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RESEARCH ARTICLE

Anti-Toxigenic Effect of Lactic Acid Bacteria Against *Aspergillus spp* Isolated from Wheat Grains

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Abstract:

Introduction:

Many fungi infect the wheat grains. Under field and or storage conditions from temperature and humidity, some fungi can produce aflatoxins (AFs), which may cause acute or chronic diseases. Therefore, there is a necessary and urgent need to find an effective and safe way to reduce or remove AFs.

Objective:

The objective of this study was the evaluation of *Lactobacillus rhamnosus*, *Lactobacillus gasseri,* and *Lactobacillus plantarum* for their ability to reduce and or remove AFs produced by *Aspergillus flavus* and *Aspergillus parasiticus,* which were isolated from wheat grains, as well as control of AFs produced on affected wheat grain by *A.parasiticus* spores only.

Methods:

LAB, isolated from some local dairy products, were cultured in MRS for the evaluation of their ability to remove AFs, produced by *A. flavus* and *A. parasiticus* on (YES) media, in addition to the treatment of wheat grains by LAB cells to prevent AFs produced by *A. parasiticus*.

Results:

The *L. rhamnosus* strain gave the highest reduction rates of AFs produced by *A. parasiticus* that were 62.6, 44.4, 43.3, and 52.2% for AFG_1, AFB_1, AFG_2, and AFB_2, respectively. While in the case of *A. flavus,* the reduction was 50.4, 42.7, 40.6, and 36.8% in the same order of toxins. When applied, these strains with wheat grains were affected by *A. parasiticus*, the inhibition rates of AFs were ranged between 61.4 and 75.8% with *L. rhamnosus* strain and 43.7 to 52.1% with *L. gasseri*, while *L. plantarum* strain ranged from 55.5 to 66.9%.

Conclusion:

According to this study, *L. rhamnosus* is considered one of the best strains in this field. Therefore, the present study suggests applied use of LAB as a treatment to prevent AFs production in wheat grains.

Keywords: Lactic acid bacteria (LAB), Aflatoxins (AFs), Reduction and wheat, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus plantarum*.

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1. INTRODUCTION

Aflatoxins (AFs) are a group of secondary metabolites produced by *Aspergillus flavus, A.parasiticus*, A. *nomius*, A. *bombycis*, and A. *pseudotamarii* [1, 2]. High temperature and high relative humidity are conducive for the growth of fungi producing AFs on stored grains. The incidence of AFs in foods and feeds is relatively high in the tropical and sub-tropical regions due to the warm and humid weather conditions that provide ideal conditions for the growth of the aflatoxigenic molds. The four major naturally produced AFs are known as aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). AFs have many bad effects on humans and animals. If ingested, AFs may cause acute or chronic diseases, such as carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, nephrotoxic, hepatotoxic, neurotoxic,

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as well as immunosuppressive effects [3 - 5]. Numerous studies around the world indicated the contamination of most cereal products, with one or more types of AFs, inducing large economic losses [6, 7]. Once the contamination has occurred by AFs, many methods must be established and applied for controlling or reducing AFs for the prevention and detoxification in order to preserve the safety of products intended for human consumption. Many microorganisms, including bacteria, yeasts, and molds, are capable of reducing toxins in foods and feeds, therefore, many studies used lactic acid bacteria (LAB) to bind toxins in *vitro* and in *vivo* [8 - 10].Therefore, this study aimed for the isolation and identification of fungi from wheat grains then investigated the ability of three strains from LAB (*L. rhamnosus, L. gasseri and L.plantarum*) isolated from some local dairy products for the reduction of AF s produced by *A. parasiticus* and *A. flavus,* which were isolated from wheat grains, in addition to the treatment of wheat grains by these strains for inhibition AFs produced by *A. parasiticus*.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Wheat Grain Samples

Fifty (50) samples for wheat grains were collected from local markets of Cairo and Giza. Samples were subjected to mycological examination.

2.1.2. Lactic Acid Bacteria Strains

In this study, three strains from LAB (*Lactobacillus rhamnosus, Lactobacillus gasseri and Lactobacillus plantarum*) were used which were isolated from some local dairy products. These strains were identified according to their morphological and cultural characteristics [11] and using -50CHLAPI- identification system (BioMerieux). [12].

2.2. Methods

2.2.1. Isolation of Fungi from Wheat Grains

Potato dextrose agar (PDA) medium was used for the isolation of fungi according to the method described by Pitt and Hocking [11] for un-sterilized wheat grain, while sterilized wheat grain, with the help of sterile forceps, were surface sterilized for 5 min in 1% sodium hypochlorite, then rinsed three times with sterilized distilled water. These grains were dried on sterilized filter paper.

2.2.2. Purification and Identification of Fungal Isolates

All the isolated fungi, as well as the microsocial structures on PDA medium, were identified by studying the cultural characteristics, according to previous studies [13, 14]. The frequency of fungi and relative percentage of particular species within a genus of fungi was calculated using the formula of Ghiasian *et al*. [15].

$$\text{Frequency (\%)} = \frac{\text{Number of samples infected with fungi}}{\text{Total Number of samples analysis}} \text{X100}$$

$$\text{Relative percentage (\%)} = \frac{\text{Number of fungal species isolated}}{\text{Total Number of Fungi}} \text{X100}$$

2.2.3. Determination of Toxicity for Some Isolated Fungi

The isolated *Aspergillus* spp. (*A. parasiticus* and *A. flavus*) were tested for their ability to produce AFs by cultivation on Yeast Extract Sucrose Broth (YESB) broth for 8 days at 28˚C, according to Frisvad *et al* [16].

2.2.4. Preparation of Bacteria Culture Cells

The isolates of LAB were propagated overnight (16 h) in 100 overnight (16 h) in 100 ml MRS broth (pH 6.8).A cell free solution was obtained by centrifugation of culture at 5000 rpm at 4°C 20 min, followed by filtration of supernatant through a sterilized filter (0.2 µm, Millipore), to take all cells.

2.2.5. Effect of LAB Cells on AFs Production

A loopful of LAB from the Man-Rogosa-Sharpe medium was separately incubated in tryptic soy broth at 37 °C for 18 h. Then, one mL of culture medium (density equivalent to 0.5 MacFarland standard) was added to a 100 mL Erlenmeyer flask containing 25 mL of yeast extract sucrose broth with 10^5 spores/mL each for *A. parasiticus* and *A. flavus*. Mycelial mass and AFs production rates were measured according to the method described previously [17]. After incubation, a medium containing LAB and fungus was filtered, then mycelia weight was measured, as AFs were extracted from filtrates according to the method described previously [18, 19], where extraction was carried out using 20 mL of chloroform (with 20 ml filtrate), and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through Whatman filter paper with sodium sulphate anhydrous and concentrated to dryness using a hot plate then determined using HPLC.

2.2.6. Preparation of Treated Wheat Grains Samples

Autoclaved wheat grains were used in the following seven experimental groups (100gin each): (1) Positive control: wheat grains inoculated with a spore suspension of *A. parasiticus* were prepared according to Sharma *et al.* [20]. Groups 2, 3, and 4 negative controls: wheat grains inoculated with 2.5mL for each of *L. rhamnosus*, *L. gasseri,* and *L. plantarum*, respectively; groups 5, 6, and 7 wheat grains infected by *A.parasiticus* spore suspension plus *L. rhamnosus*, *L. gasseri,* and *L. plantarum*, respectively. All the inoculations were performed by the sprinkling of grains with microbial suspensions, followed by incubation for seven days at 28 ºC [21]. On the other hand, the quantity of AFs in wheat grains samples was measured by using the Immun affinity column (Aflatest®-P affinity column) for extraction as well as HPLC for the determination of AFs concentrations [22].

2.2.7. Statistical Analyses

All data were statistically analyzed using the General Linear Model procedure of the SPSS ver. 18 (IBM Corp, NY). The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio. All statements of significance were based on the probability of P-value <0.05, which was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Isolation and Identification of Fungi

The results recorded in Table **1** revealed that the fungi-contaminated wheat grains samples were grown on PDA disinfected and non-disinfected grains. The Total Fungi Count (TFC) isolated were 382 and 312 isolates in non-sterilized and sterilized wheat grain, respectively. Also, data showed that the most isolated fungal species were identified as *Aspergillus, Pencillium, Fusarium,* and *Alternaria*.

The frequency of isolated fungi from non – sterilized and sterilized wheat grain samples shown in Fig. (**1**) revealed that the highly frequent species were *A. flavus* (88%) and *A.parasiticus* (72%) as well as *A. niger* (74%) in non–sterilized wheat grains. On the other hand, the results indicated that the highly frequent species in sterilized wheat grains samples were *A. ochraceous* (32%) and A. *parasiticus* (30%). These results are in agreement with previous studies [12, 23]. This is mostly related to the physiology of fungi and their adaptation to the different matrices and environmental conditions [24]. Fungal spoilage is caused by two factors: (biotic) living, which include insects, birds, rodents, and microorganisms, and (non-biotic), non-living, which include temperature, humidity, and time [25]. Deterioration of grain is mainly affected by moisture content, temperature, relative humidity, storage conditions, fungal growth, and insect pests. In grain, the occurrence of fungi, especially *A. flavus* and *Fusarium spp*., facilitated by hot and humid conditions, poses a risk through the production of mycotoxins. In order to maintain high quality maize for both short- and long-term storage, wheat must be protected from weather, growth of microorganisms, and pests [26].

In Egypt, the weather gives a chance for the growth of *Aspergillus* species and other fungi on grains as it is characterized by high temperature and high relative humidity. Also, contamination occurs through small amounts of spores contaminating the grain as it goes into storage from the harvest in handling and storage equipment or from spores already present in storage structures. However, wheat grains were cultivated in the winter season (November /December) and harvested in the summer, which enhanced the chances of pre-harvest contamination. Also, the farmers have used old traditional farming practices, which can enhance fungal infections [27].

3.2. Screening of AFs Production by Some Isolated Strains

In this study, the isolates of *A. flavus* and *A.parasiticus* from surface-sterilized wheat grains were investigated to produce of AFs. The results in (Table **2**) clarify that 6 and 8 strains of *A. flavus* and *A.parasiticus* were producers for AFs, respectively.

Table 1. Fungal contamination in non -sterilized and sterilized wheat grain (n=50).

Name of the Fungi	Non –sterilized Wheat Grain			Sterilized Wheat Grains		
	No. of Samples Infected	No. of Isolates	R.P (%)	No. of Samples Infected	No. of Isolates	R.P (%)
A.flavus	44	98	25.6	8	14	10.1
A.parasiticus	36	56	14.6	15	22	15.8
A.niger	37	77	20.1	11	25	17.9
A.ochraceous	12	14	3.7	16	16	11.5
Alternaria	38	44	11.5	14	28	20.1
Pencillium spp.	33	55	14.4	13	13	9.3
Fusarium spp.	29	38	9.9	9	21	15.1
TFC count/5seeds	**382**			**139**		

R.P= relative percentage; **Relative percentage (%) = (Number of fungal species isolated / Total Number of fungi isolated) x 100.**

Table 2. Toxicity of *A. flavus* and *A. parasiticus* isolated from wheat grains samples.

Type of Fungal	Numbers	Concentration of AFs (ng/100ml)							
		AFG_1		AFB_1		AFG_2		AFB_2	
		Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
A. flavus	14(6)*	35.15	8.31	75.23	21.77	24.19	13.8	32.15	6.23
A. parasiticus	22(8)*	37.38	6.4	57.42	8.21	33.1	11.22	20.8	7.77

***Number of +ve samples.**

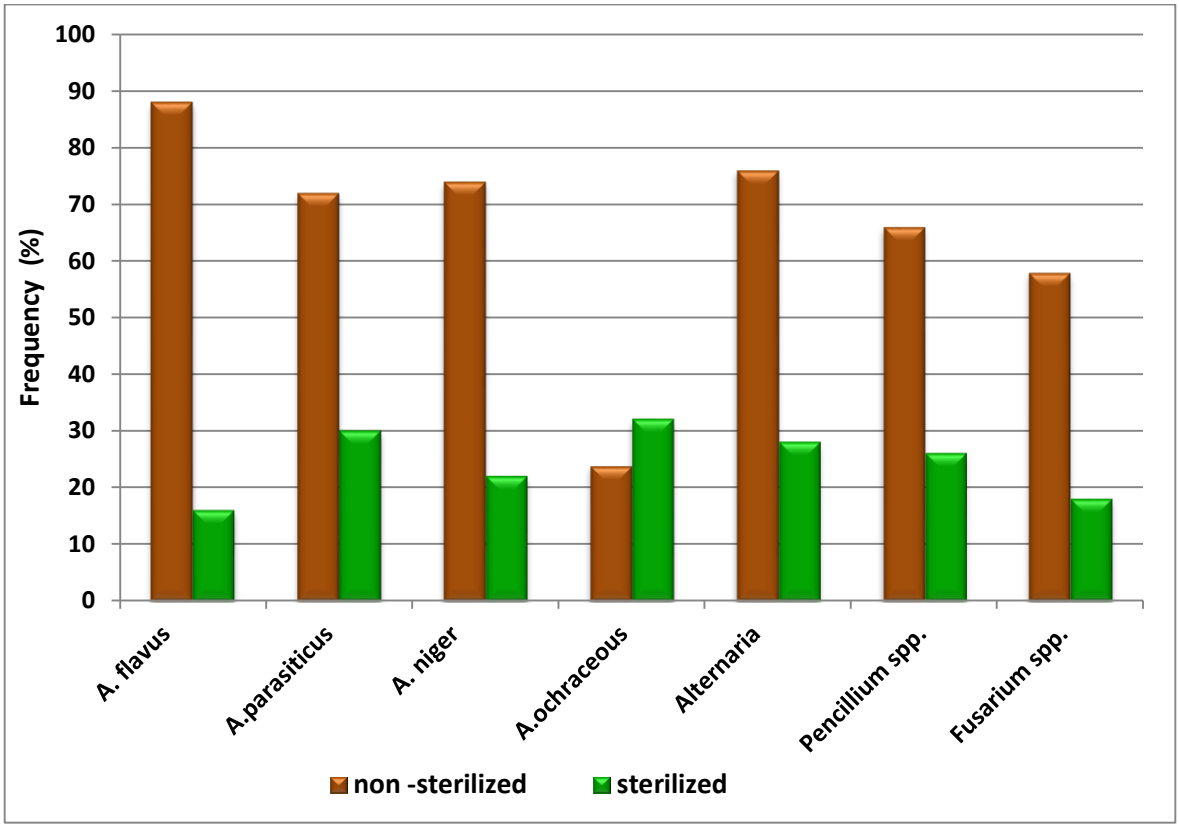

Fig (1). The frequency for fungi in non-sterilized and sterilized wheat samples.

The production of AFs by *A. flavus* and *A.parasiticus* depends on the possession of AFs genes. The isolates varied widely due to the prescience of seven toxigenic gene (aflR, aflS, aflQ, aflP, aflD, aflM, and aflO). The isolates of toxigenic *A.flavus* possessed at least 5 (out of 7) of these genes that were not detected in most of the isolates, as there were variations in AFs type [28]. In *A. flavus* and *A. parasiticus*, AFs pathway genes are clustered within a 75-kb region of the fungal genome on chromosome III [29, 30]. AFs biosynthesis is coded by an 80 kb long DNA sequence, as a cluster containing 30 putative genes characterized in both *A. flavus* and *A. parasiticus*.The variations in Toxin Type among aflatoxigenic fungi this due to the metabolic behavior of strains according to the molecular genetics and phylogenetic relationships. *A. flavus* differs from *A. parasiticus* by loss of a portion of the gene, aflU (cypA), involved in G type AFs production as well as *A. flavus* and *A. parasiticus* group forms a polyphyletic assemblage containing isolates of different morph types and having the ability to produce AFs [31].

3.3. Control of AFs Produced in Liquid Media by Addition Some LAB Strains

In this study, three strains from LAB were used to control the AFs produced on Yeast extract sucrose (YES) media by *A. flavus* and *A. parasiticus*. The ability of LAB strains to reduce AFs produced by *A. parasiticus* ranged from 20% to 62.6%. The results in Fig. (**2**) indicated that the highest rate of reduction of AFs were with *L. rhamnosus* strain that removed AFG_1, AFB_1, AFG_2, and AFB_2 up to 62.6, 44.4, 43.3, and 52.2%, respectively. While in the case of *L. gasser,* the strain rate of reduction of toxins was decreased to 29.1, 25.0, 20.6, and 26.03% with AFG_1, AFB_1, AFG_2, and AFB_2, respectively. However, *L. plantarum* strain reduction was 42.5% and 31.5% for AFG_1 and AFG_2, respectively.

The data in Fig. (**3**) showed the impact of LAB on AFs produced by *A.flavus* isolated from wheat grains. When treated with the YES media by *Lrhamnosus,* AFG_1 and AFG_2 reduced to 50.4 and 40.6%, respectively. The production of AFB_1 and AFB_2 decreased to 42.7 and 36.8%, respectively. The highest rate reduced with *L. plantarum* was 34.2% with AFG_1. The results showed that *L.gasseri* has the ability to degrade AFs contents in medium ranging from 17.9 to 23.6%. These LAB strains used in this study have a significantly higher reduction on AFs when used in liquid media. Also, there were significant differences depending on the type of toxin. Analysis of variance and Duncan analysis showed significant of ($p \leq 0.05$) on the reduction of AFs (Tables **3** and **4**).

The interaction between ingredients of LAB cell wall with AFs may be due to the adsorbance of AFs through treatments affecting LAB wall polysaccharides, lipids, and proteins causing an increase in the binding with AFs, whereas LAB cell wall binds the toxin with non-covalent weak bonds accompanied by some electrostatic attraction through lactinine

like protein, polysaccharides, and peptidoglucan. The binding between AFs and bacterial cell wall ingredients modifies AFs structures and gets a new structure form. This mechanism was with four types of AFs because the activity of this type of AFs depends on the same active groups, such as double bonds, OH, CHR3R, *etc* [32 - 34].

Table 3. ANOVA for the effect of LAB on AFs produced by from *A. parasiticus* in YES media.

Source	SS	df	MS	F	*P*
Intercept	49387.65	1	49387.65	4272.239	0.0000
LAB	3949.242	2	1974.621	170.813	0.0000
Toxin	871.5945	3	290.5315	25.13219	0.0000
LAB*toxin	182.8627	6	30.47711	2.636398	0.0415
Error	277.4432	24	11.56013	-	-
Total	54668.8	36	-	-	-

SS: Sum of Squares, df: degree of freedom, MS: mean square, P: probability at confidence 0.95.

Table 4. ANOA for the effect of LAB on AFs produced by from *A. flavus* in YES media.

Source	SS	df	MS	F	*P*
Intercept	36627.58	1	36627.58	3665.609	0.0000
LAB	2810.304	2	1405.152	140.6246	0.0000
Toxin	201.1808	3	67.06028	6.711248	0.0000
LAB*toxin	192.4517	6	32.07528	3.210024	0.01853
Error	239.8133	24	9.992222	-	-
Total	40071.33	36	-	-	-

SS: Sum of Squares, df: degree of freedom, MS: mean square, P: probability at confidence 0.95.

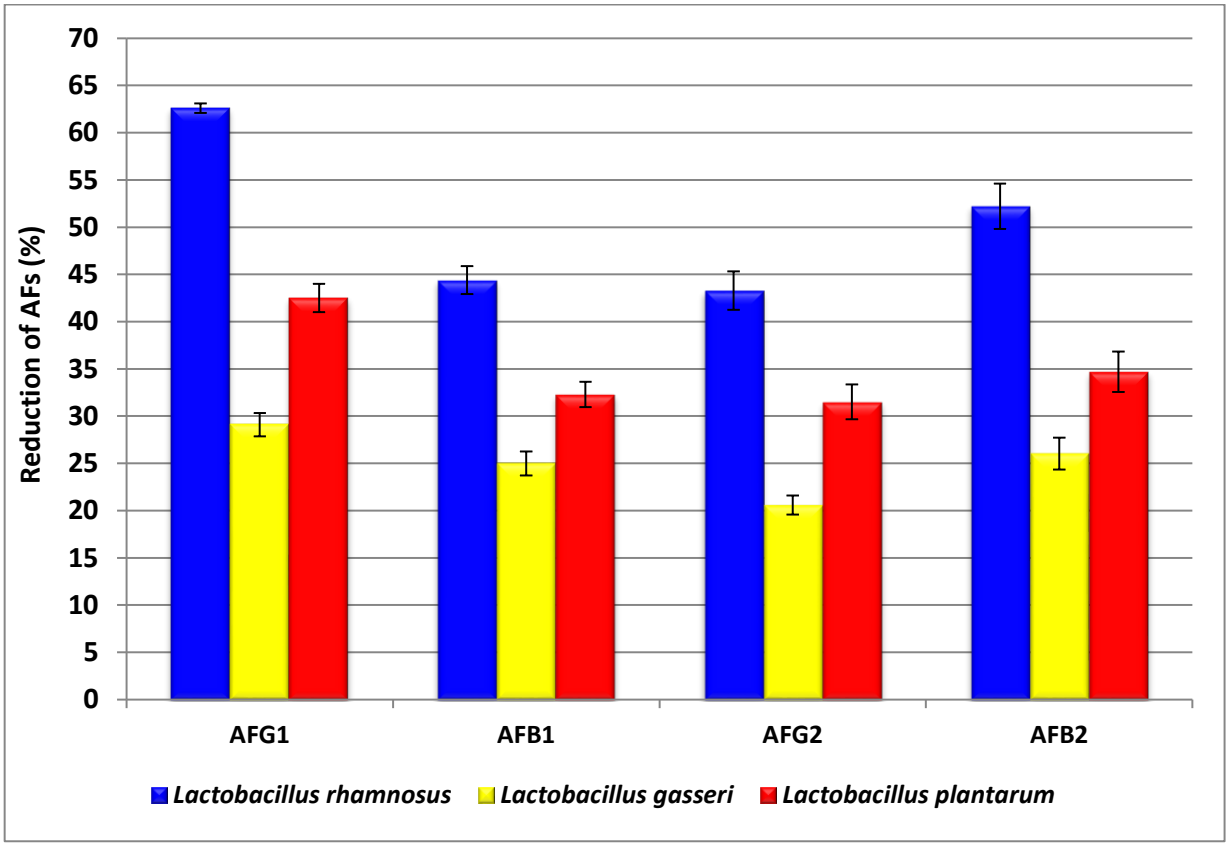

Fig (2). The percentages of reduction of AFs produced by *A. parasiticus* after treatment liquid media by LAB.

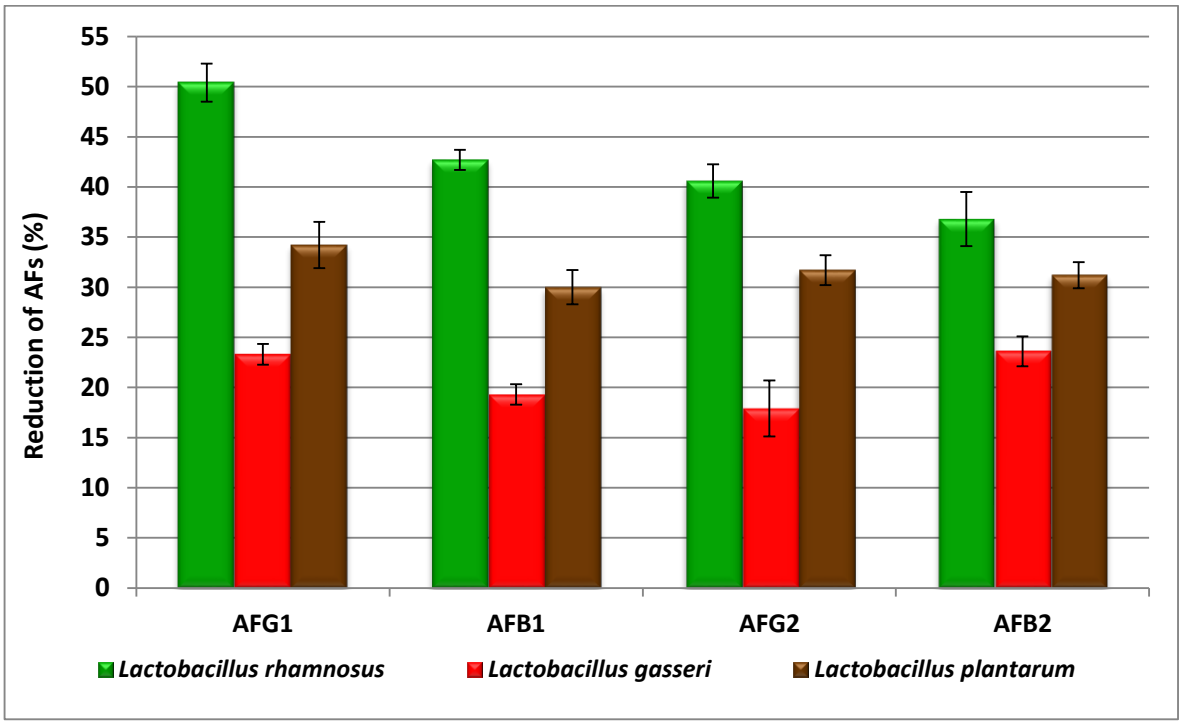

Fig (3). The percentages of reduction AFs produced by A. *flavus* after treatment liquid media by LAB.

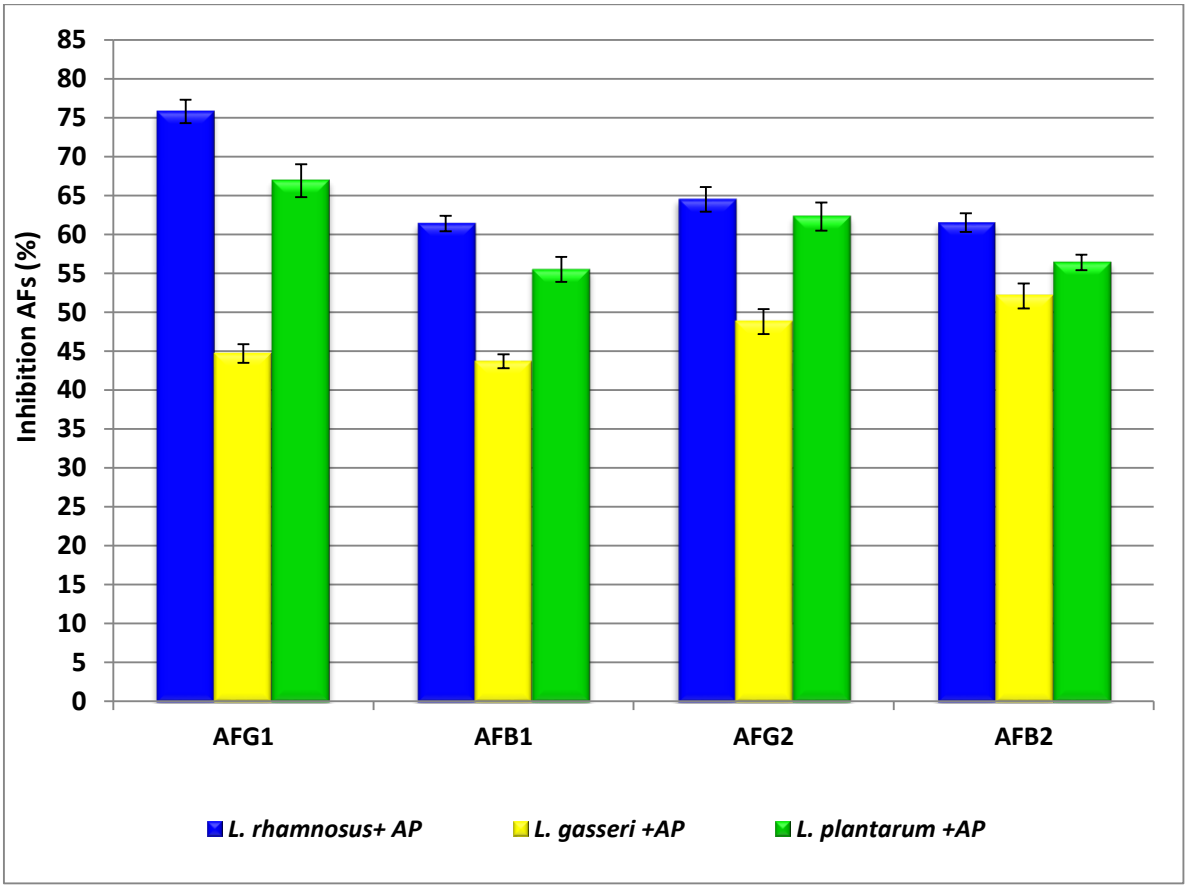

Fig (4). The percentages of inhibition of AFs in affected wheat grains after treatment with some LAB strains.

3.4. Control of AFs Production in Affected Wheat Grains with Spores of *A. parasiticus*

In this study, three strains for LAB were used for inhibition of the production of AFs on wheat grains. Data presented in Fig. (**4**) showed the percentages of inhibition of AFs in wheat affected by *A. parasiticus* spores after treatment (incubation) with LAB cells. When wheat grains were treated with *L.rhamnosus* strain, the reduction in AFG_1, AFB_1, AFG_2, and AFB_2 was 75.8±1.5, 61.4±1.0, 64.5±1.6, and 61.5±1.2%, respectively. While in the case of wheat treated with *L. gasseri,* the reduction of AFs was 44.7±1.2, 43.7±1.0, 48.8±1.6, and 52.1±1.6% in AFG_1, AFB_1, AFG_2, and AFB_2, respectively. AFs were removed by *L. plantarum* to 66.9±2.1and 62.3±1.0% with AFG_1 and AFG_2, respectively. On the other hand, AFB_1 and AFB_2 inhibition is 55.5±1.9 and 56.4±1.0%, respectively. The obtained data reflected that *L.rhamnosus* strain was the most efficient organism in inhibition of toxins then treatment by *L .plantarum*. The inhibition of AFs in wheat grains may be due to the biotransformation potential for toxins by LAB strains as well as the interaction between LAB and the accumulation of toxin through inhibition of their biosynthesis,whereas the production of AFs by *A. parasiticus* depends on expression genes responsible for producing of AFs which may be due to the effect of adding LAB strains [35 - 37].

CONCLUSION

The present study investigated the ability of *L.rhamnosus*, *L.gasseri,* and *L.plantarum* isolated from some local dairy products to control AFs produced by *A.flavus* and *A.parasiticus,* which were isolated from wheat grains. According to this study, L. *rhamnosus* is considered to be one of the best strains in this field. So that the use of LAB, which already has Generally Regarded as Safe (GRAS) status, should be encouraged for reduction and or removal of AFs. Moreover, the present study suggests applied use of LAB as a treatment to inhibit and or prevents AFs production in wheat grains.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this research are available in the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Peterson SW, Ito Y, Horn BW, Goto T. Aspergillus bombycis, a new aflatoxigenic species and genetic variation in its sibling species, A. nomius. Mycologia 2001; 93(4): 689-703.

[2] Siciliano I, Dal Bello B, Zeppa G, Spadaro D, Gullino ML. Static hot air and infrared rays roasting are efficient methods for aflatoxin decontamination on hazelnuts. Toxins (Basel) 2017; 9(2): 72. [http://dx.doi.org/10.3390/toxins9020072] [PMID: 28230792]

[3] Kelly JD, Eaton DL, Guengerich FP, Coulombe RA Jr. Aflatoxin B1 activation in human lung. Toxicol Appl Pharmacol 1997; 144(1): 88-95. [http://dx.doi.org/10.1006/taap.1997.8117] [PMID: 9169073]

[4] Abd El-Rahim AH, Abd El-Kader HAM. AbdElMoneim OM, El-Desouky TA, Farag IM. Evaluation of Protective Role of Activated Charcoal against DNA Damage, Cytogenetic Changes and Reproductive Toxicity Induced by Aflatoxin B1 in Mice. Int J Pharm Sci Rev Res 2014; 28(2): 69-82.https://globalresearchonline.net/journalcontents/v28-2/15.pdf

[5] Supriya C, Girish BP, Reddy PS. Aflatoxin B1-induced reproductive toxicity in male rats: possible mechanism of action. Int J Toxicol 2014; 33(3): 155-61. [http://dx.doi.org/10.1177/1091581814530764] [PMID: 24728861]

[6] Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. Int J Environ Res Public Health 2017; 14(6): 632. [http://dx.doi.org/10.3390/ijerph14060632] [PMID: 28608841]

[7] Blanksona GK, Mills-Robertsonb FC, Ofosu IW. Survey of occurrence levels of aflatoxins in selected locally processed cereal based foods for human consumption from Ghana. Food Control 2019; 95: 170-5. [http://dx.doi.org/10.1016/j.foodcont.2018.08.005]

[8] Haskard CA, El-Nezami HS, Kankaanpää PE, Salminen S, Ahokas JT. Surface binding of aflatoxin B(1) by lactic acid bacteria. Appl Environ Microbiol 2001; 67(7): 3086-91. [http://dx.doi.org/10.1128/AEM.67.7.3086-3091.2001] [PMID: 11425726]

[9] Lahtinen SJ, Haskard CA, Ouwehand AC, Salminen SJ, Ahokas JT. Binding of aflatoxin B1 to cell wall components of Lactobacillus rhamnosus strain GG. Food Addit Contam 2004; 21(2): 158-64. [http://dx.doi.org/10.1080/02652030310001639521] [PMID: 14754638]

[10] Hamidi A, Mirnejad R, Yahaghi E, *et al.* The aflatoxin B1 isolating potential of two lactic acid bacteria. Asian Pac J Trop Biomed 2013; 3(9): 732-6. [http://dx.doi.org/10.1016/S2221-1691(13)60147-1] [PMID: 23998015]

[11] Pitt JI, Hocking AD. Fungi and Food Spoilage. 2nd ed. London, UK: BlackieAcademic and Professional 1997. [http://dx.doi.org/10.1007/978-1-4615-6391-4]

[12] Sneath PHA, Mair NS, Sharpe M. Elizabeth and Holt, JG Bergy's manual of systematic bacteriology. (Eds.) Pub.Williams and Wilkins 2009; p. 2605.

[13] Filtenborg O, Frisvad JC, Svendsen JA. Simple screening method for molds producing intracellular mycotoxins in pure cultures. Appl Environ Microbiol 1983; 45(2): 581-5. [http://dx.doi.org/10.1128/AEM.45.2.581-585.1983] [PMID: 6338829]

[14] Samson RA, Hoekstra ES, Frisvad JC, Eds. Introduction to FoodandAirborne Fungi. 7th ed. Utrecht, Netherlands: CentraalbureauvoorSchimmelcultures 2004.

[15] Ghiasian SA, Kord-Bacheh P, Rezayat SM, Maghsood AH, Taherkhani H. Mycoflora of Iranian maize harvested in the main production areas in 2000. Mycopathologia 2004; 158(1): 113-21. [PMID: 15487329]

[16] Frisvad JC, Thrane U. Important mycotoxins,and fungi which produce them. In: Hocking , JI.Pitt RA Samson, Thrane U, Eds. advances in Food Mycology AD. Springer, New York 2006; pp. 3-25.

[17] Ghazvini RD, Kouhsari E, Zibafar E, Hashemi SJ, Amini A, Niknejad F. Antifungal activity and aflatoxin degradation of

Bifidobacteriumbifidum and Lactobacillusfermentum against toxigenic Aspergillusparasiticus. Open Microbiol J 2016; 10: 197-201. [http://dx.doi.org/10.2174/1874285801610010197] [PMID: 28077976]

[18] El-Desouky TA, May MA, Naguib K. Effect of fenugreek seeds extracts on growth of aflatoxigenic fungus and aflatoxin B1 production. J Appl Sci Res 2013; 9: 4418-25. Available at: http://www.aensiweb.com/jasr/jasr/201

[19] El-DesoukyTA and Ammar HAM. Honey mediated silver nanoparticles and their inhibitory effect on aflatoxins and ochratoxin A. J Appl Pharm Sci 2016; 6(06): 83-90. [http://dx.doi.org/10.7324/JAPS.2016.60615]

[20] Sharma A, Padwal-Desai SR, Nair PM. Aflatoxin-producing ability of spores of Aspergillus parasiticusexposed to gamma radiation. J Food Sci 1990; 55: 275-6. [http://dx.doi.org/10.1111/j.1365-2621.1990.tb06076.x]

[21] da Silva JFM, Peluzio JM, Prado G, *et al.* Use of probiotics to control aflatoxin production in peanut grains. ScientificWorldJournal 2015; 2015959138 [http://dx.doi.org/10.1155/2015/959138] [PMID: 26221629]

[22] El-Desouky TA, Sharoba AMA, El-Desouky AI, El-Mansy HA, Naguib K. Effect of Ozone Gas on Degradation of Aflatoxin B1 and AspergillusFlavus Fungal. J Environ Anal Toxicol 2012; 2: 128. [http://dx.doi.org/10.4172/2161-0525.1000128]

[23] El Sayed TI, El Desouky TA, Abd El Aziz AM. Investigation of fungus associated within cooccurrence of aflatoxins and ochratoxin a in cereals from Egypt. MOJ Toxicol 2019; 5(3): 92-9. [http://dx.doi.org/10.15406/mojt.2019.05.00161]

[24] Frisvad JC, Thrane U. Mycotoxin production by food-borne fungi In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, Eds. Introduction to Food Borne Fungi. Centraalbureauvoor Schimmelcultures, CBS, Delft 2002; pp. 251-61.

[25] Amadi JE, Adeniyi DO. Mycotoxin production by fungi isolated from stored grains. African Journal of Biotechnology 2009; 8(7): 1219-21. [http://dx.doi.org/10.4314/AJB.V8I7.60075.]

[26] Suleiman RA, Rosentrater K, Aand Bern CJ. Effects of Deterioration Parameters on Storage of Maize: A Review. J Nat Sci Res 2013; 3(9): 147-65. [http://dx.doi.org/10.13031/aim.20131593351]

[27] Baka M, Awad Z. Plant extract control of the fungi associated with different Egyptian wheat cultivars grains. J Plant Prot Res 2014; 54(3): 231-7. [http://dx.doi.org/10.2478/jppr-2014-0035.]

[28] Fakruddin M, Chowdhury A, Hossain MN, Ahmed MM. Characterization of aflatoxin producing Aspergillus flavus from food and feed samples. Springerplus 2015; 4(1): 159. [http://dx.doi.org/10.1186/s40064-015-0947-1] [PMID: 25883886]

[29] Yu J, Bhatnagar D, Cleveland TE. Completed sequence of aflatoxin pathway gene cluster in Aspergillus parasiticus. FEBS Lett 2004; 564(1-2): 126-30. [PMID: 15094053]

[30] Chang PK, Horn BW, Dorner JW. Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic Aspergillus flavus isolates. Fungal Genet Biol 2005; 42(11): 914-23. [http://dx.doi.org/10.1016/j.fgb.2005.07.004] [PMID: 16154781]

[31] Cary JW, Ehrlich KC. Aflatoxigenicity in Aspergillus: Molecular genetics, phylogenetic relationships and evolutionary implications. Mycopathologia 2006; 162(3): 167-77. [http://dx.doi.org/10.1007/s11046-006-0051-8]

[32] Peltonen K, el-Nezami H, Haskard C, Ahokas J, Salminen S. Aflatoxin B1 binding by dairy strains of lactic acid bacteria and bifidobacteria. J Dairy Sci 2001; 84(10): 2152-6. [http://dx.doi.org/10.3168/jds.S0022-0302(01)74660-7] [PMID: 11699445]

[33] Gratz S, Mykkänen H, El-Nezami H. Aflatoxin B1 binding by a mixture of Lactobacillus and Propionibacterium: *in vitro versus ex vivo.* J Food Prot 2005; 68(11): 2470-4. [http://dx.doi.org/10.4315/0362-028X-68.11.2470] [PMID: 16300092]

[34] Huang L, Duan C, Zhao Y, *et al.* Reduction of aflatoxin BR1R toxicity by Lactobacillusplantarum C88: A Potential probiotic strain isolated from chinese traditional fermented food "Tofu.". PLoS One 2017; 12(1)0170109 [http://dx.doi.org/10.1371/journal.pone.0170109]

[35] Gomaa EZ, Abdelall MF, El-Mahdy OM. Detoxification ofaflatoxin B1 by antifungal compounds from Lactobacillusbrevis andLactobacillusparacasei, isolated from Dairy Products. Probiotics Antimicrob Proteins 2018; 10(2): 201-9. [http://dx.doi.org/10.1007/s12602-017-9350-2] [PMID: 29150754]

[36] Ghanbari R, Aghaee EM, Rezaie S, *et al.* The inhibitory effect of lacticacid bacteria on aflatoxin production and expression of aflR gene inAspergillusparasiticus. J Food Saf 2018; 38e12413 [http://dx.doi.org/10.1111/jfs.12413]

[37] Fochesato AS, Cuello D, Poloni V, Galvagno MA, Dogi CA, Cavaglieri LR. Aflatoxin B_1 adsorption/desorption dynamics in the presence of Lactobacillus rhamnosus RC007 in a gastrointestinal tract-simulated model. J Appl Microbiol 2019; 126(1): 223-9. [http://dx.doi.org/10.1111/jam.14101] [PMID: 30188600]