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Estimation of Aflatoxins in Different Samples of Rice *Oryza Sativa L.*

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Abstract: The purpose of this investigation was to see if total aflatoxins (B1 + B2) were present in unpacked and packed rice samples. A total of 70 rice samples were obtained from the markets of Lahore, Pakistan. This including different kinds of Basmati rice, Sella rice, and brown rice, then tested using TLC technique and photographed under UV light. AFs were found in 27% (n = 19) of the rice samples, with concentrations ranging from 7.9 to 21.3 µg kg^{-1}. The amount of AFs over the extremities was found in 5.7% (n = 4) of basmati rice samples, 5.7% (n = 4) of Sella rice samples, and 15.7% (n = 11) of brown rice samples. The whole positive samples evaluated had AFs above the EU's ML for total aflatoxin. It was found that there is a need to establish a strict and continuous national monitoring plan to improve the safety and quality of rice in Pakistan.

Keywords: Aflatoxins; Oryza Sativa; TLC Technique; UV Light

1. Introduction

Aflatoxins are a significant public health issue because they have the potential to induce hepatitis, haemorrhage, edema, immunosuppression, and liver cancer due to their potent hepatotoxic, tetratogenic, and mutagenic properties. It is primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* either before or after yield.[1] Plants naturally contain both poisonous and non-toxic compounds. Harmful compounds often find their way into plants from the atmosphere. Microorganisms are everywhere in our world, and depending on when they occur, they may be harmful or non-toxic. Fungi are essential plant pathogens that contain a variety of toxic by-products. Mycotoxins are natural toxic and secondary metabolites made by filamentous fungi as a result of a chemical or enzymatic reaction. Plants, poultry, and humans are also at risk from these microorganisms. Every year, mycotoxins contaminate a vast number of crops, causing them to spoil. As a result, millions of tons of food are thrown away from crops and stores in order to avoid harmful effects on the environment.[2]

Molds from the genera *Aspergillus*, *Penicillium*, and *Fusarium* contain mycotoxins, which are naturally poisonous food pollutants. Aflatoxins (AFs) are mostly produced by fungi of the *Aspergillus* genus. *The most common species are Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, and Aspergillus tamari.*[3] These fungi commonly contaminate cereal yields such as wheat, walnuts, maize, cotton, peanuts and tree nuts,[4] posing serious health risks to humans and animals. Hepatotoxicity, teratogenicity, and immunotoxicity are only a few examples.[5]

There are over 20 known types of aflatoxins, but the main four are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 with aflatoxin M1 (AFM1) and M2 (AFM2) being the hydroxylated metabolites of AFB1 and AFB2[6] or dermatological approaches that cause the inflammatory response to become more active.[7] Fig. 1 shows the structure of aflatoxins AFB1, AFB2, AFG1, AF.

The most important staple food crop is rice (*Oryza sativa* L.), and the majority of rice is cultivated in the autumn or rainy season. Floods and heavy rains, particularly near harvest in coastal areas of the country's eastern, southern, and western regions, wet the crop and make flower clusters more susceptible to *Aspergillus* sp. Infestation.[8] A *flavus* isolates from rice grains were found to be capable of producing AFB1 in a preliminary analysis.[9] Rice is another essential grain that promotes the production of mycotoxins. Pakistani rice is known all over the world for its rich fragrance and flavour. Aflatoxins (AFs) are particularly toxic and carcinogenic fungal by-products that have been found in a variety of foods, including grains. Thin layer chromatography (TLC) was used to look for aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) in rice samples obtained in 2008-2009. The specialty of this research is to determine the aflatoxins in different types of rice samples are as following.

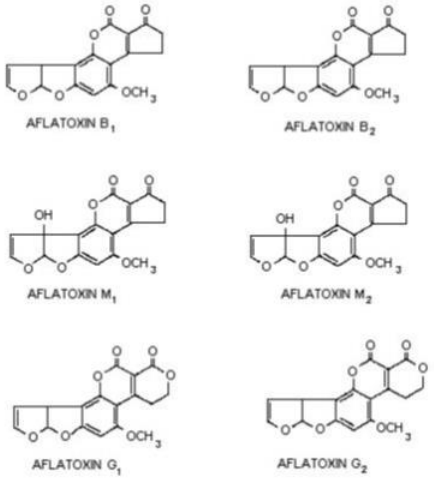

Fig. 1. Structure of Aflatoxin: AFB1, AFB2, AFG1, AF.

- To determine the aflatoxins (B1, B2) in the rice samples by using the thin layer chromatography (TLC) technique and observe it under UV radiation
- To estimate the presence of aflatoxin range in different rice samples.

2. Materials and Methods

2.1. Collection of Samples

2.1.1. Food Samples

Around 70 samples of rice were collected from different areas of Lahore where the storage condition of rice was good and not. The areas consisted of Ichra, Liberty Market, Qainchi, Bhatti Gate, Chuburgi, Ghazi Chowk, Model Town, Bank Stop, Anarkali, Samanabad, Nishtar colony, Shalmi, Township, Jail Road, Abid Market, Johar Town, Mazangchungi, Defence, Wapda Town, Youhanabad, Garden Town, and Faisal Town.

2.2. Grinding of Sample

The AOAC technique does not work to obtain homogenous samples. The number 977.16 was utilized. Approximately 200 g of each sample was taken and thoroughly mixed for 10 minutes. To achieve a homogenous and representative sample, all rice samples were ground in a sample mill. To calculate AFs, a total of 50 g of each sample was obtained. All samples were sealed in airtight polyethylene bags until they were used in the experiment.

2.3. Extraction of Aflatoxin

A 500 ml flask was filled with 50 g of crushed rice, 25 ml of water, 25 g of diatomaceous earth, and 150 ml of chloroform. For 30 minutes, the solution is completely mixed on the wrist.

2.4. Filtration

The rice mixture was filled by Whattman No. 1 filter paper to obtain 50 ml of chloroform ($CHCL_3$) in a beaker. 50 ml was evaporated in steam bath at the temperature of 60°C.

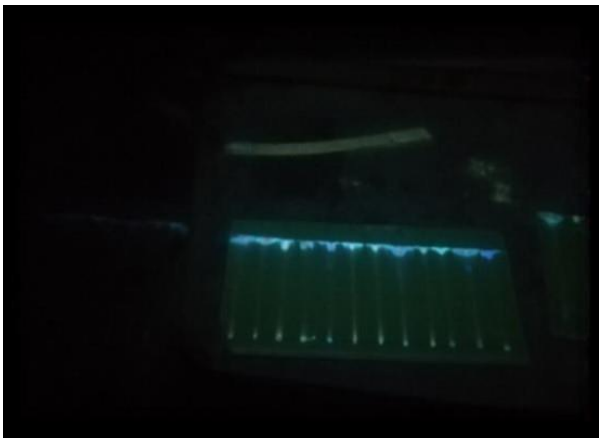

Fig. 2. TLC plate under UV light.

2.5. Thin Layer Chromatography (TLC)

On a TLC plate, spotting was done (about 1.5 cm from the base). To avoid the stain dissolving in the solvent, the solvent should be placed below it. On a TLC plate, spots of 2, 5, and 10 µl were formed using sample micro-syringes. As a benchmark, 2, 5, and 10 were chosen. The development was done in two tanks, one with anhydrous ether and the other with acetone-chloroform. Place the TLC plate first. Reduce the TLC plate to half its original size. Remove the plate from the tank after a time and dry it on the hot plate. Place the TLC plate in tank II, which is filled with acetone- chloroform (1:9, v/v). According to Rf, the ratio is modified. The presence or absence of aflatoxin can be detected by viewing the dried plate under 365 nm ultraviolet light. The fluorescence intensity is used to calculate the amount of aflatoxin (Fig. 2). On a TLC plate, spotting was done (about 1.5 cm from the base). To avoid the stain dissolving in the solvent, the solvent should be placed below it. On a TLC plate, spots of 2, 5, and 10 µl were formed by using sample micro- syringes. As a benchmark, 2, 5, and 10 µl were chosen. The development was done in two tanks, one with anhydrous ether and the other with acetone-chloroform. To begin, put the TLC plate in anhydrous ether tank I. in tank remove the plate after a time and dry it on the hot plate. TLC tank II should be placed here, which is filled with acetone-chloroform (1:9, v/v). According to Rf, the ratio is modified. The presence or absence of aflatoxin can be detected by viewing the dried plate under 365 nm ultraviolet light. The fluorescence intensity is used to calculate the amount of aflatoxin.

2.6. Quantitative Determination

The test solution was thoroughly dried before being evaluated quantitatively for aflatoxin. Then, to test the solution, add benzene acetonitrile (98:2). 2 µl, 5 µl, and 10 µl of the test solution should be placed on the TLC plate. Put stain and the stain should be of the same size. Now, in the succeeding volumes of 2, 5, and 10 µl, use the same standard of topical aflatoxins.

2.7. Interpretation of the Plate

The comparison is conducted with the standard aflatoxin spot to examine how similar the test solution spot and the standard are. Under fluorescent light, the spots were studied. The presence of

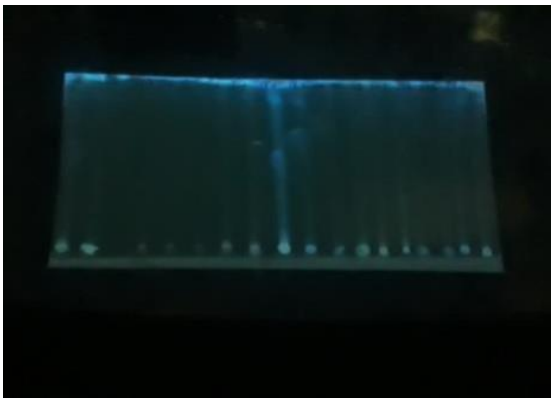

Fig. 3. Different samples on plate viewed under UV light.

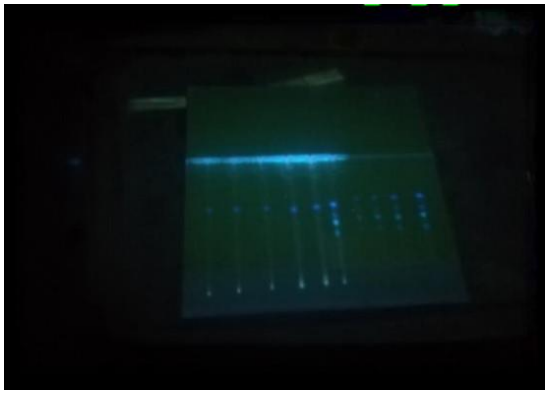

Fig. 4. Standards on Positive samples.

aflatoxin in the sample is shown when the test spot and the standard spot are placed on one other. The Rf value and color of the sample are comparable to that of aflatoxin (Fig. 3).

2.8. Detection

The fluorescence intensity of the aflatoxin-containing sample spot and the reference spot were compared. Each volume has been captured on tape. The spot should not be so tiny that it prevents a precise reading. For a better outcome, dilute the solution and repeat the TLC method if the stain is too tiny.

2.9. Calculation

The following formula may be used to calculate aflatoxin absorption in a sample:

$$Aflatoxins\ contents\ (mg/kg) = \frac{S*Y*V}{W*Z}. \quad (1)$$

Where,

- S: volume of aflatoxin standard in millilitres with density equal to Z = millilitres of sample
- Y stands for the standard aflatoxin concentration in milligrams per millilitre.
- Z: the amount of sample extract (in millilitres) necessary to achieve same fluorescence intensity. For standard aflatoxin, S = ml
- V: The amount of solvent necessary to dilute the final extract in millilitres.
- W: The original sample's weight in grams contained in the final extract.

3. Results

Mycotoxins issues are not limited to the industrialized or developing nations; they are a concern that affects many countries' agricultural industries. Aflatoxins are strong carcinogens that occur naturally as toxic fungus metabolites and cause substantial health concerns and acute toxic effects in people and animals. The aflatoxin found in rice has the potential to cause serious health problems and, if not correctly recognized, death. Aflatoxin was found in several rice samples in this investigation, and it was discovered that the rice samples were contaminated with aflatoxin owing to inappropriate storage conditions and a variety of other causes (Fig. 4).

3.1. Screening of Samples for Positive Result

For qualitative and quantitative analysis of aflatoxin in rice, the collected rice samples were analyzed using thin layer chromatography (TLC). The TLC samples were examined using a UV spectrophotometer. The presence or absence of aflatoxin in rice samples was tested in an experiment. The presence and absence of aflatoxin in the samples are quantified using thin layer chromatography.

3.2. Standards for Aflatoxin

The sample on the TLC plate was detected using a tiny syringe. As a benchmark, 2, 5, and 10 µl were chosen. According to Rf, the ratio is modified. The presence or absence of aflatoxin can be detected by viewing the dried tablet under 365 nm ultraviolet light. The fluorescence intensity is used to calculate the amount of aflatoxin.

3.3. Quantitative Detection of Aflatoxin

Aflatoxin was found in 19 of the 70 rice samples treated with natural substances, but aflatoxin was not found in the other 51 rice samples. When seen under UV light, a TLC plate produces a blue flash that corresponds to the spot of a standard solution. For qualitative investigation of fungal spores, sulphuric acid is sprayed right away.

3.4. Mathematical Formula

The following formula can be used to calculate aflatoxin absorption in rice samples

$$Aflatoxins\ contents\ (mg/kg) = \frac{S*Y*V}{W*Z}. \quad (2)$$

All the concentrations of aflatoxin in the rice samples can be easily estimated by putting some values in formula. Here the maximum and the minimum value determined by the use of above formula that are as following:

Table 1. Rice samples were indicating the detection of Aflatoxin AFB1

S.No	Area	Rice Sample	Aflatoxins Concentration (ppb)
1	Ichra	Super Basmati	Not detected
2	Ichra	Sella	Not detected
3	Ichra	Brown	Detected
4	Liberty Market	Super Karnal Basmati	Not detected
5	Liberty Market	Sella	Not detected
6	Liberty Market	Brown	Not detected
7	Qainchi	Super Basmati	Detected
8	Qainchi	Sella	Not detected
9	Qainchi	Brown	Detected
10	Bhatti Gate	Super Basmati	Not detected
11	Bhatti Gate	Sella	Not detected
12	Bhatti Gate	Brown	Detected
13	Chuburgi	Basmati	Detected
14	Chuburgi	Sella	Detected
15	Chuburgi	Brown	Not detected
16	Model Town	Basmati	Not detected
17	Model Town	Sella	Not detected
18	Model Town	Brown	Detected
19	Ghazi Chowk	Basmati	Not detected
20	Ghazi Chowk	Sella	Detected
21	Ghazi Chowk	Brown	Not detected
22	Bank Stop	Basmati	Not detected
23	Bank Stop	Sella	Detected
24	Bank Stop	Brown	Detected
25	Anarkali	Basmati	Not detected
26	Anarkali	Sella	Not detected
27	Anarkali	Brown	Not detected
28	Samanabad	Basmati	Not detected
29	Samanabad	Sella	Not detected
30	Samanabad	Brown	Not detected
31	Nishtar Colony	Basmati	Detected
32	Nishtar Colony	Sella	Not detected
33	Nishtar Colony	Brown	Detected
34	Shalmi	Basmati	Not detected
35	Shalmi	Sella	Not detected
36	Shalmi	Brown	Not detected
37	Town Ship	Basmati	Not detected
38	Town Ship	Sella	Not detected
39	Town Ship	Brown	Detected
40	Jail Road	Basmati	Not detected
41	Jail Road	Sella	Not detected
42	Jail Road	Brown	Detected
43	Abid Market	Basmati	Not detected
44	Abid Market	Sella	Detected
45	Abid Market	Brown	Detected
46	Johar Town	Basmati	Not detected
47	Johar Town	Sella	Not detected
48	Johar Town	Brown	Not detected
49	Mazang Chungi	Super Basmati	Detected
50	Mazang Chungi	Sella	Not detected
51	Mazang Chungi	Brown	Detected
52	Defence	Basmati	Not detected
53	Defence	Sella	Not detected
54	Defence	Brown	Not detected
55	Wapda Town	Basmati	Not detected
56	Wapda Town	Sella	Not detected
57	Wapda Town	Brown	Not detected
58	Youhanabad	Basmati	Not detected
59	Youhanabad	Sella	Not detected
60	Youhanabad	Brown	Detected
61	Garden Town	Basmati	Not detected
62	Garden Town	Sella	Not detected
63	Garden Town	Brown	Not detected
64	Faisal Town	Basmati	Not detected
65	Faisal Town	Sella	Not detected

Table 2. Positive rice samples were indicating the concentrations of Aflatoxin AFB1

S.No	Area	Rice Sample	Aflatoxins Concentration(ppb)
1	Ichra	Brown	7.9
2	Qainchi	Super Basmati	9.5
3	Qainchi	Brown	10.8
4	Bhatti Gate	Brown	17.2
5	Chuburgi	Basmati	13.5
6	Chuburgi	Sella	15.8
7	Model Town	Brown	9.1
8	Ghazi Chowk	Sella	8.8
9	Bank Stop	Sella	11.9
10	Bank Stop	Brown	9.4

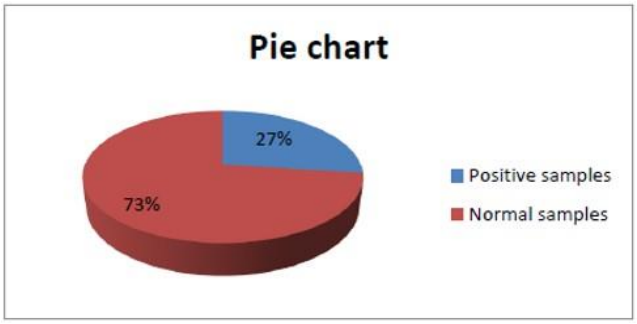

Fig. 5. Pie chart showing contamination of rice samples. In this 27% aflatoxin was found in total rice sample.

3.4.1. For Ichra Brown Rice Sample

$$Aflatoxins\ contents\ (mg/kg) = \frac{S*Y*V}{W*Z} = \frac{0.75\times2.01\times500}{10.1923\times9.25} = 7.99(ppb). \quad (3)$$

3.4.2. For Mazang Chungi Super Basmati Sample

$$\text{Aflatoxins contents (mg/kg)} = 2.0 \times 2.01 \times 500/10.1923 \times 9. = 21.3(\text{ppb}). \quad (4)$$

The minimum value is $7.99(\text{ppb})$ while maximum value is $21.3(\text{ppb})$ by calculations.

3.5. Percentage

The percentage of result can be easily determined by putting the values in the given formula of percentage:

$$Percentage = Value/Total\ Value \times 100 = 19/70 \times 100$$
$$\%\ of\ aflatoxin\ in\ total\ rice\ samples = 27\%$$

4. Discussion

Aflatoxins are amongst the most potent carcinogens, naturally occurring fungal toxic metabolites and cause momentous health risks and acute toxicological effects to human beings as well as animals. Aflatoxin in rice may harm the health to greater extent and if not properly determined; may cause death. Here in this study the aflatoxin in various rice samples was determined and it was found that due to improper storage conditions and many other factors the rice samples were contaminated with aflatoxins. The result shows that the study of presence of aflatoxin in various rice samples

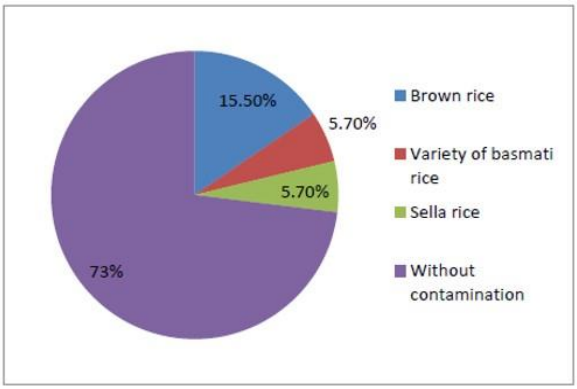

Fig. 6. Different samples with different percentages. In this 15.50% aflatoxin found in brown rice, 5.70% aflatoxin found in variety of basmati rice, 5.70% aflatoxin found in sella rice but 73% aflatoxin did not found in rice.

collected from different areas of Lahore. Some of them were positive samples and contaminated with aflatoxin B1 (Fig. 5). There were 70 samples collected from different areas consisted of Ichra, liberty market, Qainchi, Bhatti gate, Chuburgi, Ghazi chowk, model town, Anarkali, Samanabad, Nishtar colony, Shalmi, jail road, Abid Market, Township, Johar town, Mazangchungi, Defence, Wapda town, Garden town, Youhanabad and Faisal town. The samples of rice were basmati, super basmati, super Karnel basmati, sella and brown. Three samples of rice from each place collected and analysed by TLC technique and observed under UV light. Different limits of aflatoxin tolerance are set by European Union for different food products and commodities. The aflatoxin tolerance limit for rice is 4 ppb more than this limit it is not suitable for human consumption according to FDA. Our results in rice samples showed contamination ranging between 7.9-21.3 ppb. AFBI detected in rice samples that were contaminated and their tolerance limit exceed from the European standards. Detection range of aflatoxin in brown rice was between 7.9-17.2 ppb in 11 samples. The range for variety of basmati rice was between 8.2-21.3 ppb in about 4 samples and for sella rice the range was between 8.1-15.8 ppb. Total 19 rice samples were exceeding FDA approval and their range above than the permissible limit. The lowest amount of aflatoxin detected in brown rice (7.9 ppb) sample was collected from Ichra. The highest amount of aflatoxin detected in one of the variety of basmati rice (super basmati rice 21.3 ppb) from Mazang Chungi which was beyond the regulations set by FDA and WHO. WHO recommended limit for white rice is 2-4 ppb. However, there was no contamination and G2 was not detected in samples. There were no AFG2 in all samples of rice (brown, sella and broken) from the year of 2006-2010 as referred.[10] According to table (1) there were 70 samples of rice collected from different areas of Lahore and determined the aflatoxin from samples. 19 samples were contaminated with aflatoxins B1. According to table (2) the contamination present in brown samples n=11 (15.7%) their values were (7.9, 10.8, 17.2, 9.1, 19.4, 10.6, 16.7, 11.5, 9.6, 8.9 and 16.8) ppb. The contamination occurs in variety of basmati rice were n=4 (5.7%) their values were (9.5, 13.5, 8.2 and 21.3) ppb. The samples of sella rice contained n=4 (5.7). All of 19 samples were 27.1% their values were (15.8, 8.8, 11.9 and 8.1) ppb (Fig. 6). According to Fig. 2 the rice samples were visualized on TLC plate under UV light. In the plate sprayed with H_2SO_4 and dry it. After drying the more and better results found related to contaminated aflatoxin samples. According to (fig. 4) the standards were applied on the contaminated samples then placed it on TLC plate for the further clarification and the detection of aflatoxin in samples. Through that better results were found. It was observed that brown rice contained more contamination of aflatoxins with respect to other rice samples that were either be B1, B2, G1 and G2. White rice (basmati and sella) were less contaminated with aflatoxin in numbers. The contamination occurs due to problems during harvesting or in storage by fungus and its growth. Occurrence of toxic fungus in food commodities is not a healthy sign. Therefore, its reduction is necessary to minimize toxic effects on human health.

5. Conclusions

The present status of the aflatoxins (AFs) level in Pakistani brown rice presents a potential risk to the human health. The initial approach to control AFs is to take precaution and proper action such as better harvesting practices, handling, packaging, storage and as well as transportation. Our investigation demonstrates that rice market represents a significant source of exposure to aflatoxin. The presence of aflatoxins beyond limits has a serious bearing on the safety and ultimately can reduce the quality and marketability of rice. Mycotoxins are a major cause of deterioration and spoilage in stored crops. So it is necessary to monitor and control contamination of these commodities from aflatoxins in both domestic and international trade. The extra care should be employed both in post and pre harvest measures to reduce the risk of aflatoxin contamination. Contamination can originate in the field and increase dramatically during grain Storage. The Proper management of the crop, careful handling and proper storage of the grain are very critical in preventing aflatoxin contamination. It is recommended that the consumers buy rice from authentic retailers. Furthermore, it is advised that the items be stored in cool and dry conditions and any dirty, opened and damaged packaging must be rejected.

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Conflicts of Interest

The authors declare no conflict of interest.

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