Research Journal of Recent Sciences ISSN 2277-2502
Vol. **2(ISC-2012),** 20-23 **(2012)** *Res.J.Recent.Sci.*

Factors Influencing the Interaction of three Fungi and Mycotoxin Production

Surekha M., Kiran S., Naresh A. and Reddy S.M.
Toxicology Laboratory, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, INDIA

Available online at: www.isca.in
Received 19th October 2012, revised 31st December 2012, accepted 28th January 2013

Abstract

The interaction of three fungi viz, Penicillium griseofulvum P. crustosum and Aspergillus terreus in relation to mycotoxins production was studied. Production of penitrem A, CPA, patulin and terreic acid by above fungi varied with the environmental conditions. In general glucose, sucrose and potassium nitrate were favoured carbon and nitrogen sources. The biomass production also varied with environmental factors.

Keywords: Interaction of fungi, *Penicillium griseofulvum*, *P. crustosum*, *Aspergillus terreus*, cyclopiazonic acid, penitrem A, patulin and terreic acid.

Introduction

In nature organisms compete either for space or for nutrients which may result in the elimination of weak organism or may settle down in coexistence due to a balance of power. In the process of interaction one organism affects the other in variety of ways. In recent times, some attempts have been made to understand such phenomena with a hope that such information may be helpful in formulating biocontrol agent for prevention and control of mycotoxins. A part from interacting fungi, a third factor i.e. environment play a significant role exerting its influence on individual or both the fungi. Though, the interaction of different fungi in relation to mycotoxins production have studied by some earlier workers[1-8] but practically no information is available on factors influencing interaction of mycotoxigenic fungi and mycotoxin production. Therefore, in the present investigations influence of different factors on the interaction of fungi and mycotoxin production was studied.

Material and Methods

Monosporic cultures of *Penicillium griseofulvum P. crustosum* and *Aspergillus terreus* isolated from paddy straw were grown in 50ml buffered Czapek's dox medium (Sucrose 30g; $NaNo_3$ 3.0g; KH_2Po_4 1g; KCl 0.5g; $FeSo_4$ 7 H_2O 0.01g; $MgSo_4$ $7H_2O$ 0.5g; distilled water 1000ml; pH 5.5) contained in 250 ml Erlenmeyer conical flasks for 15 days at 27 ±2ºC and amount of mycotoxins production by respective fungi was assessed. The pH changes and biomass produced was also assessed.

The influence of carbon and nitrogen source on interaction of above mycotoxic fungi was studied by substituting sucrose and sodium nitrate of basal medium by different carbon and nitrogen sources so as to supply equivalent amount of carbon and nitrogen sources respectively.

The pH of the basal medium was adjusted to desired level (2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5) with the help of 6N HCl/NaOH for studying the influence of pH, and temperature (15, 20, 25, 30, 35 and 40ºC) on interaction of mycotoxigenic fungi and its impact on mycotoxin elaboration was also studied.

At the end of 15days incubation period, the cultures were harvested on previously dried and weighed Whatman No. 42 filter paper to determine the biomass of the fungus. The filter paper along with mycelial mat was dried at 70ºC for 2 days and weighed to a constant weight after cooling to room temperature in a desiccator. The culture filtrate was employed for extraction and estimation of different mycotoxins. Patulin[9], terreic acid[10], cyclopiazonic acid[11] and penitrem A[12] were estimated by standard methods. Mycelial mat was used for extraction and estimation of penitrem A. The experiments were conducted in triplicate. The results obtained are statistically analysed using SPSS software (Version 12.0). The details of extraction and estimation of other mycotoxins were similar to those described earlier[13]. The results are presented in table 1 and 2.

Results and Discussion

A critical study of results presented in Table 1 reveals that *P. griseofulvum, P. crustosum* and *A. terreus* interacted intimately during their growth resulting in disturbances in their metabolism. Sucrose favoured the production of CPA by *P. griseofulvum*, while xylose was ideal carbon source for production of penitrem A by *P. crustosum.* On the other hand, patulin production by *A. terreus* was maximum in medium containing starch, where as sucrose and starch were responsible for increased production of terreic acid. Lactose inhibited the production of all mycotoxins by the fungi under investigations. Glycerol inhibited the production of penitrem A and patulin by *P. crustosum* and *A. terreus* respectively. Sorbitol exerted varied influence on three fungi under study for production of respective mycotoxins.

Table-1
Statistical analysis of effect of different carbon and nitrogen sources on production of biomass and mycotoxins by three mycotoxigenic fungi

Source	Statistical Parameters	Final P^H	*P. griseofulvum*	*P. crustosum*	*A. terreus*		Dry weight (mg/ml)
			CPA (µg/ml)	Penitrem A (mg/g)	Terreic acid (ppb)	Patulin (ppb)	
Carbon source							
Glucose	Mean±St.d S.E	6.00±0.70 0.50	106.5±16.2 11.5	1.05±0.71 0.50	152.5±24.7 17.5	36.5±9.19 6.50	20.8±0.98 0.70
Xylose	Mean±St.d S.E	6.35±1.20 0.85	103.0±21.2 15.0	1.34±0.31 0.22	132.5±53.0 37.5	30.5±17.6 12.5	18.3±4.52 3.20
Sucrose (C)	Mean±St.d S.E	5.65±0.21 0.15	112.0±8.48 6.00	1.15±0.57 0.40	155.0±21.2 15.0	33.0±14.1 10.0	20.8±0.98 0.70
Lactose	Mean±St.d S.E	6.25±1.06 0.75	73.0±63.6 45.0	0.89±0.94 0.66	112.5±81.3 57.5	29.0±19.7 14.0	17.6±5.51 3.90
Starch	Mean±St.d S.E	6.25±1.06 0.75	76.5±58.6 41.5	1.02±0.76 0.59	164.0±81.3 57.5	31.5±16.2 11.5	19.1±3.39 2.40
Sorbitol	Mean±St.d S.E	6.00±0.70 0.50	91.5±37.4 26.5	0.96±0.84 0.60	125.0±63.6 45.0	34.0±12.7 9.00	19.3±3.04 2.15
Glycerol	Mean±St.d S.E	5.45±0.07 0.50	84.0±48.0 34.0	0.92±0.90 0.64	115.0±77.7 55.0	29.0±19.7 14.0	20.3±1.69 1.20
Nitrogen source							
Sodium nitrate (C)	Mean±St.d S.E	5.65±0.21 0.15	111.0±9.89 7.00	1.12±0.62 0.44	153.0±24.0 17.0	31.5±16.2 11.5	20.8±0.98 0.70
Potassium nitrate	Mean±St.d S.E	5.55±0.70 0.50	102.0±22.6 16.0	1.28±0.39 0.28	142.5±38.8 27.5	32.5±14.8 10.5	20.5±2.05 1.45
Ammonium nitrate	Mean±St.d S.E	5.00±0.70 0.50	72.5±64.3 45.5	0.85±0.99 0.70	102.5±95.4 67.5	27.5±21.9 15.5	16.8±6.57 4.65
L-aspartic acid	Mean±St.d S.E	5.90±0.56 0.40	107.0±15.5 11.0	0.96±0.84 0.60	121.0±69.2 49.0	29.0±19.7 14.0	19.3±3.04 2.15
L-asparagine	Mean±St.d S.E	6.25±1.06 0.75	114.0±5.65 4.00	0.94±0.87 0.62	117.5±74.2 52.5	30.5±17.0 12.5	20.3±1.69 1.20
L-tyrosine	Mean±St.d S.E	5.25±0.35 0.25	102.5±21.9 15.5	0.79±1.08 0.76	155.0±21.2 15.0	26.5±23.3 16.5	18.4±4.38 3.10
Urea	Mean±St.d S.E	5.75±0.35 0.25	69.5±68.5 48.5	0.90±0.92 0.65	110.0±84.8 60.0	29.0±19.7 14.0	17.3±5.93 4.20

S.E= Standard Error, St.d= Standard Deviation.

Table-2
Effect of temperature and pH on biomass and mycotoxin production by three mycotoxigenic fungi

Temperature (ºC)	Final pH	*P. griseofulvum*	*P. crustosum*	*A. terreus*		Dry weight (mg/ml)
		CPA (µg/ml)	Penitrem A (mg/g)	Terreic acid (ppb)	Patulin (ppb)	
15	5.8	58.0	0.21	20.0	0.00	15.7
20	6.2	80.0	0.32	120.0	0.00	18.1
25	5.8	105.0	0.70	142.0	25.0	20.0
30	6.8	90.0	0.52	135.0	20.0	19.1
35	6.5	85.0	0.22	50.0	15.0	15.1
40	5.6	20.0	0.00	25.0	0.00	13.1
pH						
2.5	5.0	0.00	0.00	28.0	0.00	3.15
3.5	5.5	75.0	0.15	38.0	5.00	10.1
4.5	5.0	90.0	0.20	100.0	8.00	13.1
5.5 (C)	5.8	105.0	0.72	140.0	23.0	21.1
6.5	7.0	82.0	0.32	143.0	20.0	20.1
7.5	7.8	50.0	0.18	138.0	6.00	18.1
8.5	8.5	70.0	0.00	120.0	0.00	16.2
9.5	8.5	0.00	0.00	40.0	0.00	5.12

Out of seven nitrogen sources tested, *L*-asparagine supported maximum amount of CPA production by *P. griseofulvum*, while *P. crustosum* opted potassium nitrate for penitrem A production. *L*-tyrosine induced the maximum production of terreic acid. However, it supported minimum amount of penitrem A and patulin by *P. crustosum* and *A. terreus* respectively. The present fungi also exhibited preferential response towards *L*-aspartic acid and *L*-asparagine. Amino form of nitrogen was preferred for production of CPA and patulin by *P. griseofulvum* and *A. terreus* respectively. On the other hand, for production of penitrem A and terreic acid, the acid form was preferred. Such preferential utilization of acid and amino forms of nitrogen has also reported for other fungi also[14,15,16]. Urea inhibited the production of CPA by *P. griseofulvum* to a significant level. Out of three nitrates tested ammonium nitrate was poor as it supported least amount of growth and respective mycotoxin production. Sodium nitrate followed by *L*-asparagine favoured the growth of the present three fungi. On the other hand, urea and ammonium nitrate inhibited the biomass production by the fungi under investigations.

It is evident from the results presented in table 2, that production of CPA and penitrem A production by *P. griseofulvum* and *P. crustosum* respectively was maximum at 20-25 ºC, while *A. terreus* preferred 25-30ºC for patulin and terreic acid production. *P. crustosum* failed to produce penitrem A at 40ºC, while production of CPA by *P. griseofulvum* was adversely affected to a significant level at this temperature. *A. terreus* failed to produce patulin at incubation temperature of 15, 20 and 40ºC. The amount of CPA produced by *P. griseofulvum* was almost same at 20 and 35ºC. The biomass production was maximum at incubation temperature of 25ºC to 30ºC, while 40ºC temperature inhibited the growth of interacting fungi to some extent. Temperature below and above 25^0C affected the biomass production by the present fungi.

It is also evident from the table that all the three fungi understudy opted pH 5.5 for production of all the mycotoxins. The amount of toxin produced by respective fungi decreased both with the increase of alkalinity or acidity. At pH 2.5 and 9.5 production of CPA, penitrem A and patulin production by *P. griseofulvum*, *P. crustosum* and *A. terreus* respectively was totally inhibited, while *A. terreus* could produce a little amount of terreic acid, at pH 2.5. pH 5.5 to 7.5 was found to be favourable for biomass production, which decreased with further increase in acidity or alkalinity. The final pH of the medium varied with the environmental conditions. No positive correlation could be observed between mycotoxin production and pH of the medium.

Conclusion

From the present investigations it is clear that fungi living in an environment encounter interact and influence the living of other fungi. Further, the fungal interaction is also influenced by nutrients (carbon and nitrogen sources) present, pH of the substratum and temperature prevailing. When D-xylose favoured the production of penitrem A by *P. crustosum*, starch stimulated the production of terreic acid. On the other hand, patulin produced by *A. terreus* remained unaffected. *L*-tyrosine and sodium nitrate were responsible for maximum production of Terreic acid, while *L*-asparagine favoured production of CPA by *P. griseofulvum*. pH of 5.5 and incubation temperature of 25±2˚C were favourable for production respective of mycotoxins by the fungi understudy. Thus situation in an open environment is complex and needs to be studied more critically.

Acknowledgement

Thanks are due to Head, Department of Botany, Kakatiya University for providing necessary laboratory facilities and University Grant Commission, New Delhi financial assistance.

Reference

1. Kim Y.K. and Roh J.K. Survey for zearalenone in imported corn, *Korean J Anim Sci.,* **28,** 29-32 **(1986)**

2. Cuero R.G., Smith J.E. and Lacey J., Interaction of water activity, temperature and substrate in mycotoxin production by *A. flavus*, *P. viridicatum* and *F. graminearum* in irradiated grains, Trans. British, *Myco. Sco*., **89,** 221-226 **(1987)**

3. Roy A.K. and Chourasia H.K. Inhibition of aflatoxins production by microbial interaction, *J Gen Apl Microbiol*., **36,** 59–62 **(1990)**

4. Lee H.B. and Magan N., Impact of environment and inter specific interactions between spoilage fungi and *Aspergillus ochraceus* on growth and ochratoxin production in maize grain, *Int. J. Food Microbiol*., **61,** 11–16 **(2000)**

5. Simpson D.R., Thomsett M.A. and Nicholson P., Competitive interactions between *Microdochium nivale* var. *majus*, *M. nivale* var. *nivale* and *Fusarium culmorum* in planta and in vitro, *Environmental Microbiology.*, **6(1),** 79–87 **(2004)**

6. Bacon C.W., Hinton D.M. and Hinton Jr A., Growth-inhibiting effects of concentrations of fusaric acid on the growth of *Bacillus mojavensis* and other biocontrol *Bacillus* species, *J. Appl. Microbiol*., **100,** 185–194 **(2006)**

7. Maren A., Klich. Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*, *Mycoscience*., **48,** 71-80 **(2007)**

8. Jeffrey D. Palumbo and Teresal L. O'Keeffe, Microbial interactions with mycotoxigenic fungi and mycotoxins, *Toxin Reviews*., **27,** 261-285 **(2008)**

9. Subramanian T., Colorimetric determination of patulin produced by *Penicillium patulum*, *J. Assoc. Off. Anal. Chem*., **61,** 581-583 **(1982)**

10. Subramanian T., Kuppuswamy M.N. and Shanmuga Sundaram, E.R.B. Colorimetric determination of terreic acid produced by *Aspergillus terreus*, *J. Assoc. Off. Anal. Chem.*, **61,** 581-583 **(1978)**

11. Rathinavelu A. and Shanmuga Sundaram, E.R.B. Simple colorimetric estimation of cyclopiazonic acid in contaminated food and feeds, *J. Assoc. Off. Anal. Chem*., **67,** 38-40 **(1984)**

12. Hou C.T., Ciegler A. and Hesseltine C.W., Tremorgenic toxins from penicillia colorimetric determination of tremortims A and B, *Anal Bio Chem*., **37,** 422-428 **(1970)**

13. Surekha M. and Reddy S.M., Effect of carbon and nitrogen sources on the production of penitrem B by Penicillium aurantiogriseum, *Foila Microbiol*., **37(1),** 47-49 **(1992)**

14. Sakai Y., Yoshida N., Yurimoto H., Takabe K. and Kato N., Sub cellular localization of fructosyl amino acid oxidases in peroxisomes of *Aspergillus terreus* and *Penicillium janthinellum. J. Biosci. Bioeng*., **87,** 108-111 **(1999)**

15. Yoshida N., Sakai Y., Serata M., Tani Y. and Kato N., Distribution and properties of fructosyl amino acid oxidase in fungi, *Appl. Environ. Microbiol*., **61**, 4487-4489 **(1995)**

16. Joanne M., Kingsbury Alan L. and Goldstein John H., Mc Cusker. Role of Nitrogen and Carbon Transport, Regulation and Metabolism Genes for *Saccharomyces cerevisiae* Survival In Vivo, *Eukaryot Cell*., 5(**5),** 816–824 **(2006)**