Natural Occurrence of Moulds and Mycotoxins in *Synadenium glaucescens* Extracts (SGE) under Different Storage Conditions

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Abstract

Fungal growth and mycotoxin contamination in value-added medicinal plants products are quality and safety attributes that negatively affect entry to the market. This research aimed at investigating the occurrence of spoilage fungi and mycotoxins in Synadenium glaucescens extracts (SGE) from different plant parts and storage conditions. Laboratory whole water extraction method was used to prepare SGE from root-wood, root bark, leave, stem-bark and stem-wood. SGEs were subjected to storage temperature (25°C and 4°C refrigeration) and light (light and dark) conditions for 21 days. Samples were evaluated weekly to enumerate the occurrence of spoilage fungi and identified. In a follow-up experiment, pure cultures of Fusarium moniliforme and Aspergillus flavus were inoculated in SGEs and incubated for 14 days to allow production of mycotoxins. Aflatoxin and fumonisins were quantified using LC-MS/MS. It was established that 70% of samples of SGE contained Fusarium moniliforme and 60% Rhizopus spp. SGE samples stored under full light illumination were spoiled by Rhizopus species (35%), F. moniliforme (30%), F. pallidoroseum (3%), Cladosporium leguminicola (5%), C. sphaerospermum (2%), Alternaria alternate (6%) and Curvularia lunata (4%). The highest isolation frequency of F. moniliforme was in SGE from root wood (42%) and stem (42%). The highest (38%) isolation frequency of Rhizopus sp. was in SGEs from stem wood followed by root bark (32%) and 30% in both stem and root. Aflatoxin B1 was not detected in any sample. Fumonisin B1 (FB1) was detected in 80% of the samples and the concentration varied from 0.01µg/Kg to 6.33 µg/Kg. Among the samples contaminated with FB1, SGEs made from roots were contaminated by FB1 in a range of 0.03 to 0.04 µg/Kg, stem wood from 1.52 to 6.33 µg/Kg while in the root bark varied from 0.01 to 1.83 µg/Kg. SGE made from stem bark had FB1 ranging from 1.03 to 4.04 µg/Kg. Since fungal contamination was noted after 21 days of incubation, the source of spoilage fungi could be from the environment during postharvest handling. Therefore, it can be recommended that SGE safety can be ensured if good manufacturing practices (GMP) are maintained during preparation. Moreover, the Leaf SGEs were less vulnerable to fungal growth and fumonisin contamination at room temperature. Therefore, where the efficacy is the same, the leaf of S. glaucescens is possibly a better source of SGE formulations. These findings provide a benchmark of future investigations for more innovative GMP and safety measures to protect consumers against risks of exposure to mycotoxins.

Keywords: *Synadenium glaucescens* syrup, fungi, spoilage, aflatoxin, fumonisin

Introduction

Mycotoxin contamination of food and feed is a worldwide safety concern in public health (Miedaner, Gwiazdowska, and Waśkiewicz 2017). The presence of mycotoxins in the food and feed chain causes great fear over human health because mycotoxins can induce severe toxicity effects at low dose levels (Eskola *et al.* 2020). The toxins are produced by some toxigenic fungal species that belong mainly to genera *Aspergillus, Fusarium, Claviceps, Penicillium,* Stachybotrys, and *Altenaria*

(Rocha-Miranda and Venâncio 2019; Wild and Gong 2010). The proliferation of toxigenic fungi and production of mycotoxins on food and feed occur at any point along the product chain from farm to folk (Gaddeyya *et al.* 2012). Some of the most common mycotoxins are aflatoxins, fumonisins, and patuline. Liquid extracts of plants like edible fruits and medicinal plants are among the substrates that are likely to favour the growth of mycotoxigenic fungi and the production of mycotoxins.

Like many other sub-Saharan countries, Tanzania has been using medicinal plants for many centuries (Elsie *et al.* 2016; Suma *et al.* 2018). The people of Tanzania believe herbal products are closer to nature and free of any side effects. There is a wide variety of herbs in Tanzania due to her rich biodiversity in varied ecological conditions. Synadenium glaucescens (Euphorbiaceae) is one among many medicinal herbal plants that are indigenous to East Africa including Tanzania (Elkarim *et al.,* 2020; Mabiki *et al.,* 2019). The plant is known to be of great importance to mankind, especially cherished for its effectiveness in the treatment of both animal and human illnesses such as excessive menstruation, skin conditions, sickle-cell, diabetes, hypertension, sores and wounds (Mabiki *et al.,* 2013). Medicinal decoctions are prone to deterioration due to impurities that can cause attack by biological contaminants including fungi. It has been established that whether used fresh or dried, some of these medicinally important plants are facing serious problems of attack by various phytopathogens. Various phytopathogens adversely affect medicinal plant parts and decrease the medicinal value of the part. It may be harmful to the human body while using these infected parts as a medicine. Previous studies have revealed significant contamination of *A. niger*, *Penicillium janthinellum, Aspergillus flavus, Aspergillus brassicae, Aspergillus pullulans, Drechslera* sp. and *P. janthinellum* in various species of medicinal herbal products (Probst *et al.* 2011; Chilaka *et al.* 2016; Adetuniji *et al.* 2014; Chilaka *et al.* 2017). Discolourations, quality deteriorations, reduction in commercial values as well as in therapeutic potential and mycotoxin production have been linked to mouldy contaminated herbal drugs (Gautam and Bhadauria 2009; Rocha-Miranda and Venâncio 2019).

The contamination of herbal products with mycotoxins does not only cause health hazards but also results in economic losses, especially for products meant for export. The mycotoxins most commonly found in fresh and their processed plant products are aflatoxins, fumonisins, ochratoxin A, and patulin (Rocha *et al.* 2009). Toxins produced by *Alternaria* sp. Include alternariol, alternariol methyl ether and attenuate (Rocha *et al.* 2009). These toxins may be found in syrups resulting from the use of contaminated raw material or invading toxigenic fungi in the final manufactured product.

To protect consumers against these toxins, over 100 countries globally including Tanzania established maximum tolerable limits (MTL) for different food and feed products (Kimanya *et al.* 2008; Kimanya *et al.* 2012). In Tanzania, the ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC) through its council for Traditional medicine has set a condition that a registered traditional medicine should be free from aflatoxins (Kheir *et al.,* 2020). To limit, mycotoxin contamination below the MTL, preventive measures like good agricultural practices (GAP) and good manufacturing practices (GMP) are forefront strategies (Amoa-Awua *et al.* 2007). The practices must aim to create unfavourable conditions for the growth of fungi and the production of mycotoxins. Under storage conditions, fungal proliferation and production of mycotoxin are favoured by certain environmental factors such as humidity, illumination, and temperature, as well as certain biotic conditions like the type of substrate.

Even with such numerous studies regarding the processing and storage of plant extracts of Synadenium glaucescens, findings of occurrence of fungi and their mycotoxins in Synadenium glaucescens extracts from different parts of the plant are currently lacking in the literature. The present study aimed at optimizing storage conditions for good quality and safety of extracts prepared from Synadenium glaucescens plant parts.

Methodology

Source of *Synadenium glaucescens* extract (SGE)

Twenty (20) samples (10ml each) of SGE were obtained from the chemistry laboratory of the College of Natural and Applied Sciences (CoNAS), Sokoine University of Agriculture. Of the 20 samples, 5 samples were extracts from each of the five plant part categories (root-wood, root bark, leaf, stem-bark and stem-wood). Harvesting of Synadenium glaucescens from the wild, sample preservation, preparation of extract from different parts of the plant were as per Mabiki *et al.* (2013).

Experimental design

A triplicated completely randomized design experiment involving two storage temperature conditions (4°C and 25°C) and illumination (dark and light) was established under laboratory conditions. The experiment aimed to determine the influence of these factors on the growth of mycotoxin producing fungi and the presence of mycotoxins in SGE from root-wood, root bark, leaf, stem-bark and stem-wood. In a follow-up experiment, pure cultures of Aspergillus flavus and Fusarium moniliformin were artificially inoculated in the SGEs from root-wood, root bark, leave, stem-bark and stem-wood to evaluate growth and production of aflatoxins and fumonisins, respectively. Extracts for natural and artificial inoculation were kept in sterile glass petri dishes (90mm) for seven days before they were subjected to mycological analysis and quantification of mycotoxins.

Observation of spoiled SGE

After seven days, observations were made to identify spoiled SGE and data counts were recorded including the nature of spoilage. The observation was made for 21 days, three times at 7 days interval. After 21 days, spoiled and unspoiled samples were subjected to mycological and mycotoxin analysis.

Identification of Spoilage fungi

After 21 days of observation, the SGE samples were transferred to the African Plant and Seed Health laboratory of the Sokoine University of Agriculture for mycological analysis. To develop pure cultures for fungal identification, one milliliter (1ml) of each of the tested SGE was transferred aseptically into sterilized petri dishes using a heat sterilized isolation loop. The petri dishes contained 12-15ml of glucose Czapek's Agar medium with the following compositions per litre; 2g $NaNO_3$, 1g $K2HPO_4$, 0.5g $MgSO_4.7H_2O$, 0.5g Yeast extract, 10g glucose and 15g Agar (Menaka *et al.,* 2011). Before inoculation, the media was autoclaved at 121°C, 15pa for 15 minutes and cooled to just above solidifying temperature before they were dispensed into petri dishes. While adding the SGE, the dishes were rotated by hand in broad swirling motion so that the SGE is dispersed in the agar. The plates were incubated at room temperature for 7 days and sub-cultured to a fresh medium for culture purification using a method described in Degraeve *et al.* (2016). Where possible, fungi were identified to species level directly from colonies on Potato Dextrose Agar (PDA) media using well-established techniques of macroscopic and microscopic examination and standard reference books for the identification of moulds using lactophenol blue stain (Gautam and Dill-Macky 2012; Popovski and Celar 2013). A portion of the obtained culture was placed and teased out into a clean glass slide upon a drop of lactophenol cotton blue using sterile inoculating needles and covered with a clean coverslip (Morufat and Muhammad 2018). The light microscope depended on studying the morphological characteristic and microscopic characteristics which was compared to the mycological atlas for confirmatory identification (Ahmad *et al.* 2014). The relative density (RD) and isolation frequency (Fr) of species were calculated as per equations 1 and 2, respectively.

$$RD = \frac{Number\ of\ isolates\ of\ a\ species\ or\ genus}{Total\ number\ of\ fungal\ isolates} \times 100 \quad (1)$$

$$Fr = \frac{Number\ of\ samples\ with\ a\ species\ or\ genus}{Total\ number\ of\ samples} \times 100 \quad (2)$$

Artificially inoculated fungi in SGEs

A follow-up experiment was performed to find out mycotoxigenic fungi's ability to produce mycotoxins in SGEs under artificial inoculation. Pure cultures of F. moniliforme and

A. flavus were artificially inoculated in SGEs maintaining uninoculated samples as control. The pure cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in the laboratory at 25±2°C. Such mother culture slants were preserved at 5°C in the refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies. To inoculate in SGE, petri plates containing 20 ml of PDA medium were inoculated with 9 mm mycelial disc from 10-day old culture of different isolates. Disks of mycelium were cut with a flamed corkborer and transferred to petri dishes containing PDA media. These plates in triplicate were incubated at 25°C for 10 days.

Mycotoxin analysis

For the quantification of mycotoxins in SGE, samples were sent to an ISO accredited laboratories of the Tanzania Bureau of Standards (TBS) (ISO 9001:2015 Certified). A method developed by Arroyo-Manzanare *et al.* (2015) for the determination of mycotoxin in cereal syrup was used with little modification. In sub sections 2.6.1 to 2.6.4 below, we describe the used chemicals and reagents, instruments and equipment, sample treatments and the LC-MS/MS used for quantification of fumonisins and aflatoxins in SGE.

Chemicals and reagents

Fumonisin and Aflatoxin standards were obtained from Supelco (Bellefonte, PA). Ethyl acetate was obtained from Merck (Darmstadt, Germany), n-hexane and perchloric acid from JT Baker (Deventer, Netherlands). Acetonitrile was bought from LGC Promochem (Wesel, Germany), acetic acid from Carl Roth (Karlsruhe, Germany), sodium hydrogen carbonate from Alkaloid (Skopje, Macedonia), and sodium sulphate anhydrous from Lach-ner (Neratovice, Czech Republic). Ultrapure water was produced by GenPure Water Purification System (Thermo Scientific, Thermoelectron LED, Langenselbold, Germany).

The reagents used were of analytical grade. Solvents were of HPLC grade and standard aflatoxins and fumonisins were of analytical grade. Formic acid was used as a mobile phase additive for HPLC methanol (MeOH), ammonium formate and individual standards of aflatoxins and fumonisins were obtained from Sigma Aldrich (St Louis, MO, USA). For sample treatment, formic acid (analysis grade) was procured from Merck (Darmstadt, Germany), and an HPLC grade acetonitrile (MeCN) from Panreac (Madrid, Spain) were used. Ultrapure water (18.2 MO cm1, Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout the analysis. Mycotoxin extraction kits; SampliQ QuEChERS consisting of either buffered QuEChERS extraction packed (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate and 0.5 g disodium hydrogen citrate sesquihydrate) or non-buffered QuEChERS extraction packed (4 g $MgSO_4$, 1 g NaCl) were acquired from Agilent Technologies Inc. (Wilmington, DE, USA). Acrodisc 13 mm syringe filters with 0.2 lm nylon membrane (Pall Corp., MI, USA) were used for filtration of extracts prior to the injection into the chromatographic system.

Instruments and equipment

Mycotoxin analyses were carried out using an Agilent 1290 Infinity LC (Agilent Technologies, Waldbron, Germany) furnished with a binary pump, online degasser, autosampler (5 lL loop) and a column thermostat. The MS measurements were performed on a triple quadrupole MS API 3200 (AB SCIEX, Toronto, ON, Canada) with electrospray ionisation (ESI). As chromatographic column, the A C18 Zorbax Eclipse Plus RRHD (50 2.1 mm, 1.8 lm) was used. For sample preparation, a Universal 320R centrifuge (Hettich Zentrifugen, Tuttlingen, Germany), a vortex-2 Genie (Scientific Industries, Bohemia, NY, USA) and an evaporator System (System EVA-EC, from VLM GmbH, Bielefeld, Germany) were used. Instrumental data were collected using the Analyst Software version 1.5 with Schedule MRM TM Algorithm (AB Sciex).

Sample treatment

Two grams of each sample of Synadenium glaucescens extract (SGE) and 8 mL of 30 mM $NaH2PO_4$ pH 7.1 were placed into a 50 mL screw-cap test tube with conical bottom, which was shaken by vortex for 10 s. Subsequently, 10 mL of 5% formic acid in MeCN was added

to the tube, and it was shaken again by vortex for 2 min. Agilent SampliQ EN QuEChERS extraction kit (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate and 0.5 g disodium hydrogen citrate sesquihydrate) was added and the tube was quickly shaken vigorously for 1 min. Then, the samples were centrifuged at 4500 rpm for 5 min followed by transferring 2 mL of the upper MeCN layer to a vial which was evaporated to near dryness under a gentle stream of nitrogen and reconstituted with 1 mL of MeOH:H_2O (50:50, v/v). The samples were filtered with a 0.2 lm filter before injection and aflatoxins and fumonisins were determined by LC-MS/MS.

LC-MS/MS analysis

The chromatographic method was used for the determination of the selected mycotoxins involved UHPLC separations which were performed in a C18 column (Zorbax Eclipse Plus RRHD 50 2.1 mm, 1.8 lm). The separation performance used a mobile phase consisting of 0.3% aqueous formic acid solution with 5 mM ammonium formate (solvent A), MeOH with 0.3% formic acid and 5 mM ammonium formate (solvent B) at a flow rate of 0.4 ml min-1. The eluent gradient profile was set as follows: 0 min: 5% B; 1 min: 50% B; 2 min: 72% B; 4 min: 80% B and 6 min: 90% B. Lastly it was back to 5% B in 0.2 min and maintained for 1.8 min for column equilibration. The temperature of the column was 35°C and the injection volume was 5 lL (full loop). The MS worked with ESI in positive mode and under the multiple reaction monitoring (MRM) conditions which were optimized as in Arroyo-Manzanares *et al.* (2015). The ionisation source parameters were: source temperature 500 C; curtain gas (nitrogen) 30 psi; ion spray voltage 5000 V; and GAS 1 and GAS 2 (both of them nitrogen) were set to 50 psi.

Statistical analysis

The data were statistically analysed using the Xlstat (version 2017.5) and the SPSS software (version 20). Descriptive statistics and the ANOVA calculates whereby if the test revealed a significant effect (significance level α=0.05), a posthoc Tukey test was performed.

Results

Fungal spoilage of stored SGE

The incidence of fungal growth in SGE samples stored under refrigeration and room temperature were significantly different (p=0.001) (Fig. 1). SGE samples stored at room temperature (25°C) had higher (35%) spoilage incidences compared to 0% spoilage of samples under refrigeration (4°C) (Fig. 1A). Whether the SGE samples were stored under light or darkness, findings in Figure 2 show that these factors had no significant (p=0.001) influence on the spoilage incidence (Fig. 1B). Spoilage frequency also varied (p=0.001) with parts of *Synadenium glaucescens* plant from which SGE was obtained. Root wood, stem bark, and root bark had spoilage incidence between 38-40% while all other parts had spoilage incidence less than 20% (Fig. 1C). Combined analysis shows that all combinations involving refrigeration storage did not show fungal spoilage symptoms while all combinations with room temperature storage showed contamination at varying frequencies depending on whether they were exposed to light or darkness and also regardless of the type of plant part used (Fig. 1D)

Occurrence of Fungal species

Effects of temperature

The results in Table 1 shows that the temperature conditions in which SGEs were stored significantly influenced on the occurrence of fungal species (p=0.001) No fungal species were isolated from samples stored under refrigeration but numerous of them were recovered from SGEs stored at room temperature. About seven in every ten samples of SGE contained *Fusarium moniliforme* (relative density = 40%), while about 60% of these samples had *Rhizopus* species (relative density = 35%). Isolation frequency of less than 10% was observed for *F. palidoroseum*, *C. sphaerospermum*, *Curvalaria lunata* and *Alternaria alternate* while *Cladosporium leguminicola* had an isolation frequency of 13.81% (relative density 9%).

Effects of light

Observations in this study show that darkness significantly initiated the growth of spoilage

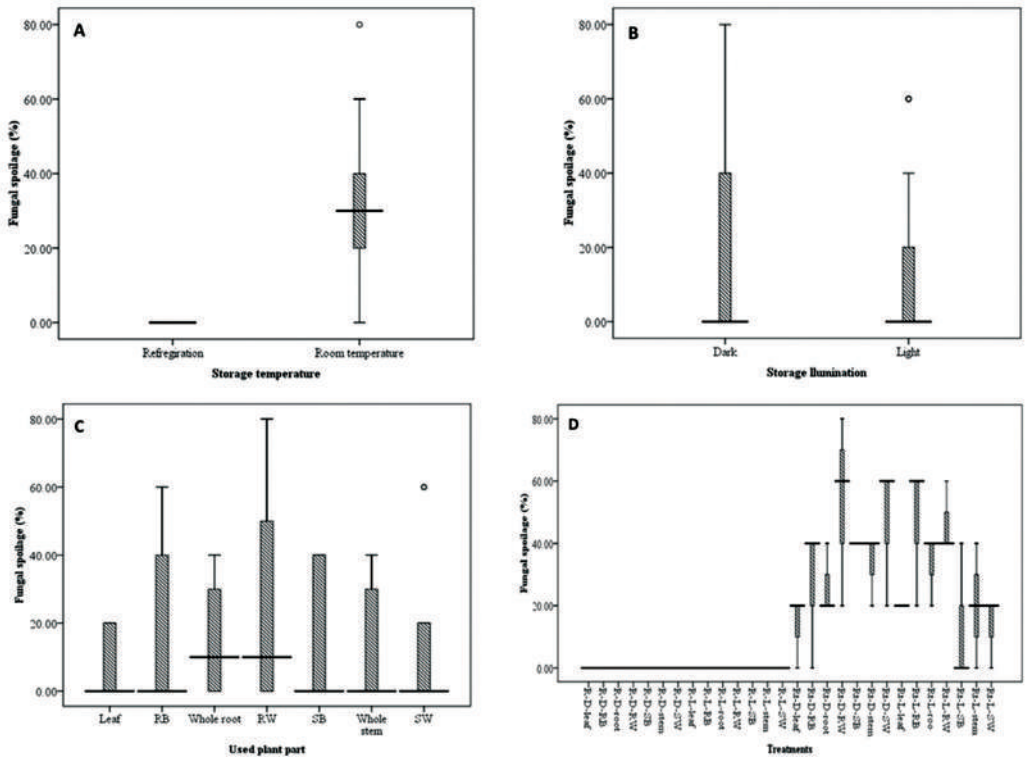

Figure 1: Effects of temperature (A), illumination (B), plant parts (C) and treatment combinations on the incidence of fungal spoilage of SGE (D) interaction of the three factors: R=Refrigeration, Rt=Room temperature, D=Darkness, L=Light, RB=root bark, RW=Root wood, SB- stem bark, SW=Stem wood

fungi and made SGE samples more vulnerable to spoilage than samples that were exposed to normal light illumination. At varying levels, all SGE samples stored under full light illumination were infected by spoilage fungi notably *Rhizopus* species (35%), *Fusarium moniliforme* (30%), *F. pallidoroseum* (3%), *Cladosporium leguminicola* (5%), *C. sphaerospermum* (2%), *Alternaria alternate* (6%) and *Curvularia lunata* (4%) (Fig. 2). Regarding fungal species relative density, the results show that, of all the isolates recovered from SGE samples, 20% and 19% were *F. moniliforme* in darkness and light stored SGEs respectively. Around 15% and 18% of the isolates were *Rhizopus* sp. in SGEs stored in darkness and light respectively. The rest 38% of the isolates constituted *F. palidoroseum, Cladosporium leguminicola,*

Table 1: Occurrence of spoilage fungi in SGEs stored under a different temperature conditions

	Isolation frequency (Fr) (%)		Relative density (RD) (%)	
	Rt (25°C)	Rf (4°C)	Rt (25°C)	Rf (4°C)
Rhizopus sp.	58.57	0	35.19	0
Fusarium moniliforme	69.05	0	39.54	0
Fusarium pallidoroseum	7.62	0	4.15	0
Cladosporium leguminicola	13.81	0	8.99	0
Cladosporium sphaerospermum	4.76	0	2.77	0
Curvularia lunata	6.67	0	3.66	0
Alternaria alternata	9.52	0	6.34	0

Key: *Rt = Room temperature storage, Rf = Refrigeration cold storage*

C. sphaerospermum, Curvalaria lunata and *Alternaria alternate* (Fig. 2B).

lunata and *Alternaria alternata*. Isolation frequency of these spoilage fungi across SGEs

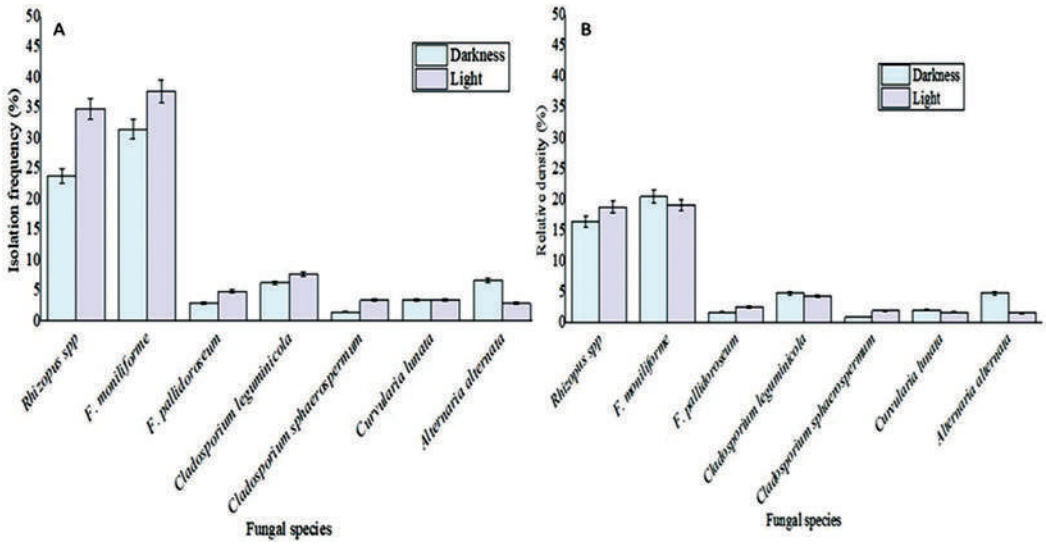

Figure 2: Isolation frequency and relative density of fungal species in SGEs under different storage light regimes

Fungal species in SGEs from different plant parts

Figure 3 shows that SGEs from all *Synadenium glaucescens* plant parts used in this study contained *Rhizopus* sp., *Fusarium moniliforme, F. palidoroseum*, *Cladosporium leguminicola, C. sphaerospermum*, *Curvalaria* of different plant parts varied significantly (p = 0.002). *Fusarium moniliforme* was the most abundant in all SGEs. The highest isolation frequency of *F. moniliforme* was in SGE from root wood (42%) and stem (42%). This was followed by 36% Fr in stem wood, stem bark and stem wood (Fig. 3A). *Rhozopus* sp. was the

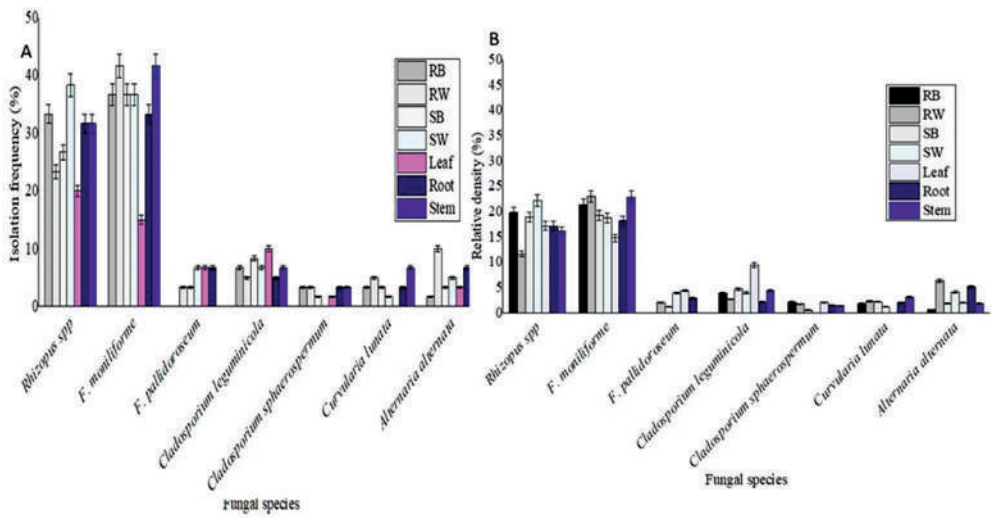

Figure 3: Isolation frequency (A) (p=0.002) and relative density (B) (p=0.004) of spoilage fungi isolated from different parts of SGEs. SB=stem bark, RB=root bark, SW=stem wood, RW=root wood

second most abundant fungi in all SGEs. The highest (38%) isolation frequency of *Rhizopus* sp. was in SGEs from stem wood followed by root bark (32%) and 30% in both stem and root. Less than 10% isolation frequency was observed for other fungal species. With reference to the numbers of isolates, species identification showed that relative density varied (p=0.004) with type SGE. For SGEs of RB, SB, and stem, in every 10 isolates, 2 were *F. moniliforme*. Similarly, for SGEs of RB, SB, SB and SW, for every 10 isolates, 2 were *Rhisopus* sp. (Fig. 3B).

Interaction effects

Figures 4A and 4B present the findings of the occurrence of spoilage fungi in SGEs that were stored under room temperature conditions. There were no fungal species isolated from SGEs that were stored under cold conditions. The occurrence of spoilage fungi and species relative density in SGEs from different parts of a plant was significantly (p=0.002) dependent on the storage light and storage temperature (Fig. 4A and B).

78%Fm). The *Synadenium glaucescens* extracts treated as RT-L-SW and Rt-L-RB were equally contaminated by *F. moniliforme* at an occurrence of 98% and 96% respectively. This was followed by the occurrence of the *F. moniliforme* in Rt-D-RB, Rt-D-SB and Rt-D-stem at equally 80%. Low occurrence of *F. moniliforme* was observed in Rt-L-leaf (20%) and Rt-D-leaf (40%). Seventy-five percent of each of the *Synadenium glaucescens* extracts were treated with R-D-RB, Rt-D-SW, Rt-L-SW, Rt-L-root and Rt-L-stem contained *Rhizopus* sp. The least (25%) occurrence of *Rhizopus* sp. was recorded in *Synadenium glaucescens* extracts that were treated with Rt-D-leaf (Fig. 4A).

Fungal species relative frequency varied significantly (p=0.002) between treatments. For *F. moniliforme*, the lowest (20%) relative density (RD) was recorded in SGEs treated with Rt-L-leaf while the highest (48%) RD of the same was in Rt-D-RB, Rt-D-SB, Rt-D-stem, Rt-L-RB and Rt-L-SW. *Rhizopus* sp. had the lowest (18%) RD in *Synadenium glaucescens* extracts treated with Rt-D-SW and the highest (50%)

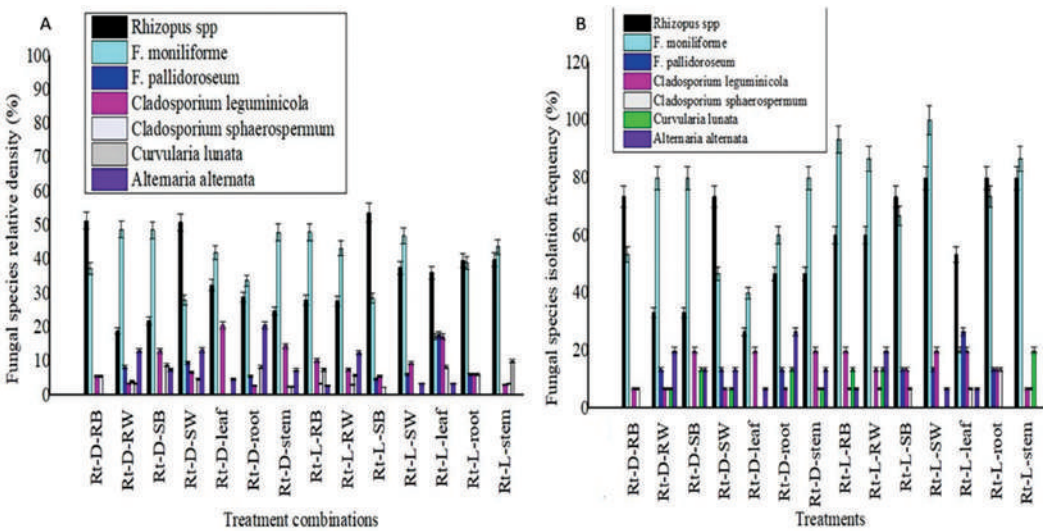

Figure 4: Isolation frequency and fungal species relative density in SGE samples under different treatment combinations (p=0. 002). SB=stem bark, RB=root bark, SW=stem wood, RW=root wood

Both *Rhizopus* sp. (Rh) and *F. moniliforme* (Fm) were the most abundantly occurring in almost every sample. The two equally occurred in samples of RT-L-SB (60%Rh, 63%Fm), Rt-L-root (70%Rh, 76%Fm) and Rt-L-stem (76%Rh, was in each of the Rt-D-RB, Rt-D-SW and Rt-L-SB samples.

Occurrence of aflatoxins and fumonisins

Table 2 shows the level of fumonisin

associated with the SGE samples analyzed. In all the samples, aflatoxins were not detected. The results suggest that SGEs that were kept at room temperature were all positive for fumonisins except samples of Rt-D-leaf and Rt-L-leaf. For the positive samples, the toxins were detected during the second week following storage and the mean concentration varied from 0.01µg/Kg to 6.33 µg/Kg. Among the samples contaminated with FB1, SGEs made from roots had consistently the lowest FB1 concentration ranging from 0.03 to 0.04 µg/Kg. The concentration was high in SGEs made from stem bark stored in both light and darkness. In the stem wood, the concentration varied from 1.52 to 6.33 µg/Kg while in the root bark the FB1 concentration varied from 0.01 to 1.83 µg/Kg. Mycotoxigenic fungi were also inoculated in SGE made from stem bark where the FB1 concentration ranged from 1.03 to 4.04 µg/Kg.

Discussion

Occurrence of spoilage fungi in SGE

For the first time this study is reporting the occurrence of mycotoxins and mycotoxigenic fungi in herbal syrups. The use of these plant

Table 2: Occurrence of fumonisins and aflatoxins in SGE samples analyzed (Nd = Not detected)

Treatments	Fumonisin B1 (µg/kg)	Aflatoxins B1 (µg/kg)
R-D-SB	Nd	Nd
R-D-SW	Nd	Nd
R-D-leaf	Nd	Nd
R-D-root	Nd	Nd
R-D-stem	Nd	Nd
R-L-RB	Nd	Nd
R-L-RW	Nd	Nd
R-L-SB	Nd	Nd
R-L-SW	Nd	Nd
R-L-leaf	Nd	Nd
R-L-root	Nd	Nd
R-L-stem	Nd	Nd
Rt-D-RB	0.01	Nd
Rt-D-RW	0.60	Nd
Rt-D-SB	1.03	Nd
Rt-D-SW	6.33	Nd
Rt-D-leaf	Nd	Nd
Rt-D-root	0.04	Nd
Rt-D-stem	0.06	Nd
Rt-L-RB	1.83	Nd
Rt-L-RW	2.38	Nd
Rt-L-SB	4.06	Nd
Rt-L-SW	1.52	Nd
Rt-L-leaf	Nd	Nd
Rt-L-root	0.03	Nd
Rt-L-stem	4.67	Nd
Control (uninoculated)	Nd	Nd

products have been useful medicinal herbs for decades in Tanzania and other sub-Saharan African countries (Sánchez-Rangel *et al.,* 2012; Suma *et al.* 2018; Mabiki *et al.* 2013). The identified fungal contaminants of herbal syrups were *Rhizopus* species, *Fusarium moniliforme, F. pallidoroseum, Cladosporium leguminicola, C. sphaerospermum, Alternaria alternata* and *Curvularia lunata*. These fungi occurred as isolates from SGE samples made from the leaves, stems, stem-bark, stem-wood, root-bark and root-wood of *Synadenium glaucescens*. This suggests that no part of *Synadenium glaucescens* can be considered immune from fungal attacks. However, the study elucidates that, SGEs made from leaves were less vulnerable to most of the spoilage fungi than SGE samples made from the rest of plant parts. Previous studies have established that compared to any other part of the plant, leaves, seeds and to lesser extent fruits are commonly preferred herbal extracts. Reasons to why leaves are of choice could not be established in this study. Similar reports have established that such fungi are common fungi that cause spoilage of strawberries, fruits, and vegetables (Gautam and Bhadauria 2009; Ahmad *et al.* 2014; Bankole and Adebanjo 2003).

Levels of spoilage and frequency of fungal occurrence were closely related to storage conditions. The SGE samples stored at room temperature were highly contaminated by almost all the identified fungal species. However, *Fusarium moniliforme* (also known as *Fusarium verticillioides*) attacked the SGEs the highest suggesting they are vulnerable to this fungi. The occurrence of *F. moniliforme* in fruit and other plant products is also reported by Rocha-Miranda and Venancio (2019). Apart from deteriorating the quality of the spoiled product, *Fusarium moniliforme* produces fumonisin, a mycotoxin associated with various illnesses to humans and animals including cancer (Sánchez-Rangel *et al.,* 2012; Madege *et al.* 2018).

Rhizopus species which were also isolated in great proportions of SGEs are also known to be spoilage fungi of many foods and feed products. Kumar *et al.* (2015) reported that *Rhizopus* sp. is important spoilage fungi of bread. In this study, the prevalence of *Cladosporium* species in the SGEs was varying with different incubation temperatures and light regimes under invitro conditions. *Cladosporium* sp. are mostly known as potentially pathogenic fungus frequently occurring in outdoor environments (Alwatban *et al.,* 2014). This implies that these fungi possibly were brought in the laboratory as systemic fungi through plant tissues. Since the literature sources do not offer enough to compare these postharvest spoilage fungi, it suggests that possibly this is one of the few studies reporting on the occurrence of *Cladosporium leguminicola, C. sphaerospermum* as spoilage fungi of herbal syrups under storage environment. *Cladosporium* sp. are known mycotoxin secreting fungi which pose a safety concern to the use of SGEs if control measures are not considered throughout the production chain.

Alternaria alternate was another fungal species discovered in SGE spoiled samples. These findings are in line with previous studies which report that Alternaria infects fruits and vegetables (Feng and Zheng 2007). The observed infection in SGE confirms the previous reports that *Alternaria alternata* is a common pathogen that infects many plant species under field conditions as well as postharvest plant-based products (Feng and Zheng 2007). The importance of *A. alternata* is not only yielding loss, but also the cause of food and feed safety worries as these fungi produce mycotoxins like alternations, altenuene, tentoxin, and tenuazonic acid (Blandino *et al.* 2017).

Storage practices can also be associated with increased or reduced occurrence of spoilage fungi in stored products. In this study, SGE samples were subjected to different storage temperatures and light regimes. Hence, the study established that *Synadenium glaucescens* extracts were differentially vulnerable to spoilage fungi depending on the part of the plant and storage conditions. It is established that SGEs stored under the cold condition at a temperature of 4^{o}C were completely not attacked by all spoilage fungi. This proves that a cold chain of fresh products is critical because fungi invasion in stored products causes loss, colour change, flavour, and degradation of nutritive value

The occurrence of spoilage was high in samples stored at room temperature. Room temperature (25-27^{o}C) is a suitable condition for the growth of most fungal species (Lahouar *et al.* 2017; Dantigny 2021). The vulnerability of SGEs under different light conditions were also tested. Findings suggest that there was little difference in fungal spoilage between samples kept in darkness and light, an observation which conforms to previous studies (Beyer *et al.* 2004; Mallikarjunan *et al.* 2016). Therefore, it is concluded that whether mould grows faster in a light or dark environment depends on other environmental factors (Dantigny 2021; Liu *et al.* 2016).

Occurrence of fumonisin B1 and Aflatoxin B1 in SGEs

Fumonisin B1 (FB1) is a mycotoxin produced by the fungus *Fusarium verticillioides* (*F. moniliforme*), which commonly infects maize and other agricultural products. From artificial inoculation of *F. moniliforme* in SGEs, fumonisin B1 was detected in many samples which were stored at room temperature but no FB1 was detected in SGEs that were kept under refrigeration (4^{o}C). The result suggests that on one hand cold storage is not an optimal condition for fungal secondary metabolism, on the other hand, room temperature storage will not only increase product deterioration but also worsen the safety of the stored product through contamination of mycotoxins (Drakulic *et al.,* 2017). Previous studies have established that fumonisin production by *F. verticillioides* starts at a temperature of 15^{o}C and maximum is attained at 25^{o}C (Madege *et al.* 2018)

The study has established that, among the SGE samples that were kept at room temperature, FB1 was not detected in SGEs from leaves of *Synadenium glaucescens*. The reason for this observation could not be established in this study. A similar study involving Chenopodium album plant established that roots and leaves of the plant had numerous chemical compounds (2(3H)-furanone, dihydro-4,4-dimethyl; 9-octadecenoic acid (Z), methyl ester; 9,12-octadecenoic acid (Z), methyl ester; 6-methylene bicyclo (3.2.0) hept-3-en-2-one., 1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester and hexadecanoic acid, methyl ester) with possible antifungal effects (Alkooranee *et al.* 2020). Possibly, this is the reason SGEs from roots of *Synadenium glaucescens* stored in darkness had 158 times lower concentrations of FB1 than SGE from stem bark under the same storage conditions. Similarly, FB1 concentration in SGE from roots of *Synadenium glaucescens* stored under darkness was 51 times lower than the concentration in SGE made from stem bark and stored in light. In the different storage conditions, spoilage incidences at room temperature were observed after seven days of incubation suggesting that these fungi likely were a result of postharvest handling and not inherent in plant tissues from the wild.

Conclusion

It is clear from this study that SGEs made from different parts of *Synadenium glaucescens* have different vulnerabilities to spoilage fungi. The spoilage can be aggravated by storage conditions such as room temperature since it makes the syrups more susceptible to spoilage fungi. The study did not establish clear evidence on whether the storage of syrup in light or darkness changed the spoilage potential. The fact that spoilage and fungal growth happened after twenty-one days of incubation, we can conclude that the fungi did not come with plants from the field but likely were introduced during post-harvest handling in the laboratory. It is recommended that the GMP be emphasized. Furthermore, where efficacy is the same, the leaf of *S. glaucescens* is possibly a better source of SGE formulations.

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