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RESEARCH ARTICLE

BIOLOGICAL IMPACT OF THE HOST PLANTS METABOLITES OF *ASPERGILLUS FLAVUS* ON ITS GROWTH AND ITS AFLATOXINS BIOSYNTHESIS

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ABSTRACT

The present review outlines the antifungal activity of several secondary metabolites against growth of *Aspergillus flavus* as well as production of aflatoxins. There are different types of secondary metabolites such as terpenes, terpenoids, flavonoids, cyanogenic glycosides, saponins etc. These secondary metabolites are produced by cereal (host) crops including many other plants. *A. flavus* is a pathogenic fungus produces aflatoxins, which are considered as toxigenic, carcinogenic, mutagenic and teratogenic organic compounds. Here, we have discussed about the effects of essential oils of *Origanum vulgare*, *Melaleuca cojeputi* (white wood), *Curcuma longa* (turmeric) leaves, and extracts of *Camellia sinensis* (pure tea), chlorophyllous plants, *Cymbopogon citratus*, *Moringa oleifera*, *Ocimum gratissimum* and *Clerodendrum volubile* against the mycelial growth of *A. flavus* as well as production of aflatoxins. We also tried to correlate their biological properties with secondary metabolites, which are found in host plants of *A. flavus*. We concluded that above mentioned natural compounds have good potential to arrest the mycelial growth of *A. flavus* as well as production of aflatoxins, and there might be possibility that plants secondary metabolites are playing a key role behind the anti-aflatoxigenic as well as fungicidal activities of these natural compounds. Because of this, we can say that these plants secondary metabolites can be used as substitute against the infectivity of *A. flavus* as well as aflatoxins production.

Key words: Secondary metabolites, *Aspergillus flavus*, Aflatoxins, Carcinogenic, Mutagenic, Teratogenic, Essential oils.

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INTRODUCTION

The cereals such as rice, maize, wheat, sorghum, barely cover two -third of the world population as food items. These crops are the major diet for human beings. Fungal pathogens are considered as greatest threat to global cereal production. They are main host of *Aspergillus flavus*, and are being damaged by this fungus at large scale (1, 2).

Pathogenic fungus is considered as greatest threat to global cereals production. *A. flavus* is a pathogenic fungus which affects both humans and animals (3). The popular 'ear root disease' in corn and yellow mold disease in peanuts are basically caused by *A. flavus* in both pre-harvest and post-harvest conditions. These diseases are best known examples of ill effects of this mold (4). When corn is affected by ear-rot disease, it rapidly reduces weight as well as nutrient value. *A. flavus* produces aflatoxins, which are organic compounds. These are extremely carcinogenic and mutagenic contaminants (5, 6). Aflatoxins are considered as naturally occurring mycotoxins; they cause greater harm to these cereal crops. It

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has been reported that about 25% of the world staple food items are contaminated by mycotoxins annually (7). Aflatoxins have the prospectively responsible of acute hepatitis and even liver implications in animals and humans, and can lead to death if untreated (8-10). There are four main types of aflatoxins: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2) and among these, AFB1 is most potent mycotoxin (11). International Agency for Research on Cancer (IARC) has named and grouped them as human carcinogens (12). Fungal and plant interaction show complex and challenging scenario that are being studied by metabolomic approaches (13). Host plants of pathogens have various types of defence mechanisms to avoid the pathogens attack. These defence mechanisms include primary metabolites signalling, phytoalexin production, production of reactive oxygen species, cross linking of cell wall polymers, synthesis of pathogenesis related proteins etc. (14). Physical defence mechanism of host plants also play an important role during pathogenic attack by strengthening the cells wall with lignins (15). However, there is a direct relation between aflatoxins production, free radical generation and oxidative stress (16). Plants synthesize various types of secondary metabolites which play an important role against the infection of wide range of pathogens (17). Plant secondary metabolites are found accumulated in particular tissues in a highly sophisticated form, because some secondary metabolites are even toxic to the plants themselves if they are miss-localized (18). Hypersensitive response plays an important role during pathogen attack by forming the layer of dead cells that restricts spread of pathogen (19). Hypersensitive response also leads to the production of secondary metabolites, which are not only necessary for survival of plants but play a critical role during pathogen infection or in other defence mechanisms (20). Secondary metabolites categorized as many useful compounds like allelopathic compounds, antimicrobial agent (21), phytoanticipans and phytoalexins (22). It gives a perfect indication that secondary metabolites can be employed to arrest the growth of *A. flavus* as well as aflatoxins production. Many researchers and scholars around the world have been conducting research for a long time to recognize and characterize more numbers of secondary metabolites of cereal crops to discover possible potential as antifungal compounds, which can be employed against the contamination of *A. flavus* as well as production of aflatoxins to save the cereal crops.

The aflatoxins biosynthesis is initiated by biological activities of polyketide synthase (PKS) enzyme. The whole biosynthesis controls the aldol cyclization and aromatization of polyketide compound, which is the first stable compound in aflatoxin production. The *in silico* inhibition of PKS enzyme was formulated by using many plant secondary metabolites. It gives clear ideas about the environmental friendly prevention of aflatoxin biosynthesis in toxigenic species (6, 23).

Effect of different plant metabolites

Terpenes and Terpenoids

The terpenes and terpenoids are most commonly found secondary metabolites and over 40000 compounds have been described till now (24). Terpenes are synthesised from isoprene unit (C_5H_8) through mevalonate or non-mevalonate pathway (Figure 1). Isoprene units are added by condensation reaction and form a complex compound of terpenes such as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, tetraterpenes and polyterpenes. Terpenoids were defined as oxidised terpenes (25). These compounds have a tremendous role in plants such as plants hormones, electron careers, pigments, vitamins and also play crucial role during plant pathogen interaction (26). Both terpenes and terpenoids are stored in specialized glandular or secretary structure of cells to protect the host plant from potential toxicity of compounds (26, 27). Rice is well known host plant of *A. flavus*. Many studies revealed that concentration of terpenoids compounds inversely proportional to the pathogen infection in rice (28). The diterpenoid compounds: momilactones A and momilactones B, are secreted in rice leaves against the infection of *Magnoporthe grisea* fungus (28, 29). Other studies revealed new diterpenoid compound, oryzalexin A-D, which is found in rice and known as rice phytoalexins (30). Later, other three compounds of diterpenoids have been identified in rice and named orzyalexin S, orzyalexin E and oryzyalexin F. All of these three compounds show good antifungal activity (31, 32). Monoterpenes in essential oils play the role of neurotoxic against insect pests, here, they block the activity of neurotransmitter acetyl cholinestrase (33). Sometime terpenes work as electron donor and react with free radical to make more stable product, therefore it can terminate radical chain reaction (34).

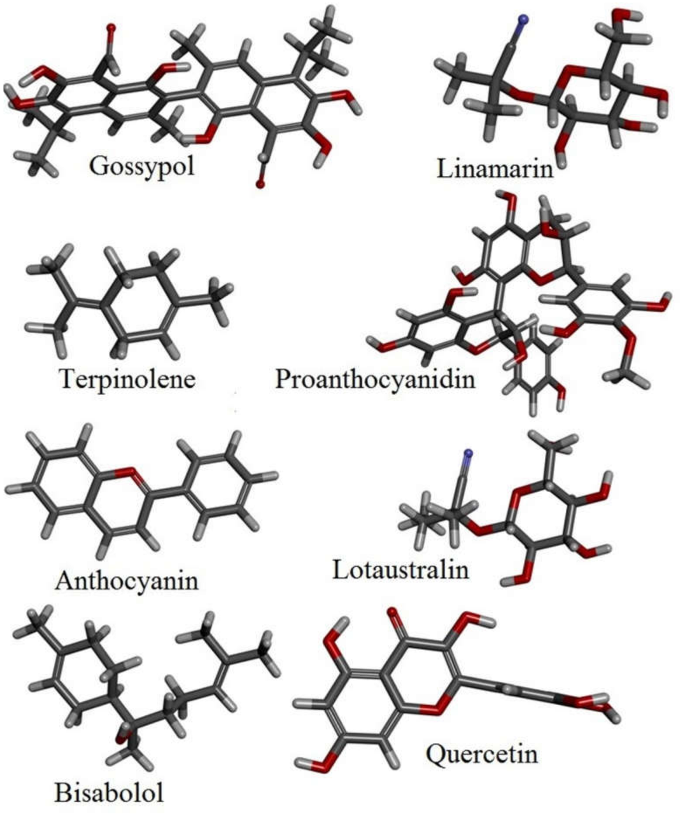

Figure 1. List of secondary metabolites which behave as inhibitors in blocking the mycelial growth of *A. flavus* and its aflatoxin biosynthesis

Terpenes and Terpenoids are major constituents of essential oils and determine the biological properties of essential oils (35). Many researches were done regarding the roles of essential oils against the growth of *A. flavus* along with its aflatoxins production. Researchers have found that essential oils can behave as good inhibitors against the growth of *A. flavus* as well as aflatoxin biosynthesis (36-38). Essential oils inhibit the growth of *A. flavus* by destroying the mycelium as well as prevent the growth of new mycelium development

(39). The essential oil of *Origanum vulgare* shows strong inhibitory effect on the growth of *A. flavus* as well as production of aflatoxins, and main fraction of this oil is constituted by monoterpenes, which covers 90.5% of total oils composition (40). Dusanee and his colleagues (2007) observed the effects of 16 essential oils of cerematic plants against *A. flavus* infection , and found that the essential oil of white wood (*Melaleuca cojeputi*) has the highest inhibitory effect on the growth of *A. flavus* than other common essential oils (41). The essential oil of white wood plant contains monoterpenes compound as a major constituents of oil, here, monoterpenes are terpinolene and γ-terpinene (42). In another experiment, essential oil of turmeric leaves inhibited the growth of *A. flavus*, here, terpinolene is also a major ingredient of turmeric leaves oil (43). Cotton produces terpenes compounds Gossypol, a type of disesquiterpene that shows inhibitory effect on the growth of *A. flavus* (44). Monocyclic sesquiterpenes, α-bisabolol also shows strong antifungal activities (45, 46). Terpenes act as an antioxidant to kill the microbes. It has been proved by studies on the essential oil of *Boswellia socotrana,* which has higher antioxidant activity due to presence of higher concentration of oxygenated monoterpenes (47). Figure 1 show the structures of main classes of secondary metabolites which have been discussed in this review.

Flavonoids

Flavonoids are a large class of secondary metabolites. These are synthesised through shikimate pathway (Figure 2). There are different types of flavonoids: flavonols, flavones, flavanones, anthocyanidins, proanthocyanidins and chalcones. Flavonoids play a vital role throughout the life cycle of many plants. These compounds show various useful roles such as antioxidant activity, role in signalling pathway, antimicrobial activity, flower pigmentation and UV protection (48, 49). Flavonoids have become more significant secondary metabolites due to their use in pharmaceutical industries as anti-inflammatory and anticancer agents at large scale (50). To save the host plants during pathogens attack flavonoids show various types of defense mechanisms such as inhibition of cellulases and other microbial enzymes, cross linking of important enzymes, chelation of metals to deactivate the microbial enzyme and formation of crystalline structure which works as a physical barrier during pathogen attack (51). In vitro studies showed that flavonoids inhibit the growth of fungus and these secondary metabolites are secreted by plants during initial stage of fungal infection, when plant cells come in direct contact with fungus cells (1). Most of the flavonoids need compartmentalization inside the cell to ignore the mutagenic and oxidative effects of active compounds. It was seen that synthesis of the flavone saponarin in mesophyll protoplasts was inhibited in the absence of vacuoles, it indicated that activation of vacuoles are very important for the production of flavone (52). In *A. flavus* host plants, viz. maize, barley and rye , various types of pathways have been identified for vascular transport such as multidrug resistance associated proteins like ABC transporter, vacuolar ATP binding cassette (ABC) transporter and pH-dependent vacuolar flavonoid/H+ antiporters (52-54). More attention are being given on the properties of flavonoids to implement their properties in metabolic engineering of crop plants to make disease resistance plants (55). It has been purposed that flavonoids at higher concentration inhibit the production of aflatoxins (56, 57). Anthocyanidins, a class of flavonoids , have good inhibitory effect against the growth of *A. flavus* as well as production of aflatoxins (57). Pure tea extract inhibits the production of aflatoxins by down regulating the transcription of *aflR* and *aflS* genes (58). Flavonoids are the major constituent of tea extract (59). Proanthocyanidins, a type of flavonoids, have been reported to make a physical barrier when plants are attacked by fungus species (51). Sinha (1990) conducted an experiment to prevent the growth of *A. flavus* along with its aflatoxins production in cereals and oil-seeds plants. For this, he observed the effect of aqueous extracts of 25 chlorophyllous plant, and found that the aqueous extract containing flavonoids as common chemical ingredients inhibited the production of aflatoxins Later, Mallozzi et al. (1996) studied the effect of other flavonoids such as kaempherol, quercitin, kaempheritin and naringinin, against the growth of *A. flavus* as well as aflatoxins production. He obsorbed that these flavonoids compounds also have good inhibitory effect against the growth of *A. flavus* as well as aflatoxins production, and among these four flavonoids compounds, kaempheritrin has the greatest inhibition effect in respect to the production of the aflatoxin B1(51). Other researchers reported about sakuranetin (a flavonoids compound), which is secreted in rice against the fungus attack (60). It has also been reported that some members of flavonoids work as phytoanticipans, which inhibit the growth of fungus *M. grisea* in rice (61).

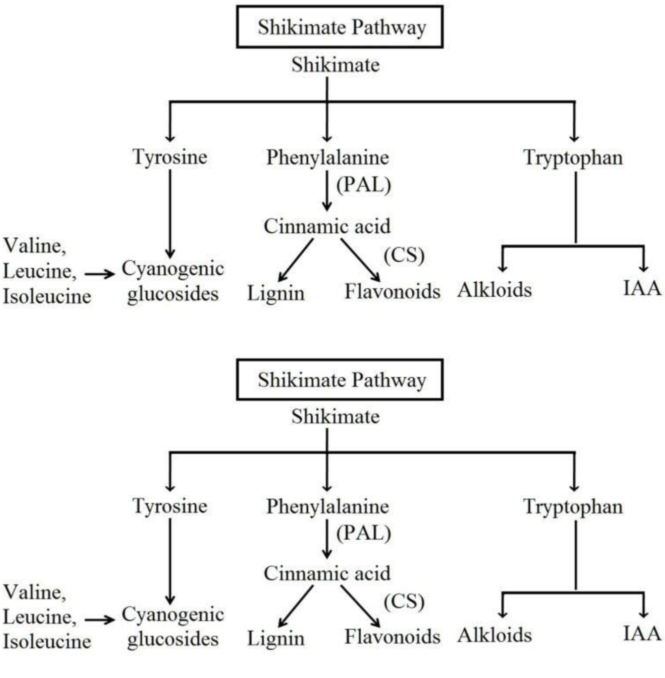

Figure 2. Flavonoids and cyanogenic glucosides (Secondary metabolites) are synthesised through shikimate pathway

Cyanogenic Glycosides

Cyanogenic glycosides are important secondary metabolites , which are found in more than 2600 plant species and also in many cereal crops, viz. wheat, maize, rice, barley and oat (62). These compounds are synthesised through shikimate pathway (Figure 1) and here, amino acid: lucine, isolucine, valine, phenylalanine, tyrosine and the non-protein amino acid cyclopentenyl- glycine play the role of precursors compounds (63). Cyanogenic glycosides are compartmentalized separately from β-glucosidases to inhibit the release of toxic compound HCN in normal condition, because cyanogenic glycosides are activated by β-glucosidase depended hydrolysis and form an unstable compound aglycone, which is further converted into a ketone or an aldehyde group and toxic compound HCN (64, 65). For example, in sorghum plants cynogenic glycosides dhurrins are found in epidermal layers of leafs but β-glucosidases and α-hydroxynitrile lyase (which also activates cyanogenic glycosides and release HCN) are found in mesophyll cells (66). Cyanide is highly toxic because it inhibits oxidative function of cytochromes oxidase of mitochondria that reduces the ability of cells to use oxygen for aerobic respiration (67, 68). It has been reported that cyanogenic glycosides can play an important role in life of plants against the infection of pathogen. Cyanogenic glycosides such as linamarin and lotaustralin (methyl linamarin) play a vital role during pathogen attacks. Both linamarin and lotaustralin are found abundantly in cassava plant, and this plant has low level of infection of *Aspergillus flavus* along with its aflatoxins production (69, 70). Barely plants have five types of leucine derived cyanogenic glycosides but β-glucosidase which hydrolyses and activates cyanogenic glycosides are found only in germinating barely, because of this cyanide potential of barely is not harnessed during fungal attack (63). This situation makes barely plant prone to the infection of *A. flavus*. Lotus corniculatus is a type of flowering plants, when this plant is attacked by fungal species or pathogen then leaves of this plant start to secrete HCN to arrest the growth of fungal species or pathogen (71).

Saponins

Saponins are a class of secondary metabolites which are found in many plants (72). They are glycosylated triterpenes steroids and steroidal alkaloids, which are synthesised through mevalonate or non-mevalonate pathway (Figure 3) (1). These secondary metabolites show a high degree of antimicrobial, insecticidal allelopathic activity (72, 73). Saponins are considered as natural inhibitors of pathogen attack (74, 75). These compounds reside in plant cells in inactive form, but when plant is attacked by pathogen then inactive form of saponins are converted into active form with the help of plants enzymes (76). Saponins are generally used to make drugs, foaming agents, cosmetics, sweeteners and taste modifiers (72). Cereal plant oat contains two types of saponins compounds: four triterpenoids avenacins and two steroidal avenacosides, are found in roots and leaves respectively (74, 77). Avenacins are found active in their natural glycosylated form but avenacosides become active in their aglycone forms (78, 79). Inactive avenacosides are stored in plant vacuoles and become active when pathogenic fungi disturb the cell membrane. Thereafter, β- glucosidase hydrolyses the D-glucose unit of avenacosides to form active aglycone (80). Active aglycone forms complexes with sterols of plasma membrane of fungi and disturbs this membrane by pore formation that leads to death of fungi (1). Avenacins, which are found active in their glycosylated form, are also stored in appropriate plants vacuole in highly sophisticated manner and protect themselves from their own toxic effect by different membrane sterols compositions (73, 74, 81). Researchers reported that plant extracts of *Cymbopogon citratus*, *Moringa oleifera*, *Ocimum gratissimum* and *Clerodendrum volubile* have inhibitory effect against the growth of *A. flavus* and also hypothesised that this inhibitory effect may be possible due to presence of saponins (82). In another experiment, researchers isolated CAY -1 (steroidal saponins, molecular weight 1243.35 Da) from the plant *Capsicum frutescens* and found that this saponin has high inhibitory effects against the germinating conidia of *A. flavus* (83). Osbourn (1996) has also reported that saponins can act as chemical defence barrier against the infection of fungi (73). When *A. flavus* infects the plants, then plants come into stress and hypersensitive response develops. Hypersensitive response leads to the synthesis of secondary metabolites (Figure 4) (20). During this, hexose is formed from starch. Thereafter, shikimate pathway (SM), mevalonate pathway (MVA), methylerythritol pathway (ME) and synthesis of PR proteins begin to encounter the *A. flavus*. Flavonoids, cyanogenic glucosides (CG), phytoalexins and lignins are synthesised from aromatic amino acid through SM pathway but terpenes and saponins are synthesised from MVA/MEP pathway. Lignins solidify the cells wall and make a nondegradable barrier against the pathogen infection (23, 84).

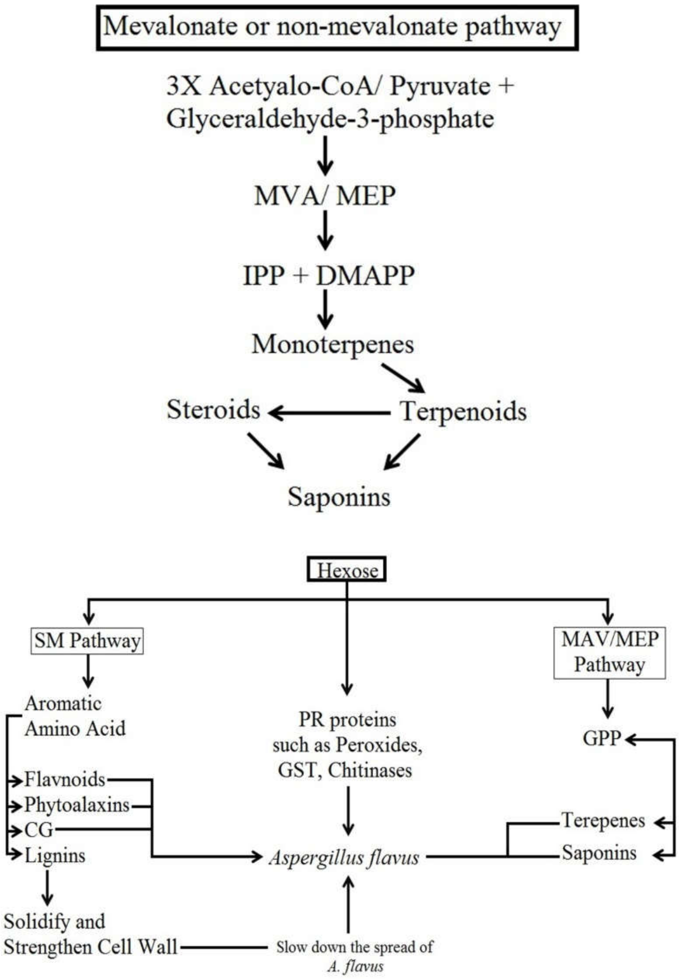

Figure 4. Hypothetical schematic presentation of plants defense mechanism during the infection of *Aspergillus flavus*

RESULTS AND DISCUSSION

Essential oils of *Origanum vulgare*, white wood (*Melaleuca cojeputi*) and turmeric leaves show inhibitory effect on the growth of *A. flavus*, and terpenes are the major constituent of these oils. Bakkali *et al*. (2008) also reported that terpenes and terpenoids are main components of essential oils and determine the biological properties of these oils (85). Later, the work of J.E. Mellon *et al*. (2011) proves that terpenes have inhibitory effects against the growth of *A. flavus*. Other researchers also told that terpenes and terpenoids have antifungal properties. So, according to these studies we can hypothesise that terpenes and terpenoids have potential to inhibit the growth of *A. flavus* as well as biosynthesis of aflatoxins (44). Pure tea extracts and aqueous extracts of chlorophyllous plants inhibit the production of aflatoxins and both plants extracts have flavonoids as major components. Experiment of Mallozzi *et a*l. (1996) has given an evidence that flavonoids have potential to inhibit the production of aflatoxins (51). Later, Robert (1999) proves that anthocyanidins (a class of flavonoids) can inhibit the growth of *A. flavus* as well as production of aflatoxins. Many researchers also reported that flavonoids can inhibit the production of aflatoxins and also have antifungal properties. These studies are revealing that flavonoids can be a good inhibitor to prevent the growth of *A. flavus* along with its aflatoxins production (57). Many researchers reported that cyanogenic glycosides have good antifungal properties. The cassava plants are more rich in cyanogenic glycosides and less susceptible to infection of *A. flavus* while cyanogenic glycosides are also found in barely plant but they are not activated during fungal attack, because their activators β-glucosidases are found only in germinating barley plants not in adult barely plants (63). This condition makes adult barley plants more susceptible to infection of *A. flavus*. These studies are indicating that cynogenic glycosides can be employed to inhibit the growth of *A. flavus* as well as production of aflatoxins. The aqueous extracts of *Cymbopogon citratus*, *Moringa oleifera*, *Ocimum gratissimum* and *Clerodendrum volubile* considerably inhibit the mycelial growth of *A. flavus*. Jeff-Agbolla and Awe (2016) concluded that inhibitory effect of these plants extract might be possible due to presence of saponins (82). This hypothesis can be supported by the experiment of De lucca *et al*. (2002), here, researchers observed the effect of pure form saponin compound on the rowth of *A. flavus* and found that this saponin compound has potential to arrest the growth of this mold (83). Osbourn (1996) also supported the antifungal properties of saponins (73). According to above reports we can say that saponins can be used as a good agent to arrest the growth of *A. flavus* and its aflatoxins production.

Conclusion

In this review, we have discussed about the major classes of secondary metabolites, which are found in cereal crops and other plants. As seen from these studies, we concluded that secondary metabolites such as terpenes, terpenoids, flavonoids, cyanogenic glycosides and saponins have good antifungal properties. They can arrest the growth of *A. flavus* along with its aflatoxins production. All of these secondary metabolites are also found in host plants of *A. flavus*. So, we can hypothesize that if expressions of these secondary metabolites are increased in host plants (cereal crops) of this mold then we can save these host plants (cereal crops) from the harmful effects of *A. flavus* and aflatoxins. In future, this hypothesis can help genetics and plant breeders to develop *A. flavus* resistance cereal crops plants, which would be fruitful for the world's economy.

Conflict-of-Interest

There is no any conflict of interest while submitting this manuscript for publication.

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