Journal of Advances in Microbiology

13(1): 1-11, 2018; Article no.JAMB.44730
ISSN: 2456-7116

Phytochemical Screening and Antifungal Activities of *Zingiber officinale* (Roscoe) on Mycotoxigenic Fungi Associated with the Deterioration of *Pennisetum glaucum* Grains

Olubunmi A. Ayodele[1*], Felix A. Akinyosoye[1], Daniel J. Arotupin[1], Oluwole O. Owoyemi[1] and Ayodele B. Oyindamola[1]

[1]Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author OOO performed the statistical analysis. Authors FAA, DJA and OOO managed the analyses of the study. Authors OAA and ABO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/44730
Editor(s):
(1) Dr. Pratibha Vyas, Assistant Professor, Department of Microbiology, School of Biotechnology and Biosciences, Lovely Professional University, Punjab, India.
Reviewers:
(1) Mohamed M. Deabes, National Research Centre, Egypt.
(2) Ferenc Bagi, University of Novi Sad, Serbia.
(3) Oyetayo Adedayo Michael, Rufus Giwa Polytechnic, Nigeria.
(4) Silvia D. Peña Betancourt, University UAM-X, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/27335

Original Research Article

Received 16 August 2018
Accepted 30 October 2018
Published 21 November 2018

ABSTRACT

The control of mycotoxigenic fungal isolates associated with deterioration of *Pennisetum glaucum* grains using *Zingiber officinale* (Roscoe) roots extracts were investigated in this study. Aqueous, Methanol and acetone solvents were used in the extraction of *Z. officinale* root extracts. The extracts were screened for phytochemicals which revealed the presence of alkaloids, cardiac glycoside, tannin, saponin, flavonoids and terpenoids. A total of fourteen (14) fungi were isolated from pearl millet grains using standard microbiological method. These include: *Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Aspergillus fumigatus, Penicillum frequentans, Penicillum chrysogenum, Penicillum italicum, Penicillum oxallicum, Fusarium oxysporum, Fusarium*

**Corresponding author: E-mail: olubunmi.dele10@gmail.com;*

subglutinans, Trichoderma harzanium, Beauveria bassiana, Mucor mucedo, Saccharomyces cerevisiae. The antifungal activities of *Z. officinale* root extracts on fungal isolates from deteriorating pearl millet grains were carried out using agar well diffusion technique. Findings from the study revealed that *Z. officianalis* extracts demonstrated a varying degree of antifungal activity against the fungal isolates from pearl millet grains. Methanollic extract of *Z. officinale* displayed the highest antifungal activity against the isolates compared to aqueous and acetone extracts. The result of the commercial antifungal agents revealed the great effectiveness of fluconazole over griseofulvin in the control of mycotoxigenic fungi associated with the deterioration of pearl millet grains. In light of these findings, *Z. officianalis* extracts can be employed in the control of mycotoxigenic fungi associated with the deterioration of Pearl millet grains and can also be developed fully into a biocontrol agent upon purification and used in the control of mycotoxigenic fungi associated with the infection of cereal grains.

Keywords: Zingiber officinale; mycotoxigenic; Pennisetum glaucum; antifungal; extracts; phytochemical.

1. INTRODUCTION

1.1 Mycotoxigenic Fungi

Mycotoxigenic fungi are the species of fungi that have the ability to produce mycotoxins. Among these are the genera *Aspergillus, Fusarium,* and *Penicillium* [1]. Mycotoxin-producing moulds species are extremely common, and they can grow on a wide range of substrates under a wide range of environmental conditions. The degree of contamination is based on a number of factors like weather, temperature of storage and moisture content of the grain [2]. An early and brisk detection of mycotoxin producing moulds in foods is important, so that the level of contamination of food materials can be reduced to the barest minimal which ultimately secures food for human and animal consumption and prevent wastage [3].

1.2 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by some fungi. They have been implicated in degradation of quality in many agricultural products especially cereals. Mycotoxins have also been reported by many researchers to cause diseases in humans and animals [4,5]. Nearly all mycotoxins are cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis [6]. Furthermore, they are also toxic to the cells of higher plants and animals, including humans. Toxigenic moulds vary in their mycotoxin production depending on the substrate on which they grow [7]. There are eight (8) groups of mycotoxins found frequently in foods; they include aflatoxin, ochratoxin, fumonisin, zeralenone, deoxynivalenol, T-2 toxin, trichothecenes and the ergot alkaloids [8]. Three groups of mycotoxigenic fungi have been reported to produce the bulk of these mycotoxin; they are the *Penicillium* spp*, Fusarium* spp and the *Aspergillus* spp. [9]. The species of the *Aspergillus* and *Penicillium* genus are the ones that proliferate easier in stored grain [10].

1.3 Antimicrobial Activity of Ginger (*Zingiber officianale* (Roscoe)

Zingiber officinale (Roscoe) is a flowering plant in the family Zingiberaceae whose root, is widely used as a spice or a medicine. Ginger is indigenous to southern China, other parts of Asia and subsequently to West Africa and the Caribbean Kim et al. [11]. Ginger is often cooked as an ingredient in many dishes. They are consumed worldwide as flavouring agent which is used extensively in food, beverage, and confectionary industries in the products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, and other bakery products Wang et al. [12]. It has a number of essential nutrients such as mineral manganese, vitamins, proteins, energy and so on and so forth. Ginger has been used in the treatment of stomach upset, nausea, vomiting, sea sickness and chemotherapy Hassan et al. [13]. The essential oil from the ginger root is applied topically as a pain reliever Bhargava et al. [14].

In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor Sasidharan and Nirmala, [15]. Reports have shown that *Zingiber officianle* has incredible antimicrobial properties. Sebiomo et al. [16] and

Hassan et al. [13] reported that *Zingiber officianale* showed antibacterial properties against *Staphylococcus aureus, Klesbiella* spp*, Enterococcus* spp*, Pseudomonas* spp and antifungal properties were equally shown in *Candida albicans.* Ponmurugan and Shyamkumar [17] also found that *Zingiber officianale* showed antibacterial properties in *Enterobacter* spp. The antimicrobial activities possessed by *Zingiber officianale* makes it suitable for the preservation of food and prevention of spoilage by food spoilage microorganisms.

The objectives of this research are to isolate, characterise mycotoxigenic fungi on deteriorating pearl millet (*Pennisetum glaucum*) grains and determine the antifungal effects of ginger (*Zingiber officinale*) root extracts on the mycotoxigenic fungal isolates.

2. MATERIALS AND METHODS

2.1 Collection of Samples (Pearl Millet Grains and *Z. Officinale* [Roscoe] Root)

Eighty samples of Pearl millet grains were collected from hawkers at Akure main market, Ondo state. The millet grains were stored at 30 ± 2°C in an atmosphere of low relative humidity with a moisture content of 10%. The samples were analysed at the Postgraduate Microbiology Laboratory of The Federal University of Technology, Akure, Ondo state.

A basket of ginger roots was collected from Sabo market in Akure metropolis. The roots were washed and dried at room temperature (25 ± 2°C) for fourteen (14) days. The roots were then pulverised using a high speed Rico grinder (FP-101 model).

2.2 Extraction of Plant Materials

2.2.1 Methanolic, acetone and aqueous extractions

Five hundred grams (500 g) of the crushed ginger roots were soaked separately in 500 mL of methanol, water and acetone in a sterile container. The mixtures was rocked gently to form a homogeneous suspension and allowed to stand for three (3) days. The mixtures were filtered through a sterile muslin cloth followed by the Whatman filter paper. The filtrates were then evaporated to dryness using a rotary evaporator. Phytochemical screening was carried out on all the *Z. officinale* extracts to determine the active principles in them following the procedures of Owoyemi and Oladunmoye [18].

2.3 Isolation of Mycotoxigenic Fungal Isolates

The Pearl millet grains collected from Akure main market, Ondo state were examined on a weekly basis for the first four weeks, and every two weeks until the tenth week using direct plating (pour plate) technique in potato dextrose agar. The grains were surface sterilised in 2.5% sodium hypochlorate and crushed in a mortar using a pestle. One gram (1 g) of the crushed millet was aseptically weighed and put into 9 ml of sterile distilled water. The serial dilution of the crushed grains was done using a dilution factor of 10^3. One millilitre (1 ml) of the diluted mixture was transferred into sterile Petri dishes for each dilution factor. Twenty millilitres (20 ml) of sterilised agar was added to the Petri dishes containing the samples. The samples were incubated at 25°C for five (5) days according to the methods of Wangara et al. [19].

2.4 Identification and Characterisation of Mycotoxigenic Fungal Isolates

The isolated organisms were identified using their cultural and morphological characteristics in the media. Distinct colonies of isolates that grew on the culture plates were observed for their cultural and morphological features, this was then followed by microscopic examination of the mycotoxigenic fungal isolates on the glass slide placed under the microscope. The cultural features examined included shape, elevation, surface edge, consistency, texture, pigmentation and reverse pigmentation. Microscopic characterisation was done by analysis of microscopic morphology. This includes the examination of conidia morphology (especially septation, shape, size, wall texture and colour), the type of conidiogeneous cell (non-specialised or hypha like) as described by Pitt, [20].

2.5 Determination of Antifungal Activities of *Z. officinale* Leaf Extracts on Mycotoxigenic Fungal Isolates from Pearl Millet Grains

The agar well diffusion method described by Abass et al. [21] was employed in the

determination of the antifungal activities of *Z. officinale* root extracts on fungal isolates from pearl millet grain.

2.6 Determination of Commercial Antifungal Agents Activities on Fungal Isolates from Pearl Millet Grains

The disc diffusion method was employed in the determination of the activities of antifungal agents. Potatoes dextrose agar medium and 50 mg/mL of Fluconazole and Griseofulvin were used in this study following the procedures of Owoyemi and Oladunmoye, 2017 [18].

3. RESULTS

3.1 Phytochemical Composition of Ginger (*Zingiber officinale)* Roots

Table 1 shows the qualitative phytochemical composition of ginger (*Zingiber officinale)* roots. Fig. 1 and 2 shows the quantitative phytochemical composition of ginger (*Zingiber officinale)* roots using water, methanol and acetone as an extraction solvent. The findings revealed the presence of alkaloids in the aqueous extract (20.8%), methanolic extract (40.0%), and acetone extract (30.7%). Cardiac glycoside in aqueous extract (10.7 mg/mL), methanolic extract (27.3 mg/mL) and acetone extract (12.5 mg/mL). Anthraquinone was present in aqueous extract at 1.0 mg/mL, methanolic extract (1.8 mg/mL) and in acetone extract (1.0 mg/mL). Tannin was present in aqueous extract at 0.4 mg/mL, methanolic extract (1.5 mg/mL) and acetone extract (1.0 mg/mL). Phlobotannin was present in aqueous extract at 4.7 mg/mL, methanolic extract (7.3 mg/mL) and acetone extract (2.5 mg/mL). Saponin was present in aqueous extract at 19.9 mg/mL, methanolic extract (40.8 mg/mL) and acetone extract (22.0 mg/mL). Flavanoid was present in aqueous extract at 10.7 mg/mL, methanolic extract (28.8 mg/mL) and acetone extract (13.5 mg/mL). Terpernoid was present in aqueous extract at 1.2 mg/mL, methanolic extract (2.8 mg/mL) and acetone extract (1.4 mg/mL).

3.2 Antifungal Activities of Ginger (*Zingiber officinale*) Roots Aqueous Extracts on Isolated Fungi from *Pennisetum glaucum* during Storage

Aqueous extract of ginger (*Zingiber officinale*) roots displayed low antifungal activities against the isolated fungi. No inhibition was observed using 0.1 g/ml of the extract against the isolated fungi. *Aspergillus flavus* had zones of inhibition of 1.37 mm at 0.3 g/ml, 3.07 mm at 0.4 g/ml and 6.10 mm at 0.5 g/ml of the extract. *Aspergillus oryzae* was also observed to have inhibition zones of 2.10 mm at 0.4 g/ml and 5.10 mm at 0.5 g/ml. The aqueous extract *Zingiber officinale* against *A. fumigatus,* had no inhibition observed at 0.2 g/ml. Zones of inhibition of 2.07 mm was observed at 0.3 g/ml, 5.13 mm at 0.4 g/ml and 7.07 mm at 0.5 g/ml. *Penicillium chrysogenum* had zones of inhibition of 1.13 mm at 0.2 g/ml, 3.07 mm at 0.3 g/ml, 4.03 mm at 0.4 g/ml and 6.50 mm at 0.5 g/ml. *Penicillium oxalicum* also had zones of inhibition of 2.17 mm at 0.4 g/ml, and 4.10 mm at 0.5 g/ml. Zones of inhibition observed in *Penicillium italicum* were recorded to be 1.03 mm at 0.3 g/ml, 3.03 mm at 0.4 g/ml and 5.07 mm at 0.5 g/ml but, no inhibition was observed at 0.2 g/ml*. Fusarium oxysporum* had zones of inhibition 1.10 mm at 0.2 g/ml, 1.07 mm at 0.3 g/ml, 4.10 mm at 0.4 g/ml and 6.07 mm at 0.5 g/ml. *F. subglutinans* had zones of inhibition of 2.47 mm at 0.3 g/ml, 4.73 mm at 0.4 g/ml, 6.63 mm at 0.5 g/ml. No inhibition was observed at 0.2 g/ml. Aqueous extract *Zingiber officinale* demonstrated no antifungal property against *A. niger, P. frequentans, Beauveria bassiana* and *Trichoderma harzianumn* as illustrated in Table 2.

Table 1. Qualitative phytochemical composition of ginger (*Zingiber officinale)* roots

Bioactive principles	Aqueous extracts of ginger	Methanol extracts of ginger	Acetone extracts of ginger
Alkaloids	++	++	++
Tannins	+	+	+
Glycosides	++	++	++
Saponins	++	++	++
Flavonoids	++	++	++
Terpenoids	+	+	+
Phlobatannins	+	++	+

Legend. + = Present, ++= Abundantly present

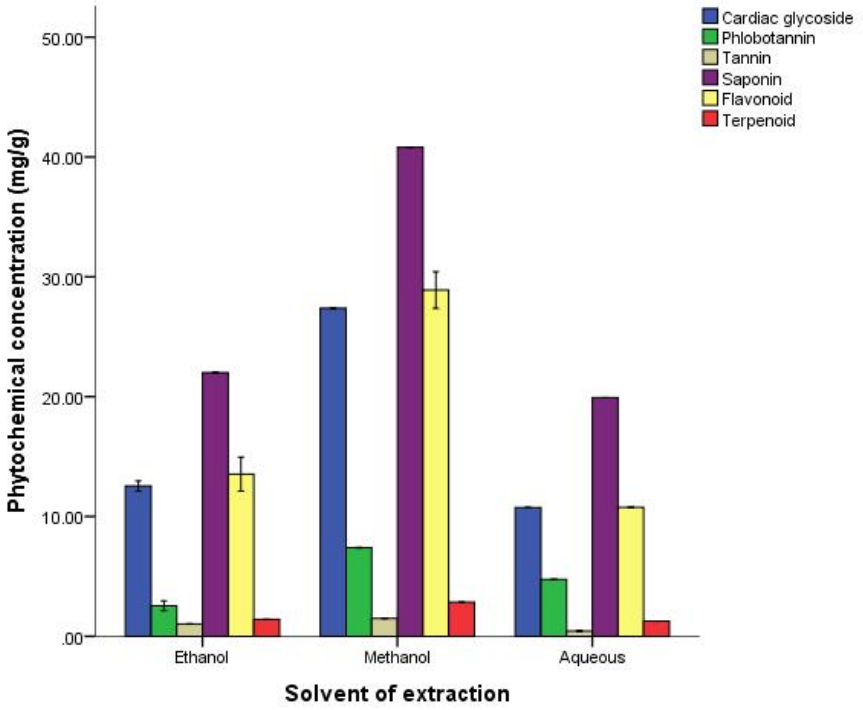

Fig. 1. Quantitative Phytochemical Components of *Z. officinale*

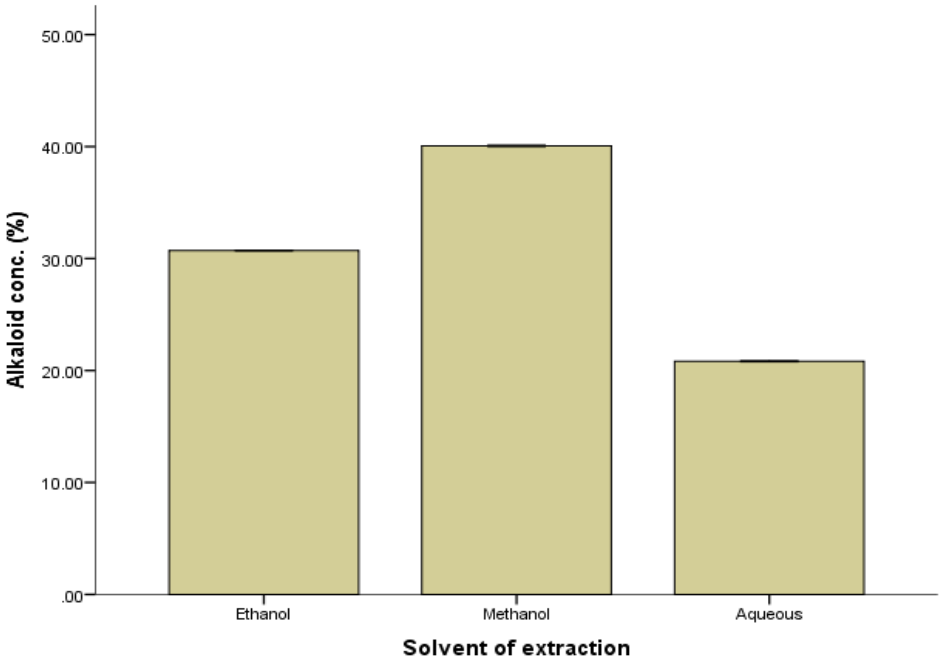

Fig. 2. Phytochemical Quantitative Alkaloid Components of *Z. officinale*

Table 2. Antifungal activities of ginger (*Zingiber officinale)* roots (Aqueous Extract)on fungal isolates from Pearl millet grains

	Conc. of extract (g/ml)				
Selected isolates	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Aspergillus flavus	0.00±0.00^{a}	0.00±0.00^{a}	1.37±0.07^{c}	3.07±0.07^{c}	6.10±0.10^{d}
Aspergillus niger	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00a
Aspergillus oryzae	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.10±0.06^{b}	5.10±0.06^{c}
Aspergillus fumigatus	0.00±0.00^{a}	0.00±0.00^{a}	2.07±0.07^{d}	5.13±0.09^{f}	7.07±0.07^{f}
Penicillium frequentans	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Penicillium chrysogenum	0.00±0.00^{a}	1.13±0.09^{b}	3.07±0.07^{f}	4.03±0.03^{d}	6.50±0.12^{e}
Penicillium oxalicum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.17±0.09^{b}	4.10±0.10^{b}
Penicillium italicum	0.00±0.00^{a}	0.00±0.00^{a}	1.03±0.03^{b}	3.03±0.03^{c}	5.07±0.03^{c}
Fusarium oxysporum	0.00±0.00^{a}	1.10±0.10^{b}	1.07±0.07^{b}	4.10±0.10^{d}	6.07±0.07^{d}
Fusarium subglutinans	0.00±0.00^{a}	0.00±0.00^{a}	2.47±0.03^{e}	4.73±0.15^{e}	6.63±0.09^{e}
Trichoderma harzianum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Beauveria bassianna	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Table 3. Antifungal activities of ginger (*Zingiber officinale)* roots (Methanolic extract) on fungal isolates from Pearl millet grains

Conc. of extract (g/ml)					
Selected isolates	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Aspergillus flavus	0.00±0.00^{a}	3.03±0.03^{e}	6.17±0.09^{g}	10.50±0.28^{i}	14.17±0.09^{i}
Aspergillus niger	0.00±0.00^{a}	2.13±0.09^{d}	3.03±0.03^{d}	6.17±0.09^{g}	10.20±0.12^{h}
Aspergillus oryzae	0.00±0.00^{a}	1.03±0.03^{b}	4.10±0.06^{f}	6.50±0.06^{h}	8.07±0.07^{g}
Aspergillus fumigatus	0.00±0.00^{a}	2.50±0.06^{e}	3.03±0.03^{d}	5.10±0.10^{e}	6.07±0.07^{e}
Penicillium frequentans	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Penicillium chrysogenum	0.00±0.00^{a}	1.40±0.06^{c}	2.03±0.03^{c}	4.10±0.06^{d}	6.07±0.07^{e}
Penicillium oxalicum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.10±0.06^{b}	4.20±0.12^{c}
Penicillium italicum	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.06±0.06^{b}	3.10±0.06^{b}
Fusarium oxysporum	0.00±0.00^{a}	2.10±0.06^{d}	3.47±0.09^{e}	5.47±0.15^{f}	7.07±0.07^{e}
Fusarium subglutinans	0.00±0.00^{a}	0.00±0.00^{a}	1.07±0.07^{b}	4.07±0.07^{d}	5.07±0.07^{d}
Trichoderma harzianum	0.00±0.00^{a}	0.00±0.00^{a}	2.03±0.03^{c}	3.47±0.03^{c}	4.10±0.10^{c}
Beauveria bassianna	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

3.3 Antifungal Activities of Ginger (*Zingiber officinale*) Roots Methanolic Extracts on Isolated Fungi from *Pennisetum glaucum* during Storage

Methanolic extract of ginger (*Zingiber officinale*) roots displayed the highest antifungal action against the isolated fungi. No inhibition was observed using 0.1 g/ml of the extract against the isolated fungi. *Aspergillus flavus* had zones of inhibition of 3.03 mm at 0.2 g/ml, 6.17 mm at 0.3 g/ml, 10.50 mm at 0.4 g/ml and 14.17 mm at 0.5 g/ml of the extract. Inhibition zones in *A. niger* were 2.13 mm at 0.2 g/ml, 3.03 mm at 0.3 g/ml, 6.17 mm at 0.4 g/ml and 10.20 mm at 0.5 g/ml. *A. oryzae* was also observed to have inhibition zones of 1.03 mm at 0.2 g/ml, 4.10 mm at 0.3 g/ml, 6.50 mm at 0.4 g/ml and 8.07 mm at 0.5 g/ml. The methanolic extract *Zingiber officinale* against *A. fumigatus,* had a moderate inhibition of 2.50 mm which was observed at 0.2 g/ml, 3.03 mm at 0.3 g/ml, 5.10 mm at 0.4 g/ml and 6.07 mm at 0.5 g/ml. *Penicillium chrysogenum* had zones of inhibition of 1.40 mm at 0.2 g/ml, 2.03 mm at 0.3 g/ml, 4.10 mm at 0.4 g/ml and 6.07 mm at 0.5 g/ml. *P. oxalicum* also had zones of inhibition of 2.10 mm at 0.4 g/ml, and 4.20 mm at 0.5 g/ml. Zones of inhibition observed in *P. italicum* were recorded to be 2.06 mm at 0.4 g/ml and 3.10 mm at 0.5 g/ml No inhibition was observed at 0.2 g/ml and 0.3 g/ml. *Fusarium oxysporum* had zones of inhibition 2.10 mm at

0.2 g/ml, 3.47 mm at 0.3 g/ml, 5.47 mm at 0.4 g/ml and 7.07 mm at 0.5 g/ml. *F. subglutinans* had zones of inhibition of 1.07 mm at 0.3 g/ml, 4.07 mm at 0.4 g/ml, 5.07 mm at 0.5 g/ml. *Trichoderma harzianum* had inhibition zones of 2.03 mm at 0.3 g/ml, 3.47 mm at 0.4 g/ml and 4.10 mm at 0.5 g/ml. No inhibition was observed at 0.2 g/ml. The extract demonstrated no antifungal property against *P. frequentans, Beauveria bassiana* and as shown in Table 3.

3.4 Antifungal Activities of Ginger (*Zingiber officinale*) Roots Acetone Extracts on Isolated Fungi from *Pennisetum glaucum* during Storage

Acetone extract of ginger (*Zingiber officinale*) roots displayed a wide spectrum of antimicrobial property against the isolated fungi. No inhibition was observed using 0.1 g/ml of the extract against the isolated fungi. *Aspergillus flavus* had zones of inhibition of 2.20 mm at 0.2 g/ml, 4.07 mm at 0.3 g/ml, 6.10 mm at 0.4 g/ml and 7.23 mm at 0.5 g/ml of the extract. Inhibition zones in *A. niger* were 1.53 mm at 0.3 g/ml, 3.17 mm at 0.4 g/ml and 6.10 mm at 0.5 g/ml. *A. oryzae* was also observed to have inhibition zones of 1.03 mm at 0.2 g/ml, 3.03 mm at 0.3 g/ml, 5.17 mm at 0.4 g/ml and 7.03 mm at 0.5 g/ml. The acetone extract *Zingiber officinale* against *A. fumigatus,* had a moderate inhibition of 2.07 mm was observed at 0.2 g/ml, 3.07 mm at 0.3 g/ml, 6.13 mm at 0.4 g/ml and 8.23 mm at 0.5 g/ml. *Penicillium chrysogenum* had zones of inhibition of 2.03 mm at 0.3 g/ml, 3.03 mm at 0.4 g/ml and 5.10 mm at 0.5 g/ml. *P. oxalicum* also had zones of inhibition of 1.03 mm at 0.3 g/ml, 4.10 mm at 0.4 g/ml, and 5.20 mm at 0.5 g/ml. Zones of inhibition observed in *P. italicum* were recorded to be 3.10 mm at 0.3 g/ml, 5.43 mm at 0.4 g/ml and 6.23 mm at 0.5 g/ml No inhibition was observed at 0.2 g/ml. *Fusarium oxysporum* had zones of inhibition 2.03 mm at 0.3 g/ml, 4.47 mm at 0.4 g/ml and 6.03 mm at 0.5 g/ml. *F. subglutinans* had zones of inhibition of 1.07 mm at 0.2 g/ml, 1.53 mm at 0.3 g/ml, 2.10 mm at 0.4 g/ml, 4.07 mm at 0.5 g/ml. *Trichoderma harzianum* had inhibition zones of 1.13 mm at 0.2 g/ml, 3.07 mm at 0.3 g/ml, 4.23 mm at 0.4 g/ml and 7.00 mm at 0.5 g/ml. The extract demonstrated no antifungal property against *Penicillium frequentans, Beauveria bassiana* and as shown in Table 3.

3.5 Antifungal Activities of Commercial Antifungal Agents on Isolated Fungi from *Pennisetum glaucum* during Storage

Commercial antifungal agents Fluconazole and Griseofulvin were used as control in this study. The results indicated that Fluconazole showed a higher antifungal capacity on the isolates while Griseofulvin demonstrated a relatively weak antifungal activity on the isolates. Using Fluconazole at 50 mg/mL, 29.4 mm zone of inhibition was observed on *A. niger,* 28.5 mm on *F. subglutinans*, 26.9 mm on *T. harzanium* and 25.0 mm on *P. frequentans.* Using Griseofulvin at 50 mg/mL, 18.8 mm zone of inhibition was observed on *B. bassiana*, 4.4 mm on *P. chrysogenum*, 6.5 mm on *A. oryzae* and 5.2 on *A. fumigatus* as represented in Fig. 3 below.

Table 4. Antifungal activities of Ginger (*Zingiber officinale)* roots (Acetone extract) on fungal isolates from Pearl millet grains

	Various conc. of extract (g/ml)				
Selected isolates	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Aspergillus flavus	0.00±0.00^{a}	2.20±0.20^{d}	4.07±0.12^{f}	6.10±0.10^{h}	7.23±0.25^{e}
Aspergillus niger	0.00±0.00^{a}	0.00±0.00^{a}	1.53±0.06^{c}	3.17±0.15^{c}	6.10±.17^{d}
Aspergillus oryzae	0.00±0.00^{a}	1.03±0.06^{b}	3.03±0.12^{e}	5.17±0.21^{f}	7.03±0.06^{e}
Aspergillus fumigatus	0.00±0.00^{a}	2.07±0.12^{d}	3.07±0.12^{e}	6.13±0.12^{h}	8.23±0.25^{f}
Penicillium frequentans	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Penicillium chrysogenum	0.00±0.00^{a}	0.00±0.00^{a}	2.03±0.06^{d}	3.03±0.06^{c}	5.10±0.17^{c}
Penicillium oxalicum	0.00±0.00^{a}	0.00±0.00^{a}	1.03±0.06^{b}	4.10±0.17^{d}	5.20±0.26^{c}
Penicillium italicum	0.00±0.00^{a}	1.53±0.06^{c}	3.10±0.17^{e}	5.43±0.21^{g}	6.23±0.25^{d}
Fusarium oxysporum	0.00±0.00^{a}	0.00±0.00^{a}	2.03±0.06^{d}	4.47±0.06^{e}	6.03±0.06^{d}
Fusarium subglutinans	0.00±0.00^{a}	1.07±0.12^{b}	1.53±0.06^{c}	2.10±0.10^{b}	4.07±0.12^{b}
Trichoderma harzianum	0.00±0.00^{a}	1.13±0.06^{b}	3.07±0.12^{e}	4.23±0.21^{d}	7.00±0.00^{e}
Beauveria bassianna	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different

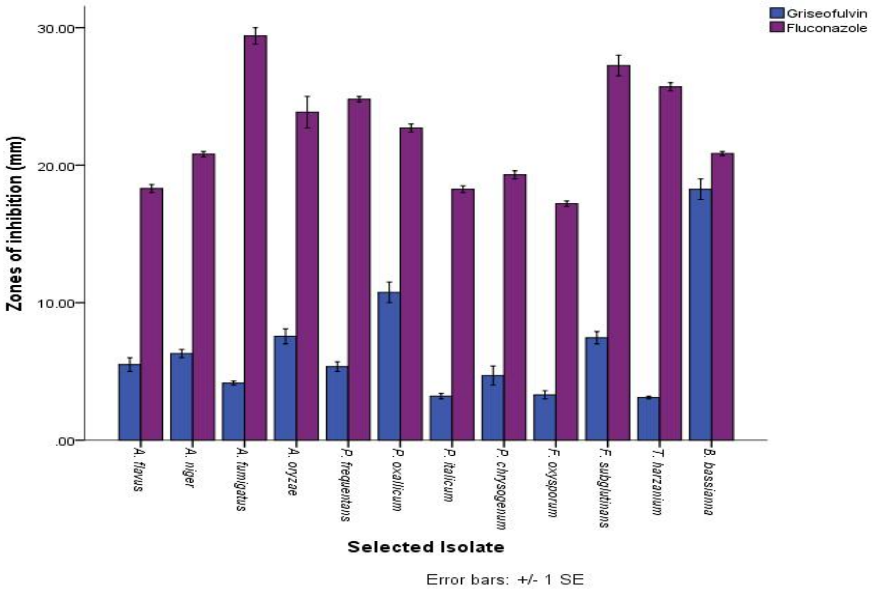

Fig. 3. Antifungal activities of Commercial Antifungal agents on isolated fungi from *Pennisetum glaucum* during storage

4. DISCUSSION

The phytochemical compostition and antifungal activities of aqueous, methanolic and acetone extracts of *Zingiber officinale* on mycotoxigenic fungi isolated from deteriorating Pearl millets grains were evaluated in this study. A total of twelve (12) mycotoxigenic fungi were isolated from the Pearl millet grains which include: *Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Aspergillus fumigatus, Penicillum frequentans, Penicillum chrysogenum, Penicillum italicum, Penicillum oxallicum, Fusarium oxysporum, Fusarium subglutinanas, Trichoderma harzanium,* and *Beauveria bassiana.* Moulds are generously endowed with proteolytic and lipolytic enzymes and therefore, can cause softening of food products. They change the appearance of food and also cause off flavour developments in them [22]. *Aspergillus* spp, *Penicillium* spp and *Fusarium* spp have been reported worldwide as known mycotoxin producers [23,1,24]. Their presence in foods suggests potential mycotoxin contamination. Mycotoxins are heat stable and when consumed, they may result in food intoxication despite undergoing heat treatment. The main toxic effects of mycotoxin ingestion in humans and animals are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression [25,26]. *Beauveria bassiana* isolated from this study is not a regular contaminant of cereal grain, but mostly found in soil. Hence, its presence in the pearl millet (*Pennisetum glaucum*) grains is an indication of improper handling by the farmers and marketers leading to contact with soils which habour the organism.

Phytochemical screening of *Z. officinale* roots revealed the presence of tannin, cardiac glycosides, saponins, flavonoids alkaloids and phlobatanins, this is in agreement with the findings of Bhargava et al. [14]. In particular the saponins, flavonoids, cardiac glycosides and alkaloids were detected in rich fractions while tannins and terpernoids were present in small fractions of ginger extracts evaluated in this study. *Z. officinale* present such potential of high antifungal activities against the fungal test organisms isolated from deteriorating pearl millet grains. In this study, the aqueous extracts of *Z. officinale* showed low inhibitory activities against the fungal isolates. However, moderate inhibitory capacity was observed for the following microorganisms: *A. fumigatus, P. chrysogenum* and *F. subglutinans.* with inhibition zones of 7.07 mm, 6.50 mm and 6.63 mm using 0.5 g/ml of the extract. The methanolic extracts of *Z. officinale* roots exhibited good antifungal activities on *A. flavus* with inhibition zones of 14.17 mm and 7.23 mm respectively using 0.5 g/ml of the extract *and* this agrees with the findings of Mostafa et al. [27] and Sharma et al. [28]. Methanolic and acetone extracts were also effective as they displayed good antifungal activities against *A. niger* with inhibition zones of 10.20 mm and 6.02 mm using 0.5 g/ml of the extract, this finding corroborates

the submission of Bansod and Rai [29]. Methanolic extracts of *Z. officinale* also displayed antifungal capacities towards *A. oryzae, A. fumigatus, F. oxysporum, P. chrysogenum* with inhibition zones of 8.07 mm, 6.07 mm, 7.07 mm and 6.07 mm at 0.5 g/ml respectively. This disagrees with the findings of Jadon and Dixit [30] who reported that *Z. officinale* demonstrated no antifungal property against *P. chrysogenum*. Acetone extracts of *Z. officinale* showed a significant inhibitory capacity towards *T. harzanium*, *A. fumigatus, A. oryzae* and *A. flavus*. No inhibitory activity was displayed using 0.1 g/ml of all three extracts; low inhibitory actions were experienced using 0.2 g/ml, 0.3 g/ml and 0.4 g/ml of the extracts. The most remarkable and significant antifungal activities of *Z. officinale* was observed at the concentration of 0.5 g/ml of the extracts on the isolates. The growths of *P. frequentans and Beauveria bassiana* were not inhibited having used all the three distinct extracts of *Z. officinale.* Methanolic extracts of *Z. officinale displayed* a higher antifungal activity compared with aqueous and acetone extracts, the credit of methanolic extraction could be due to the fact that methanol is an organic solvent that will dissolve organic compound better; hence, librates the active component required for antimicrobial activity [18]. The low antifungal activity observed in the aqueous extraction could be due to low liberation of the active constituents of the plant materials considering the extraction solvent. This reveals that the solvent of extraction affects the degree of antifungal activity of the extracts. In all, methanolic extract of ginger (*Zingiber officinale*) demonstrated the highest antimicrobial property on the isolates. It is noteworthy that the antifungal activities of plants are dependent on the concentration of the extract. Commercial antifungal agents (griseofulvin and fluconazole) activities on the fungal isolates were investigated in the study. Findings revealed that fluconazole exhibited a good antifungal activities against the test isolated fungi compared to griseofulvin as illustrated in Fig. 2. In comparison between the antifungal activities of *Z. officinale* extracts and the commercial antifungal agents, the latter produced a better result. However, the antifungal activities of the former can be improved upon by purification of the extracts.

5. CONCLUSION

This study clearly indicated that *Aspergillus* spp, *Penicillium* spp and *Fusarium* spp were predominant mycotoxigenic fungal contaminants of Pearl millet grains. Methanolic and Acetone extracts of Ginger (*Zingiber officinale*) roots possess good antifungal activity against *Aspergillus flavus, A. oryzae, A. fumigatus*, *Penicillium italicum, P. chrysogenum, P. oxalicum, Fusarium oxysporum, F. subglutinans, Trichoderma harzianum*. This confirms the presence of bioactive compounds such as alkaloids, tannins, flavanoids, and saponins which when purified can be used as a biocontrol agent in the prevention of deterioration in agricultural products and could also be explored as a therapeutic agent in the control of mycotoxigenic fungi associated diseases in humans.

In vivo assessment at other concentrations, using other microorganisms will be helpful in determining the full potential of this plant in the control of pathogenic and toxigenic microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reddy C, Reddy K, Kumar R, Laha G, Muralidaran K. Exploration of aflatoxia contamination and its management in rice. Journal of Mycology and Plant Pathology. 2004;34:816–820.
2. Doster M, Michailides T, Morgan D. *Aspergillus* species and mycotoxins in figs from California orchards. Plant Diseases Journal. 1996;80:484–489.
3. Lawlor P, Lynch B. Mycotoxin management. African Farming and Food Processing. 2005;46:12–13.
4. Lewis L, Onsongo M, Njapau H. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environmental Health Perspectives. 2005; 113(12):1763–1767.
5. Boonen J, Malysheva S, Taevernier L, Diana Di Mavungu J, De Saeger S, De Spiegeleer B. Human skin penetration of selected model mycotoxins. Toxicology. 2012;301(1-3):21–32.
6. Mohammed K, Atef A. Monograph on mycotoxigenic fungi and mycotoxins in foods and feed. Toxin Reviews. 2013;29:3 – 26.

7. Jarvis M. Surface binding of aflatoxin B_1 by lactic acid bacteria. Applied Environmental Microbiology. 1990;67:3086–3091.
8. Reddy K, Reddy C, Abbas H, Abel C, Muralidharan K. Mycotoxigenic fungi, mycotoxins, and management of rice grains. Toxin Reviews. 2008;*27*(3):287–317.
9. Council for Agricultural Science and Technology [CAST]. Mycotoxins: Risks in plant, animal and human systems. Ames, Iowa, USA: Council for Agricultural Science and Technology Press; 2003.
10. Pozzi D. Storage and Grain Supply. Campinas: Campineiro Institute of Agricultural Education. 1986;603.
11. Kim M, Miyamoto S, Yasui Y, Oyama T, Murakami A, Tanaka T, Zerumbonem P. A tropical ginger sesquiterpene, inhibits colon and lung carcinogenesis in mice. International Journal of Cancer. 2009; 124(2):264–71.
12. Wang J, Yang Z, Liu L, Zhanqin L, Zhao L, Xuezhong Z, Liu L. Protective effect of naringenin against lead-induced oxidative stress in rats. Biological Trace Element Research. 2012;146:354-359.
13. Hassan H, Raauf A, AbdRazik B, Hassan B. Chemical composition and antimicrobial activity of the crude extracts isolated from *Zingiber officinale* by different solvents. *Pharmaceutica Analytica Actam*. 2012;**3**: 9-12.
14. Bhargava S, Dhabhai K, Batra A, Sharma A, Malhotra B. *Zingiber officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. Journal of Chemical and Pharmaceutical Research. 2012;4(1):360-364.
15. Sasidharan I, Nirmala A. Comparative chemical composition and antimicrobial activity fresh and dry ginger oils (*Zingiber officinale* Roscoe). International Journal of Current Pharmaceutical Research. 2010; 2:40-43.
16. Sebiomo A, Awofodu A, Awosanya A, Awotona F, Ajayi A. Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. Journal of Microbiology and Antimicrobials. 2011;3: 18-22.
17. Ponmurugan K, Shayamkumar R. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific Journal of Tropical Biom. 2012;edicine:2(8):597–601
18. Owoyemi O, Oladunmoye M. Phytochemical screening and antibacterial activities of *Bidens pilosa* L. and *Tridax procumbens* L. on Skin Pathogens. International Journal of Modern Biology and Medicine. 2017;8(1):24–46.
19. Wangara I, Matasyoh J, Nakavuma J. Isolation of mycotoxigenic molds contaminating maize and groundnut in selected districts of Kenya. Research Journal of Microbiology. 2014;9:177–188.
20. Pitt J. Toxigenic fungi and mycotoxins. British Medical Bulletin. 2000;56:184-192.
21. Abass S, Hussain K, Hussain Z, Ali R, Abass T. Antibacterial activity of different soaps available in local market of Rawalpindi (Pakistan) against daily encountered bacteria. Pharmaceutica Analytica Acta. 2016;7:522.
22. Saleh A, Zhang Q, Chen J, Shen Q. Millet grains: Nutritional quality, processing, and potential health benefits. Comprehensive Reviews in Food Science and Food Safety. 2013;12:281–295.
23. Abass H, Cartwright R, Xie W, Mirocha C, Richard J, Dvora J, Sciumbato G, Shier W. Mycotoxin production by *fusarium proliferatum* isolates from rice with *fusarium sheath* rot disease. Mycopathologia. 1999;147:97–104.
24. Makun H, Gbodi T, Akanya H, Sakalo A, Ogbadu H. Fungi and some mycotoxin contamination in rice (*Oryza sativa*) in Niger state, Nigeria. African Journal of Biotechnology. 2007;6:99–108.
25. Lacey J. The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. Journal of Stored Product Research. 1988;24:39 – 50.
26. Desjardins A, Manandhar G, Plattner R, Maragos C, Shrestha K, McCormick S. Occurrence of *Fusarium* species and mycotoxins in nepalese maize and wheat and the effect traditional processing method on mycotoxin levels. Journal of Agricultural and Food Chemistry. 2000;48:1377–1383.
27. Mostafa A, Al-Rahmah A, Abdel-Megeed A. Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. Journal of Medicinal Plants Research. 2011;5(17):4231–4238.
28. Sharma N, Tiwari R, Prakash-Srivastava M. *Zingiber officinale* roscoe. Oil: A

preservative of stored commodities against storage mycoflora. International Journal of Current Microbiology and Applied Sciences. 2013;2:123–134.

29. Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *Aspergillus niger*. World Journal of Medical Sciences. 2008;3(2): 81–88.

30. Jadon R, Dixit S. Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains. Journal of Medicinal Plants Studies. 2014;2:58–63.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27335