Effect of Some Factors Including Irradiation on the Ergot Alkaloids Production by Members of *Penicillium*

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Abstract: Six hundred isolates belonging to 30 species of the genus *Penicillium* were screened for ergot alkaloids production. Ergot alkaloids were confined to 67 isolates of 17 species. *P. corylophilum* (isolate No. 2760) produced 750 mg of ergot alkaloids per litter of culture medium and was selected as the experimental organism. Biosynthesis of alkaloids was markedly affected when the experimental organism was cultured on basal liquid medium of different types and levels of carbon and nitrogen sources, pH values, phosphate concentration and incubation temperature. The mycelial dry weight of *P.corylophilum* and ergot alkaloids accumulation decreased by increasing the irradiation dose levels and the fungus was completely inhibited at 3.0 kGy. Chromatographic and chemical analysis showed that mycelial extract of *P. corylophilum* (isolate No. 2760), cultivated as static culture under optimal conditions for alkaloids formation produced 1.380 g/l. Alkaloids analysis of the crude extract clearly show that it is composed of three components, namely ergokryptine, agroclavin and elmoclavine. The identity of the compounds was confirmed by high performance liquid chromatography and ultraviolet and infrared analysis.

Key words: Ergot, alkaloids, production *penicillium*, HPLC

Introduction

The pharmacological effects make ergot alkaloids so toxic in some cases but also make them pharmaceutically useful in others. Due to its vasoconstrictive effects, ergotamine is prescribed to control migraine headaches and to stop postpartum bleeding (Shelby and Kelley, 1990). Other potentially useful properties of these compounds are related to their ability to induce labor contractions, inhibit lactation by prolactin suppression, act as an adrenergic blocking agent and sorotonin antagonist, terminate early pregnancy and inhibit mammary tumors (Floss *et al.*, 1973; Masurekar, 1992; Flieger *et al.*, 1997).

Several independent investigations have shown the production of ergot alkaloids by members of *Claviciptaceae* such as all species of the genus *Claviceps, Endophytic claviciptaeous* fungi of

the genus *Epichloe (Acremonium)* and *Ballansia* as well as *Epiphytic atkinsonella* (Kelleher, 1970; Mantle, 1973, 1975; Bacon *et al.*, 1979; Robbers, 1984; Bacon, 1985; Flieger *et al.*, 1997). Spilsbury and Wilkinson (1961). Screening fungi for alkaloids production made a dramatic discovery that ergot alkaloids can be produced by fungi other than *Claviceps* and *Balanasia* species. Now it is well known that ergot alkaloids are produced by members of *Penicillium, (P. aurantiovirens, P. chermisinum, P. ciricavirde, P. sizovae, P. roquefortii, P. corylophilum, P. kapuscinkii, P. regulosum* and *P.concavorugulosum)* (Kozlovsky *et al.*, 1982; Kozlovsky and Reshetilova, 1984a and b; Hong and Robbers, 1985) and the members of *Aspergillus (A. fumigatas, A. tamarii* and *A. flavus)* (Nadia Naim, 1964; Rao *et al.*, 1977; Flieger *et al.*, 1997).

Because of the wide spectrum of ergot applicability, a continuous and still growing interest in these fungal metabolites exists. Current studies are directed mainly toward optimize the fermentation processes involved, particularly the genetics and physiology of alkaloids formation. In the last 10 years, investigations in these directions have received much attention but much work remains to be done, especially in the domain of regulatory mechanisms governing alkaloids biosynthesis. Moreover the influence of ionizing radiation on the fungal metabolites biosynthesis has been established by many investigators (Aziz and Abd EL-Aal, 1990, Aziz *et al.*, 1990; EL-Far *et al.*, 1992).

When endeavouring to plan this work two lines of approach presented themselves. The first was intended to prospect the presence of ergot alkaloids in representatives of locally isolated penicilli, the second to elucidate the role of some factors concerned in the synthesis of alkaloids by the selected organism.

Materials and Methods
Source of fungal isolates

The different fungi tested were obtained from Assiut University Culture Collection (AUCC), Botany Department, Faculty of Science, Assiut University, Egypt. They were isolated previously from different sources in Egypt (Moubasher, 1993).

Cultivation

The cultures were maintained on malt agar. Spores from a 5-7 days-old culture were transferred into three 250 ml Erlenmeyer flasks containing 50 ml of the following medium (g/l): mannitol, 50; sodium nitrate, 2.0; succinic acid, 5.4; $MgSO_4.7H_2O$, 0.3; KH_2PO_4, 1.0; distilled water, to 1 litre. The pH of the medium was adjusted to 5.2 with concentrated ammonia. The fungal isolates were cultivated and incubated at $28\pm2^{\circ}C$ as static cultures for 21 days.

Preparation of cultures for determination of alkaloids

After the desired period the content of each flask (medium + mycelium) was disintegrated in a high-speed Braun MSK cell homogenizer (200 ml flask containing 50 ml culture medium and 50

g glass beads of 0.5 mm diameter). The extent of cell breakage was examined by light microscopy. Disintegration of the material until at least 95% of all cells were disrupted (about 5 min). The suspension was separated from the glass beads by filtration through a nylon cloth (pore size 200 μm), centrifuged (85 Hz, 10 min) to remove cell debris and the clear supernatant was collected and diluted to 50 ml.

Determination of alkaloids

Total alkaloids content of the culture filtrate was assayed by adding 2 ml of Van Urk reagent (Van Urk, 1929), as modified by Smith (1930), to 1 ml culture filtrate and measuring the absorbance at 590 nm using a Supertonic 2000. The amount of alkaloids present was calculated from a standard curve of ergotamine. The culture filtrate (50 ml) was brought to pH 8-9 with aqueous ammonia and extracted with 50 ml portions of chloroform three times. The chloroform extracts were pooled, dried over anhydrous Na_2SO_4 and evaporated to dryness at 40-50°C under vacuum. The residue was analyzed by thin-layer chromatography (TLC) on silica gel G using a chloroform-methanol (80:20) solvent system. Pure samples of chanoclavine, elymoclavine, festuclavine, agroclavine, ergokryptine, fumigaclavine A, and fumigaclavine B were employed as references to identify the alkaloids spectrum. Fumigaclavine A used as the reference sample was prepared by acetylation of a sample of fumigaclavine B according to the method described by Spilsbury and Wilkinson (1961). Spots were developed by spraying the TLC plates with Ehrlich's regent (I gm of p-dimethylaminobenzaldehyde dissolved in 10 ml of water and 20 ml of HCl) (Stahl, 1969; Abou-Charr *et al.*, 1972). The alkaloids content of the mycelium was determined by a similar procedure using 1 g dried and powdered mycelium.

High-performance liquid chromatograph (HPLC)

Extracts of the culture filtrate and mycelium were subjected to high-performance liquid chromatography (HPLC), under the following conditions: HPLC equipment, Waters Associates ALC/GPC 244; stationary phase, μ-Bondapak NH_2, 30 cm×3.9 mm internal diameter (ID); mobile phase, chloroform-isopropanol (80:20); elution, isocratic; flow rate, 1 ml/min; detection, ultraviolet (uv) at 280 nm; temperature, 25°C; pressure, 1400 psi (approximately) (1 psi = 6.895 kPa); to confirm the identification of alkaloids authentic samples of chanoclavine, elymocavine, festuclavin, agroclavine, ergokryptine, fumigaclavine A and fumigaclavine B were used as references for comparison.

Effect of some environmental factors and γ-irradiation on the production of ergot alkaloids by
P. corylophilum (isolate No. 2760)
Factors affecting ergot alkaloids production
Carbon source

In order to obtain the highest level of ergot alkaloid by the selected isolate *P. corylophilum,*

different carbon sources were used in the basal medium instead of mannitol. The used carbon sources were glycerol, glucose, factors, maltose, lactose, sacrose sorbitol, mannitol and starch.

Nitrogen sources

For the same purpose different nitrogen sources were used instead of sodium nitrate. The used nitrogen sources were potassium nitrate, ammonium nitrate, ammonium chloride, ammonium oxalate and ammonium citrate.

Effect of inorganic phosphate

Basal medium with different concentrations of $H_2 KPO_4$ (0.25 to 5.0 mg/L) were used to establish the effect of phosphate on ergot alkaloid production by the select isolate.

pH

The pH of the basal medium was adjusted to different pH values ranging from 2 to 7 by using 0.1N NaOH and 0.1N HCl.

Incubation temperature

Flasks inoculated with the selected isolate were incubated at different temperatures ranging from 20 to 45°C.

Sodium chloride concentrations

Flasks containing basal medium with different concentrations of NaCl up to 10% were used to establish the effect of salinity on the ergot alkaloid production by the selected isolate.

Radiation effects

Spore from 7 days old cultures of *P. chorylophilum* (isolate No.2760) grown at 25°C on slants of malt agar were harvested in sterile 0.1%. Tween 80 filtered through four layers of sterile cheesecloth pelleted by centrifugation at 12.000 Kg for 5 min at 5°C. The spore suspension was dispensed in appropriate volume into saline solution (0.85% NaCl) to give final concentrations of conidia of 10^5 conidia/ml. These preparations were irradiated in air with a ^{60}Co irradiation unit (Gamma Cell model 220 apparatus, NCRRT, Cairo, Egypt) by using doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy at a dose rate of 200 Gy/min. Unirradiated and irradiated conidia were transferred into three 250 ml Erlenmeyer flasks containing 50 ml of the fermentation medium, the pH of the medium was adjusted to 5.5 and the flasks were incubated at 25°C as static cultures for 21 days. At the end of the incubation period the fungal mycelial dry weight and ergot alkaloids were determined.

Results and Discussion

Six hundred isolates belonging to 30 species of the genus *Penicillium* were screened for ergot alkaloids accumulation. The results clearly showed that the formation of ergot alkaloids was confined only to members of 17 species (Table 1). The present finding, is the first report in the production of ergot alkaloids by members of 14 species of fungi namely, *P. brevi-compactm, P. chysogenium, P. citrinum, P. jensenii, P. funiculosum, P. griseofulvum, P. isolandicum, P. italicum, P. oxalicum, P. purpurogenum, P. purpureum, P. variabile, P. viridicatum* and *P. waksmanii.*

P. corylophilum (isolate No. 2760) which recorded as the most highly ergot alkaloids producer (750 mg/L) was selected as the experimental organism for further investigations of the different physiological and biochemical aspects of ergot alkaloids biosynthesis.

Effect of carbon sources

Attempts have been made to evaluate the effect of different carbon sources and suitable concentration of the most favourable carbon source for supporting both fungal growth and stimulating alkaloids production by the selected experimental organism. Ten different carbon sources were tested (Fig. 1). Glycerol, glucose, fructose and galactose permitted better fungal growth rather than disaccharides (maltose, lactose and sucrose). However, disaccharides (sucrose, maltose, and lactose) as well as sugar alcohols (mannitol and sorbitol) supported high yield of alkaloids. However, sucrose when used as the sole carbon source proved to be superior to the other disaccharides tested, giving a maximum yield of alkaloids (0.800 gm/1). These results are in harmony with that obtained by Arcamone *et al.* (1961) and Socic and Gaberc-Porekar (1992). They reported that in ergot submerged fermentations, the slowly metabolized carbon sources such as sucrose, mannitol and sorbitol are preferentially used.

The influence of sucrose level in the nutritive medium on the growth of the experimental organism and alkaloids formation was investigated (Fig. 2). Although the growth of *P. corylophilum* No. 2760 was promoted with the increase of the sucrose concentration, yet maxima of alkaloids (825 mg/1) yield was obtained at 80 gm of sucrose per litre.

Glucose and other rapidly metabolized carbon sources (fructose and galactose) have been found to suppress markedly the production of alkaloids, a phenomenon being quite general in secondary metabolites production (Drew and Wallis, 1983).

Effect of nitrogen source

The experimental organism was able to utilize a wide range of nitrogen sources (Fig. 3). Highly fungal growth (320 and 345 mg/50 ml) and low yield of alkaloids were occurred with potassium and sodium nitrate. A similar result was previously recorded by Taber and Vining (1958) using *Claviceps purpurea*. They suggested that nitrate had a negative effect on the alkaloids production.

Comparatively low growth values (230-250 mg/50 ml) were recorded with ammonium nitrate, ammonium chloride, ammonium oxalate and ammonium citrate. Although the growth of the experimental isolate, using ammonium chloride, was comparatively low (270 mg/50 ml) yet the fungus was able to synthesize the highest yield of alkaloids (1.200 gm/l) compared with the other nitrogen sources tested. A similar result was obtained by Gaberc-Porekar *et al.*, (1987) who reported that ammonia being rapidly utilized nitrogen source, which is usually almost depleted from the medium until a high production of ergot alkaloids starts.

The nitrogen level of the culture medium proved to be an important factor influencing growth and alkaloid formation. Thus, it was shown that a certain level of nitrogen (1 g/1, ammonium chloride) supported maximal growth (270 mg/50 ml) value and high alkaloids yield (1.200 g/l). The increase of nitrogen level was accompanied by a corresponding decrease of both growth value and alkaloids formation (Fig. 4).

Effect of inorganic phosphate

Variation of KH_2PO_4 content from 0.0 to 5 g/l affected both fungal growth and alkaloid formation (Fig. 5). Gradual increase of phosphate concentration regularly increased fungal growth to reach maximum (315 mg/50 ml) at 2.0 g/l of potassium dihydrogen phosphate, followed by a rapid decrease of growth at higher phosphate concentrations. On the other hand alkaloids formation was maximal (1.220 g/l) at phosphate concentration of 0.5 gm/l.

Conflicting data on the importance of phosphate on the alkaloids biosynthesis by *Claviceps* species have been previously reported. Thus, although Windisch and Bronn (1960) have noted that limitation of phosphate in carbohydrate rich medium appeared to give best results. Taber and Vining (1958) and Brady and Tyler (1960) have shown that phosphate is an important nutrient factor for alkaloids formation. Gram *et al.* (1980) using a growth model reported that in case of alkaloids production, by *Claviceps purpurea*, inorganic phosphate was determined as limiting factor for growth and alkaloid production.

For many years it has been known that inorganic phosphate concentrations, which are optimal for microbial growth often, inhibit secondary metabolism, and ergot alkaloid biosynthesis has also been shown to be subject to this phosphate regulation (Vaidya and Desai, 1982). One of the most important cellular activities in which phosphate is involved is the storage of energy, and one of the possible explanations for phosphate regulation is its influence on the energy state of cell or the concentration of particular phosphonucleotides.

De Waart and Taber (1960), Mary *et al.* (1965) and Taber (1967) noted that a high concentration of inorganic phosphate inhibits alkaloids synthesis in saprophytic cultures of fungi. It can be postulated that excess phosphate, required for production of protein and nucleic acids, prolongs the growth phase of the organism. During growth, amino acids such as tryptophan are utilized predominantly in the synthesis of protein. With the termination of the growth phase,

tryptophan becomes available to serve as an inducer of alkaloid synthesizing enzymes and as a precursor in the synthesis of alkaloids (Weygand and Floss, 1963; Robbers *et al.*, 1972), On the other hand, prolongation of the growth phase results in the inhibition of alkaloids synthesis due to lack of tryptophan accumulation.

Effect of pH

The pH value of the nutritive medium exerted a marked influence on alkaloid production (Fig. 6). Using initially adjusted medium, the results revealed that mycelial growth and alkaloids biosynthesis increased gradually with the increase of pH values reaching maxima (260 mg/50 ml and 1.280 gm/l) at pH ranging from 2.0 to 5.0. This is in harmony with the finding of Mizrahi and Miller (1970) who reported that pH optima for growth and alkaloid synthesis are in the range of 5.5.

Effect of incubation temperature

Incubation temperature proved to be an important factor in alkaloids synthesis. Temperature 35 and 25°C were the optima for the mycelial growth (315mg/ 50 ml) and alkaloids accumulation (1.380 g/l) by *P.corylophilum* isolate No. 2760 (Fig. 7). Several reports indicated that optimum temperatures for alkaloids synthesis usually near 25°C. Socic and Garberc-Porekar (1992) using different strains of *Claviceps* for ergot alkaloids production recorded that optimal temperature lies between 24 and 27°C depending on the strain.

Effect of sodium chloride concentrations

The production of ergot alkaloids by *P. corylophilum* was clearly affected by the NaCl concentration in the medium. The production of ergot alkaloid by the tested isolate reached its maximum at 6% NaCl concentration (Fig. 8). Thereafter sharply decreased by increasing the concentration of sodium chloride. This due to the activity of microorganisms since its growth also decreased by increasing the sodium chloride concentration over 5%.

Norkrans (1966) reported that, a decrease in growth rate and prolongation of the log phase generally runs parallel with an increase in NaCl concentration above 2 or 4%. Adler and Gustafsson (1980) mentioned that the sensitivity of cells towards exposure to high salinity was measured in term of length of the log phase, which decreased with increases of intracellular polyals concentration. On other hand Shevckuk *et al.*, (1987) stated that the presence of inorganic salts as NaCl and KCl in the medium acted as salting-out for glyphosphate in aqueous solution. Abou EL-Hawa *et al.* (1993) reported that increasing salt concentration up to 8% was correlated positively with *A. Candidus*. EL-Gwally *et al.* (2001) found that the highest productivity of glycerol by *Zygosaccharomyces roucii* was obtained at 14% NaCl in the medium.
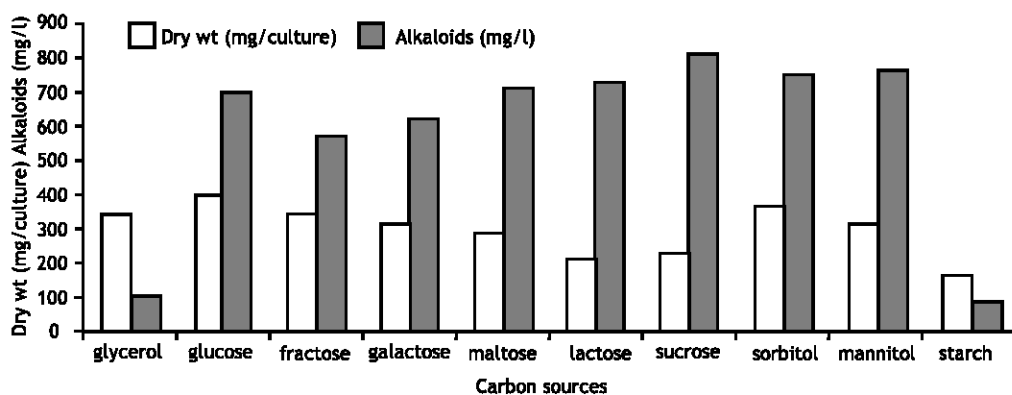
Fig. 1: Effect of different carbon sources on growth and ergot alkaloid production by *P. corylophilum*
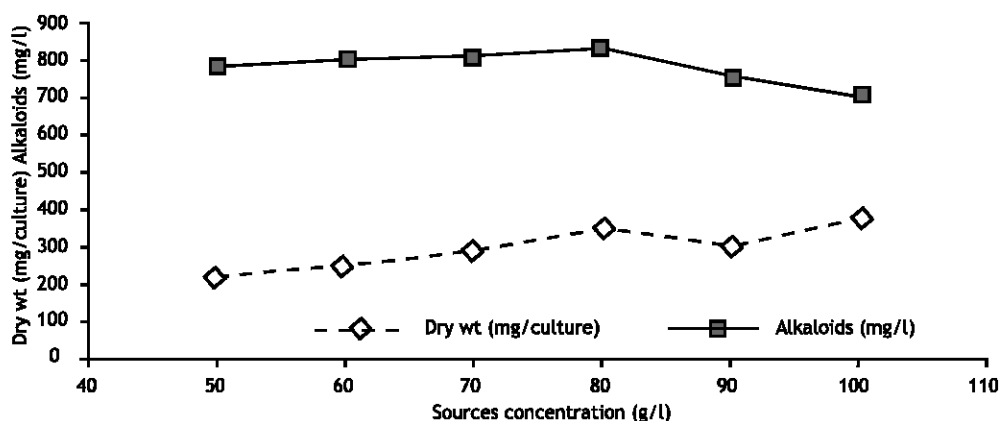

Fig. 2: Effect of sucrose concentration on growth and ergot alkaloid production by *P. corylophilum*

Effect of gamma irradiation

Through this present experiments Fig (9), it was revealed that the mycelial dry weight of *P. chorylophilum* and alkaloids accumulation decreased by increasing the radiation dose levels and the fungus was completely inhibited at 3.0 kGy. The sensitivity of fungi to ionizing radiation has been established by several investigators. Shahin (1986) mentioned that the growth of *A. flavus, A. niger Fusarium solani and P. chrysogenum* in saline solution was gradually decreased with increasing the dose levels of gamma-rays from 0.5 to 3.0 kGy Aziz *et al.* (1990) and EL-Far *et al.*, (1992) reordered that the dose required for complete inhibition of natural fungal flora ranged from 4 to 6 kGy. There are a number of conflicting reports, which suggest that the production
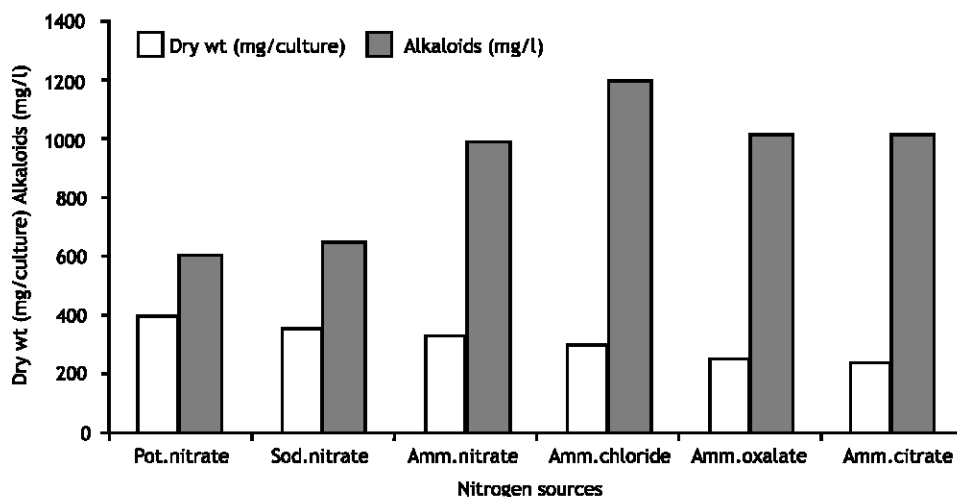
Fig. 3:   Effect of different nitrogen sources on growth and alkaloids production by *P. corylophilum*
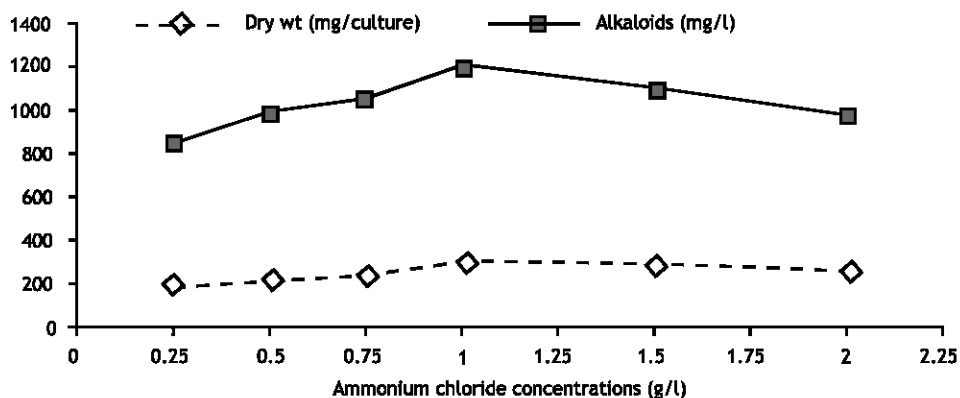

Fig. 4:   Effect of ammonium chloride concentrations (g/L) on growth and ergot alkaloid production by *P. corylophilum*

of toxic fungal metabolites is either increased (Paster *et al.*, 1985) or decreased (Sharma *et al.*, 1990) or unaffected (Paster and Bullerman, 1988) after irradiation of fungal conidia under various laboratory conditions. From the present results, it could reported that no production of alkaloids by *P. chorylophilum* at 3.0 kGy (Fig. 10). It appears from the present study that pH, incubation temperature, sodium chlorides, ammonium chloride, concentration of phosphate and irradiation dose, affect mould growth and ergot production.

It is sincerely hoped that the work comprised in this work may be a worth while contribution for the biosynthesis of alkaloids by fungi and to participate in any future microbial production
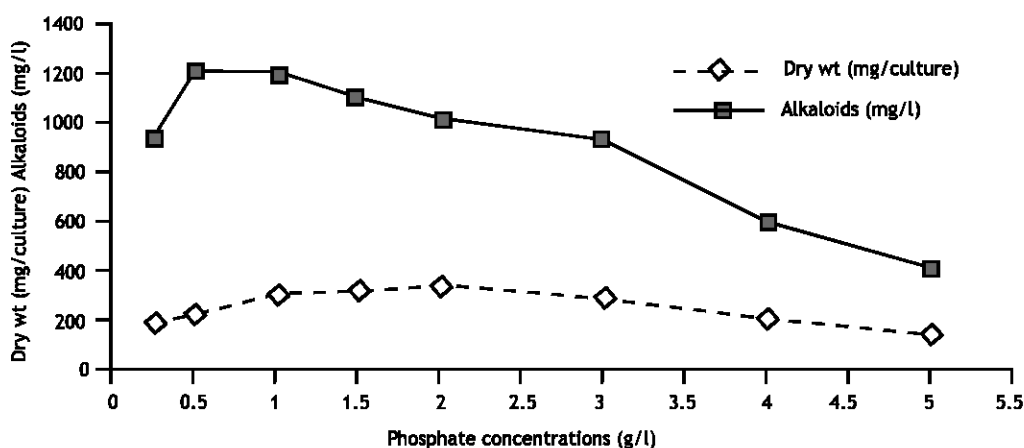
Fig. 5: Effect of phosphate concentrations (gm/l) on growth and ergot alkaloids production by *P. corylophilum*
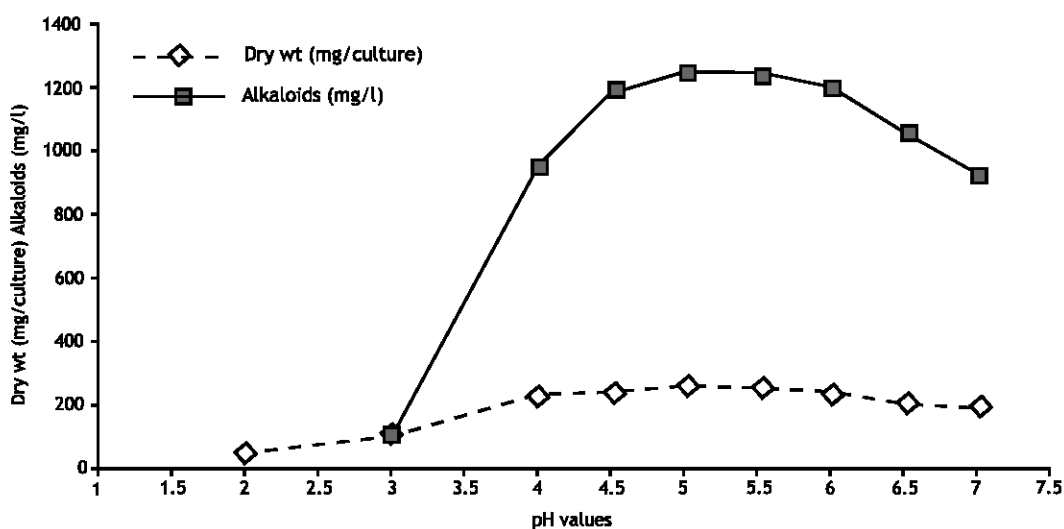

Fig. 6: Effect of pH values on growth and ergot alkaloids production by *P. corylophilum* of that group of alkaloids in this country.

Isolation and identification of ergot alkaloids produced

In an attempt to study the composition of the alkaloids produced by the experimental organism the alkaloids fraction was analyzed. The identity of each isolated alkaloid was determined by comparing their relative mobilities with the corresponding alkaloid references using Thin Layer Chromatographic (TLC). The identity of the peptide-type alkaloids was
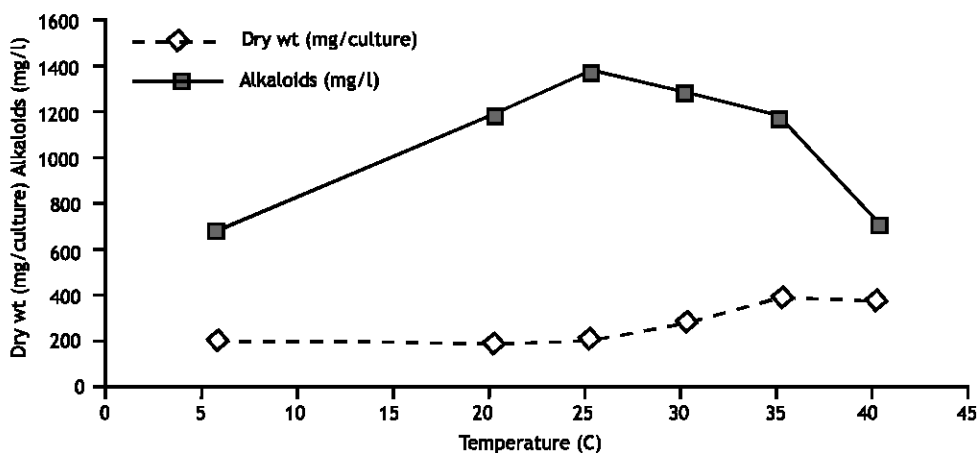
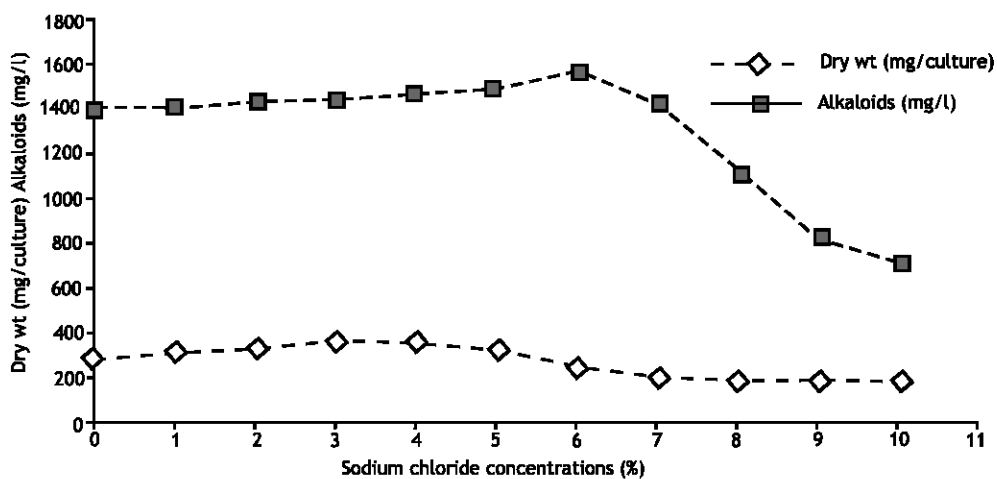Fig. 7: Effect of incubation temperature on growth and ergot alkaloids production by *P. corylophilum*

Fig. 8: Effect of sodium chloride concentrations on growth and ergot alkaloids production by *P. corylophilum*

further verified by acidic hydrolysis and investigation of the nature of the hydrolytic products.

TLC analysis of the extract of mycelium of the experimental organisms revealed three Ehrlich's reagent positive spots (produced a characteristic pinkish-blue colour). The major spot was identified as ergokryptine (Rf = 0.70). Irradiation of this spot with U.V. lamp induced blue fluorescence. The acidic hydrolysis of the compound corresponding to ergokryptine and chromatography of the produced amino acids revealed the presence of proline and leucine. The other two spots were not fluorescent under U.V. irradiation. Hence, they belong to clavine
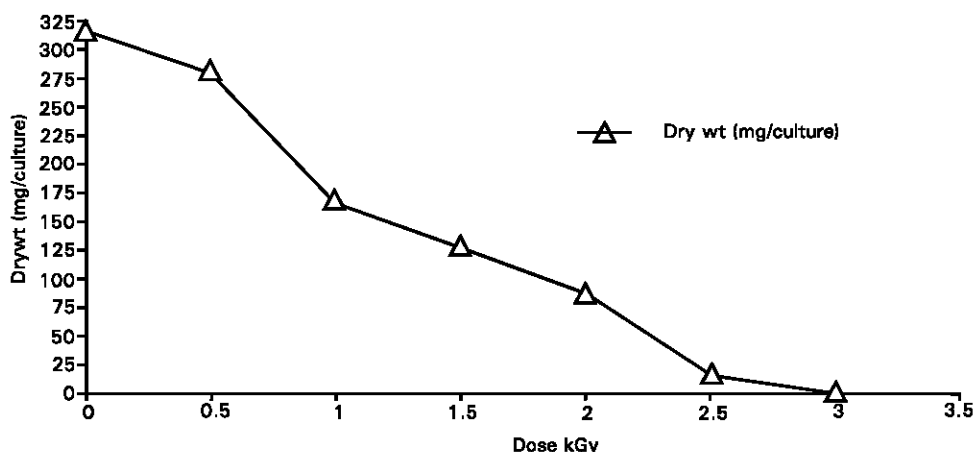
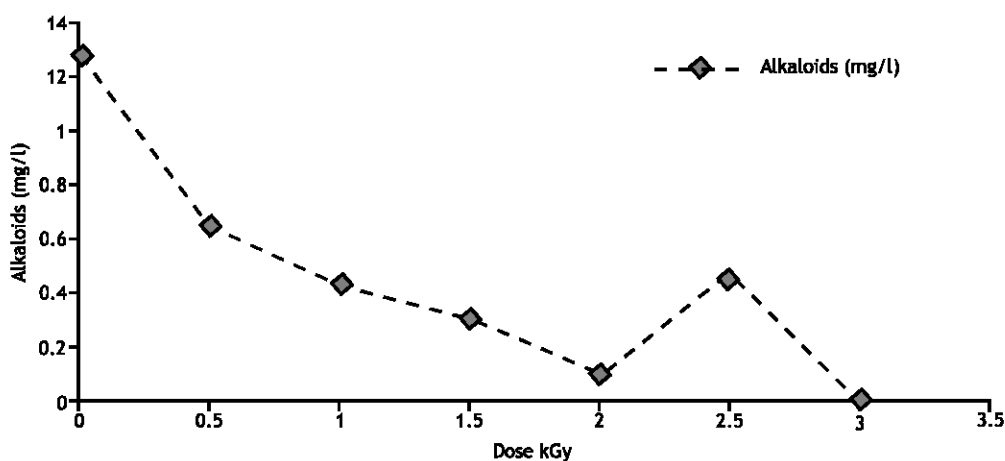Fig. 9: Influence of gamma-irradiation on the growth of *P. corylophilum*

Fig. 10: Influence of gamma-irradiation on the biosynthesis of ergot alkaloids from *P. corylophilum*

group. One spot (Rf=0.40) was identified as agroclavine and the other (Rf=0.28) as elmoclavine. HPLC analysis confirmed the identification of the alkaloid constituents of the mycelium extract of the experimental organism as ergyptine, agroclavine and elmoclavine.

Although, most ergot alkaloids have been isolated from the genus *Claviceps* and the genus *Balansia*, a few ergoline derivatives, especially those belonging to the clavine group, have been reported from other fungi as well. Festuclavine, agroclavine, elymoclavine, chanoclavine and fumigaclavine A, B and C have been isolated from *A. flavus, A. fumigatus* and *A. tamarii* (Spilsbury and Wilkinson 1961; Yamano *et al.*, 1962; Nadia Naim, 1964; Cole *et al.*, 1977; Janardhanan *et al.*, 1983). Paper chromatographic evidence of the production of clavine alkaloids by a strain of *Penicilium requeforti* was observed by Taber and Vining (1958). Constaclavine was isolated from

Table 1: Productivity of 600 isolates of *Penicillium* species of ergot alkaloids

Organisms tested	No. of isolates tested	No. of producer	Range of alkaloids production (mg/l)
P. aurantiogriseum	20	-	-
P. brevikompactum	120	14	100-425
P. Corylophilum	0	4	200-750
P. Chrysogenum	130	16	200-675
P. Citrinum	62	7	200-675
P. duclauxii	10	--	-
P. decumbens	5	--	-
P. digitatum	2	--	-
P. expansum	11	--	--
P. funiculosum	12	1	100
P. griseofulvum	4	--	-
P. glaucum	10	2	-
P. islandicum	30	3	100
P. italicum	5	--	100
P. janthinellum	4	1	375-200
P. jensenii	5	1	500-600
P. lanosum	5	--	-
P. miczynskii	4	2	100
P. nigricans	5	--	-
P. oxalicum	23	7	250-650
P. purpurogenum	9	1	650
P. purpureum	21	3	100-275
P. roqueforti	9	1	200
P. rugulosum	5	-	-
P. simplicissimum	5	-	-
P. steckii	4	--	-
P. variabile	5	1	200
P. verruculosum	10	--	-
P. viridicatum	15	1	550
P. waksmanii	30	2	600-700
Total	600	76	

P. chermesinum chanoclavine I and two new alkaliods, rugulovasine A and B, from *P. concavo-rugulovasum* and cycloclavine from *A. japonicus* (Agurell, 1964; Abe *et al.*, 1969; Yamatodani *et al.*,1970; Furuta *et al.*, 1982).

References

Abe, M., S. Ohmomo, T. Obasha and T. Tabucla, 1969. Isolation of chanclavine (1) and two new infeterconvertible alkaloids rugulovsinc A and B. from the culture of *Penicillium concarn-rugulovanam*. Agri. Biol. Chem., 33: 469-471.

Abou-Charr, C.I., H.F. Guenther, M.F. Manuel, J.F. Robbers and H.G. Floss, 1972. Biosynthesis of ergot alkaloids. Incorporation of (5R)-and 4(S)-mevalonate-5. T.

Abou-EL-Hawa, M., N. Awny, M.Z. EL-Fouly and A.A. Abou Zeid, 1993. Degradation of malathion by indol-3-acetic acid producing soil microflora under influence of different environmental conditions. Egypt. J. Microbiol., 28: 355-372.

Adler, L. and L. Gustafsson, 1980. Polyfric alcohol production and intracellular amino acid pool in relation to halotolerance of the yeast *Debaryomyces hansenii*. Arcj Micorbiol., 124: 123-130.

Agurell, S., 1964. Costaclavine from *Penicillium chernusimum*. Experientia. 20: 25-26.

Arcamone, F., E.B. Chain, A. Ferretti, A. Minghetti, P. Pennella, A. Tonolo and L. Vero, 1961. Production of a new lysergic derivative in submerged culture by a strain of *Claviceps paspali* Stevens and Hall. Proc. R. Soc., Ser. B., 155: 26-54.

Aziz, N.H. and S.S. Abd EL-Aal, 1990. Influence of potassium sorbate and sodium benzoate on gamma irradiation conidia of *Aspergillus ochraceus, Penicillium chrysogenium* and fusarium moniliform. Isotope and Rad. Res., 22, 2: 113-150.

Aziz, N.H., M.R. Refai and S.S. Abd EL-Aal, 1990. Occurrence of aflatoxin and aflatoxigenic moulds in coffee beans and decontamination by gamma irradiation. J. Egypt. Vet. Med. Ass., 502: 257-263.

Bacon, C.W., 1985. A chemically defined medium for the growth and synthesis of ergot alkaloids by species of *Baloansia*. Mycologia, 77: 418-423.

Bacon, C.W., J.K. Porter and J.D. Robbins, 1979. Laboratory production of ergot alkaloids by species of *Baloansia*. J. Gen. Microbiol., 113: 119-126.

Brady, L.R. and V.E. Tyler, 1960. J. Am. Pharm. Assoc. Sci. Ed., 49: 332.

Cole, R.J., J.E. Kirksey, J.W. Donnor, D.M. Wilson, J.C. Johnson, Jr.A.N. Johnson, D.M. Bedell, J.P. Springer, K.K. Chexal, J.C. Cardy and R.H. Cox, 1977. Mycotoxins produced by *Aspergillus fumigatus* species isolated from molded silage. J. Agri. Food. Chem., 25: 826-830.

De Waart, C. and W.A. Taber, 1960. Some aspects of phosphate metabolism of *Claviceps purpurea*. (Fr.) Tul. Can. J. Microbiol., 6: 675-678.

Drew, S. and D.A. Wallis, 1983. Regulation of secondary metabolism and keys to its manipulation. In Secondary Metabolism and Differentiation in Fungi, (J. W. Bennet and A. Ciegler, eds.). Marcel Dekker, New York, 5: 35-54.

EL-Far, F., N.H. Aziz and S. Hegazy, 1992. Inhibition by gamma-irradiation and antimicrobial food additives of aflatoxin B$_1$ production by *Aspergillus falvus* in poultry diet. Die Nahrung, 362: 143-149.

EL-Gwally, I.M., M.A. Abo-State, M.Z. EL-Fouly, G.E. Mohamed and H.A. Khalil, 2001. Factors affecting glycerol production by halotolerant yeast *Zygosaccharomyces roucii*. Az. J. Microbiol., 52: 219-232.

Flieger, M., M. Wurst and R. Shelby, 1997. Ergot alkaloids-sources, structures and analytical methods. Folia Microbiol., 42: 3-30.

Floss, H.G., J.M. Cassady and J.E. Robbers, 1973. Influence of ergot alkaloids on pituitary prolactin and propactin-dependent processes. J. Pharm. Sci., 62: 699-715.

Furuta, T., M. Kolke and M. Abd, 1982. Isolation of cycloclavino grom the culture broth of *Aspergillus faponicus* Sito. Agric. Biol. Chem., 64: 1921-1992.

Gaberc-Porekar, V., H. Socic and E. Pertot, 1987. Metabolic changes in a conidia induced *Claviceps paspali* strain during submerged fermentation. Can. J. Microbiol., 33: 602-606.

Gram, B., M. Mele and M. Kremser, 1980. Model of growth and ergot alkaloids production by *Claviceps purpurea*. Biotechnol. Bioeng., 22: 255-270.

Hong, S.L. and J.E. Robbers, 1985. Genetics of ergoline alkaloid formation in *Penicillium roqueforti*. Appl. Environ. Microbiol., 50: 558-561.

Janardhanan, K.K., Abdul Sattar and A. Husain, 1983. Production of fumigaclavine a by *Aspergillius tamarii* kita. Can. J. Microbiol., 30: 274-250.

Kelleher, W.J., 1970. Ergot alkaloid fermentations. Adv. Appl. Microbiol., 11: 211-244.

Kozovsky, A.G. and T.A. Reshetilova, 1984a. Regulation of the biosynthesis of ergot alkaloids by *Penicillium sizovae*. Folia Microbiol., 29: 301-305.

Kozlovsky, A.G. and T.A. Reshetilova, 1984b. Biosynthesis of roquefortine by growing fungi of the fungus *Penicillium*. Mikrobiologiya., 53:1.

Kozlovsky, A.G., T.F. Soloviyova, V.G. Sakharovsky and V.M. Adaxix, 1982. Ergot alkaloids argroclavine-1 and epoxyagroclavine-1metabolites of *Penicillium corylophilum*. Prikel. Biokhim. Mikrobiol., 18: 535.

Mantle, P.G., 1973. Production of ergot alkaloids in vitro by *Sphacelia sorgi*. J. Gen. Microbiol., 75: 275-281.

Mantle, P.G., 1975. Industrial exploitation of ergot fungi. In: The Filamentous Fungi, (J. E. Smith and D. R. Berry, eds.), Edward Arnold, London, 1: 281-300.

Mary, N.Y., W.J. Kelleher and A.E. Schwarting, 1965. Production of lysergic acid derivatives in submerged culture. III. Strain selection on defined media. Lloydia, 28: 218-229.

Masurekar, P.S., 1992. Therapeutic Metabolites (Ergot alkaloid) in Biotechnology of Filamentous fungi-Technology and Products (Finkelsrein D. B. and Ball, C.). pp: 265-228.

Mizrahi, A. and G.A. Miller, 1970. A defin medium for the production of lysergic acid alkaloids by *Claviceps paspali*: Biotechnol. Bioeng., 12: 641-644.

Moubasher, A.H., 1993. Soil fungi in Qatar and other Arab countries Published by the Centre of Scientific and Applied Research, University of Qatar, Doha, Qatar. pp: 566.

Nadia Naim, A., 1964. Alkaloid formation in fungi M. Sc. Thesis, Botany Department, Faculty of Science, Cairo University, Egypt.

Norkrans, B., 1966. Studies on marine occurring yeast: growth related to pH, N_2Cl concentration and temperature. Arch. Microbial., 54: 374-392.

Paster, N., R. Barlcai-Golan and R. Podons, 1985. Effect of gamma radiation on ochratoxin production by Fungus *Aspergillus ochraceus*. J. Sci. Food Agri. 36: 445-449.

Paster, N. and L.B. Bullerman, 1988. Mould spoilage and mycotoxin formation in grains as collected by physical means. Int. J. Food Microbial., 7: 257-265.

Rao, K.K., Gupta, A.R. and V.K. Singh, 1977. Effect of phosphate on ergot alkaloid synthesis in *Aspergillus fumigatus*. Folia Microbiol., 22: 415-419.

Robbers, J.E., 1984. The fermentative production of ergot alkaloids. In Advances in Biotechnological Processes, Vol. 3 (A. Mizrahi and A. L. Wezel, eds.). Alan R. Liss, New York, pp: 197-239.

Robbers, J.E., L.W. Robertson, K.M. Hornemann, A. Jindra and H.G. Floss, 1972. Physiological studies on ergot: further studies on the induction of alkaloid synthesis by tryptophan and its inhibition by phosphate. J. Bacteriol., 112: 791-796.

Shahin, A.M. Azza, 1986. Studies on the Phytoimmunity of some irradiated garlic bulbs against fungal rot. M. Sc. Thesis Faculty of Agriculture, Cairo, University, Cairo, Egypt.

Sharma, A., S.R. Badwal-Desai and P.M. Nair, 1990. Aflatoxin production ability of spores of *Aspergillus parasitcus* exposed to gamma radiation. J. Food Sci., 55: 275-276.

Shelby, R.A. and V.C. Kelley, 1990. An immunoassay for ergotamine and related alkaloids. J. Agric Food Chem., 38: 1130-1134.

Shevckuk, I.A., Y.G. Dubchenko and G.E. Grudneva, 1987. Solvent extraction isolation and separate determination of glyphosphate and dichlorous in aqueous solution. J. Analyt. Chem. USSR., 42: 267-270.

Smith, M.L., 1930. Quantitative colorimetric reaction for ergot alkaloids and its application in the chemical standardization of ergot preparations. Public Health Rep., 45: 1466-1481.

Socic, H. and V. Gaberc-Porekar, 1992. Biosynthesis and physiology of ergot alkaloids. Hand Book of Applied Mycology. Fungal Biotechnology, (Arora, D.K., Elander, R.P. and Mukerji, K. G.) Edit. Marcel Dekker Inc. Publisher, New York, U.S.A, 4: 475-515.

Spilsbury, J.F. and S. Wilkinson, 1961. The isolation of festuclavine and two new clavine alkaloids from *Aspergillus fumigatus* Fres. J. Chem. Soc., pp: 2085-2091.

Stahl, E., 1969. Thin layer chromatography, a laboratory hand book. Springer Verlag, Inc., New York. Stain No. 4, pp: 689.

Taber, W.A., 1967. Fermentative production of hallucinogenic indole compounds. Lloydia, 30: 39-66.

Taber, W.A. and L.C. Vining, 1958. Influence of certain factors on one *in vitro* production of ergot alkaloids by *Claviceps purpurea*. Can. J. Microbiol., 4: 611-626.

Vaidya, H.C. and J.D. Desai, 1982. Effect of phosphate on growth, carbohydrate catabolism and alkaloid biogenesis in *Calviceps* sp. strain SD 58. Indian J. Exp. Biol., 20: 475-478.

Van Urk, H.W., 1929. A new sensitive reaction for the ergot alkaloids, ergotamine, ergotoxine and ergotinine and its adaptation to the examination and colorimetric determination of ergot preparations. Pharm. Weekbl., 66: 473-481.

Weygand, F. and H.G. Floss, 1963. The biogenesis of ergot alkaloids. Angew. Chem. Intern. Ed., 2: 243-247.

Windisch, S. and W. Bronn, 1960. U.S. Patent 2, 936, 266. German Patent, 1, 073, 689, and 1, 077, 826.

Yamano, T., K. Kishino, S. Yamatodami and M. Abe, 1962. Research on ergot alkaloids occurred in culture of *Aspergillus fumigatus* Fres. Annu. Rep Takeds Res. Lab., 21: 95-101.

Yamatodami. S., T. Asahi, T. Matsukura, S. Ohmono and M. Abc, 1970. Structure of rugulovasine A. B and their derivatives. Agri. Biol. Chem., 34: 485-487.