

Review

Aflatoxin B1 in Food and Feed: An Overview on Prevalence, Determination and Control Tactics

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Abstract

Aflatoxin B1 studies have attracted too much attention because of serious illness caused by its presence in food or feed; it is the most impactful existing aflatoxin among the group of many different mycotoxin, which was proved to contaminate immense percentage of the global food supply therefore yielding in food insecurity problem all over the world. The present review emphasize on crops mostly infected by AFB1 in different countries, decontamination technologies and recent rapid detection techniques used to determine AFB1, a short comparison between aptamer method and molecularly imprinted technology used in screening aflatoxin B1 as well. Several detoxification strategies have been discussed and demonstrated that in order to make them more efficient it is recommended to combine two or more methods, moreover regarding detection of aflatoxin B1 there are many ways of detecting but not all are advantageous so easy, rapid, portable, high selective and sensitive, cheap techniques are the mostly preferred, aptamers and MIPs are commonly used since they possess the requirements listed above. the combination of good decontamination methods and good detection methods is required to fight against many difficulties such as food insecurity, different cancers, malnutrition, growth problems and also reduce a lot of money wasted.

Keywords: Aflatoxin B1, control strategies, aptamers, molecularly imprinted polymers, food insecurity.

Introduction

Recently the world is facing a big problem of increasing population, food safety and security are the main problem that are affecting the entire world population, food security and safety are mainly defined by (i) enough food availability, (ii) access to safe food and (iii) utilization of the food in terms of quality, nutritional and cultural purposes for a healthy life (Walls *et al.*, 2019). Food insecurity and malnutrition can be caused by lack of any of these intents, leading to serious troubles to human health and also economy. Aflatoxin B1 has a great impact on food security because it contaminates huge number of food and feed giving raise to food insecurity worldwide (Kumar *et al.*, 2017). Aflatoxin B1 is generated by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, which are capable to infect enormous amount of food commodities (Schabo *et al.*, 2020) furthermore aflatoxin B1 is the highest toxic among all mycotoxins, AFB1 the lethal dose (LD50) is 0.36 mg kg⁻¹ (body weight), which fall within the highly toxic poison (Nonaka *et al.*, 2009). Various research have been done in order to study the toxicity of AFB1 based on results obtained there were enough human epidemiological and animal experiments proving the carcinogenicity of AFB1

(Fedeles and Essigmann, 2018), mutagenicity and teratogenicity (Do *et al.*, 2020), even more reports have also demonstrated that AFB1 can induce many cancers attacking different organs for instance liver, stomach, lung etc. cooking and industrial process cannot eradicate aflatoxin B1 considering its high heat stability, therefore its control became a matter of great importance (Rushing and Selim, 2019). *Aspergillus* producing aflatoxin B1 are present mostly in regions where the temperatures are about 28°C optimum thus high occurrence of aflatoxin B1 in tropical and subtropical regions (Mahato *et al.*, 2019). Due to grave health problems for human and animals caused by contaminated food and feed intake, different countries have set strict regulations for aflatoxin B1 in order protect their population. The European Union is very strict regarding to aflatoxin B1, the standard level for aflatoxin B1 is 2 mg/kg, US food safety regulations have set a maximum permitted level of 20 µg/kg for aflatoxin B1 in all foods, but milk is exceptional with a maximum permitted level of 0.5 µg/kg. In addition Food and Agriculture Organization (FAO) maximum tolerated levels of aflatoxin B1 range between 1–20 µg/kg in food, and 5–50 µg/kg in dietary cattle feed (Egmond *et al.*, 2007).

Moreover, in order to strengthen sustainable agricultural productivity numerous novel techniques and decontamination strategies are employed for pre and post-harvest management of AFB₁. However there are many reported studies talking about aflatoxin B₁ in food and feed, the newness of this review rely on comparison of different rapid detection methods for aflatoxin B₁, furthermore this review also highlight occurrence, control strategies of aflatoxin B₁ in food and feed to guarantee food safety and security.

Occurrence of Aflatoxin B₁ in food and feed

Aflatoxin B₁ (AFB₁) is an unavoidable food contaminant, global food supply is greatly dominated by cereals and others grains for instance rice, wheat, corn, Groundnuts, Fruits, spices, Sorghum and it is well known that aflatoxin B₁ highly contaminate those products. Aflatoxin B₁ naturally presented in cereals particularly in rice and maize has become troublesome due to changing agricultural technology, Cereals contamination is not a problem of one special geographic place or any climate region it is globally, it can be generated when the cereals are still in the field and in storage; they attack both the grain and the whole plant (Ok *et al.*, 2007).

Rice

Many countries have reported the occurrence of afB₁ in their rice currently including Sweden, India, Malaysia, Pakistan, Ecuador, Brazil, China, and Canada. Study conducted in Saudi Arabia used imported samples from India, Pakistan, USA, Egypt and Australia; the rice samples presented Concentrations of aflatoxin B₁ and total AFS ranging between ≤ 0.123 and ≤ 2.58 $\mu\text{g}/\text{kg}$, respectively which were within the permissible amounts of the EU and other international legislations (Al-Zoreky and Saleh, 2019). About 230 rice samples Congregated during beriberi epidemic in 2007–2009 from different places of Brazil were tested for presence of aflatoxin B₁, the obtained results concluded that those samples had aflatoxin B₁ concentration of up to 180.74 $\mu\text{g}/\text{kg}$ (Almeida *et al.*, 2012). Study in Canada has shown that 99 out of 200 samples of rice imported from Asia and the United States were having AFB₁, the mean concentration was ranging between 0.34 – 0.39 $\mu\text{g}/\text{kg}$ (Bansal *et al.*, 2011; Shahbaz *et al.*, 2020). In Colombia aflatoxin B₁ is a very serious problem as demonstrated in a research conducted using 40 samples of rice and rice products among those 40 samples 4 samples were tested positive with a concentration of 7.1 $\mu\text{g}/\text{kg}$ (Diaz *et al.*, 2001). During July 2006 to June 2011, brown rice from different regions of Pakistan were tested for aflatoxins, 262 samples were employed, the (95.4%) of samples that means 250 samples showed presence of aflatoxin B₁, aflatoxin B₁ contamination was ranging between 1.07 – 24.65 $\mu\text{g}/\text{kg}$ (Asghar *et al.*, 2014).

Paddy rice from Ecuador was used during aflatoxin B₁ study 43 samples were studied 3 samples presented aflatoxin B₁ with a mean level of 20.6 $\mu\text{g}/\text{kg}$. in addition 208 samples of brown rice were employed in order to study aflatoxins level in Pakistan and the results showed that 19% samples were having a high content of aftoxin B₁ compared to EU limits the highest content of AFB₁ was 8.91 $\mu\text{g}/\text{kg}$ (Iqbal *et al.*, 2016).

Groundnuts

Aflatoxin B₁ presence in groundnuts have been studied and many reports came out. Among all the groundnuts types the most contaminated are peanuts but not only peanuts, others like pistachios and hazelnuts contained AFB₁ too. A survey conducted in japan for the purpose of investigating mycotoxins contaminating different retail foods, in 21 peanuts butter 10 were having elevated level of AFB₁, 2.59 $\mu\text{g kg}^{-1}$ (Kumagai *et al.*, 2008). In DRC there are many ways of preparing peanuts such as roasting or boiling or mixed with others food in powder form as well as eating raw peanuts that is why in DRC peoples have high possibly of eating contaminated food, 60 samples were used in a study conducted in Kinshasa 70% of samples had higher concentration of AFB₁ more than World Health Organization (WHO) limits (Kamika and Takoy, 2011). AFB₁ was high in Zimbabwe peanut and peanut butter as demonstrated by a report from Bulawayo markets aflatoxin B₁ was the most predominant with mean level of 51.0 ng/g , and range, 3.7 to 191 ng/g the total sample was 29 samples (Mupunga *et al.*, 2014). Aflatoxin B₁ level were examined in Chinese raw peanuts 1040 samples were utilized in this study, the results have indicated that 25% of the whole samples had aflatoxin B₁ with concentration between 0.01 and 720 $\mu\text{g}/\text{kg}$ (Ding *et al.*, 2012). 101 samples of peanuts and peanut products marketed in the State of Rio Grande do Sul, Brazil were analyzed and 14% tested positive for aflatoxin B₁ in peanuts samples with concentrations of 24.0 to 87.5 $\mu\text{g}/\text{kg}$, in samples of peanuts products the level were from 22.0 to 84.6 $\mu\text{g}/\text{kg}$ (Hoeltz *et al.*, 2012). Different groundnuts were tested in Zimbabwe for aflatoxin B₁ presence, the results showed that 12.5% of samples owned 175.9 $\mu\text{g}/\text{kg}$ AFB₁, in cowpeas samples 4.3% were positive and 1.4 to 103.4 $\mu\text{g}/\text{kg}$ was the concentrations range (Maringe *et al.*, 2017).

Maize

Many investigations have been done about AFB₁ contaminating maize those reports showed that maize is highly infected, numerous countries have set their own limit for aflatoxin B₁ in maize and other maize products. For instance researches carried out a research in Vietnam to check the frequency of AFB₁ contamination in 378 maize samples (54.0%) which means 204 samples presented AFB₁ at more than 5 $\mu\text{g}/\text{kg}$ (Nguyen *et al.*, 2018).

In Iran 373 samples of maize at harvest stage were brought together over 3 years from 2006–2008, AFB₁ was in 146 samples (43.6%), the total mean level were 30.94, 95.72, 192.13 $\mu\text{g}/\text{kg}$ in 2006, 2007 and 2008 respectively (Osboo *et al.*, 2012). Aflatoxin B₁ was found in cooked corn meal dog food were contaminated by aflatoxin B₁ after the signs of sickness a study were conducted in order to investigate the root cause of illness, in dog food investigators have come out with results showing presence of 1,640 ppb of AFB₁ (Wouters *et al.*, 2013). A study in Croatia employed 633 maize samples to assess AFB₁ level in maize, the obtained results have showed the occurrence of AFB₁ with 81 $\mu\text{g}/\text{kg}$, and the maximal value in maize was 2072 $\mu\text{g}/\text{kg}$ (Pleadin *et al.*, 2014). 114 maize samples from two south African districts Vhembe and Gert Sibande samples were tested for Aflatoxin B₁ (AFB₁) and the concentration was between 1 and 133 $\mu\text{g kg}^{-1}$ in Vhembe District and in Gert Sibande was 1.0 $\mu\text{g kg}^{-1}$ (Mngqawa *et al.*, 2016).

Wheat/Sorghum/Cereals

Besides maize, groundnuts and rice aflatoxin B₁ are also found in other commodities such wheat, sorghum and cereals for example a survey performed in Iran 34 wheat samples were employed in those 34 samples, according to the results 10 samples were AFB₁ positive and there was no concentration higher than permitted aflatoxin levels in Iran (15 ng/g). the highest level of AFB₁ in this survey was 7.08 ng/g (Namjoo *et al.*, 2016). Indian, 1,646 samples of wheat grain were analyzed for aflatoxin B₁ (AFB₁). AFB₁ level $\geq 5 \mu\text{g kg}^{-1}$ were found in 40.3% of the samples, and 16% were having concentration greater than Indian permissible regulatory limit of 30 $\mu\text{g kg}^{-1}$ (Toteja *et al.*, 2006). Even if high incidence of AFB₁ was present in wheat (44.8%), wheat samples owned 6.0 $\mu\text{g}/\text{kg}$ which was the least maximum limits (Elbashir and Ali, 2014). antithetically cereals presented a minimum rate of contamination (26.0%) and a tolerable highest concentration AFB₁ at 32.9 $\mu\text{g}/\text{kg}$ (Serrano *et al.*, 2012; Iqbal *et al.*, 2014).

Fruits/spices

Although currently there are no numerous studies done about AFB₁ contamination in fruits and spices, some few researches have confirmed the existence of AFB₁ in fruits and spices. Many countries incorporate spices such as cumin, black pepper, and chili pods/powder within every dish therefore a high contamination of AFB₁ can occur. In Malaysia spices like dried chilli, fennel, cumin, turmeric, black and white pepper, poppy seed, coriander, 'garam masala', and mixed spices were examined for AFB₁ existence the mean concentration of AFB₁ were 7.31 ng g⁻¹ (Ali *et al.*, 2015). About 18 spices samples were used in a study that took place in Pakistan to explore the level of contamination, findings showed that 12 samples were

positive, and the concentration was above the level set by European Commission (Hussain and Sohail, 2012). An investigation was carried out in turkey to examine the level of aflatoxins in spices sold in local market in turkey 27 samples of different spices including paprika, all the chilli powder and four ground black pepper and the results showed AFB₁ concentration ranging between 0.5-116.4, 1.6-80.4 and 0.3-1.2 $\mu\text{g}/\text{kg}$ respectively (Bircan, 2005). Regarding fruits many studies reported many cases of AFB₁ contamination, for instance in Greece 26 samples of dried vine fruits, 23% of the samples indicated the presence of AFB₁ with mean of 1.4 $\mu\text{g kg}^{-1}$ (Kollia *et al.*, 2014). Various Survey performed using Dried fruits (including figs, raisins, currants, sultanas, plums, dates, and apricots) provided the second lowest frequency of occurrence (36.0%) and the second lowest concentration of 16.3 $\mu\text{g}/\text{kg}$ (Heshmati *et al.*, 2017).

Detoxification methods

Aflatoxin B₁ is affecting food safety and security because it attacks plenty of commodities therefore many efficient techniques to avoid or remove AFB₁ in contaminated commodities have been developed, some control techniques are used before harvesting the crop and others are used after the harvesting (pre-harvesting and post harvesting). In pre-harvesting there are strategies like applying pesticides on crop in field, crop rotation, timing of planting, use of genetically modified seeds resistant to *Aspergillus* infection and environmental stressors. Post-harvest strategies that can be employed are proper drying, packaging, storage, and preservatives/pesticide usage. those measures can be used to prevent the contamination but studies have proved that they are not efficient that is why other advanced strategies are being use once the crop are already contaminated those techniques contain additives that can be biological or chemical that are added to AFB₁ contaminated crop to minimize or transform AFB₁, in addition physical processes usage. In this review we are highlighting strategies used to detoxify the contaminated crops using some different techniques including Physical treatment, Biological treatment, Chemical treatment, and Sorbent additives

Physical strategies

Detoxification by physical means include numerous ways such as Heating treatment, Irradiation, Electrolyzed water (EOW), Pulsed light technology to remove AFB₁.

Heat treatment

In modern industries heating is mostly used technique in many processing units, it is also used for decontaminate food/feed from aflatoxin B₁ but this strategy is not efficient since aflatoxins have high resistance to heat, in order to remove interesting quantity of AFB₁ rough heating is required.

Temperatures between 150–200°C can remove huge amounts of AFB₁ when the humidity is high (Lee *et al.*, 2015). Heating treatment has some advantages such as it is cheap, quick and easily performed. But it has also disadvantages like the temperatures used can affect other nutrients presented in food. In addition the degree of AFB₁ reduction is founded on initial mycotoxin concentration, the range of binding between mycotoxin and food or feed products, heat penetration, moisture content, and processing conditions.

Irradiation treatment

γ -radiation is also a way of detoxifying AFB₁ contaminated food, this method is common and mostly used in groundnuts, grains, palm juice, soybean, and animal feed. Current studies have demonstrated that during this technique a γ -ray source (such as ⁶⁰Co) is compulsory and it is used to irradiate food products until an ionizing radiation ranging from 6 to 60 kGy is achieved. Moreover this technique showed reduction effectiveness of 65% at high irradiation (Schindler *et al.*, 1980). Gamma irradiation is efficacy to moderately fungi-damaged feedstuffs. Current study conducted in Sudanese peanut oil involving titanium dioxide (TiO₂) whereby photocatalysis $\geq 99.4\%$ of AFB₁ were removed in 4 minutes of irradiation. This technique is more favorable compare to γ radiation because it does not cause any change in other food nutrients (Magzoub *et al.*, 2019).

Electrolyzed water (EOW) treatment

The newly developed technique that employs electrolyzed water to decontaminate the AFB₁ contaminated food or feed are being used currently. The EOW contain many OH groups which play a significant role in killing fungus like *A. flavus* by this way AFB₁ is reduced when EOW is used. A study conducted using peanuts samples in order to investigate the effectiveness of electrolyzed oxidizing water for removal of aflatoxin B₁ showed that after 15 minutes of treatment AFB₁ was mostly eliminated (Xiong *et al.*, 2012). Two types of water are produced and can be used in this treatment: neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW). The antimicrobial mechanism of both water rely on three properties: pH, oxidation-reduction potential (ORP) and available chlorine concentration (ACC).

Pulsed light technology

Bacteria, viruses, fungi, and spores presented on surface of food and material can be devastated by Pulsed light, hence this strategy is an effective way of removing aflatoxin B₁ (Elmnasser *et al.*, 2007). A non-thermal technology that was used to kill and stop mutagenic activity of mycotoxins have been developed and the obtained results have shown that this technique is powerful for mycotoxins degradation in

this study about 98% of AFB₁ was destroyed through eight flashes of pulsed light (Moreau *et al.*, 2013).

Chemical treatment

AFB₁ contaminated food can be decontaminated using chemical additives this strategy has become one of the most popular. Mostly used additives include Ammonia, Hydrochloric acid, Lactic acid and citric acid, Ozone.

Ammonia treatment

Ammonization is another way used to remove aflatoxins in different food products, this technique can employ gaseous ammonia at atmospheric pressure or NH₄OH. Ammonization demonstrated its efficacy in decontaminating AFB₁ contaminated products such as corn (Bagley, 1979). Multiple reports have proved the effectiveness of ammonia treatment to lower the aflatoxin B₁ content within 1 hour treatment, the temperature has big effect on the result of decontamination for instance as shown in different studies when NH₄OH at high temperature or gaseous NH₃ were used the above 99% of aflatoxin B₁ were eliminated on the other hand at low temperature like 25°C there is not significant removal of AFB₁ (Martinez *et al.*, 1994). This strategy is influenced by water content of the product and also the temperature.

Acids treatment

During HCl treatment hydrolysis reaction took place and is more powerful reaction to degrade AFB₁. A survey to examine degradation of AFB₁ by HCl was carried out; the results showed that HCl concentration, temperature and time are the key factors influencing the efficiency of HCl. At beginning the conditions were 1 mol L⁻¹ HCl at 110°C for 4 h degradation was 27.7% resulted in degradation of AFB₁ by 27.6% (33.07 $\mu\text{g kg}^{-1}$) after 4 h when the conditions changed the results improved to 42.5% after 8 h using 3 mol L⁻¹ finally full elimination was observed when 5 mol L⁻¹ HCl after 4 h at 110°C were employed (Aly and Hathout, 2011). In addition, organic acids have also proved their roles in crop decontamination; its activity is influenced by same factors as said previously. Lactic acid presented highest efficiency during a study carried out employing other organic acid such as acetic and citric acid in degrading AFB₁. The out comings showed that 85% of AFB₁ were removed after 2 hours of heating (Aiko *et al.*, 2015). The combination of acidification with high temperatures (80-120°C) has demonstrated a significant degradation of AFB₁.

Ozonation treatment

Food contain AFB₁ can be decontaminated using ozone, the utilization of ozone does not have any side effects based on The United States Food and Drug Administration (FDA), because it is mostly applied in food processing as oxidizing agent and is well known (GRAS).

In this process molecular ozone and hydroxyl groups react with aflatoxin B₁ resulting in degradation to reduced molecular products, therefore lowering its toxicity. Ozone concentration and exposure time are the two main factors influencing ozonation for example a study reported that employing 6–90 mg/L presented significant eradication of AFB₁ shortly. Moreover a study conducted using 60 mg/L of Ozone, 8 hours of exposure time and 1 kg maize sample has given considerable decrease of AFB₁ up to 57.0% (Porto *et al.*, 2019).

Biological treatment

Biological ways of removing or reducing aflatoxin B₁ in crop or food products have been studied. There are many microorganisms that are being used in this technique. The usage of biological methods are more advantageous over non biological methods since they are eco-friendly, specific, efficient as well. In this technique bacteria, fungi, yeasts, extracts, cold plasma technology have been utilized. Bacteria can detoxify contaminated commodity within metabolism or direct connecting on AFB₁. Bacteria like *Flavobacterium aurantiacum* NRRL B-184 from soil have played an important role to remove AFB₁ from contaminated stuff. Even though aflatoxins are produced by fungi, there are some fungi strains that can be employed to degrade AFB₁ such as *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus* sp., and nonaflatoxin-producing *A. flavus*. Biological techniques took longtime that is its main weakness (Hamad *et al.*, 2018). Numerous plant extracts have been used to degrade aflatoxin B₁, this approach showed good results but it is time consuming it takes between one and three days, *Adhatoda vasica* Ness and *Corymbia citriodora* extracts had presented a capacity to degrade more than 95%. Although, the obtained efficiency of this method, the active components in charge of degrading is not proved yet (Iram *et al.*, 2014).

Enzymes degradation capacity have been studied, results showed that this technique and other biological techniques discussed above they are time consuming and some still not clearly demonstrated its effectiveness on food because there are not been use in food. Lactone ring of AFB₁ were cleaved by aflatoxin-detoxifzyme an enzyme. that enzyme showed high detoxification capacity and the optimum conditions were 35°C and a pH of 6.8 (Liu *et al.*, 2001). Lastly Cold plasma technology to remove AFB₁ has been investigated several studies have been carried out using cold plasma technology to detoxify the contaminated food products. It owns advantageous characteristics like high efficiency and short treatment time, no residue, and low impact on the quality of treated food products. A study using microwave-induced argon plasma stated that AFB₁ could be completely eliminated within 5 seconds of treatment (Park *et al.*, 2007).

Sorbent additives

Interestingly addition of sorbent in food or feed have shown a significant role in elimination or reduction of aflatoxin B₁ based diseases, those sorbent do not degrade or attack aflatoxins in food or feed conversely those sorbent they limit absorption of the ingested aflatoxin B₁, chlorophyll and chlorophyllin are the two most used sorbent additives in this process. A study using Chlorophyllin showed that when it is added to animal feed there was a diminish of 37% AFB₁-DNA adduct in addition employing that sorbent can cause decline of 77% of tumor rate (Breinholt *et al.*, 1995).

Detection Techniques

To ensure food safety, detection methods are needed, there are many technique of aflatoxin B₁ determination; those techniques differ from each other in term of sensitivity, selectivity, accuracy, reproducibility and other many properties. The mostly used techniques are chromatographic based methods and immunosorbent based techniques those techniques are traditional in other words they present many disadvantages therefore with developing day by day technology detection techniques must also be updated hence there are numerous changes that have been made to improve the aflatoxin B₁ screening. Traditional methods own drawbacks such as inadequate for quick and real-time utilization in food and feed samples because they are tedious, labor-intensive, and need skilled personnel (Shukla *et al.*, 2017). Because of huge amount of existed detection techniques in this part authors are more focused on recently rapid detection techniques such as aptamers and molecularly imprinted techniques (Wang *et al.*, 2020). Rapid detection methods have become of great importance currently in order to overcome difficulties presented by traditional techniques. Aptamers are produced through Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and they are short in size made of artificial single-stranded oligo (deoxy) nucleotides that can bind various targets (Zhong *et al.*, 2020). Aptamers are used in numerous field such as food safety, environment monitoring, diagnosis of diseases, drug delivery that is due to aptamers properties like significant selectivity and great affinity to targets, can detect broad targets, poor immunogenicity, nanoscale size and easily modified (Vorobyeva *et al.*, 2018). Aptamer target complex is formed through different forces depending on target of interest those forces can be hydrogen bonding, stacking of aromatic rings, salt bridges, Van der Waals forces and other electrostatic links, as well as shape complementarity (Rahimizadeh *et al.*, 2017). Despite advantages presented by aptamers, there are also some disadvantages such as time consuming, expensive, stability problem, more screening cycles, any injury on target cells would affect screening outcomes all those drawbacks are due to the fact that

target proteins used for SELEX are extracted and purified from live cells overexpressing targets, moreover drawbacks differ based on which SELEX type used to generate aptamer. Recently aptamers have been extensively used in detection of aflatoxin B₁ and this approach has provided significant results. In 2015 aflatoxin B₁ was screened using aptamer based biosensor composed of single stranded amino-modified DNA aptamers specific to AFB₁ fixed by a mixture of Poly (amidoamine) dendrimers of fourth generation (PAMAM G₄) and cystamine on electrode, this probe showed a sensitivity ranging between 0.1–10 nM and limit of detection was 0.40 ± 0.03 nM, in addition stability is another factor showing the quality of a biosensor when this sensor was stored at 4°C it was still showing same results for 60 h (Castillo *et al.*, 2015). Aptamer was efficiently used to determine aflatoxin B₁, aptasensor was based on catalytic effect of aptamer/G-quadruplex DNAzyme complex, this sensor owned high sensitivity with low limit of detection 0.02 ng/mL, in this research authors proved that magnetic oxidized multiwall carbon nanotubes can also be employed to reduce elevated background (Wang *et al.*, 2019).

Taking into account various advantages of DNAzymes and aptamers, authors tried to develop selective and sensitive AFB₁ sensor by combining aptamer specific for AFB₁ and DNAzymes, the reported results of the optimized conditions exhibited a detection limit of 10 ng/mL (Jafari *et al.*, 2017) Because of high toxicity of aflatoxin B₁ on human health therefore on field screening would be helpful. Regarding to on field detection a portable aptasensor has been proposed employing a pH meter as readout, this probe used the change in pH as indicator of aflatoxin B₁ concentration, The reaction between AFB₁ and AFB₁ aptamer resulted in breakdown of hydrogel network and this induced the discharge of urease which is responsible for urea hydrolysis thus the pH increase, the linearity was 0.2–20 μM and limit of detection was 0.1 μM (Zhao *et al.*, 2018). Another common and largely employed detection technique for aflatoxin B₁ is molecularly imprinted polymers (MIPs), generally MIPs manifest advantages and disadvantages as other detection methods but when choosing an approach to be used in detection depend on many criteria, up to date research is an expensive sector so any cheap, non-sophisticated, accurate, selective detection technology is welcomed that is why molecularly imprinted technology is one of the widely used in many sectors like detection, environment monitoring, drug delivery and so on. MIPs are man-made biomimetic recognition elements capable of definitely bind a target molecule of interest. They are composed of functional monomers, cross-linkers and a target molecule. Functional monomers and cross linking agent are co-polymerized around a target molecule, resulting in a greatly cross-linked three-dimensional

complex polymer (Habimana *et al.*, 2018). At the end target is washed away leaving openings that are able to specifically fit with target, for that reason MIPs own capacity of selectively recognize target molecules; plenty reports have demonstrated the benefits of molecularly imprinted polymers compared to other detection methods. Molecularly imprinted polymer technique exhibit remarkable advantages like easy synthesis and handle, high stability, high specificity, high selectivity and sensitivity, excellent reproducibility as well as low fabrication cost, highly resistant to heat, acids and bases (Zor *et al.*, 2015), furthermore they are stay stable at high pH and temperatures. However in spite of these pertinent benefits, MIP exhibits some limitations. For instance misdistribution and unfair embedding of binding sites, as well as low accessibility for target molecule, weak binding power, and troubles to modernize the MIP polymers. In order to overcome these difficulties Magnetic molecularly imprinted polymers (MMIPs) played an important role as the answer by providing a method of immobilizing and renewing MIP on the solid support (Sedeño *et al.*, 2017).

MIPs have been extensively used for aflatoxin B₁ determination and showed significant results for instance recently AFB₁ was screened using MIPs combined with covalent organic framework composite and gold nanoparticles utilizing Quartz crystal microbalance sensor, authors combined advantages of Covalent organic frameworks (COFs), AuNPs and MIP to produce a highly sensitive and selective probe, target was eluted using methanol/water solution. The suggested sensor had linearity of $0.05\text{--}75$ ng mL⁻¹, low detection limit (2.8 pg mL⁻¹) (Gu *et al.*, 2019). Molecularly imprinting was mixed with quantum dot to develop a fluorescent sensor for rapid and accurate determination of aflatoxin B₁ (AFB₁). Instead of aflatoxin B₁, 5, 7-dimethoxycoumarin (DMC) acted like a dummy template, 3-Aminopropyltriethoxysilane was functional monomer, and the cross-linking agent were tetraethyl orthosilicate. Linear range was from 80 to 400 ng/g, limit of detection of 4 ng/g, whichever was lesser compared to that set by the US Food and Drug Administration (Guo *et al.*, 2019). A highly-Sensitive biosensor to detect aflatoxin B₁ employed MIPs synthesized through electropolymerization technique; the sensor was designed using electropolymerisation of p-aminothiophenol-functionalized AuNPS around AFB₁ which was the target of interest. The sensor exhibited linearity of 3.2 fM to 3.2 μM and quantization level of 3 fM (Jiang *et al.*, 2015). On field screening methods in food safety is a major concern, therefore scientists tried to develop different detection techniques to control food safety. Recent report demonstrated the production of smartphone-based optical mimic life sensor using free-standing molecularly imprinted polymer (MIP)

membranes to detect aflatoxin B1. Detection technique was based on selective bond between MIP and aflatoxin B1 which initiate the fluorescence of aflatoxin B1 with intensity linearly related to its concentration, 20 ng mL^{-1} was the detection limit; the storage stability was one year at 22°C (Sergeyeva et al., 2019). In this part, quick detection methods were discussed for their uses in detection of aflatoxin according to reviewed studies MIPs are more advantageous than aptamers because of its stability even at high temperatures in other words as demonstrated above aptamers are made of DNA which are well known for their low resistance to harsh conditions such as high temperatures since they can be denatured on the contrary MIPs do not be change because of high temperatures and other conditions like concentrated acid, aptamers production is more expensive than MIPs, in addition one developed MIP can be used several times without big difference in results but for aptamers that may not happen, MIPs can be stored for longtime than aptamers as shown in example of a stored MIP for one year at 22°C .

Conclusion

Serious measures should be considered when implementing food safety and security, so the crops will not be infected easily by aflatoxin B1 in field and even after harvesting. Good agricultural practices (GAPs), good manufacturing practices (GMPs) and good storage practices (GSPs) all those systems in conjunction with newly processing systems comprising heat, UV, pulsed light, electrolyzed water, cold plasma, ozone, gamma (γ) irradiation along with either biological, or chemical approach revealed high aflatoxin B1 detoxification capacity. In addition when decontaminating food and feed it is worthy to keep their nutritional value. Before decontamination detection is the first step to be conducted in order to make sure that the commodities have aflatoxin B1 therefore very sensitive, selective and quick detection techniques are required they exist huge number of rapid methods can be employed in aflatoxin B1 detection but in this review we took in consideration only aptamers technology and molecularly imprinted polymers both have good reputation in sensing but when comparing them molecularly imprinted polymers own more advantages over aptamers thus many sensors have incorporated MIPs in their sensing platform.

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