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Original Article

Control of the Cyclopiazonic Acid Level in *Aspergillus flavus*-Contaminated Wheat Flour

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Abstract
Introduction: Cyclopiazonic acid (CPA) is a mycotoxin produced by various fungal species such as *Aspergillus flavus* (*A. flavus*). This study aimed to limit and control the level of CPA production in *A. flavus*-contaminated wheat flour.
Materials and Methods: Wheat flour samples (35 samples) were collected from various locations in Egypt. The fungal contaminations were determined and identified. Pure colonies of *A. flavus* were maintained and tested for CPA production. Different procedures like ultraviolet (UV) treatment, heat treatment, materials adsorption, and biosorption by *Lactobacilli* spp. were applied to control and reduce the CPA level.
Results: Among 24 samples, 14 *A. flavus* isolates (58.33%) were able to produce CPA. Yeast sucrose broth was the most favorable medium for CPA production, yielding 290.6 µg/100 ml dry biomass. UV light had an impact on the synthesis of CPA at different exposure times, decreasing by 45.5% after 60 minutes of exposure. CPA levels decreased with increasing temperature and exposure time, with a maximum reduction of 71.1% achieved at 100 °C for 30 minutes. Charcoal was the most effective adsorption material, removing 53.3% of CPA. *Lactobacillus acidophilus* (*L. acidophilus*) was the most effective biosorbent, removing over 96.0% of CPA. Increasing the inoculum of *L. acidophilus* cells by 5 × 107 reduced CPA levels by 82.1%.
Conclusions: The diversity of abiotic and biotic control measures and their effectiveness may provide new hope for controlling and reducing CPA levels.

Keywords: *Aspergillus flavus*, Cyclopiazonic Acid, *Lactobacilli* spp., Ultra Violet
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Introduction

Certain filamentous fungi create secondary metabolites called mycotoxins.[1,2] Aspergillus species are among the most frequently occurring poisonous fungi that impact the food chain. *Aspergillus flavus* (*A. flavus*) is a significant generator of aflatoxins, which are extremely toxic and carcinogenic substances that are of concern for food safety.[3] Some isolates of *A. flavus* can also produce cyclopiazonic acid (CPA).[4] Many fungus species in the Ascomycete genera like Penicillium and Aspergillus generate the indole-hydranitramic acid mycotoxin known as alpha-cyclopiazonic acid (α-CPA), which is an ergot-like alkaloid.[5,6]

CPA can specifically inhibit the calcium-dependent ATPase (SERCA) in the sarcoplasmic or endoplasmic reticulum, which is necessary for calcium absorption in the muscle contraction-relaxation cycle. CPA has been linked to altered normal intracellular calcium influx and increased muscle contraction.[7,8] It has been reported to cause damage to the liver, kidneys, pancreas, salivary glands, spleen, and cardiac and skeletal muscles of animals.[9] CPA-contaminated millet led to human poisoning.[10] In addition, CPA was also found in various feed, livestock and poultry meat products.[11] It has been reported that animals exhibit severe gastrointestinal upset and neurological disorders after ingesting CPA-contaminated food.[9] The CPA contamination of food and feed poses a great threat to human health as well.

Ultra-violet (UV) light has been used for sterilizing and germicidal irradiation the agricultural products, such as stored grain for food or animal feed. While it is well established that UV treatment works to destroy harmful molds that infect grain surfaces, it is still unknown how and to what extent this type of radiation can remove mycotoxins.[12]

In milk, CPA does not change much when processed or stored normally. Only 2.8%, 2.9%, and 5.8% of the CPA level was removed in homogenized, pasteurized milk kept at 4 °C after 7, 14, and 21 days, respectively. Similar results were obtained when homogenized, pasteurized milk stored at 4 °C was frozen after 7, 14, and 21 days, respectively.[13]

Large molecular weight substances known as "mycotoxin-adsorbing agents" should be able to bind the mycotoxins present in the contaminated products without breaking down in the animals' digestive system. Inorganic compounds based on silica or organic polymers based on carbon can be used as mycotoxin-adsorbing agents.[14] The capacity of a number of organisms, including yeasts and bacteria, to reduce mycotoxin contamination has been investigated. It is well known that lactic acid bacteria (LAB) have bio-preservative capacities and exhibit antibacterial and antifungal characteristics.[15,16]

This study investigates various techniques as antifungal treatments to control and reduce the levels of *A. flavus* and CPA in wheat flour samples. Food and feed can be decontaminated from *A. flavus* and CPA using UV light, heat treatment, charcoal adsorption, and *Lactobacilli* spp. as a biosorption technique.

Materials and Methods

Isolation, Purification, and Identification of A. flavus Isolates

Thirty-five samples (each weighing 500 g) of wheat flour were gathered between February and September 2023 from various locations in Egypt. The samples were transferred to the lab in sterile plastic bags and stored at 4 °C for further fungal isolation and purification processes. Each sample (10 g) was homogenized in sterile saline solution, diluted in 10^{-1} to 10^{-4}, pipetted into sterile petri dishes with Czapek's-Dox medium containing a bacteriostatic agent, and incubated for 4 to 7 days at 28 °C.[17] A single pure colony was obtained from each isolate for additional research. The slants containing pure colonies were maintained at 4-5 °C.[18] Pure fungal colonies were identified down to the species level using the identification keys mentioned before.[19,20]

Dry Biomass and CPA Production by A. flavus

Sucrose yeast extract was used for this purpose.[21] Following the necessary incubation period, the culture flasks were filtered using pre-weighed filter paper (Whatman No. 1). The filter papers were dried for 48 hours at 80 °C in an electric oven and weighed.[22] The dry biomass was recorded as g/100 ml.

Extraction and Determination of CPA

A comparable volume of chloroform was added to the filtrated broth. The chloroform layer containing CPA was dried over anhydrous Na_2SO_4. To screen for CPA, thin-layer chromatography (TLC) plates were prepared[23] and placed in development solvents (formic acid, ethyl acetate, and toluene).[24] The plates were air-dried and sprayed with Ehrlich reagent (p-dimethylaminobenzaldehyde 10 g, hydrochloric acid 100 ml, and acetone 400 ml). The violet spots were identified, and the CPA-containing chloroform layer was dried over anhydrous Na_2SO_4 and evaporated. Each sample was prepared by adding 0.5 ml methanol, 1 ml Ehrlich reagent, and 5 ml hydrochloric acid "5N". The absorbance was measured by a spectrophotometer at a wavelength of 560 nm.[25]

Counting of Live and Dead Microbial Cells

The bacterial suspension turbidity was adjusted to 0.5 McFarland standard using a spectrophotometer set to a 600 nm wavelength.[26] Yeast and fungal spore counts were performed with a hemocytometer. Plate count techniques confirmed the number of bacteria, yeast, and mold.

UV Radiation

A. flavus (count 5 x 10^7 cfu/ml) was grown on plates and incubated at 28 °C for 5 days. Meanwhile, the plates were exposed to UV radiation (UV-C, λ 254 nm, 60 cm lamp 20 watt) at a height of 30 cm and different exposure times (20, 40, and 60 minutes). Three identical flasks containing the production medium were prepared and infected with 1 ml of untreated (control) and 1 ml of UV-treated spores. The dry biomass and CPA were measured after being cultured for seven days at 28 °C.[27]

Heat Treatment

Triplicate tubes containing 10 ml of culture filtrates were placed in a water bath at different temperatures (40, 60, 80, and 100°C) for varying durations (10, 20, and 30 minutes).[13] The dry biomass and CPA were measured as previously described.

Adsorption Materials Application

Adsorption materials (clay, charcoal, or silicate) were used for 30 minutes and then filtered. A comparison was made between the two parts (one without adsorption materials and another with adsorption materials) to determine the percentage (%) of CPA adsorbed or removed by the adsorption materials.

Biosorption and Degradation of CPA by Certain Microorganisms

The pellets of Lactobacillus spp. were obtained by centrifuging the suspension of live cells (7 × 10^7 cells/ml). Then, 2 ml of phosphate buffer saline (PBS) with varying pH values was added to the tubes. CPA was added under aseptic conditions. The tubes were incubated at 37 °C for 48 hours or 100 °C for 20 minutes, then CPA was extracted and quantified.[28,29]

Reduction of CPA production by biotic control (*Lactobacillus acidophilus*) was measured in triplicates. The sterilized polished rice grains (50 gr) were packed in plastic bags and 10% water was added. *A. flavus* (3 × 10^7 cells) for CPA production and *L. acidophilus* as biotic control at different counts (5 × 10^1: 5 × 10^7) were added.[28,29] All bags were incubated at 28 °C for 7 days. Then, 100 ml of chloroform was added and shaken for 30 minutes, to extract and detect CPA

Statistical Analysis

The statistical analysis was carried out using SPSS v.29.0.10 (IBM, USA). Data were expressed as mean ± SD. The student t-test assessed the normality of all variables' distribution, and we applied Pearson correlation to compare the means between the two groups Data were recorded as significant when the prevalence values were calculated at 0.05 or less ($p<0.05$).

Results

A. flavus Isolates Screening for their CPA Production

Twenty-four wheat flour samples out of thirty-five were found contaminated with *A. flavus*. The total count of fungal isolates in wheat flour ranged between 3 × 10^2 to 17 × 10^2 cfu. Fourteen *A. flavus* isolates (58.33%) produced CPA (Table 1 and Figures 1 & 2).

Table 1. Detection of CPA and Dry Biomass Produced by Fungal Isolates on Culture Medium

Fungal isolates (No.)	Dry biomass (g/100 ml culture medium)	CPA (µg/100 ml culture medium)
1	1.09 ± 0.08	0.0
2	0.62 ± 0.04	77.6 ± 0.07
3	0.33 ± 0.04	0.0
4	0.91 ± 0.07	0.0
5	0.26 ± 0.02	71.4 ± 0.09
6	0.53 ± 0.05	0.0
7	0.50 ± 0.03	32.4 ± 0.13
8	0.46 ± 0.08	280.8 ± 0.04
9	0.47 ± 0.04	0.0
10	0.38 ± 0.02	75.8 ± 0.11
11	0.38 ± 0.05	39.2 ± 0.15
12	0.94 ± 0.11	182.2 ± 0.06
13	1.01 ± 0.04	71.4 ± 0.09
14	0.62 ± 0.04	0.0
15	0.29 ± 0.06	70.4 ± 0.05
16	0.34 ± 0.08	77.9 ± 0.09
17	0.60 ± 0.04	73.6 ± 0.07
18	0.89 ± 0.08	0.0
19	0.51 ± 0.06	0.0
20	0.61 ± 0.11	169.2 ± 0.07
21	0.53 ± 0.09	34.4 ± 0.10
22	0.99 ± 0.07	253.5 ± 0.07
23	0.37 ± 0.08	37.4 ± 0.09
24	0.42 ± 0.03	0.0

$^*p<0.05$, statistically significant

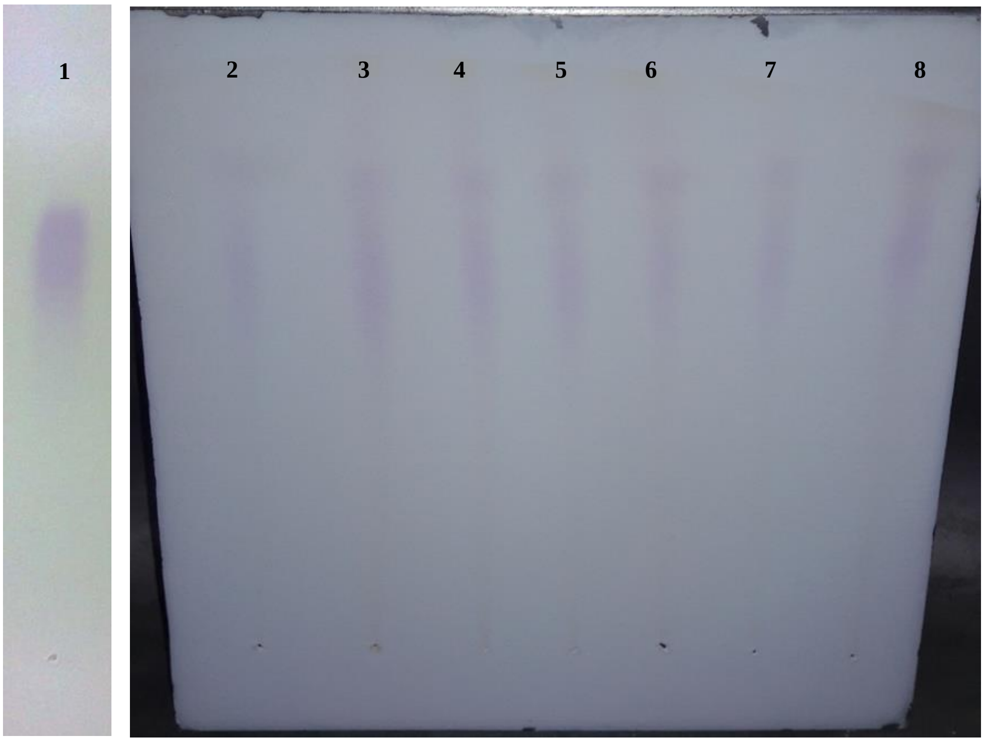

Figure 1. CPA Spots (violet spots) on TLC for some *A. flavus* Isolates Positive Samples Compared with the Standard CPA. (1) Standard, (2) isolate No. 2, (3) isolate No. 5, (4) isolate No. 7, (5) isolate No. 8, (6) isolate No. 10, and (7) isolate No. 11.

Figure 2. *A. flavus* Isolate No. 8 on Potato Dextrose Agar.

The most active sample was isolate No. 8, which produced 280.8 µg/100 ml of CPA in the culture medium, with a dry biomass of 0.46 g/100 ml. The second most active was isolate No. 22, which produced 253.5 µg/100 ml of CPA in the culture medium, with a dry biomass of 0.99 g/100 ml. The least active was isolate No. 7, which produced 32.4 µg/100 ml of CPA in the culture medium with a dry biomass of 0.5 g/100 ml.

On the other hand, A. flavus isolate No. 1 had the maximum dry biomass of 1.09 g/100 ml in the culture medium but did not produce any CPA (0.0 µg/100 ml). The second highest dry biomass was from *A. flavus* isolate No. 13, which had a dry biomass of 1.01 g/100 ml and produced 71.4 µg/100 ml of CPA in the culture medium.

Table 2. Effects of Different UV Doses on Dry Biomass and CPA Production by *A. flavus* Isolate No. 8

Time of exposing to UV radiation (minutes)	Dry biomass (g/100 ml culture medium)	Cyclopiazonic acid (µg/100 ml culture medium)	Percentage of cyclopiazonic acid reduced (%)
0.0 (Control)	3.06 ± 0.04	899.6 ± 0.08	
20.0	0.88 ± 0.06	612.9 ± 0.04	31.8
40.0	0.81 ± 0.04	555.1 ± 0.06	38.3
60.0	0.79 ± 0.06	490.7 ± 0.04	45.5

Control = *Aspergillus flavus* with no UV radiation exposure, $^{*}p<0.05$, statistically significant

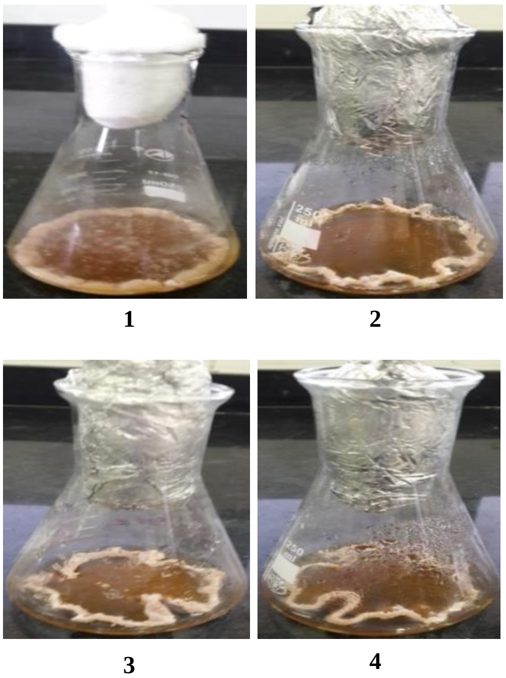

Figure 3. Images of conidia cultures of *A. flavus* exposed to different UV doses (1: control 0 min, 2: 20 min, 3: 40 min, and 4: 60 min.).

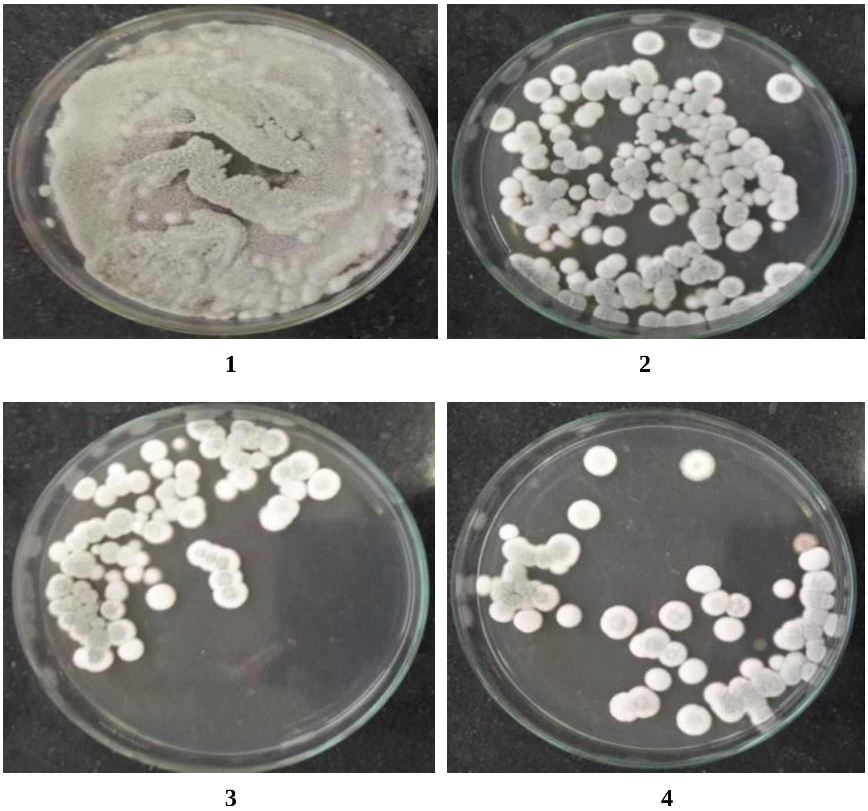

Figure 4. Images of Colonies Resulted from Conidia of *A. flavus* Exposed to Different UV Doses (1: control 0 min., 2: 20 min., 3: 40 min, and 4: 60 min.).

Control of CPA Production by Different Methods

The capacity of the following control parameters to limit and regulate *A. flavus* production of CPA was examined. These control factors included: UV radiation, heat treatment, adsorption by charcoal, silica, and clay, and biosorption by *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus rhamnosus*.

Reduction of CPA Amount by UV Radiation

UV light could decrease the growth of the experimental isolate and the synthesis of CPA at different exposure times. At 20, 40, and 60 minutes, it decreased the quantities of CPA by 31.8%, 38.3%, and 45.5%, respectively (Table 2 and Figures 3 & 4). A significant difference was observed between UV radiation exposure time and CPA groups ($p<0.05$).

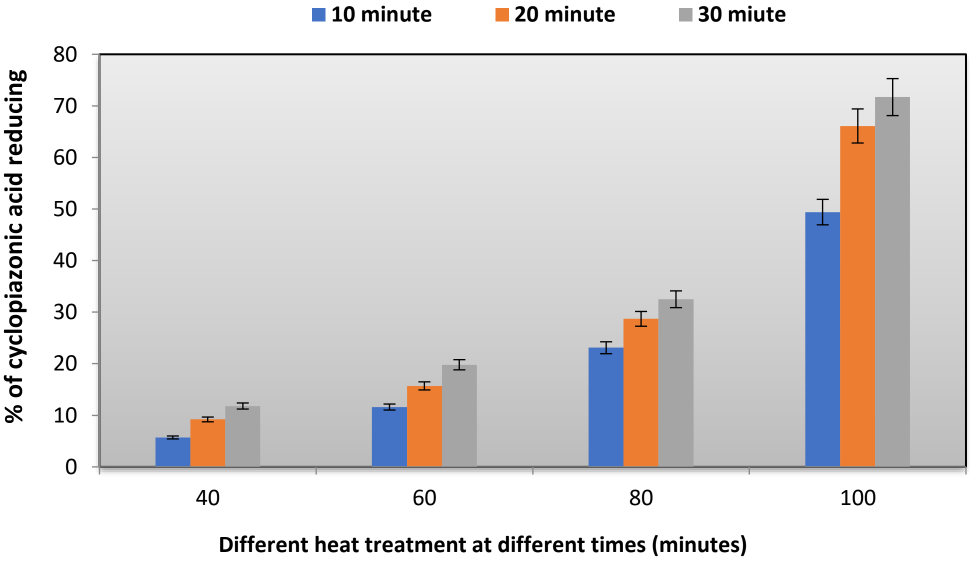

Figure 5. Percentages of CPA Reduction by Heat Treatment at Different Times.

Table 3. Adsorption of CPA Produced by *A. flavus* Isolate No. 8 Using Different Adsorption Materials

Adsorbed materials	Cyclopiazonic acid (µg/100 ml culture medium)	Cyclopiazonic acid (µg/100 ml culture medium)	Cyclopiazonic acid adsorption (%)
	Before adsorption	After adsorption	
Clay	218.1 ± 0.08	128.4 ± 0.03	41.2
Charcoal		101.9 ± 0.05	53.3
Silicate		151.4 ± 0.05	30.6

Dry biomass: 1.04 ± 0.04 g/100 ml culture medium; $^{*}p$<0.05, statistically significant

Reduction of CPA of A. flavus Isolate No. 8 by Heat Treatment

The data in Figure 5 showed a decrease in CPA levels with increasing temperature and exposure time. The maximum and minimum reductions were observed as 71.1% at 100 ºC for 30 minutes and 5.7% at 40 ºC for 10 minutes.

CPA Adsorption by Different Adsorption Materials

All tested adsorption materials were able to remove large amounts of CPA. The most active material was charcoal, followed by clay and silicate, which removed CPA by about 53.3%, 41.2%, and 30.6%, respectively (Table 3). A significant difference was observed between the CPA groups before and after adsorption ($p<0.05$).

Biosorption of CPA by living and dead microorganisms

L. acidophilus was the most active in biosorbing CPA, with a rate of over 96.0%, followed by *L. rhamnosus* and *L. bulgaricus*, which biosorbed CPA at rates of over 93.0% and 92.0%, respectively (Figure 6). Among dead cells, *L. acidophilus* was the most active in biosorbing CPA, with a rate of over 94.0%, followed by *L. rhamnosus* and *L. bulgaricus*, which biosorbed CPA at rates of over 92.0% and 91.0%, respectively (Figure 7).

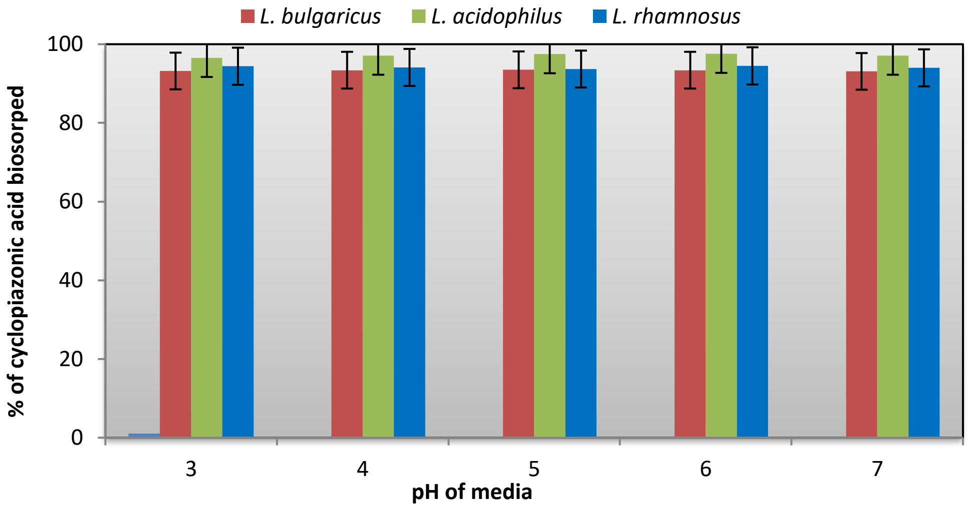

Figure 6. Percentage of CPA Biosorbed by Different Living *Lactobacillus* spp.

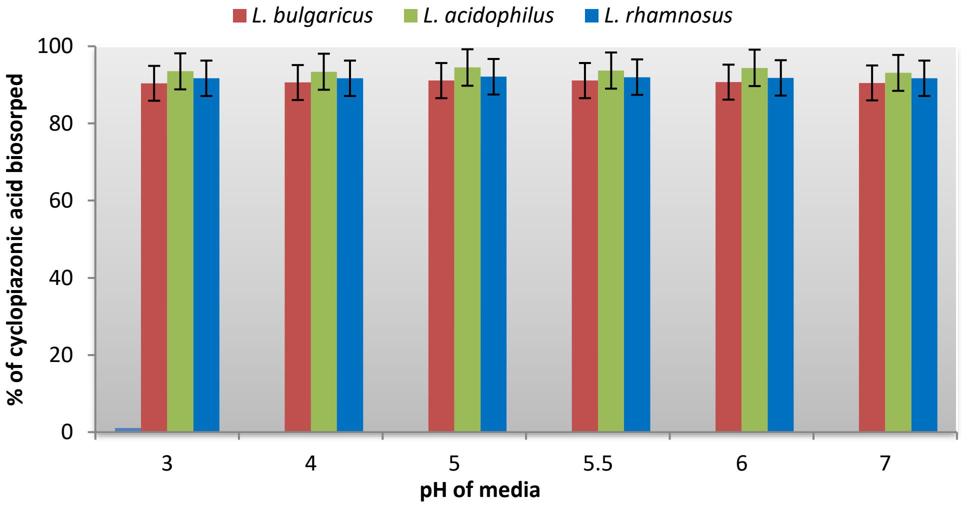

Figure 7. Percentage of CPA Biosorbed by Different Boiled *Lactobacillus* spp.

Table 4. Reduction of CPA Production by *A. flavus inocula* Rice by Adding *Lactobacillus acidophilus*

Treatment	***L. acidophilus*** **count No. (ml)**	**Final CPA concentration after treatment (µg/100 g)**	**Percentage of CPA reduction (%)**
-ve control	0.0	0.0	---
+ve control	0.0	71.5 ± 0.03	---
Group 1	5×10^1	71.1 ± 0.09	0.6
Group 2	5×10^3	61.3 ± 0.07	14.3
Group 3	5×10^5	31.1 ± 0.05	56.5
Group 4	5×10^7	12.8 ± 0.09	82.1

-ve control = Sterilized rice grains plus 10% water without *A. flavus inocula* (0.0 µg/100 g).

+ve control = Sterilized rice grains plus 10% water plus 3×10^7 *A. flavus inocula* inocula of *L. acidophilus* (71.5 ± 0.03 µg/100 g).

Biotic Control of CPA by Lactobacillus acidophilus

Increasing the inoculum of *L. acidophilus* cells decreased the amount of CPA produced by *A. flavus* inocula compared to the sample without *L. acidophilus* (+ve control) (Table 4). No significant difference was observed between *L. acidophilus* count and reduction of CPA ($p>0.05$).

Discussion

Aspergillus, Penicillium, and Fusarium are the main mycotoxin-producing fungi found in nature; however, only a small number of species in these genera can produce toxins such as ochratoxin A, patulin, fumonisins, aflatoxins, CPA, trichothecenes, or zearalenone.[30]

In terms of human nutrition, wheat is a strategic basic ingredient. Mold can grow on a variety of foods, including wheat flour. An investigation on mold contamination in all wheat flour samples revealed the abundance and significant species and genus variations of mold. Penicillium, Aspergillus, and Rhizopus mold species were found to be predominant in the wheat flour samples.[31]

The total qualitative and quantitative mycobiota of whole wheat flour and white wheat flour (type 405) were examined. The total fungal counts of the whole wheat flour were 1833 molds, whereas the white wheat flour had 1730 CFU/2 g. Aspergillus species accounted for 84% and 77.3% of the isolations in the mycobiota of the whole wheat and white wheat flours, respectively.[31]

While CPA was not found in the SGS agro-ecological zone, it was found in the samples taken from the DS agro-ecological zone, which included garri, maize, millet, rice, sorghum, and wheat. With the exception of garri, all samples for SS had CPAs between 1.283 and 9.601 µg/kg. Agro-ecological zones collected samples with the following percentages of positives: 100%, 0%, 83.3%, 67.4%, 67.4%, and 32.4% for DS, SGS, SS, NG, SuS, MA, and HF, respectively. The average for all zones was 57.1%, which can be regarded as a significant CPA occurrence for staple commodities. The samples had CPA concentrations ranging from 1.027 µg/kg to 14.918 µg/kg.[50]

Among the mycotoxins, CPA is one of the most significant naturally occurring contaminants. It is produced by several Aspergillus and Penicillium species and found in a variety of agricultural goods, such as peanuts, pistachios, corn, barley, and millet. As it enters into the food chain, it poses a health risk to both humans and animals.[9,32]

Numerous physical, chemical, and biological methods are used to decontaminate food and feed. Moreover, many techniques are combined to eliminate and degrade mycotoxins without compromising the quality of the raw materials. To eliminate or reduce the quantity of mycotoxins, the food and feed industry employs chemical processes such as reduction, ammoniation, oxidation, acidification, and alkalization.[33,35] Mycotoxins can be partially removed by physical techniques such as dry cleaning, milling, color sorting, irradiation, floating, water washing, and removing damaged grains.[34,35] Nevertheless, the use of hundreds of microorganisms, such as bacteria, yeast, and fungi, has been suggested as a very promising alternative for the biological decontamination of mycotoxins. LAB are the most abundant microbes for the breakdown of mycotoxins among all other microorganisms.[35]

It has been established that radiation is a safe and effective form of treatment. It can reduce mycotoxin levels and eliminate microbial contamination from food and feed without raising the temperature.[36,39]

An irradiation method with germicidal properties is ultraviolet (UV). UV light, which ranges widely from 100 to 400 nm in the electromagnetic spectrum, is non-visible with three types based on the wavelength: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm).[37,39] The most potent germicidal radiation is UV-C, which can be used to lower the surface contamination from food contact during post-harvest storage.[37,39] *A. flavus* can be successfully inactivated by UV irradiation without compromising the quality of food, however, the effectiveness of inactivation varies substantially between irradiation techniques.[38,39]

The most significant manipulation of mycotoxin content in a final food product during industrial processing depends on the combination of time and temperature. Since most mycotoxins are thermally and chemically stable, conventional food preparation at temperatures as high as 100 °C has little effect on most mycotoxins; however, higher temperatures during frying, roasting, toasting, and extrusion may reduce the contamination of mycotoxins.[34] Although some additional CPA degradation occurred from more intense heat treatments (2 hours at 100 °C), 40% to 50% of the initial concentration was still present.[13]

One of the methods used to overcome the negative effects of mycotoxins in animal feed is to add toxin-sequestering agents to the feed. However, the effects of toxin-sequestering agents are variable, depending on the toxin binders, target mycotoxins, and animal species.[40] The capacity of activated carbon to bind aflatoxins has been investigated. In an *in vitro* gastrointestinal model, the activated carbon decreased the availability of both nivalenol and deoxynivalenol.[41] The activated carbon is chemically composed of silicates and/or aluminosilicates, and clays are regarded as natural adsorbents.[42]

Lactic acid bacteria (LAB) are the most recommended microorganisms for the degradation of mycotoxins because they have a strong safety record in food applications. LAB are chosen over other microbes because they exist in a variety of strains that are easy to grow and maintain, are highly safe to use in food, and naturally thrive in the human gut, which enables them to effectively eliminate mycotoxins.[35,43] There are two ways that LAB can detoxify mycotoxins from the diet. Food detoxification by LAB is achieved using the live cells of the microorganisms and/or the enzymes produced by certain LAB strains. Food has long been preserved using LAB to keep it safe from decaying bacteria like fungus.[35,43]

The adsorption of mycotoxins by the cell wall of LAB strains has been suggested as an additional technique to remove mycotoxins from specific foods. Nevertheless, this effect has been linked to the presence of proteins, peptidoglycans, and polysaccharides in the cell wall of LAB strains.[35,44]

Five Lactobacillus strains, including *L. rhamnosus* GG, *L. rhamnosus* LC705, *L. acidophilus*, *L. gasseri*, and *L. casei*, can extract Aflatoxin B1 (AFB1) from liquid media. Additionally, these strains have shown that the probiotic strains *L. rhamnosus* GG and *L. rhamnosus* LC705 are highly effective in eliminating up to 80% of AFB1.[45,47] Furthermore, it was found that *L. rhamnosus* GG could extract up to 70% of the ZEN chemical structure from liquid medium.[46,47] Compared to other strains, *L. acidophilus* ATCC4495 exhibited greater antifungal efficacy against *A. flavus* and *A. parasiticus*, which are producers of aflatoxin.[48]

Total damage continuously grew by extending the storage period to the end. Compared to the other treatments, the original 100% supernatant of *L. acidophilus* ATCC4495 treated (negative control) non-infected maize grains and showed the lowest infection percentage.[48] As the supernatant concentration was lowered, the overall damage percentage of both *L. acidophilus* strains increased progressively. Notably, the original 100% supernatant treatment exhibited the lowest total damage percentage compared to the 50% and 25% dilution treatments. Decrease in the overall percentage of damage in maize grains infected with lactic acid bacteria may be attributed to the treatment ability to block the formation of various Aspergillus species mycelial sporulation, as previously indicated.[49]

The generation of aflatoxins was reduced by the presence of *L. acidophilus* strains following storage at room temperature for 30 days. The lowest concentration of aflatoxin was observed when moisture was modified in uninfected maize grains to 20% using 100, 50, and 25% of the cell free supernatant (negative controls).[48]

While CPA was not found in the SGS agro-ecological zone, it was found in the samples taken from the DS agro-ecological zone, which included garri, maize, millet, rice, sorghum, and wheat. With the exception of garri, all samples for SS had CPAs between 1.283 and 9.601 µg/kg. Agro-ecological zones collected samples with the following percentages of positives: 100%, 0%, 83.3%, 67.4%, 67.4%, and 32.4% for DS, SGS, SS, NG, SuS, MA, and HF, respectively. The average for all zones was 57.1%, which can be regarded as a significant CPA occurrence for staple commodities. The samples had CPA concentrations ranging from 1.027 µg/kg to 14.918 µg/kg.[50]

Biological control approaches continue to face problems in terms of effectiveness and safety. Antifungal probiotics (LAB, yeast, Bacillus) are thought to provide an answer.[51,52] Finding the right microbe is therefore essential and involves procedures including collecting samples containing possible target microorganisms, isolating them, screening and identifying them, and assessing strain safety.[52,53]

Conclusion

Many techniques can be used to control and reduce the growth of *Aspergillus flavus* and the production of cyclopiazonic acid (CPA). Increasing the exposure time to UV light can reduce the growth of *Aspergillus flavus*, which in turn reduces the production of CPA. Heat treatment also contributes to the reduction of CPA. Conventional techniques using the adsorption of charcoal, clay, and silica are also used to reduce CPA levels. Along with them, biological control using *Lactobacilli* spp., is also efficient in inhibiting *A. flavus* proliferation and metabolite production. All of these techniques can be used on crops before and after harvest, food, and feed.

Authors' Contributions

All authors have the same contribution in Data analysis, Methodology, Investigation, Software, Writing-Reviewing and Editing.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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