Malaysian Journal of Microbiology, Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Molecular characterization of mycotoxin-producing *Aspergillus parasiticus* and sensitivity pattern to different disinfectants

Zunira Mughis[1], Muhammad Asad Ali[1]*, Aftab Ahmad Anjum[1], Muhammad Ovais Omer[2], Mateen Abbas[1] and Maria Najeeb[1]

[1]Institute of Microbiology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan.
[2]Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, Pakistan.
Email: asad.ali@uvas.edu.pk

Received 30 March 2022; Received in revised form 23 August 2022; Accepted 31 August 2022

ABSTRACT

Aims: The study was aimed to isolate and characterize the mycotoxin-producing filamentous *Aspergillus parasiticus* from the feed samples. The sensitivity pattern of the isolates was assessed against different disinfectants.
Methodology and results: Fifty different feed samples were screened for *A. parasiticus* isolation. Isolates were subjected to macroscopic and microscopic characterization. Polymerase chain reaction was performed to confirm the isolates at the genomic level. Mycotoxin producing potential of the isolates was assessed by thin-layer chromatography (TLC). To quantify the toxins, high performance liquid (HPLC) was employed. The antifungal potential of disinfectants was determined by the well diffusion method followed by minimum inhibitory concentration (MIC) calculation. Out of twenty isolates of *A. parasiticus,* 11(55%) isolates were observed positive for toxin production. Three toxigenic isolates (AspP2, AspP4 and AspP8) were selected to evaluate their susceptibility against disinfectants by well diffusion method. AspP2 produced maximum (5.90 ng/mL) toxin, followed by AspP4 (3.11 ng/mL) and AspP8 (18.47 ng/mL). Terralin showed maximum fungicidal activity with 29.66 ± 8.08 mm zone of inhibition at 0.42 µg/mL MIC. Hypochlorite and Instru Star showed 99% disinfection with 30, 60 and 90 min contact time (6 mean log reduction) for all *A. parasiticus* isolates. Alpha Guard inhibited growth after 15 min contact time for all the isolates.
Conclusion, significance and impact of study: This study provides data indicating the contamination of feed samples with mycotoxin-producing *A. parasiticus* isolates and their sensitivity against commercially available disinfectants. Use of these disinfectants in appropriate concentrations and time could help prevent the contamination of food, feed and healthcare settings with the fungal species.

Keywords: Aspergillus parasiticus, disinfectants, minimum inhibitory concentration, mycotoxin, zone of inhibition

INTRODUCTION

Mycotoxins are secondary metabolites produced by certain filamentous fungal species responsible for serious toxic diseases in humans and animals (Tola and Kebede, 2016). Accumulation of these toxins in crops causes a significant decrease in yield; hence, economic loss (Udomkun *et al.*, 2017). *Aspergillus* spp. including *A. flavus* and *A. parasiticus* are the primary contaminants of crops and animal feeds (Yogendrarajah *et al.*, 2016; Sana *et al.*, 2019). *Aspergillus flavus* and *A. parsasiticus* have various applications in the biotechnology and food industries (Frisvad *et al.*, 2019). On the other hand, they can contaminate food and feeds by producing toxins, including aflatoxins and ochratoxin A (Varga *et al.*, 2004). These species produce aflatoxins as secondary metabolites that often contaminate commodities, including peanuts, corn and cottonseed (Begum and Samajpati, 2000). Aflatoxins are the most potent mycotoxins that cause genomic mutations and cancer (Ahmed *et al.*, 2017). In humans and animals, these toxins severely damage the liver (Wen *et al.*, 2016). Consumption of toxin-contaminated food results in poisoning and even death in severe cases (Wen *et al.*, 2016).

Fungal pathogens are widely distributed in our environment; *Aspergillus* spp. are considered the main source of nosocomial infections (Seeliger and Schröter, 1984). In healthcare settings and pharmaceutical and food industries, these fungal pathogens often contaminate medicines and food products (Adeyeye, 2020). However, eradication of this contamination is a challenging task. Various disinfectants are widely used in hospitals, pharmaceutical and food industries, and research laboratories (Gupta *et al.*, 2002). They have broad-

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496

spectrum antimicrobial activity and help to prevent hospital-acquired infections (Abdolrasouli *et al.*, 2017). Many of these formulations are considered to have a broadly based antifungal activity; however, the evidence for their efficacy against medically important fungi is unclear in the literature.

In hospital settings, quaternary ammonium compounds and other chemical agents are often used; however, appropriate exposure time is the critical factor for proper disinfection. Several studies have reported the bactericidal activity of commercially available disinfectants. Nevertheless, their fungicidal activity has not been well highlighted (Okungbowa and Usifo, 2010). There is a considerable difference in antifungal potential among commercially available quaternary ammonium compounds against different fungal species (Ohta *et al.*, 1996). *Aspergillus parasiticus* produces aflatoxins and mostly contaminates crops and animal feed. However, literature about the efficacy of disinfectants against this fungus is not available. The purpose of the present study was to isolate the indigenous *A. parasiticus* and evaluate their sensitivity pattern against commonly used disinfectants.

MATERIALS AND METHODS

Feed samples

Fifty feed samples were collected from different places in Punjab, Pakistan. Sampling was carried out following an organized sampling procedure under the project entitled "Purification and standardization of mycotoxins extracted from indigenous fungi under optimized experimental conditions'' at the Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore.

Isolation of *A. parasiticus* from feed

For isolation, the feed samples were grounded and diluted in 0.2% agar (10-fold dilution). Approximately 0.1 mL of each dilution was spread on Sabouraud's dextrose agar plates containing chloramphenicol and incubated at 25 °C for 3-5 days. For identification, macroscopic (color of colony, texture, diameter and presence of folds, diffused pigment, and ridges) and microscopic (type of spores, type of hyphae, presence of vesicle, metulae, phialides and unique structures such as foot cell) characters were evaluated by following methods described by Watanabe (2010).

Inoculum preparation

Aspergillus parasiticus spore suspension was prepared by inoculating a loop full of spores in 10 mL of sterile normal saline. Spores were mixed in standard saline water through gentle mixing and a few drops of tween 80 were added. Spore suspension (10 µL) was added into Neubauer chamber. Spores were counted in 5 boxes of the chamber under a microscope at 40×. The number of spores/mL was calculated by using the following formula: Spores/mL = (A+B+C+D+E) × 2000. Spores were adjusted to 10^6/mL for further experiment.

Mycotoxin-producing potential of *A. parasiticus*

Mycotoxin-producing species of *A. parasiticus* were screened using thin-layer chromatography (TLC) following a previously conducted study (Singh and Cotty, 2019). Standard spore suspension (10^6) was inoculated into Sabouraud's dextrose broth (SDB) and placed in the dark at 25 °C for 15 days. Broth culture was autoclaved and homogenized. Homogenized fungal culture (12.5 g) was taken in a beaker and chloroform (45 mL), methanol (5 mL), water (5 mL) and sodium chloride (2.5 g) were added. The mixture was placed at 37 °C for 30 min, followed by filtration through Whatman filter paper. The filtrate was concentrated and 1 mL chloroform was added, followed by spotting on the TLC plate. The plate was placed in a tank filled with 95 mL chloroform and 5 mL acetone that covered a 3/4 distance of the plate. Results were observed under ultraviolet light at 365 nm.

High-performance liquid chromatography (HPLC)

Mycotoxins were quantified by high-performance liquid chromatography (HPLC) (Beyene *et al.*, 2019). A sample of poultry feed (25 g) was added to a mixture containing 80 mL acetonitrile, 20 mL water and 5g NaCl and homogenized by continuous shaking for 1 h. After homogenization, the solution was filtered and 9 mL filtrate was transferred to Mycosep Glass Tube, added 70 µL of acetic acid and vortexed for 30 sec. The mixture was then passed through the Mycosep column. A volume of 2 mL was taken and water was evaporated to obtain a dry filtrate. Hexane (200 µL) was added to the dried filtrate to re-dissolve mycotoxins and vortexed for 30 sec. Then, 50 µL tri-Fluro acetic acid was added, vortexed for 30 sec and placed in the dark for 5 min. One milliliter solution of water and acetonitrile (8:2) was filtered through a syringe filter and run through HPLC to purify the mycotoxin further. The concentration of mycotoxins was calculated using following formula: Concentration = Area of sample/Area of standard*Concentration of standard

Molecular identification

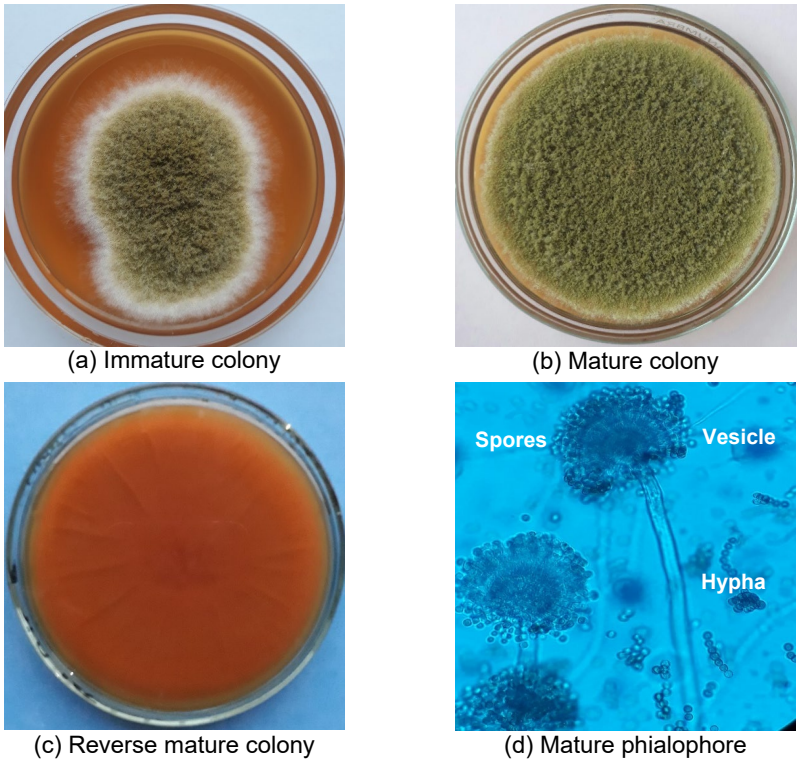
Toxigenic isolates of *A. parasiticus* were confirmed by polymerase chain reaction (Sardiñas *et al.*, 2010). DNA was extracted by using the kit method (Gene all). PCR was performed using species-specific forward (5'-GTCATGGCCGCCGGGGGCGTC-3') and reverse primers (5'-CCTGGAAAAAATGGTTGTTTTGCG-3'). Amplicons were analyzed by gel electrophoresis.

Well diffusion method

The antifungal potential of disinfectants was determined by the well diffusion method (Bocate *et al.*, 2019). Sabouraud's dextrose agar seeded with spore suspension (10^6 spores/mL) was poured into sterilized

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496



(a) Immature colony (b) Mature colony

(c) Reverse mature colony (d) Mature phialophore

Figure 1: Macroscopic and microscopic characteristics of *A. parasiticus* isolates. (a) Immature parsley green, granular colonies with white edges; (b) Mature colonies with pale-yellow edges; (c) the reverse side of mature colonies and (d) Mature phialophores under 40×.

Petri dishes and allowed to solidify. Wells were punctured in the agar and sealed with molten agar. Recommended concentrations of disinfectants including Terralin (0.05%), Alpha Guard (0.15%), Instru Star (0.1%), Hypochlorite (0.05%), Endo Terra (0.3%), Distel (0.1%), Descocid-N (0.1%) and Isopropanol (7%) were prepared and 10 uL was added into wells separately. Plates were incubated in an upright position at 25 ± 3 °C for 3-5 days and the diameter of the zone of inhibition (ZOI) was measured in millimeters (mm).

MIC

The lowest concentration of disinfectant, which can inhibit the visible growth of the fungus, was calculated by the broth microdilution method (Tarhriz *et al.*, 2020). Two-fold serial dilutions of disinfectants were prepared in microtitration plates. Standard spore suspension (10^6 spores/mL) was added up to the 11^{th} well. Optical density (OD) was measured and recorded by an ELISA reader at 595 nm wavelength and MIC was calculated.

Log reduction

The antifungal activity of disinfectants against toxigenic species of *A. parasiticus* was calculated by the log reduction method (Sickbert-Bennett *et al.*, 2005). Spores and disinfectants were mixed together in a 1:1 ratio and collected at an interval of 0, 15, 30, 60 and 90 min. Ten-fold serial dilutions were prepared in sterile normal saline and 100 µL was spread on Sabouraud's dextrose agar plate, followed by incubation at 25 °C for 3 days. Colony forming units (CFU/mL) were counted using the formula: Percentage Log reduction = Log_{10} CFU at T_0 - Log_{10} CFU at T_f.

Statistical analysis

The data were analyzed by analysis of variance (one-way ANOVA) with a level of significance of 0.05, followed by Duncan's multiple range tests (DMRT) using the Statistical Package for Social Sciences (SPSS) version 20.

RESULTS

Identification of isolates

Aspergillus parasiticus isolates were identified by observing macroscopic and microscopic characteristics. The colony characters were observed daily on Sabouraud's dextrose agar. Macroscopically, the colonies were parsley green, granular and had pale-yellow edges. Microscopically, septate and hyaline hyphae, conidiophores of varying lengths and spherical vesicles were observed (Figure 1).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496

Table 1: Screening of toxigenic isolates of *A. parasiticus* by thin-layer chromatography.

Isolate	Thin-layer chromatography (TLC)				
	Aflatoxin				Ochratoxin
	B1	B2	G1	G2	
AspP1	-ve	+ve	-ve	-ve	-ve
AspP2	-ve	+ve	-ve	-ve	-ve
AspP3	-ve	+ve	-ve	-ve	-ve
AspP4	-ve	+ve	-ve	-ve	-ve
AspP5	-ve	+ve	-ve	-ve	-ve
AspP6	-ve	+ve	-ve	-ve	-ve
AspP7	-ve	+ve	-ve	-ve	-ve
AspP8	-ve	+ve	-ve	-ve	-ve
AspP9	-ve	+ve	-ve	-ve	-ve
AspP10	-ve	-ve	-ve	-ve	-ve
AspP11	-ve	+ve	-ve	-ve	-ve

+ve denotes production of toxin; -ve denotes no production of toxin.

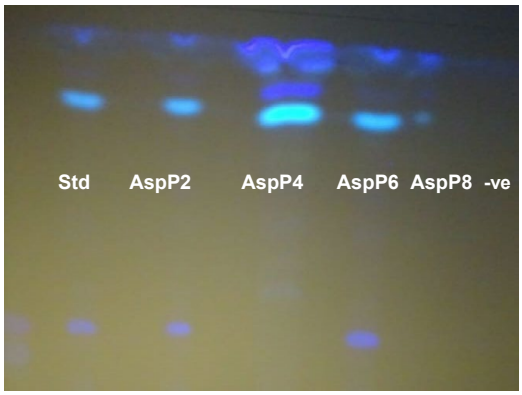


Figure 2: Thin layer chromatography plate of *A. parasiticus* showing mycotoxin production. Std: Standard of aflatoxin; AspP: *A. parasiticus*; -ve: Negative control of aflatoxin.

Mycotoxin-producing potential of *A. parasiticus*

Isolates of *A. parasiticus* were screened for mycotoxin production potential by thin-layer chromatography (TLC). The TLC plate was observed under ultraviolet light at 365 nm and the mycotoxin-producing isolates of *A. parasiticus* appeared fluorescent. Out of 20 tested isolates, 11(55%) were positive for toxin production (Figure 2, Table 1).

High-performance liquid chromatography

The mycotoxins were quantified by HPLC and the final volume was calculated using the above-mentioned formula. The AspP2 produced 5.90 ng/mL of toxin, AspP4 3.11 ng/mL and AspP8 18.47 ng/mL (Figure 3).

Molecular identification

The *A. parasiticus* isolates were confirmed by polymerase chain reaction using species-specific primers. A band of 430 bp was observed for all isolates that confirmed the selected isolates (Figure 4).

Well diffusion method

Disinfectants Alpha Guard, Instru Star, Isopropanol, Terralin, Distel, Endo Terra, Descocid-N and Hypochlorite were tested for their antifungal activity by well diffusion method against toxigenic isolates of *A. parasiticus*. Zone of inhibitions were measured and recorded (Table 2; Figure 5a, 5b). The highest zone of inhibition (ZOI) was observed for Terralin (29.66 ± 8.08 mm), followed by Alpha Guard (19.00 ± 8.88 mm), Endo Terra (17.00 ± 7.00 mm), Instru Star (16.66 ± 4.93 mm) and Hypochlorite (13.66 ± 7.23 mm) against isolates of *A. parasiticus*. Statistically, the zone of inhibitions differed significantly.

MIC

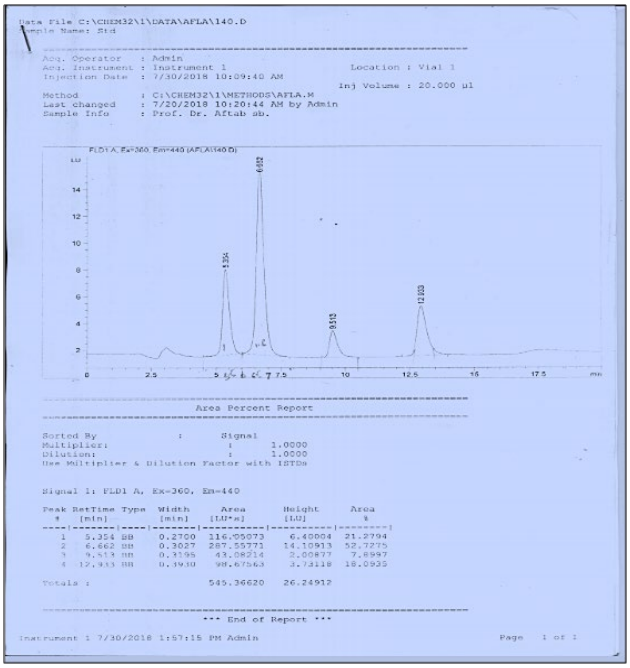
By the broth microdilution method, the lowest disinfectant concentration that inhibited the fungus's visible growth was observed and MIC was calculated. MIC was performed for those disinfectants who showed a zone of inhibitions. Highest mean MIC was of Hypochlorite (1.56 µg/mL) followed by Terralin (0.42 µg/mL), Instru Star (0.4 µg/mL), Alpha Guard (0.32 µg/mL) and Endo Terra (0.3 µg/mL). Statistically, Terralin, Alpha Guard, Instru Star and Endo Terra differ non-significantly, while hypochlorite differs significantly from Terralin, Alpha Guard, Instru Star and Endo Terra (Table 3).

Log reduction

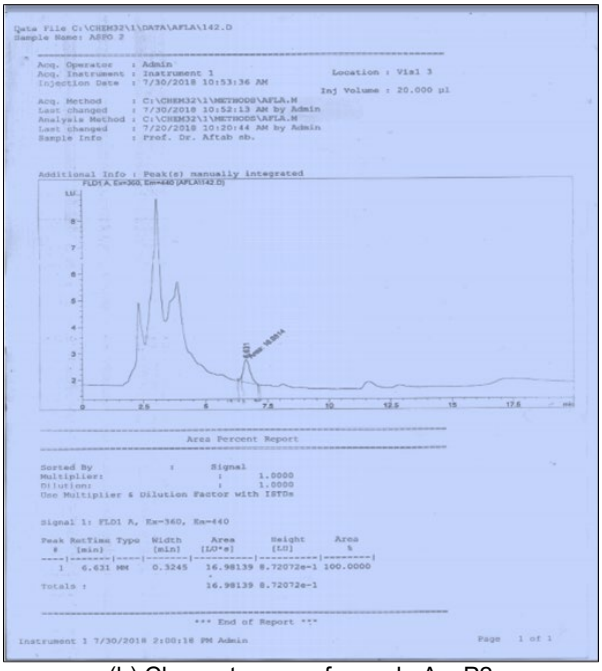
Antifungal activity of disinfectants, including Alpha Guard, Instru Star, Hypochlorite, Endo Terra and Terralin was calculated by log reduction method against toxigenic species of *A. parasiticus* (Table 4). At different incubation times, colonies were counted and colony-forming units CFU/mL were calculated. Hypochlorite and Instru Star showed 99% disinfection with 30, 60 and 90 min contact time (6 mean log reduction) for all *A. parasiticus* isolates. Terralin also showed a similar pattern for AspP2 and AspP8; however, for AspP4 fungicidal activity was observed after 15 min contact. Alpha guard inhibited growth after 15 min contact time for all the isolates.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

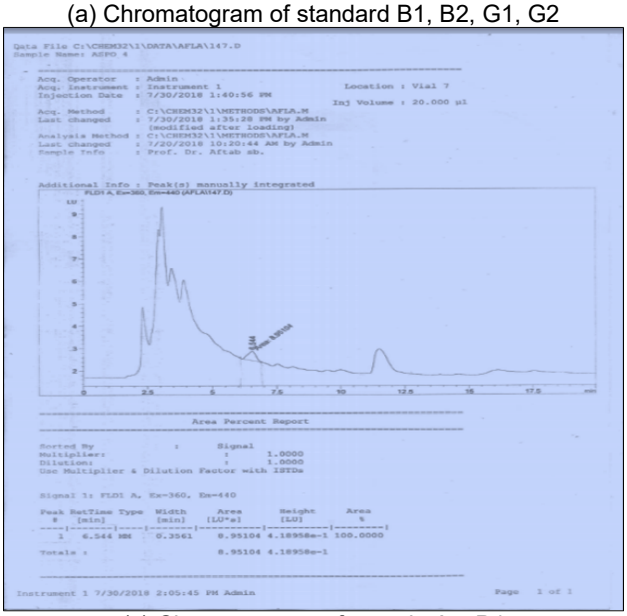
Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496



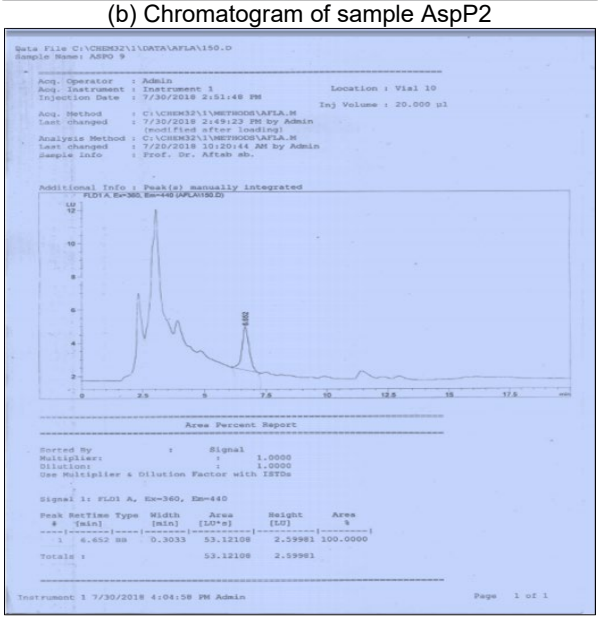
(a) Chromatogram of standard B1, B2, G1, G2



(b) Chromatogram of sample AspP2



(c) Chromatogram of sample AspP4



(d) Chromatogram of sample AspP8

Figure 3: Chromatogram of standard and sample by high-performance liquid chromatography. AspP: *A. parasiticus*.

DISCUSSION

In this study, *A. parasiticus* was isolated from feed samples and identified by microbiology and molecular biology techniques. Mycotoxin-producing potential of the isolates was evaluated and their sensitivity pattern against different disinfectants was assessed. The growth was obtained after 3-5 days of incubation at 25 °C on Sabouraud's dextrose agar plates. The mature colonies were parsley green in color with yellow edges. Similar results were in a previous study conducted by Nikolić *et al.* (2021). These results confirm the contamination of animal feed with *A. parasiticus*. In some other studies, growth was observed at 42 °C; this shows that this fungal specie can thrive at a broad temperature range (Ehrlich *et al.*, 2007).

Pitt and Hocking (2009) studied microscopic characteristics of *Aspergillus* species extensively. The

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496

Table 2: Mean zone of inhibition of disinfectants against *A. parasiticus*.

Sr.no	Disinfectant	Composition	Mean ± standard deviation
1	Terralin	Alkyl dimethyl benzyl ammonium chloride, phenoxyethanol, amines, alkyltrimethylene	29.66 ± 8.08[c]
2	Alpha Guard	Polyhexamethylenebiguanide hydrochloride, didecyldimethyl ammonium chloride	19.00 ± 8.88[b]
3	Instru Star	Cocospropylenediammonium alkyl amines	16.66 ± 4.93[b]
4	Isopropanol	Alcohol	0.00 ± 0.00[a]
5	Hypochlorite	Hypohlorous acid, free chlorine	13.66 ± 7.23[b]
6	Distel	Polymeric biguanidehydrochloride, alkyl dimethyl benzyl ammonium chloride, dodecyl dimethyl ammonium chloride	0.00 ± 0.00[a]
7	Endo Terra	Alkyl dimethyl ethylammoniumethosulphate, polyhexamethylenbiguanide, cocospropylene diamiguanidium acetate	17.00 ± 7.00[b]
8	Descocid-N	Benzylkonium chloride, didecyldimethyl ammonium chloride, formic acid	0.00 ± 0.00[a]

Values having different superscripts are significantly different. Values with the same superscripts differ non-significantly.

Table 3: Mean minimum inhibitory concentration of disinfectants against *A. parasiticus* isolates.

Fungal isolate	AspP2	AspP4	AspP8
MIC disinfectant	µg/mL	µg/mL	µg/mL
Hypochlorite	1.5625	1.5625	1.5625
Terralin	0.7813	0.0975	0.39
Instru Star	0.0497	0.39	0.7813
Alpha Guard	0.195	0.39	0.39

Table 4: Log reduction of *A. parasiticus* isolates.

Disinfectant	Isolate	Contact time (Mean log reduction)				
		0	15	30	60	90
Hypochlorite	AspP2	5.47 ± 0.76[a]	5.62 ± 0.57[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP4	5.46 ± 0.78[a]	5.85 ± 0.32[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP8	5.52 ± 0.69[a]	5.84 ± 0.34[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
Terralin	AspP2	5.57 ± 0.64[a]	5.70 ± 0.45[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP4	5.63 ± 0.54[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP8	5.51 ± 0.71[a]	5.84 ± 0.36[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
Instru Star	AspP2	5.71 ± 0.64[a]	5.47 ± 0.63[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP4	5.41 ± 0.85[a]	5.77 ± 0.51[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP8	5.52 ± 0.69[a]	5.84 ± 0.36[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
Alpha Guard	AspP2	5.72 ± 0.64[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP4	5.75 ± 0.55[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]
	AspP8	5.71 ± 0.64[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]	6 ± 0.000[a]

Values having different superscripts are significantly different. Values with the same superscripts differ non-significantly.

authors noted that the color and shape of phialides and the size and shape of spores are not reliable for the identification of *Aspergillus* species. Nevertheless, conidium wall ornamentation could be a reliable factor for distinguishing morphologically similar species. The conidia of *A. parasiticus* are spherical and have heavy spiny or bristly thick walls.

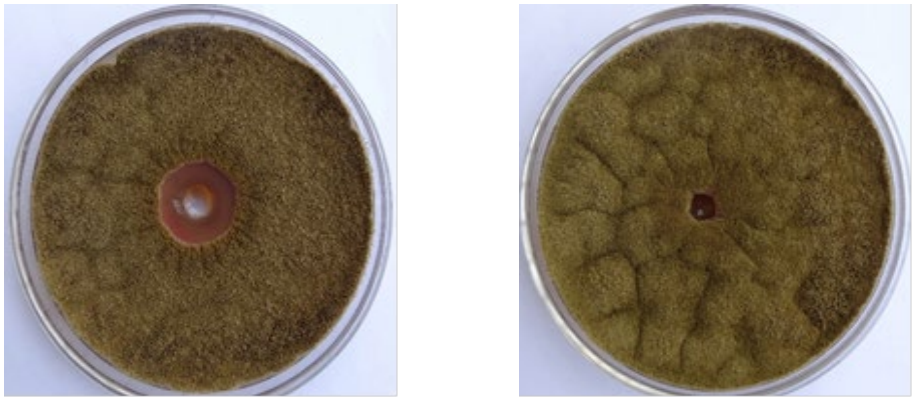
In the present study, *A. parasiticus* isolates were identified microscopically by cellophane tape (Harris, 2000) and slide culture method (Wijedasa and Liyanapathirana, 2012). In this study, rough, thin-walled conidia with long stripes were observed in all the isolates. The present study's microscopic results were comparable with Klich and Pitt (1988).

Macroscopic and microscopic methods for identification are authentic but time taking, so molecular techniques such as PCR are used for species confirmation. Khoury *et al.* (2011) employed the PCR-RFLP method to separate the isolates from a pure culture on the basis of molecular characteristics. Two species *A. flavus* and *A. Parasiticus* were observed. Ahmad *et al.* (2014) identified and differentiated *A. flavus* and *A. parasiticus* isolates from peanuts using a similar method. In this study, the isolates of *A. parasiticus* were confirmed by PCR by targeting the toxin genes.

Many methods are used for the determination of mycotoxins, such as solid-phase extraction, high-performance liquid chromatography (HPLC), gas

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 524-532
DOI: http://dx.doi.org/10.21161/mjm.221496



(a) Disinfectant showing zone of inhibition (b) Disinfectant showing no zone of inhibition

Figure 5: Anti-*A. parasiticus* activity of disinfectants by well diffusion assay.

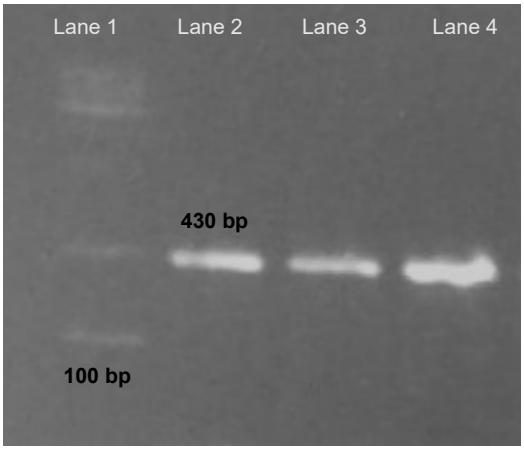


Figure 4: Agarose gel electrophoresis of *A. parasiticus*. Lane 1: Ladder; Lane 2: AspP2; Lane 3: AspP4; Lane 4: AspP8.

chromatography (GC), capillary electrophoresis and thin-layer chromatography (TLC) (Turner *et al.*, 2009). This study assessed the mycotoxin-producing potential of the *A. parasiticus* isolates (qualitative and quantitative analysis) using the TLC and HPLC methods. To differentiate between toxigenic and nontoxigenic species of *A. parasiticus*, thin layer chromatography was performed. Out of 20 tested isolates, 11(55%) were observed positive for toxin production. Chauhan *et al.* (2016) also used TLC to differentiate the toxin-producing isolates from non-producing ones.

HPLC analysis showed that AspP8 produced 18.47 ng/mL toxin followed by AspP2 (5.90 ng/mL) and AspP4 (3.11 ng/mL). Donner *et al.* (2009) observed the ability of *A. parasiticus* isolates to synthesize aflatoxins and recorded the average concentrations of AFB1 and AFG1 to be 90-2092 µg/kg and 99-450 µg/kg, respectively.

Disinfectants are chemical agents that have antimicrobial potential against a broad range of microbes. However, previous studies have reported these disinfectants ineffective against fungi. In this study, toxigenic isolates were selected and assessed their sensitivity pattern against different disinfectants by well diffusion method. The highest zone of inhibition (ZOI), 29.66 ± 8.08 mm was obtained with Terralin, while the lowest zone of inhibition (ZOI) with Hypochlorite 13.66 ± 7.23 mm. While Isopropanol, Distel and Descocid-N showed no zone of inhibition 0.00 ± 0.00. Basaran (2011) conducted a similar study and showed that hypochlorite exhibits antifungal activity with a 16 mm zone of inhibition.

Further, the broth microdilution method assessed the MIC of disinfectants. The highest MIC was of Hypochlorite 1.56 µg/mL, while the lowest mean MIC was of Endo Terra 0.32 µg/mL for all the isolates of *A. parasiticus*. Antifungal activity of disinfectants Alpha Guard, Instru Star, Hypochlorite, Endo Terra and Terralin was calculated by the log reduction method. After different incubation periods, colonies were counted and colony-forming units CFU/mL were calculated. Hypochlorite and Instru Star showed 99% disinfection with 30, 60 and 90 min contact time (6 mean log reduction) for all *A. parasiticus* isolates. Terralin also showed a similar pattern for AspP2 and AspP8; however, for AspP4 fungicidal activity was observed after 15 min contact. Alpha Guard inhibited growth after 15 min contact time for all the isolates.

Sen *et al.* (1999) reported that 5% NaOCl alone showed antifungal activity after 30 min. These results are in accordance with our study. In this study, Hypochlorite was a combination of hypochlorous acid and free chlorine. Studies have reported that the presence of free chlorine disrupts microbial cell membrane permeability and impairs membrane function, hence, cell death (Freiberg, 1957). Quaternary ammonium compounds (QACs) are active ingredients in disinfectant products and are less toxic with bactericidal efficacy. According to a study, a product prepared with didecyl dimethyl ammonium chloride, a QAC, was active against multiple fungal strains (including *A. brasiliensis*) (Polarine, 2012). In today's market, quaternary ammonium compounds are the major ingredient of disinfectant formulations. In the current study, Alpha Guard (QAC) showed maximum fungicidal activity in a shorter contact period.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

CONCLUSION

It was concluded that disinfectants have the potential to inhibit *A. parasiticus* growth. Hypochlorite, Instru Star and Terralin exhibits 99% disinfection for all tested contact times in *in vitro* testing. So, these disinfectants could be effective in inhibiting and preventing contamination of crops and feed with toxins produced by *A. parasiticus*.

REFERENCES

Abdolrasouli, A., Armstrong-James, D., Ryan, L. and Schelenz, S. (2017). *In vitro* efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with *Candida auris*. *Mycoses* **60(11), 758-763.**

Adeyeye, S. A. O. (2020). Aflatoxigenic fungi and mycotoxins in food: A review. *Critical Reviews in Food Science and Nutrition* **60(5), 709-721.**

Ahmad, M. M., Ahmad, M., Ali, A., Hamid, R., Javed, S. and Abdin, M. Z. (2014). Detection of *Aspergillus flavus* and *Aspergillus parasiticus* from aflatoxin-contaminated peanuts and their differentiation using PCR-RFLP. *Annals of Microbiology* **64, 1597-1605.**

Ahmed, A. M. A., Tabana, Y. M., Musa, K. B. and Sandai, D. A. (2017). Effects of different mycotoxins on humans, cell genome and their involvement in cancer. *Oncology Reports* **37(3), 1321-1336.**

Basaran, P. (2011). Antifungal effect of acids and surface active compounds for post-harvest control of *Aspergillus parasiticus* growth on hazelnut. *Journal of Food Processing and Preservation* **35(2), 236-246.**

Begum, F. and Samajpati, N. (2000). Mycotoxin production on rice, pulses and oilseeds. *Naturwissenschaften* **87(6), 275-277.**

Beyene, A. M., Du, X., Schrunk, D. E., Ensley, S. and Rumbeiha, W. K. (2019). High-performance liquid chromatography and enzyme-linked immunosorbent assay techniques for detection and quantification of aflatoxin B_1 in feed samples: A comparative study. *BMC Research Notes* **12, 492.**

Bocate, K. P., Reis, G. F., de Souza, P. C., Junior, A. G. O., Durán, N., Nakazato, G., Furlaneto, M. C., de Almeida, R. S. and Panagio, L. A. (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. *International Journal of Food Microbiology* **291, 79-86.**

Chauhan, N. M., Washe, A. P. and Minota, T. (2016). Fungal infection and aflatoxin contamination in maize collected from Gedeo zone, Ethiopia. *SpringerPlus* **5, 753.**

Donner, M., Atehnkeng, J., Sikora, R. A., Bandyopadhyay, R. and Cotty, P. J. (2009). Distribution of *Aspergillus* section Flavi in soils of maize fields in three agroecological zones of Nigeria. *Soil Biology and Biochemistry* **41(1), 37-44.**

Ehrlich, K. C., Montalbano, B. G. and Cotty, P. J. (2007). Analysis of single nucleotide polymorphisms in three genes shows evidence for genetic isolation of certain *Aspergillus flavus* vegetative compatibility groups. *FEMS Microbiology Letters* **268(2), 231-236.**

Freiberg, L. (1957). Further quantitative studies on the reaction of chlorine with bacteria in water disinfection. *Acta Pathologica et Microbiologica Scandinavica* **40(1), 67-80.**

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S., Nováková, A., Chen, A. J., Arzanlou, M., Larsen, T. O., Sklenář, F., Mahakarnchanakul, W., Samson, R. A. and Houbraken, J. (2019). Taxonomy of *Aspergillus* section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology* **93, 1-63.**

Gupta, A. K., Ahmad, I. and Summerbell, R. C. (2002). Fungicidal activities of commonly used disinfectants and antifungal pharmaceutical spray preparations against clinical strains of *Aspergillus* and *Candida* species. *Medical Mycology* **40(2), 201-208.**

Harris, J. L. (2000). Safe, low-distortion tape touch method for fungal slide mounts. *Journal of Clinical Microbiology* **38(12), 4683-4684.**

Khoury, A. E., Atoui, A., Rizk, T., Lteif, R., Kallassy, M. and Lebrihi, A. (2011). Differentiation between *Aspergillus flavus* and *Aspergillus parasiticus* from pure culture and aflatoxin-contaminated grapes using PCR-RFLP analysis of aflR-aflJ intergenic spacer. *Journal of Food Science* **76(4), M247-M253.**

Klich, M. A. and Pitt, J. I. (1988). Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Transactions of the British Mycological Society* **91(1), 99-108.**

Nikolić, M., Savić, I., Nikolić, A., Jauković, M., Kandić, V., Stevanović, M. and Stanković, S. (2021). Toxigenic species *Aspergillus parasiticus* originating from maize kernels grown in Serbia. *Toxins* **13, 847.**

Ohta, S., Makino, M., Nagai, K. and Zenda, H. (1996). Comparative fungicidal activity of a new quaternary ammonium salt N-alkyl-N-2-hydroxy-ethyl-N,N-dimethylammonium butyl phosphate and commonly used disinfectants. *Biological and Pharmaceutical Bulletin* **19, 308-310.**

Okungbowa, F. I. and Usifo, A. O. (2010). Radial growth of *Aspergillus* and *Penicillium* spores exposed to laboratory disinfectants. *Micología Aplicada International* **22(2), 59-61.**

Pitt, J. I. and Hocking, A. D. (2009). *Aspergillus* and related teleomorphs. *In:* Fungi and Food Spoilage. Springer, Boston, MA. **pp. 275-337.**

Polarine, J. (2012). Fungicidal activity of globally acceptable quaternary ammonium disinfectants. *Journal of Validation Technology* **18(4), 53.**

Sana, S., Anjum, A. A., Yaqub, T., Nasir, M., Ali, M. A. and Abbas, M. (2019). Molecular approaches for characterization of aflatoxin producing *Aspergillus flavus* isolates from poultry feed. *Pakistan Veterinary Journal* **39(2), 169-174.**

Sardiñas, N., Vázquez, C., Gil-Serna, J., González-Jaén, M. T. and Patiño, B. (2010). Specific detection of *Aspergillus parasiticus* in wheat flour using a highly

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

sensitive PCR assay. *Food Additives and Contaminants: Part A* **27(6), 853-858.**

Seeliger, H. P. and Schröter, G. (1984). Prevention of fungal infections in hospitalized patients. *Immunitat und Infektion* **12, 143-150.**

Sen, B. H., Safavi, K. E. and Spångberg, L. S. (1999). Antifungal effects of sodium hypochlorite and chlorhexidine in root canals. *Journal of Endodontics* **25(4), 235-238.**

Sickbert-Bennett, E. E., Weber, D. J., Gergen-Teague, M. F., Sobsey, M. D., Samsa, G. P. and Rutala, W.A. (2005). Comparative efficacy of hand hygiene agents in the reduction of bacteria and viruses. *American Journal of Infection Control* **33, 67-77.**

Singh, P. and Cotty, P. J. (2019). Characterization of *Aspergilli* from dried red chilies (*Capsicum* spp.): Insights into the etiology of aflatoxin contamination. *International Journal of Food Microbiology* **289, 145-153.**

Tarhriz, V., Eyvazi, S., Shakeri, E., Hejazi, M. S. and Dilmaghani, A (2020). Antibacterial and antifungal activity of novel freshwater bacterium *Tabrizicola aquatica* as a prominent natural antibiotic available in Qurugol Lake. *Journal of Pharmaceutical Sciences* **26(1), 88-92.**

Tola, M. and Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food and Agriculture* **2(1), 1191103.**

Turner, N. W., Subrahmanyam, S. and Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: A review. *Analytica Chimica Acta* **63(2), 168-180.**

Udomkun, P., Wiredu, A. N., Nagle, M., Bandyopadhyay, R., Müller, J. and Vanlauwe, B. (2017). Mycotoxins in Sub-Saharan Africa: Present situation, socio-economic impact, awareness, and outlook. *Food Control* **72, 110-122.**

Varga, J., Juhász, A., Kevei, F. and Kozakiewicz, Z. (2004). Molecular diversity of agriculturally important Aspergillus species. *In*: Molecular Diversity and PCR-detection of Toxigenic *Fusarium* Species and Ochratoxigenic Fungi. Mulè, G., Bailey, J. A., Cooke, B. M. and Logrieco, A. (eds.). Springer, Dordrecht. **pp. 627-640.**

Watanabe, T. (2010). Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. CRC Press, Boca Raton.

Wen, J., Mu, P. and Deng, Y. (2016). Mycotoxins: Cytotoxicity and biotransformation in animal cells. *Toxicology Research* **5(2), 377-387.**

Wijedasa, M. H. and Liyanapathirana, L. V. C. (2012). Evaluation of an alternative slide culture technique for the morphological identification of fungal species. *Sri Lankan Journal of Infectious Diseases* **2(2), 47-52.**

Yogendrarajah, P., Vermeulen, A., Jacxsens, L., Mavromichali, E., De Saeger, S., De Meulenaer, B. and Devlieghere, F. (2016). Mycotoxin production and predictive modelling kinetics on the growth of *Aspergillus flavus* and *Aspergillus parasiticus* isolates in whole black peppercorns (*Piper nigrum* L). *International Journal of Food Microbiology* **228, 44-57.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538