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VARIATION IN AFLATOXIN CONTAMINATION OF RICE WITH RESPECT TO POST HARVEST CONDITIONS

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Abstract

Rice *(Oryza sativa*) is a precious cash crop which plays vital role in the wealth of Pakistan. In Pakistan, due to unawareness, lack of information, and mismanagement of staple foods like rice encouraged aflatoxin contamination and caused huge financial losses. Because it is the most compelling human carcinogen known compound therefore critically assessed before export to foreign countries. The current study was formulated to investigate the effect of humidity and temperature on total aflatoxin contamination of Pakistani rice (IRRI-6) variety at post harvesting stage condition. Total 120 samples were collected at different rice mills in Karachi and used to determine aflatoxin contamination through ELISA Kit method. Results revealed the highest (2.5 ug/kg) aflatoxin content observed in rice sampled in July while the lowest (1.4 ug/kg) content reported in rice stored in March. In both condition the value showed within the acceptable ranges of the standards of Pakistan (PSQCA) and United State (FDA and FAO) for food exports (MTL<20µg/kg). Similarly, it was lower than the maximum tolerated level (MTL, 4µg/kg) recommended by European Union for rice import in EU countries. Among the three variables grain moisture content showed positive correlation as compared to temperature and humidity. Therefore, it is concluded that the total aflatoxin level in IRRI-6 rice is minimum and ideal for human consumption however the grain moisture content must be less than 10-13%.

Key words: Aflatoxins, Food, Health, Carcinogen, Rice, ELISA.

Introduction

Food security and food safety are amongst the foremost challenges in the existing climate of expanding inhabitants worldwide. These are primarily governed by three major elements viz., (i) availability of sufficient food, (ii) gain access to protected food and (iii) nutritional and quality bases consumption for a healthful living (Anon., 1996). Moreover, to human health, the socio-economic part of the humanity severely influenced owing to the failure of any of the facets mentioned above through the rout of malnutrition and food insecurity. One of the important reasons liable for building food insecurity is the contamination of feed and food mycotoxins which affected 1/4th of the world's crop (Udomkun *et al*., 2017; Pankaj *et al*., 2018; Wu, 2007). Fusarium, Aspergillus and Penicillium, are the three fundamental genera of fungi responsible for mycotoxins production (Kumar *et al.,* 2008; Reddy *et al*., 2010). Including several kinds of mycotoxins, aflatoxins (AFs) are extremely lethal and are well known to pollute a wide range of foodstuffs such as rice, milk-based products, meat, dried fruits, groundnuts, and maize, (Iqbal *et al*., 2015; Perrone *et al*., 2014; Mutegi *et al*., 2009). According to the production aspects, the major AFs are made by the species of Aspergillus mainly *A. parasiticus*, *A. flavus,* and *A. nomius* and (Mishra & Das, 2003; Payne & Brown, 1998). As compared to some other species of *Aspergillus* these fungi mostly multiply in the humid and warm environments of subtropical and tropical territories (Battilani *et al*., 2011; Reiter *et al*., 2009; Magan, 2007). Owing to their resistant nature towards high temperature currently available food handling procedures are not adequate to eradicate the contamination of AFs from feed and food items (Medina *et al*., 2017).

The utilization of AFs infected food has posed critical health problems in animals and humans (Fung & Clark, 2004; Sherif *et al*., 2009; Binder *et al*., 2007). To sustain the health of humans and other animals, many countries have applied stringent guidelines for AFs (Juan *et al*., 2012). Usually, the safe range of AFs content in the edible food items for human are 4–30 µg/kg. According to Wu, (2006), the maximum tolerable limit for AFs has been set to 20 µg/kg in the United States of America (USA). However, the European Union applied strictest specification for any product intended for human consumption with total AFs and AFB1 level not further than 4 µg/kg and 2 µg/kg, respectively. Therefore, it is very important to explore the diversity of Pakistan rice towards AFs contamination. Because rice (*Oryza sativa* L.) is an essential food ingredient and an excellent basis of foreign exchange earnings therefore it possesses very critical position in national economy of Pakistan (Ali *et al*., 2018). Most of the farmer depend on rice farming which is their main basis of income and employment however the export value of rice heavily depends on the quality of as mentioned earlier. Therefore, protective measures should be taken during post-harvesting of rice to increase Pakistan's global competitiveness in rice trade. Because aflatoxin contamination is one of the major factors of sample rejection during rice export. The major risk factor of aflatoxin contamination is the storage systems of rice in Pakistan which have been found significantly correlated with aflatoxin contamination. Therefore, this study was designed to check the average aflatoxin contamination range in IRRI-6 of Pakistani rice cultivars and their correlation with temperature, humidity, and grain moisture content.

Material and Methods

Sample collection: Polished IRRI-6 rice samples collected from the various rice mills in located in Karachi, Pakistan. Samples were taken about 5000 pp bags-10,000 pp bags (each bag 25-50 kg) in lots shape. Approximately, 10 % bulk samples collected and well mixed and reduced about 2kg samples were brought to laboratory for aflatoxin analysis from each rice mills.

AF isolation: Appropriate amount (50 gm) of samples were blended with of 70% methanol (250 ml) in a high-speed blender for 1 minute. The extract (5 ml) was filtered by pouring through a Whatman filter and collected the filtrates samples for aflatoxin quantification.

AF quantification: Approximately, 100ul conjugate were added up to the red marked and mixed. Similarly, samples (100ul) and control (100ul) were added up to the red marked and mixed wells. The mixed (conjugate + control and conjugate + sample) were then transferred to the antibody containing well and incubated for 2 minutes followed by dumping the liquid from wells containing antibody. Washed wells thoroughly for 5 minutes with demonized water. Later the substrate (100ul) was transferred using 12 channel pipettor from reagent boat to antibody wells and incubated for 3 minutes. Transferred 100ul reaction stop reagents from reagent boat to antibody wells followed by reading the result using a micro well reader with a 650 nm filter.

Statistical analysis

The results were subjected to statistical analysis using excel software.

Results and Discussion

Distribution pattern of total aflatoxin contamination in local rice (IRRI-6) samples collected from various places (storage facilities) of Karachi monthly with varying conditions (average temperature, relative humidity, grain moisture content) given in the table (Table 1). According to the data analysis the highest (2.5 ug/kg) content of aflatoxin was reported in rice samples stored in the month of July while the lowest (1.4 ug/kg) content of AF contamination was found in rice samples collected in the month of March. Generally, the ratio of aflatoxin content was low in samples stored during the months of January, February, Mach, October, November, and December while the samples stored in the months of April, May, June, July, August, September showed high content (Figs. 2 and 3). But the average value of aflatoxin contaminated samples was found below than Food and Drug Authorities (FDA) of United State, Food and Agriculture (FAO) and Pakistan Standards and Quality Control Authority (PSQCA) set values (20ug/kg). The current study showed similar results as reported for AF contamination in Pakistan. According to Hussain *et al*, (2011), the range of AFs contamination in 70% of AF contaminated food (40 samples) were 3.7µg/kg to 4.9µg/kg. In another report from Pakistan, the aromatic rice showed low level of AF contamination (Muhammad *et al*., 2013). Similar study conducted using 599 samples of rice which showed the average concentration of AFB1 and AFB2, in white, brown, and sella rice were 0.49, 0.56, 0.73µg/kg and 0.03, 0.03, 0.02µg/kg, respectively. Comparative analysis manifested that the climatic conditions of March, July and August seems to be related to beneficial environmental requirements to control AF contamination in rice (Firdous *et al*., 2012). As mentioned above, 599 collected samples of brown, white and sella rice showed 0.02ug/kg to 16.65µg/kg concentrations of aflatoxin (Firdous *et al*., 2012). But the current study was designed to analyze approximately 1200 (total) rice samples from different storage conditions for aflatoxins contamination in IRRI-6 rice. The result analysis of internal as well as external growth of Aspergillus in stored rice grains manifested that *A. flavus* and *A. niger* were dominated in almost all the seed samples as reported earlier (Reddy, 2008). While aflatoxin contamination in rice was ranged between 0.5 to 3.5µg/kg which is the normal range of AF contaminated rice (Reddy *et al*., 2006, 2008 & 2009). According to the present results, it is concluded that the status of total aflatoxin contamination level in Pakistani IRRI-6 rice does not exceed the maximum level assigned by PSQCA and FDA. Among the three variables grain moisture content showed positive correlation as compared to temperature and humidity (Fig. 4). Therefore, it is concluded that the total aflatoxin level in IRRI-6 rice is minimum and ideal for human consumption however the grain moisture content must be less than 10-13%.

Table 1. Distribution pattern of total aflatoxin in IRRI-6 rice in accordance with storage conditions (temperature and humidity) and grain moisture content.

Months	Temperature (°C)	Humidity (%)	Grain moisture (%)	Aflatoxin (ug/kg)	Aflatoxin (ug/kg)	Aflatoxin (ug/kg)	Average	SD	SE
January	36.2	49.3	13.3	1.4	1.7	1.5	1.53	0.15	0.09
February	30.6	67.6	12.7	0.9	1.95	1.5	1.45	0.53	0.30
March	30.6	67.6	13.1	0.5	1.4	1.5	1.17	0.59	0.34
April	36.3	34.6	13.9	0.8	1.5	1.4	1.23	0.38	0.22
May	31.6	50	13.9	2.5	2.2	2.8	2.50	0.30	0.17
June	29.2	44.6	13.8	2.6	2.45	3.2	2.75	0.40	0.23
July	28.6	30.3	13.7	3	2.5	3.5	3.00	0.50	0.29
August	22.1	33.3	13.8	2.5	2.55	3.5	2.85	0.56	0.33
September	25.4	57.6	13.2	2.1	2.6	3.1	2.60	0.50	0.29
October	32.3	67.3	13.4	1.6	2.4	2.6	2.20	0.53	0.31
November	35.2	56.4	13.7	0.8	1.9	1.6	1.43	0.57	0.33
December	31.3	67.3	12.9	1.2	1.5	1.2	1.30	0.17	0.10

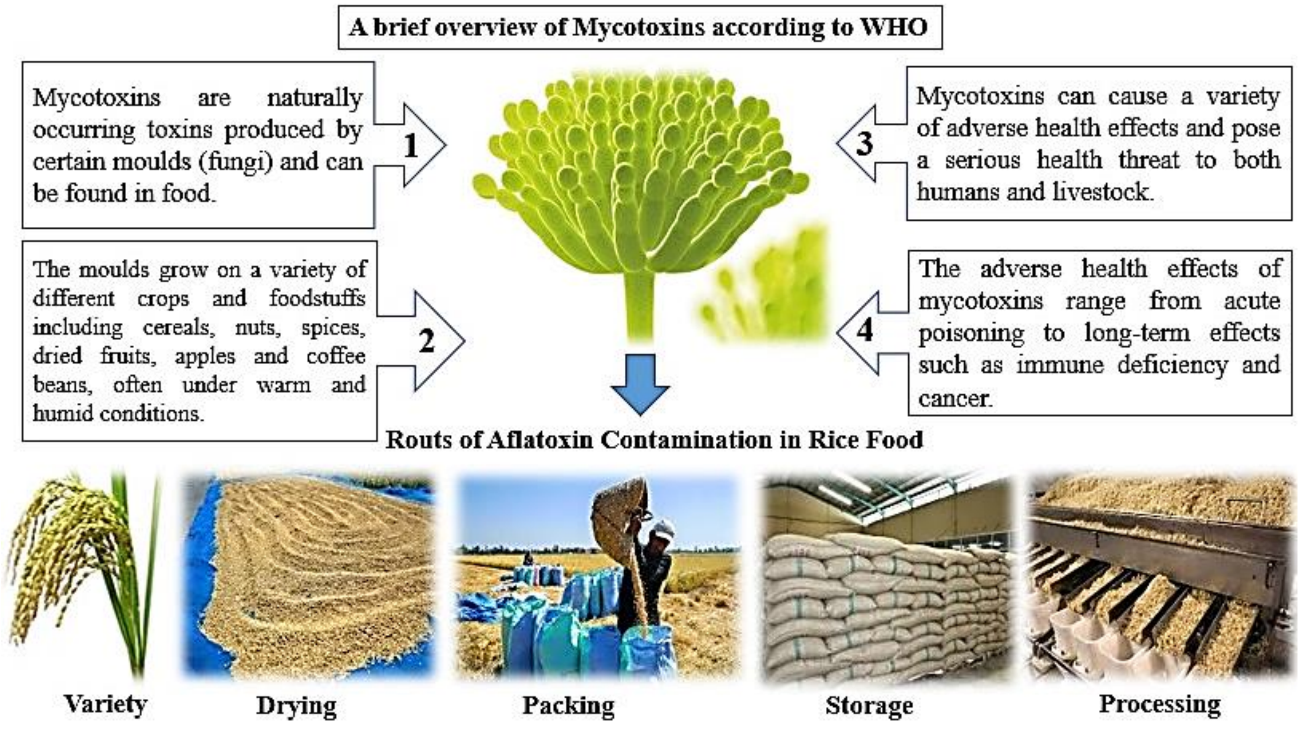

Fig. 1. An overview of mycotoxin and possible ways of aflatoxin contamination in rice food.

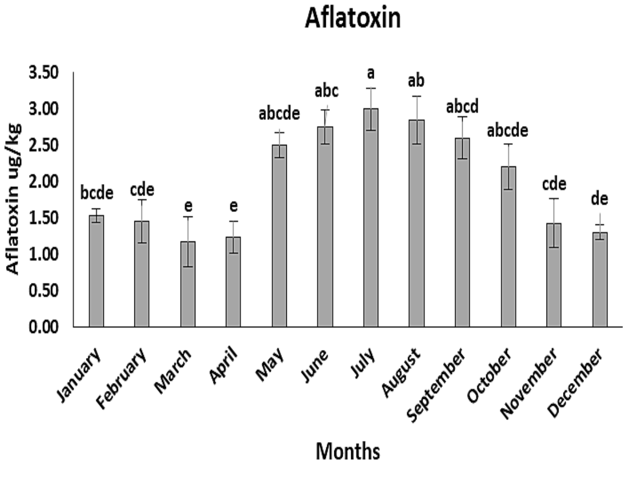

Fig. 2. Aflatoxin content of IRRI-6 rice samples collected from January to December at different rice mills Karachi, Pakistan.

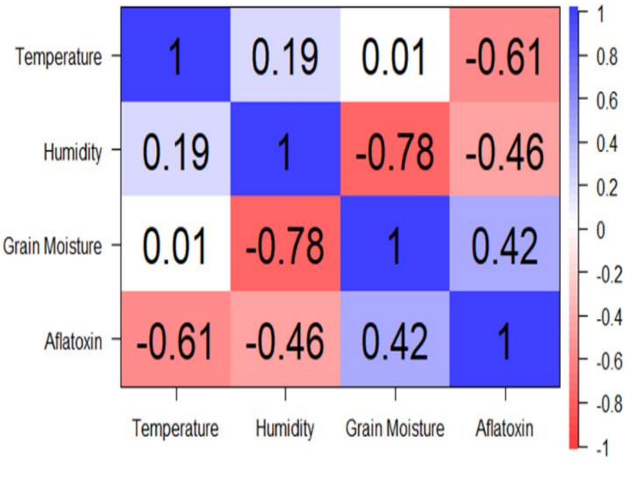

Fig. 4. Correction analysis among temperature, humidity, and grain moister effect on aflatoxin contamination.

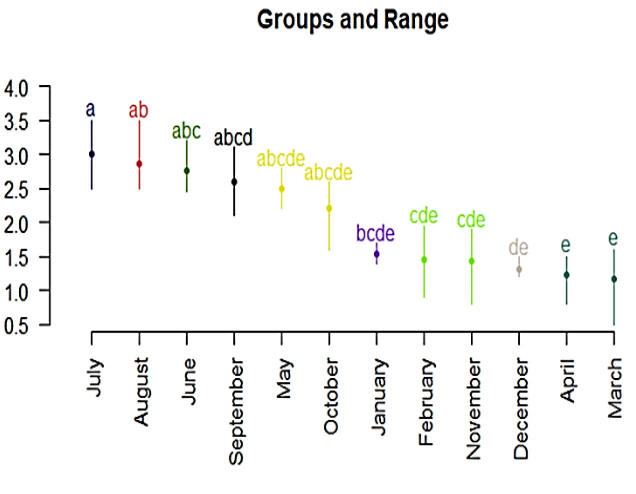

Fig. 3. Group ranges from highest to lowest values.

Rice is an important supply of food and an excellent resource for foreign exchange earnings, but Pakistan is experiencing huge economic especially at farm level losses owing to the unavailability of adequate storage facilities. During the last year, approximately 120 samples were analysed, they were obtained from different rice mills in Karachi, Pakistan. There is limited information and awareness about aflatoxin contamination in under developing countries like, Pakistan, India, and Africa etc. They don't analysis aflatoxin contamination for local consumption except exportable rice. It has been reported that most farmers and traders in Pakistan store grains like rice, maize, and wheat in polypropylene bags, which do not shield aflatoxin contamination in the stored grains (Desai & Ghosh, 2003; Anon., 2004).

Aflatoxin contamination chances are 100% in those grains which are stored under the verandah and stored or heaped on the floor (unshelled). The short overview of mycotoxin and the possible routes of entry of aflatoxins in rice food chain is presented in (Fig. 1). Among other methods, storage above fire racks showed maximum protection against aflatoxin contamination in cereal grains, however it is not feasible to storage large amounts of seeds. Therefore, outdoor storage facilities were adopted by some farmers to store maize, rice and wheat linked with silos and granaries which do not guarantee protection from insect infestation, molds infection and moisture pick-up. Similarly, the storage conditions in the retail market promotes environmental influence and cause AF contamination. Therefore, poor packaging procedures make it susceptible to infection by mycotoxin molds. In addition to rice, wheat, and shelled kernels, pounded/ milled flour of maize, showed highly contaminated by aflatoxin due to their improper storage at form level. There are numerus deaths reported due to aflatoxins contamination in food items (Moss, 1996; Kotsonis *et al.*, 2001). Therefore, most of the international buyers (clients) reject the import of rice from Pakistan which is a big barrier to penetrate in the international market. According to the Pakistan Standard and Quality Control Authority (PSQCA), and Food and Drug Association (FDA), the acceptable level for total aflatoxin in rice is 20ug/kg while European Union has decided 4ug/kg as the maximum level for total aflatoxin contamination. The present study was carried out to check the aflatoxin contamination in IRRI-6 and correlate with storage conditions.

Conclusion

Rice is an important cash crop for Pakistan however it can generate more foreign exchange if it is processed and stored properly. One of the major postharvest losses is food contamination with mycotoxins. Aflatoxin is most common mycotoxin therefore strictly monitored before export. IRRI-6 is the most common non-basmati rice in Pakistan which export to most of the foreign countries however it gets contamination during postharvest processing. The study revealed AF contamination variation in rice samples collected in different conditions however the contamination in IRRI-6 rice is in acceptable range. Although the collected samples were safe to export however proper storage facility is highly demanding to make it more profitable. The first factor before storage is the proper drying mechanism to reduce grain water content. Later the other factors including temperature and humidity should be strictly monitored during storage time.

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