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SIGNIFICANT REDUCTION OF AFLATOXIN LEVEL BY SOLAR IRRADIATION IN STORED WHEAT (*TRITICUM AESTIVUM L.*)

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ABSTRACT

Aflatoxin are the type of mycotoxin which are known to be highly carcinogenic and their presence in wheat lessen the quality of grain, during storage aflatoxin increase their growth due to improper storage and moisture control. The main objective of this study was to design a method that help to reduce level of aflatoxin from the wheat grain during storage. wheat exposed under sun was found to reduce aflatoxin level significantly. Solar irradiation causes aflatoxin reduction from wheat grain when exposed as opengrain or grain packed in the sack for specific time duration of 0, 5, 15 and 30 hours. The results revealed that aflatoxin level were decreases more under long length of time of exposure that is after 30 hours 75 -81 % reduction was observed in open grain and 50 – 63 % was in wheat grain packed in sack, reduction after 5 an 15 hours were noted 22-34% and 52 -64% respectively in open grain moreover in wheat grain that was exposed packed in sack showed reduction 6- 18% after 5 hours and 33-45% after 15 hours.Residues remain after solar irradiation treatment was recorded under maximum residue limit set by FDA (20ng/g), residues was noted to reduce more after 30 hour of exposure time (open grain 2.7 -5 and grain in sack 6 -11 ng/g). Natural sun light was found to be effective method in regard of aflatoxin decontamination and

provide safe wheat for food and feed purpose and helpful for the Pakistan to compete in the national and international demands.

Keywords: Wheat, solar, irradiation, aflatoxin, decontamination, residues

INTRODUCTION

Wheat (*Triticum aestivum L.*) is principle diet and on the 1st rank among all cereals in Pakistan (1), Over the past three decades increased wheat production occurred largely due to the deployment of high-yielding varieties and increased fertilizer use thatcontributing to the value added in agriculture and Gross domestic product that furnish the economy of Pakistan (2,3). Many methods has applied for improvement and quality control of wheat that muddle productivity (4) beside this 20% of wheat has lost every year (5). Wheat can be contaminated by various microscopic fungi during its development that can affect grain quality, chemical properties as well as its rheological characteristics (6). Contamination caused by the fungal secondary metabolite called 'Mycotoxin' that can grow due to environmental and ecological factors, during growth at field, after harvest, during transportation or during storage. Among mycotoxins aflatoxins are the naturally produced mycotoxin and is known to be most potent toxin and is highly carcinogenic placed in 1st group by International Agency for Research on Cancer (7). Due to aflatoxins 40% of the productivity lost in developing countries (8). Aflatoxins well grow under favourable temperature 13–40°C (optimum 30 °C) and humidity, even grains stored under high moisture/humidity or at warm temperatures and/or inapt dried can be at risk of aflatoxin colonization (9).These toxins effect human and animal health globally because these are mutagenic, teratogenic, carcinogenic and immunosuppressive and are responsible for deterioration of agricultural products (7,10,11).

These toxins are environmentally stable as resistant towards thermal changes and many processing (12). Decontamination of aflatoxin at pre harvest level is quite impossible therefor many efforts has been done at the postharvest stage particularly during storage, various methods have been reported by many scientists for the decontamination of mycotoxins that are chemical, physical and biological (13,14). In present study natural, simple and cost effective method as well as the method that ensure to retain the nutritive value have been employed for the decontamination purpose and can be easily applied for the bulk quantity of wheat during storage at godowns the common wheat store houses.

MATERIALS AND METHODS

Wheat sampling

Sampling was done from all government godowns of Hyderabad division (Bolhari, Hali road, Fatah chowk, Hala city, Matiyaari, Thatta, Tandpallhyaar, Dadu, K.N shah, Sehwan and Aarazi), during the visits temperature and humidity of all godowns were noted i-e 37-52°C and 37-52% respectively. Sampling was done by selecting one specific stack of the wheat sacks and were taken from the upper, central and bottom sack.

Mycological Study

Mycoflora was determine by the Agar plate method described by Mathur *et al.*(2003) (15) and relative isolation frequency (Fq.) of *Aspergillus parasiticus and Aspargillus flavus* (aflatoxin producing fungi)was calculated by the method described by Fatma Bensassi *et al.*,2011 (16).

Decontamination method

In present study the solar radiation was used for the aflatoxin level reduction from stored wheat. Natural Sun light is consisting of different rays of different wavelengths that has been used as germicidal and for drying purpose of various products from many centuries. Keeping this concept in vision the study was design, that would be helpful in maximizing the storage duration of wheat and reducing the moisture content and stop aflatoxin colonization and decrease aflatoxin level as well.Treatment of wheat under sun light was time dependent that is the wheat was exposed under open sun light for 0, 5, 15 and 30 hours. Wheat was exposed under sun as open grain and also the grain was exposed as packed in sack (common packaging material used at godowns for wheat storage) for the same proposed time duration.

Sample Preparation

After irradiation wheat samples was ground and 25ml of 70% methanol was added and shake for three minutes by hand or mechanical shaker then filter through whatman no.1 filter paper and filtrate was collected and saved for the analysis.

Sample analysis

Collected filtrate was used for the analysis by Enzyme linked immunosorbent assay (ELISA) technique and commercially immunoassay kit i-e Neogen ELISA Kit (Veratox , Product no. 8030) was used, the kit was based on competitive direct enzyme linked immunosorbent assay format. Concentration was calculated by ELISA 'state fax 2100' (Awareness technology).

Effect on crude Protein and Crude fat

Protein and fat contents was analysed according to standard method of AACC, 2000 (17).

Data Analysis

All the experiments had three replicates. Data was analyzed for one-way analysis of variance followed by Student-Newman-Keuls multiple test at 0.05 level using compare means procedure of SPSS 16 (18).

RESULTS AND DISCUSSION

Effect on reduction of aflatoxin under sun light described in table 1 and results revealed that reduction percentage was increased with the increase in exposure time after 30 hour, grain showed higher rate of decontamination of aflatoxin in both open grain (73 to 82%) and wheat grain packed in sacks (50 to 63%), whereas the aflatoxin reduction of open grain exposed for 5 and 15 hours recorded 21-34%, 52 - 68% respectively and thereduction in packed grain was noted 6-18% after 5 hour exposure and 33-45% after 15 hour of solar irradiation.

Figure 1 and 2 showing positive correlation among exposure time and reduction of aflatoxin level. The decontamination rate increases with increase in exposure time therefore the samples exposed for 30 hours under open sun rays was showed more reduction in aflatoxin percentage in open grain and same results was noted for the wheat grain packed in sack.

Data in table 2 shows the aflatoxin residues remaining after solar irradiation, residues was observed within the permissible range of aflatoxin (20 ng/g) set by FDA.After 5 hours exposure residues become under the permissible ranges in open grain (10-17ng/g) and grain packed in sack (10-20ng/g) and the 30 hours sun exposure shows more residues reduction in open grain and grain in sack i-e 2.7 -5 and 6 -11 ng/g respectively.

Wheat are known to be the rich source of protein and protein are sensitive against irradiation and become denatured under long exposure, due to aflatoxin attack proteins was noted altered from their standard value and found average 11.9%, and fat was also analyse due to wheat exposure under open environment to observe lipid peroxidation. Figure 3 and 4 shows the protein and fat content before and after solar treatment and No effect was found in protein and on fat after the solar treatment.

Table 1: Reduction percentage (%) of Aflatoxin after Solar Irradiation

Godown	Hour (0)	Hours (5)	Hours (15)	Hours (30)
Open Grain				
Bolhari	0	22[ab]	54[a]	81[a]
Hali road	0	26[ab]	52[a]	73[a]
Fatah Chowk	0	23[ab]	55[a]	77[a]
Hala city	0	27[ab]	61[a]	83[a]
Matiyaari	0	26[ab]	60[a]	80[a]
Thatta	0	25[ab]	62[a]	75[a]
Tando allahyaar	0	23[ab]	61[a]	79[a]
Dadu	0	27[ab]	54[a]	77[a]

K.N Shah	0	21[a]	52 [a]	82 [a]
Sehwan	0	34[b]	65 [a]	82 [a]
Aarazi	0	31 [ab]	68 [a]	81 [a]
F-Statistics at df = 32		2.2	1.3	1.9
Grain in Sack				
Bolhari	0	13 [a]	40[ab]	63 [a]
Hali road	0	15 [a]	42 [ab]	57 [a]
Fatah Chowk	0	11 [a]	38 [ab]	50 [a]
Hala city	0	16 [a]	44 [ab]	55 [a]
Matiyaari	0	13 [a]	33[a]	60 [a]
Thatta	0	12 [a]	43[b]	56 [a]
Tando allahyaar	0	6 [a]	38 [ab]	61 [a]
Dadu	0	12 [a]	36 [ab]	59 [a]
K.N Shah	0	13 [a]	45[b]	60 [a]
Sehwan	0	15 [a]	43 [ab]	52 [a]
Aarazi	0	18 [a]	45 [ab]	63 [a]
F-Statistics at df = 32		0.7	2.3	1.6

*Values followed by the same letter are not significantly different at 0.05 level Student-Newman-Keuls test.

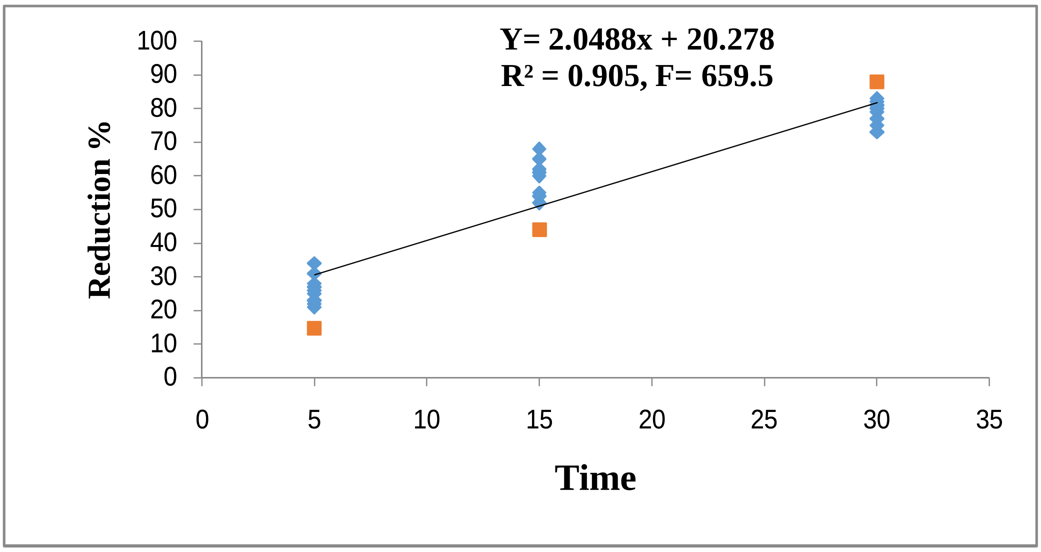

Figure 1: Regression curve of Aflatoxin level reduction (%) in relation withsolar exposure time of open wheat grain

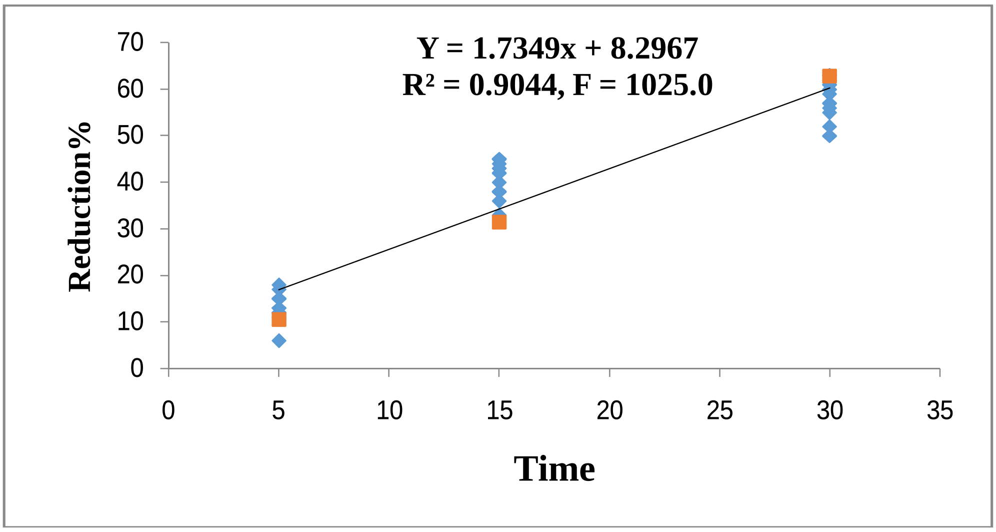

Figure 2: Regression curve of Aflatoxin level reduction (%) in relation withSolar exposure time of wheat grain packed in sack

Table 2: Residues remaining after Solar Irradiation (ng/g)

Godowm	Hour (0)	Hours (5)	Hours (15)	Hours (30)
Open Grain				
Bolhari1	22^{bcd}	17^{bc}	10^{bc}	4^{a}
Hali road2	19^{abcd}	14^{ac}	9^{abc}	5^{a}
Fatah Chowk3	18^{abc}	13.8^{abc}	8^{abc}	4^{a}
Hala city4	18^{ab}	13^{ab}	7^{ab}	3^{a}
Matiyaari5	15^{a}	11^{a}	6^{a}	3^{a}
Thatta6	16^{a}	12^{a}	6^{a}	4^{a}
Tando allahyaar7	13^{a}	10^{ab}	5^{ab}	2.7^{a}
Dadu8	22^{cd}	16^{c}	10^{c}	5^{a}
K.N Shah9	23^{cd}	18^{c}	11^{b}	4^{a}
Sehwan10	23^{cd}	15^{abc}	8^{abc}	4^{a}
Aarazi11	22^{cd}	15^{bc}	7^{abc}	4^{a}
F-Statistics at df = 32	8.3	5.8	4.5	1.9
Grain in sack				
Bolhari	22^{a}	19^{a}	13^{a}	8^{a}
Hali road	19^{a}	16^{a}	11^{a}	8^{a}
Fatah Chowk	18^{a}	16^{a}	11^{a}	9^{a}
Hala city	18^{a}	15^{a}	10^{a}	8^{a}
Matiyaari	15^{a}	13^{a}	10^{a}	6^{a}
Thatta	16^{a}	14^{a}	9^{a}	7^{a}
Tando allahyaar	13^{a}	12.12^{a}	8^{a}	5^{a}
Dadu	22^{a}	19.2^{a}	14^{a}	9^{a}
K.N Shah	23^{a}	20^{a}	12.5^{a}	9^{a}
Sehwan	23^{a}	19.5	13^{a}	11^{a}
Aarazi	22^{a}	18	12^{a}	8^{a}
F-Statistics at df = 32	2.0	2.0	2.1	1.2

*Values followed by the same letter are not significantly different at 0.05 level Student-Newman-Keuls test.

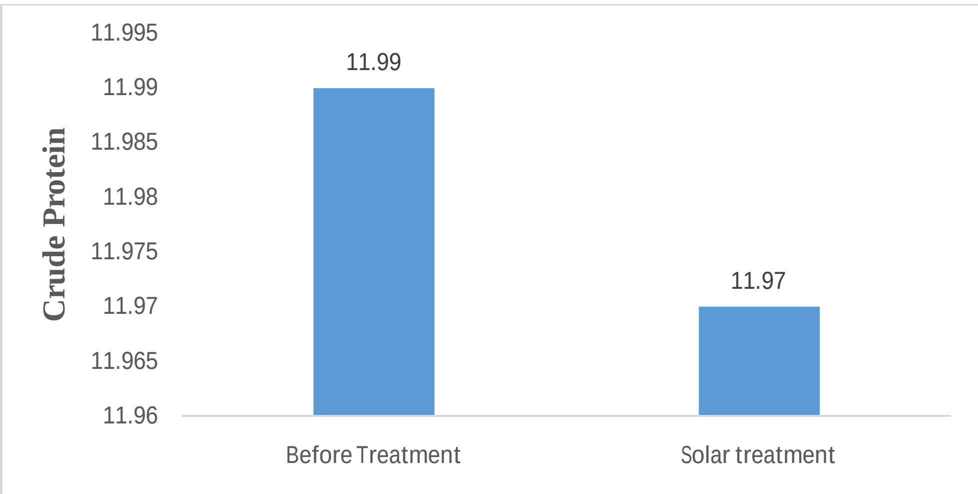

Figure 3: Crude protein after solar irradiation solar treatment

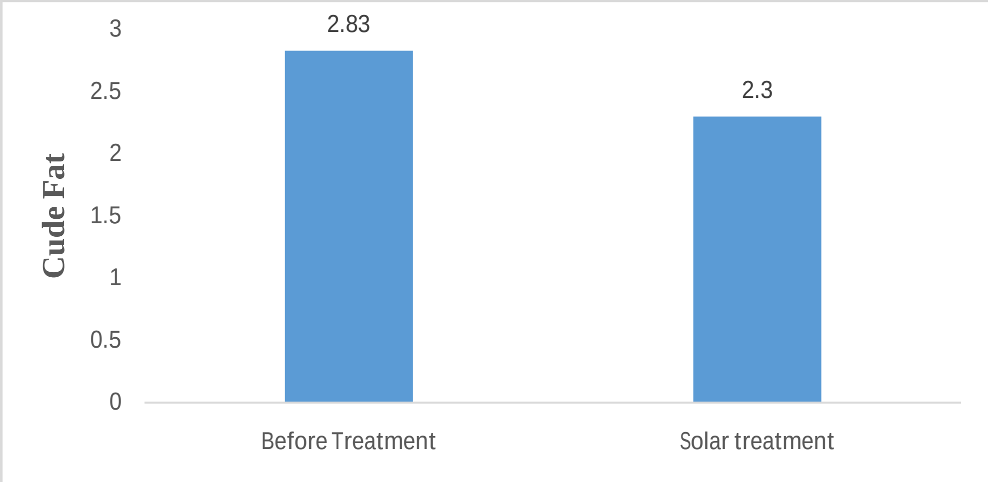

Figure 4: Crude fat after solar irradiation solar treatment

DISCUSSION

Aflatoxin B1 and total aflatoxin were decreased in feed when exposed under direct sunlight for the 3 and 30 hours exposure time and more than 60% reduction was noted in samples with long exposure length i-e 30 hours (19), the results are in consistent with sumbal *et al.* (2015) found 70 to 95 ug/kg of ochratoxin was reduce in poultry feed after 8 hours of sunlight exposure(20). Aflatoxin B1 were significantly reduce upto 75% under 10 mints sun light exposure from crude ground nut oil (Shantha and Sreenivasa Murthy, 1977), Shantha *et al.*(1986) detected 77 and 90 % aflatoxin B were destroyed under sunlight (21, 22). Previously reported results also indicate that the solar radiation could not completely destroy aflatoxin because penetration power into aflatoxin containing particle is limiting factor in decontamination process (Basappa & Sreenivasa Murthy, 1977; Shantha & Sreenivasa Murthy, 1988) (23, 24). Results are also supported by Samarjeewa *et al.*,1990 (25).

Sunlight is found to be effective in elimination of many contaminates and microbes supported by many researchers, Amvrazi, (2010) reported the Photodegradation effect by ultraviolet radiations of sunlight and elaborated that all contaminates are susceptible to photo degradation to some degree(26).Sun drying of peeled onions reduced residues up to 90.88% of Endosulfan and 94.56% 0f Profenofos pesticides which are known as potent hazardous pesticides if its residus above MRLs remain in food. (27)

In a laboratory investigation, the effect of natural sunlight and UV light exposure on dissipation of fipronil insecticide from two soils (clay loam and sandy clay loam) and the effect of pH on the persistence of fipronil in aqueous medium were studied. Dissipation of fipronil insecticide under sunlight followed biphasic first order kinetics in both soils. The half-life of the insecticide in sandy clay loam type soil was found to be 5.71 days for the first faster phase and 23.88 days for the second slower phase, whereas, in clay loam soil, the corresponding half-lives were 4.02 and 8.38 days, respectively (28).

Growth of natural populations of planktonic marine bacteria was observed upon exposure to natural sun light In Narragansett Bay seawater with most of the phototrophic and bacteria grazing nanoflagellates removed by filtration, a slight increase in bacterial populations began after 24 to 36 h in the dark, in populations exposed to sunlight, growth was delayed further Diluting the bacteria-dominated size fraction (< 0 6 pm) with 0 2 pm filtered seawater resulted in a shorter lag period and more rapid growth than undiluted filtrates Sunlight caused a significant lengthening of the lag period but did not affect the exponential growth rate suggesting that the bacteria were susceptible to sunlight. (29). Exposure time showed direct proportionality with aflatoxin reduction in present work and show correlation with previous reported results that photodegradation of aflatoxins was found to increase with increased duration of exposure time (19).

Results of present study suggested that the aflatoxin residues were in permissible level and are consistent with Taheri *et al.* (2012) reported that summer and winter wheat was contaminated with aflatoxin but the concentration was recorded within permissible level [30]. Out of 83 samples of wheat contaminated with Aflatoxin B1 only 1 sample was exceeding MRLs that is 25.6 µg/Kg (31) same results were also reported by Trombete *et al.,* 2014 (32)

Protein and fat concentration were not altered after solar treatment, results are in agreement with previously reported data that Concentration of proteins in samples after contaminatio with *A. Flavus* were noted 12.04% (6). It was observed from the results that no effect was found on protein after radiation treatment. Garg *et al.* (2013) reported no effect were seen in the nutritional values of peanuts when treated with UV irradiation for decontamination of aflatoxin (33).

CONCLUSION

It was sum up from the present study that aflatoxin level reduction rate increase with increase in exposure time and residues were noted in the range of permissible level. More aflatoxin reduction was observed in wheat after 30 hours exposure in both open grain and in wheat grain packed in sack as well therefore wheat can be protected from the contamination by exposing the wheat to direct sun light at the godowns which reduces the moisture content of grain and provide conditions unsuitable for growth of aflatoxin producing fungi and wheat can be stored for maximum period of time with lowest contamination ratio.

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