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Mycotoxin Contamination of Dietary and Medicinal Wild Plants in the Eastern Cape Province of South Africa

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Nineteen dietary and 30 medicinal wild plants used by residents of the Eastern Cape Province of South Africa were investigated for the presence of fumonisin B_1 and aflatoxin B_1. The plants were extracted in water, and cleanup was undertaken on immunoaffinity cartridges; analysis was by HPLC using fluorescence detection. None of the plant extracts contained detectable levels of aflatoxin B_1; however, eight plants, four dietary and four medicinal, were positive for fumonisin B_1 at levels ranging from 34 to 524 μg/kg and from 8 to 1553 μg/kg, respectively. The presence of fumonisin B_1 was confirmed by LC-MS/MS using positive ion electrospray ionization. Fumonisin B_1 provided characteristic fragment ions at *m*/*z* 704, 686, 546, 528, 370, and 352 corresponding to sequential loss of H_2O and tricarboxylic acid moieties from the alkyl backbone. These results indicate that exposure to fumonisin B_1 is much more widespread than initially thought and is the first report of mycotoxin contamination in South African medicinal and dietary wild plants.

KEYWORDS: ***Fusarium*; fumonisins; tandem mass spectrometry; dietary wild plants; medicinal plants; aflatoxins; *marog*; *imifino***

INTRODUCTION

Mycotoxins are secondary metabolites produced by a wide range of fungi known to contaminate a variety of food and agricultural commodities worldwide (*1*) and thus have great significance in human health. There are five mycotoxins, or groups of mycotoxins, that frequently occur in food: deoxynivalenol/nivalenol; zearalenone; ochratoxin; fumonisins; and aflatoxins. The foodborne mycotoxins of most interest in South Africa are the fumonisins and aflatoxins, and reports from the literature indicate that these toxins can also be found in a variety of medicinal plants.

The fumonisins (diesters of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-polyhydroxy-eicosanes) are toxic and carcinogenic secondary metabolites, produced primarily by the fungus *Fusarium verticillioides* (Sacc.) Nirenberg (*2*). These mycotoxins have been implicated in several animal disease syndromes as well as nephrotoxicity, hepatotoxicity, and hepatocellular carcinoma in rats (*3*). Currently, 28 structural fumonisin analogues are known (*4*), but most research has focused on the most commonly and widely occurring natural form, fumonisin B_1, which has been classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC) (*5*).

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The consumption of fumonisin-contaminated corn has been statistically associated with the development of hyperendemic levels of human esophageal cancer in the Transkei region of the Eastern Cape Province of South Africa (*6*) and in Hebei and Henan Provinces in China (*7*). Furthermore, investigations have also shown an association with primary liver cancer in Henan Province (*8*).

Although it has been well established that fumonisin B_1 is a natural contaminant primarily in maize, research undertaken in Portugal has indicated the natural occurrence of fumonisin B_1 (**Figure 1**) in medicinal plants as well (*9*). In a study of 69 samples of 4 medicinal plants, namely, *Citrus sinensis*, *Tillia grandifollia*, *Stigmata maydis*, and *Matricaria chamomilla*, 59% of these samples contained fumonisin B_1 ranging from 20 to 700 μg/kg. In a subsequent study by Omurtag and Yazicioglu (*10*) several herbal teas and medicinal plants consumed regularly in Turkey were analyzed for fumonisins B_1 and B_2. Two samples were found to contain fumonisin B_1 at levels of 0.160 and 1.487 μg/g.

The aflatoxins, on the other hand, are secondary metabolites produced by some strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* in areas of the world with hot, humid climates and are known to contaminate rice, cottonseed, peanuts, peanut products, maize, and maize products (*11*). Members of this group of metabolites have closely similar structures and form a unique group of highly oxygenated,

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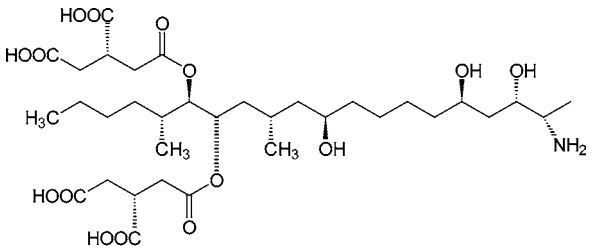
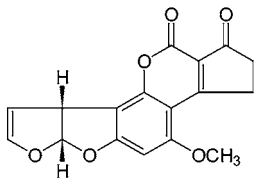


Fumonisin B_1 [1]



Aflatoxin B_1 [2]

Figure 1. Chemical structures of fumonisin B_1 and aflatoxin B_1.

naturally occurring heterocyclic compounds with specific forms designated B_1 (6-methoxydifurocoumarone), B_2, G_1, G_2, M_1, and M_2.

Because aflatoxins, especially aflatoxin B_1 (**Figure 1**), are potent carcinogens, there is interest in the effects of long-term human exposure to low levels of these important mycotoxins. In 1987, the IARC placed aflatoxin B_1 on the list of human carcinogens (*12*). This is supported by a number of epidemiological studies done in Asia and Africa that have demonstrated a positive association between dietary aflatoxins and liver cell cancer (LCC). Additionally, the expression of aflatoxin-related diseases in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation.

Although the highest concentrations of aflatoxin are produced as a result of postharvest spoilage of commodities stored under warm moist conditions, significant concentrations may also be produced in the field before harvest (*13*). A recent survey demonstrated that relatively low levels of aflatoxin can occur in herbs and spices, and these levels are not reduced by domestic cooking (*14*). An investigation into the aflatoxin contamination of plants used as medicines in Bihar, India, showed that 14 of 15 of the samples analyzed were positive for aflatoxin B_1 (*15*). The highest level of contamination was detected in the seeds of *Piper nigrum* (1.20 μg/g), followed by seeds of *Mucuna prurita* (1.16 μg/g), and the lowest level was detected in the bark of *Acacia catechu* (0.09 μg/g). Of the 158 isolates of *A. flavus* obtained from these plants, 49 were found to be toxigenic. In another study conducted in Andhra Pradesh, India, chilies (*Capsicum annuum*) were shown to contain aflatoxin B_1 (*16*). Of the 182 chili samples tested, 59% of the samples were contaminated with aflatoxin B_1 and 18% contained the toxin at nonpermissable levels (>30 μg/kg). *Aspergillus* spp. were also detected in six Asian medicinal plants (*17*); however, only one, that is, *Aerva lanata*, contained aflatoxin B_1 at a level of 0.5 μg/kg. Mycotoxin-producing fungi, namely, *A. flavus*, *Alternaria alternata*, *Penicillium chrysogenum*, and *Fusarium verticillioides*, have also been found in the leaves of *Catha edulis* in Yemen (*18*). Aflatoxin B_1 contamination was also found in six crude herbal drug preparations in Nigeria (*19*), where levels as high as 0.8 μg/g were detected.

These studies provide evidence that wild plants can be naturally contaminated with mycotoxins, and it is ironic if the treatment of one disease is the unintended cause of another. Southern Africa is exceptionally rich in plant diversity with some 30000 species of flowering plants, accounting for almost 10% of the world's higher plants, many of which, through cultural diversity, are used daily as either food or medicine (*20*). As a source of food, the leaves of various plants called *imifino* are cooked traditionally with other herbs to form a relish that is served with maize meal. In addition to food, over 3000 species are used in the informal systems of medicine with over 350 species being the most commonly used and traded medicinal plants.

Given the importance of the use of wild plants in South Africa and the concerns regarding mycotoxin contamination, the present investigation was undertaken to determine the presence of fumonisin B_1 and aflatoxin B_1, two known carcinogenic mycotoxins, in plants consumed by residents of the Eastern Cape Province of South Africa.

MATERIALS AND METHODS

Reagents and Standards. A pure standard of fumonisin B_1 was isolated at PROMEC Unit according to the method of Cawood et al. (*21*), and a stock solution was prepared at a concentration of 250 μg/mL in an acetonitrile/H_2O mixture (1:1). This solution was used to prepare a working solution at a concentration of 50 μg/mL. Aflatoxin B_1 was purchased from Sigma (St. Louis, MO) and prepared at a stock solution concentration of 125 μg/mL. From this, a working solution of 1.25 μg/mL was used in the analytical determination. Formic acid (analytical grade), KH_2PO_4, NaCl, and methanol were obtained from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was obtained from Romil (Cambridge, U.K.), whereas water for general laboratory use and HPLC mobile phase was deionized on a Milli-Q water purification system (Millipore, Bedford, MA).

Collection of Plants. Nineteen dietary and 30 medicinal plants identified by 2000 patients attending three referral hospitals in the Eastern Cape Province of South Africa were collected from Flagstaff (Hala location), Tsolo (Unga location), along the banks of the Umzimvubu River in Port St. Johns (Eastern Cape), and the Silverglen Medicinal plant nursery (KwaZulu-Natal) during November 2002. Elderly men and women familiar with local botany and potential collection locations assisted during this component of the study. The identities of the plants were authenticated by comparison with reference specimens at the Kei Herbarium (University of Transkei) and Natal Herbarium (National Botanical Institute, KwaZulu-Natal). Voucher specimens were also deposited in these herbariums for future reference.

Preparation of Plant Extracts. The plants were washed in fresh running water to eliminate dust, dirt, and possible parasites and then washed again with deionized water. The bulb of *Brunsvigia* sp. and the leaves of all other plants were air-dried and then ground into a fine powder. A ground sample of each plant (2 g) and sodium chloride (0.4 g) was homogenized in a 30 mL mixture of methanol/water (80:20) for 5 min. The extract was then filtered through Whatman no. 4 filter paper and the filtrate collected in a clean vessel. Ten milliliter quantities of this extract were each taken for fumonisin B_1 and aflatoxin B_1 cleanup, respectively.

Cleanup for Fumonisin B_1 Determination. The filtered extract (10 mL) was diluted with a solution of phosphate-buffered saline (PBS, pH 7.0) (40 mL) containing 0.5% Tween 20 solution. The extract was then filtered through a microfiber filter (Schleicher & Schuell) and the filtrate transferred into a polypropylene syringe barrel, which was attached to the FumoniTest immunoaffinity (IA) column (Vicam). The extract was passed through the IA column at a rate of about 1−2 drops/s until air passed through the column. Thereafter, PBS (15 mL) was passed through the column at a rate of 1−2 drops/s. The fumonisin B_1 was eluted from the IA column under gravity by passing HPLC grade methanol (3 mL) through the column at a rate of 1 drop/s, and the eluate was collected into a glass vial. The eluate was dried under a stream of nitrogen at 60 °C and concentrated at the base of a small vial (4 mL capacity).

Table 1. Levels of Fumonisin B_1 Found in Indigenous Dietary Wild Plants

common name[a]	botanical name	family	level of fumonisin B_1 (μg/kg)
idolo lenkonyana (X)	*Rumex lanceolatus* Thunb.	Polygonaceae	524
idumbe (lomfula), idumbi (Z)	*Colocasia esculenta*	Araceae	–[b]
iGuzu (X)	*Physalis viscosa* L.	Solanaceae	–
imbabazane, uralijane (X)	*Urtica urens* L.	Urticaceae	–
imbilikikane (X), iMbicane	*Chenopodium album* L.	Chenopodiaceae	–
inlaba (X)	*Sonchus oleraceus* L.	Asteraceae	–
intebe (X, Z)	*Zantedeschia aethiopica* (L.) Sg	Araceae	105
isinama (X)	*Amaranthus asper* L.	Amaranthaceae	–
isiqwashumbe (X)	*Raphanus raphinastrum*		69
lwatane (X)	*Raphanus nasturtio aquatica* L.	Brassicaceae	–
isithate (Z)	*Oxalis corniculata* L.	Oxalidaceae	–
umdiza wethafa (Z)	*Sida dregei* Burtt. Davy	Brassicaceae	–
umhlabangubo (X), uqadolo (Z)	*Bidens pilosa* L.	Asteraceae	–
umsobosobo, umsobo wenja (X), umsobo wehlati, umsobo (Z)	*Solanum nigrum* complex	Solanaceae	34
Umtyutyu, unodlomboyi (X), umbhido (Z)	*Amaranthus hybridus* L.	Amaranthaceae	–
uNongotyozana, nonyongwana, inyongo (X), icukudwane (Z)	*Centella asiatica* L.	Umbelliferae	–
uqupose (X), imbuya (Z)	*Amaranthus thunbergii* Moq.	Amaranthaceae	–
uselwa-iwenyoka (Z)	*Coccinia rehmanii* Cogn.	Cucurbitaceae	–
uvevane (X, Z)	*Sida rhombifolia* L.	Malvaceae	–

[a] X, Xhosa; Z, Zulu. [b] Not detected ($\leq$8 μg/kg).

Cleanup for Aflatoxin B_1 Determination. The filtered extract (10 mL) was diluted with a solution of 10% Tween 20 (20 mL). The extract was then filtered through a microfiber syringe filter into a polypropylene syringe barrel, as above, which was attached to the AflaTest-P IA column (Vicam). The extract was passed through the IA column at a rate of about 1−2 drops/s until air passed through the column. Thereafter, water (15 mL) was passed through the column at a rate of 1−2 drops/s. Aflatoxin B_1 was eluted from the IA column under gravity by passing HPLC grade methanol (3 mL) through the column at a rate of 1 drop/s and the eluent collected into a glass vial. The eluent was dried under a stream of nitrogen at 60 °C and concentrated at the base of a small vial (4 mL capacity).

HPLC Analysis. Fumonisin B_1 analysis was carried out by reconstituting the residue into methanol (200 μL), and aliquots were derivatized with *o*-phthaldialdehyde (OPA) prior to reversed-phase HPLC separation and fluorescence detection according to the method of Sydenham et al. (*22*). Aflatoxin B_1 was determined after reconstituting the residue into methanol (200 μL) and injecting 5 μL aliquots onto the column according to the method of Thiel et al. (*23*). In the latter method, the HPLC mobile phase was adjusted to contain 0.01 M KH_2PO_4/acetonitrile/methanol in the ratio 690:220:75 (v/v/v).

HPLC-MS Analysis. HPLC-MS analysis was performed using a SpectraSERIES P2000 HPLC pump equipped with an AS 1000 autosampler containing a 20 μL injection loop. The HPLC column was attached on-line to a Finnigan MAT LCQ ion trap mass spectrometer set up for positive ion electrospray ionization (ESI). The test samples were filtered through a 0.45 μm syringe filter prior to injections. Binary gradient reversed-phase HPLC was performed on a 150 × 4.6 mm i.d., 5 μm, ODS Luna C_{18} column (Phenomenex, Torrance, CA). Solvents A and B consisted of water/acetonitrile/formic acid in the ratios 90:10:0.1 and 10:90:0.1, respectively, and pumped at 0.7 mL/min. Full scan MS/MS between *m*/*z* 330 and 730 was undertaken to monitor fumonisin B_1 in the plants. A collision energy of 34% was used to fragment the protonated molecular ions, and the resulting product ions for fumonisin B_1 were monitored as diagnostic indicators for its presence. The HPLC eluate entered the mass spectrometer without splitting at a source voltage of 4.5 kV and a capillary voltage of 40 V, while the heated capillary temperature was maintained at 220 °C and the sheath to auxiliary gas ratio was set at 4:1.

Statistical Analysis. Basic statistics comprising the mean and relative standard deviation for the spiked samples were computed using Microsoft Excel software (Microsoft Corp.) following duplicate experiments.

RESULTS AND DISCUSSION

The results of this study indicated that although none of the plants contained detectable levels of aflatoxins B_1, 8 of 49 (16%) of the plants analyzed contained FB_1 ranging between 8.3 and 1553 μg/kg (**Tables 1** and **2**). Four of the plants were identified as dietary supplements, whereas the remaining four were frequently used as traditional medicine. The plant *Rumex lanceolatus*, which is cooked as a vegetable, contained 524 μg/kg fumonisin B_1 and was consumed by an equal number of males and females (21%). Although *Solanum nigrum* complex was consumed by 48% males and 70% females, it contained the lowest levels of fumonisin B_1 among the dietary wild plants investigated (**Figure 2A**). The plants *Zantedeschia aethiopica* (L.) Sg and *Raphanus raphinastrum*, which also contained low levels of fumonisin B_1, were consumed by 10 and 53% males and by 13 and 76% females, respectively. More females were observed to consume these traditional plants and were the ones to identify many of these plants because the females are generally responsible for the preparation of the meals and are thus the major collectors of these plants from the veld.

Indigenous edible plants continue to play a significant role in the rural areas of the Eastern Cape Province of South Africa. The leaves of the wild plants are chopped and mixed with maize meal and traditionally called “isigwampa”. The leaves are also used as side dishes to give flavor, and others provide a bitter taste to the meals. Although they are seasonal, the variety of plants used ensures that some will be available throughout the year. In addition, many of the wild plants can be dried and stored in airtight containers for use during the off-season, so as to prevent a shortage in the supplies of food resources.

The popular traditional spinach plants, such as *Amaranthus hybridus* (and other *Amarathus* species), *Chenopodium album*, *Bidens pilosa*, and *Sonchus oleraceus*, collectively referred to as *imifino*, did not contain detectable levels of fumonisin B_1. The amaranths commonly known as marog are widely used as spinach. The fact that the name *marog* is also used for other relishes testifies to the popularity of the plant in rural diets. Commercial scale farming has become popular in recent years, and nowadays the leaves are also processed and canned. *A. hybridus* is the most commonly cultivated marog, although

Table 2. Levels of Fumonisin B_1 Found in Indigenous Medicinal Wild Plants

common name[a]	botanical name	family	level of fumonisin B_1 (μg/kg)
iGqwaka (X), umhlawazizi, umhlawazi (Z)	*Catha edulis* (Vahl.) Forsk ex Endl.	Celastraceae	38
ikhwane (X)	*Mariscus congestus* (Vahl.) C.B.Cl.	Cyperaceae	–[b]
imbilikikane (X), iMbicane	*Chenopodium album* L.	Chenopodiaceae	–
imPepha (X), impepho	*Helichrysum cymosum* (L.) D.Don	Asteraceae	–
iNdlebe ye-bokwe (X), amanzemnyama, ishwaga (Z)	*Pelargonium* sp. cf. *inqinans* (L.) L'Herit	Geraniaceae	–
iNxinene (X), inhlungunyembe (Z)	*Acokanthera oppositifolia* (Lam.) Codd	Apocynaceae	–
inzininiba (X), umsuzwane (Z)	*Lippia javanica* (Burm. F.) Spreng.	Verbenaceae	–
itolofiya (X)	*Opuntia vulgaris* Mill.	Cactaceae	–
uBobo (X)	*Dalbergia obovata* E. Mey.	Fabaceae	8.6
Ubuhlungu bethafa (X), isihlungu (Z)	*Teucrium reparium* Hochst.	Labiatae	–
Ubuvima (X), ibuvimba (Z)	*Withania somnifera* (L.) Dun	Solanaceae	–
uhlololwane (X), idolo-lenkonyane-elimhlophe, uhladlwana olukhulu (Z)	*Hypoestes aristata* (Vahl) Soland. Ex Roem. & Schult. var. *alba* Balkwill	Acanthaceae	–
umAsibele (X), umBangabanga, uAasibele, idololenkawu, umaSibe, umAsibe, uMasibele (Z)	*Deinbollia oblongifolia* (E. Mey. ex Arn.) Radlk.	Sapidaceae	–
umathunga (X)	*Eucomis autumnalis* (Mill.) Chitt. subsp. *autumnalis*	Amaryllidaceae	–
umayime (X)	*Brunsvigia* sp.	Amaryllidaceae	1553
umbhanga bhanga (X), uboqo, ugwanya (Z)	*Solanum mauritianum* Scop.	Solanaceae	–
umbhewe, umhlashosana (Z)	*Pteridium aquilinum* subsp. *aquilinum*	Dennstaedtiaceae	–
umHlaba (X), uNomaweni, iKhala (Z)	*Aloe Ferox* Mill.	Aloeaceae	–
umHlonyane (X)	*Artemisia afra* Jacq. ex Wild	Asteraceae	–
umnyamanzi (X), umtoli, umNgamanzi (Z)	*Acacia caffra* (Thumb.) Willd	Mimosaceae	–
umTentekwane (X), iNtenendkiwane, inTentekiwane, isiThende, isidhende (Z)	*Maesa lanceolate* Forsk. var. *rufescens*	Myrsinaceae	–
umThombe (X), inTendekwane (Z)	*Ficus craterostoma* Warb. ex Mild Br. & Burr.	Moraceae	–
umuNga (X)	*Acacia karroo*	Mimosaceae	–
umvumbangwe (X)	*Datura stramonium* L.	Solanaceae	42
umyama (X), umnama, umZungulwa (Z)	*Maytenus acuminata* (L.f) Loes	Celastraceae	–
uNongotyozana, nonyongwana, inyongo (X), icukudwane (Z)	*Centella asiatica* L.	Umbelliferae	–
uSingalamaxegwazana (X), umkhokha wehlathi, umkoka (Z)	*Convolvulus farinosus* L.	Convolvulaceae	–
uTywala bentaka (X), ubukhwezane, uguguvama, umphema (Z)	*Lantana rugosa* Thunb.	Verbenaceae	–
u-vuma (X), umlahlama, umhlatholan (Z)	*Turraea floribunda* Hochst.	Meliaceae	–
uxobo (X, Z), uklenya (Z)	*Gunnera perpensa* L.	Gunneraceae	–

[a] X, Xhosa; Z, Zulu. [b] Not detected ($\leq$8 μg/kg).

several others are also widely used as green vegetables, including *A. thunbergii*, *A. spinosus*, and *A. deflexus*, which grow naturally as weeds in southern Africa.

The bulb of *Brunsvigia* sp., which is used medicinally as a purgative and emetic (*24*), was found to contain 1553 μg/kg fumonisin B_1 (**Figure 2B**). Bulb decoctions of this plant are also administered as enemas for renal and liver complaints (*25*), thus arousing serious concerns because fumonisin B_1 has been shown to be nephrotoxic (*26*) and hepatotoxic and to induce hepatocellular carcinoma in rats (*27*). Despite such results, where the need for caution is of paramount importance, the use of traditional remedies continues to play a significant role in the well-being of rural South Africans. Apart from folklore norms, the services and advice of indigenous practitioners are valued because they are offered in terms that patients can understand and in the context of cultural values and practices that are shared by both patients and healers alike.

To confirm the presence of fumonisin B_1 in the eight plant samples, HPLC-MS/MS analysis was undertaken. With the MS/MS mode it was possible to selectively excite the protonated molecular ion of fumonisin B_1 resident in the ion trap to produce diagnostic fragment ion spectra and hence obtain unequivocal confirmation of fumonisin B_1 presence in these plant samples. The fragmentation pathway for the fumonisins following collision-induced dissociation (CID) consisted of sequential losses of water and tricarboxylic acid side chains from the alkyl backbone resulting in the fragments $[M + H - H_2O]^+$, $[M + H - 2H_2O]^+$, $[M + H - TCA]^+$, $[M + H - H_2O - TCA]^+$, $[M + H - 2TCA]^+$, and $[M + H - 2TCA - H_2O]^+$ at *m*/*z* 704, 686, 546, 528, 370, and 352, respectively. A typical HPLC-MS/MS result is indicated in **Figure 3**.

Whereas the HPLC analytical method was developed and validated in previous studies, the extraction method for aflatoxin B_1 and fumonisin B_1 was validated prior to the analysis of the plants. This was done to ensure that matrix interferences and analyte losses during extraction were minimal so that the results would reflect the true level of contamination. In this aspect of the study, samples of the plant *R. lanceolatus* were each spiked in duplicate with aflatoxin B_1 at the 0.1 and 0.02 μg/g levels, whereas fumonisin B_1 was spiked at the 0.25 and 1 μg/g levels. Recoveries of the toxins were calculated after extraction and HPLC analysis and adjustment for the quantity of toxins present in the background matrix. Mean recoveries of 93% (%RSD = 7.78) and 85% (%RSD = 5.66) were obtained for aflatoxin B_1 at the 0.02 and 0.1 μg/g levels, respectively. Fumonisin B_1 showed mean recoveries of 98% (%RSD = 7.78) and 73% (%RSD = 4.10) at the 0.25 and 1 μg/g levels, respectively. The results indicated that the extraction method yielded acceptable recoveries, thus making it suitable for mycotoxin determination in plant matrices.

The level of fumonisin B_1 found in *R. lanceolatus* is comparable with the mean level of fumonisin B_1 found in the leaves of the orange tree (*Citrus sinensis*) (537 μg/kg) harvested in Lisbon, Portugal (*9*), and the 1989 harvest of white corn from South African commercial farms (570 μg/kg) (*1*). The level is also similar to the mean levels found in commercial corn-based

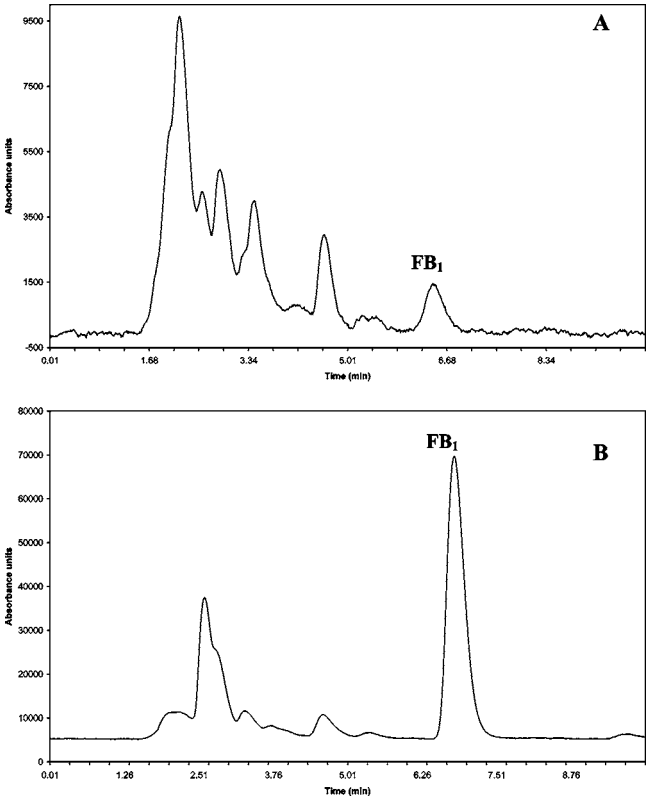


Figure 2. Reversed-phase HPLC chromatogram of (**A**) *S. nigrum* complex and (**B**) *Brunsvigia* extract.

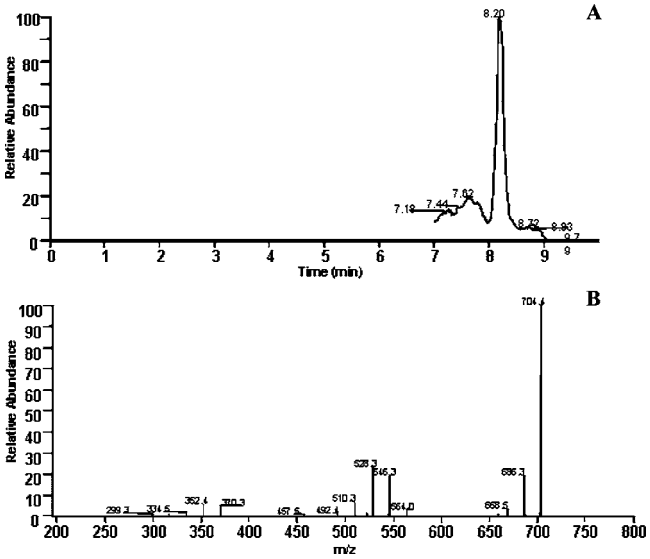


Figure 3. (**A**) Total ion chromatogram of fumonisin B_1 in *R. lanceolatus* extract; (**B**) product ion mass spectra produced by collision-induced dissociation of the protonated molecular ion of fumonisin B_1 serving as a diagnostic indicator.

human foods sampled in retail outlets in Canada and South Africa (corn flour, 550 μg/kg) as well as the United States (white corn meal, 550 μg/kg). The level is, however, much higher than the levels found in black tea (*Camellia sinensis*), corn silk (*Zea mays*), chamomile (*Matricaria chamomilla*), and the leaves of the linden tree (*Tillia grandifolia*) (*9*). In contrast, the bulb of *Brunsvigia* sp. contained fumonisin B_1 at a level comparable to the levels found in naturally contaminated "good" maize samples from the high esophageal cancer incidence areas of the Transkei region of South Africa (1600 and 1840 μg/kg in the 1985 and 1989 harvest seasons, respectively) (*1*). A provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of body weight (bw)/day of fumonisin B_1 alone or in combination with two other analogues, fumonisins B_2 and B_3, was set by the 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (*28*). Such data only serve to highlight the severity of the problem because rural residents of the Eastern Cape Province and, in particular, the former Transkei region are already exposed to high levels (probable daily intake = 47 μg/kg of bw/day for good maize and 355 μg/kg of bw/day in the case of moldy maize) (*29*) of this toxin via their consumption of contaminated maize as a dietary staple. Findings of fumonisin B_1 presence in dietary and medicinal wild plants raise further concerns with regard to the frequency and magnitude of exposure of these populations to this toxin.

Although fumonisin B_1 is not genotoxic and does not respond to any of the tests for mutagenicity with or without microsomal activation (*30*), the available evidence suggests that fumonisin B_1 may exert its cancer-promoting and toxic effects by inhibiting key enzymes involved in the de novo synthesis and turnover of sphingolipids (*31*), which are important compounds for the structural and regulatory integrity of cells (*32*). The dietary wild plants investigated in this study form part of the traditional diets, and although some of the plants had low levels of fumonisin B_1 contamination, it could be argued that low levels of contamination are not going to be toxicologically significant and remain dependent on the frequency of intake and variation in levels. However, the frequency and quantity of intake of these plants can result in prolonged exposures to such levels of toxin, which can have a significant impact on the health status of people consuming these plants.

The control of fumonisin production in the field is an intrinsically difficult problem. This study indicates that exposure to fumonisin B_1 is much more widespread than initially thought and certainly places the residents of the Eastern Cape Province, who are heavily dependent on these indigenous plants for their dietary and medicinal value, into a very high category of exposure to fumonisin B_1. This study reports for the first time the presence of fumonisin B_1 in dietary and medicinal wild plants of South Africa, and although the scope of this study was purely on the analytical determination of mycotoxins, the potential fumonisin B_1-producing *Fusarium* species in these plants have not yet been identified, thus warranting follow-up mycological investigations.

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