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Zearalenone production in sabouraud dextrose broth and rice culture by various species of *Fusarium* fungi

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ABSTRACT

Zearalenone (ZEN) is an estrogenic mycotoxin produced by *Fusarium* fungi. Pure preparations of the toxin should be available for *in vitro* and *in vivo* studies. The growth medium affects the activity of the fungus and the production of the toxin. ZEN producing capacity of three *Fusarium* species (*F. oxysporum, F. graminearum,* and *F. solani*) and the effects of Sabouraud Dextrose Broth (SDB) and rice culture on the ZEN production were investigated. The *Fusarium* fungi were incubated in SDB and rice culture at a constant temperature of 25°C for 30- and 48-day periods respectively. HPLC with fluorescence detection was used to measure the concentrations of ZEN. The amounts of ZEN produced by various *Fusarium* species on SDB were not statistically different. However, the quantity of ZEN produced on rice culture was significantly higher for *F. graminearum* compared to the amounts produced by *F. oxysporum* and *F. solani* ($P \leq 0.001$). *F. graminearum* produced significantly higher amounts of the toxin ($P \leq 0.001$) on rice culture than on SDB. In fact, the highest amount of ZEN production was seen by *F. graminearum* on rice culture. The amount of ZEN produced by *F. oxysporum* and *F. solani* were not different between SDB and rice cultures. The *in vitro* ZEN production by *Fusarium* fungi may be different on SDB and rice culture. *F. graminearum* can produce higher amounts of ZEN on rice medium compared to SDB. The activities of *F. oxysporum* and *F. solani* appear to be almost similar on these media.

Keywords: *Fusarium* species, Rice, media culture, SDB, Zearalenone

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INTRODUCTION

Zearalenone (ZEN) is an estrogenic mycotoxin produced by *Fusarium* fungi (Placinta *et al*., 1999 and Mirocha *et al*., 2013) that contaminate various cereal crops such as corn, rice, wheat, barley, oats, sorghum, and millet. In addition to ZEN, trichothecene group and fumonisins are the other most occurring Fusarium mycotoxins. There are potential health risks for humans and animals with widespread presence of fungi and mycotoxins in infected plants and foods (Ismaiel and Papenbrock, 2015; Adeyeye, 2016). Pure preparations of the toxins should be available for *in vitro* and *in vivo* studies. Compared to other economically important mycotoxins, less study are available on ZEN production by fungi. The growth medium affects the activity of the fungus and the production of the toxin. A wide range of cultures have been used for growing fungi *in vitro*. Cultures generally contain carbohydrate and nitrogen sources and vitamins required for growth of fungi. Many fungi may deteriorate in the culture if the food supply is too rich or certain accessory factors are absent (Basu *et al*., 2015). Sabouraud dextrose broth (SDB) is an enrichment broth medium containing peptones and dextrose to provide amino acids and energy for growth of fungi. Cereal grains including rice, wheat and corn are good nutritional sources for growth of fungi (Mannaa and Kim, 2017). Production of

ZEN by *Fusarium* spp. on rice cultures has been shown by (Richardson *et al*., 1985) *in vitro*. Mycotoxigenic potentials of some *Fusarium* species on rice, maize and potato dextrose agar (PDA) media were compared by Shi *et al*. (2017).The aim of the study was to evaluate the ZEN producing capacity of three *Fusarium* species (*F. oxysporum, F. graminearum* and *F. solani*) and to assess the effect of SDB and rice culture on the ZEN production.

METHODOLOGY

Fungal source

F. oxysporum PTCC 5115 and *F. solani* PTCC 5284 were obtained from the stock collection maintained at Iranian Research Organization for Science and Technology (IROST). *F. graminearum* was obtained from the stock of the Department of Agriculture, Golestan University, Gorgan, Iran. The stock cultures were kept on SDB (Merck®, Germany) at 25°C at the Department of Animal Health Management, School of Veterinary Medicine, and Shiraz, Iran

Preparation of culture media

SDB and rice media were prepared for inoculation and incubation of the *Fusarium* fungi. The SDB medium was prepared according to the manufacturer's instruction by dissolving 1.08 grams of SDP powder in 36 ml distilled water in nine separate tubes. The rice flour used for preparation of the rice medium was purchased from local suppliers (Shiraz, Iran) and was tested by high performance liquid chromatography (HPLC) to be ZEN-free. Rice flour (270 grams) was mixed with 135 ml distilled water (9 separate flasks) and was vigorously shaken to prevent clumping. The cultures were autoclaved at 121ºC for 15 minutes. Prior to inoculation, the stock fungi were cultured separately on PDA for 7 days at 25°C to become active. Each fungus was inoculated on each medium in three replicates according the method described previously (Shi *et al*., 2017; Medina and Magan, 2011). The SDB and rice cultures were incubated at 25°C, respectively for 30- and 48-day periods. Then, the cultures were autoclaved to terminate the fungal growth and ZEN biosynthesis. The SDB cultures were centrifuged (750 g, 10 minutes) and the supernatant was separated. Rice cultures were dried at 80ºC for 72 h. Then, one gram of the ground culture was added to10 ml of distilled water and was shaken at 200 rpm for 180 minutes (Lab tech Shaking Incubator/LSI-3016A, South Korea) and the supernatant was separated. The supernatants were kept at -80ºC until analysis (Borzekowski *et al*., 2018).

Measuring of ZEN

The quantities of ZEN were measured in the supernatants of the cultures (three replicates for each fungus) by HPLC according to the instruction number 9239 of Iranian National Standard Organization (Agilent®, USA). The HPLC system consisted of a pump and a fluorescence detector. ZEN was separated in HPLC column (C_{18} octadecylsilane; 5 µm × 10 cm × 4.6 mm) with a mobile phase of water: methanol: acetonitrile (34:56:10, v/v/v). The fluorescent detection was performed at both 275 nm and 450 nm wavelengths to show the excitation and emission respectively. ZEN retention times with 1 mL/min flow rate were 7–8 minutes. The total run time was 10 minutes. The total recovery of ZEN was 85%. The ZEN amounts were reported as ppb.

Statistical Analysis

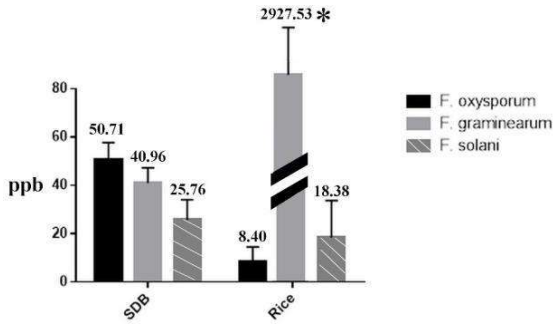
The SPSS statistical software (version 21.0) was used to analyze the data. The differences between the amounts of ZEN produced by various *Fusarium* species in the SDB and rice media were compared using two-way analysis of variance (ANOVA) and Bonferroni post-hoc test at $P \leq 0.05$. Data were presented as mean±standard deviations (SD) of the means.

RESULTS

The amounts of ZEN produced by various *Fusarium* species on SDB were not statistically different ($P>0.05$; Fig. 1; 50.71±6.97 ppb, 40.96±6.35 ppb and 25.76±8.36 ppb for *F. oxysporum*, *F. graminearum* and *F. solani,* respectively). However, the quantity of ZEN produced on rice culture was significantly higher for *F. graminearum* (2927.53±1789.82 ppb) compared to the amounts produced by *F.*

oxysporum (8.40±6.20 ppb) and *F. solani* ($P \leq 0.001$). *F. graminearum* produced significantly higher amounts of the toxin ($P \leq 0.001$) on rice culture than on SDB (Figure 1). In fact, the highest amount of ZEN production was seen by *F. graminearum* on rice culture. The amount of ZEN produced by *F. oxysporum* and *F. solani* were not different between SDB and rice cultures.

Fig 1. Comparison of ZEN (ppb) production by *Fusarium* species on Sabouraud dextrose broth (SDB) and rice culture.***F. graminearum* produced significantly higher amounts of the toxin on rice culture than on SDB and other fungi on rice culture ($P \leq 0.001$).



According to the clinical laboratory standards, good growth of the microorganisms must be provided by an optimal nutrient medium (Pfaller *et al*., 2005). According to the results of the present study and based on the amounts of ZEN detected in the cultures, rice culture may be a better medium for growth and activity of *F. graminearum* compared to SDB. Thus, rice culture may be an inexpensive alternative for other media to grow *F. graminearum* and to produce ZEN. The activity of *F. oxysporum* and *F. solani* may not be different on the two cultures. Ravimannan *et al.* (2014) suggested different formulation of some seeds as alternative cultures media to grow fungi. The toxin producing capacity of some selected *Fusarium* species at different culture conditions were studied by Kokkonen *et al*. (2010) with the highest ZEN production by *F. graminearum* in all tested media. In another study, significantly more mycotoxins were produced by some *Fusarium* species in rice and maize media compared to PDA medium (Shi *et al*., 2017). In accordance with the findings of the present study, ZEN was produced by *F. graminearum* with highest levels in rice media (Shi *et al.*, 2017). The temperature of 25°C was adopted from some related experiments investigating multiple *Fusarium* species *in vitro* (Kokkonen *et al*., 2010; Richard *et al*., 2007). Brennan *et al*. (2003) found the optimum temperature for growth of *F. graminearum* was 25 °C *in vitro*. Also, the highest ZEN production by *F. graminearum* and *F. oxysporum* was achieved in incubation at 25°C (Milano and López, 1991). The findings of this study revealed that *in vitro* ZEN production by *Fusarium* fungi may be different on SDB and rice culture. *F. graminearum* can produce higher amounts of ZEN on rice medium compared to SDB. The activities of *F. oxysporum* and *F. solani* appear to be almost similar on these media. Rice culture may be a good and inexpensive medium to promote ZEN production by *F. graminearum*.

AUTHORS' CONTRIBUTION

AO and MS designed the study work, performed the experimental work, and provided samples. AO, AR and MM prepared manuscript and reviewed the work. MM revised the final edition of the manuscript.

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ETHICAL APPROVAL

Ethical approval for the study was obtained from the Committee for the study of scientific research at the School of Veterinary Medicine, Shiraz University and Shiraz, Iran (9430049).

CONFLICT OF INTEREST **148**

The authors declare that they have no conflict of interest.

REFERENCES

Adeyeye, S. A. 2016. Fungal mycotoxins in foods: A review. *Cogent Food and Agriculture*. **2**(1): 1213127

Basu, S., Bose, C., Ojha, N., Das, N., Das, J. and Pal, M. 2015. Evolution of bacterial and fungal growth media. *Bioinformation*, **11**(4): 182.

Brennan, J., Fagan, B., Van Maanen, A., Cooke, B. and Doohan, F. 2003. Studies on in vitro growth and pathogenicity of European Fusarium fungi. *European Journal of Plant Pathology*. **109**(6): 577-587.

Ismaiel, A. and Papenbrock, J. 2015. Mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture*. **5**(3): 492-537.

Kokkonen, M., Jestoi, M. and Laitila, A. 2012. Mycotoxin production of *Fusarium langsethiae* and *Fusarium sporotrichioides* on cereal-based substrates. *Mycotoxin Research*, **28** (1): 25-35.

Kokkonen, M., Ojala, L., Parikka, P. and Jestoi, M. 2010. Mycotoxin production of selected Fusarium species at different culture conditions. *International Journal of Food Microbiology*. **143** (1-2): 17-25.

Mannaa, M. and Kim, K. D. 2017. Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*. **45**(4): 240-54.

Medina, A. and Magan, N. 2011.Temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae* strains from north European countries. *International Journal of Food Microbiology*. **28**(3): 392-398.

Milano, G. and López, T. A. 1991. Influence of temperature on zearalenone production by regional strains of *Fusarium raminearum* and *Fusarium oxysporum* in culture. *International Journal of Food icrobiology*. **13**(4): 329-333.

Mirocha, C., Christensen, C. and Nelson, G. 2013. F-2 (zearalenone) estrogenic mycotoxin from Fusarium. *Microbial Toxins*, **7**: 107-138.

Pfaller, M. A., Diekema, D. J. and Rinaldi, M. *G.* 2005. Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biological fluids. In: *Antibiotics in laboratory edicine*, 5th edition, *Lippincott Williams & Wilkins, Philadelphia,* 226-265 **PP.**

Placinta, C., D'mello, J. and Macdonald, A. 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium mycotoxins*. *Animal Feed Science and Technology*. **78** (1-2): 21-37.

Ravimannan, N., Arulanantham, R., Pathmanathan, S. and Niranjan, K. 2014. Alternative culture media for fungal growth using different formulation of protein sources. *Annals of Biological Research*. **5** (1): 36-39.

Richard, E., Heutte, N., Sage, L., Pottier, D., Bouchart, V. and Lebailly, P. 2007. oxigenic fungi and mycotoxins in mature corn silage. *Food and Chemical Toxicology*. **45** (12): 2420-2425.

Richardson, K. E., Hagler, Jr. W. M. and Mirocha, C. J. 1985. Production of earalenone, alpha-and beta-zearalenol and alpha-and beta-zearalanol by Fusarium spp. in rice culture. *Journal of Agricultural and Food Chemistry*. **33**(5): 862-866.

Shi, W., Tan, Y., Wang, S., Gardiner, D., De Saeger, S. and Liao, Y. 2017. Mycotoxigenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling. *Toxins*. **9** (1): 6.

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