Production profile and comparison analysis of main toxin components of Fusarium oxysporum f. sp. sesami isolates with different pathogenicity levels

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Abstract

Fusarium wilt is a critical fungus disease for sesame, which is induced by *Fusarium oxysporum* f. sp. *sesami* (*FOS*). In order to determine the toxin production profiles of the *FOS* isolates with different pathogenicity levels under various culture conditions, we evaluated the content variation of fusaric acid (FA) and 9, 10-dehydrofusaric acid (9, 10-DFA) produced by the 4 representative *FOS* isolates. Results indicated that the maximum amount of FA reached to 2848.66 μg/ml in Czapek medium, while 9, 10-DFA was mainly produced in Richard and Low-carbon Richard medium. The concentration of 9, 10-DFA on Richard culture medium varied from 0 μg/ml to 716.89 μg/ml. Of the 5 kinds of culture media, Czapek culture medium was the most conductive to produce FA. FA production was significantly affected by culture medium, culture time, and their interaction (***, $P < 0.001$). The results suggest that there is no correlation between toxin production and pathogenicity level of *FOS* isolates. The findings give key information for the mechanism analysis of *FOS*- sesame interaction and pathogen control.

Introduction

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop known to humans and is valuable for the high oil quality of seeds (Ashri 1998). Sesame is mainly grown in the tropical and sub-tropical areas of the world (Ashri 1998). However, the inherent low resistance to biotic stresses (e.g., *Fusarium* wilt and charcoal rot diseases) and abiotic stresses (e.g., waterlogging, logging, and low temperature) often results in low productivity of the world sesame (Jyothi et al. 2011; Zhang et al. 2019).

Sesame *Fusarium* wilt disease (SFW) is one of the most critical fungus diseases for sesame, which is caused by *Fusarium oxysporum* f. sp. *sesami* (*FOS*). *FOS* pathogen commonly infects sesame roots from seedling stage to flowering stage. The susceptible plants would wilt to death (Su et al. 2012; Miao et al. 2020). Recently, a number of *FOS* isolates have been identified from wilted sesame plants and their morphological and pathogenic characteristics were systematically described (Su et al. 2012; Qiu et al. 2014; Duan et al. 2020). Based on the pathogenicity level and diversity of effector genes of *FOS* isolates, Duan et al. (2020) grouped the 69 *FOS* isolates collected from major sesame-growing areas in China into three subgroups using three hosts (i.e., Yuzhi 11, Ji 9014, and HJ 16) according to the pathogenicity level for the first time. As to the subgroup with high pathogenicity, all tested sesame cultivars presented susceptible. Only wild species such as *S. radiatum* and *S. angustifolium* exhibited the stable and high resistance to SFW (Miao et al. 2022). Thus, deficiency of breeding materials with high resistance to *FOS* limits the progress in sesame disease resistance breeding. Indistinct relationship between pathogenic variation and toxin production of different *FOS* isolates also impedes the mechanism analysis of *FOS* pathogen and sesame interaction and the technical development of *FOS* pathogen control.

Reports show that many *Fusarium* species produce mycotoxin FA as secondary metabolites and exhibit the virulence during inoculation with crops (Bacon et al. 1996; Abouzeid et al. 2004; Bani et al. 2014; Ding et al. 2018). FA can promote many physiological responses in plant cells such as cell growth, mitochondrial activity, and membrane permeability (Bouizgarne et al. 2006; Jiao et al. 2013). In previous years, sesame scientists detected FA and FA-2H, FA + O, and FA + 2O-H as *FOS* toxins from culture media (Zhu et al. 2016; Li et al. 2017). Li et al. (2017) successfully isolated FA and FA analogue 9, 10-DFA and analyzed the virulence of them to sesame seedings for the first time. Inoculation results indicated that *FOS* filtered solutions containing FA and FA analogues inhibited the growth and development of sesame seedlings (Zhu et al. 2016).

In this study, we compared toxin amounts produced by four *FOS* isolates with different pathogenic levels and analyzed the influence of culture medium types on toxin composition and production. We determined the relationship between the amount of FA and 9, 10-DFA and the virulence of *FOS* isolates. The findings give meaningful information for revealing the inoculation mechanisms of *FOS* with sesame and *Fusarium* wilt control techniques.

Materials And Methods

Fungal strain and culture medium

Four *FOS* isolates including HSFO 07011, HSFO 07021, HSFO 10001, and HSFO 10008 were chosen from *FOS* isolates reservoir for toxin production analysis (Table 1). Microconidia of all isolates were stored in 30% glycerol suspension at -70ºC. All *FOS* isolates were kindly provided by Henan Sesame Research Center, Henan Academy of Agricultural Sciences (HRSC, HAAS), China.

Table 1
Origin and comparison of growth characters of the 4 *FOS* isolates

FOS isolate no.	Collection origin	Collection year	Colony diameter for 4 days culture (cm)	Colony diameter for 4 days culture (cm)	Colony surface color	Colony bottom color	Colony texture	Microconidium size (length × width) (μm)	Macroconidium size (length × width) (μm)	Chlamydospore diameter (μm)
HSFO 07011	Henan	2007	4.79 ± 0.03	5.88 ± 0.21	Pink white	Pink white	Cotton wool	(5.00-11.94) × (2.19–3.69)	(19.61–36.73) × (3.07–4.64)	8.79 ± 1.33
HSFO 07021	Henan	2007	4.94 ± 0.14	6.37 ± 0.23	Pink	Red	Wool-like	(7.24–12.58) × (2.56–3.94)	(19.36–28.03) × (2.91–4.35)	7.66 ± 0.80
HSFO 10001	Liaoning	2010	4.23 ± 0.05	5.60 ± 0.18	Red	Red	Felted	(5.79–14.25) × (1.84–3.04)	(18.52–29.86) × (2.37–4.48)	8.73 ± 0.92
HSFO 10008	Liaoning	2010	4.04 ± 0.26	5.31 ± 0.43	White	Pink white	Sparse	(6.21–10.13) × (1.96–2.86)	(18.22–28.84) × (2.11–4.25)	---

Values represent the mean value ± SD (Standard Deviation).

Five kinds of culture media used to analyze *FOS* culture condition include: (1) PD culture medium: potato 150 g, glucose 20 g, distilled water fixed volume to 1000 ml. (2) Richard medium: KNO_3 10 g, KH_2PO_4 5 g, $MgSO_4{\cdot}7H_2O$ 2.5 g, $FeCl_3$ 0.02 g, sucrose 50 g, distilled water fixed volume to 1000 ml. (3) Low-carbon Richard medium: KNO_3 10 g, KH_2PO_4 5 g, $MgSO_4{\cdot}7H_2O$ 2.5 g, $FeCl_3$ 0.02 g, glucose 18 g, distilled water fixed volume to 1000 ml. (4) Czapek medium: $NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g, sucrose 30 g, distilled water fixed volume to 1000 ml. (5) Armstrong medium: KH_2PO_4 1.1 g, $MgSO_4{\cdot}7H_2O$ 0.4 g, KCl 1.6 g, $Ca(NO_3)_2$ 5.9 g, $ZnSO_4$ 0.2 mg, $FeCl_3$ 0.2 mg, $MnSO_4$ 0.2 mg, glucose 20 g, distilled water fixed volume to 1000 ml.

Morphological and pathogenic characteristics investigation of FOS isolates Morphological and growth characteristics of the 4 *FOS* isolates were investigated on PDA medium according to Su et al. (2012). Pathogenicity of *FOS* isolates were evaluated with four sesame varieties including Yuzhi 11, Zhengzhi 98N09, Ji 9014 and Rongxian black sesame according to the method of Duan et al. (2020). Ten seeds of each variety were sowed in a 12-cm-diameter plastic pot with a mixture of conidial suspensions, sterilized vermiculite, and soil (1:2:6, v/v/v). Initial concentration of *FOS* isolates was set at 1×10^6 conidium/ml. Sterile distilled water was used as control. Four replications for each variety were set.

Pathogenicity level of *FOS* isolates was divided into four levels according to the grade scale described by Qiu et al. (2014). Level 0: nonpathogenicity, DI = 0. Level 1: weak pathogenicity, $0 \leq DI \leq 20$. Level 2: moderate pathogenicity, $20 < DI \leq 50$. Level 3: high pathogenicity, $50 < DI \leq 100$.

Sesame resistance to each *FOS* isolate was evaluated according to the method of Miao et al. (2019). Growth status of seedlings for each treatment was recorded after 4 weeks inoculation. The categorical disease severity scale was applied as follows: S0 = no visible symptom; S1 = slight wilting on leaf, slight stem shrinking, and reddish brown spots on taproot; S2 = yellow and drying leaves, whole plant wilting or death. The disease index (DI) was calculated using the following formula:

$$DI = \frac{\sum(S_i \times n_i)}{2N} \times 100.$$

Where S_i - disease grade; n_i - corresponding number of diseased plants; N - total number of plants investigated.

Toxin culture and HPLC assay of FA and 9, 10-DFA contents Before performing toxin culture, 0.5 ml microconidia suspension of each *FOS* isolate was pre-cultured on Potato dextrose agar (PDA) (Difco, Detroit, MI) medium. Then all assayed isolates were individually inoculated with the above five specific culture media in dark at 28°C with a shaking culture condition at 120 rpm. After the pathogen was filtered with aseptic lens paper each time, each sample of culture media was collected. Three replicates for each treatment were set in this study.

Standard FA sample (Sigma, USA) and the isolated and purified 9, 10-DFA from HSFO 07021 cultured on Richard culture medium by HSRC, HAAS were used to set up the standard curves of FA and 9, 10-DFA. Amount of FA and 9, 10-DFA were individually dissolved in pure water and the concentration of the two standard samples ranged from 25 μg/ml to 800 μg/ml. HPLC linear regression curves (absolute amount against chromatographic peak area) were obtained based on the weighted values of the standard samples.

Two main toxins, FA and 9, 10-DFA were extracted from culture media according to the methods of Li et al. (2017). During analysis on HPLC system (Waters, America), the mobile phases were CH_3CN (eluent A) and H_2O added with 0.1% acetic acid (eluent B). Each sample was washed and filtered with disposable water (0.22 μm) for concentration analysis. Ten μL aliquot per treatment was assayed with three replicates. Concentration of FA and 9, 10-DFA in culture media was calculated according to the above calibration curve obtained with pure FA and 9, 10-DFA, respectively.

Data analysis

The curve plot was visualized using Origin. All statistical analyses were performed using SPSS 16.0. Univariate analysis of variance was employed to explain the effects of culture medium, culture time and pathogenicity level of *FOS* strains on FA production.

Results

Morphological and pathogenic variation of the 4 FOS isolates

In this study, the 4 representative *FOS* isolates including HSFO 07011, 07021, 10001, and 10008 were chosen from *FOS* reservoir to explore the toxin production of *FOS* under different culture conditions. All the 4 isolates exhibited the specific and diverse morphological characters of *Fusarium oxsporum* on PDA media (Fig. 1; Table 1). The texture of the 4 colonies changed from cotton-like, wooly, felted to sparse on PDA medium. The density and length of hyphae of the 4 isolates evidently varied (Fig. 1a). The surface color of colonies varied from white, pink white, pink to red, while the bottom color presented pink white or red (Fig. 1b).

In addition, we measured and compared the size of conidia of the 4 isolates on PDA. Macroconidia showed *Fusarium*-like shape (Fig. 1d), while microconidia presented oval, rod-shaped, oblong or sickle-shaped (Fig. 1c). The sizes of microconidium and macroconidium ranged from 5.00-14.25 μm in length and 1.84–3.94 μm in width and 18.22–36.73 μm in length and 2.11–4.64 μm in width, respectively (Table 1). Meanwhile, three isolates except for HSFO 10008 produced chlamydospores on PDA, SNA or sesame stalk medium (Fig. 1e).

For the 4 isolates, the growth rate of colonies obviously varied (Table 1). The colony diameter for 4 days culture ranged from 4.04 cm (HSFO 10008) to 4.94 cm (HSFO 07021), while the diameter for 6 days culture ranged from 5.31 cm (HSFO 10008) to 6.37 cm (HSFO 07021).

To determine the pathogenicity level of the 4 *FOS* isolates, we performed pathogen inoculation and evaluated the disease index (DI) values with the four sesame varieties, i.e., Yuzhi 11, Zhengzhi 98N09, Ji 9014 and Rongxian black sesame (Table 2). Four grades were applied to determine the pathogenicity level of the *FOS* isolates (Qiu et al. 2014). The results confirmed that HSFO 10001 and HSFO 07011 belong to high pathogenic pathogens with grade 3 level on all the 4 sesame varieties. HSFO 10008 showed the weak pathogenicity on the 3 sesame varieties (i.e., Zhengzhi 98N09, Ji 9014 and Rongxian black sesame). The DI value ranged from 2.22 ± 2.32 (Rongxian black sesame) to 20 ± 7.80 (on Ji 9014). HSFO 07021 presented the lowest pathogenicity with grade 1 only on Zhengzhi 98N09, as the DI value was low to 7.5 ± 7.50.

Table 2
Pathogenicity level of the four *FOS* isolates on sesame hosts

FOS isolate no.	Yuzhi 11		Zhengzhi 98N09		Ji 9014		Rongxian black sesame	
	DI	Pathogenicity	DI	Pathogenicity	DI	Pathogenicity	DI	Pathogenicity
HSFO 07011	97.50 ± 2.50	3	95.00 ± 2.88	3	97.50 ± 2.50	3	100.00 ± 0.00	3
HSFO 07021	0.00 ± 0.00	0	7.50 ± 7.50	1	0.00 ± 0.00	0	0.00 ± 0.00	0
HSFO 10001	97.78 ± 2.40	3	98.00 ± 2.39	3	100.00 ± 0.58	3	100.00 ± 2.34	3
HSFO 10008	0.00 ± 0.00	0	6.67 ± 4.75	1	20.00 ± 7.80	1	2.22 ± 2.32	1

Values represent the mean value ± SD.

FA production profile of the 4 FOS isolates under different culture conditions Before evaluating the toxin production of *FOS* isolates with different pathogenic levels, we firstly filtered the culture media of the 4 *FOS* isolates and measured the toxin components in the culture media (Fig. 2). Results indicated that three toxin components including FA (Fig. 2a-1), 9, 10-DFA (Fig. <link rid="fig2">2</link>a-2) and 10-hydroxyfusaric acid (Fig. 2a-3) were detected from the culture media. Of which FA and 9, 10-DFA are the main toxin components produced by *FOS* isolates (Fig. 2b).

Considering the concentration of 10-hydroxyfusaric acid could not be measured for lack of standard sample, we measured the production of FA and 9, 10-DFA to reflect the toxin production capability of *FOS* isolates in this study. Each of the *FOS* isolates was grown for 45 days in 125 ml Erlenmeyer flasks containing 50 ml culture medium for toxin extraction. Four culture media, i.e, Armstrong medium (A), Czapek medium (C), Low-carbon Richard medium (L) and Richard medium (R) were also applied to reflect the effects of culture medium on toxin production of the 4 *FOS* isolates (Fig. 3).

The results showed that the highest amount of FA for each *FOS* isolate presented during 20–45 days in Czapek medium (C) (Fig. 3c). Under Czapek medium culture, HSFO 10008 with weak pathogenicity produced the top amount of FA at 2848.66 μg/ml (on the 20th day). Followed were HSFO 07021, 07011 and 10001, respectively. For HSFO 10001 with high pathogenicity, FA could be detected in 5 days cultured medium, while the top amount (2072.72 μg/ml) of FA presented on 25 days culture medium. As to HSFO 07021 and HSFO 07011 with different pathogenicity levels, the FA production tendency was similar under the same culture medium, with the top amount of 2837.40 μg/ml (on the 45th day) and 2386.64 μg/ml (on the 35th day), respectively.

Under Armstrong medium (A) culture condition, all the 4 *FOS* isolates produced the low amount of FA (Fig. 3d). The top amount of the two highly pathogenic isolates HSFO 10001 and HSFO 07011 reached to 192.16 μg/ml and 192.82 μg/ml, respectively. The weak pathogenic isolates HSFO 07021 and 10008 produced 90.86 μg/ml and 210.93 μg/ml FA, respectively. For Richard medium, the FA content of the four isolates almost all reached the high value with 1338.79 μg/ml, 1035.28 μg/ml, 1021.45 μg/ml, and 429.96 μg/ml, respectively on the 10th day (Fig. 3a). The results illustrated that there was no correlation between FA yield and the pathogenicity level of *FOS* strains.

9, 10-DFA production profile of the 4 FOS isolates under different culture conditions

Under the 4 culture media, the yield profiles of 9, 10-DFA of the 4 FOS isolates were also plotted and compared (Fig. 4). Interestingly, we did not detected 9, 10-DFA from HSFO 07011 with high pathogenicity in any of the 4 culture media. HSFO 07021, HSFO 10001 and HSFO 10008 secreted 9, 10-DFA under Czapek medium (C), Low-carbon Richard medium (L) or Richard medium (R). The peak amount of 9, 10-DFA reached to 716.89 μg/ml for HSFO 07021 in Richard medium (R) at the middle culture stage (for 25 days). For HSFO 10001 and HSFO 10008, the largest amount of 9, 10-DFA was 411.71 μg/ml and 251.15 μg/ml, respectively and closed to each other, even though the pathogenic level evidently differed.

In Low-carbon Richard medium, the concentration of 9, 10-DFA slowly increased with the culture time. The maximum amount (206.12 μg/ml) presented on HSFO 07021 isolate, while HSFO 10008 and HSFO 10001 exhibited the peak amount at 159.36 μg/ml and 75.72 μg/ml, respectively. The results showed that both culture medium and *FOS* isolate genotype had effects on 9, 10-DFA production.

Analysis of Variance (ANOVA) of FA production with pathogenicity level of *FOS* isolate and culture conditions

Subsequently, we combined FA and 9, 10-DFA production of each isolate under Richard medium (R) and Low-carbon Richard medium (L) (Fig. 5). The results showed that the change rule of total toxin was consistent with that of FA. In Low-carbon Richard medium (L), the total toxin content of weakly pathogenic strain HSFO 07021 was always higher than that of strongly pathogenic strain HSFO 07011, and the change trend of the total toxin production was similar (Fig. 5b). However, in Richard medium (R), there was an opposite rule, that is, the total toxin content of HSFO 07021 was always lower than that of HSFO 07011, except for the 25th day (Fig. 5a).The results also indicated that toxin production capability of a *FOS* isolate could be affected by culture conditions and have no direct correlation with its pathogenicity level.

In order to explore the effect of medium type, culture time and pathogenicity level of FOS strain on FA production, we thus performed the ANOVA (Table 3). The results showed that the medium type, culture time and their interaction had significant effect on FA production (p value < 0.001), while pathogenicity level of FOS strain was not. Based on the F value, medium type had the largest effect on FA yield, followed by culture time and interaction of both medium type and culture time (Table 3).

Table 3 Analysis of the effects of medium type, culture time, pathogenicity level of *FOS* strain and their interaction on FA production

Source of variation	df	F value
MT	3	94.492***
CT	8	5.163***
MT × CT	24	3.511***
MT × PLS	3	3.958*
CT × PLS	8	0.624
CT × MT × PLS	24	0.738
PLS	1	0.052

*, *** indicate the significance (F-test) at 0.05 and 0.001 level, respectively. Abbreviations: MT, medium type; CT, culture time; PLS, pathogenicity level of *FOS* strain.

Discussion

Sesame *Fusarium* wilt is a critical fungal disease in sesame production. In order to reveal fusaric acids production profiles of *FOS* isolates with different pathogenicity levels and determine the main factors affecting mycotoxin amount, we evaluated the content variation of the two main toxins, FA and 9, 10-DFA secreted by the 4 representative *FOS* isolates. Results indicated that the maximum amount of FA from the 4 *FOS* isolates reached to 2848.66 μg/ml in Czapek medium. The second toxin 9, 10-DFA was mainly produced in Richard and Low-carbon Richard medium, with the concentration range from 0 μg/ml to 716.89 μg/ml. Czapek culture medium was the most conductive to produce FA. FA production was significantly affected by culture medium, culture time, and their interaction (***, P < 0.001). No correlation between toxin production and pathogenicity level of *FOS* isolates existed. The findings give key information for further mechanism analysis of *FOS* - sesame interaction and pathogen control.

Relationship of toxin production and pathogenicity level of FOS **isolates**

Soil-born *Fusarium* wilt is one of the most serious diseases on more than 100 crops. The related fungus *Fusarium oxpsorum* has been ranked into the top 10 fungal pathogens based on scientific and economic importance (López-Díaz et al. 2018). FA is the first fungal phytotoxin isolated from infected host plants (Niehaus et al. 2014). Being a non-specific toxin, FA has been detected from the sesame, cotton, flax, watermelon, tomato and other so many cash crops infected by *Fusarium* species (Verma et al. 2005; Zhu et al. 2016; Li et al. 2017). FA has phytotoxicity and contributes the infection and colonization of *Fusarium oxsporum* pathogens in hosts. Zhou et al. (2017) compared the FA production of FOC colonies under different nitrogen forms and levels, and found that FA content was positively correlated with FOC (*Fusarium oxsporum* f. sp. *cucumerinum*) number or relative membrane injury in cucumber. López-Díaz et al. (2018) analyzed the virulence of *FOS* mutants lacking *fub1* and found that the *FOS* mutants were unable to produce FA and its derivatives. The vascular wilt symptoms on the tomato plants infected by *fub1* mutants significantly reduced.

Meanwhile, some reports showed that the production of FA and 9, 10-DFA is not related to the pathogenicity level of the *Fusarium* strain (Abouzeid et al. 2004; Dor et al. 2007). For example, Abouzeid et al. (2004) compared the production of the phytotoxic compounds of 53 strains from 15 *Fusarium* species. The concentration of FA and 9, 10-DFA varied from 4-165 mg/L and from 9-204 mg/L, respectively. Two weakly pathogenic strains, AP05 and ACb2, produced high concentration of FA and 9, 10-DFA. However, a strong pathogenic strain, FT2 produced quite low amounts of toxins. The results suggested that the virulence was not consistently positively correlated with the production of FA and 9, 10-DFA.

As to *FOS*, previous studies demonstrated that *FOS* isolates could produce FA, 9, 10-DFA and other derivatives (Zhu et al. 2016; Li et al. 2017). The extracted toxins of *FOS*, FA and 9, 10-DFA exhibited phytotoxic and inhibiting character on sesame seedlings, even though 9, 10-DFA had lower toxic effects on the growth of sesame seedlings than that of FA (Li et al. 2017). Abouzeid et al. (2004) found that all the tested strains produced FA and 9, 10-DFA, while 9, 10-DFA presented the higher amount than FA. For the 4 *FOS* isolates in this study, the peak concentration of FA varied from 2072.72 μg/ml (HSFO 10001) to 2848.66μg/ml (HSFO 10008) under Czapek medium condition. Meanwhile, the peak amount of 9, 10-DFA reached to 251.15μg/ml (HSFO 10008) to 716.89 μg/ml (HSFO 07021) in Richard medium (R). The maximum concentration of FA was 3.97 times as that of 9, 10-DFA. The results demonstrated that FA was thus the main and key toxin component for *FOS*. However, the FA concentration of *FOS* isolates was not significantly correlated with the pathogenicity level (Table 3). Both highly pathogenic and weakly pathogenic *FOS* strains could produce high concentrations of FA and 9, 10-DFA, reflecting the similar phenomenon to some other *Fusarium* species (Kachlicki and Jedryczka 1997). We thus believe the infection of *FOS* colonization in sesame plants presented both *FOS*-sesame interaction and FA stress effects.

Effects of culture nutrients on toxin production of FOS **isolates**

In the study, the contribution of the 4 culture media to FA production of *FOS* isolates ranked as follows: Czapek, Richard, Low-carbon Richard and Armstrong culture medium. Different from the other three media, *FOS* strains in Armstrong culture medium produced the lowest FA content. The maximum values of FA content of strains HSFO 10001, HSFO 10008, HSFO 07011 and HSFO 07021 were 192.16 μg/ml, 217.70 μg/ml, 192.81 μg/ml and 90.86 μg/ml, respectively (Fig. 3d). Moreover, no 9, 10-DFA was detected for the 4 isolates (Fig. 4). Therefore, culture nutrients significantly affected the FA production of *FOS* isolates. Compared with the other culture media, Armstrong medium contained 0.2 μg/ml Zn^{2+}.

Previous studies reflected that the addition of Zn ions into the culture broth could drastically reduce the FA production of mid strain (Chakrabarti and Chaudhary 1980; Duffy and Défago 1997; Saikia et al. 2009). Duffy and Défago (1997) found zinc, copper or their mixture added into Czapek-Dox medium resulted in the increase of the number of microconidia and the total fungal biomass of *F. oxysporum* f. sp. Radici slycopersici (FOR), but significantly decreased the FA production. Saikia et al. (2009) added different trace amounts of zinc and copper ions into liquid medium for FOC (*F. oxysporum* f. sp. *ciceri*) culture and determined that 10 μg/ml zinc and copper ions significantly reduced the FA production. In order to reveal the inhibition function of mineral nutrients on *Fusarium* pathogens, Wang et al. (2020) grew cucumber plants with zinc and copper treatments. The results showed that addition of Zn decreased FA transportation to shoots and induced the increase of antioxidant enzymes activity. Meanwhile, addition of Cu decreased the absorbance of FA and mitigated the toxicity of FA possibly through chelation. Thus, we believe that the decrease of FA of *FOS* isolates in Armstrong medium should result from the addition of Zn^{2+}. Functional analysis and application of zinc, copper and other mineral nutrients should be studied for sesame *Fusarium* wilt control in near future.

In a total, we assayed the concentration variation of FA and 9, 10-DFA produced by *Fusarium oxysporum* species for 45 days continuous culture under the same culture conditions in the study. Comparison analysis indicated that FA was produced in diverse culture media (Fig. 3). For highly pathogenic isolate HSFO 10001, the FA concentration in the Czapek culture medium significantly increased on 25th day and was higher than that in Richard culture. As shown in Fig. 3, the FA production curves of HSFO 10001 and HSFO 10008 were similar, even though the FA content in Czapek and Richard culture medium fluctuated. Meanwhile, the FA concentration curves of strain HSFO 07011 and HSFO 07021 were similar. We inferred that the production of FA was also influenced by *FOS* isolate origin. However, the pathogenicity level of a *FOS* isolate was the most important trait during disease resistance mechanism analysis and new variety breeding in sesame.

Duan et al. (2020) divided the 54 pathogenic *FOS* isolates into three subgroups with various pathogenic levels on the three sesame hosts for the first time. Ten *secreted-in xylem (SIX)* genes, belonging to one family of effectors, were identified and applied to group the *FOS* isolates. With the aid of high pathogenic *FOS* isolates, Miao et al. (2020) evaluated the resistance level of 42 sesame germplasm to *Fusarium* wilt during the vegetative stage. The results showed that only several wild species, such as *S. radiatum* and *S. angustifolium* presented the stable high resistance (DI = 0), consistent with previous results (Nimmakayala et al. 2011; Zhu et al. 2016). Of the 40 assayed cultivars, 55% were highly susceptible, and 27.5% were resistant to

FOS. No accessions with high resistance were found. Thus, the virulence of *FOS* isolates on sesame hosts needs to be studied further. Interaction mechanism of *FOS* and elite sesame germplasm with high resistance should be enhanced in the future (Miao et al. 2022).

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests.

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Figures

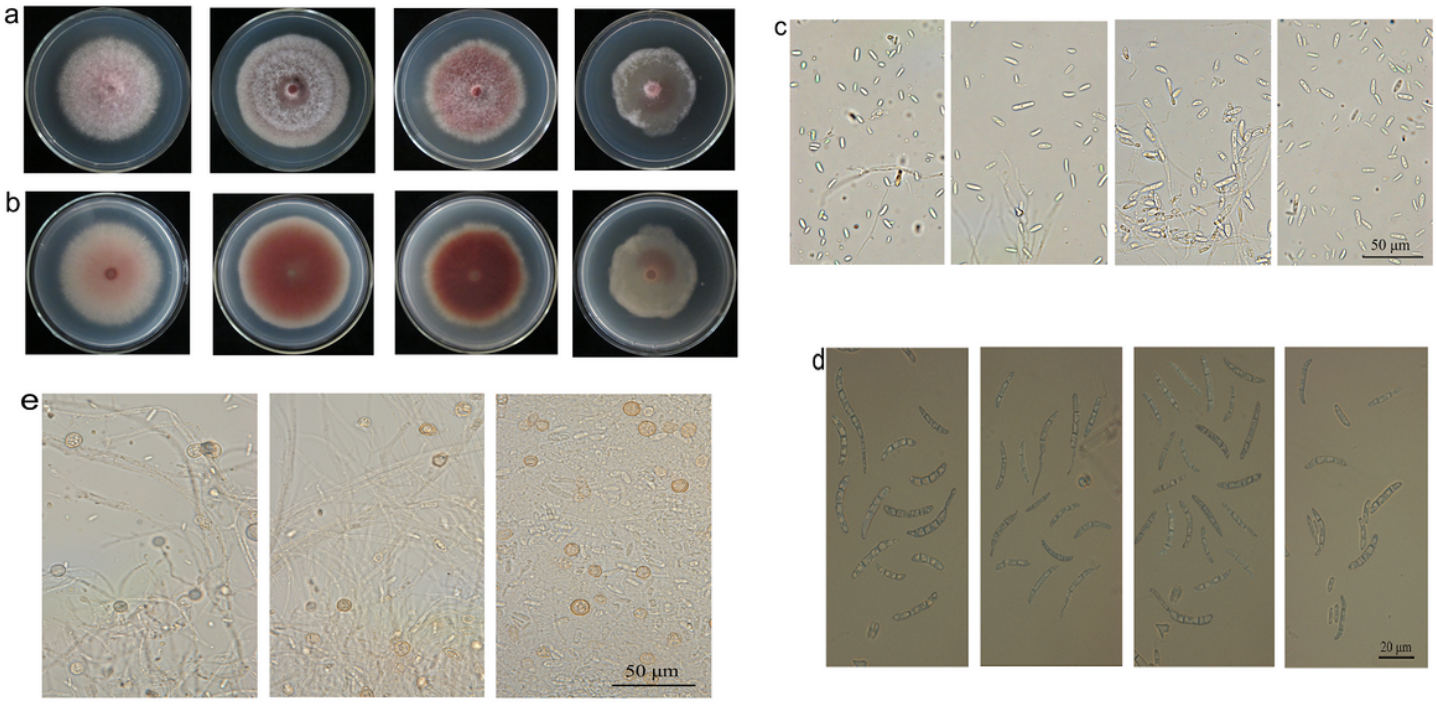

Figure 1

Morphological characteristics of the 4 *FOS* isolates. (a) Colony surface image. (b) Colony bottom surface image. (c) Microconidia. (d) Macroconidia. (e) Chlamydospores. All the images are arranged according to the rank of HSFO 07011, HSFO 07021, HSFO 10001 and HSFO 10008, respectively.

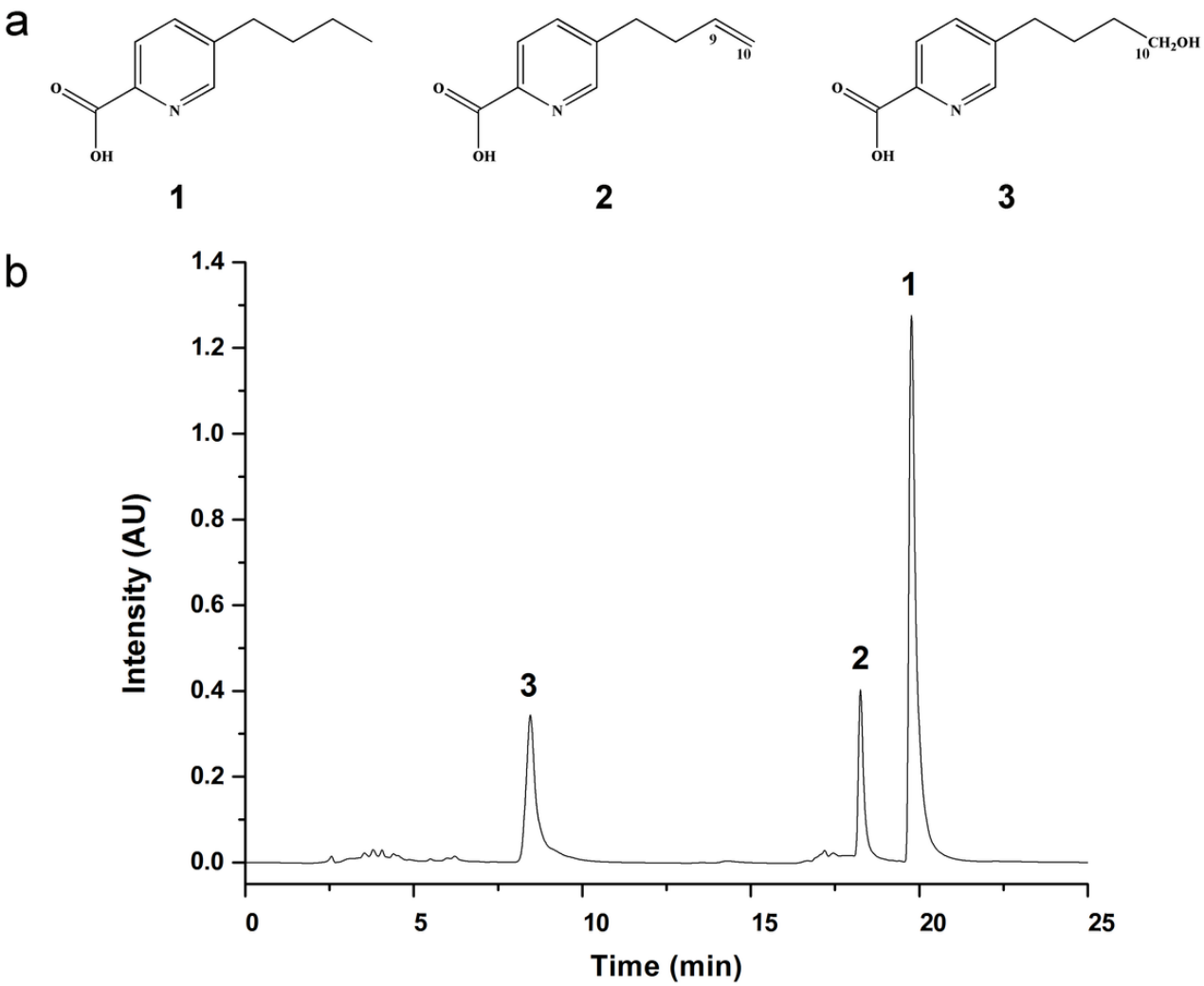

Figure 2

Toxin components produced by *FOS* isolates. (a) Structures of FA (1), 9, 10-DFA (2) and 10-hydroxyfusaric acid (3). (b) HPLC profile of Richard culture filtrates of HSFO 07021 isolate.

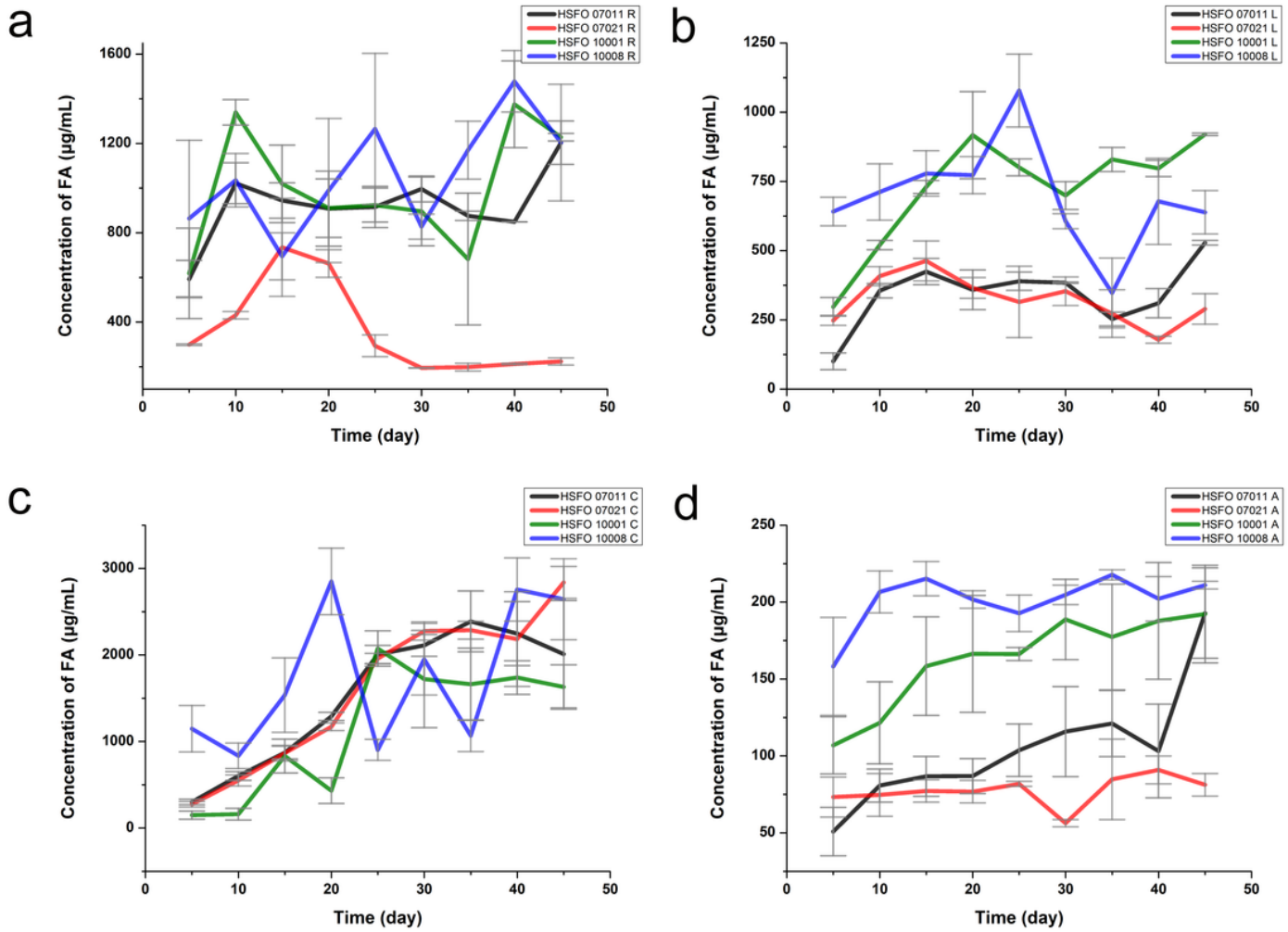

Figure 3

FA production profiles of the 4 *FOS* isolates on different culture media. (a) FA Production of 4 *FOS* isolates in Richard medium (R). (b) FA Production of 4 *FOS* isolates in Low-carbon Richard medium (L). (c) FA Production of 4 *FOS* isolates in Czapek medium (C). (d) FA Production of 4 *FOS* isolates in Armstrong medium (A). FA concentration in PD cultures was too low to be shown in the figure.

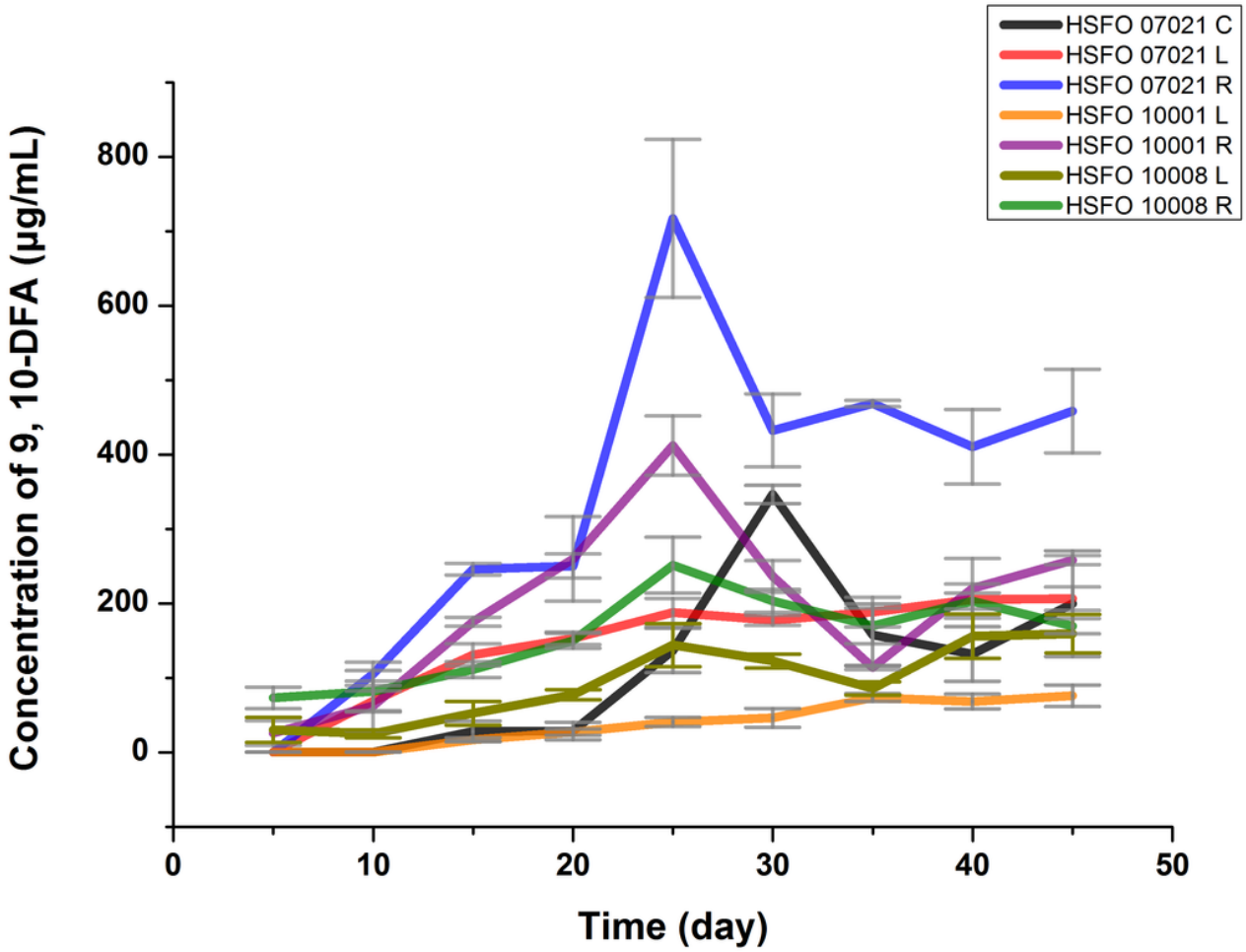

Figure 4

9, 10-DFA production profile of the 4 *FOS* isolates on different culture media. A, C, L and R represent Armstrong medium, Czapek medium, Low-carbon Richard medium and Richard medium, respectively.

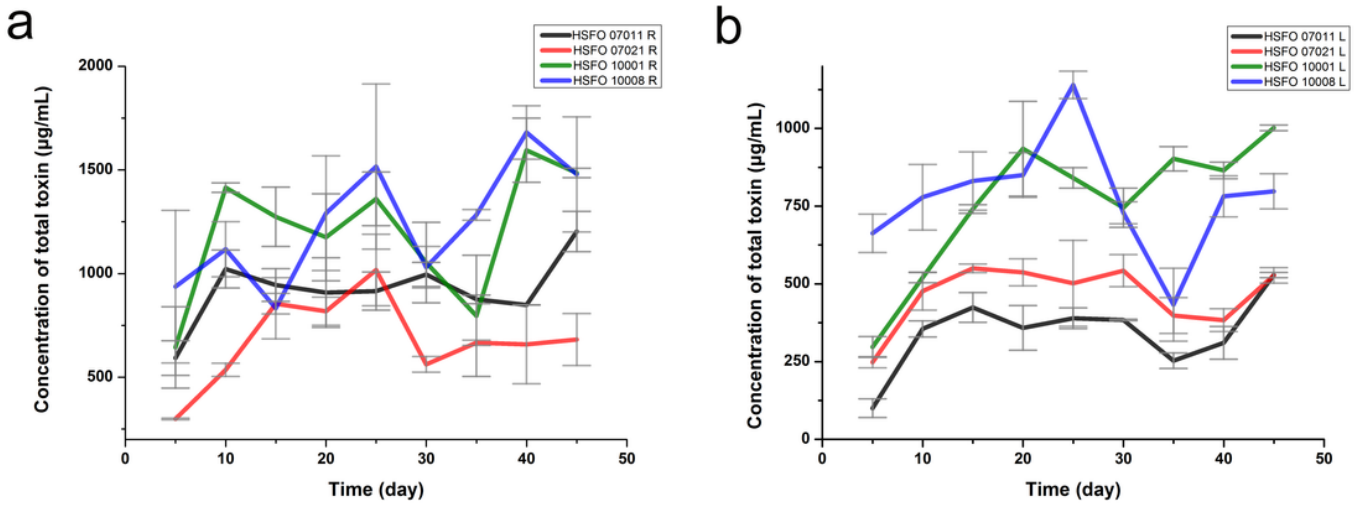

Figure 5

Toxin production profile of the 4 *FOS* isolates on different culture media. (a) Total toxin production of 4 *FOS* isolates in Richard medium. (b) Total toxin production of 4 *FOS* isolates in Low-carbon Richard medium. L and R represent Low-carbon Richard medium and Richard medium, respectively.