Recent Trends on Utilization of Phytochemicals against Mycotoxin producing *Aspergilli* and *Penicillium* species

Dissertation submitted in partial fulfillment of the requirement for the degree of

MASTERS OF SCIENCE

IN

BIOTECHNOLOGY

BY

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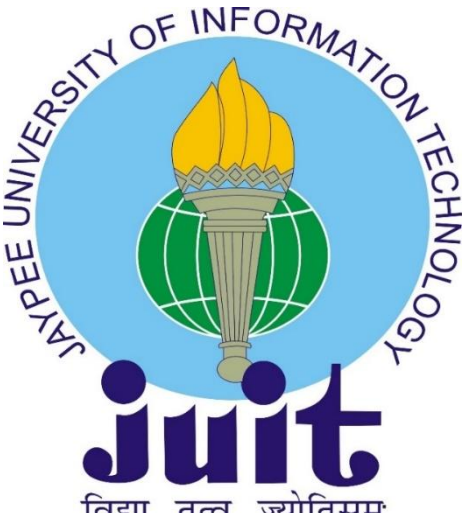
UNDER THE GUIDANCE OF

Dr. Jata Shankar



Jaypee University of Information Technology, Waknaghat

May 2021

Declaration

I hereby declare that the work reported in the Master's thesis entitled "**Recent Trends on Utilization of Phytochemicals against Mycotoxin producing *Aspergilli* and *Penicillium* species**" submitted at Jaypee University of Information Technology, Waknaghat India, is an authentic record of my work that was carried out under the supervision of **Dr. Jata Shankar**. I have not submitted this work elsewhere for any other degree or diploma.

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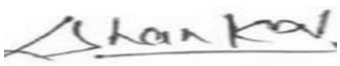
Certificate

This is to certify that the work which is presented in the project title, "**Recent Trends on Utilization of Phytochemicals against Mycotoxin producing *Aspergilli* and *Penicillium* species**" submitted in the partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology at Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by **Aanchal Bansal** (197806) during a period from August 2020 to May 2020 under the supervision of Dr. Jata Shankar, Associate Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat.

The above statement is correct to the best of my knowledge.



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Acknowledgment

Completing and submitting the thesis work seemed very illusionary. It was a resilient journey that would not have been possible without the enormous support of few people. I want to thank Jaypee University of Information Technology, Waknaghat for providing me this opportunity. I would like to extend my deepest gratitude towards my supervisor throughout this project, Dr. Jata Shankar, Associate professor. His constant support and guidance have helped me complete my thesis. His insightful and constructive comments, suggestions, warm encouragement, and extreme tolerance are invaluable. His meticulous feedbacks have helped to grow as a researcher and as a person. He has been diligent in editing and correcting my documents which is tiresome and I owe him for that.

I would like to show my special appreciation to my parents who have supported and encouraged me throughout to stay focused on continuing my literature research.

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Abstract

Mycotoxins had been diagnosed as critical toxins affecting animal species and human beings after the discovery of aflatoxin B1 in 1960. Mycotoxigenic fungi are pervasive and are held chargeable for monetary loss as they lower crop yield and the peculiarity of food. The presence of fungal species and their mycotoxins are now additionally found in medicinal herbs and pre-packaged foods. Since prevention isn't always usually possible, cleansing of mycotoxins had been tried by using other means. In addition to recognized mycotoxins, for which limit levels in food are imposed, unregulated, so-called “emerging mycotoxins” had also been proven to arise often in agricultural products. Significant demanding situations nevertheless exist due to the complicated heterogeneous nature of meal composition. The capacity of blended omics techniques inclusive of metabolomics, transcriptomics, genomics, and proteomics might contribute to our information in identifying, isolating, and characterizing mycotoxins pre and post-harvest. One of the promising techniques is the usage of bioactive plant metabolites. According to the World Health Organization (WHO, 2001), about 80% of the populace of the sector still rely on conventional plant-based drug treatments for primary healthcare. Plants generate secondary metabolites as a resistance mechanism towards pathogenic microorganisms, bugs, and negative environmental situations. These metabolites are referred to as phytochemicals. These might include essential oils but are non-nutritive. However, they could guard human beings and animals against diseases resulting from microbes or toxins related to them because of the antimicrobial characteristics they possess. These metabolites are one of the most encouraging agents for upcoming drug discovery and development. Based on their chemical structures, phytochemicals have been divided into major groups including phenolics, coumarins, flavonoids, etc. These compounds may act directly or diffusely to defend against illnesses or pathogens.

Keywords: Aflatoxin, Patulin, Toxicity, Phytochemicals, *Aspergillus flavus*

Objectives

1. **To study the factors affecting biosynthesis of Aflatoxin and Patulin, and their toxicity.**
2. **To study the effect of phytochemicals on mycotoxins produced by Ascomycetes**
3. **To analyze the anti-tumor activity of Patulin**

Literature Search Strategy

Search terms	AND, OR
Keywords searched	Aflatoxins, patulin, biosynthesis, phytochemicals, toxicity, cancer, degradation, ascomycetes
Databases searched	*NCBI- PubMed* *Google Scholar* *PubChem* *SDME*
Years of research included	*1943-2020*
Language	*English*
Types of studies to be included	*Qualitative studies, Quantitative studies*
Figure construction	*Microsoft*

Chapter 1
Inhibition of Aflatoxins by Phytochemicals

Introduction

Aflatoxins are a predominant class of mycotoxins produced in *Aspergillus* species, specifically *A.flavus* and *A.parasiticus*. They are known to suppress the immune response [1]. Aspergillus are saprophytic, opportunistic pathogens that infect the food, feed, herbs, and different commodities after harvest at some point of storage. This is because of the humid surroundings and moisture present. Therefore, to avoid such infection, vegetation has to be dried nicely earlier than storage or positive bio-preservatives have to be used. Certainly, herbal compounds like phytochemicals that don't have any adverse outcomes medically and physiologically, are taken into consideration for secure human consumption [2]. Most phytochemicals have antioxidative residences that help to neutralize ROS in vegetation due to aflatoxin production. AFB1, AFB2, AFG1, and AFG2 are the principal forms of aflatoxins [137]. AFB1 is the most poisonous and declared as a group 1 carcinogen by International Agency for Research on Cancer (IARC) [3]. AFB1 specifically objectives liver and is related to hepatocellular carcinoma. It also can cause stomach pain, vomiting, edema, and death [4]. Aflatoxin infection may also arise at once from food/vegetation formerly infected by aflatoxin, or circuitously with the aid of using milk, hen, and meat because of preceding infection of animal feed. Aflatoxins are furanocoumarins [5]. Their activation is catalyzed by cytochrome p450 enzymes in the liver and transformed into highly reactive epoxides. The toxicity of AFB1 is due to the lactone ring and the difuran ring moiety with double bonds [6]. It was discovered that the cleavage of the lactone ring yields a non-fluorescent compound that has decreased mutagenicity and toxicity. B2 and G2 groups are much less poisonous than AFB1 due to the fact they lack the double bond ring moiety.

Occurrence

Food and Feed

Mycotoxins are natural contaminants observed everywhere in the World. They are observed in a variety of commodities of plant origin and additionally in animal-derived meals. Environmental elements like temperature and moisture content material are the important

thing factors contributing to mycotoxin synthesis [7]. Toxins produced by Aspergillus species are usually referred to as storage mycotoxins due to the fact they have an effect on the vegetation after harvest in storage because of flawed drying and warm humid environment. Few micrograms per kilogram of aflatoxins in peanuts, dried fruits, oilseeds, cereals, tree nuts, and processed ingredients are declared secure for human intake [8]. However, continual intake can result in bioaccumulation. Similarly, in animal feed, the quantity of AFB1 is considered secure in unique concentrations for distinct animals, which include 10-20 ug/kg for younger animals, round 100ug/kg for breeding cattle, swine, and chicken animals [9]. However, extended exposure to aflatoxins can purpose primary health issues from acute demise to continual disease. Animal intoxication results in gastrointestinal dysfunction, anemia, jaundice, reduced egg or milk manufacturing, etc in poultry [10]. AFM1 and AFM2 are thought to be secreted in animal milk that's then transferred to feeding animals and humans [11]. The author additionally claimed that meat gathered from a nearby marketplace in Jordan exhibited AFB1, AFB2, AFG1, and AFG2. Aspergillus and AFB1 had been always observed in rice samples in India [12], China and Nigeria, and UAE. High quantities of aflatoxins are observed in Maize merchandise that is used each as meals and feed. Aflatoxins had been additionally observed in Maize primarily based products in the UK [13].

Herbal Medicines

Herbal drug treatments additionally recognized as phytomedicines or botanical drug treatments had been winning withinside the international for many years. These drug treatments make contributions to a big part of healthcare in international locations like India, China, and Africa [14]. Extrinsic elements might also additionally contaminate the herbs which must be severely monitored. As the idea of natural medication is growing worldwide, fungal infection has come to be a major thing of concern. Toxigenic fungal species like Aspergillus produce mycotoxins which can unfold from soil and plant life at once to the herbs. Aflatoxins are carcinogenic, teratogenic, and mutagenic. The presence of mycotoxins in natural drug treatments additionally lowers the drug interactions and efficiency besides presenting toxicity [15]. Several kinds of research in India stated the presence of AFB1 and citrinin in natural and medicinal plant life. These mycotoxins had been additionally determined in herbs from Argentina [16]. AFB1 additionally infected the medicinal plant ginseng [17]. *Aspergillus* and *Penicillium* species generating aflatoxins, ochratoxin A and citrinin had been determined in medicinal herbs in Brazil [18]. According to reviews in Saudi

Arabia [19], AFB1 was observed frequently in Pimpinella anisum, Piper nigrum, Mentha piperita, and Origanum majorana. A net concentration of 13.6% aflatoxins had been detected in spices in Korea [20]. Many of the herbs had been infected with a mixture of mycotoxins as consistent with researches carried out in Spain [21]. In international locations like Morocco, flavoring meals like pepper, paprika, cumin, saffron, and ginger are dried in open areas on the ground. Due to flawed environmental conditions, the hazard of mycotoxin manufacturing could be very common. Aflatoxins had been stated in high abundance in 14 samples out of fifty-five samples of 4 sorts of spices in this study [22].

Biological Fluids

Aspergillus species mainly A.flavus and A.parasiticus produce danger-associated mycotoxins which include aflatoxin. They are associated with hepatocellular carcinoma and may be as critical as inflicting death. The severity of the impact of mycotoxin relies upon on general quantity ingested, period of exposure, and synergism. Studies had been carried out to test the concentration of mycotoxins in organic samples which includes urine and different fluids, tissues, and organs []. The general extraction method used turned into LLE. These experiments had been carried out on humans, pigs, rats, and chickens. Most quantities of mycotoxins had been observed in urine samples and aflatoxins shape a prime class of those mycotoxins [23].

Toxicity

The toxicity of aflatoxins is due to the lactone ring and difuran ring moiety with double bonds. Long-time period intake of aflatoxins results in bioaccumulation in the liver [6]. The liver has cytochrome p450 enzymes that convert AFB1 to AFB1-8,9-exo-epoxides. These epoxides are surprisingly reactive. They are transformed into reactive dialdehydes that adjust the lysyl residues in proteins. They shape derivatives with DNA, RNA, and proteins, and react with p53 tumor suppressor genes [24]. Epoxides are carcinogenic that purpose mutations in codon 249 in humans, and codon 12 and 13 of Ras oncogenes [25]. During this procedure, AFB1-N7-Gua is generated, which converts into its greater stabilized shape AFB1-formamidopyrimidine (AFB1-FABY) adduct. This leads to the transformation in nucleotides from Guanine to Thymine and consequences into mutations and malignant transformation [26]. This in addition ends in inhibition of cellular growth, increase in LDH activity, ROS generation, retardation, immunosuppression, and genotoxicity [27]. Phytochemicals may be used to offer resistance towards AFB1. They initiate the aldo-keto

reductase activity and glutathione S-transferase (GST) activity in opposition to epoxide. AFB1-aldehyde reductase (AFAR) prevents the binding of dialdehyde to primary amino acids of proteins and therefore prevents cytotoxicity. The toxicity mechanism has been illustrated in [137]. AFAR gene is positioned on chromosome 1 in humans. However, in the course of colorectal/liver and most cancers, this place is typically deleted [25].

Biosynthesis of Aflatoxin

Biosynthesis of aflatoxin takes place in *A.flavus* and *A.parasiticus*. The first precursor withinside the biosynthesis of AF is Norsoloronic Acid (NOR), a brick pink pigment. NOR ends in the formation of the next intermediates. The biosynthetic pathway includes several intermediating genes, enzymes, and mechanisms. Nearly 30 genes are recognized to be involved, clustered in seventy-five (75) kb region on chromosome III of the fungal genome. This vicinity is placed 80kb far from telomere [28]. The genes have been isolated from *A.parasiticus*. The homologous genes have been additionally determined in *A.nidulans*, however, aflatoxin manufacturing does take place on this because of the absence of gene encoding for transferase enzyme. Transferase is needed for the switch of a methyl group to Sterigmatocystin in step 11 [137]). The complete biosynthetic pathway was defined by Jiujiang Yu [29]. The first step is the formation of precursor unit NOR from a starter hexanol. Hexanol is first transformed into a polyketide catalyzed via fatty acid synthase enzymes and polyketide synthase (PksA). The fatty acid synthases that catalyze the reaction are fas-1 and fas-2 that encodes for fatty acid synthase-1 and the alpha subunit of fatty acid synthase respectively. Polyketide synthase gene has 4 conserved domain names in its amino-acid sequence, that is, beta-ketoacyl synthase (KS), acyltransferase (AT), and an acyl carrier protein (ACP), and thioesterase (TE).

The practical domains are involved withinside the formation of a polypeptide, which is then transformed into noranthrone through PksA. The oxidation of noranthrone to NOR is

catalyzed through monooxygenase i.e., noranthrone oxidase encoded through hypC gene. The subsequent step is the reduction of NOR. The nor1 gene encoding for ketoreductase reduces the primary keto group of NOR hydroxyl institution and results in the formation of Averantin (AVN). Other genes that may be used for this redox reaction are homologous norA and norB that codes for short-chain aryl alcohol dehydrogenase. This next step is an oxidation reaction catalyzed through p-450 monooxygenase. This enzyme is encoded through the ord1 gene. The product is 5-hydroxyaverantin (HAVN). This is a reversible response and NADPH is used as a cofactor. The subsequent step is the formation of Averufin (AVF) which is a key intermediate. During the conversion of AVN to AVF, other intermediates are formed. One of them is HAVN as mentioned above. The different one is Averufanin (AVNN) catalyzed via alcohol dehydrogenase encoded through adhA gene. AVNN is a shunt metabolite. HAVN may be at once transformed to AVF or through an intermediate 5-oxoaverantin (OAVN) catalyzed through to cytosolic enzymes. Averufin (AVN) is transformed to Versiconal Hemiacetal Acetate (VHA) in an oxidation reaction catalyzed through cytochrome p450 monooxidase. The proteins encoded through different genes avfA and cypX are additionally required in the formation of hydroxy versicolorone that is useful in the ring closure step.

A cluster gene estA encodes for esterase which catalyzes the conversion of VHA to Versiconal (VHOH/VAL). Another intermediate Versiconal acetate (VOAc) is likewise catalyzed through esterase to be transformed to VOH, versiconal. The subsequent key step in aflatoxin biosynthesis is the side chain cyclo-dehydration of versiconal to shape Versicolorin B (VER B). This step is catalyzed through VHOH cyclase and VER B synthase. This is a crucial step because it closes the bifuran ring of aflatoxin which contributes to toxicity and carcinogenicity. VER B includes tetrahydrobifuran ring and leads to formation of AFB2/AFG2 while VER A includes dihydrobifuran ring and results in formation of AFB1/AFG1. Interconversion between VER B and VER A requires desaturation of bifuran ring, catalysed through microsomal enzymes cytochrome P 450 monooxygenase withinside the presence of NADPH as a cofactor. In the following step, VERA and VER B are transformed to demethylsterigmatocystin (DMST) and demethyldihydrosterigmatocystin (DMDHST) respectively. Ketoreductase encoded through ver1, and cyt P450 monooxygenase encoded through verB are concerned in the production of DMST from VER A. The different enzymes encoded through a hypA and ordB have additionally been advised to play a function in this response. The 43 kD O-methyltransferases aid the transfer of methyl group from S-adenosyl-methionine (SAM) to the hydroxyl group of DMST and DMDHST

which results in the production of sterigmatocystin (ST) and dihydro sterigmatocystin (DHST), respectively. The corresponding gene dmtA encodes for a protein containing SAM binding motif which has been isolated from *A. parasiticus*. Another O-methyl transferase A enzyme catalyzes the formation of O-methylsterigmatocystin (OMST) and dihydro OMST from ST and DHST respectively. This enzyme is encoded by omt1 gene and has substrate-specific activity and isn't always capable to further methylate DM ST or DM DHST. The subsequent step is the formation of AFB1 and AFG1 from OMST, and AFB2 and AFG2 from DH OMST [137]. The principal enzyme required for this monooxygenase reaction is encoded through ordA gene. However, for G-group aflatoxins, an extra set of genes is likewise required. These consist of cypA gene, nadA and nor1 genes. AFB1 and AFB2 are produced in *A.flavus*, however, all four agencies AFB1, AFB2, AFG1, AFG2 are produced through *A.parasiticus*. As *A.flavus* does not cause the production of G-group aflatoxins, it lacks nadA and norB genes. Once AFB1 and AFB2 enter the body, the liver cytochrome p450 enzymes convert them into notably reactive epoxides or they may be hydroxylated into much less dangerous AFM1 and AFM2 [29].

Factors affecting Aflatoxin Production

Aflatoxin biosynthesis is stricken by diverse biotic and abiotic environmental elements. These encompass dietary elements like carbon and nitrogen availability, physiological like pH, environmental factors like temperature and moisture content, and different bio-reactive agents. These elements may be manipulated to lessen the aflatoxin biosynthesis and fungal growth [29].

Nutritional Factors

Nutritional assets like carbon and nitrogen incredibly affect the aflatoxin biosynthesis pathway. *A. parasiticus* has genes for sugar consumption that are required for AF production. They use simple sugars along with glucose, fructose, sucrose, and maltose. *A. flavus* and *A. parasiticus* have lipA gene for the processing of lipid substrate that may be the right carbon

source [30]. Nitrogen reasserts like asparagine, aspartate, alanine, sulfates, proline, glutamine guide AF production, whereas, sodium nitrate and sodium nitrite downregulate the averufin and AF formation. Nitrates ends in overexpression of aflR gene that has a poor impact on the biosynthetic pathway [31]. However distinctive amino acids have distinctive results on mycotoxin production.

Environmental and Physiological Factors

The optimum temperature required for aflatoxin synthesis is 30°C, whilst the temperature rises above 36°C the pathway genes can't characteristic nicely and influences mycotoxin production. Patel et al demonstrated that the production of aflatoxin was highest at 27°C and at pH 6.5-7 [32]. Outbreaks are maximum throughout warm and humid climates and drought situations. This can be due to the water pressure situations advanced in flora that weakens their defence system, or accidents withinside the tissues that allow fungal spores to enter [33]. Another issue is culture pH. Acidic medium favours the toxin synthesis, whereas alkaline medium inhibits this pathway. The pacC gene regulates the pH homeostasis by binding to the aflR transcriptional factor and altering its expression [34].

Phytochemicals

Phytochemicals are secondary metabolites produced via floral species for defense mechanisms. Many phytochemicals are recognized to work in opposition to aflatoxin production [29]. Since they are herbal metabolites without any damaging effects, they were taken into consideration to be used as anti-aflatoxigenic in humans and animals. AF synthesis is correlated with oxidative stress. Antioxidants like ascorbic acid, caffeine, gallic acid, quercetin, and different phenolic agents are used for the manipulation of Aspergillus species generating mycotoxins. Hence, those antioxidative herbal compounds, with minimum clinical or environmental danger may be used as antifungals.

Quercetin

Quercetin falls in the flavonoid group of polyphenols. It is the plant pigment observed in crimson wine, green tea, apples, onions, berries, and different foods. In silico results revealed that quercetin may be used to inhibit AFB1 biosynthesis in Aspergillus flavus [138]. To evaluate the impact of quercetin on AFB1 biosynthesis in vivo, integrated proteome and HPLC analyses were conducted [35]. A.flavus was cultured in cornflour with and without quercetin and labeled as CFq and CF respectively. After forty-eight hours of incubation

AFB1 biosynthesis reduced by 51% in CFq. Quercetin is a green and economically viable anti-aflatoxigenic agent. The proteomic evaluation discovered that the culture plate with quercetin (CFq) had a low count of proteins i.e., 705, compared to CF which had 843 proteins. However, transmembrane proteins have been found in CFq and not in CF. AFB1 production is correlated with oxidative stress, and quercetin produces antioxidant proteins like catalyze A, peroxidases, fatty acid oxygenase, hsp70, etc. to fight the oxidative strain and decrease the production of reactive oxygen species (ROS). The subculture without quercetin exhibited MAPK pathway that makes use of carbon for aflatoxin biosynthesis. On the alternative hand, CFq lacks those proteins and led to the CAMP/Pka pathway. The enzymes required for preliminary steps of mycotoxin synthesis are found in both cultures. However, CFq lacks acetyl CoA synthetase that's the predominant enzyme for the synthesis of precursor Norsoloronic acid (NOR). Zhao et al (2015) said that 800ug/ml of quercetin can inhibit ROS manufacturing in mice [36]. Choi et al (2010) studied the impact of quercetin on AFB1 mediated liver harm in mice [37]. The impact was discovered on HepG2 cells dealt with AFB1. AFB1 increased oxidative stress and therefore ROS production in HepG2 cells. Quercetin being an antioxidant confirmed superoxide dismutase activity, decreased serum LDH levels, and multiplied GSH degrees that assist in detoxifying the reactor electrophiles. It has additionally been proven that isorhamnetin used alongside quercetin has better potential in inhibiting lipid peroxidation. Hence, it became evident that quercetin does not provide absolute protection against aflatoxin-mediated liver damage, however, it promotes the antioxidant defense system.

Gallic Acid

Gallic acid is likewise known as trihydrobenzoic acid. It is a phenolic acid present in gallnuts, witch hazel, sumac, tea leaves, barks, and different plants. Gallic acid may be used to inhibit aflatoxin biosynthesis in a dose-based manner [38]. Gallic acid has specific inhibitory pathways -

1) inhibit the expression of farB gene that encodes for proteins employed in fatty acid beta-oxidation. FarB gene has subunits fox A and acuJ, which encodes for proximal beta-oxidation multifunction enzyme and acetyl-carnitine transferase respectively. All the above-mentioned genes are downregulated because of the presence of gallic acid.

2) downregulation of creA gene, that is, carbon repression regulator encoding gene. This gene regulates the gene expression through binding to the promoter in the target gene and results in aflatoxin biosynthesis.

Gallic acid suppresses this gene and downregulates the AF synthesis. It reduces oxidative stress via glutathione and thioredoxin-based system in *A. flavus*. In *A.flavus*, a gene cluster of fifty-six genes is needed for AF production. The management of gallic acid affects sixteen of those genes and additionally impacts the carbon utilizing pathway, therefore, promoting the pentose phosphate pathway. Gallic acid increases the expression of genes like catalase, peroxidases, and different genes related to the antioxidative effect.

Trans-cinnamic acid (CA) and Ferulic acid (FA)

Trans-cinnamic acid and ferulic acid is additionally known as hydroxy acid are natural compounds located in plants. They are wealthy antioxidants. These compounds on their own and in the mixture have been examined to inhibit aflatoxin biosynthesis at distinct water activities [39]. This examination was carried out on maize grains. 25mM CA and 30mM FA, alone and in mixture CA + FA (25mM + 30mM) confirmed maximum inhibition on Aspergillus species at distinct water activities (aw) - 0.99, 0.97, 0.95 and 0.93. The test was carried out at specific levels of water activity due to the fact aflatoxin infection is maximum after harvest, in the course of storage due to flawed drying procedures and the presence of moisture content. As maize is harvested moist, proper drying strategies ought to be followed. Therefore, CA and FA are taken into consideration as green fungi-toxicants to manipulate AF biosynthesis in *A.flavus* and *A.parasiticus* at different water activities ranging from 0.99 to 0.93.

Carvacrol (CR) and Trans-cinnamaldehyde (TC)

Carvacrol is a monoterpenoid phenol possessing aromatic properties. Trans-cinnamaldehyde is a phenylpropanoid that gives flavor to cinnamon. These compounds had been determined to decrease AF precipitated toxicity in chicken embryos [40]. Mycotoxins from Aspergillus species can also additionally contaminate the poultry feed and cause deleterious outcomes in chickens. These outcomes result in decreased egg production which can also additionally deliver big financial loss as well. Embryo viability and hatchability are also affected. These contaminants also can be transmitted to the embryos. It turned into an obvious discussion that

chickens injected with phytochemicals could transfer them to embryos, so CR and TC had been injected individually to one-day-old eggs at a concentration of 1%. After 18 days of incubation, it was seen that AF production was inhibited and, embryo weight and tibia length had been increased. The entire mechanism at the back of the effectiveness of CR and TC isn't known, however, it became evident that they decreased the oxidative stress and modulated the expression of apoptotic genes.

Essential oils of turmeric

Alpha phellandrene, p-cymene, and terpinolene are the primary components present in turmeric leaf oil. The essential oil in turmeric rhizomes has antifungal activity in opposition to numerous fungi [41]. Terpinolene, which's the predominant element is a monotespenic constituent and is present in different oils too [42]. The constituents of turmeric essential oil respond in synergism to reveal fungistatic and fungicidal impact in opposition to Aspergillus species and different fungal genera. To apprehend the impact of turmeric essential oil on aflatoxin biosynthesis in *A.flavus*, distinct concentrations of oil have been inoculated in yeast extract sucrose (YES) broth infected with aflatoxin [43]. Essential oil at 1% and 1.5% confirmed 95.3 percent and 100% aflatoxin inhibition respectively. The mechanism at the back of this activity is the alteration withinside the enzymatic reactions for cellular functions, which impacts morphogenesis and growth of fungus. Thus, phytochemicals present in turmeric leaves may be efficaciously utilized in synergism as bio preservatives in storage to minimize aflatoxin contamination.

Coumarin

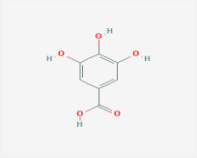
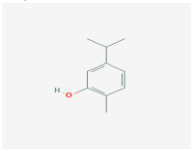
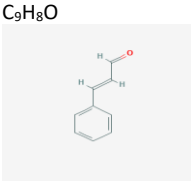
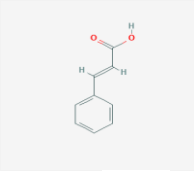
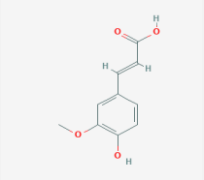
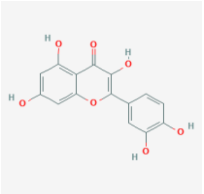
Coumarin is a fragrant natural compound discovered in lots of plants. It well-known for its antibacterial and antifungal properties. Antifungal and anti-aflatoxigenic abilities of 4-hydroxy-7-methyl-3-phenyl coumarin and its structurally associated compound 2,3-dihydro benzofuran have been studied with the use of HPLC and fluorescence detection [44]. Coumarin was observed to suppress the numerous genes required for aflatoxin biosynthesis. It downregulates aflD, aflK, aflQ, and aflR genes that encode for ketoreductase, versicolorin

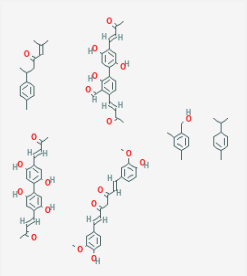
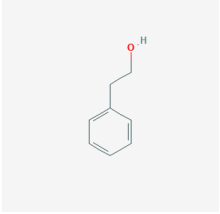
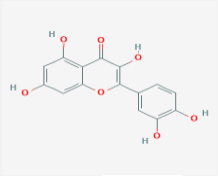
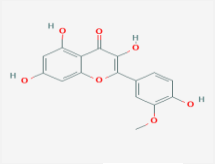
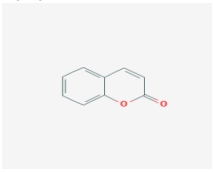
B synthase, p450 monooxygenase respectively, in A.flavus. On the opposite hand, 2,3-dihydro benzofuran also inhibits the transcription factor (aflS) in AF biosynthetic pathway. These compounds confirmed 50% inhibition at 10ug/ml and entire inhibition at 1000ug/ml.

2-phenylethanol (2-PE)

2 phenyl ethanol produced via *Pichia anomola* has the potential of decreasing A.flavus and inhibit AF production whilst implemented in pistachio trees [45]. This phytochemical is deadly for Aspergillus flavus at excessive concentration. A study was carried out to decipher the impact of 2-PE at low concentration (1ul/ml) through the use of RNA Sequencing Technology. After incubation of 72 hours, the alcohol was observed to affect 131 genes of general 13,485 genes of A.flavus, out of which 82 genes had been downregulated. Some of those downregulated genes belonged to the gene cluster involved in aflatoxin biosynthetic pathway. Many of those genes were concerned in the degradation of branched-chain amino acids to provide building blocks and ATP for mycotoxin production. Downregulation of those genes decreased the degradation of branched-chain amino acids and upregulated the genes for translation. It became cited that aflatoxin biosynthesis occurred at 30°C temperature. However, 37°C was not optimum for the growth of A.flavus. It became evident that low ranges of 2-phenyl ethanol did not have an effect on fungal growth but reduced aflatoxin production. However, at increased concentration, each of the activities is rendered.

Table 1. Phytochemicals having inhibiting properties on aflatoxin production

	PHYTOCHEMICAL	CHEMICAL FORMULA AND STRUCTURES	SOURCE OF AFLATOXIN	HOST FOR STUDY	MECHANISM	REFERENCE
1	Gallic Acid	$C_7H_6O_5$ or $C_6H_2(OH)_3COOH$  PubChem- 370	A.flavus	-	Inhibiting the expression of FarB and CreA genes	Zhao et al,2018
2	Carvacrol and trans-cinnamaldehyde	$C_{10}H_{14}O$  PubChem- 10364 C_9H_8O  PubChem- 637511	A.flavus, A.parasiticus	Chicken embryos	Reduced oxidative stress Modulation of expression of apoptotic genes	Yin et al,2017
3	Trans-cinnamic acid and Ferulic acid	$C_9H_8O_2$  PubChem- 444539 $C_{10}H_{10}O_4$  PubChem- 445858	A.flavus, A.parasiticus	Maize	OH groups in phenolic compounds make hydrogen bonds with target enzymes. Block cell wall structures	Nesci et al, 2007
4	Quercetin	$C_{15}H_{10}O_7$  PubChem- 5280343	A.flavus	Maize/ Maize related products	Reduced oxidative stress ACoA synthetase gene disruption	Tiwari et al, 2018

5	Essential oil of turmeric leaves (Curcuma longa L.)	$C_{96}H_{104}O_{20}$  PubChem- 11980944	A.flavus	Yeast Extract Sucrose	Reduced availability of moisture Cell wall alterations	Sindhu et al,2010
6	2-Phenylethanol	$C_8H_{10}O$ or $C_6H_5CH_2CH_2OH$  PubChem- 6054	A.flavus	Pistachio trees	Downregulation of biosynthetic genes Stimulated growth of branched-chain amino acids	Chang et al,2015
7	Quercetin and isorhamnetin	$C_{15}H_{10}O_7$  PubChem- 5280343 $C_{16}H_{12}O_7$  PubChem- 5281654	Aspergillus	Mice- AFB1-treated HepG2 cells	Inhibit the production of ROS and cytotoxicity block the decrease of reduced glutathione (GSH) levels	Choi et al,2010
8	4-Hydroxy-7-methyl-3-phenylcoumarin	$C_9H_6O_2$  PubChem- 323	A.flavus	(lab)	Downregulation of aflK Expressing Versicolorin B Synthase	Moon et al, 2017

Conclusion and future prospects

The overview was primarily based on reading the biosynthetic pathway of Aflatoxins production, the mechanism of its toxicity, and the way specific phytochemicals are used to inhibit the mycotoxin production which may acts as an important virulent factor in pathogenesis (46). Biosynthetic machinery involved in the production of myotoxin during favorable or unfavorable conditions needs investigation to unpin their role in the biology of the fungi (47). Particular to Aflatoxin, they are synthesized during hyphae or mycelia stage,

and involvement of signaling pathway such to calcineurin-Crz1, MAPK, and cAMP/PKA during the morphogenesis or the stress condition (48-49). We concluded that phytochemicals may be used alone, or synergistically with other metabolites to inhibit signaling pathways or generating ROS to inhibit the growth proliferating conidia or production of secondary metabolites (50). The fundamental mechanism outlining the effectiveness of phytochemicals is their anti-oxidative nature and capacity to down-regulate the expression of genes involved AF biosynthesis. Some of those plant metabolites won't offer complete resistance to the AF, however, allows lessening the further production. These biological methods of mycotoxin control can be a promising approach to mitigate certain health problems.

Chapter 2
Overview of Patulin Toxicity and Degradation

Introduction

Patulin is a toxic secondary metabolite produced by numerous species of molds, specifically within the genera *Penicillium*, *Aspergillus*, and *Byssochlamys*. Among all the patulin-producing species, *Penicillium expansum* is most commonly associated with patulin and fruit decay. It is found in soil in the natural environment [51] and also in indoor air [52]. It is known to infect agricultural commodities during harvest, storage, or processing. This is because pome fruits like apples and pears, or stone fruits like cherries, plums and peaches are frequently damaged during harvest and transport. As a result, *P. expansum* penetrates inside the fruit through these injuries and eventually starts the production of secondary metabolites [53]. Intact fruits almost lack patulin [54]. This species has also been found to grow on building materials [52] and humid wooden blocks [55].

Patulin was first isolated from *P. griseofulvum* and *P. expansum* in 1943 by Birkinshaw et al. in an attempt to find new fungal metabolites with antimicrobial properties [56]. However, the medicinal properties of patulin were overseen due to its toxicity to humans and animals [57]. Patulin is known to cause neurological, immunological, and gastrointestinal toxicity. Diarrhea, hematemesis, and extreme weakness are a few of the symptoms seen in different animal species as a result of acute patulin poisoning [58].
Due to the adverse effects of mycotoxin consumption, a limit was set on the patulin level by the US food and drug administration (FDA) and the European commission (EC) as 50 micrograms per liter for fruit-derived products and 10 ug/L for infant food [8]. Over the last two decades, surveys have been conducted on the occurrence of patulin in food in different countries as summarised (Table 2.1).

Table 2.1 Occurrence of Patulin in food in different countries based on surveys

COUNTRY	COMMODITY	PERCENTAGE OF SAMPLES EXCEEDING PATULIN LIMIT	REFERENCE [118 – 126]
ARGENTINA	Apples and pears by-	5.88%	Funes and Resnik

	products (solid and semi-solid consistency)		(2009)
AUSTRALIA	Apple, pear, and mixed fruit products	22%	Burda (1992)
CHINA	Apple products	16%	Yuan et al. (2010)
INDIA	Apple juices	16%	Saxena et al. (2008)
IRAN	Apple juices	33%	Cheraghali et al. (2005)
	Apple juice concentrates	56%	
ITALY	Fruit juices	13%	Bonerba et al. (2010)
SPAIN	Apple juices	11%	Murillo-Arbizu et al. (2009)
TURKEY	Concentrated apple juices	43.5%	Gokmen and Acar (1998)
USA	Apple juice and cider	2.2%	Harris et al. (2009)

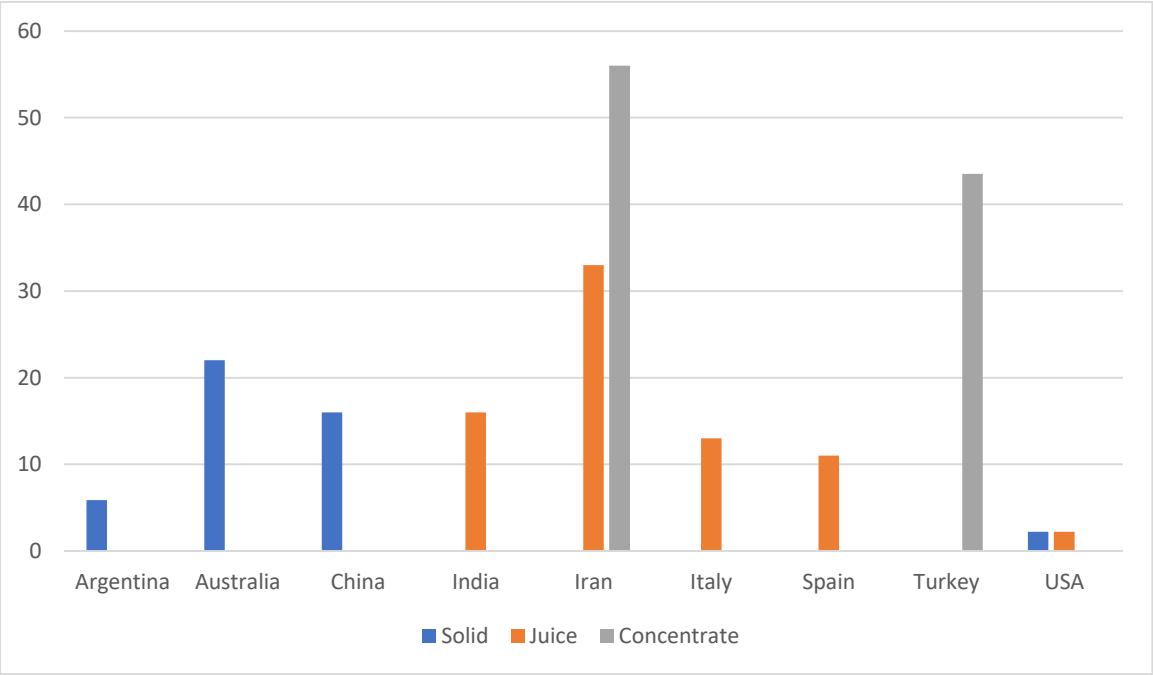
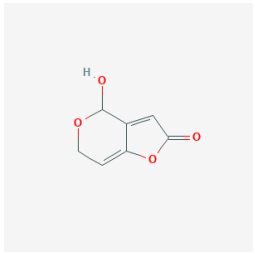


Figure 1.1 Graphical representation of surveys conducted in different countries for Patulin

To mitigate the adverse health and socio-economic impact of patulin contamination, various physical and chemical strategies have been employed. But due to insufficient effect of physical methods and resistance development by excessive use of fungicides, biological control has come to light. Bioactive plant secondary metabolites exhibit antifungal properties that can limit exposure to mycotoxins without any environmental or health hazard [59].

Quercetin, umbelliferone, cinnamon aldehyde are few of the phytochemicals that inhibit biosynthesis of patulin.



Solid
Odorless
Melting point- 111.0 °C
Vapour pressure- 6.8X10-6 mm Hg at 25 °C (est)

Figure 2.2 Structure and properties of Patulin

Biosynthesis

Patulin is a polyketide synthesized in 10 enzymatically driven steps. The genes encoding these enzymes are present in a cluster on the chromosome [60]. In *Aspergillus clavatus*, a cluster of 15 genes encoding enzymes involved in biosynthetic pathway of patulin are present in 40 KB region[61].

The first step is the condensation of acetyl Co A and malonyl Co A to 6 methyl salicylic acid (6-MSA) catalyzed by 6 methyl salicylic acid synthase (6msas). The gene encoding this enzyme was isolated from *Penicillium griseofulvum* [62]. This was the first polyketide synthase gene to be studied in vitro [63]. 6msas is a multifunctional enzyme with four identical polypeptide chains [64].

Decarboxylation of 6 MSA leads to the formation of m-cresol [65]. The methyl group of m-cresol gets oxidized to the aldehyde group followed by two hydroxylation reactions. These two reactions are catalyzed by two cytochrome p450 enzymes [61] that lead to the formation of m- hydroxybenzyl alcohol and toluquinol. The enzyme responsible for the production of toluquinol also converts m-hydroxybenzyl alcohol to gentisyl alcohol. Toluquinol is a co-metabolite of patulin that is metabolized to deoxyepoxydon. It is not considered as an intermediate of this pathway[66].

The next epoxidation reaction results in the formation of one of the patulin precursor isoepoxydon [67]. Isoepoxydon dehydrogenase (idh) converts isoepoxydon to phyllostine followed by the formation of neopatulin catalyzed by neopatulin synthase. GMC (glucose-methanol-choline) oxidoreductase catalyze the last step of the patulin biosynthesis pathway [68].

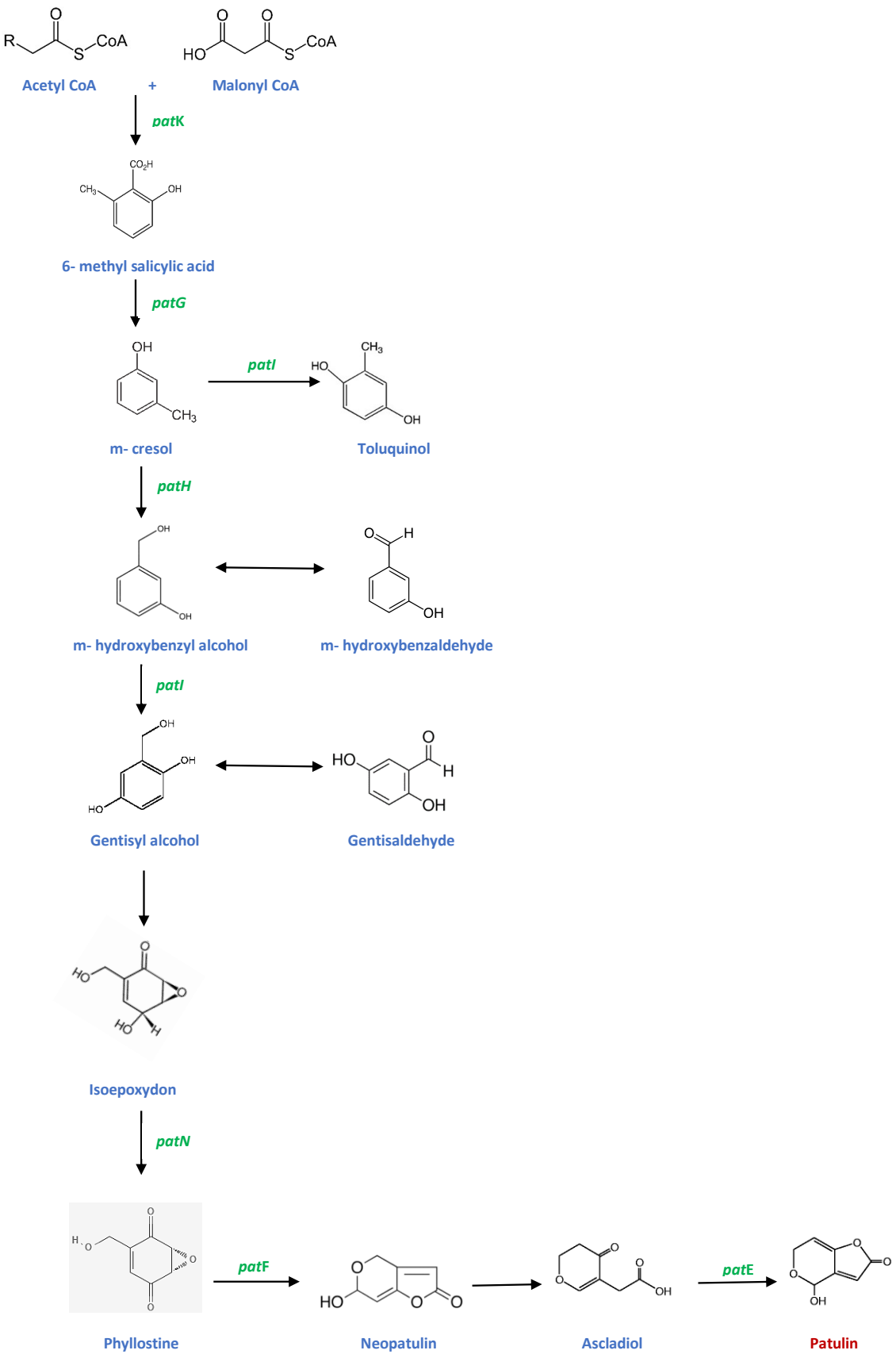


Figure 2.2 Biosynthetic pathway of Patulin

The gene cluster also contains three transporter genes [61] - Acetate Transporter, MSF (Major Facilitation Superfamily) transporter, and one ABC (ATP-binding cassette) transporter. It also encodes a transcription factor that upregulates the other genes for patulin production.

Table 2.2 Genes encoding enzymes involved in the biosynthesis of patulin

	ENZYME	FUNCTION	GENE	REFERENCE [127 – 136]
1.	Putative acetate transporter	Cell metabolism	*pat*A	Artigot et al. (2009), Tannous et al. (2014)
2.	Putative carboxyl esterase	Unknown	*pat*B	Artigot et al. (2009), Tannous et al. (2014)
3.	Putative MFS (major facilitator) transporter	Membrane transport	*pat*C	Artigot et al. (2009), Tannous et al. (2014)
4.	Putative Zn-dependent alcohol dehydrogenase	Interconversion between gentisyl alcohol and gentisaldehyde	*pat*D	Artigot et al. (2009), Tannous et al. (2014)
5.	GMC (glucose-methanol- choline) oxidoreductase	Catalyses the ring closure step from ascadiol to patulin	*pat*E	Tannous et al. (2017)
6.	Neopatulin synthase	Converts phyllostine to neopatulin	*pat*F	Tannous et al. (2015b)
7.	6-msa decarboxylase	Decarboxylation of 6-msa to m-cresol	*pat*G	Light et al. (1969)
8.	Cytochrome p450 monooxygenase	Hydroxylation of m-cresol to m-hydroxybenzyl alcohol Formation of gentisyl alcohol	*pat*H *pat*I	Murphy et al. (1974)
9.	Putative deoxygenase		*pat*J	Artigot et al. (2009), Tannous et al. (2014)
10.	6- methylsalycylic acid synthase	Catalyse the first step in patulin pathway	*pat*K	Lynen et al. (1961)
11.	Encodes for transcription factor	Upregulates the pathway genes	*pat*L	Snini et al. (2016)
12.	ABC (ATP binding cassette) transporter	Acts as an efflux pump, provide protection to plant pathogens	*pat*M	Puel et al. (2007)

13.	Isoepoxidon dehydrogenase	Converts isoepoxidon to phyllostine	*pat*N	Fedeshko et al. (1992)
14.	Isoamyl alcohol oxidase	Unknown	*pat*O	Dombrink et al. (2008)

The presence of nitrogen suppresses the dehydrogenase enzyme which leads to decreased secondary metabolite production 69[]. Glucose is used as the carbohydrate source for patulin production[57]. Manganese is known to enhance the expression of idh gene [70]. For patulin production in vitro, the optimum pH is 5.0 [71]

Paster et al 1995 [72] claimed that oxygen requirement for the pathway was negligible. It was concluded from several studies that refrigeration was not an appropriate method to control patulin contamination [47].

Toxicity

The strong affinity of patulin for sulfhydryl groups inhibits the functioning of enzymes. Patulin activates the reactive oxygen species (ROS) generation by phosphorylation of protein kinases [73]. Rodents are more sensitive to patulin as compared to poultry animals [74]. The administration of patulin in rats induced gastrointestinal changes, weight loss, and changes in renal function [75]. Prolonged ingestion led to neurotoxicity in mice, hamsters, and chickens [76]. However, monkeys did not react to small doses of patulin for four weeks. Slight changes in behavior were observed by continuous injection of 5 mg of patulin per kg b.w/day for 14 days [77].

Brand et al 2018 [78] demonstrated the effect of patulin on the enteric nervous system. It is called the brain in the gut and controls intestinal functions. The extract of *Penicillium copribium*, patulin was analyzed for its effect on cell viability and functionality in human neuronal cell lines (SH-SY5Y). Patulin decreased the cell viability at an EC50 of 0.23ng/ul and increased the ROS production at a very low concentration of 1ng/ul as well. Microscopic analysis revealed altered morphology of cells accompanied by altered calcium signaling that can cause neuronal damage and initiate chronic diseases such as Alzheimer's disease. Patulin showed ATP reduction in enteric neurons in a dose-dependent manner. Patulin ingestion can cause hormonal changes. Selmanoglu and Kockaya [79] claimed that testosterone levels were increased in mice due to patulin consumption. It also induced

mutagenic effects in Bacillus subtilis [80]. Patulin was declared genotoxic by WHO [81] and known to impair DNA synthesis in mammals. International Agency for Research on Cancer (IARC) classified patulin as group 3, i.e, not carcinogenic to humans [80].

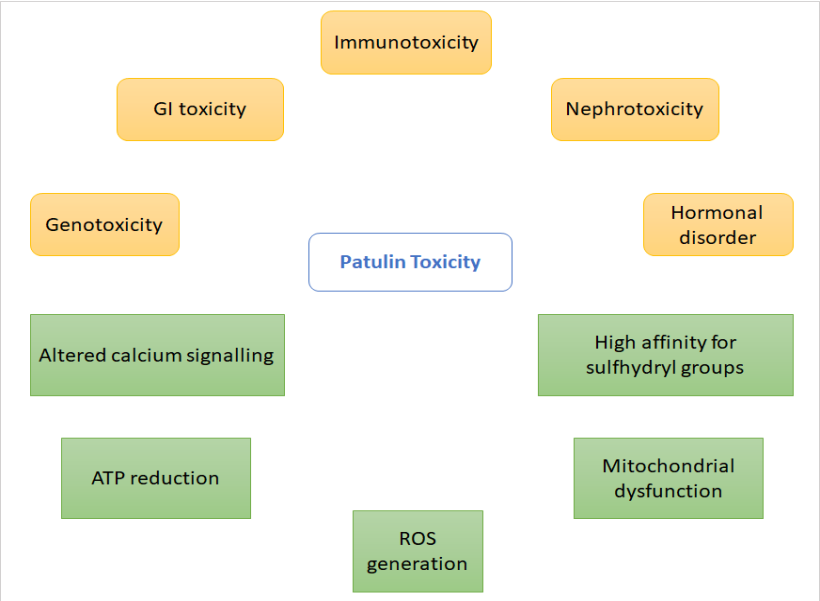


Figure 2.3 Types of Patulin toxicity and, mechanism

Han et al 2020 [82] described the mechanism behind apoptotic effect of patulin in human embryonic kidney (HEK293) cells. The data concluded that patulin triggered apoptosis by disrupting the mitochondrial function, decreasing the energy production, release of cyt C to cytosol from mitochondria, and gradually starting the caspase signaling pathway. The transcriptomic and proteomic analysis revealed differentially expressed genes and proteins in HEK293 cells induced by patulin. Many of these were related to other functional diseases like Parkinson's disease and Alzheimer's disease. Tsai et al [83] demonstrated that patulin can suppress the immune system. It causes mitochondrial dysfunction and downregulates cytokine production. In vitro exposure to patulin inhibited macrophage functioning in rats [84]. In another study, secretion of IFN-Y and IL- 4 was reduced in human macrophages by patulin [85]. The proposed mechanism behind the cytokine secretion effect was intracellular glutathione (GSH) depletion [86]. In-vivo studies of patulin on immune response conducted on mice showed an increase in the number of splenic T lymphocytes and decreased immunoglobulin concentration. Patulin also affected the microbiological activity in mice [87].

Degradation

Due to the toxic effects of patulin, the presence of mycotoxin in food should be controlled if not eliminated. Few preventive measures such as protecting the fruit from physical damage or injury should be employed during harvest, transportation, and storage. Activated charcoal treatment is one of the oldest physical treatments for patulin detoxification [88]. However, due to the deteriorative effects of charcoal on fruit juices the usage is limited [89]. Patulin decontamination by UV radiation did not cause any physiological damage in food products but a high level of suspended particles in unfiltered juices can absorb UV rays before patulin [90]. Funes et al investigated the effect of pulsed light (PL) on apple-based food products to reduce patulin contamination [91]. Certain antifungals like benzimidazole and other chemicals like potassium phosphate can inhibit the growth of patulin-producing fungi [92]. The excessive use of fungicides and chemicals is restrained in many countries due to the development of resistant strains and their health and environmental impact [93]. The utilization of natural antifungals and safe chemicals are encouraged. The growth and secondary metabolite production in *P. expansum* were inhibited by a plant resin made by honey bees and is called propolis [94]. Jayashree et al studied the antioxidant and hepatotoxicity-related effects of green tea leaves against patulin [95]. The extract of green tea leaves reduced the liver damage induced by patulin in mice. Combined oxidative treatment with safe chemicals can completely inhibit patulin production and prevent apples from blue rot disease. Sodium hypochlorite (NaClO), copper sulphate ($CuSO_4$), and hydrogen peroxide (H_2O_2) can be combined for this treatment [96]. Oporto et al suggested that patulin toxicity can be reduced by microbial agents by two pathways [97] - 1) by cell wall absorption and 2) by an unknown degradation mechanism that converts patulin into non-toxic E-ascadiol. Five strains of *Saccharomyces cerevisiae* that is, Wine/ European (WE) and Sake (SA), North America(NA), Chinese, Malaysian, and West African(WA) were tested. The effect of these strains (both active and inactive form) on patulin accumulation was investigated at 3h and 24h incubation. All the strains belonging to different regions had different transcriptional profiles that lead to a difference in their efficacy. WE strain was the most effective and expressed genes for sulphur accumulation pathway. The genes responsible for beta D-glucan and mannoproteins in cell wall that cause adsorption were also differentially expressed in all strains. Yang et al [98] demonstrated that the effect of *Rhodotorula mucilaginosa* on patulin was

based on lysine crotonylation (Kcr). Quantitative lysine Crotonylone analysis revealed that proteins involved in gluconeogenic pathway and tricarboxylic acid cycle were overexpressed, whereas the KCR proteins for ribosome and, carbohydrate transport and metabolism were downregulated. Hence it is evident that certain microbial species have antimicrobial properties.

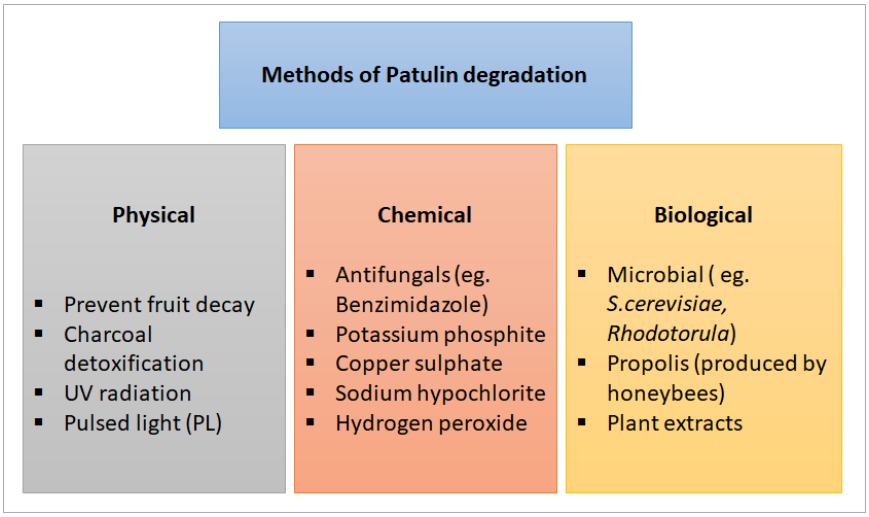


Figure 2.4 Methods of Patulin degradation

Phytochemicals

Phytochemicals are the secondary metabolites produced by plants as their defense system against pathogens. They do not have any nutritive value [99]. Due to their antimicrobial, anti-inflammatory, and antioxidant properties, studies have estimated that plant metabolites can protect humans and animals against certain microbe-originated diseases [100]. There are different categories of phytochemicals that can be used against the production of patulin. Two of these categories, flavonoids, and coumarins were discussed by Sanzini et al [101] to observe the effect on genes involved in patulin by synthetic pathway at the transcriptional level. Quercetin (flavonoid) and umbelliferone (coumarin) were added to the culture medium in in-vitro trials to observe the reduction in patulin accumulation after 14 days of incubation. These phytochemicals did not affect the mycelial growth of fungi, indicating that primary fungal growth was not reduced. However, secondary metabolite production was reduced by 42% with quercetin, 45% with umbelliferone, and 68% with a combination (table 2.3). The genes involved in the biosynthetic pathway were downregulated at the transcriptional level. Five of these genes- idh, msas, p450-1, p450-2, and paeb1 were first amplified by RT-PCR to

experiment and then analyzed. The ATP-binding cassette (ABC) transporter encoded by paeb1 provides a defense mechanism in *Penicillium expansum* against fungicides. The downregulation of this gene increases the effect of phytochemicals. Due to the suppression of all five genes, it was concluded that both these phenolics can protect against blue rot caused by patulin.

Table2.3 Effect of Quercetin and umbelliferone based on Sanzini et al

PHYTOCHEMICAL	CONCENTRATION (MG/L)	INCUBATION PERIOD	PATULIN REDUCTION (%)
QUERCETIN (QUE)	10	8 days	42
UMBELLIFERONE (UMB)	10	8days	45
COMBINATION (QUE + UMB)	20	8 days	68

In another study by Yang et al, experimental data was collected for the effectiveness of quercetin against patulin and other mycotoxins [102]. This data was generated in relevance to four human cancer cell lines ie, HeLa, HepG2, PC-3, and SK-N-MC. Cell viability and lactate dehydrogenase activity (LDH) were altered, suggesting the positive effect of quercetin against cell toxicity. As conducting continuous in-vivo experiments can generate errors, precise modeling of the cytotoxicity studies was done using artificial neural networks (ANN). ANN works similar to biological neural networks and is a concept of artificial intelligence (AI) based on neuro modeling [103].

Cinnamon oil is called GRAS (Generally Recognised as Safe Compound) because of its fat solubility, less toxicity, permeability, biodegradability, and biocompatibility. The phytochemical present in cinnamon oil, ie. trans-cinnamaldehyde, eugenol, and linalool have broad-spectrum antifungal properties [104]. Jeong et al exhibited the effectiveness of cinnamon oil against Penicillium fungal communities in cheese [105].
In another study, cinnamon oil was found effective against the growth and development of patulin-producing species at a concentration of 0.25mg/L [106]. Spore germination, conidial production, and mycelial accumulation and expression were inhibited. This study concluded that even in the presence of the most favorable carbon source, CCR (carbon catabolite repression) the expression of CreA gene was downregulated with prolonged incubation with cinnamon oil. The expression of other genes involved in carbohydrate metabolism in P. expansum were also suppressed at both mRNA and protein level. Microscopic results

indicated the decrease of mitochondria and hence ATP levels. Cinnamon oil also invaded the cell wall synthesis process and caused the leakage of small electrolytes in Fusarium and E. coli respectively [107, 108].

In a study by Wang et al, the effect of cinnamaldehyde and citral combination were analyzed at transcriptional level in P. expansion [109]. RNA Sequencing revealed 1713 differentially expressed genes (DEGs) that were involved in patulin biosynthesis. The antifungal test concluded the negative impact of Cin/Cit on mycelial growth and spore germination in a dose-dependent manner. The mycelia and spores appeared wrinkled and depressed when analyzed by scanning electron microscope (SEM). The physiological effects of this combination exhibited dysfunction of mitochondrial membrane, decreased energy and increased ROS generation. As an indication of oxidative stress, the levels of hydrogen peroxide were increased.

Tragni et al used a combined in-silico, in-vitro and in-vivo strategy to identify small molecules that had a high affinity for GMC oxidoreductase [110]. This enzyme catalyzed the ring closure step in patulin synthesis from E-ascadiol. 6-hydroxycoumarin (structurally similar to umbelliferone) and metricane were found to reduce patulin production by 60 and 41% respectively when applied on apples at a concentration of 100ug/ wound in vivo. In vitro analysis proved the effectiveness of 6-hydroxycoumarin in a dose-dependent manner. This strategy can be employed for identifying small molecules having a high affinity for certain proteins involved in the production of several mycotoxins produced by *A. terreus* [111]..

Anti-tumour activity

Cancer is one of the leading causes of high mortality worldwide mainly due to late diagnosis. The disadvantages of the anticancer drugs and chemotherapy need to be corrected with new formulations and lower side effects. The neurotoxic effects of patulin make it a viable candidate for cancer therapies.

The response of the innate immune system to any infection or tissue injury causes inflammation. The activation of leukocytes and neutrophil degranulation releases reactive oxygen species (ROS) [112]. Chronic inflammation may lead to disease progression like inflammation-associated hepatocellular carcinomas. Tsai et al demonstrated that cell toxicity by patulin can become a promising approach for immunomodulation [113]. Patulin reduces

the cytokine production by mitochondrial dysfunction and activating the p62 dependent mitophagy pathway. Due to the similar effects on normal cell physiology, the concentration of patulin was limited to 1uM in this experiment.

The protein members of NF-KB family (Nuclear Factor Kappa) are the key regulators of chronic inflammation, observed to be overexpressed in most tumors[114]. The dimers of NF-kB family bind to the nf-kb inhibitor and inactivates them. This pathway causes cell proliferation, invasion, survival, angiogenesis, and metastatis of tumor cells. Monteillier et al investigated the efficiency of patulin as a lung cancer chemopreventive drug [115]. Patulin was found as an effective NF-kB inhibitor at a concentration of 0.25uM. TNF-alpha-induced p65 nuclear translocation was the target o patulin in inhibiting the activation of nf-kb pathway. It also downregulated the Wnt pathway and exhibited proapoptotic and anti-migration activities in lung cancer. These dozes of patulin did not show any side effects but the concentration should be regulated.

Abastabar et al investigated the effect of patulin on cervical and colon cancer utilizing three different cell lines- HeLa, SW48, and MRC-5 [116]. The utilization of different concentrations of patulin (0.5,1,2 and 4uM) revealed that patulin induced cytotoxicity in a dose-dependent manner. Cell proliferation and growth of tumors were reduced.

Table 2.4 Comparison of pharmacological properties of Patulin and anti-cancer drugs

	PATULIN	**MITOMYCIN**	**LETRAZOLE**
FORMULA	$C_7H_6O_4$	$C_{15}H_{18}N_4O_5$	$C_{17}H_{11}N_5$
MOLECULAR WEIGHT	154.12 g/mol	334.33 g/mol	285.30 g/mol
LIPOPHILICITY: CONSENSUS LOW $P_{O/W}$	-0.00	-0.80	2.31
WATER SOLUBILITY	Highly soluble	Soluble	Moderately soluble
GI ABSORPTION	High	Low	High
BBB PERMEANT	No	No	Yes
CYP INHIBITOR	No	No	No/Yes
LOG K_P (SKIN PERMEATION)	-7.94 cm/s	-8.62 cm/s	-6.10 cm/s

DRUGLIKENESS: BIOAVAILIBILTY SCORE	0.55	0.55	0.55
LEADLIKENESS	No	Yes	Yes
SYNTHETIC ACCESSIBILITY	3.58	4.80	2.58

Discussion

Patulin is known to cause apoptosis in several cell lines by ROS generation, covalently binding to sulfhydryl groups in proteins, and phosphorylation of protein kinases [117]. Phytochemicals can be utilized to reduce patulin toxicity in cells. As patulin can cause apoptosis and reduce tumor growth in cancer cell lines, it is an extreme candidate to be utilized in targeted anticancer therapy [116]. Targeted therapy is different from chemotherapy as it does not affect normal cells. It is targeted to block certain proteins are enzymes specific to cancer cells. Due to the toxic effects of patulin on neuronal cells, the immune system, and other cells, its dose it should be regulated. In the given table the pharmacological properties of patulin have been compared with few anticancer drugs (Table- 2.4). This information was extracted by using SDME.

Summary

Fungi make up 0.1% of the total microbiome. The presence of fungal species in crops may lead to the production of mycotoxins. Mycotoxins are secondary metabolites generated by fungi. Aflatoxins are one of the major classes of mycotoxins among ochratoxins, zearalenone, fumonisins, etc, produced by the Ascomycota division of fungi. Aspergillus is mainly associated with the biosynthesis of aflatoxin. It infects and affects the crops worldwide leading to huge monetary loss and other health problems. Another mycotoxin that is not studied enough is Patulin, which causes similar damage. It was discovered as an anti-microbial substance after penicillin, but due to its huge toxic effects on humans and animals, this concept has been discarded. To mitigate the toxicity of mycotoxins, certain methods of prevention, control, and degradation have been employed. Phytochemicals are the secondary metabolites of plants, produced as a defense mechanism against pathogens. These plant metabolites have also been proven to be effective against fungal species and mycotoxins in mammals. Quercetin, coumarin, essential oils in turmeric, cinnamaldehyde, umbelliferone, etc, have been demonstrated to have anti-mycotoxin effects at different concentrations. These phytochemicals can be used alone or in synergism to extract overall positive results. However, patulin is being researched for its pharmacological properties due to its immune and neuro-toxic effects. It can be used as an immunomodulatory drug and also in the treatment of cancer. As it can cause apoptosis in normal healthy cells also, its concentration and usage should be regulated. It can be an effective candidate in targeted cancer therapy and precision medicine.

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List of Publications (unpublished)

Book Chapter

Aanchal Bansal, Megha Sharma, Aakanksha Pandey, Jata Shankar. Insight into Phytochemicals as anti-aflatoxigenic agent to control contamination in crops. In Sustainable utilization of Fungal Resources: Applications on plants and animal health. Springer's (2021) **– In production**