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Patulin Production by Fungi Isolated from Barley Locally Grown in Saudi Arabia

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Abstract. Patulin is a fungal toxin that can occur in contaminated feeds, particularly barley. The influence of seventeen fungal species isolated from barley produced in Saudi Arabia, to produce patulin was tested *in vitro* using three different media i.e., barley (BM), potato dextrose agar (PDA) and yeast-extract sucrose (YES) broth. Patulin was produced by 70.6% of the tested isolates. The best medium for production of patulin by the fungi is PDA followed by BM, but none of the tested fungi grown on YES has yielded patulin. In BM, the quantities of patulin produced varied from 948 µg/kg barley produced by *Eurotium herbariorum* to 2448 µg/kg barley produced by *Aspergillus parasiticus*. The biological studies undertaken using chick embryos and *Bacillus subtilis* have confirmed the toxicity of the barley medium extracts.*

Introduction

Patulin is a mycotoxin produced by some species of *Aspergillus* and *Penicillium* species [1, pp. 273-355]. Some of these fungi are contaminats of feed and potentially toxic to animals [1, pp. 273-355; 2; 3]. Such mycotoxicoses are among the major causes of high mortality in poultry in Saudi Arabia [4] and has been linked to poultry feeds that contain barley as a major constituent. Hence, the present study was undertaken to evaluate the potential of patulin production by some fungal species isolated from locally produced barley.

Materials and Methods

Mycological study

Seventeen species of fungi belonging to the general *Aspergillus, Eurotium* and *Penicillium* were isolated and identified by the authors from locally grown barley seeds (*Hordeum vulgare* L. 'Gustoe') according to Raper and funnell [5, pp. 1-686], Raper and

Thom [6, pp. 1-875], and Barnett and Hunter [7, pp. 1-225]. Isolates of *Aspergillus* spp and *Penicillium* spp were also sent to Ms. Kemin Europa N.V., Industriezone Wolfstee, 2410 Herentals, Belgium for identification. Six replicates of each pure isolate were transferred on PDA slants and incubated for 10 to 14 days at 25°C. Those fungi which were suspected of producing patulin were grown on autoclaved barley seeds medium (BM), potato dextrose agar (PDA Difco) and yeast extract sucrose broth (YES) media for the production of patulin.

Table 1. Production of patulin from isolated fungi grown on various media for 7 days at 25°C

Isolated fungi	Media		
	PDA	YES	BM
Aspergillus flavus	+	-	+ (1680)
A.fumigatus	+	-	+ (1680
A. glaucus	-	-	-
A. nidulans	-	-	-
A.niger	-	-	-
A. ochraceus	-	-	-
A. parasiticus	+	=	+ (2448)
Aspergillus sp.	+	-	+ (211)
A. terreus	+	-	-
Eurotium amestelodami	-	-	-
E. herbariorum	+	-	+ (948)
Eurstium sp.	+	-	+ (2116)
Penicillium auruntigriseum	-	-	-
P. chrysogenum	+	-	-
P. citrinum	+	-	+ (2091)
P. corylophilum	+	-	+ (1954)
Penicillium sp.	-	-	-

* PDA : Potato dextrose agar medium.
** YES : Yeast extract sucrose broth medium.
*** BM : Barley medium, the figures in parenthesis are μg patulin per kg barley.
Patulin detected (+) or not detected (-) in media.

Screening and confirmation

All media were screened for patulin production using the methods of Al-Julaifi and Al-Khaliel [8]. The BM was dried, ground and extracted. The purified, extract was analyzed using thin-layer chromatography (TLC) and its patulin content was assessed by the methods of Williams; Scott *et al.* and Waldi [9, pp. 426-438; 10; 11, p. 909].

The toxicity of the mold extracts was biologically assessed using the chickembryo test accepted by Verret *et al.*, [12]. Microbiological test using *Bacillus subtilis* as a test organism according to Burmeister and Hesseltine [13] and paper disc agar diffusion technique of Olivigni and Bullerman [14] were also used to show the effect of patulin.

Results and Discussion

None of the fungi species grown on YES has produced patulin, while 10 (58.8%) of those grown on PDA and 8 (47.1%) grown on BM have yielded the toxin. This clearly indicates that PDA is the best medium for the production of patulin followed by BM. The lowest quantity of patulin on BM was obtained from *Eurotium herbariorum* and the highest (2448 µg/kg) was recorded from *Aspergillus parasiticus* (Table 1). With the exception of *A. terreus* and *Penicillium chrysogenum* that did produce patulin only in PDA, whereas several species of fungi have produced patulin in both of PDA and BM (Table 1). This might indicate that PDA could provide the fungal species with all of the chemical components that enable it to produce toxin. On the other hand, the main constituents of barley (BM), maltose did not stimulate patulin formation in the same way

Table 2. The results of toxicity confirmation tests of patulin produced by isolated fungi grown on barley medium for 7 days at 25 °C

Isolated fungi	Microbiological test		Chick embryo test
	Inhibition off *Bacillus subtilis* inhibition zone (mm)		% Mortality of chick embryo
	25 µl	50 µl	
Aspergillus flavus	-	5.22	10
A. fumigatus	-	5.28	15
Aspergillus sp.	-	5.50	10
Eurotium harbariorum	5.15	5.24	10
Eurotium sp.	6.65	7.50	20
A. parasiticus	-	-	-

* - = Not determined.

as dextrose. Nevertheless, the presence of some glucose in the barley initiated the production of the quantities of patulin detected in BM. Similar observations were made by Bullerman [15]. Both glucose and sucrose have been regarded as suitable sources of carbon for patulin production by most isolates of memebrs of the genera *Aspergillus* and *Penicillium* [16].

The undertaken toxicity confirmation tests as indicated by the mortality rate of chick embryos and by the size of the inhibition zones of *Bacillus subtilis* have confirmed the toxicity of the detected patulin (Table 2). However, not all of the results can solely be explained in this way. This is because the extract from *Euortium* sp. that resulted in a high mortality rate and a large inhibition zone had far less patulin concentration than *A. parasiticus* which gave neither mortality nor an inhibition zone. This might be explained by the possibility that species of *Eurotium* might also be producing other mycotoxins or some other unknown toxic substances.

References

[1] Abraham, E.P. and Florey , H.W. "Antibiotics from Fungi. 2. Substances Other Than Penicillic." In: Florey, H.W. *et al. Antibiotics*. London: Oxford University Press, 1973.

[2] Dickens, F. and Jones, H.E.H. "Carcinogenic Activity of a Series of Reactive Lactones and Related Substances." *Brit. J. Cancer*. 15 (1961), 85-100.

[3] Dustin, P. Jr. "New Aspects of Pharmacology of Antimitotic Agents." *Pharmacol. Rev.* 15 (1963), 449-480.

[4] Eissa, Y. M., Al Ogaily, Z., and Al Jardan, S. "Causes of Mortality in Broiler Chicken in Saudi Arabia. Workshop on Poultry Production in Saudi Arabia. April 6-8, 1987. Regional Agriclture and Water Research Center, Ministry of Agriculture and Water. Kingdom of Saudi Arabia, 1987, 68-73.

[5] Raper, K.B. and Fennell, D.I. *The Genus Aspergillus*. Baltimore, MD: The Williams and Wilkins Company, 1965.

[6] Raper, K.B. and Thom, C.A. *Manual of the Penicillia*. New York: Hafner Publishing Company, 1968.

[7] Barnett, H.L. and Hunter, B.B. *Illustrated Genera of Imperfect Fungi*. Minneapolis, MN: Burgess Publishing Company, 1972.

[8] Al-Julaifi, M.Z. and Khaliel, A.S. "Incidence of Mold Growth and Aflatoxin Production in Locally Grown Barley Used as Ingredient for Poultry Feeds." *Arab Gulf J. Sci. Res.*, 10, No. 2 (1992), 87-97.

[9] Williams, E. "Natural Poisons." In: *Official Methods of Analysis*. Washington City: Association of Official Analytical Chemists, 1970.

[10] Scott, P.M., Lawrence, J.W., and Van Walbeek, W. Detection of Mycotoxins by Thin Layer Chromatography: Application to Screening of Fungal Extracts. *Appl. Microbiol.* 20, No. 5 (1970), 839-842.

[11] Wadi, D. "Spray Reagents for Thin-layer Chromatography." In: Stahl. E. (Ed), *Thin-layer Chromatography Laboratory Handbook*. Springer International Student Education. Heidelberg: Springer-Verlag Berlin, 1969.

[12] Verret, M.J., Marliac, J.P., and Laughlin, J. Use of the Chicken Embryo in the Assay of Aflatoxin Toxicity." *J. Assoc. Off. Anal. Chem.* 47 (1964), 1003-1006.

[13] Burmeister, H.R. and Hesseltine, C.W. "Survey of the Sensitivity of Microorganisms to Aflatoxin." *Appl. Microbiol.* 14, No. 3 (1966), 403-404.

[14] Olivigni, F.J. and Bullerman, L.B. "A Microbiological Assay for Penicillic Acid." *J. Food Protection.* 41, No. 6 (1978), 432-434.

[15] Bullerman, L.B. "A Screening Medium and Method to Detect Several Mycotoxins in Mold Cultures." *J. Milk and Food Technol.* 37, No. 1 (1974), 1-3.

[16] Wienser, B.P. "Bacterial Effects of *Aspergillus clavatus.*" *Nature* (London), 149 (1942), 356-357.

البَتيولين الناتج من الفطريات المعزولة من الشعير المحلي بالمملكة العربية السعودية

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(سُلِّم في ٤ رمضان ١٤١٤هـ؛ وقُبل للنشر في ١٤ شعبان ١٤١٥هـ)

ملخص البحث. يعتبر البتيولين من السُمَيَّات الفطريّة التي يمكنها أن تلوث الأغذية لا سيما الشعير. ولقد تمّت دراسة تأثير سبعة عشر فطرا معزولة من الشعير المنتج محليًا بالمملكة العربية السعودية في إنتاج البتيولين وذلك باستخدام ثلاثة بيئات هي بيئة الشعير، بيئة دكستروز البطاطس وبيئة مرق دكستروز مستخلص البطاطس. لوحظ أن ٦, ٧٠٪ من العزلات قيد الدراسة أنتجت البتيولين وأن أفضل بيئة لإنتاجه كانت بيئة دكستروز البطاطس تليها بيئة الشعير بينما لم تنتج أي من العزلات الفطرية التي تمّت تنميتها على بيئة مرق دكستروز مستخلص البطاطس مادة البتيولين. وتراوحت كميَّات البتيولين في بيئة الشعير من ٩٤٨ ميكروجرام/كيلوجرام شعير أنتجتها فطرة *Eurotium herbariorum* إلى ٢٤٤٨ ميكروجرام/كيلوجرام شعير أنتجتها فطرة *Aspergillus parasiticus* وأثبتت الدراسات البيولوجية التي أجريت باستخدام اختبار الأجنة وبكتيريا *Bacillus subititis* سُمِّيَّة مستخلصات بيئة الشعير.