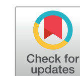
Oil Crop Science 6 (2021) 81–86

Contents lists available at ScienceDirect

Oil Crop Science

journal homepage: www.editorialmanager.com/ocsci/default.aspx

Effect of non-aflatoxigenic strains of *Aspergillus flavus* on aflatoxin contamination of pre-harvest peanuts in fields in China



Liying Yan [*], Wanduo Song, Yuning Chen, Yanping Kang, Yong Lei, Dongxin Huai, Zhihui Wang, Xin Wang, Boshou Liao [**]

Key Laboratory of Biology and Genetics Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, Wuhan 430062, China

A R T I C L E I N F O

Keywords:
Peanut
Non-aflatoxigenic *Aspergillus flavus*
Aflatoxin reduction
Field trial

A B S T R A C T

Aflatoxin contamination of peanuts is one of the most concerns in peanut production in China. Applying non-aflatoxigenic *Aspergillus flavus* strains, based on competitive exclusion, has been proved to be a promising strategy to reduce aflatoxin contamination in pre-harvest peanuts. Two non-aflatoxigenic *A. flavus* strains collected in China, which have been proved effectively reducing aflatoxin in the laboratory, were mixed with high aflatoxin producer to the soil in peanut growing season. The two non-aflatoxigenic strains significantly ($P < 0.05$) reduced aflatoxin contamination in peanut kernels under both normal and drought stresses in two fields. Compared to control, the total aflatoxin (sum of aflatoxin B_1 and B_2) was reduced 26.7–99.12% in field 1, and 84.96–99.33% in field 2. The aflatoxin was reduced 84.96–99.33% under drought stress in two fields. The present study indicated the non-aflatoxigenic *A. flavus* strains could be potential biocontrol agents for reducing aflatoxin contamination under field condition.

Introduction

Peanut (*Arachis hypogaea* L.) is a very important economic and oil crop in China, with above 500 million hectares of planting area and approximately 17 million tons of annual production (Liao et al., 2020). Aflatoxin contamination is a serious problem in peanut production in the world. Aflatoxin is well known as a secondary metabolite, which is produced by *Aspergillus* fungi including *A. flavus* and *A. parasiticus* (Payne and Brown, 1998). It is carcinogenic, highly toxic, mutagenic, teratogenic compounds that have been classified as group I carcinogens by the International Agency for Research on Cancer (IARC, 2002). Those peanuts contaminated with excessive levels of aflatoxin are harmful to human health and cannot be consumed. The occurrence of aflatoxin contamination is now clear. During pod maturation or after harvest, peanut pods are easily infected by *Aspergillus* species and contaminated by aflatoxin. And the most significant contamination usually occurs before harvest because fungi invasion and aflatoxin accumulation become accelerated when peanut is usually under high temperature and drought stress in later growing season (Sanders et al., 1984; Dorner and Cole, 1992).

Through several years' investigation, peanuts harvested from the Yangtze River region, where is hot and dry at the late-season, accumulated the highest level of aflatoxin contamination compared with peanuts from other planting areas in China (Ding et al., 2015). Therefore, it is essential to develop effective strategies to manage aflatoxin contamination of peanut in this region.

Among the approaches of aflatoxin contamination management, applying competitive and non-aflatoxigenic strains of *A. flavus* and/or *A. parasiticus* to soil has been proved to be an effective way to reduce pre-harvest aflatoxin contamination of crops (Cotty 1990; Dorner and Cole 2002; Dorner et al., 1999; Abbas et al., 2011). This approach is based on the premise that when high numbers of spores of the non-aflatoxigenic *Aspergillus* strains are applied to crops, they will win the competition with naturally occurring toxigenic strains on fighting for infection sites and nutrients. Reductions of aflatoxin by 88–90% using non-aflatoxigenic *A. flavus* strains have been demonstrated in peanut in USA, Argentina, Australia and Africa countries (Dorner 2003; Pitt and Hocking 2006; Alanis Zanon et al., 2016; Probst et al., 2011). Biocontrol agents have their own adaptive niches, potential biocontrol strains must be selected from the similar ecological niches and have great competitive ability to native toxigenic strains (Dorner and Cole 1992; Moradi et al.,

* Corresponding author.
** Corresponding author.
E-mail addresses: yanliying2002@126.com (L. Yan), liaoboshou@163.com (B. Liao).

https://doi.org/10.1016/j.ocsci.2021.04.004
Received 6 April 2021; Received in revised form 29 April 2021; Accepted 29 April 2021
Available online 6 May 2021
2096-2428/© 2021 the Oil Crop Research Institute, Chinese Academy of Agriculture Sciences. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2020). To manage the aflatoxin contamination of peanut in China, it is essential to collect the native non-aflatoxigenic *A. flavus* strains from peanut. Earlier investigators found that the non-aflatoxigenic *A. flavus* strains collected from China could efficiently reduce aflatoxin production of toxigenic strains in the laboratory (Yin et al., 2009; Zhou et al., 2015; Yan et al., 2016), but no cases on utilizing them in the field condition was reported before.

In the previous study, we found NAFFHB 396, a non-aflatoxigenic strain, can reduce aflatoxin content of a high aflatoxin producer AF2202 over 90% in the laboratory condition (Yan et al., 2016). To test the competitive ability of non-aflatoxigenic strains in the field, NAFFHB 396 and another non-aflatoxigenic strain 707 were separately applied with AF2202, and the aflatoxin mitigation of both NAFFHB396 and 707 were evaluated. Meanwhile, we also try to identify whether the colonization and competitively exclusion ability of these two non-aflatoxigenic strains would be influenced by the drought stress. The aim of the present study was to evaluate the efficacy of two native non-aflatoxigenic *A. flavus* strains NAFFHB396 and 707 to reduce aflatoxin production in peanuts under field conditions in China.

Material and method

Inoculum preparation

Aspergillus flavus strains including a high aflatoxin producer AF2202 (Wang et al., 2016; Yan et al., 2016), two non-aflatoxigenic strains: NAFFHB396 (Yan et al., 2016), and 707 (Yan et al., 2018) were used in the present study. The peanut used in this study was a susceptible cultivar (Zhonghua12), developed by Oil Crops Research Institute, CAAS (Wang et al., 2016). For inoculum preparation, AF2202, NAFFHB396 and 707 were inoculated on potato dextrose medium and cultured at 30 °C in the dark for 7 days, respectively. Conidia of each strain were collected, suspended in 0.1% Tween 20 water and adjusted to 2×10^5 conidia per mL. Dry corn grits were autoclaved at 121 °C for 1 h, transferred to sterilized plastic boxes after cooled down, inoculated with conidia suspension (500 mL conidia suspension per kg) of each strain separately, and mixed thoroughly in the hood. The cultures were maintained at 30 °C for 24 h, then stored at 4 °C.

Field experiments

The competitive ability of NAFFHB396 and 707 when challenged with equal conidial density of toxigenic *A. flavus* AF2202 was evaluated in field. Toxigenic strain AF2202 was added to soil alone as control. Experiments were performed in two fields at Wuchang in 2015 and 2016. The previous crop was rice for field 1, and peanut for field 2. Each treatment consisted of 3 plots and each plot with 200 plants. The experiment was conducted by a completely randomized design with 3 replicates. Peanut was sowed on 27 April in 2015, and on 30 April in 2016. A 10 g row^{-1} of *A. flavus* inocula were added to the soil at flowering stage (50–60 days after sowing). In addition, a rain exclusion shelter was placed over the *A. flavus* treated plots one month before harvest, those plots without a shelter were set as control. In 2015, plants were harvested at 132 DAP (days after planting). In 2016, plants were harvested at 128 DAP. Soil samples (every sample was collected from 5 spots of each plot, and approximately 200 g in total) were collected just before peanut harvest, transferred to a paper bag, dried immediately at 50 °C for 72 h. Soil samples were passed through a No.4 sieve (250 μm), then kept under 4 °C. Peanut pod samples were collected, air dried and kept at 4 °C.

Microflora analysis

To ascertain the density of *A. flavus* soil population of the mixed inocula containing non-aflatoxigenic strains with toxigenic strain and inoculum of toxigenic strain alone, a series of assays were conducted in the field under normal and drought stress in 2015 and 2016. *Aspergillus* section *Flavi* fungi were recovered from soil by the dilution plate technique on modified dichloran rose bengal chloramphenicol agar (DRBC) (Pitt et al., 1993). Ten gram of soil sample was suspended in 100 mL 0.1% peptone water, shaken for 30 min at room temperature. The soil suspension was decimally diluted from 10^{-1} to 10^{-5}. A 100 μL aliquot of each dilution from 10^{-2} to 10^{-5} was plated on the surface of DRBC medium with 3% NaCl (Alaniz Zanon et al., 2013), each dilution with 3 replicates. The cultures were kept at 30 °C in the dark for 3–7 days. Colonies showing pink reverse color and producing olive-green conidia were identified as *A. flavus*. Colony number between 25 and 250 per plate was recorded. The density of *A. flavus* population in the soil was indicated as colony forming units per gram (cfu g^{-1}).

Determination of kernel infection by A. flavus

A total of 18 plots were tested for *A. flavus* infection, and 9 plots were under drought stress and 9 plots were under normal condition. There was approximately 2 kg of kernels randomly taken from each plot, and 100 kernels (3 replicates) were selected for *A. flavus* infection determination. The remaining kernels were ground and kept for aflatoxin analysis. Peanut kernels were surface sterilized for 30 s with 75% ethyl alcohol, 2 min with 1% sodium hypochlorite (NaClO), rinsed with sterile water for 3 times and soaked in water for 8 min. Kernels were transferred to sterile petri dishes (9 cm in width), 10 kernels per petri dish. The petri dishes with kernels were placed gently into plastic boxes, sealed with covers and incubated at 30 °C for 7 days. Kernels which surface covered by olive-green *Aspergillus* conidia were regarded as infection. The percentage of infection was calculated by the formula as follows, percentage of infection = infected kernels/total tested kernels × 100.

Aflatoxin analysis

The aflatoxin analysis was performed based on Chinese method standards (Wang et al., 2007) with minor modification. Five gram of ground kernel was transferred to 100 mL flask and suspended in 15 mL 4% NaCl-methanol-water. The mixtures were treated with ultrasonic wave for 15 min at room temperature, passed through two layer of filter paper (Waterman No.1). The filtrate (3 mL) was transferred to a 15 mL glass tube, diluted with 8 mL of sterile water and mixed thoroughly by shaking for 1 min, then stayed overnight. The mixture was filtered through a Millipore filter (0.45 μm), then passed through immune-affinity column and washed twices with 10 mL deionized water. The pure extract was obtained by eluted the column with 1 mL methanol for HPLC profiling. HPLC analysis was conducted at room temperature on a reverse phase C18 column of 4.6 mm × 250 mm (Angilent Technologies, Santa Clara, CA, USA) with a mobile phase that consisted of methanol/H_2O (55:45, *V/V*) at a flow rate of 0.7 mL min^{-1} on an Angilent 1200 system (Angilent Technologies, Santa Clara, CA, USA), and 10 μL of each sample was loaded.

Statistics analysis

Mean and variance of density of *A. flavus* population in the soil, percentage of infection and aflatoxin content of kernels were analyzed by SPSS 22.0, variance analysis was determined by General Linear Model using year, fields, strains, and drought stress treatment as fixed factors, density of *A. flavus* population, percentage of infection and aflatoxin content of kernel were set as dependent variable, respectively.

Results

Density of A. flavus population in soil

Colonies of *A. flavus* appeared on the DRBC medium after 3 days incubation with pink reverse color. Olive green conidia emerged on the colonies 5 days later (Fig. 1A and B).

Investigation of soil microfloral was conducted in 2015 and 2016. For the plots treated with AF2202 alone, the density of AF2202 in soil population was approximately 3.2×10^5 cfu g^{-1} in field 1, and up to 4.18×10^5 cfu g^{-1} in field 2 in 2015. The density of population of AF2202 was over 5.17×10^5 cfu g^{-1} in field 1, respectively, and was up to 1.25×10^6 in field 2 in 2016. For the plots treated with toxigenic and non-aflatoxigenic strains together, the density of AF2202 + 707/NAFFHB396 was 2.88×10^5–3.72×10^5 cfu g^{-1} under normal condition and was 3.42×10^5– 4.43×10^5 cfu g^{-1} under drought stress condition in 2015. Similar density of *A. flavus* was found in 2016, with 1.39×10^5–1.16×10^6 cfu g^{-1} under normal condition and 3.54×10^5–1.81×10^6 cfu g^{-1} under drought stress condition.

The density of *A. flavus* soil population was not significantly influenced by strains and fields, but was significantly influenced by years and drought stress by SPSS analysis (Table 1). The population of *A. flavus* under drought stress was significant higher than that under normal condition both in 2015 and 2016 ($P < 0.05$) (Fig. 2A and 2B). Meanwhile, the population of *A. flavus* in 2015 was significantly different with those in 2016 under both normal condition ($P < 0.05$), and drought stress condition ($P < 0.01$) (Fig. 3A and 3B).

Fungi colonizing peanut kernels

Peanut kernels of all treatments were infected by *A. flavus* under both normal and drought stress condition in two years. In 2015, percent of infection of peanuts by AF2202 alone was 2.0% and 12.3% in field 1 and field 2 under normal condition, and was 12.0% and 80.5% in field 1 and field 2 under drought stress. For plots treated with AF2202 + 707 and AF2202+NAFFHB396, the percent of infection of peanuts was 3.5%–64% under normal condition, and 12.0%–92.5% under drought stress condition. In 2016, percent of infection of peanuts by AF2202 alone ranged from 60.34% to 88.67%, percent of infection ranged from 83.28% to 93.67% by AF2202 + 707 and NAFFHB396. Percent of infection of peanuts by *A. flavus* was significantly affected by years and drought stress, but was not significantly affected by strains and fields by SPSS analysis (Table 1). Percent of infection of peanuts by *A. flavus* was significantly different between normal condition and drought stress both in 2015 and in 2016 (Fig. 4). Infection percentage of peanut by *A. flavus* in 2015 and 2016 was significantly different under normal condition, but not significant different under drought stress condition (Fig. 5).

Aflatoxin reduction

Total aflatoxin (sum of aflatoxin B_1 and B_2) of peanuts was detected in 2016. The aflatoxin content of peanuts was significantly influenced by non-aflatoxigenic *A. flavus* strains, but not by fields and drought stress analyzed by SPSS (Table 2). For treatment of AF2202 alone, the total aflatoxin content was 10.66 μg kg^{-1}, 584.29 μg kg^{-1} in field 1, and 667.14, 913.76 μg kg^{-1} in field 2 under normal and drought stress condition, respectively. For treatment of AF2202 + 707, it was 7.81 μg kg^{-1}, 24.5 μg kg^{-1} in field 1, and 5.08 μg kg^{-1}, 6.06 μg kg^{-1} in field 2, meanwhile, for treatment of AF2202 + NAFFHB396, it was 6.21 μg kg^{-1}, 5.12 μg kg^{-1} in field 1 and 7.11 μg kg^{-1}, 137.38 μg kg^{-1} in field 2 under normal and drought stress condition respectively (Table 3). Compared to co-treated with non-aflatoxigenic strains, peanuts treated with AF2202 alone accumulated 1.37–131 folds of aflatoxin under normal condition in field 1 and 2, and 6.65–150 fold of aflatoxin under drought stress condition in field 1 and 2, respectively.

In comparison with peanuts treated with AF2202 alone, the aflatoxin concentration in the plots treated with non-aflatoxigenic and AF2202 together was significantly reduced under drought stress in field 1 and under both drought stress and normal condition in field 2. There was no significant difference in aflatoxin of peanuts collected in soil added by AF2202 either with NAFFHB396 or with 707. Compared to AF2202, the non-aflatoxigenic strains resulted in over 26.7% reduction of aflatoxins under normal condition, and more than 95.8% reduction under drought stress in field 1. The non-aflatoxigenic strains resulted in approximately 99% reduction of aflatoxin under normal condition and more than 84% reduction of aflatoxin under drought stress condition in field 2. No significant ($P > 0.05$) differences in abilities of reducing total aflatoxin between 707 and NAFFHB396 was shown under both normal and drought stress in field 1, and under the normal condition in field 2 (Table 3).

Table 1
Analysis of variance for density of *Aspergillus flavus* soil population and percentage of peanut infected by *A. flavus*.

	Source of variation	Sum of squares	DF	Mean square	F value	P value
Colonies in soil	Year	7944.18	1	7944.18	7.228	0.015
	Field	3289.667	1	3289.667	2.993	0.101
	Strain	2518.033	2	1259.016	1.146	0.340
	Drought	6572.41	1	6572.41	5.980	0.025
Percentage of infection	Year	9695.436	1	9695.436	21.398	0
	Field	82.288	1	82.288	0.182	0.675
	Strain	3107.205	2	4533.603	3.429	0.055
	Drought	4672.692	1	4672.692	10.313	0.005

Discussion

Aflatoxins do harm to both the safety and value of foods and feeds. Management of aflatoxin contamination of food and feed is required to

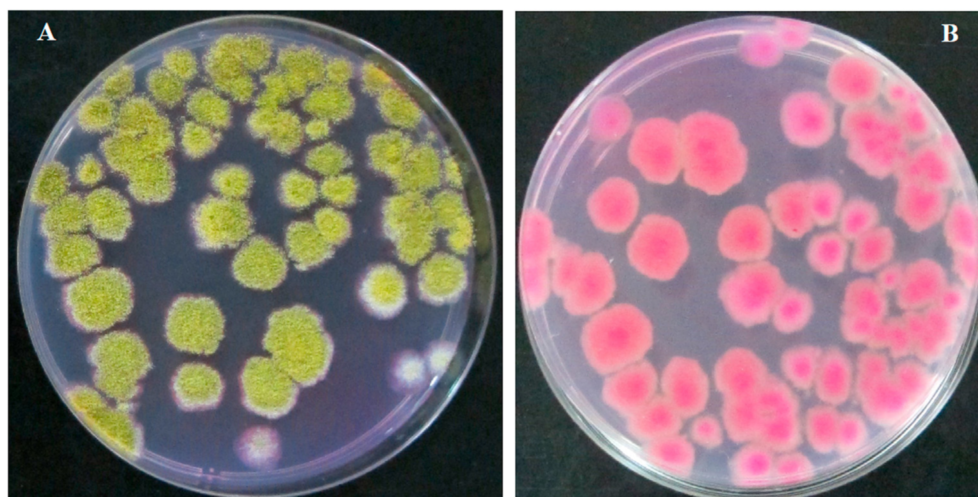


Fig. 1. Colonies of *A. flavus* on DRBC medium. A, olive green conidia of *A. flavus* on DRBC medium; B, pink reverse color of *A. flavus* on DRBC medium.

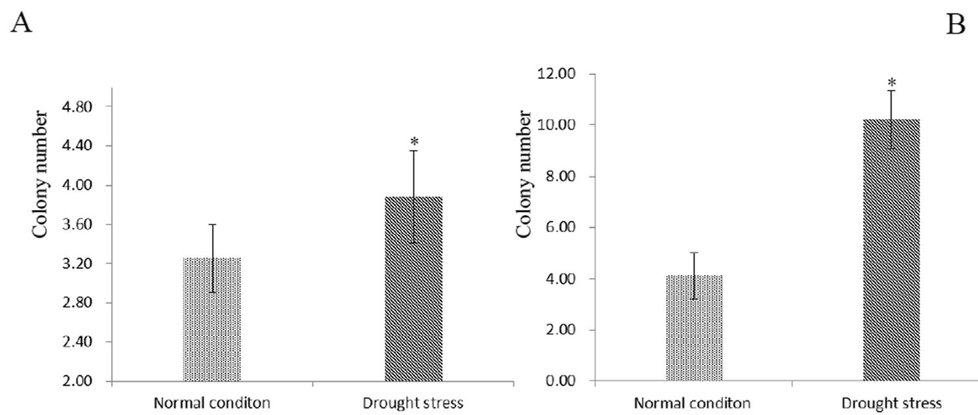


Fig. 2. Density of *A. flavus* population in soil in different treatments A, density of *A. flavus* population in the soil in 2015; B, density of *A. flavus* population in the soil in 2016. * indicates the difference reaching a significant level ($P < 0.05$).

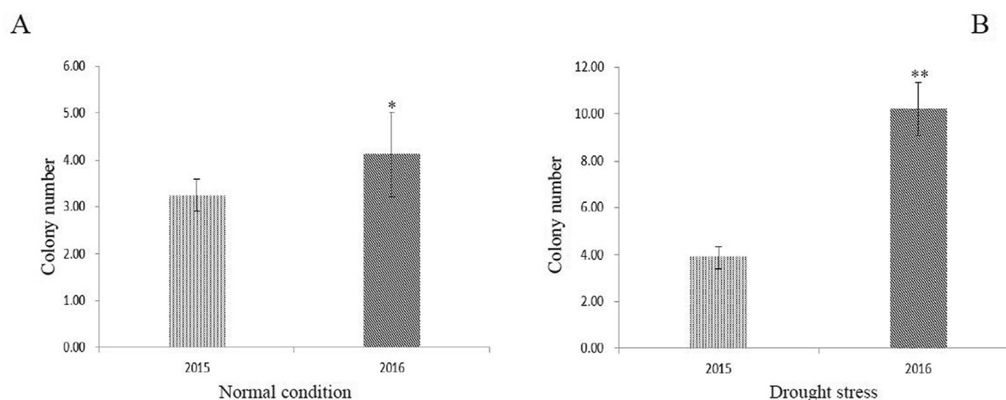


Fig. 3. Density of *A. flavus* population in the soil in different years. A, density of *A. flavus* population in the soil under normal condition; B, density of *A. flavus* population in the soil under drought stress. * shows difference reaching a significant level ($P < 0.05$); ** indicate difference reaches a significant leve ($P < 0.01$).

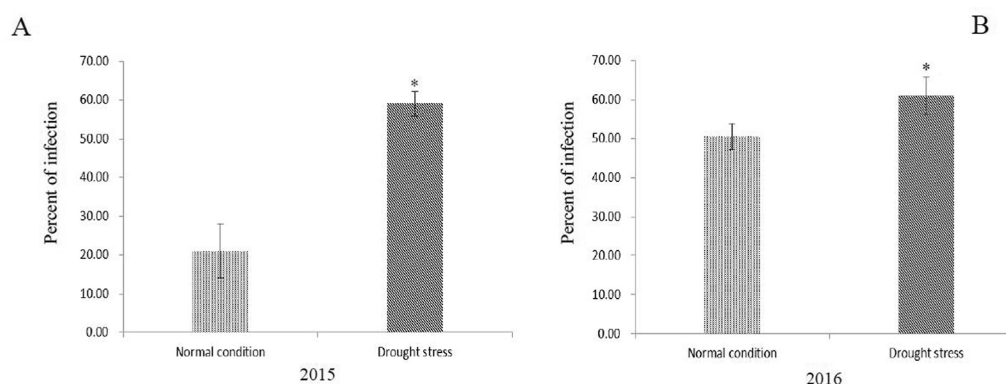


Fig. 4. Percent of peanuts infected by *A. flavus* in different treatments. A, percent of peanuts infected by *A. flavus* in 2015; B, percentage of peanuts infected by *A. flavus* in 2016. * shows the difference reaching a significant level ($P < 0.05$).

food security (Wu et al., 2010). A biological control strategy that utilizing naturally occurring non-aflatoxigenic isolates of *A. flavus* to competitively exclude aflatoxin producers, has been employed successfully in many crops, including corn, cotton, peanut and pistachio (Dorner et al. 2003, 2009; Jaime et al., 2017; Cotty 1994; Doster et al., 2014). A formulation of non-aflatoxigenic *A. flavus* strains NRRL 21882 was first developed and named Afla-Guard for use in peanut fields in USA (Dorner and Lamb 2006). Later on, several biocontrol formulations consisted of non-aflatoxigenic *A. flavus* strains were developed in USA, Italy, Serbia, Nigeria, and Turkey for prevention of aflatoxin contamination in peanut and corn (Mauro et al., 2018; Savic et al., 2020; Atehnkeng et al., 2014; Moral et al., 2020). Though in some previous studies, some non-aflatoxigenic strains have been collected from China and exhibited aggressive nature against toxigenic strains in the laboratory (Yin et al., 2009; Zhou et al., 2015; Yan et al., 2016), there is no report on utilization of non-aflatoxigenic *A. flavus* in field until now. This study firstly demonstrated the effect of non-aflatoxigenic *A. flavus* strains native to China in reducing aflatoxin contamination of peanut in the fields.

Application of non-aflatoxigenic *A. flavus* strains in the field was highly efficient in reduction aflatoxin contamination in peanuts.

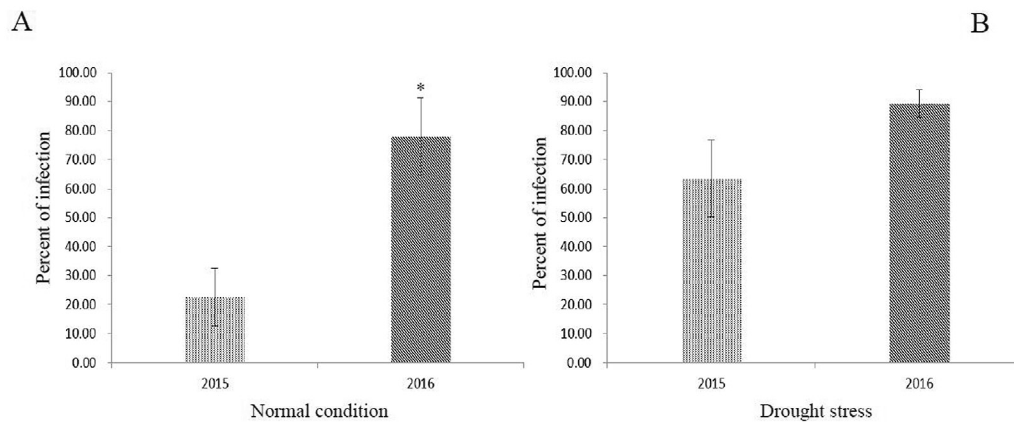


Fig. 5. Percent of peanuts infected by *A. flavus* in different years. A, percent of peanuts infected by *A. flavus* under normal condition; B, percentage of peanuts infected by *A. flavus* under drought stress. * shows the difference reaching a significant level ($P < 0.05$).

Table 2
Analysis of variance for aflatoxin of peanuts.

	Source of variation	Sum of squares	DF	Mean square	F value	P value
Aflatoxin	Field	100456.020	1	100,456	2.578	0.152
	Strain	720023.165	2	360011.6	9.240	0.011
	Drought	77940.201	1	77940.2	2.000	0.200

Aflatoxin contamination of peanuts in the field in USA could be reduced by 77–98% with non-aflatoxigenic strains *A. flavus* NRRL 21882 and *A. parasiticus* NRRL 21369 (Horn and Dorner 2009). Reductions in aflatoxin levels of peanuts averaging 71% were detected in treated plots with an non-aflatoxigenic *A. flavus* (AFCHG2) in different inoculation treatments in Argentina (Alanis Zanon et al., 2013). Aflatoxin reductions of peanuts ranged from 58.3% to 100% by Aflasafe SN01 in Senegal (Senghor et al., 2020). In the present study, the reduction in aflatoxin by NAFFHB396 and 707 was from 84.96% to 99.33% under drought stress condition, and also attained 98.93–99.24% reduction under normal condition. It suggested that non-aflatoxigenic strains NAFFHB396 and 707 were efficient in reducing aflatoxin contamination of peanuts in the field. Both strains could be potential biocontrol agents for exclude toxigenic *A. flavus* in the field.

Drought stress during maturation of peanut pods increased the ability of *A. flavus* to infect peanuts (Horn, 2003; Alanz Zanon 2013). The present study also confirmed this conclusion. Though the soil population of *A. flavus* in the soil were similar in the plots both under drought stress and normal condition in 2015 and 2016, the percentage of peanut infection were higher in the plots treated by drought stress than those under normal condition, and aflatoxin content of peanuts collected from plots treated with drought stress also higher than those collected from plots under normal condition. Njoroge et al. (2018) argued that biological control was ineffective when drought prevailed in peanut. Dorner (2009) demonstrated that biocontrol was particularly effective when aflatoxin conductive situation was promoted by drought stress. In the present study, we found that the aflatoxin reduction of toxigenic AF2202 were over 84% by non-aflatoxigenic strains under drought stress condition, and over 26.7% under control condition. The results were in according with those from Dorner (2009). It suggested that non-aflatoxigenic strains used in present study have good competitiveness under both normal and drought stress condition, and it is promising to reduce aflatoxin under drought stress condition.

The worldwide accepted levels for aflatoxin B_1 and total aflatoxin (the sum of aflatoxin B_1, B_2, G_1, and G_2) range from 1 to 20 mg kg^{-1} and from 0 to 35 mg kg^{-1}. The regulatory limit for aflatoxin B_1 contamination is 20 μg kg^{-1} in China (Ding et al., 2012). The aflatoxin can attain a safe level after treated with non-aflatoxigenic *Aspergillus flavi* strains (Alanz Zanon 2013). Similar result was also found in the present study. Peanuts harvested from plots treated with a mixture of toxigenic and non-aflatoxigenic *A. flavus* strains accumulated less aflatoxins compared with those treated by AF2202 alone, significantly less aflatoxins were detected under drought stress condition. Less than 20 μg kg^{-1} aflatoxin was detected in the plots treated with non-aflatoxigenic strain under normal condition and also in the plots treated with NAFFHB396 in field 1 and 707 in field 2 under drought stress condition. It suggested that utilization of non-aflatoxigenic *A. flavus* strains could efficiently reduce aflatoxin content of peanuts both under normal condition and under drought stress condition, and in most cases the peanuts collected in the plots treated with non-aflatoxigenic strains could attain a safe level.

The two non-aflatoxigenic strains were collected from different provinces along the Yangtze River region, NAFFHB396 was from Hubei Province, 707 was from Guizhou Province. The two strains shared similar genes deletion pattern in aflatoxin synthesis cluster (Yan et al., 2018) and demonstrated similar aflatoxin reduction efficiency in the laboratory condition (data not published). In the present study, the two strains revealed similar high aflatoxin reduction efficiency under both normal

Table 3
Aflatoxin content of peanuts and reduction rate by non-aflatoxigenic *A. flavus* strains.

Treatment	Field 1				Field 2			
	Control	Reduction rate (%)	Drought stress	Reduction rate (%)	Control	Reduction rate (%)	Drought stress	Reduction rate (%)
707 + AF2202	7.81 ± 4.65a	26.7	24.5 ± 5.28a	95.8	5.08 ± 1.80a	99.24	6.06 ± 0.44a	99.33
396 + AF2202	6.21 ± 1.14a	46.7	5.12 ± 0.89a	99.12	7.11 ± 2.89a	98.93	137.38 ± 12.16b	84.96
AF2202	10.66 ± 1.31a		584.29 ± 23.58b		667.14 ± 72.34b		913.76 ± 48.62c	

Notes: Different lowercase letter after data indicates difference reaches a significant level ($P < 0.05$).

and drought stress condition in field 1 and field 2. It suggested that the two strains could be used as biocontrol potential agents to control aflatoxin contamination in the field condition along the Yantzge River region.

The present study showed the good competitive ability of two atoxigeinc *A. flavus* strains native in China to exclude the toxigenic strain from peanuts in field. Both strains have the potential to be biocontrol agents to manage toxigenic *A. flavus* of peanut in China.

Funding

This research was supported by the Central Public-interest Scientific Institution Basal Research Fund (2021-2060302-061-019), the National Natural Science Foundation of China (Grant Nos. 32001510 and 31461143022), China Agricultural Research System (CARS-13), the Innovation Program of the Chinese Academy of Agricultural Sciences (2021-2060302-049-031).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Jianbin Guo for suggestion of data analysis.

References

Abbas, H.K., Zablotowicz, R.M., Horn, B., Phillips, N.A., Johnson, B.J., Jin, X., Abel, C.A., 2011. Comparison of major biocontrol strains of non-aflatoxigenic *Aspergillus flavus* for the reduction of aflatoxins and cyclopiazonic acid in maize. Food Addit. Contam. 28 (2), 198–208.

Alanis Zanon, M.S., Chiotta, M., Giaj-Merlera, G., Barros, G.G., Chulze, S.N., 2013. Evaluation of potential biocontrol agent for aflatoxin in Argentinean peanuts. Int. J. Food Microbiol. 162, 220–225.

Alanis Zanon, M.S., Barros, G.G., Chulze, S.N., 2016. Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. Int. J. Food Microbiol. 231, 63–68.

Atehnkeng, J., Ojiambo, P.S., Cotty, P.J., Bandyopadhyay, R., 2014. Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link: FR vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.). Biol. Contr. 72, 62–70.

Cotty, P.J., 1990. Effect of atoxigenic strains of *Aspergillus flavus* on aflatoxin contamination of developing cottonseed. Plant Dis. 74, 233–235.

Cotty, P.J., 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology 84 (11), 1270–1277.

Ding, X.X., Li, P.W., Bai, Y.Z., Zhou, H.Y., 2012. Aflatoxin B_1 in post-harvest peanuts and dietary risk in China. Food Contr. 23, 143–148.

Ding, X., Wu, L., Li, P., Zhang, Z., Zhou, H., Bai, Y., Chen, X., Jiang, J., 2015. Risk assessment on dietary exposure to aflatoxin B_1 in post-harvest peanuts in the Yangtze River ecological region. Toxins: Baseline. 7, 4157–4174. https://doi.org/10.3390/toxins7104157.

Dorner, J.W., 2009. Development of biocontrol technology to manage aflatoxin contamination in peanuts. Peanut Sci. 36 (1), 60–67.

Dorner, J.W., Cole, R.J., 2002. Effect of application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanuts in storage. J. Stored Prod. Res. 38 (4), 329–339.

Dorner, J.W., Lamb, M.C., 2006. Development and commercial use of afla-Guard(VR), an aflatoxin biocontrol agent. Mycotoxin Res. 22 (1), 33–38.

Dorner, J.W., Cole, R.J., Blankenship, P.D., 1992. Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. J. Food Protect. 55 (11), 888–892.

Dorner, J.W., Cole, R.J., Wicklow, D.T., 1999. Aflatoxin reduction in corn through field application of competitive fungi. J. Food Protect. 62 (6), 650–656.

Dorner, J.W., Cole, R.J., Connick, W.J., Daigle, D.J., McGuire, M.R., Shasha, B.S., 2003. Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol. Contr. 26 (3), 318–324.

Doster, M.A., Cotty, P.J., Michailides, T.J., 2014. Evaluation of the atoxigenic *Aspergillus flavus* strain AF36 in pistachio orchards. Plant Dis. 98 (7), 948–956.

Horn, B.W., 2003. Ecology and population biology of aflatoxigenic fungi in soil. J. Toxicol. Toxin Rev. 22 (2–3), 351–379.

Horn, B.W., Dorner, J.W., 2009. Effect of nontoxigenic *Aspergillus flavus* and *A. parasiticus* on aflatoxin contamination of wounded peanut seeds inoculated with agricultural soil containing natural fungal populations. Biocontrol Sci. Technol. 19 (3), 249–262.

IARC, 2002. Monographs on the evaluation of carcinogenic risks to humans: some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation. 82, 171–215.

Jaime, R., Antilla, L., Liesner, L., Cotty, P.J., 2017. Options to reduce costs of aflatoxin control in commercial cotton with area-wide programs utilizing atoxigenic biocontrol agents. Phytopathology 107, S129.

Liao, B.S., 2020. A review on progress and prospects of peanut industry in China. Chin. J. Oil Crop Sci. 42 (2), 161–166.

Mauro, A., Garcia-Cela, E., Pietri, A., Cotty, P.J., Battilani, P., 2018. Biological control products for aflatoxin prevention in Italy: commercial field evaluation of atoxigenic *Aspergillus flavus* active ingredients. Toxins. 10 (1), e30.

Moradi, M., Rohani, M., Fani, S.R., Mosavian, M.T.H., Probst, C., Khodaygan, P., 2020. Biocontrol potential of native yeast strains against *Aspergillus flavus* and aflatoxin production in pistachio. Food Addit. Contam. 37 (11), 1963–1973.

Moral, J., Garcia-Lopez, M.T., Camiletti, B.X., Jaime, R., Michailides, T.J., Bandyopadhyay, R., Ortega-Beltran, A., 2020. Present status and perspective on the future use of aflatoxin biocontrol products. Agronomy. 10 (4), e491.

Njoroge, S.M., 2018. A critical review of aflatoxin contamination of peanuts in Malawi and Zambia: the past, present, and Future. Plant Dis. 102, 12.

Payne, G., Brown, M., 1998. Genetics and physiology of aflatoxin biosynthesis. Annu. Rev. Phytopathol. 36, 329–362.

Pitt, J.I., Hocking, A.D., 2006. Mycotoxins in Australia: biocontrol of aflatoxin in peanuts. Mycopathologia 162, 233–243. https://doi.org/10.1007/s11046-006-0059-0.

Pitt, J.I., Hocking, A.D., Bhudhasamai, K., Miscamble, B.F., Wheeler, K.A., Tanboon-Ek, P., 1993. The normal mycobiota of commodities from Tailand. 1. Nuts and oilseeds. Int. J. Food Microbiol. 20, 211–226.

Probst, C., Bandyopadhyay, R., Price, L.E., Cotty, P.J., 2011. Identification of atoxigenic *Aspergillus flavus* isolates to reduce aflatoxin contamination of maize in Kenya. Plant Dis. 95, 212–218.

Sanders, T.H., Blankenship, P.D., Cole, R.J., Hill, R.A., 1984. Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination. Mycopathologia. 86, 51–54. https://doi.org/10.1007/BF00437229.

Savic, Z.T., Dudas, M., Loc, M., Grahovac, D., Budakov, I., Jajic, S., Krstovic, T., Barosevic, R., Krska, M., Sulyok, et al., 2020. Biological control of aflatoxin in maize grown in Serbia. Toxins. 12 (3), e162.

Senghor, L.A., Ortega-Beltran, A., Atehnkeng, J., Callicott, K.A., Cotty, P.J., Bandyopadhyay, R., 2020. The atoxigenic biocontrol product Aflasafe SN01 is a valuable tool to mitigate aflatoxin contamination of both maize and groundnut cultivated in Senegal. Plant Dis. 104 (2), 510–520.

Wang, J., Liu, X.M., 2007. Assessment of dietary aflatoxins exposure in Chinese residents. Chin. J. Food Hyg. 3, 238–240.

Wang, H.M., Lei, Y., Wan, L.Y., Yan, L.Y., Lv, J.E., Dai, X.F., Ren, X.P., Guo, Wei, Jiang, H.F., Liao, B.S., 2016. Comparative transcript profiling of resistant and susceptible peanut post-harvest seeds in response to aflatoxin production by *Aspergillus flavus*. BMC Plant Biol. 16, 54.

Wu, F., Khlangwiset, P., 2010. Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: case studies in biocontrol and postharvest interventions. Food Addit. Contam. 27, 496–509.

Yan, L.Y., Kang, Y.P., Lei, Y., Wan, L.Y., Huai, D.X., Jiang, H.F., Ren, X.P., Liao, B.S., 2016. Biocontrol potential of reducing aflatoxin contamination by an atoxigenic strain of *Aspergillus flavus* isolated from peanut. Oil Crop Sci. 1, 45–51.

Yan, L.Y., Kang, Y.P., Lei, Y., Wan, L.Y., Huai, D.X., Jiang, H.F., Ren, X.P., Liao, B.S., 2018. Genetic diversity of atoxigenic *Aspergillus flavus* isolates from peanut kernels in China. Oil Crop Sci. 3 (1), 42–49.

Yin, Y., Lou, T., Yan, L., Michailides, T.J., Ma, Z., 2009. Molecular characterization of toxigenic and atoxigenic *Aspergillus flavus* isolates, collected from peanut fields in China. J. Appl. Microbiol. 107, 1857–1865.

Zhou, L., Wei, D.d., Selvaraj, J.M., Shang, B., Zhang, C.S., Xing, F.G., Zhao, Y.J., Wang, Y., Liu, Y., 2015. A strain of *Aspergillus flavus* from China shows potential as a biocontrol agent for aflatoxin contamination. Biocontrol Sci. Technol. 25 (5), 583–592.