Screening and optimization of some inorganic salts for the production of ergot alkaloids from *Penicillium* species using surface culture fermentation process

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Abstract: The present study deals with the production of ergot alkaloids from *Penicillium commune* and *Penicillium citrinum,* using surface culture fermentation process. Impact of various inorganic salts was tested on the production of ergot alkaloids during the optimization studies of fermentation medium such as impact of various concentration levels of succinic acid, ammonium chloride, $MgSO_4$, $FeSO_4$, $ZnSO_4$, pH and the effect of various incubation time periods was also determined on the production of ergot alkaloids from *Penicillium commune* and *Penicillium citrinum*. Highest yield of ergot alkaloids was obtained when *Penicillium commune* and *Penicillium citrinum* that were grown on optimum levels of ingredients such as 2g succinic acid, 1.5 and 2g NH_4Cl, 1.5g $MgSO_4$, 1g $FeSO_4$, 1 and 1.5g $ZnSO_4$ after 21 days of incubation time period using pH 5 at 25°C incubation temperature in the fermentation medium. Ergot alkaloids were determined using Spectrophotometry and Thin Layer Chromatography (TLC) techniques.

Keywords: Ergot alkaloids, *Penicillium commune, Penicillium citrinum,* culture conditions, spectrophotometry, TLC.

INTRODUCTION

Natural products synthesized by microorganisms have played a major role in the discovery and manufacturing of drugs, which are in-use for the treatment of several human ailments. These natural products are commonly called as secondary metabolites, and these constitute an important group of bioactive compounds that can be used in pharmaceutical, cosmetic and food industry (Devi and Prabakaran, 2014). Alkaloids are the largest group of natural products synthesized as secondary metabolites in plants, animals and in fungi (Polak and Rompala, 2007). Alkaloids have been reported as a group of organic substances, containing at least one nitrogen atom in the ring structure of their molecule. Alkaloids in fungi were initially recognized in *Claviceps purpurea* (member of Clavicipitaceae family) the agent causing ergot of rye. Generally, the members of Clavicipitaceae family infest grass species, including cereal grains, and these members are capable of producing a number of different important ergot alkaloids (Danicke and Diers, 2013). These compounds have proven to be important pharmacologically and agriculturally (Wallwey and Li, 2011; Ryan *et al.,* 2013). *Penicillium* is well known all over the world, for producing secondary metabolites and commercially valued extracellular enzymes (Gulliamon *et al.,* 1998; Tiwari *et al.,* 2007, 2011). The secondary metabolites produced by *Penicillium* include alkaloids, antibiotics, hormones and mycotoxins, etc. Therefore, many species of genus *Penicillium* and *Aspergillus* are also the potential candidates for the production of ergot alkaloids, including *Penicillium sizovae, Penicillium chermisinum, Penicillium roquefortii, Penicillium corylophilum, Penicillium regulosum* (Moussa, 2003), *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus tamari*, respectively (Flieger *et al.,* 1997). The most prominent ergot alkaloids produced by *Claviceps* and *Penicillium* species are ergometrine, ergotamine, ergosine, ergocristine, ergocriptine, ergocornine, festuclavine, epicostaclavine, fumigaclavine and isofumigaclavine (Kozlovsky *et al.,* 2013).

The toxicity of ergot alkaloids make them pharmacologically very useful. All of the types of ergot alkaloids have been used in making different medicines to cure migraine, to reduce postpartum bleedings, for inducing labour contractions, for the inhibition of lactation, to terminate pregnancy and to inhibit mammary tumors (Floss *et al.,* 1973; Masureker, 1992; Fleiger *et al.,* 1997; Moussa, 2003). Taking into consideration the above-mentioned scenario and the day by day increasing demand of ergot alkaloids as pharmacological and therapeutic agents, a strong need has been felt to develop a cost-effective process for the biosynthesis of ergot alkaloids for commercial use in Pakistan using some novel sources. Hence, the present study was designed and conducted on the optimization of culture conditions for the enhanced production of ergot alkaloids using surface culture fermentation technique.

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MATERIALS AND METHODS

Procurement of fungal species

Penicillium commune and *Penicillium citrinum* were obtained from Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, New Campus, Lahore and Institute of Industrial Biotechnology, GC University, Lahore.

Maintenance of fungal cultures

The cultures were maintained on malt extract agar (MEA) medium slants (slants were prepared by taking 2g of malt extract and 2g of agar in 250ml Erlenmeyer flask and dissolved in 100ml of distilled water). Spores from 7-10 days old culture slant were transferred to the freshly prepared slants and placed in incubator at 25°C for 10 days for the growth of mycelium. The slants having proper growth after incubation were stored in refrigerator at 4°C for further studies.

Composition of growth medium and fermentation studies

Various self constructed fermentation media were screened for the production of ergot alkaloids and M5 fermentation medium was selected for the maximum production of ergot alkaloids. Surface culture fermentation technique was used for the production of ergot alkaloids in M5 fermentation medium. The growth medium contained (g/100ml) ingredients such as sucrose 5, yeast extract 0.5, succinic acid 0.5, tryptophan 0.5, asparagine 0.5, NH_4Cl 0.2, KH_2PO_4 0.5, $MgSO_4$ 0.03, $FeSO_4$ 0.001 and $ZnSO_4$ 0.002. pH of the medium was adjusted to 5.2 and after autoclaving 5ml of spore suspension (10^{6-7} spores/ml) was transferred to the respective flask containing self constructed fermentation medium. After inoculation, the growth medium was incubated at 25°C for 21 days.

Optimization of fermentation condition

The self-constructed fermentation medium was further optimized to enhance the production of ergot alkaloids. Effect of following inorganic salts and fermentation condition was studied for the maximum yield of ergot alkaloids:

Effect of concentration levels of succinic acid

In order to get the maximum yield of ergot alkaloids, various concentrations i.e. 0.5, 1, 1.5, 2, 2.5 and 3 g were employed in the fermentation medium to find the suitable concentration of succinic acid for mycelium growth and ergot alkaloids production by *Penicillium commune* and *Penicillium citrinum*.

Effect of concentration levels of ammonium chloride

Different NH_4Cl concentrations such as 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% were used in the fermentation medium to find out the suitable concentration for mycelium growth and ergot alkaloids production by *Penicillium commune* and *Penicillium citrinum*.

Effect of concentration levels of $MgSO_4$

Effect of different concentration levels of $MgSO_4$ i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% were studied to find out the suitable $MgSO_4$ concentration level for the growth of mycelium and maximum yield of ergot alkaloids by *Penicillium commune* and *Penicillium citrinum*.

Effect of concentration levels of $FeSO_4$

Effect of different concentration levels of $FeSO_4$ i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% were evaluated to find out the suitable $FeSO_4$ concentration level for the maximum mycelial growth and yield of ergot alkaloids by *Penicillium commune* and *Penicillium citrinum.*

Effect of concentration levels of $ZnSO_4$

Effect of different concentration levels of $ZnSO_4$ i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % were evaluated to find out the suitable $ZnSO_4$ concentration level for maximum growth of mycelium and production of ergot alkaloids by *Penicillium commune* and *Penicillium citrinum*.

Effect of pH

The optimum pH level was evaluated for the growth of mycelium and production of ergot alkaloids by *Penicillium commune* and *Penicillium citrinum* by adjusting the pH of the fermentation medium at different pH levels i.e. 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0. The pH of each growth medium was adjusted before sterilization with 0.1N HCl and ammonia solution.

Effect of various incubation time periods

Effect of various incubation times such as 7, 14, 21 and 30 days was determined on growth of mycelium and production of ergot alkaloids by *Penicillium commune* and *Penicillium citrinum* by incubating the fermentation media for above mentioned time range in incubator.

Determination of Ergot Alkaloids

Each flask culture after 21 days of incubation was filtered under aseptic condition to separate the grown mycelial mass (intracellular) and culture liquid filtrate (extra cellular). The collected mycelial mass and liquid culture medium was stored at 4°C for further analysis. The amount of ergot alkaloids present in extra cellular and intracellular filtrates was measured by drawing standard curve of reference salts such as ergotamine, bromocriptine mesylate and dihydroergotamine methane sulfonate salts.

Assay for extra cellular ergot alkaloids (culture liquid filtrate)

The extra cellular filtrates separated from mycelium were centrifuged at 5000rpm/min for 5min at 4°C. After centrifugation the supernatants were collected and named as culture liquid filtrates. The extra cellular filtrates of

ergot alkaloids obtained from *Penicillium commune* and *Penicillium citrinum* were purified using chloroform extraction method (Naude, 2005). In this procedure, ergot alkaloids were extracted three times in 50ml of chloroform in a separating funnel. The extracts were poured in separate glass bottles. The remaining water in the extracts was removed by rotary evaporator. After purification, 1ml of extra cellular ergot alkaloids was taken in a test tube, and 2ml of Van Urk reagent was added into it (Van Urk, 1929; Smith, 1930). The same process was adopted for all the samples. All the test tubes were incubated at 37°C for 30min and OD (optical density) was measured at 590nm by Spectrophotometer (Hitachi U2900/U2910 double beam) against blank.

Assay for Intracellular ergot alkaloids (mycelial filtrate)

All the mycelial mass separated from the flask cultures was analyzed for ergot alkaloids yield. All the mycelial samples were dried in oven at 40°C for 24 hours and their dry weights were noted. The dried mycelial samples were mixed with chloroform for three hours and after that these samples were subjected to cell lysis by sonication process using Ultrasonic Generator at 200rpm/ min for 15min to release the contents of ergot alkaloids. All the sonicated material was homogenized again in a homogenizer for 15 min for complete cell lysis so that all of the contents of ergot alkaloids may be released in the chloroform solution. Mycelial filtrate extracts collected after filtration were assayed with Van Urk reagent as mentioned above and OD was measured by Spectrophotometer (Hitachi U2900/U2910 double beam) at 590 nm against blank.

RESULTS

Penicillium commune and *Penicillium citrinum* were grown on the self-constructed fermentation medium for 21 days using surface culture fermentation process. It was found that maximum ergot alkaloids were produced from extra cellular filtrate of *Penicillium citrinum* (1.54mg/ml) and intracellular filtrate of *Penicillium commune* (0.96 mg/ml). The self-constructed fermentation medium was proved to be the most suitable medium for the growth of mycelium and production of ergot alkaloids as presented in table 1.

Optimization of culture conditions for the production of ergot alkaloids

Effect of concentration levels of succinic acid

Table 2 represents ergot alkaloids yield i.e. 1.35mg/ml from *Penicillium commune* and from *Penicillium citrinum* obtained from extra cellular filtrates at 2% concentration level of succinic acid. Least ergot alkaloids concentration in extracellular filtrate was measured at 0.5% level i.e. 0.35mg/ml and 0.45mg/ml from *Penicillium commune* and *Penicillium citrinum* respectively. Mycelial growth of both of the fungi influenced by the concentration levels of succinic acid was recorded as described in the fig. 1. Ergot alkaloids yield produced by *Penicillium commune* and for *Penicillium citrinum* in intracellular filtrate was quantified and highest yield (0.98mg/ml) was observed at 2% of succinic acid. It was also observed that with concentration of succinic acid beyond 2% in the fermentation medium, a decrease in the production of ergot alkaloids and mycelial growth occurred.

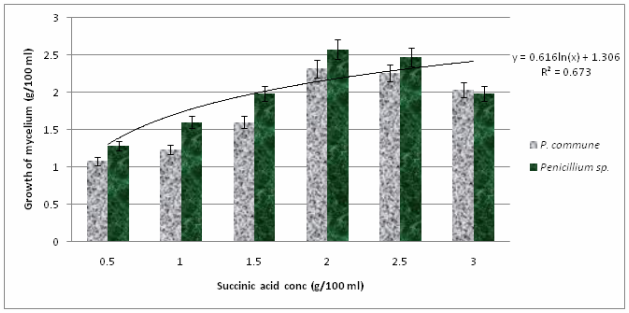

Fig. 1: Effect of different concentrations of succinic acid on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

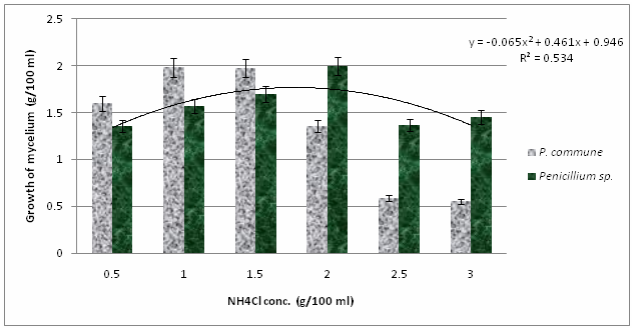

Fig. 2: Effect of different concentrations of NH_4Cl on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

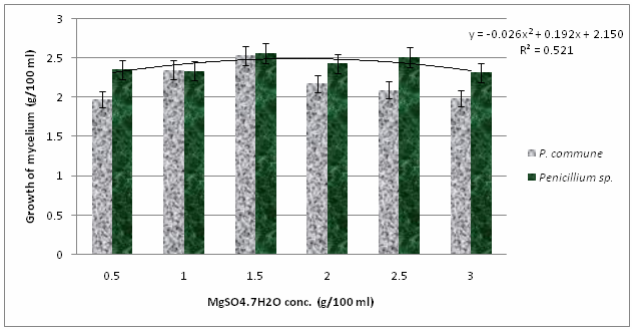

Fig. 3: Effect of different concentrations of $MgSO_4.7H_2O$ on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

Effect of concentration levels of ammonium chloride

Highest yield of ergot alkaloids (1.25mg/ml) in extra cellular filtrate of *Penicillium commune* was observed at 1.5% level of NH_4Cl and at 2% level for *Penicillium citrinum* (1.15mg/ml). Least ergot alkaloids yield was measured at 0.5% level i.e. 0.35mg/ml and 0.25 mg/ml by *Penicillium commune* and *Penicillium citrinum* respectively from their extra cellular filtrates (table 3).

Mycelial growth of both of the fungi greatly influenced by the varied concentration levels of NH_4Cl as presented in the fig. 2. Assessment of production of ergot alkaloids from intracellular filtrate indicated that highest yield was observed at 1.5% level of *Penicillium commune* (1.25 mg/ml) and 2% of *Penicillium citrinum* (1.00mg/ml). Ammonium chloride in growth medium greatly influenced the growth of mycelium and increased the ergot alkaloids production but above 2% concentration hindered the production of alkaloids.

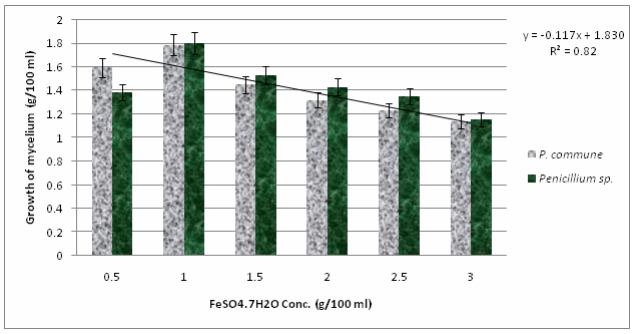

Fig. 4: Effect of different concentrations of $FeSO_4.7H_2O$ on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

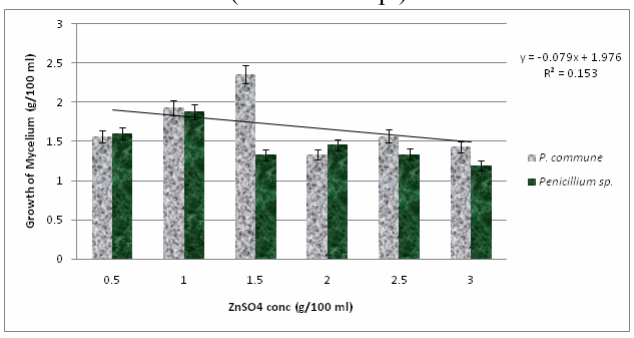

Fig. 5: Effect of different concentrations of $ZnSO_4.7H_2O$ on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

Effect of concentration levels of $MgSO_4.7H_2O$

Table 4 shows the ergot alkaloids production in the extra cellular and intracellular filtrates of *Penicillium commune* and *Penicillium citrinum.* It was found that, the considerable yield of ergot alkaloids was obtained in extra cellular filtrates of *Penicillium commune* (1.53mg/ml) and *Penicillium citrinum* (0.95mg/ml) at 1.5% concentration level of $MgSO_4.7H_2O$ in the fermentation medium. Lowest yield of ergot alkaloids was quantified at 0.5% level of $MgSO_4.7H_2O$ i.e. 0.59mg/ml and 0.32mg/ml from *Penicillium commune* and *Penicillium citrinum* respectively in their extra cellular filtrates. Mycelial growth of the fungi was significantly influenced by the concentration levels of $MgSO_4.7H_2O$ which is presented in the fig 3. Maximum ergot alkaloid yield was obtained from intracellular filtrates of *Penicillium commune* (0.93mg/ml) and *Penicillium citrinum* (0.33mg/ml) at 1.5% level of $MgSO_4.7H_2O$.

Effect of concentration levels of $FeSO_4.7H_2O$

Maximum ergot alkaloids yield in the extra cellular filtrates of *Penicillium commune* and *Penicillium citrinum* was obtained at 1.0% concentration level of $FeSO_4.7H_2O$ i.e. 0.79mg/ml and 0.93mg/ml, respectively. Lowest yield of ergot alkaloids i.e. 0.25mg/ml and 0.32mg/ml in extra cellular filtrates of *Penicillium commune* and *Penicillium citrinum* respectively was quantified at 3% concentration level in the fermentation medium. Mycelial growth of both fungal species was significantly influenced by the different concentrations of $FeSO_4.7H_2O$ (fig. 4). Ergot alkaloid yield of *Penicillium commune* and *Penicillium citrinum* was found to be best at 1% level of $FeSO_4.7H_2O$ i.e. 0.75mg/ml and 0.83mg/ml, respectively (table 5).

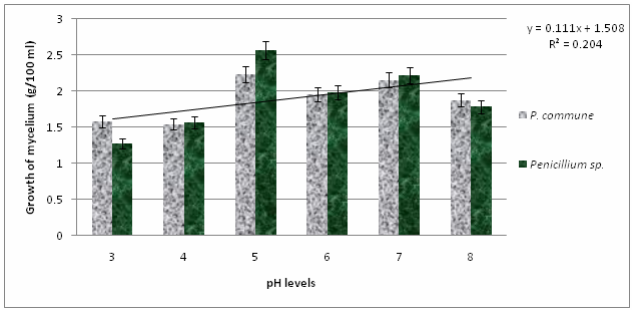

Fig. 6: Effect of different concentrations of pH on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

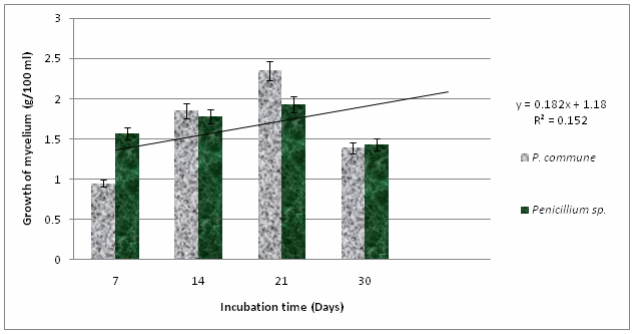

Fig. 7: Effect of various incubation time periods on the mycelial growth of *Penicillium commune* and *Penicillium citrinum* (*Penicillium* sp.)

Effect of concentration levels of $ZnSO_4.7H_2O$

Table 6 shows that, maximum ergot alkaloid yield was measured in the extracellular filtrates of *Penicillium commune* (1.00mg/ml) at 1.5% addition of $ZnSO_4$ in culture medium and of *Penicillium citrinum* (0.78mg/ml) at 1% concentration level. Lowest yield of ergot alkaloids in extra cellular filtrate was obtained at 3% $ZnSO_4$ i.e. 0.43 mg/ml and 0.13mg/ml from *Penicillium commune* and *Penicillium citrinum* respectively. Mycelial growth of both of the fungi has significantly influenced by the varied concentration of $ZnSO_4$ (fig. 5). Ergot alkaloid yield in intracellular filtrates was found highest at 1% and 1.5% concentration level in culture medium of *Penicillium citrinum* (0.63mg/ml) and *Penicillium commune* (0.85mg/ml).

Table 1: Production of ergot alkaloids using surface culture fermentation process

Fungal species	Extra cellular Ergot Alkaloids (mg/ml)	Intracellular Ergot Alkaloids (mg/ml)
Penicillium commune	1.35±0.02	0.96±0.01
Penicillium citrinum	1.54±0.001	0.635±0.002

Each value is an average of three replicates and ± indicates the standard deviation of these replicates.

Table 2: Effect of concentration levels of succinic acid on the production of ergot alkaloids

Succinic Acid (g)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
0.5	0.35±0.01	0.05±0.01	0.45±0.06	0.35±0.01
1	0.45±0.02	0.25±0.02	0.58±0.02	0.49±0.01
1.5	0.98±0.001	0.76±0.03	0.95±0.03	0.57±0.04
2	1.35±0.02*	0.98±0.01*	1.35±0.03*	0.98±0.02*
2.5	1.00±0.05	0.63±0.02	0.99±0.01	0.75±0.01
3	1.00±0.02	0.62±0.03	0.75±0.01	0.73±0.02

Table 3: Effect of concentration levels of NH_4Cl on the production of ergot alkaloids

NH_4Cl (g)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
0.5	0.35±0.04	0.35±0.02	0.25±0.02	0.07±0.06
1	0.69±0.03	0.58±0.01	0.29±0.06	0.35±0.02
1.5	1.25±0.01*	1.25±0.001*	1.05±0.02	0.95±0.01
2	1.05±0.04	1.05±0.06	1.15±0.03*	1.00±0.003*
2.5	0.95±0.01	1.03±0.05	1.07±0.02	0.76±0.02
3	0.85±0.01	0.95±0.02	0.91±0.01	0.65±0.02

Table 4: Effect of concentration levels of $MgSO_4.7H_2O$ on the production of ergot alkaloids

$MgSO_4.7H_2O$ (g)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
0.5	0.59±0.01	0.35±0.03	0.32±0.02	0.03±0.05
1	1.35±0.01	0.74±0.01	0.56±0.01	0.23±0.01
1.5	1.53±0.005*	0.93±0.01*	0.95±0.01*	0.33±0.01*
2	0.95±0.01	0.65±0.02	0.43±0.005	0.21±0.02
2.5	0.75±0.01	0.54±0.01	0.33±0.02	0.13±0.01
3	0.32±0.01	0.34±0.04	0.13±0.006	0.03±0.003

Table 5: Effect of concentration levels of $FeSO_4.7H_2O$ on the production of ergot alkaloids

$FeSO_4.7H_2O$ (g)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
0.5	0.35±0.005	0.43±0.02	0.45±0.01	0.32±0.01
1	0.79±0.005*	0.75±0.02*	0.93±0.02*	0.83±0.01*
1.5	0.56±0.01	0.58±0.01	0.74±0.02	0.79±0.03
2	0.54±0.02	0.43±0.02	0.63±0.005	0.63±0.01
2.5	0.49±0.03	0.35±0.03	0.53±0.01	0.53±0.003
3	0.25±0.02	0.13±0.02	0.32±0.03	0.34±0.02

Where, EEA=Extra cellular Ergot Alkaloids, IEA= Intracellular Ergot Alkaloids Each value is an average of three replicates and ± indicates the standard deviation of these replicates.

Table 6: Effect of concentration levels of $ZnSO_4$ on the production of ergot alkaloids

$ZnSO_4$ (g)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
0.5	0.53±0.02	0.43±0.01	0.59±0.01	0.43±0.01
1	0.79±0.01	0.63±0.02	0.78±0.01*	0.63±0.02*
1.5	1.00±0.03*	0.85±0.01*	0.65±0.02	0.59±0.03
2	0.95±0.01	0.42±0.01	0.53±0.03	0.43±0.02
2.5	0.79±0.02	0.32±0.03	0.32±0.01	0.33±0.02
3	0.43±0.03	0.10±0.03	0.13±0.03	0.03±0.06

Table 7: Effect of pH on the production of ergot alkaloids on the production of ergot alkaloids

pH	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
3	0.82±0.01	0.65±0.02	0.71±0.02	0.54±0.01
4	1.71±0.02	0.96±0.01	1.35±0.01	0.98±0.01
5	2.14±0.001*	1.95±0.01*	1.99±0.01*	1.89±0.02*
6	1.74±0.01	1.57±0.02	1.54±0.02	1.05±0.01
7	1.85±0.02	0.96±0.01	1.01±0.03	0.95±0.02
8	1.34±0.01	0.44±0.01	1.00±0.04	0.65±0.03

Table 8: Effect of incubation time periods on the production of ergot alkaloids

Incubation time (Days)	*Penicillium commune*		*Penicillium citrinum*	
	EEA (mg/ml)	IEA (mg/ml)	EEA (mg/ml)	IEA (mg/ml)
7	1.99±0.01	0.96±0.01	1.92±0.01	0.64±0.01
14	2.21±0.01	1.86±0.01	2.00±0.01	0.81±0.02
21	2.96±0.005*	2.35±0.01*	2.43±0.02*	0.98±0.002*
30	1.23±0.02	1.39±0.02	1.55±0.02	0.41±0.01

Where, EEA=Extra cellular Ergot Alkaloids, IEA= Intracellular Ergot Alkaloids Each value is an average of three replicates and ± indicates the standard deviation of these replicates.

Effect of pH

Table 7 shows the best ergot alkaloids yield in the extra cellular filtrates of *Penicillium commune* (2.14mg/ml) and *Penicillium citrinum* (1.99mg/ml) at pH 5 of the fermentation medium. Lowest yield of ergot alkaloids in extra cellular filtrates was measured at pH 3 i.e. 0.82 mg/ml and 0.71mg/ml of *Penicillium commune* and *Penicillium citrinum* respectively. Mycelial growth of both of the fungi greatly influenced by the varied pH levels as mentioned in the fig. 6. Ergot alkaloid yield was estimated in intracellular filtrates too and highest yield was measured at pH 5 of *Penicillium commune* (1.95 mg/ml) and *Penicillium citrinum* (1.89mg/ml) and as presented in table 7.

Effect of incubation time periods

Table 8 shows, the presence of maximum ergot alkaloids yield after 21 days of incubation period in the extra cellular filtrates of *Penicillium commune* (2.96mg/ml) and *Penicillium citrinum* (2.43mg/ml). Lowest yield of ergot alkaloids in extra cellular filtrate was obtained after 30 days i.e. 1.23mg/ml and 1.55mg/ml of *Penicillium commune* and *Penicillium citrinum* respectively. Mycelial growth of fungi was influenced by different incubation time periods (fig. 7). Ergot alkaloid yield was estimated in intracellular filtrates and highest yield was observed after 21 days of incubation of *Penicillium commune* (2.35mg/ml) and *Penicillium citrinum* (0.98mg/ml) and as presented in the table.

DISCUSSION

Fungal organisms have been recognized as a warehouse of novel secondary metabolites and many of them show significant biological activities. These fungal organisms are ubiquitous in nature and are a potential source to meet the demand of new drugs in pharmaceutical industry (Devi and Prabakaran, 2014). Typical alkaloids are derived from plants and they contain one or more nitrogen atom in their structures and are called as amino-alkaloids. These are also known as protoalkaloids. True alkaloids such as i.e. lysergic acid alkaloids and gliotoxins are rarely present in lower plants but can be found in various species of fungi (Roberts and Wink, 1998). Ergot alkaloids are produced by several fungi, such as *Claviceps, Epichole, Neotyphodium, Aspergillus* and *Penicillium*, mostly representing two different orders of Ascomycetes i.e. Hypocreales and Eurotiales. These

species can produce a wide variety of significant ergot alkaloids in fermentation medium (Clay and Shardl, 2002).

Different culture conditions were optimized during the present study to investigate the effect of various organic and inorganic compounds on the ergot alkaloids yield during fermentation conditions. Reshetilova and Kozlovsky (1990) described that production of ergot alkaloids depends on many factors in which, composition of culture medium, pH, temperature and other organic and inorganic compounds play significant role to enhance the yield of the product in artificial media. The organic and inorganic compounds exhibit some regulatory effects or behave as structural elements that influence the biosynthesis of secondary metabolites during fermentation studies.

The nitrogen level of the fermentation medium greatly influences the growth of mycelium and production of ergot alkaloids. Effect of different concentration levels of NH_4Cl on the mycelial growth and ergot alkaloids production was studied during the optimization studies (table 3). The results are in consonance with Moussa (2003) who reported that the addition of high concentration of NH_4Cl in the fermentation medium partially supported the synthesis of ergot alkaloids. Gaberc-Porekar *et al.* (1987) also reported that ammonia is rapidly utilized nitrogen source and it depletes from the growth medium readily until a high production of ergot alkaloids starts. The effect of different salts such as $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and $ZnSO_4$ was determined on the mycelial growth and production of ergot alkaloids during the present study. It was investigated that in the presence of all these salts there was a remarkable increase in the production of ergot alkaloids as described in the tables 4, 5 and 6. These ions help in the growth of fungal organism and act as cofactors to trigger and initiate metabolic processes of the organism as described by Fujiwara and Yammato (1987).

The production of ergot alkaloids was strongly controlled by pH of the fermentation medium during the present study. *Penicillium commune* and *Penicillium citrinum* were active on different pH levels ranged from pH 3 to 8 in which pH 5 was found as optimum for the production of ergot alkaloids in extra cellular and intracellular filtrates of *Penicillium commune* and *Penicillium citrinum* respectively (table 7). Similar findings have been reported by Mizrahi and Miller (1970), Bogo *et al.* (2003) and Moussa (2003) for the production of ergot alkaloids from different fungal species.

Incubation temperature and incubation time period significantly influenced the growth of mycelium and production of ergot alkaloids *in vitro* fungal cultures of test species. It was observed that incubation temperature of 25°C proved as the best suitable temperature for mycelial growth and ergot alkaloids production as described by Socic and Garberc-Porker (1992), in which they described that 24°C to 27°C temperature range supported maximum ergot alkaloid production. Different incubation periods for the growth and production of mycelium was observed during the present study and maximum quantity of ergot alkaloids was produced after 21 days incubation of *Penicillium commune* and *Penicillium citrinum* (table 8). It was also noted that with the increase in incubation temperature above 30°C and incubation time above 30 days slowed down the mycelial growth and ergot alkaloids yield. This may be due to the inhibition of mycelial development in the high temperature which was not suitable for the production of ergot alkaloids and growth inhibition in incubation period longer than 21 days. This may also be due to the depletion of nutrients in fermentation medium as described by Zerdani (2004) in his experiments.

CONCLUSION

Ergot alkaloids are not only produced in plants but in fungi too. However, the amount of ergot alkaloids produced by fungal species is comparatively less but it can be enhanced by focusing on the nutritional requirements of the new fungal species having the ability to produce ergot alkaloids. The literature survey indicated that this aspect has not yet been focused across the globe. Therefore, *Penicillium commune* and *Penicillium citrinum* were evaluated for its ability to produce ergot alkaloids in culture liquid (extra cellular) and mycelial (intracellular) filtrates and these species can be a potential candidate for the better production of ergot alkaloids. The present study also revealed that the surface culture fermentation technique is comparatively better and cost effective for the production of ergot alkaloids.

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