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Fungal microbiome related to mycotoxin contamination in medicinal and edible seed Semen Persicae

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ABSTRACT

Medicinal and edible seed Semen Persicae is susceptible to mycotoxin and fungal contamination. However, the occurrence of mycotoxin contamination and fungal infection is still unclear. In this paper, ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry and high-throughput sequencing were conducted to determine the mycotoxin contents and fungal abundances of Semen Persicae. 42.86% of samples were positive for aflatoxin B1 (26.48–48.37 μg/kg) and 28.57% of samples were positive for aflatoxin B2 (1.47–4.82 μg/kg). Ochratoxin A and fumonisin B1 were only detected in one sample (91.02 and 34.61 μg/kg, respectively). Chao 1 and Shannon indices were significantly higher in the Dalian of Liaoning, Baotou of Innermongolia and Langfang of Hebei regions than in other groups. Ascomycota, Basidiomycota, *Wallemia*, *Candica*, *Saccharomyces* and *Aspergillus* were the predominant fungi and they were significantly region-specific. Simultaneously, the diversity, composition and co-occurrence network complexity in the mycotoxin-free group were significantly higher than those in the mycotoxin-contaminated group. Spearman correlation analysis showed aflatoxins, ochratoxin A and fumonisins contents were positively and significantly correlated with the abundances of *Aspergillus*, *Rhodotorula*, *Wallemia* and *Candida*. In conclusion, this study reported the prevalence of mycotoxin contamination and the great diversity of fungi associated with Semen Persicae for the first time, providing an early warning for subsequent potential mycotoxin biosynthesis.

1. Introduction

Approximately 70%–80% of people living in developing countries rely on traditional herbal medicines for their primary healthcare [1]. In China, traditional Chinese medicine (TCM) plays an important role in the treatment of major epidemic diseases, especially

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COVID-19 [2]. TCM is mainly produced in China and exported to countries, such as Japan, the United States and the European Union [3]. In TCM, medicinal parts (such as roots, leaves, flowers and seeds) are used separately to achieve the best clinical effect. Up to now, more than 60 medicinal seeds have been recorded in the Chinese Pharmacopeia Commission [3].

As medicinal seeds contain rich nutrients (such as starch, fat and protein), they are susceptible to fungal contamination in the field or in storage, leading to the production of mycotoxins [4]. Mycotoxin contamination in medicinal seeds is a common problem affecting the quality and safety of medicinal seeds. At present, more than 400 mycotoxins in herbal medicines have been discovered and their occurrence has been reported [5,6]. Aflatoxin B1 (AFB1) content levels ranged from 1.22 μg/kg to 23.17 μg/kg and the total AFs ranged from 1.22 μg/kg to 25.01 μg/kg in Platycladi Semen [7]. AFB1 and ochratoxin A (OTA) were only detected in platycladi seeds (52.0 μg/kg) and tangerine seeds (92.3 μg/kg), respectively [4]. In another study, high AFB1 levels (5.61–27.8 μg/kg) in platycladi seeds were detected [8]. The Chinese Pharmacopeia has set the maximum acceptable level of 5 μg/kg for AFB1 and 10 μg/kg for the sum of AFs (AFB1, AFB2, AFG1 and AFG2) in 24 herbal medicines [3]. However, no standard level is available for OTA and fumonisins (FBs) contents. Therefore, simultaneous determination of mycotoxins (AFB1, AFB2, AFG1, AFG1, OTA, FB1 and FB2) in medicinal seeds should be performed.

The toxigenic fungi belonging to *Aspergillus*, *Penicillium* and *Fusarium* could produce AFs, OTA and FBs [9]. At present, comprehensive analysis of the isolation and molecular identification of mycotoxigenic fungi have been widely used in herbs [10,11]. However, the culture and isolation of fungi are always involved in complicated steps and time-consuming. Moreover, this process is low-throughput and it leads to the loss of unculturable strains. High-throughput sequencing can provide mass data of the microbial community in low abundances and has been extensively applied in fungal studies [12,13], thereby providing a new promising prospect to study the fungal diversity and composition of surface microbiome in herbs.

Semen Persicae (Tao Ren in Chinese), the dry mature fruit-kernel of peach (*Prunus persica* L. or *Prunus davidiana* CARR.), is one example of medicinal and edible seeds. Except for its edible usages, Semen Persicae is a representative herb for invigorating blood circulation and eliminating stasis, such as anticoagulant, antiphlogistic, and anodyne [14]. Semen Persicae is commonly produced in Hebei, Shanxi, Shandong, Henan, Sichuan and Yunnan provinces of China, and it is sold worldwide. It is commonly used in many herbal medicine formulations, such as 50 Chinese patent medicine preparations in National Essential Drugs and 47 preparations in the Pharmacopeia of the People's Republic of China [15]. Semen Persicae samples are prone to fungal and mycotoxin contamination if they are improperly processed and stored. However, the relationship between mycotoxin contents and fungal abundances in Semen Persicae has not reported.

In this study, ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry (UPLC-QQQ-MS) and high-throughput sequencing (HTS) were conducted to determine the contents of mycotoxins and the abundances of fungal communities in Semen Persicae samples. This study aimed to (i) evaluate the occurrence of mycotoxin contamination in Semen Persicae samples; (ii) observe significant differences in the fungal diversity, composition and network amongst different Semen Persicae samples; and (iii) predict the fungi that potentially correlated with mycotoxin contents. This work systematically investigated the occurrence of mycotoxins and fungal communities in Semen Persicae, which could provide useful information for early effective prevention and control of mycotoxin contamination in Semen Persicae samples.

2. Methods and materials

2.1. Sampling

Twenty-one Semen Persicae samples were collected from main producing areas throughout China (Table 1). All collected samples were placed in sterile paper bag as soon as possible to avoid second contamination. The Semen Persicae samples were used to determine mycotoxin contents and fungal abundances. These samples were divided into seven groups, including Dalian of Liaoning (TR4), Baotou of Innermongolia (TR41), Langfang of Hebei (TR43), Linyi of Shandong (TR52), Xian of Shanxi (TR54), Chifeng of

Table 1
Voucher information and levels of mycotoxins in Semen Persicae samples (x ± S).

Voucher number	Sources	AFB1 (μg/kg)	AFB2 (μg/kg)	AFG1 (μg/kg)	AFG2 (μg/kg)	OTA (μg/kg)	FB1 (μg/kg)	FB2 (μg/kg)	Total mycotoxins (μg/kg)	Mycotoxins profile
TR4	Dalian, Liaoning	35.67 ± 2.17	4.82 ± 0.36	–	–	–	–	–	40.49 ± 4.35	MC
TR41	Baotou, Innermongolia	–	–	–	–	–	–	–	–	MR
TR43	Langfang, Hebei	–	–	–	–	–	–	–	–	MR
TR52	Linyi, Shandong	–	–	–	–	–	–	–	–	MR
TR54	Xian, Shanxi	48.37 ± 3.28	–	3.65 ± 2.89	–	–	34.61 ± 2.16	–	86.63 ± 5.52	MC
TR71	Chifeng, Innermongolia	–	–	–	–	–	–	–	–	MR
TR95	Yantai, Shandong	26.48 ± 3.62	1.47 ± 0.08	–	–	91.02 ± 3.20	–	–	118.97 ± 4.79	MC

Note:- , MC and MR represent not detected, mycotoxin-contaminated and mycotoxin-free, respectively.

Innermongolia (TR71) and Yantai of Shandong (TR95), on the basis of production area. Meanwhile, these samples were further divided into two groups, namely, mycotoxin-contaminated (MC) and mycotoxin-free (MR), in accordance with the presence of mycotoxins.

2.2. Mycotoxin content analysis of Semen persicae samples

Six standard substances AFB1 (1162-65-8), AFB2 (7220-81-7), AFG1 (1165-39-5), AFG2 (7241-98-7), FB1 (116,355-83-0), and FB2 (116,355-84-1) were prepared. The stock solution of AFB1, AFB2, AFG1, AFG2, FB1, and FB2 were 1 mg/mL, then diluted to 100 μg/mL for the calculation of the mycotoxin content in our samples.

Approximately 5 g of Semen Persicae sample was weighed and 25 mL of 75% (v/v) methanol was added; the mixture was sonicated for 40 min and centrifuged for 10 min at 10,000 r/min [16]. Then, 5 mL of the supernatant was accurately extracted, diluted with 20 mL of water and passed through the HLB column at the flow rate of 2 mL/min. The column was rinsed with 10 mL of distilled water. The eluate was diluted with 2 mL of methanol, passed through 0.22 μm filter and injected into UPLC-MS/MS to determine the mycotoxin contents.

Chromatographic separations were performed with an ZORBAX Eclipse Plus C18 column (100 mm × 3 mm, 1.8 μm, Agilent, USA). The column temperature for AFs and OTA was 30 °C, the injection volume was 1 μL and the flow rate was 0.4 mL/min [17]. The mobile phase consisted of 0.1% HCOOH methanol (A) and 0.1% HCOOH water (B). The gradient elution program was as follows: 0–3.5 min, 45% A; 3.5–4.5 min, 45%–75% A; and 4.5–10 min, 75% A. The column for FBs was 35 °C, the flow rate was 0.3 mL/min and the injection volume was 1 μL [18]. The mobile phase was composed of methanol–acetonitrile (v/v, 50/50; A) and 0.1% HCOOH water (B) and the gradient was set as follows: 0–2.3 min, 30%–70% A; 2.3–4.0 min, 70% A; and 4–4.2 min, 70%–100% A.

Multiple reaction monitoring mode and the positive electrospray ion polarity were employed. High-purity nitrogen was used as the drying and ionisation gas. The ionisation source conditions were as follows: the capillary voltage was 2.50 kV, the source temperature was 150 °C and the desolvation temperature was 350 °C. The parameters on the *m/z* and collision energy of parent ions, primary daughter ions and secondary daughter ions of mycotoxins are shown in Table S1.

2.3. HTS analysis of Semen persicae samples

Approximately 5 g of Semen Persicae sample was placed into 50 mL sterilized centrifuge tube, added with 10 mL of sterilized water and shaken for 30 min. Then, the mixture was filtered to remove epidermis and centrifugated for 20 min at 7830 rpm to collect surface microorganisms (5920 R, Eppendorf, Germany) [19].

Total DNA was extracted using PowerWater DNA Isolation Kit (MPBIO, USA) in accordance with the manufacturer's instruction and the DNA quality and quantity were determined with NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) [20]. The internal transcribed spacer (ITS) sequences were amplified with fungal primers ITS7F/ITS4R. The amplification procedure was as follows: 30 s of initial denaturation at 98 °C; followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min. The PCR products were purified and sequenced on an Illumina PE 250 platform (Illumina, USA) and 250 bp paired-end reads were generated [21]. The raw sequencing data are publicly available in the National Center for Biotechnology Information with the accession number SAMN26902968-SAMN26902988.

Raw sequences with ambiguous bases were discarded and quality filtering and trimming were conducted by Quantitative Insights into Microbial Ecology (QIIME2, version 1.7.0) with the following parameters: truncLen = 210 and 177 for forward and reverse reads, respectively [22]. The obtained amplified sequence variants (ASVs) were subjected to the following taxonomic assignment. The BLASTN algorithm was used to conduct taxonomic classification and the classification of the top hit with an e-value of $\leq 10^{-5}$ and an identity of ≥98% was accepted as the classification of each ASV [23]. The phylogenetic affiliation of ITS representative sequences was investigated against the Unite (version 8.2) databases (release 132) [24].

The obtained ASVs were used to analyze the α and β diversities. The α-diversity indices (Chao 1 and Shannon) were calculated by mothur (version 1.30.1) [25]. A Venn diagram was constructed to explore the unique and shared ASVs amongst different groups [26]. Principal coordinate analysis (PCoA) was performed to estimate the taxonomic dissimilarity on the basis of Bray–Curtis distance matrices [27]. The significant differences in community dissimilarity were calculated by the permutational multivariate ANOVA using the Adonis function [28]. A Circos diagram was established to show the distribution proportion of predominant microbiota [21,29]. Linear discriminant analysis effect size (LEfSe) analysis was conducted to dig for the biomarkers amongst different region groups [$P < 0.05$ and linear discriminant analysis (LDA) > 4] and mycotoxin content groups ($P < 0.05$ and LDA >3) [30]. Co-occurrence analysis was performed on the basis of robust correlations (different region groups, Spearman's $|r| > 0.8$ and $P < 0.05$; different mycotoxin content groups, Spearman's $|r| > 0.8$). Gephi software was used to exhibit the network visualization and property measurements [31]. Spearman correlation analysis was conducted on SPSS 24.0 to explore the relationship between fungi abundances and mycotoxin contents.

3. Results

3.1. Mycotoxin content analysis of Semen Persicae samples

The contents of mycotoxins (AFs, OTA and FBs) in Semen Persicae samples were determined (Table 1). Three (42.86%) of seven Semen Persicae samples were positive for AFB1 and the levels ranged from 26.48 μg/kg to 48.37 μg/kg, exceeding the permissible limits of AFB1 (5 μg/kg) in the Chinese Pharmacopeia. Two (28.57%) of seven Semen Persicae samples were positive for AFB2 and the

levels ranged from 1.47 μg/kg to 4.82 μg/kg. OTA and FB1 were only presented in one sample (91.02 and 34.61 μg/kg, respectively). TR4 samples were simultaneously contaminated with AFB1 (35.67 μg/kg) and AFB2 (4.82 μg/kg). TR54 samples were simultaneously contaminated with AFB1 (48.37 μg/kg), AFB2 (3.65 μg/kg) and FB1 (34.61 μg/kg). TR95 samples were simultaneously contaminated with AFB1 (26.48 μg/kg), AFB2 (1.47 μg/kg) and OTA (91.02 μg/kg).

3.2. Comparison of fungal communities in Semen Persicae samples based on sampling location

A total of 1,670,548 ITS sequences (200–500 bp) were generated from 21 Semen Persicae samples. The Chao 1 and Shannon indices of fungal community were significantly higher in the TR4, TR41 and TR43 groups than in the other groups ($P < 0.05$; Fig. 1A and B and Table S2). The ITS reads were subsequently divided into 2944 ASVs after cluster analysis (Fig. 1C). Amongst the ASVs, 461, 481, 538, 299, 355, 458 and 307 were especially unique for TR4, TR41, TR43, TR52, TR54, TR71 and TR95 groups, respectively, whilst the remaining 45 ASVs were shared by the groups. For β-diversity, as revealed by PCoA, different degrees of clustering was also observed in Semen Persicae samples on the basis of sampling location (Adonis, $R^2 = 0.88$, $P < 0.001$; Fig. 1D).

ASVs were assigned to different taxonomic levels to further understand the exact composition of microbiota at different sample locations. At the phylum level, Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota were obtained (Figs. 1E and 2A and Table S3). Ascomycota and Basidiomycota were the most predominant phyla and they presented significant region-specific effect ($P < 0.05$), with an average of 75.54% and 16.16%, respectively. Ascomycota had the highest abundance in the TR95 samples and the lowest in the TR54 samples. Basidiomycota had the highest proportion in the TR54 samples and the lowest in the TR71 samples. At the genus level, *Wallemia*, *Candica*, *Saccharomyces* and *Aspergillus* were the most abundant genera and they presented significant region-specific effect ($P < 0.05$), with relative abundances of 0.23%–49.28%, 0.09%–52.43%, 0.09%–31.92% and 0.06%–16.89%, respectively (Fig. 1F and 2B and Table S3). Amongst them, *Aspergillus*, *Candida* and *Saccharomyces* were significantly enriched in the TR54, TR52 and TR95 groups, respectively.

LEfSe revealed the differences in community composition amongst different groups from phylum to species (Fig. 2C and S1). Amongst the 193 biomarkers (LDA >4), 57, 29, 43, 11, 16, 21 and 16 were enriched in the TR4, TR41, TR43, TR52, TR54, TR71 and TR95 groups, respectively. The class Sordariomycetes, the order Hypocreales, the families Saccharomycetaceae and Nectriaceae and the genus *Gibberella* were enriched in the TR4 group. The class Agaricomycetes, the families Hypocreales Incertae sedis and Botryosphaeriaceae and the genus *Trichothecium* were enriched in the TR41 group. The class Dothideomycetes, the order Pleosporales,

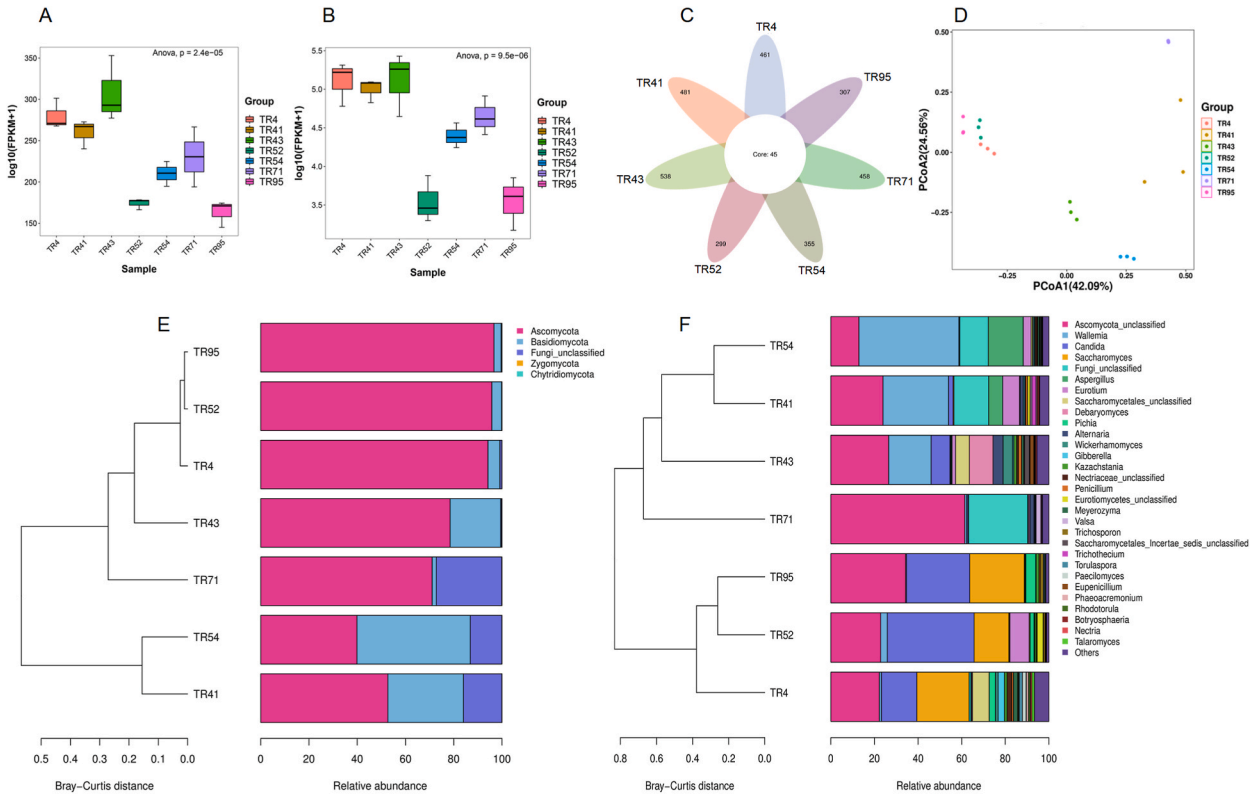

Fig. 1. Diversity and composition of fungal community in Semen Persicae samples based on sampling location. (A) Chao 1 index. (B) Shannon index. (C) Venn diagram. (D) PCoA analysis based on Bray–Curtis distance matrices. (E) Fungal composition at the phylum level. (F) Fungal composition at the genus level.

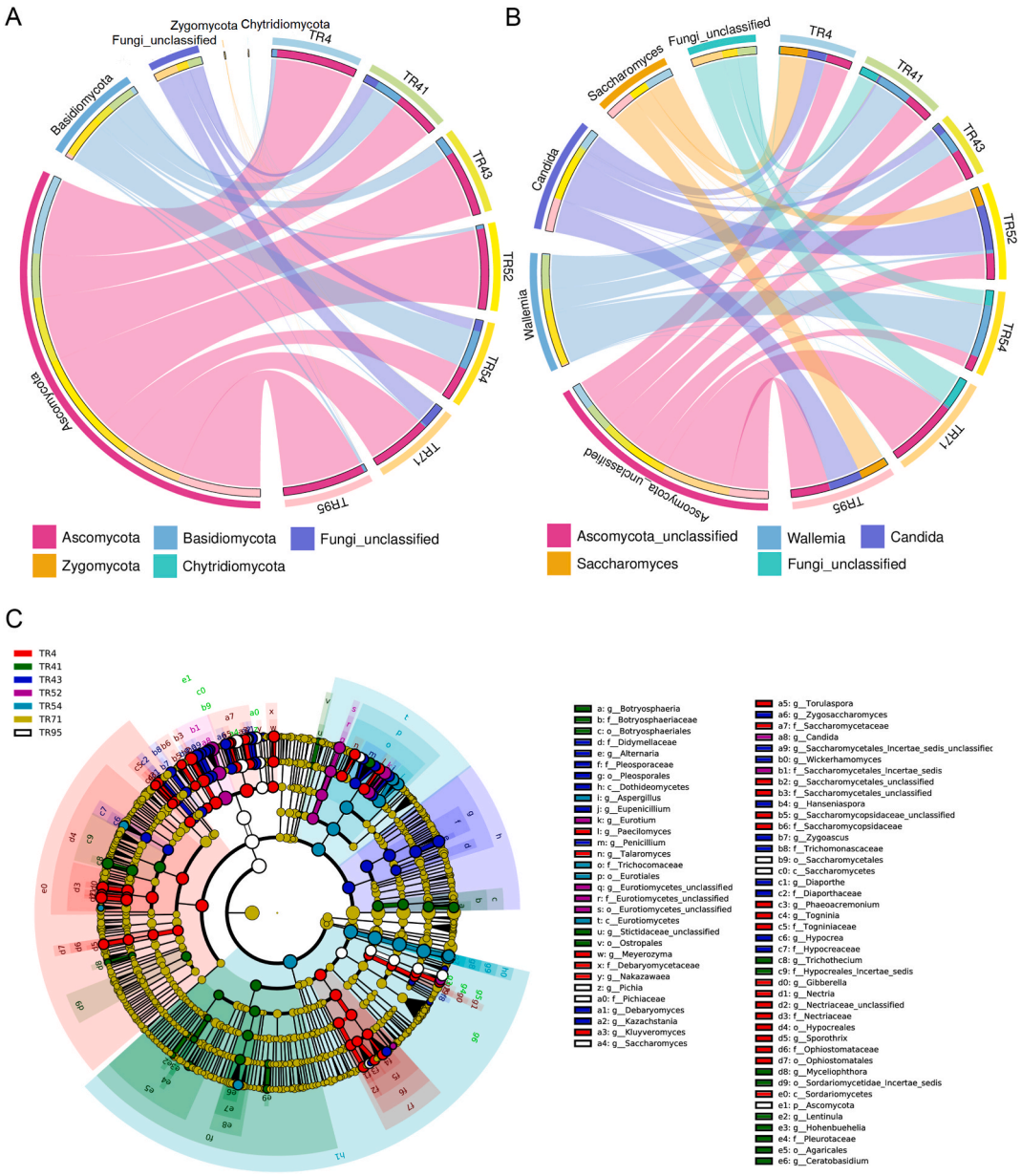

Fig. 2. Circos plot and linear discriminant effect size (LEfSe) of fungal community in Semen Persicae samples based on sampling location. (A) Circos plot of core fungal taxa at the phylum level. (B) Circos plot of core fungal taxa at the genus level. (C) LEfSe with linear discriminant analysis (LDA) score higher than 4.0 and *P*-values less than 0.05.

the genus *Debaryomyces* and the species *Debaryomyces hansenii* were enriched in the TR43 group. The family Hypocreales Incertae sedis, the genera *Candida* and *Eurotium* and the species *Candida humilis* and *Aspergillus reptans* were enriched in the TR52 group. The class Wallemiomycetes, the order Wallemiales, the family Wallemiaceae and the genus *Wallemiales* were enriched in the TR54 group. The order Diaporthales, the family Valsaceae and the genus *Valsa* were enriched in the TR71 group. The phylum Ascomycota, the class Saccharmycetes, the order Saccharomycetales and the genus *Saccharomyces* were enriched in the TR95 group.

The co-occurrence patterns of microbial communities amongst different regions showed the effects of region on network complexity (as indicated by average degree) and connectivity (Fig. 3 and Table S4). The average path length and average clustering coefficient were highest in the TR52 group. The values of topological properties (i.e. average degree, number of nodes, number of edges, positive edges, average weighted degree and total triangles) were highest in the TR43 group. Meanwhile, modularity, density and modularity with resolution were highest in the TR54 group. The value of negative edges was detected to be highest in the TR71 group.

3.3. Comparison of fungal communities in Semen Persicae samples based on the presence of mycotoxins

The diversity and composition of fungal communities were compared between the MC and MR groups. The Chao 1 and Shannon indices in the MR group (252.01 ± 45.78, 4.79 ± 0.37) were significantly higher than those in the MC group (205.79 ± 57.22, 4.06 ± 0.82; $P < 0.05$; Fig. 4A and B and Table S5). Venn profile showed that 351 ASVs were shared in the MR and MC groups, with 860 and 400 unique ASVs, respectively (Fig. 4C). Significant differences in β-diversity were observed between the MR and MC groups (Adonis, $R^2 = 0.09$, $P < 0.05$; Fig. 4D).

The community and composition at the phylum and genus levels were compared. Greater phylum and genus diversity were found in the MR group than that in the MC group (Fig. 4E, F, 5A and 5B). At the phylum level, the relative abundance of Ascomycota was significantly higher (95.56% ± 2.06%) in the MC group than that in the MR group (64.52% ± 17.02%; $P < 0.05$; Fig. 4E and 5A and Table S6). By contrast, the relative abundance of Basidiomycota were significantly higher in the MR group (25.27% ± 18.78%) than in the MC group (4.00% ± 1.93%). The top 30 abundant genera are shown in Fig. 4F and 5B and Table S6. The relative abundances of *Candida*, *Saccharomyces* and *Aspergillus* were significantly ($P < 0.05$) higher in the MC group (28.38% ± 15.03%, 21.70% ± 5.67% and 7.54% ± 6.74%, respectively) than in the MR group (2.90% ± 1.25%, 0.27% ± 0.25% and 0.24% ± 0.11%, respectively). By contrast, the relative abundance of *Wallemia* was significantly higher in the MR group (24.06% ± 3.82%) than in the MC group (1.45% ± 1.57%, $P < 0.05$).

LEfSe revealed the differences in community composition amongst the MR and MC groups from phylum to species (Fig. 5C and S2). Amongst the 79 biomarkers (LDA >3.0), 42 and 37 were enriched in the MR and MC groups, respectively. The class Wallemiomycetes, the order Wallemiales, the family Wallemiaceae, the genus *Wallemia* and the species *Wallemia sebi* were enriched in the MR group. Meanwhile, the phylum Ascomycota, the order Saccharomycetales, the class Asccharomycetes, the family Saccharomycetales Incertae sedis, the genus *Candida* and the species *Candida humilis* were enriched in the MC group.

The co-occurrence patterns of microbial communities between the MC and MR groups showed the effects of mycotoxins on network complexity (as indicated by average degree) and connectivity (Fig. 6 and Table S7). The average degree was higher in the MR group (8.32) than that in the MC group (6.06). The values of topological properties (i.e. average weighted degree, density and total triangles) were higher in the MC group (8.32, 8.35, 0.02 and 1320, respectively) than that in the MR group (6.06, 7.99, 0.02 and 1181, respectively). By contrast, the values of other topological properties (i.e. nodes, edges, positive edges, modularity, average path length and average clustering coefficient) were detected to be higher in the MR group (254, 770, 771, 0.99, 4.59 and 0.85, respectively) than that in the MC group (169, 703, 632, 0.89, 3.04 and 0.78, respectively).

3.4. Fungi related to mycotoxin contents in Semen Persicae

Spearman correlation analysis showed that mycotoxin contents were significantly correlated with the abundances of fungi ($P <$

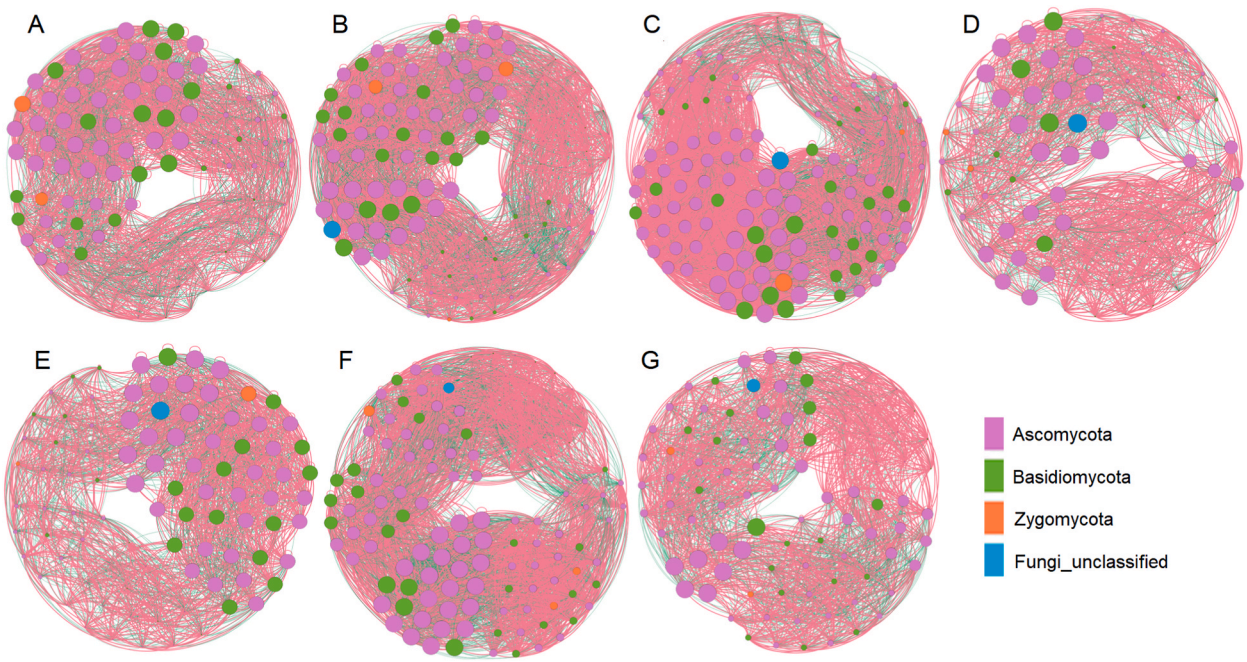

Fig. 3. Co-occurrence network analysis of microbial communities in Semen Persicae samples based on sampling location (Spearman's $|r| > 0.8$). (A) TR4 group. (B) TR41 group. (C) TR43 group. (D) TR52 group. (E) TR54 group. (F) TR71 group. (G) TR95 group. Red and green represent positive and negative links, respectively.

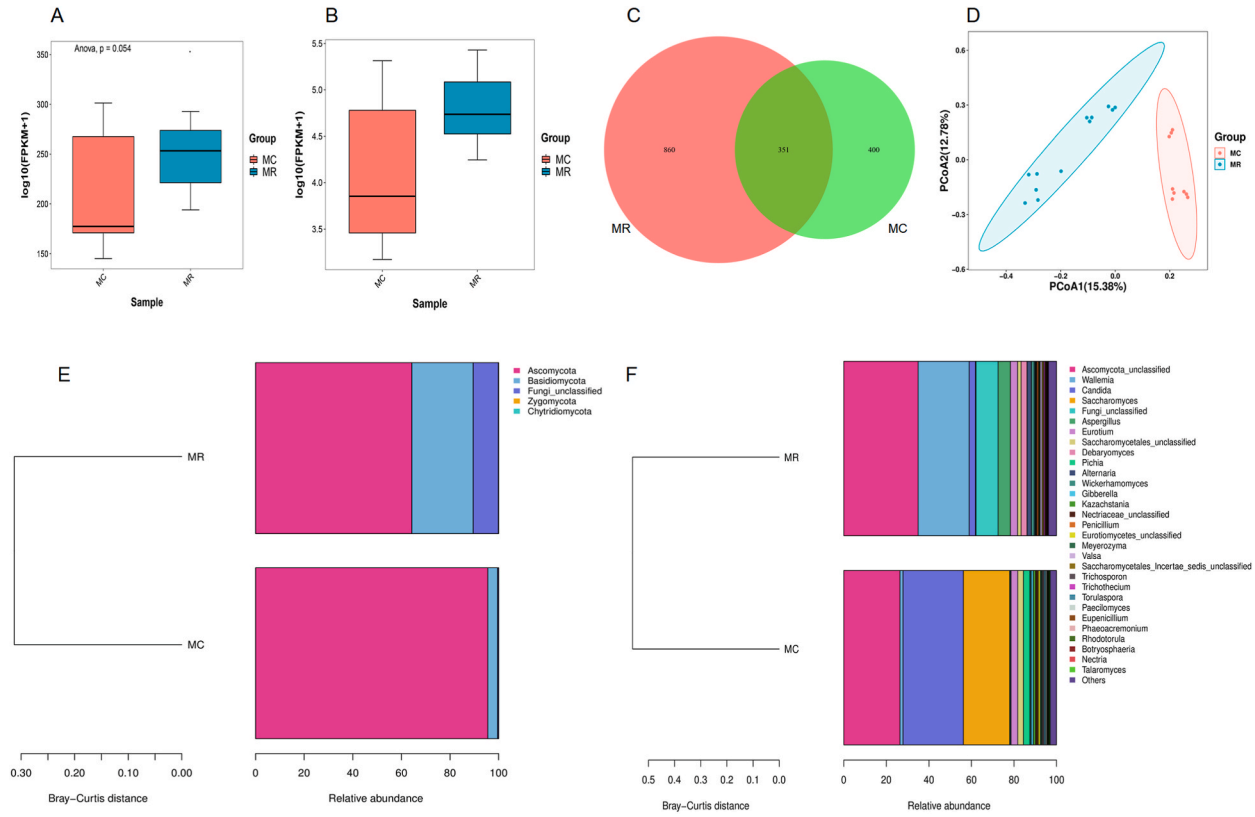

Fig. 4. Diversity and composition of fungal community in Semen Persicae samples according to the presence of mycotoxins. (A) Chao 1 index. (B) Shannon index. (C) Venn diagram. (D) PCoA analysis based on Bray–Curtis distance matrices. (E) Fungal composition at the phylum level. (F) Fungal composition at the genus level.

0.05; Fig. 7). Amongst them, AFB1 contents were positively and significantly correlated with the fungal genera *Aspergillus*, *Paecilomyces*, *Rhodotorula* and *Talaromyces* (R = 0.75, 0.63, 0.61 and 0.68, respectively). AFB2 contents were positively correlated with the fungal genera *Saccharomyces*, *Pichia*, *Torulaspora* and *Rhodotorula* (R = 0.76, 0.76, 0.80 and 0.80, respectively). AFG1 contents were positively and significantly correlated with *Wallemia*, *Candida* and *Aspergillus* (R = 0.63, 0.62 and 0.68, respectively). OTA were positively and significantly correlated with *Saccharomyces*, *Aspergillus* and *Pichia* (R = 0.67, 0.68 and 0.69, respectively). Meanwhile, positive correlations were observed between FB1 and *Wallemia* (R = 0.67) and *Aspergillus* (R = 0.61). Meanwhile, the total mycotoxin contents were positively and significantly correlated with *Torulaspora* (R = 0.52) and *Rhodotorula* (R = 0.65).

4. Discussion

Mycotoxin contamination is a considerable and public concern in medicinal herbs [32]. Mycotoxins are secondary metabolites of fungi, therefore, studying the relationship between fungi and mycotoxins is necessary. Here, UPLC-QQQ-MS and HTS were used to investigate the occurrence and frequency of mycotoxin and fungi in medicinal and edible seed Semen Persicae collected from different provinces. The data showed that 42.86% of samples were positive for AFB1 and the levels ranged from 26.48 μg/kg to 48.37 μg/kg, exceeding the permissible limits of AFB1 in the Chinese Pharmacopeia. Meanwhile, 28.57% of samples were positive for AFB2 and the levels ranged from 1.47 μg/kg to 4.82 μg/kg. OTA and FB1 were only detected in one sample (91.02 and 34.61 μg/kg, respectively). A worldwide survey of mycotoxin prevalence revealed that eight (16.67%) of 48 root herbs were simultaneously positive for AFs and OTA and seven (14.58%) and four (8.33%) samples of ginseng and polygala exceeded the permissible limits of AFB1 and AFs, respectively [33]. In another paper, the average levels (incidence) of AFB1, AFB2, AFG1 and AFG2 in herbal medicines were 1.40 (68.8%), 1.27 (50.0%), 0.50 (43.8%) and 0.94 (43.8%) μg/kg, respectively, and AFM1 was detected with maximum concentrations of 0.70 μg/kg [34]. The above results showed the urgent need to monitor mycotoxin contamination in herbal medicines.

Fungal contamination in medicinal herbs has received considerable attention from the public and it has been reported worldwide [5,35]. Next-generation sequencing, especially HTS technology, has been applied to analyze the diversity and abundance of microbiome with low abundances in herbs [36]. In the present study, Ascomycota, Basidiomycota, *Wallemia*, *Candica*, *Saccharomyces* and *Aspergillus* were the predominant fungi in Semen Persicae, which was consistent with the results of previous studies [37,38]. The Chao 1 and Shannon indices of fungal community were significantly higher in the TR4, TR41 and TR43 groups than that in other groups. The fungal community structure between herbal markets and producing areas were compared and the results showed that the community

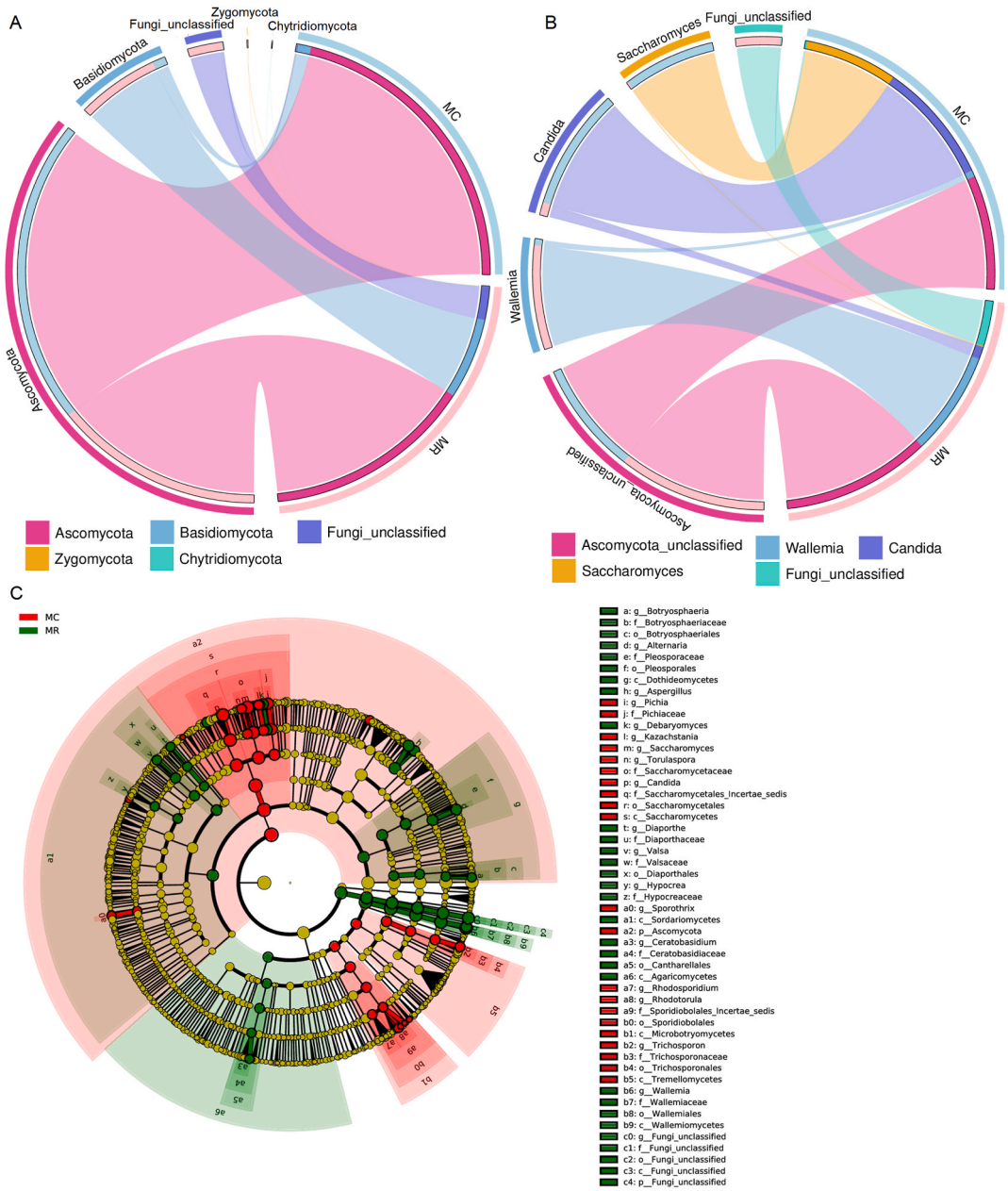

Fig. 5. Circos plot and linear discriminant effect size (LEfSe) of fungal community in Semen Persicae samples according to the presence of mycotoxins. (A) Circos plot of core fungal taxa at the phylum level. (B) Circos plot of core fungal taxa at the genus level. (C) LEfSe with linear discriminant analysis (LDA) score higher than 3.0 and *P*-values less than 0.05.

diversity and richness in producing areas were higher than those in herbal markets [38]. Ascomycota had the highest abundance in the TR95 samples, Basidiomycota and *Aspergillus* accounted for the highest proportion in the TR54 samples and *Candida* accounted for the highest proportion in the TR54 samples. The genera *Aspergillus* and *Candida* are potential mycotoxin-producing and/or human pathogenic fungi and considered possible producers of mycotoxins, including AFs and OTA [39]. This finding is the reason that these samples exhibit high mycotoxin contents. In the present study, the network profiles from the sampling location presented distinct complexity and connectivity. Network analysis was conducted to explore the relationships amongst the fungi of Myristicae Semen and the results showed numerous strong correlations amongst 20 abundant OTUs [38]. The differences in microbial diversity and composition from different regions were caused by their varying growth environments, processing procedures and storage conditions. In addition, different batches of Semen Persicae samples collected from the same sampling location may have different microbial diversity and composition. However, this study ignored the batch problem, which is the limitations of the study. For the accuracy of the experiment, the problem of batches, processing procedures and storage conditions should be considered in future experiments.

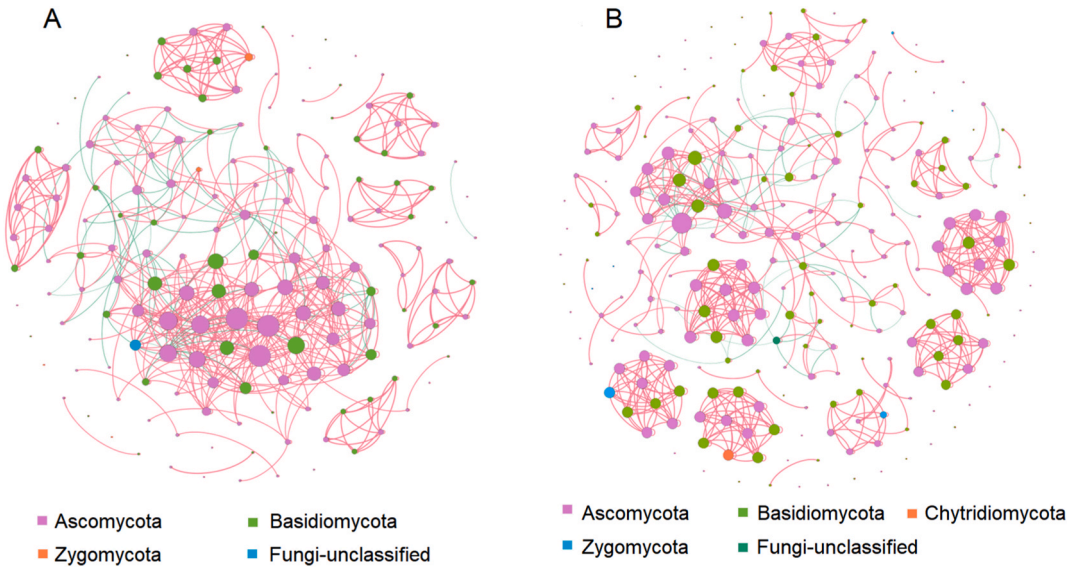

Fig. 6. Co-occurrence network analysis of microbial communities in Semen Persicae samples according to the presence or absence of mycotoxins (Spearman's $|r| > 0.8$ and $P < 0.05$). (A) Mycotoxin-contaminated group. (B) Mycotoxin-free group. Red and green represent positive and negative links, respectively.

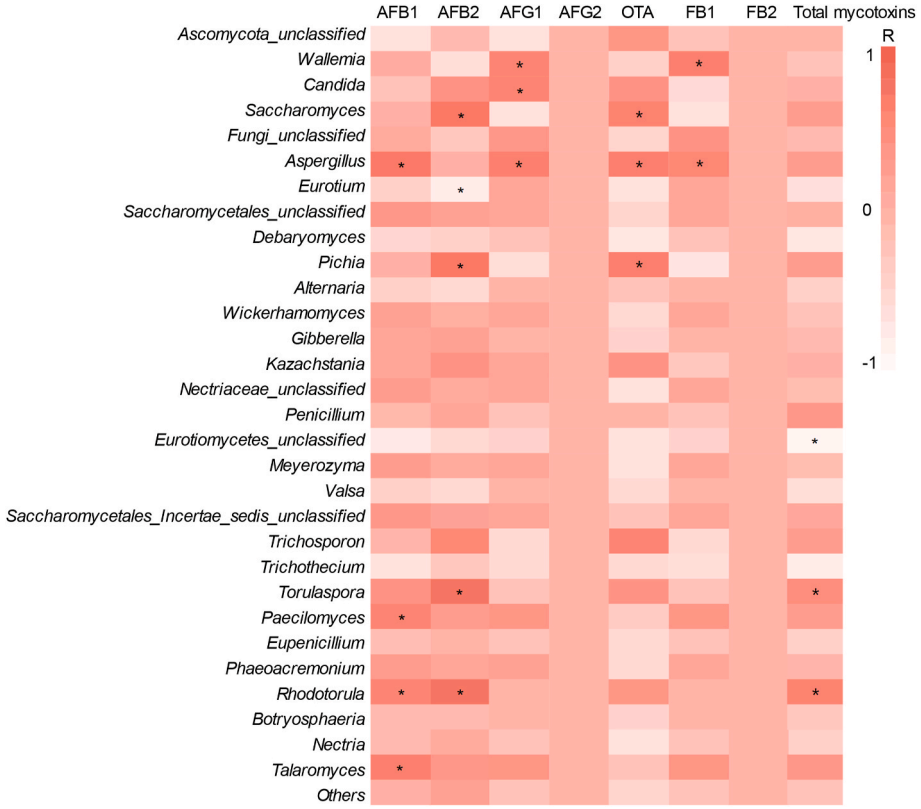

Fig. 7. Fungi related to mycotoxin contents based on Spearman's correlation analysis. * represents significant difference at $P < 0.05$.

In addition, significant differences of fungal diversity, composition and network between the MC and MR groups. The Chao 1 and Shannon indices of fungal microbiome in the MC group were higher than those in the MR group, which is consistent with the findings of other studies [7]. Fungal microbiome diversity could be reduced in the presence of AF contamination [40]. According to the LEfSe

analysis, a total of 79 biomarkers were obtained, amongst which 42 and 37 were enriched in the MR and MC groups, respectively. Ascomycota, *Wallemia* and *Aspergillus* were significantly higher in the MC group than that in the MR group. In *Platycladus orientalis*, the relative abundance of *Aspergillus* in aflatoxin-contaminated group was higher than that in aflatoxin-free group [40]. Mycotoxins were produced by fungi, such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* [41]. The reason may be the high mycotoxin contents in the MC group. In addition, the MR group exhibited more complex and highly connected network than the MC group. The links amongst genera in the network in the present study were principally positive and they showed potential for extensive cooperative interactions amongst most taxa in their respective micro-environments [42]. Therefore, mycotoxin contamination reduced the structure of microbial networks.

5. Conclusions

This work systematically provided information about the occurrence of mycotoxins and fungi in medicinal and edible seed Semen Persicae. A relatively large number of Semen Persicae was found to be contaminated by mycotoxins (AFs, OTA and FBs) and fungi (Ascomycota, Basidiomycota, *Wallemia*, *Candica*, *Saccharomyces* and *Aspergillus*). Furthermore, the mycotoxin AF, OTA and FB contents were positively correlated with the abundances of *Aspergillus*, *Rhodotorula*, *Wallemia* and *Candida.* The data may be helpful in identifying medicinal seeds that are more commonly associated with fungi and mycotoxin contamination, thus providing useful information for effective prevention and control strategies to ensure the safety of medicinal and edible seeds.

Authors contribution statement

Shilin Chen and Linlin Dong conceived and designed the research; Guangfei Wei, Bo Zhang, Yichuan Liang, and Zhaoyu Zhang performed the experiments; Conglian Liang, Lan Wu, and Huatao Yu analyzed the data; Guangfei Wei, Yongqing Zhang, and Linlin Dong wrote the manuscript. All authors read and approved the final manuscript.

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Data availability statement

The ITS rRNA gene raw sequence data were submitted to the NCBI SAR database (http://www.ncbi.nlm.nih.gov/) under accession numbers SAMN26902968-SAMN26902988.

Declaration of competing interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19796.

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