The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins

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ABSTRACT

Mycotoxins present a great concern to food safety and security due to their adverse health and socio-economic impacts. The necessity to formulate novel strategies that can mitigate the economic and health effects associated with mycotoxin contamination of food and feed commodities without any impact on public health, quality and nutritional value of food and feed, economy and trade industry become imperative. Various strategies have been adopted to mitigate mycotoxin contamination but often fall short of the required efficacy. One of the promising approaches is the use of bioactive plant components/metabolites synergistically with mycotoxin-absorbing components in order to limit exposure to these toxins and associated negative health effects. In particular, is the fabrication of β-cyclodextrin-based nanosponges encapsulated with bioactive compounds of plant origin to inhibit toxigenic fungi and decontaminate mycotoxins in food and feed without leaving any health and environmental hazard to the consumers. The present paper reviews the use of botanicals extracts and their phytochemicals coupled with β-cyclodextrin-based nanosponge technology to inhibit toxigenic fungal invasion and detoxify mycotoxins.

1. Introduction

Food and feed contamination by toxigenic fungi is of serious health concern and has been recorded since mankind initiated the cultivation and storage of agricultural commodities. These toxigenic fungi belong mainly to the genera Aspergillus, Fusarium, Claviceps, Penicillium, Stachybotrys, and Altenaria, etc. They produce toxic substances, some of which are referred to as mycotoxins. Cumulative evidence from the literature suggest that these mycotoxins do not present any apparent biochemical importance in fungal development or growth. However some secondary metabolites play a role in virulence, development and pathogenicity (Perincherry et al., 2019; Venkatesh and Keller, 2019). Mycotoxins have adverse effects on humans and animals even at low concentrations (Viladon et al., 2018; Adekoya et al., 2019), and the proliferation of toxigenic fungi on food and feed is always favoured by certain environmental factors such as humidity and temperature, as well as certain biotic conditions (i.e. substrate composition). High incidences of mycotoxin occurrence are usually reported in tropical regions such as Asia and sub-Saharan Africa, where conditions for their proliferation are optimal. Mycotoxins contaminate mainly commodities such as cereals, grains, nuts, and their by-products during production, pre-harvest and post-harvest (Milicevic et al., 2010; Zain, 2011; Nleya et al., 2018; Balendres et al., 2019; Gbashi et al., 2019). These toxins often enter the body mainly via ingestion, as well as by inhalation, parental and dermal exposure routes. The toxins can also enter the food chain through infected crops which are consumed either directly or indirectly by humans or animals as feed sources. As such, they appear in meat, milk and eggs (Hojnik et al., 2017).

Mycotoxin contamination is of serious concern for food safety and security worldwide. These toxins account for enormous economic losses in agricultural productivity and trade, with more severity in poor and developing countries. It is estimated that about 60–80% of crops worldwide can be contaminated by mycotoxins, thus resulting in significant economic losses (Eskola et al., 2019). The toxic effects of
mycotoxins are persistent, and they are difficult to completely eradicate once they enter the food/feed chain. In the agricultural industry, mycotoxins cause loss in livestock production due to reduced growth rates, decreased immunity and fertility, reduced eggs, meat and milk production, and increased mortality (Thipe et al., 2020). Furthermore, the effects associated with mycotoxins cause veterinary and healthcare costs (Marroquin-Cardona et al., 2014; Kagot et al., 2019). This contamination of food and feedstuffs by mycotoxins equally reduces the nutritional value, quality and safety of food and feed (Luo et al., 2018). Many countries have established regulatory limits on mycotoxins in agricultural commodities to limit human and animal health risks associated with them (Marroquin-Cardona et al., 2014; Haque et al., 2020). Mycotoxins cause a disease known as mycotoxicosis (Bennett and Klich, 2003; Liev and Mohd-Redzwan, 2018). The health effects of mycotoxins are extensive as they potentiate some hepatotoxic, nephrotoxic, mutagenic, genotoxic, carcinogenic, immunosuppressive and teratogenic properties. The most prevalent mycotoxins of agricultural importance include the aflatoxins (Afs), ochratoxins (OTA), fumonisins (FBs), trichothecenes and zearalenone (ZE). These have been given serious attention due to the health risks that they pose in humans and animals (Dikhoba et al., 2019; Celik, 2020).

Various approaches to control and prevent mycotoxins in food and feed have been developed. These approaches are categorised as chemical and micro-biological methods (Adebo et al., 2017; Adebiyi et al., 2019). These methods were shown to be effective in the prevention of growth of toxigenic fungi and production of associated mycotoxins during pre-harvest, after harvest food and during storage of food commodities. Chemical methods involve the use of chemicals such as ammonia, sodium hydroxide, hydrochloric acid, butylated hydroxytoluene, butylated hydroxyxyanisole and oltipraz to decontaminate mycotoxins (Galvano et al., 2001; Karlovsky et al., 2016; Colovic et al., 2019). In addition to their inefficacy in decontamination of mycotoxins, their long-term extreme use is still limited due to their residual toxic product/s, public health and environmental concerns (Abdel-Fattah et al., 2019; Alberts et al., 2019; Sadhasivam et al., 2019; Meng et al., 2020) coupled with the interference with nutrients and organoleptic properties of food and feed (Celik, 2020).

Physical methods include cleaning, dehulling, sorting, milling and ultra-violet light, pulsed light, cold plasma as well as irradiation. Other physical methods involve the use of adsorbents or binders such as activated charcoal, bentonite, zeolites and sepiolite clay. The methods have been effectively applied in decontaminating mycotoxins. However, enzymatic degradation by-products still limit their uses (Juodeikiene et al., 2012; Adebiyi et al., 2019; Lyagin and Efremenko, 2019). Therefore, there is a need to discover alternative methods that can prevent fungal colonization of agricultural commodities, detoxify or bio-transform mycotoxin residues to less- or non-toxic forms without any limitations (Iram et al., 2015; Powers et al., 2019; Haque et al., 2020).

Botanicals such as essential oils, spices, herbs and crude extracts provide outstanding alternatives for the discovery of biofungicides and nutraceuticals for mitigating mycotoxicosis and related infections. Botanicals are generally regarded as environmentally friendly and safer alternative sources of bioagents for the control of fungi and mycotoxins in food and feed (Iram et al., 2016; Adebo et al., 2020; Prakash et al., 2020). They are more affordable as opposed to other materials used for the same purpose, they provide a synergistic approach as protectants of fungal/mycotoxin contamination and further stimulate pathways that elicit the natural defence systems in plant tissues (da Cruz Cabral et al., 2013; Alberts et al., 2019; Gacem et al., 2020; Meng et al., 2020). They contain various phytochemicals with pharmacological properties against various diseases. Recent studies have been conducted on the possible application of botanicals as bio-fungicides and nutraceuticals to ameliorate the proliferation of toxigenic fungi and mycotoxin contamination in food and feed (Dikhoba et al., 2019; Ponzilaqua et al., 2019; Kavitha et al., 2020). This review aimed to elucidate the use of botanical extracts and their phytochemical compounds to prevent and detoxify mycotoxins without any adverse effect on the nutritional value of food and feed. We also review nano-encapsulation technology which can be adopted to improve bioavailability and solubility of phytochemicals used as biofungicides and nutraceuticals to prevent growth of mycotoxicogenic fungi and subsequent mycotoxin contamination.

2. Mitigation of toxigenic fungi and mycotoxins using botanical methods

2.1. Importance of botanicals

Plants have been widely used in folk medicine since time immemorial in order to treat and prevent various ailments from one generation to another (Horn and Vargas, 2008; Street and Prinsloo, 2013). Despite the advancement made in modern medicine, many populated groups in developing countries still depend on traditional medicine for preventing and treating various ailments. This is due to cultural beliefs, low cost and effectiveness (Moura-Costa et al., 2012; Ayele, 2018). According to the World Health Organization (WHO, 2001), approximately 80% of the population of the world still depend on traditional medicine for primary healthcare (Prakash et al., 2020). Recent studies have also created renewed interest in the use of botanicals and their compounds as nutraceuticals in this regard in both developed and developing countries (Galvano et al., 2001; Reddy et al., 2010; Anjorin et al., 2013; Dikhoba et al., 2019). The advantage of using plants for drug discovery is due to their abundance in nature and wide distribution geographically. A considerable number of drugs have been synthesized from plants based on their use in traditional medicine (Van Wyk et al., 1997; Dias et al., 2012). Africa is richly endowed with a wealth of medicinal plants. However, very few studies have looked into exploiting their constituents for mycotoxin detoxification (Stoev et al., 2019; Makhufele et al. unpublished data), and thus necessitate studies in this regard. The following sections provide an overview of various botanicals that exhibit antifungal activities. In addition, we also follow a nanoencapsulation approach through the fabrication and use of nanosponges to improve the stability from oxidation/degradation, availability, and demonstrate the most pronounced efficacy of botanicals.

2.2. Phytochemicals as a source of therapeutics and nutraceuticals

Plants produce secondary metabolites as a defence mechanism against pathogenic microorganisms, insects and adverse environmental conditions. These metabolites are known as phytochemicals, which are non-nutritive (Prakash et al., 2020) and to some extent essential oils. However, they can protect humans and animals against certain diseases caused by microorganisms or toxins associated with them due to the antimicrobial properties they possess (Palombo, 2011; Shin and Park, 2018; Redondo-Blanco et al., 2019). The metabolites are the most promising chemo-preventative agents for future drug discovery and development (Alabi et al., 2011). There are various major groups of phytochemical compounds discovered to date and differ according to their chemical structures (Dias et al., 2020). These major groups include phenolic compounds, flavonoids, phytosterols, carotenoids, tocols, terpenoids, alkaloids, saponins, tannins, aromatic acids, glucosinolates, carotenoids, essential oils, chlorophyll and organic acids as well as proteases inhibitors (Bhattarchar, 2011; Adebo and Gabriela Medina-Meza, 2020; Loi et al., 2020). Such compounds may act directly or indirectly to protect against ailments or pathogens, because they contain
| Plant source               | Protective agent | Model                  | Mechanism of action                                                                                                                                 | References                                                                 |
|---------------------------|------------------|------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| **Native phytochemicals** |                  |                        |                                                                                           |                                                                           |
| *Curcuma longa* L. (Turmeric) | Curcumin        | In vitro Ames assay, chicken & rodent | Hepatoprotective effects against AFB1 toxicity in vitro and in vivo                     | Soni et al. (1997); Smerak et al. (2006a); Nayak and Sashidhar (2010); Limaye et al. (2018) |
| *Curcuma amada* (Ginger)   | Ginger           | HepG2 cells & rats     | Inhibited AFB1, STE and PAT toxicity                                                       | Yang et al. (2011); Vipin et al. (2017)                                    |
| *Thymus vulgaris* (Thyme)  | Thyme oil        | Rats                   | Ameliorated oxidative stress and genotoxicity effects of AFB1                              | Abdel-Fattah et al. (2010)                                                 |
| *Syzygium aromaticum* (Clove) | Clove            | In vitro               | Inhibited AF, STE, & CIT toxicity                                                          | Hitokoto et al. (1980); Azzouz (1981); Hussain et al. (2012)               |
| *Camellia sinensis* L. (green tea) and a variety of plants | Epigallocatechin-3-gallate (EGCG) | Mice                   | Protected against DON & HT-2 toxin toxicity                                                | Smerak et al. (2006b); Yang et al. (2011); Do et al. (2015)                 |
| *Solanum lycopersicum* (tomatoes) | Lycopene       | Chicken broiler, Mice & rats | Protected against oxidative, inflammatory, hormonal & reproductive damage induced by AFB1, OTA & ZEN in mice, also inhibited T-2 toxin-induced oxidative stress | Leal et al. (1999); Aydin et al. (2013); Palabiyik et al. (2013); Wu et al. (2017a); Hedayati et al. (2018) |
| *Cyanidin*                 |                  | In vitro HepG2 & Caco-2 cells | Protected against AFB1 & OTA-induced oxidative stress                                      | Sorrenti et al. (2012); Do et al. (2015)                                    |
| *Rutin*                    |                  | Rats                   | Reversed T-2 toxin-induced lipid peroxidation in liver homogenate                           | El-sawi and Al-Seenii (2009); Wu et al. (2017a)                             |
| **Crude extracts**         |                  |                        |                                                                                           |                                                                           |
| *Prema integrifolia*       |                  | Mice                   | Inhibited AFB1 toxicity                                                                    | Singh et al. (2019)                                                       |
| *Silybum marianum*         |                  | Broiler chicken         | Inhibited OTA toxicity                                                                     | Stoey et al. (2019)                                                       |
| *Withania somnifera*       |                  | Broiler chicken         | Inhibited OTA toxicity in vivo                                                             | Stoey et al. (2019)                                                       |
| *Annona senegalensis*      |                  | In vitro               | Inhibited AFB1 genotoxicity in Ames, Vitotox and comet assays                              | Makhuwele et al. (2018a)                                                   |
| *Monanthotaxis caffra*     |                  | In vitro               | Inhibited AFB1 genotoxicity in vitro                                                       | Makhuwele et al. (2018b)                                                   |
antimutagenic, antigenotoxic, antimicrobial, anthelmintic, anticarcinogenic, antiproliferative and anti-inflammatory as well as antioxidant properties (Galvano et al., 2001; Makhalofa et al., 2017; Velu et al., 2018; Lahlou et al., 2019).

2.3. Antifungal effects and detoxification abilities of plant extracts and their phytochemicals compounds on toxigenic fungi and mycotoxin toxicity

Plants possess antimutagens, antimicrobial, antioxidants or anticarcinogens capable of compromising the toxic and genotoxic effects of mycotoxins (Madrigal-Santillan et al., 2010; Anjorin et al., 2013; Powers et al., 2019). Antioxidants protect the cell membranes and macromolecules by scavenging free radicals (Wu et al., 2017a). Furthermore, phytochemicals induce cytotoxicity in fungi by disrupting cell membrane permeability and functions; inhibiting cytoplasmic and mitochondrial enzymes; inhibiting enzymes involved in cell wall components synthesis; and altering the cell compartment, osmotic and the redox balance (Loi et al., 2020). However, plant extracts and their compounds also act by inducing xenobiotic detoxification and biotransformation pathways (Gross-Steinmeyer and Eaton, 2012; Wu et al., 2017b). Phytochemicals are capable of inhibiting enzymes that activate Phase I carcinogens as well as induce enzymes for Phase II detoxification (Galvano et al., 2001; Wu et al., 2017b). The bioactive compounds in plants have been used as additives to prevent fungal growth and aflatoxin (AF) contamination in food and feed (Table 1), thus reducing the risks of mutagenicity and carcinogenicity of such mycotoxins as AFs (Maturha and Verma, 2007).

Antifungal and anti-mycotoxigenic activities of herbal plants with potential antioxidant properties were investigated against fungal strains that are phytopathogenic, i.e. Fusarium verticilloides, A. flavus and A. ochraceus. The results reported potentials of the selected medicinal plants to be used for the discovery of biofungicides that may prevent oxidative related food spoilage (Dikhaba et al., 2019). A study by Abdel-Fattah et al. (2018) reported the antioxidant, antifungal and anti-mycotoxigenic potentials of wild stevia extracts against A. flavus, A. ochraceus, A. niger, and F. moniliforme. Furthermore, essential oils have been found to effectively modulate the growth of mycotoxigenic fungi such as A. flavus, A. oryzae, A. niger, Alternaria alternata, F. moniliforme, F. graminearum, Penicillium citrinum and P. viridicatum, etc., and their associated mycotoxins (da Cruz Cabral et al., 2013; Prakash et al., 2015; Powers et al., 2019). A study by Kocic-Tanackov et al. (2019) reported how essential oil of Carum carvi L. applied at a concentration from 1.5 μL/g demonstrated complete inhibition of the growth of A. parasiticus, and likewise 4.5 μL/g exhibited complete inhibition of the growth of A. flavus as well as the secretion of aflatoxins by the same strain in polenta. Also noted was that a concentration of 35.0 μL/g of Junipers communis L. essential oil showed strong potency against A. parasiticus and A. flavus IKB (i.e. by significantly inhibited their growth) with percentage inhibition between 42.4 and 79.8%, while 50.0 μL/g of J. communis impeded aflatoxin production by A. flavus IKB in polenta completely.

Curcumin and ellagic acid are examples of compounds isolated from plants which are used as food and feed supplements. These compounds prevent metabolism of aflatoxin B1 (AFB1) and increase the activity of glutathione-S-transferase involved in the detoxification of xenobiotics. They were equally found via Ames assay and in rat and chicken models to protect against mutagenicity induced by AFB1 with Salomonella typhimurium strain TA98 and TA100 (Son et al., 1997; Gowda et al., 2008). Smerak et al. (2006a) studied the effect of curcumin against AFB1 mutagenicity in Ames, in vivo micronucleus, comet and chemiluminescence and blast transformation methods. The results showed that curcumin was able to protect cells against mutagenicity. They also demonstrated a significant reduction in DNA damage through stimulation of DNA repairs. Curcumin has earlier been demonstrated by many authors to exhibit anticarcinogenic, antiproliferative and antimutagenic effects against various mutants in vitro and in vivo (El-Hamss et al., 1999; Inano and Onoda, 2002; Polasa et al., 2004; Hosseini and Hosseinzadeh, 2018; Khan et al., 2019). Resveratrol is another natural product isolated from grape skin which suppresses the proliferation of many tumour cells such as breast, pancreatic and prostate cancers by inhibiting xenobiotic metabolism and inducing detoxification pathways (Farombi, 2004; Thipe et al., 2019). Studies have shown that this product can protect mycotoxin-induced toxicity in vitro and in vivo (Do et al., 2015; Sridhar et al., 2015; Tabeshpour et al., 2018). Furthermore, 6-gingerol, a natural bioactive compound extracted from ginger was found to possess strong protective effects against PA-induced genotoxicity in HepG2 cells in vitro (Yang et al., 2011).

Lycopene is a natural product found in tomatoes, papaya and other red fruits and vegetables that showed to have protective effects against ZEN oxidative, reproductive and hormonal damage in mice (Aydin et al., 2013; Palabiyik et al., 2013). Lycopene also prevented T-2 toxin-induced oxidative stress and maintained GSH cellular levels in vivo (Zeal et al., 1999). Furthermore, lycopene reduced AFB1 and OTA-induced oxidative stress and apoptosis in rats (Hedayati et al., 2018). Cyanidin, a phytochemical found in various medicinal herbs, fruits and vegetables including grapes, blackberry, cherry, cranberry, raspberry, red cabbage, red onion, etc., showed a protective effect against AFB1 and OTA-induced toxicity in hepatocytes and enterocytes (Sorrenti et al., 2012). El-Sawi and Al-Seeini (2009) reported that rutin displayed strong antioxidant activity against T-2 toxin in rat liver and also decreased lipid peroxidation induced by T-2 toxin. Shebata et al. (2017) demonstrated that oil-bioactive films from three extracts of immature fig fruit, leaves, and pomegranate husks to be a novel method for post-harvest grain management against mycotoxins.

Furthermore, Negera & Washe (2019) evaluated AF degradation abilities of some selected natural food spices including garlic, ginger, black cumin, clove, sacred basil, lemon grass, thyme, fenugreek and lemon, traditionally used by the Ethiopian community for food flavoring and preservation. Electrochemical and LC-MS/MS methods were used to investigate aflatoxin degradation efficacy of the spice extracts by determining the toxin in extract-treated and non-treated samples. The results revealed that garlic had maximum AFB1 degradation activity followed by lemon and the other dietary spices during 1-hour exposure to AFB1 standard at 25 °C. The results also showed that the possible mechanism of AFB1 degradation is through chemical transformation of AFB1 parent compound to another compound by the plant extracts. Iram et al. (2015) investigated the ability of Coriaria citroidora plant extract to detoxify AFB1 and AFB2 both in vitro and in vivo. They observed that the leaf extracts had maximum detoxification at pH 8 and 30 °C temperature after 72 h of incubation. Iram et al. (2016) demonstrated that Trachyspermum ammi seed extracts can be used for the development of biologically safe herbal additives in food and feed. Ponziacquà et al. (2019) evaluated degradation capabilities of aqueous plant extracts of Rosmarinus officinalis, Origanum vulgare, Psidium cattleianum and Passiflora alata against AFB1, with R. officinalis extract exhibiting the highest degradation percentage of AFB1 (range: 49.0–60.3%) at 24–48 h, followed by O. vulgare with (range of 30.7%–38.3%) after 48 h of incubation.

Makhuvele et al. (2018a) investigated the antigenotoxicity of plant extracts against AFB1-induced genotoxicity. The results showed that most plant extracts from Arrabothys brachypetalus, Helichrysum petiolare, Hexalobus monopetalus, Friesodia obovata, Monanthotaxis caffra, Uvaria caffra, Xylopia parvifolia, Allobus monopetalus, Friesodielsia obovata, Monanthotaxis caffra, Protea plant extracts from Carum carvi L. exhibited the highest degradation per-
associated immunosuppression in broiler chicks (Stoev et al., 2019). The
same extracts also showed some hepatoprotective effects on broiler
chicks exposed to OTA with extract from S. marianum exhibiting neph-
roprotective effect against OTA toxicity. Very recently, Nerilo et al.
(2020) evaluated the use of ginger essential oils (GEO) as a fumigant
agent for stored maize grains. They reported that GEO was mainly
composed of α-zingiberene (23.85%) and geranial (14.16%). Further-
more, their results revealed that 25 and 50 μg/g, respectively, exhibited
antifungal activity against A. flavus and inhibited AFB1 and AFB2 pro-
duction. Despite the efficacy of plant extracts and their phytochemicals
in the management of toxigenic fungi and their toxins, there are various
limitations to the use of phytochemicals and their crude extracts as
biofungicides and nutraceuticals. The subsequent section elaborates on
these limitations.

2. Current limitations on the use of phytochemicals as biofungicide

There is an increased demand for the use of plant extracts and their
compounds and this raises concerns about the safety, toxicity and quality
of these products. Studies have reported the contamination of plant
materials with medicinal properties by mycotoxins (Ashitq et al., 2014;
Mulaudzi, 2019; Altyn and Twaruzek, 2020; Thipe et al., 2020).
Furthermore, research has shown that many plant extracts used in
traditional medicine and as food ingredients are toxic and mutagenic.
As such, a thorough screening of their toxicological properties is necessary.
Those that show no signs of toxicity are given high priority (Verscheave
and Van Staden, 2008; Sajid et al., 2019). The use of plant extracts,
especially in exploiting their bioactive compounds, are exploited in the
discovery and development of new antifungal and nutraceutical agents.

The high frequency of toxigenic fungal manifestation warrants the
consistent evaluation of bioactive phytochemicals and their derivatives
that exhibit high antifungal and anti-mycotoxinogenic activities as alter-
atives to conventional fungicides. Table 2 briefly presents phytochem-
icals that have shown antifungal activities and their mode of action, thus
inhibiting their respective mycotoxin biosynthesis. Moreover, nano-
encapsulation formulations are also presented, exhibiting a synergistic
effect between encapsulate and phytochemical with low side effects. The
use of natural formulations antifungal agents for safe, effective, and eco-
friendly features against fungi and mycotoxins is ideal for agricultural
practices compared to standard antifungal agents (Rai et al., 2020).
Literature precedence has proposed the notion of fully understanding the
exact principles underlying the antifungal and anti-mycotoxinogenic mode
of action of phytochemicals against mycotoxinogenic fungi (Chaudhari
et al., 2020; Xu et al., 2020), facilitated through the following:

(i) inhibition of ergosterol biosynthesis, a major sterol that regulates
plasma membrane biogenesis;
(ii) disruption of fungal cell membrane; and
(iii) production of reactive oxygen species (ROS) which results in
oxidative stress.

The nanoencapsulation (e.g. nanoemulsion, nanofibre, nanogel,
nanoliposome, nanoparticle, nanotube, and nanosponge) coupled with
phytochemicals provides promising avenues to explore possible strate-
gies for enhancing efficacy and combating fungal resistance where con-
ventional antifungal drugs prove to be ineffective (Dhakar et al., 2019;
Redondo-Blanco et al., 2019). Powers et al. (2019) emphasized on the
priority and importance of big data approaches for expediting the iden-
tification of active components. They utilized a huge data to evaluate
antifungal activity of 82 essential oils against A. niger, C. albicans, and
C. neoformans. The results obtained demonstrated that antifungal sus-
cceptibility to the essential oils was as follows: C. neoformans > C. albicans
> A. Niger. However, the use of phytochemical compounds alone limits
their full applications. This limitation is due to their instability, insolu-
bility, high volatility, low bioavailability, ability to change organoleptic
characteristics of food/feed, and limited of facilities and resources for
their extraction and purification (Sajid et al., 2019; Adebo et al., 2020;
Loi et al., 2020). To overcome these factors, cohesives research collabo-
rations between academics, research institutions, government agencies,
food and pharmacological industries, as well as international stake-
holders are required. In addition, different emerging technologies such as
nanotechnology can, in part, provide solutions to encounter some of
these limitations (Prakash et al., 2020; Thipe et al., 2020). Phytochemical
compounds can be encapsulated in edible coatings or with nanoparticles
such as nanosponges to elicit the factors listed herein (Aloabi et al.,
2019; Loi et al., 2020; Thipe et al., 2020). The subsequent section gives
brief information on nanocarriers and their encapsulated bioactive
compounds for use as antifungal and mycotoxin detoxification agents.

3. Innovative technology for mycotoxin detoxification

Frontiers in the field of nanotechnology have led to their vast appli-
cations in the field of nanomedicine, extending to the agricultural sector.
Great strides have been made in the implementation of nanotechnology
in agriculture for controlling mycotoxin contamination in food and feed
supply chain (Thipe et al., 2018). The era of green nanotechnology, with
the use of plant phytochemicals for the production of nanomaterials, has
greatly improved their safety for use in agriculture as mycotoxin detox-
yfying agents (El-Desouky & Ammar, 2016; Thipe et al., 2020). A com-
bination of nanotechnology and botanical extracts, as well as their
phytochemicals, has shown significant results in the pharmacological,
agricultural and cosmetic industries. Nano-encapsulated phytochemicals
demonstrated strong efficacy over the free form because of the increased
surface area, protection of encapsulated compounds from internal and
external environmental conditions (Prakash et al., 2020). Nanocarriers
can protect bioactive phytochemical compounds against thermal and
photodegradation and further provide controlled release of antifungal
compounds for the development of active packaging for maintaining the
integrity of food/feed during storage and protection from fungal growth
and mycotoxin contamination (Bahrami et al., 2020). They also caused
reduced toxic effects of these plant-based drugs (Pushpalatha et al., 2018;
Sajid et al., 2019). To date, there are different types of nanocarriers used
for drug delivery, including liposomes, metal nanoparticles, polymeric
nanoparticles, polymeric micelles and nanosponges. All these nano-
carriers have been reported to be effective as drug delivery systems for
plant-based products in cosmetics, agriculture and medicine (Pushpalath-
a et al., 2018; Thipe et al., 2020; Udomkun and Njukwe, 2020).
However, there is limited information in the literature on the use of
nanosponge encapsulated biofungicides, and for that, this paper high-
lights the use of cyclodextrin nanospheres as carrier vehicles to encap-
sulate phytochemicals.

3.1. Nanospheres as encapsulation system for antifungal and detoxifying
phytochemicals

Nanosponges are emerging innovative drug delivery or encapsulation
systems that result from the advancement of nanotechnology. They are
microscopic, sponge-like particles with nanometer cavities, which can
encapsulate both hydrophilic and lipophilic substances including toxins,
but when doing so, enhance their stability, bioavailability and solubility
(Ananya et al., 2020). Nanospheres are non-irritant, non-toxic,
non-mutagenic and non-allergenic agents made-up of a polyester mixed
with crosslinkers in a solution. The polyester is biodegradable and
effective in delivering the drug to the targeted site (Pawar et al., 2016;
Bhowmik et al., 2018). Nanospheres come in different forms including
cyclodextrin which is most widely used and promising in encapsulation
of phytochemicals. Cyclodextrin nanospheres are natural polymers
formed from enzymatic degradation of starch and consist of a ring of
oligosaccharides molecules. They are characterized by a highly porous
spherical, amorphous or crystalline structure (Sherje et al., 2017).
An interesting property of cyclodextrin-based nanospheres is that some
important physicochemical properties of the nanomaterials such as
| Plant source          | Protective agent | Model | Mechanism of action                                                                                                                                                                                                                                                                                                                                 | References                                                                 |
|----------------------|------------------|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| **Native phytochemicals**                                                                 |                  |       |                                                                                                                                                                                                                                                                                                                                                      |                                                                            |
| Curcuma longa L. (Turmeric) | Curcumin        | F. solani, C. albicans, P. expansum, A. flavus, and A. parasiticus | Downregulation of Δ5,6-desaturase gene (ERG3) resulting in reduced ergosterol biosynthesis leads to cell death attributed by elevated levels of reactive oxygen species (ROS) production, Reduced proteinase secretion and inhibition of H+-ATPase activity induces acidification of extracellular and intracellular matrix inhibition of hyphae development through the suppression of thymidine uptake 1 (TUP1), Curcumin is a photosensitizer (0PS): Where 0PS⁺γ→triplet excited state (3PS*)→H⁺ to a biomolecule – radicals (anion superoxide (O2·⁻), hydrogen peroxide (H2O2), hydroxyl radical (•OH), and singlet oxygen (1O2) resulting in cell death by apoptosis, necrosis, or autophagy. | Sharma et al. (2010); Neelofar et al. (2011); Moghadamtousi et al. (2014); Chen et al. (2018); Song et al. (2020); Narayanan et al. (2020) |
|                     |                  |       |                                                                                                                                                                                                                                                                                                                                                      |                                                                            |
| Curcuma amada (Ginger) | α-zingiberene, camphor, and eucalyptol | A. flavus | Fungicidal activity to destroy cellular integrity and induced alteration in mitochondrial membrane potential.                                                                                                                                                                                                                                          | Hu et al. (2015)                                                         |
|                     |                  |       |                                                                                                                                                                                                                                                                                                                                                      |                                                                            |
| Syzygium aromaticum (Clove) | Eugenyl acetate, eugenol, and β-caryophyllene | A. flavus and A. niger | Induces cell death through early apoptosis (nuclear condensation) and late apoptosis (damage of plasma membrane) in hyphae, Downregulation of metabolic genes [secondary metabolism global regulator (laeA), lipase (lipA), and metalloprotease (metP)] responsible for fungal lipid and protein metabolism. | Oliveira et al. (2020); Castellanos et al. (2020)                          |
| P. granatum (pomegranate) | Tannins        | A. alternata, A. niger F. oxysporum, F. culmorum, F. graminearum, and P. digitatum | Generation of ROS resulting in the destruction of the plasma membrane and mitochondrial dysfunction.                                                                                                                                                                                                                                            | Zhu et al. (2019)                                                        |
| C. sinensis L. (green tea) and a variety of plants | Epigallocatechin 3-O-gallate (EGCG) | Candida app. | Formation of lesions on the cell membrane caused by loss of cell membrane integrity, cellular and plasma membrane damage, Increased membrane permeability that causes osmotic imbalance which ultimately results in cell death.                                                                                                                                                   | Bebbehsani et al. (2019)                                                  |
| S. lycopersicum (tomatoes) | Lycopene       | C. albicans | Plasma membrane depolarization and cell cycle arrest (G2/M) through increased intracellular ROS, Elevated levels of cytosolic and mitochondrial Ca²⁺ homeostasis causes mitochondrial dysfunction, Facilitates cytochrome c release that results in caspase activation, Disruption of cell wall and plasma membrane, coagulation of the cytoplasm, damage cellular organelles and ergosterol biosynthesis. | Choi and Lee (2015)                                                      |
| P. nigrum L. (Pepper) | limonene, sabinene, and β-caryophyllene | F. oxysporum and A. niger. | Disruption of cell wall and plasma membrane, coagulation of the cytoplasm, damage cellular organelles and ergosterol biosynthesis.                                                                                                                                                                                                            | Castellanos et al. (2020)                                                 |
|                      |                  |       |                                                                                                                                                                                                                                                                                                                                                      |                                                                            |
| (continued on next page)                                                                 |                  |       |                                                                                                                                                                                                                                                                                                                                                      |                                                                            |
| Plant source | Protective agent | Model | Mechanism of action | References |
|--------------|-----------------|-------|---------------------|------------|
| Curcuma longa L. and Curcuma spp. | Nanovesicles (curcumin and quercetin co-membrane expansion imbalance.) | Sadeghi-Ghadi et al. (2020); Rai et al. (2020) | Inhibits the production of ergosterol followed by release of cellular ions, Inhibition of methylglyoxal, inhibition of lipid peroxidation. | |
| Curcuma longa L. and Curcuma spp. | Nanoemulsions (curcumin + piperine + honey) | Phuna et al. (2020) | Nanoemulsions possessed favorable antifungal activity (more than 80%) against the wide | |
| A. Japonicus, Gilbertella persicaria | Chitosan nanoemulsion | Chaudhari et al. (2020) | Inhibits the production of ergosterol followed by release of mitochondrial dysfunction. | |
| Botrytis cinerea, and P. expansum | Inhibits the production of ergosterol followed by release of mitochondrial dysfunction. | | | |
| Inhibits the production of ergosterol followed by release of mitochondrial dysfunction. | | | | |
| | | | | |
that encapsulation significantly decreased the irritating odour of SAEO and also improved the inhibitory effect of SAEO on Saccharomyces cerevisiae, Rhizopus stolonifer, and E. coli and its antibacterial stability in 24 h (Zhang et al., 2018).

Studies have shown that natural products encapsulated in nanospheres yielded better results than with natural products alone (Ansari et al., 2011; Kumar et al., 2018). Nanospheres have been used as a stable carrier for the encapsulation of various therapeutic agents. Different types of phytochemical bioactive compounds such as resveratrol, curcumin, quercetin, rutin, oryzanol, chologenic acid, etc. have been encapsulated in cyclodextrin nanospheres (Kumar et al., 2018; Osmanli et al., 2018; Pawar et al., 2019). A study by Ramírez-Ambrosi et al. (2014) evaluated the efficacy of β-cyclodextrin nanospheres for encapsulation of polyphenols phloridzin, rutin, and chologenic acid. The results showed that rutin had an encapsulation efficiency of 83.7 % (which was the highest) using 1,1'-carboxyldiimidazole as cross-linker in a 1:3 ratio of nanosphere/cross-linker, while phloridzin (87.2%) and chologenic acid (77.5%), with best results seen with HMDI. Radic et al. (2020) evaluated the influence of olive pomace extracts matrix and cyclodextrins (CDs) on bio-accessibility and intestinal permeability of main olive pomace polyphenols. Their results demonstrated that olive pomace polyphenols were stable during gastrointestinal digestion and encapsulation of olive pomace polyphenol with cyclodextrins significantly increased bioaccessibility of tyrosol by forming inclusion complexes and preventing tyrosol adhesion to bile salts and other macromolecules present in reaction mixtures during simulation of olive pomace extract digestion. Despite the acclaimed pharmacological efficacy, there has been no investigation on cyclodextrin-based nanospheres encapsulation of phytochemicals for applications as biofungicides and nutraceuticals against mycotoxigenic fungi and mycotoxins. Nanospheres may play crucial role in the detoxification of mycotoxins in this era of the fourth industrial revolution (4IR) due to their binding and neutralization capacity (Fliszar-Nyul et al., 2019). This is because, these cyclodextrin-based nanospheres can reduce toxic effects associated with several microorganisms including mycotoxigenic fungi by binding and neutralising attendant harmful secondary metabolites in the body without leaving any harmful effects.

Cyclodextrins as nanospheres have been extensively used in a variety of applications, and a new class of them include integrated hydrogel cellulose nanospheres referred to as nanoemulgel that have recently attracted the attention of many scientists in the discovery and development of a variety of drugs. They are polymeric emulsion systems that attracted the attention of many scientists in the discovery and development of applications, and a new class of them include integrated hydrogel nanospheres. They are polymeric emulsion systems that have been carried out on the decontamination of mycotoxins using nanoencapsulated bioactive compounds. Cyclodextrin based-nanosphere encapsulated plant extracts or bioactive compounds can improve the efficacy of plant extracts or their phytochemicals for decontamination of mycotoxins as reviewed in this paper. This approach further increases the bioavailability of benign bioactive compounds utilized in agriculture as environmentally-friendly fungicides, highly-effective at low concentration with strong antifungal and mycotoxin-inhibiting activities.

Future work should seek to develop methods that may enable the use of these agents as food and feed additives for the same purpose. Detailed studies on the mechanisms of interaction between nano-encapsulated bioactive compounds and food components, together with their effects on human and animal health, should also be explored. Indeed, the potential of such nanospheres and plant bioactive compounds to play a dual pharmacological and nutraceutical function as biofungicides and detoxifying agents in mitigating the effects mycotoxins is very interesting.

Declarations

Author contribution statement

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Additional information

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References

Abdel-Fattah, S.M., Badr, A.N., Seif, F.A.-H., Ali, S.M., Hassan, A., 2018. Antifungal and antimycotoxigenic impact of eco-friendly extracts of wild stevia. J. Biol. Sci. 18, 488–499.

Abdel-Fattah, S.M., Aborea, Y.H., Shbata, F.E., Flourage, M.R., Helal, A.D., 2010. The efficacy of thyme oil as antioxiactant of aflatoxin(s) toxicity in sheep. Journal of American Science 6, 948–960.

Adelbiyi, J.A., Kajitnesi, E., Adebo, O.A., Changwa, R., Njohb, P.B., 2019. Food fermentation and mycotoxin detoxification: an African perspective. Food Conr. 106, 106731.

Adebo, O.A., Njohb, P.B., Gbashi, S., Nwinyi, O.C., Mavumengwana, V., 2017. Review on microbial degradation of aflatoxins. Crit. Rev. Food Sci. Nutr. 57, 3206–3217.

Adebo, O.A., Gabriela Medina-Meza, I., 2020. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: a mini review. Molecules 25, 927.

Adebo, O.A., Moleleka, T., Makhubele, R., Adebojji, J.A., Gydeje, A.B., Gbashi, S., Adelbo, A.A., Ogunde, O.M., Njohb, P.B., 2020. A review on novel non-thermal food processing techniques for mycotoxin reduction. Int. J. Food Sci. Technol. 86, 960.

Adebo, O.A., Adebojji, J.A., Njobh, P., Obadina, A., Landschoot, S., Audenaert, K., Okoth, S., De Boever, M., De Saeger, S., 2019. Investigation of the metabolic profile and toxigenic
R. Makhuvele et al. Heliyon 6 (2020) e05291

Singh, C., Prakash, C., Mishra, P., Tiwari, K.N., Mishra, S.K., More, R.S., Kumar, V., Shin, B., Park, W., 2018. Zoonotic diseases and phytochemical medicines for microbial infections in veterinary sciences: current state and future perspective. Frontiers in Microbiol. 9, 1443–4428.

Thipe, V.C., Bloombaum, P., Khoobchandani, M., Katti, K.K., 2020. Green nanotechnology: nanoformulations against toxigenic fungi to limit mycotoxicosis production. In: Rai, M., Abd-Elsalam (Eds.), Nanomycotoxicology. Treating Mycotoxins in the Nano Way. Elsevier, pp. 155–188.

Tian, Y., Tan, Y., Liu, N., Liao, Y., Sun, C., Wang, S., Wu, A., 2016. Functional agents to biologically control deoxynivalenol contamination in cereal grains. Front. Microbiol. 7, 4428.

Udomkun, P., Nishio, S.K., Muller, J., Vanlaeuwe, B., Bandyopadhyay, R., 2019. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application. A review. Food Contr. 76, 127–138.

Udomkun, P., Njukwe, E., 2020. Nanotechnological methods for aflatoxin control. In: Nanomycotoxicology, pp. 385–396.

Van Wyk, B.E., Van Outelaerhoorn, B., Gerice, B., 1997. Medicinal Plants of South Africa. Briza, Publications, Pretoria.

Velu, G., Balanichamy, V., Rajan, A.P., 2018. Physiochemical and pharmacological importance of plant secondary metabolites in modern medicine. In: Roopan, S.M., Madhumitha, G. (Eds.), Bioorganic Phase in Natural Food: an Overview. Springer International Publishing AG, pp. 135–156.

Venkatesh, N., Keller, N.P., 2019. Mycotoxins in conversation with bacteria and fungi. Front. Microbiol. 10.

Verschaeve, L., Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. J. Ethnopharmacol. 119, 575–587.

Vila-Donat, P., Marín, S., Sanchis, V., Ramos, A., 2018. A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. Food Chem. Toxicol. 114, 246–259.

Vipin, A.V., Raksha, R.K., Narasimhan, S., Anu, A.K., Venkateswaran, G., 2017. Protective effects of phenolics rich extract of ginger against aflatoxin B1-induced. Biomed. Pharmacother. 91, 415–424.

World Health Organization (WHO), 2001. Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A World Review. Geneva.

Wu, Q., Wang, X., Nopovimola, E., Wang, Y., Yang, H., Li, L., Zhang, X., Kuca, K., 2017a. Antioxidant agents against trichothecenes: new hints for oxidative stress treatment. Oncotarget 8, 110708–110726.

Wu, J.-C., Lai, C.-S., Tsai, M.-J., Ho, T., Wang, Y.-J., Pan, M.-H., 2017b. Chemopreventative effect of natural dietary compounds on xenobiotic-induced toxicity. J. Food Drug Anal. 25, 176–186.

Xu, J.-L., Li, L.-W., Luo, Y.-X., Yuan, S.-H., Yuan, N.-N., 2020. Antifungal nanomaterials: current progress and future directions. Innovations in Digital Health, Diagnostics, and Biomarkers 1.

Yang, G., Zong, L., Jiang, L., Geng, C., Cao, J., Sun, X., Liu, X., Chen, M., Ma, Y., 2011. 6-Gingerol prevents putolin-induced genotoxicity in HepG2 cells. Phytother Res. 25, 1480–1485.

Yao, Y., Huang, C., Liang, X., Fang, S., Wang, J., Chen, J., 2019. Development of starch-based antifungal coatings by incorporation of natamycin/methyl-β-cyclodextrin inclusion complex for postharvest treatments on cherry tomato against Botrytis cinerea. Molecules 24 (21), 3962.

Zain, M.E., 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15, 129–144.

Zhang, G., Yuan, C., Sun, Y., 2018. Effect of selective encapsulation of hydroxypropyl-β-cyclodextrin on components and antibacterial properties of star anise essential oil. Molecules 23.

Zhu, C., Lei, M., Andarjie, M., Zeng, J., Li, J., 2019. Antifungal activity and mechanism of action of tannic acid against Penicillium digitatum. Phytol. Mol. Plant Pathol. 107, 46–50.