursun *et al*., (2002) .

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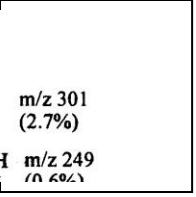




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