Pak. j. sci. ind. res. Ser. B: biol. sci. 2015 **58** (1) 23-29

Screening of *Penicillium* Species and Optimisation of Culture Conditions for the Production of Ergot Alkaloids Using Surface Culture Fermentation Process

Memuna Ghafoor Shahid[a]* and Muhammad Nadeem[b]
[a]Department of Botany, GC University, Lahore, Pakistan
[b]Food and Biotechnology Research Center, PCSIR Laboratories Complex, Roomi Road, Lahore, Pakistan

(received January 15, 2014; revised November 5, 2014; accepted December 8, 2014)

Abstract. The present study deals with the screening of fungal species and suitable fermentation medium for the production of ergot alkaloids. Various species of genus *Penicillium* were grown on different fermentation media by employing surface culture fermentation technique to achieve the most suitable medium and the best *Penicillium* sp. The results showed that medium M5 gave maximum yield with *Penicillium commune*. Different culture conditions such as effect of different carbon and nitrogen sources, their concentration levels, different pH values and sizes of inoculum on the production of ergot alkaloids were also studied to improve the yield. Maximum production of ergot alkaloids (4.32 mg/L) was achieved with 15 mL spore suspension at pH 5 in fermentation medium containing 35% (w/v) sucrose. All these results indicate that culture conditions are very much crucial to improve the yield of ergot alkaloids produced by *Penicillium commune* through surface culture process.

Keywords: ergot alkaloids, *Penicillium commune*, culture parameters, surface culture fermentation technique

Introduction

Ergot alkaloids are tryptophan derived secondary metabolites with appreciably toxicological and pharmacological applications. They have been identified in different species of fungi of Ascomycota and in many families of higher plants (Peter and Shu-Ming, 2013). The toxicity of ergot alkaloids make them pharmacologically very useful. All classes of ergot alkaloids have been used in formulating different medicines to cure various diseases e.g., migraine and to reduce post partum bleedings (Moussa, 2003). Many of the ergot alkaloids have also been used in the induction of labour contractions and for the inhibition of lactation. It can also terminate pregnancy and inhibition of mammary tumors (Fleiger *et al*., 1997; Masurekar, 1991; Floss *et al*., 1973).

Previously, only the members of family Clavicipitaceae (Hypocreales) (Genus: *Claviceps*) were used in the synthesis of ergot alkaloids. Other than genus *Claviceps*, genus *Balansia* was also used for biosynthesis of these alkaloids. Researchers are now focusing their attention to other members of class Ascomycetes e.g., species of genus *Penicillium* (*P. aurantiovirens*, *P. chermisinum, P. ciricavirde, P. sizovae, P. roquefortii, P. corylophyllum, P. kapuscinkii, P. regulosum* and *P. concavoruglosum*) (Kozlovsky *et al*., 2013; Flieger *et al*., 1997; Hong and Robbers, 1985; Rao *et al.,* 1977). *Aspergillus fumigatus* and *Penicillium commune* of the order Eurotiales are also capable of producing ergot alkaloid (Katy *et al*., 2013; Wallwey and Li, 2011; Panaccione, 2010).

The ergot alkaloids profile of Hypocreales is quite different from Eurotiales (*Penicillium* and *Aspergillus* species) (Panaccione, 2010; Lorenz *et al*., 2009). Clavicipitaceae often have ergot alkaloids profiles including clavine alkaloids, simple amides of lysergic acid and ergopeptine alkaloids. Members of Eurotiales are also capable of producing clavine alkaloids but festucaclavine, pyroclavine and fumigaclavines A, B and C alkaloids are produced by *Penicillium* and *Aspergillus* species only (Wallwey and Li, 2011). A wide range of ergot alkaloids are being used in pharmaceuticals which has made research very interesting on these species and a strong need has been felt to explore new species capable of producing significant compounds.

The present study was focused on the screening for the best fungal strain and the optimisation of culture conditions for enhanced production of ergot alkaloids using surface culture fermentation process. The presence of

*Author for correspondence;
E-mail: memunaghafoorshahid@gmail.com

ergot alkaloids was also confirmed by TLC method. This study was also focused on the use of *Penicillium commune* for the alkaloids production because this species is locally isolated and is present in the culture bank of University of the Punjab, Lahore, Pakistan. The ergot alkaloid production profile of this species is very significant, specially the ergotamine and ergocriptine. Both these alkaloids have commercial importance in pharmaceutical applications.

Materials and Methods

Fungal species. Different species of genus *Penicillium* i.e., *Penicillium commune, Penicillium italicum, Penicillium oxalicum, Penicillium digitatum* and *Penicillium* sp., IIB were obtained from Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, New Campus, Lahore and from the Institute of Industrial Biotechnology, GC University, Lahore, Pakistan.

Maintenance of the fungal cultures. The cultures were maintained on malt extract agar (2 g malt extract and 2 g agar) slants. Spores from 7-10 days old slant were transferred to the freshly prepared slants and placed in incubator at 25 °C for 10 days for the growth of mycelium.

Screening of fungal species and fermentation medium. Species of genus *Penicillium* i.e., *P. commune, P. italicum, P. oxalicum, P. digitatum* and *Penicillium* sp., IIB were screened for ergot alkaloids production. For this purpose, different fermentation media M1, M2, M3, M4 and M5 were designed for the growth and production of ergot alkaloids with each of the above mentioned fungal species as described in Table 1.

Table 1. Various fermentation media and their composition

Ingredient	Fermentation medium				
(conc. %)	M1	M2	M3	M4	M5
Sucrose	-	5	5	-	5
Mannitol	5	-	-	5	-
Peptones	-	0.1	-	-	-
NH_4OH	-	0.01	-	-	-
NH_4Cl	-	-	-	0.2	0.2
Succinic acid	0.5	-	-	0.54	0.5
Yeast extract	-	-	0.3	-	0.5
Tryptophane	-	-	-	0.025	0.5
Asparagine	-	-	-	-	0.5
K_2HPO_4	-	0.1	-	-	-
KH_2PO_4	-	-	0.1	0.5	0.5
$MgSO_4.7H_2O$	0.01	0.15	0.001	0.03	0.03
$FeSO_4$	0.01	0.001	0.001	0.001	0.01
$ZnSO_4$	0.004	0.002	0.001	0.001	0.002

pH of all the fermentation media was adjusted to 5.2 with the help of 0.1N HCl and ammonia solution. All flasks were autoclaved at 121 °C for 15 min. 5 mL of spore suspension (10^{6-7} spores/mL) of each fungal species was transferred to the respective flasks containing the above mentioned fermentation media. After inoculation these flasks were incubated at 25 °C for 21 days.

Optimisation of culture conditions for ergot alkaloids production. Fermentation medium was further optimised and following culture parameters were studied for the optimal growth of ergot alkaloids.

Effect of different carbon sources. In order to get the maximum production of ergot alkaloids by *P. commune,* different carbon sources were used e.g., glucose, fructose, maltose, sucrose, mannose and mannitol.

Effect of different nitrogen sources. Various organic and inorganic nitrogen sources were used to optimise the best nitrogen source for the maximum production of ergot alkaloids e.g., yeast extract, peptones, malt extract, meat extract, ammonium chloride and urea.

Effect of different concentrations of sucrose. Different concentrations of sucrose i.e., 10 g, 15 g, 20 g, 25 g, 30 g, 35 g and 40 g were used to optimise the best substrate concentration for the highest yield of ergot alkaloids.

Effect of different concentrations of yeast extract. Different concentrations of yeast extract i.e., 5 g, 10 g, 15 g, 20 g, 25 g and 30 g were used to optimise the best yeast extract concentration for the highest yield of ergot alkaloids.

Effect of different concentrations of phosphate. Different concentrations of KH_2PO_4 were used i.e., 0.5 g, 1 g, 1.5 g, 2.0 g, 2.5 g and 3 g in selected medium to optimise the best concentration of KH_2PO_4 for the highest production of ergot alkaloids.

Effect of different pH levels. The pH was adjusted to different pH values ranging from 3 to 8 by using ammonia solution and 0.1N HCl.

Effect of different inoculum sizes. Different inoculum sizes i.e., 5 mL, 10 mL, 15 mL, 20 mL, 25 mL and 30 mL were used to get the maximum production of ergot alkaloids in the fermentation medium.

Final batch of *P. commune* with optimised culture conditions. Ergot alkaloids production was also achieved from the final batch of fermentation medium when *P. commune* was grown on the above mentioned optimised fermentation conditions i.e., 35% sucrose, 30% yeast extract, 2 g KH_2PO_4, 15 mL inoculum size and pH 5.

Estimation of ergot alkaloids. ***Preparation of supernatant extracts*****.** The ergot alkaloid was estimated in fermented broth after centrifugation at 4 °C and at 5000 rpm for 10 min. Mycelia of all the samples were also collected separately in petri plates. The cell biomass free fermented broth was then purified through rotary evaporator.

Preparation of extracts of dry cell biomass**.** The mycelia that separated from the fermentation broth were initially weighed and placed in oven for drying at 45 °C for 24 h. Then weighed again to measure the dry weight of the mycelium. These dry mycelia were placed in methanol for 24 h and then subjected to cell lysis by sonication process for 5 cycles of 3 min at 200 rpm/min for every mycelium. After sonication, for further cell lysis, all the sonicated material was homogenised in a homogeniser for 3 cycles of 5 min so that all of the compounds of ergot alkaloids may be released from the mycelium of fungi (Naude *et al*., 2005). The mixture after homogenisation was again centrifuged and the supernatants were collected.

Assay of alkaloids**.** Chloroform extraction process was used for the extraction of ergot alkaloids made by two fungal species following method described by Moussa (2003). Ergot alkaloids were extracted 3 times in 50 mL of chloroform in a separating funnel by separating the layers. The chloroform extracts were evaporated to dryness at 40 °C in a rotary evaporator. The residues containing ergot alkaloids were estimated after Moussa (2003). 1 mL of each of the culture filtrates was added to 2 mL of Van Urk Reagent in the test tubes and reaction mixture was incubated at 37 °C for 30 min (Smith, 1930; Van Urk, 1929). The absorbance was measured at 590 nm by spectrophotometer. The amount of alkaloids present was compared with dihydroergotamine methane sulphonate salt and bromocriptine mesylate salt.

The residues were also analysed by thin layer chromatography on silica gel strips using the mobile phase of chloroform:methanol:ammonia solution in 80:20:0.5 proportion. Pure samples of methylergotamine maleate, dihydroergotamine methane sulphonate salt and bromocrptine mesylate were run as the reference to identify the possible alkaloids spectrum. Coloured spots were developed on the TLC plates by spraying Van Urk reagent according to the procedure of Stahl and Ashworth (1969).

Results and Discussion

Species of genus *Penicillium* i.e., *Penicillium commune, Penicillium italicum, Penicillium oxalicum, Penicillium digitatum* and *Penicillium* sp., IIB were obtained from Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, New Campus, Lahore and from the Institute of Industrial Biotechnology, GC University, Lahore, Pakistan.

Penicillium commune and M5 fermentation medium was recorded as the highest ergot alkaloids producer and best fermentation medium, respectively (1.36 mg/L) (Table 2).

Optimisation of some culture conditions for production of ergot alkaloids. ***Effect of various carbon sources*****.** In order to obtain the maximum yield of ergot alkaloids, various carbon sources, i.e., glucose, fructose, sucrose, maltose, mannose and mannitol were used in the M5 fermentation medium. Sucrose was optimised as the most favourable carbon source for the mycelium growth (2.21 g/L) and ergot alkaloids production (2.60 mg/L) as indicated in Table 3.

Effect of various nitrogen sources**.** In order to obtain the maximum yield of ergot alkaloids, various nitrogen sources i.e., yeast extract, peptones, malt extract, meat extract, ammonium chloride and urea were used in the

Table 2. Optimisation of fungal species and fermentation media for the production of ergot alkaloids

Fungal organism	Fermentation media									
	M1		M2		M3		M4		M5	
	Supernatant alkaloids conc	Mycelium alkaloids conc	Supernatant alkaloids conc	Mycelium alkaloids conc	Supernatant alkaloids conc	Mycelium alkaloids conc	Supernatant alkaloids conc	Mycelium alkaloids conc	Supernatant alkaloids conc	Mycelium alkaloids conc
	(mg/mL)									
P. commune	0.01	0.01	0.096	0.13	0.30	0.09	0.79	0.51	1.36	0.96
P. italicum	0.001	0.002	0.07	0.01	0.14	0.09	0.43	0.09	0.49	0.13
P. oxalicum	0.001	0.001	0.09	0.04	0.25	0.08	0.05	0.10	0.09	0.001
P. digitatum	0.010	0.01	0.096	0.05	0.96	0.08	0.09	0.14	0.14	0.095
Penicillium sp. Strain IIB	0.01	0.06	0.076	0.086	0.96	0.76	0.98	0.65	1.15	0.64

100 mL of M5 fermentation medium. Yeast extract was the most favourable nitrogen source for the mycelium growth (2.56 g/L) and ergot alkaloids (2.90 mg/L) as given in Table 3.

Table 3. Effect of carbon and nitrogen sources on the growth of *P. commune* and production of ergot alkaloids

Culture condition	Supernatant alkaloids conc. (mg/L)	Mycelial alkaloids conc. (mg/L)	Mycelium growth (g/L)
Carbon source (5g/L)			
Glucose	1.83	0.46	1.45
Fructose	1.80	0.54	1.35
Maltose	1.72	0.61	1.90
Sucrose	2.60	1.89	2.21
Mannose	1.82	1.35	1.63
Mannitol	2.15	1.60	1.84
Nitrogen sources (5g/L)			
Yeast extract	2.90	1.48	2.56
Peptones	2.31	1.40	2.35
Malt extract	1.00	1.04	2.01
Meat extract	0.61	1.05	2.00
Ammonium Chloride	0.81	0.34	1.21
Urea	0.13	0.14	1.01

Effect of different concentrations of sucrose. Various concentrations of sucrose i.e., 10 g, 15 g, 20 g, 25 g, 30 g, 35 g and 40 g were used in the 100 mL of M5 fermentation media and the best growth of mycelium (2.54 g/L). The highest yield of alkaloids production (2.72 mg/L) was obtained at 35 g of sucrose (Table 4).

Effect of different concentrations of yeast extract. Different concentrations of yeast extract i.e., 5 g, 10 g, 15 g, 20 g, 25 g and 30 g were used to optimise the best yeast extract concentration for the highest yield of ergot alkaloids in the selected optimised fermentation medium. 30 g of the yeast extract gave maximum yield of alkaloids (2.41 mg/L) and the highest growth of mycelium (2.15 g/L) (Table 4).

Effect of various concentrations of KH_2PO_4. Different concentrations of KH_2PO_4 were used i.e., 0.5 g, 1 g, 1.5 g, 2.0 g, 2.5 g, and 3.0 g in selected M5 medium. 2 g of KH_2PO_4 gave maximum production of alkaloids (1.53 mg/L) and fungus growth (2.50 g/L) as shown in the Table 5.

Table 4. Effect of sucrose and yeast extract on the growth of *P. commune* and production of ergot alkaloids

Culture condition	Supernatant alkaloids conc. (mg/L)	Mycelial alkaloids conc. (mg/L)	Mycelium growth (g/L)
Sucrose (g/L)			
10	0.14	0.03	1.60
15	0.38	0.31	1.41
20	0.70	0.68	1.92
25	0.96	0.96	1.86
30	1.66	1.23	1.68
35	2.72	1.68	2.54
40	2.33	1.34	1.99
Yeast extract (g/L)			
5	0.43	0.28	1.16
10	0.66	0.51	1.34
15	0.80	0.61	1.60
20	0.81	0.91	2.11
25	1.91	1.24	1.80
30	2.41	1.80	2.15

Table 5. Effect of various concentrations of KH_2PO_4 on the growth of *P. commune* and ergot alkaloids production

KH_2PO_4 amount (g/L)	*P. commune*		
	Supernatant alkaloids conc. (mg/L)	Mycelial alkaloids conc. (mg/L)	Mycelium growth (g/L)
0.5	0.27	0.15	1.13
1	0.54	0.25	1.60
1.5	0.96	0.45	2.34
2	1.53	0.99	2.50
2.5	1.34	0.43	2.00
3	1.34	0.84	1.96

Effect of different pH levels. The pH of the fermentation medium had a great influence on the growth of fungal mycelia and the production of ergot alkaloids by *Penicillium commune*. pH of the medium ranged from 3-8. Ergot alkaloids production (2.13 mg/L) and the growth of fungal mycelium (2.22 g/L) was found maximum at pH 5 (Table 6).

Effect of different Inoculum sizes. Different sizes of inoculums i.e., 5 mL, 10 mL, 15 mL, 20 mL, 25 mL and 30 mL, were used in the M5 fermentation media and the best inoculum size 15 mL was optimised as the best alkaloids producing inoculum size (3.34 mg/L) fungal mycelium growth (2.94 g) (Table 6).

Table 6. Effect of pH and various inoculums sizes on the growth of *P. commune* and ergot alkaloids production

Culture condition	Supernatant alkaloids conc. (mg/L)	Mycelial alkaloids conc. (mg/L)	Mycelium growth (g/L)
pH			
3	0.81	0.62	1.60
4	1.72	0.96	1.54
5	2.13	1.95	2.22
6	1.74	1.56	1.96
7	1.90	0.95	2.15
8	1.34	0.43	1.88
Inoculum sizes (mg/L)			
5	0.80	0.56	0.99
10	2.48	0.98	1.98
15	3.34	2.76	2.94
20	1.96	1.38	2.34
25	1.98	2.60	3.23
30	1.38	1.78	1.91

Final batch of *P. commune.* A yield of 4.32 mg/L of ergot alkaloids was achieved when *P. commune* was grown on the concluding fermentation medium containing all the above mentioned optimised culture condition (35% sucrose, 30% yeast extract, 2 g KH_2PO_4, 15 mL inoculum size and pH 5).

Penicillium commune was screened as the best species amongst all the above mentioned species for the production of ergot alkaloids by surface culture fermentation process. Production of ergot alkaloids was also achieved by *Penicillium concavorugulosum* (Abe *et al*., 1969) and *Penicillium chermesinum* (Agurell, 1964) and many other species of genus *Penicillium* reported by Moussa (2003).

The optimum conditions for the growth of fungal mycelium and the yield of ergot alkaloids were (a) sucrose: 35 g (b) yeast extract: 30 g (c) carbon source (sucrose) (d) nitrogen sources (yeast extract) (e) pH 5, (f) KH_2PO_4: 2 g and 15 mL inoculum size.

The fungal organism must be provided with a healthy medium in which it can grow and sporulate well and can produce maximum amount of ergot alkaloids. *Penicillium commune* produced a high amount of ergot alkaloids when it was grown in M5 fermentation medium. Different fermentation media were also used for the production of ergot alkaloids when they were inoculated by species of *Balansia* described by Bacon *et al. (*1975). In terms of sucrose and other carbon sources utilisation, sucrose was best amongst all the carbon sources for fungal species. It was seen that with the increase in the amount of sucrose there was a remarkable increase in the growth of mycelium and production of ergot alkaloids. Glucose, fructose, maltose suppressed the growth of fungus and yield of alkaloids in the fermentation medium. These results are in the harmony of conclusion given by Moussa (2003) that sucrose is the best carbon source for the production of ergot alkaloids giving a maximum yield of 0.800 mg/L in the fermentation medium. Similar results were also obtained by Socic and Gaberc-Porekar (1992) and Arcamone *et al*. (1961). On the other hand glucose and fructose had suppressed the yield of ergot alkaloids (Drew and Wallis, 1983).

The nitrogen level of the culture medium proved to be an important factor influencing the growth and yield of alkaloids. Therefore, why different nitrogen sources were also used in the experiment and they influenced the growth of the mycelium and the production of ergot alkaloids in the fermentation medium by surface culture fermentation process. Yeast extract showed a remarkable effect on the growth of the mycelium and on the yield of ergot alkaloids. Ammonium chloride and urea had a negative effect on the fungal mycelium production and ergot alkaloids production. Moussa (2003) and Taber and Vining (1958) also described a negative effect of ammonium chloride on growth of mycelium as well as on the yield of ergot alkaloids.

The pH value of the fermentation medium put a great influence on the growth of the mycelium and production of ergot alkaloids. It was shown that with the increase in pH value there was an increase in the growth of the fungus and yield of the alkaloids. The pH 5 was remarkably best amongst all pH values for the best fungal growth and production of alkaloids. Similar results were also described by Moussa (2003) and Mizrahi and Miller (1970), who reported that optimum pH 5 was proved to be the best for the maximum yield of alkaloids.

Variation of KH_2PO_4 clearly affected the fungal growth and the production of ergot alkaloids. It was noted that with the increase in the phosphate concentration there was an increase in the mycelium growth and the production of ergot alkaloids but with the maximum increase in the phosphate there was a decrease in the fungal growth and the alkaloids production. Similar results were also achieved by experiments on *Claviceps* species by Rehacek *et al*. (1971). Taber and Vining (1958) studied

the influence of low quantity of phosphate in the carbohydrate rich medium and best ergot alkaloids production was analysed but the production of ergot alkaloids was found to be inhibited with the increase in phosphate amount. Brady and Tyler (1960) also determined that phosphate is an important nutrient for alkaloids production. These results are also in harmony with the results of Taber (1967), Mary *et al*. (1965) and De Waart and Taber (1960). It can be described that the excess phosphate required for the production of protein and nucleic acids, prolongs the growth phase of the organism and during this phase amino acids e.g., tryptophane is used for the protein synthesis (Robbers *et al*., 1972; Weygand and Floss, 1963).

Different inoculum sizes in the form of spore suspension material markedly effect the production of ergot alkaloids and mycelial production in the fermentation medium. With the increase in the spores suspension size, there was an increase in the production of ergot alkaloids and when this was increased more in the fermentation media then there was a decrease in the production of alkaloids due to the limited nutrients availability in the fermentation media for the fungal spores. These results are similar with the results of Meyrath (1973) in which they described that with the increase in the inoculum size there was an increase in the alkaloids production.

Conclusion

Ergot alkaloids are pharmaceutically very significant natural alkaloids and their derivatives are very expensive. In the present study, ergot alkaloids were produced from *Penicillium commune* and ergotamine and ergocriptine alkaloids were identified which can be used to cure migraine and to stop post partum bleedings. This is a sustainable and a very cost effective way for the production of these useful alkaloids in the laboratory and this is the need of today in pharmaceutical industry.

References

Abe, M., Ohmomo, S., Ohashi, T., Tabuchi, T. 1969. Isolation of chanoclavine-(I) and two new interconvertible alkaloids, regulovasine A and B, from the cultures of *Penicillium concavorugulosum. Journal of Agricultural Biology and Chemistry*, **33:** 469-471.

Acramone, F., Chain, E.B., Ferretti, A., Minghetti, A., Pennella, P., Tonolo, A., Vero, L. 1961. Production of a new lysergic derivative in submerged culture by a strain of *Claviceps paspali*, Stevens and Hall. *Proceedings Royal Society of London, Series. B. Biological Sciences*, **155:** 26-54.

Agurell, S. 1964. Costoclavine from *Penicillium chermesinum. Experientia*, **20:** 25-26.

Bacon, C.W., Porter, J.K., Robbins, J.D. 1975. Toxicity and occurrence of *Balansia* on grasses from toxic fescue pastures. *Applied Microbiology*, **29:** 553-556.

Brady, L.R., Tyler, V.E. 1960. A note on the biosynthesis of clavine alkaloids in *Claviceps purpurea* strain 15B. *Journal of American Pharmaceutical Association,* **49:** 332.

DeWaart, C., Taber, W.A. 1960. Some aspects of phosphate metabolism of *Claviceps purpurea. Canadian Journal of Microbiology*, **6:** 675-678.

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

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

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

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

Katy, L.R., Christopher, T.M., Panaccione, D.G. 2013. Partial reconstruction of the ergot alkaloid pathway by heterologous gene expression in *Aspergillus nidulans. Journal of Toxicology*, **5:** 445-455.

Kozlovsky, A.G., Zhelifonova, V.P., Antipova, T.V. 2013. Biologically active metabolites of *Penicillium* fungi. *Journal of Organic and Bimolecular Chemistry*, **1:** 11-21.

Lorenz, N., Haarmann, T., Pažoutová, S., Jung, M., Tudzynski, P. 2009. The ergot alkaloid gene cluster: functional analyses and evolutionary aspects. *Phytochemistry*, **70:** 1822-1832.

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

Masurekar, P.S. 1991. *Therapeutic metabolites, Biotechnology of filamentous fungi. Technology and Products*, D. B. Finkelsrein and C. Ball (eds.), pp. 241-302, Butterworth-Heinemann, Boston, USA.

Meyrath, J. 1963. Influence of size of inoculum on the various growth phases in *Aspergillus oryzea. (J) Antonie van leeuwenhoek*, **29:** 57-78.

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

Moussa, L.L.A. 2003. Effect of some factors including irradiation on the ergot alkaloids production by members of *Penicillium. Online journal of Biological Sciences*, **3:** 65-81.

Naude, T.W., Botha, C.J., Vorster, J.H., Roux. C., Van Der Linde, E.J., Van Der Walt, S.I., Rottinghaus, G.E., Van Jaarsveld, L., Lawrence, A.N. 2005. *Claviceps cyperi*, a new cause of severe ergotism in dairy cattle consuming maize silage and teff hay contaminated with ergotised *Cyperus esculentus* (nut sedge) on the Highveld of South Africa. *Onderstepoort Journal of Veterinary Research*, **72:** 23-37.

Panaccione, D.G. 2010. Ergot Alkaloids. In: *The Mycota*, *Industrial Applications*, M. Hofrichter (ed.), vol. **10,** pp. 195-214, Springer-Verlag: Berlin, Germany.

Peter, M., Shu-Ming, L. 2013. Alkaloids derived from tryptophan: a focus on ergot alkaloids. In: *Natural Products*, K. G. Ramawat and J. M. Merillon (eds.), pp. 683-714, Publication of Springer-verlag, Berlin Heidelberg, New York, USA.

Rao, K.K., Gupta, A.R., Singh, V.K. 1977. Effect of phosphate on ergot alkaloids synthesis in *Aspergillus fumigates. Folia Mikrobiologica*, **22:** 415-419.

Rehacek, Z., Sajdl, P., Kozova, J., Malik, K.A., Ricicova, A. 1971. Correlation of certain alterations in metabolic activity with alkaloid production by submerged *Claviceps. Applied Microbiology*, **22:** 949-956.

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

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

Socic, H., Gaberc-Porekar, V. 1992. Biosynthesis and Physiology of ergot alkaloids. In: *Hand Book of Applied Mycology. Fungal Biotechnology*, (D. K. Arora, R. P. Elander and K. G. Mukerji (eds.), vol. **4,** pp. 475-515, Macel Dekker Inc. Publisher, New York, USA.

Stahl, E., Ashworth, M.R.F. 1969. *Thin Layer Chromatography, A Laboratory Handbook*, 104 pp., 2nd edition, Springer Verlag, Inc., New York, USA.

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

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

Van-Urk, H.W. 1929. A new sensitive reaction for the ergot alkaloids, ergotamine, ergotoxine and ergotinine and its adaptations to the examination and colorimetric determination of ergot preparations. *Pharmaceutisch Weekblad*, (Scientific Journal), **66:** 473-481.

Wallwey, C., Li, S.M. 2011. Ergot alkaloids: Structure diversity, biosynthetic gene clusters and functional proof of biosynthetic genes. *Natural Product Reports*, **28:** 496-510.

Weygand, F., Floss, H.G. 1963. The iogenesis of ergot alkaloids. *Angewandte Chemie International Edition*, **2:** 243-247.