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Growth modeling kinetics of *Alternaria alternata* in dried jujube at different temperatures

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Abstract

Alternaria alternata is a fungus that infects jujube, causes black spot disease, and produces *Alternaria* mycotoxins, such as alternariol, alternariol monomethyl ether, and tenuazonic acid. This study aimed to apply models to predict growth and mycotoxin production by *A. alternata* isolated from jujube as a function of temperature and water activity. The practicability of the model was verified on jujube agar medium. The growth conditions were water activity of 0.99 or 0.90 a_w and temperatures of 10, 15, 20, 25, 30, or 35 °C. The growth rate of *A. alternata* was highest at 0.99 a_w and 25 °C, and the lag time was the shortest at 0.90 a_w and 30 °C. The dry weight growth rate of mycelia increased gradually with the increase of temperature on PDA and jujube agar medium. Mycotoxin production by *A. alternata* was not correlated with growth, but no significant delay was detected in some cases. In general, the growth model predicted the growth of *A. alternata*.

Keywords: *Alternaria alternata*; *Alternaria* mycotoxins; predictive modeling.

Practical Application: The effects of temperature and water activity on the growth of *Alternaria alternata* were predicted by secondary models.

1 Introduction

Jujube (*Ziziphus jujuba* Mill.) is a plant in the Rhamnoidea family that contains high concentrations of sugars, fatty acids, amino acids, minerals, vitamins, polyphenols, and other antioxidants (Li et al., 2020). It has good nutritional and medicinal value (Ji et al., 2020). China is the largest jujube producer in the world, and Xinjiang is the most important jujube producing area in China. The physical and chemical indices of the jujube cultivars differ among districts (Chen et al., 2019). Therefore, it is necessary to study the physiological characteristics of microorganisms leading to jujube disease in Xinjiang. The jujube is vulnerable to black spot. *Alternaria alternata* is a fungus that infects a variety of plants and causes black spot disease in jujube (Zhang et al., 2020), pear (Sardella et al., 2018), apple (Ntasiou et al., 2015), blueberry (Wang et al., 2021), citrus (Sardar et al., 2022) and cherry tomato (Pane et al., 2016).

Alternaria produces secondary metabolites called *Alternaria* mycotoxins. The main mycotoxins detected in food are alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TeA). These mycotoxins have mutagenic, carcinogenic, and genotoxic effects (Puntscher et al., 2019). Exposure of Europeans to AOH was detected through urine tests but the risk was not characterized due to a lack of intake by a reference (Martins et al., 2019). Temperature and water activity are key factors affecting fungal growth and mycotoxin production (Lahouar et al., 2017; Thanushree et al., 2019). The shelf life of products depends on environmental conditions and exposure time (Oliveira et al., 2021).

The fungal growth rules have been quantified and predicted by mathematical models (Garcia et al., 2009). As fungal growth leads to the release of mycotoxins from the substrate, control of fungal growth is essential according to predictive modeling (Marín et al., 2021). Models have been used to evaluate the effects of different environmental factors on fungal growth and mycotoxin production in food. The effects of temperature, water activity (Bernáldez et al., 2017), and pH (Casquete et al., 2017) on the growth of *Aspergillus flavus* and the production of aflatoxin have been described by a linear model. A response surface analysis revealed an interaction between temperature and water activity on *Fusarium* mycotoxin production (Yu et al., 2021).

The purpose of this study was to describe the effects of temperature and water activity on the growth of *A. alternata* and the production of *Alternaria* mycotoxins using predictive models. The results were verified on jujube agar medium.

2 Materials and methods

2.1 Preparation of spore and culture medium

An *A. alternata* (GenBank OL989878) strain was isolated and identified from dried jujube in Xinjiang, China. The strain produced high mycotoxin levels in preliminary tests. *A. alternata* was inoculated on potato dextrose agar (PDA) at 25 °C for 5 days, sterilized 0.05% Tween80 solution was added, and the mycelial suspension was scraped on the surface of the medium with a sterile rod, through four layers of gauze to filter the mycelium. Sterile water was diluted to 10^6 spores/mL using a blood count board. The water activity of the PDA was adjusted to 0.99 and 0.90 a_w using glycerin instead of water. Water activity was

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measured with a water activity meter (HD-3A Smart Water Activity Meter, Wuxi Huake Instrument Co. Ltd., Wuxi, China). The jujube agar medium (JAM) was comprised of 3% jujube powder and 2% agar (25 °C, a_w 0.903, pH 5.19).

2.2 Growth and dry weight evaluation

Growth was assessed daily by measuring two vertical diameters of the fungal colonies. The colonies were cut from the medium and transferred to a beaker filled with distilled water (about 100 mL), which was heated in a microwave for 10 min to melt the agar. The mycelia that remained intact were collected and transferred to dry pre-weighed filter paper and dried at 80 °C for 18 h (Garcia et al., 2013). Then, the filter paper was weighed and the dry weight of the biomass was calculated as the difference.

2.3 Mycotoxin detection

Three agar plugs were taken from different parts of the medium after 7 days of cultivation using a hole punch and placed together in a vial. One mL of methanol was added, and the extract was shaken and filtered (0.22 μm organic filter membrane) into a vial for 5 s. After 60 min, the extract was placed in another vial for the high-performance liquid chromatography (HPLC) analysis. UV was detected at 254 nm and 276 nm, respectively. The AOH, AME, and TeA standards were prepared in methanol. The HPLC analysis was performed using a Shimadzu HPLC system (Tokyo, Japan) equipped with a UV detector. The HPLC detection conditions were Agilent HC-C18 column (250 × 4.6 mm, 5 μm, Agilent Technologies, Santa Clara, CA, USA), column temperature of 35 °C, and a mobile phase system of water (A) and acetonitrile (B). The gradient elution procedure was 0 min, 20% B, 1 min, 40% B, 16 min, 80% B, 18 min, 80% B, 19 min, 20% B, and 22 min, 80% B. The injection volume was 10 μL, and the flow rate was 1.0 mL/min. The recovery rate of the method was > 80% when different amounts of *Alternaria* mycotoxins were added (range 0.1-100 mg/mL).

2.4 Growth model

The growth data at each temperature were subjected to the modified Gompertz model (Zwietering et al., 1990). The maximum radial growth rate (μ_{max}, mm/d) and lag time (λ, d) were determined. The parameters obtained from the primary model were substituted into a secondary model affected by temperature, and the data were processed using IPMP (Huang, 2014).

The Ratkowsky square-root model (Ratkowsky et al., 1983).

$$\sqrt{\mu_{max}} = a\left(T - T_0\right)\left[1 - e^{b\left(T - T_{max}\right)}\right] \quad (1)$$

where μ_{max} is the maximum radial growth rate (mm/d), a and b are coefficients, T is temperature (°C), T_0 is the nominal/notational minimum temperature, and T_{max} is the maximum estimated temperature.

The Huang square-root model (Huang et al., 2011).

$$\sqrt{\mu_{max}} = a\left(T - T_{min}\right)^{0.75}\left[1 - e^{b\left(T - T_{max}\right)}\right] \quad (2)$$

The same parameters as used in Equation 1.

The Rosso cardinal model (Rosso et al., 1993).

$$\mu_{max} = \frac{\mu_{opt}\left(T - T_{max}\right)\left(T - T_{min}\right)^2}{\left[T_{opt} - T_{min}\left(T - T_{opt}\right) - T_{opt} - T_{max}T_{opt} + T_{min} - 2T\right]\left(T_{opt} - T_{min}\right)} \quad (3)$$

where μ_{max} is the maximum radial growth rate (mm/d), and μ_{opt} is the optimal radial growth rate (mm/d) at the optimum temperature (T_{opt}). T_{min} and T_{max} are the minimum and maximum growth temperatures (°C).

2.5 Model validation

$$A_f = 10^{\left(\Sigma\left|log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)\right|/n\right)} \quad (4)$$

$$B_f = 10^{\left(\Sigma log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)/n\right)} \quad (5)$$

$$\mathrm{RMSE} = \sqrt{\frac{\Sigma\left(\mu_{max,pred} - \mu_{max,obs}\right)^2}{n}} \quad (6)$$

where $\mu_{predicted}$ and $\mu_{observed}$ are the predicted growth rate and observed growth rate respectively, and $\mu_{max,pred}$ and $\mu_{max,obs}$ are the maximum predicted growth rate and the observed growth rate, respectively. The accuracy factor (A_f) and bias factor (B_f) were considered to evaluate model performance (Ross, 1996). A_f indicates how close the average predicted value is to the observed value. B_f evaluates the distance between the observed value and the prediction line (the closer A_f and B_f are to 1, the better the model fit is). The performance of the regression analysis is reported as the root mean square error (RMSE).

2.6 Statistical analysis

Growth data and dry weight were fitted using StatGraphics 18 (Statgraphics Technologies Inc., The Plains, VA, USA). The toxin data were processed with Origin 2019 (OriginLab Corp., Northampton, MA, USA). The data are expressed as mean ± standard error.

3 Results

3.1 Primary model

The maximum radial growth rate (μ) and the lag time (λ) were estimated through the modified Gompertz primary model (Table 1). Up to and including 25 °C, the radial growth rate was 0.99 a_w > JAM > 0.90 a_w, but at 30 °C and 35 °C, JAM had the greatest growth rate, at 35 °C, 0.90 a_w > 0.99 a_w, suggesting that low water activity allows better survival at high temperatures. The lag times of 0.99 a_w and 0.90 a_w were similar at 10-25 °C. The JAM lag time was much higher than the other two. These results show that *A. alternata* grew earlier on PDA than on jujube agar medium.

Table 1. Radial growth rate (μ) and reciprocal lag time (1/λ) estimates using the modified Gompertz model of *A. alternata* on PDA with different water activity values (0.99 and 0.90 a_w) and jujube agar medium (JAM).

Temperature (°C)	Medium	Radial growth rate, μ (mm/d)	Reciprocal lag time, 1/λ (1/d)	RMSE
10	PDA 0.99 a_w	4.006 ± 0.142	0.468 ± 0.034	0.354
10	PDA 0.90 a_w	1.704 ± 0.151	0.540 ± 0.116	0.349
10	JAM	3.950 ± 0.114	0.153 ± 0.012	0.302
15	PDA 0.99 a_w	7.490 ± 0.255	0.882 ± 0.096	0.673
15	PDA 0.90 a_w	3.514 ± 0.174	0.783 ± 0.066	0.428
15	JAM	6.324 ± 0.060	0.317 ± 0.063	0.624
20	PDA 0.99 a_w	10.854 ± 0.547	1.287 ± 0.077	0.810
20	PDA 0.90 a_w	5.111 ± 0.456	1.201 ± 0.186	0.314
20	JAM	8.960 ± 0.178	0.489 ± 0.072	0.521
25	PDA 0.99 a_w	12.871 ± 0.212	1.484 ± 0.022	0.758
25	PDA 0.90 a_w	5.492 ± 0.146	1.541 ± 0.000	0.403
25	JAM	11.247 ± 0.115	0.609 ± 0.009	1.097
30	PDA 0.99 a_w	10.440 ± 0.195	0.994 ± 0.051	0.741
30	PDA 0.90 a_w	8.016 ± 0.115	1.773 ± 0.000	0.444
30	JAM	10.513 ± 0.331	0.768 ± 0.078	1.114
35	PDA 0.99 a_w	4.172 ± 0.200	0.528 ± 0.120	0.360
35	PDA 0.90 a_w	6.233 ± 0.277	1.312 ± 0.077	0.319
35	JAM	6.407 ± 0.322	0.565 ± 0.076	0.734

3.2 Secondary model

The four different models were evaluated to describe the response of the fungus to the temperatures examined (Table 2). All parameters in Rosso cardinal model had physiological significance when estimating the initial parameters. However, the fitting parameters of the PDA differed greatly from the different water activity values. The optimum growth rate and optimum temperature were similar between JAM and 0.99 a_w PDA. In the Ratkowsky Square-root model, the maximum growth temperatures of *A. alternata* in the three media were 36.75, 35.38, and 37.85 °C, respectively (Equation 1). In the Huang Square-root model, the maximum growth temperatures of *A. alternata* were 36.64, 35.42, and 37.62 °C, respectively (Equation 2). In the Rosso cardinal model, the maximum growth temperatures of *A. alternata* were 36.93, 35.30, and 38.11 °C, respectively, and the fungus did not grow at temperatures > 40 °C (Equation 3). The minimum temperatures of *A. alternata* in the Rosso cardinal model were 0.46, –9.63, and –4.20 °C respectively, with relatively low values and large differences. These results show the differences in strain growth between the different water activity values and different media. The optimum growth rates were 12.34, 8.68, and 11.07 mm/d, respectively. The predicted value of JAM was close to that of 0.99 a_w PDA, and the observed growth rate of JAM was subjected to the 0.99 a_w PDA predictive model for verification.

3.3 Verification of the model

The observed and predicted values were compared intuitively, and prediction performance was evaluated numerically. The accuracy factor (A_f) and bias factor (B_f) were used as mathematical indices (Table 3). As the secondary models only evaluated the effect of temperature on the radial growth rate of *A. alternata*, there was no difference in A_f and B_f between the models. The accuracy factors were 1.1-1.2 (Equation 4), indicating large deviations between the predicted and observed values because the A_f value depended on the medium. The bias factor was close to 1 (Equation 5), indicating a good correlation between the predicted and observed values. The Rosso cardinal model fit the experimental data well, as illustrated by the low RMSE (Equation 6).

3.4 Mycelial dry weight

On PDA and JAM, the dry weight of mycelia was positively correlated with the diameter ($P < 0.05$), and the dry weight growth rate of mycelia increased gradually with the increase of temperature (15-30 °C). The dry weight growth rate was 0.99 a_w > 0.90 a_w > JAM (Figure 1), and the growth rate on jujube medium was much lower than that on PDA medium at different temperatures, which was caused by the different medium components. A significant positive correlation was observed between the colony radius and dry biomass on maize agar medium, and toxin accumulation slowed before there was a decrease in dry biomass accumulation (Garcia et al., 2013).

3.5 Alternaria ***mycotoxins***

PDA had the highest toxin content at 25 °C, JAM had the highest toxin content at 30 °C (Figure 2), and the mycotoxins produced by the *Alternaria* strains did not correlate well with maximum growth. The high a_w resulted in higher toxin content at high temperature and a low a_w resulted in higher toxin content at a low temperature on PDA. High a_w and high temperature are more conducive to the production of mycotoxins. The toxin content of all media was mostly concentrated at 20-30 °C.

Table 2. The secondary models were used to evaluate the effect of temperature on growth rate.

Secondary models	Parameters	PDA 0.99 a_w	PDA 0.90 a_w	JAM
Ratkowsky square-root model	a	0.942 ± 0.064	0.289 ± 0.015	0.572 ± 0.024
	T_{max}	36.746 ± 0.134	35.382 ± 0.001	37.851 ± 0.191
	b	0.092 ± 0.010	3.059 ± 0.000	0.151 ± 0.013
	T_0	5.427 ± 0.294	3.746 ± 0.666	3.155 ± 0.387
	RMSE	0.383	0.431	0.296
Huang square-root model	a	1.759 ± 0.077	0.695 ± 0.026	1.244 ± 0.035
	T_{max}	36.637 ± 0.142	35.421 ± 0.002	37.617 ± 0.204
	b	0.130 ± 0.012	3.109 ± 0.000	0.198 ± 0.017
	T_{min}	6.956 ± 0.229	6.656 ± 0.431	5.481 ± 0.296
	RMSE	0.427	0.427	0.330
Rosso cardinal model	T_{opt}	25.183 ± 0.175	33.358 ± 2.248	27.344 ± 0.166
	T_{max}	36.933 ± 0.131	35.295 ± 0.998	38.113 ± 0.213
	μ_{opt}	12.336 ± 0.115	8.679 ± 1.543	11.070 ± 0.084
	T_{min}	0.460 ± 0.524	−9.627 ± 2.363	−4.202 ± 0.689
	RMSE	0.336	0.480	0.267
General polynomial model	a	−0.047 ± 0.002	−0.010 ± 0.002	−0.029 ± 0.002
	b	2.169 ± 0.108	0.633 ± 0.067	1.452 ± 0.090
	c	−13.334 ± 0.999	−3.706 ± 0.638	−8.119 ± 0.900
	RMSE	0.879	0.576	0.809

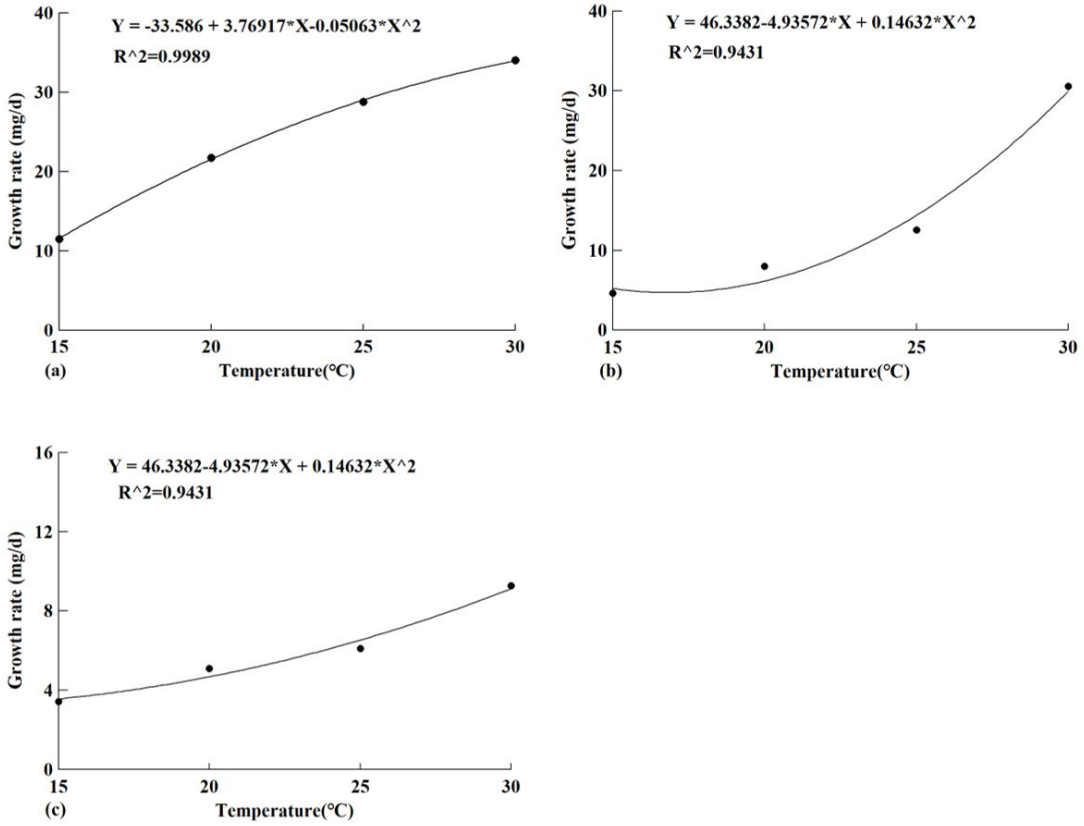

Figure 1. Effect of temperature on the growth rate (μ) of *Alternaria* isolates by mycelial dry weight (a) 0.99 a_w PDA; (b) 0.90 a_w PDA; and (c) jujube agar medium (JAM).

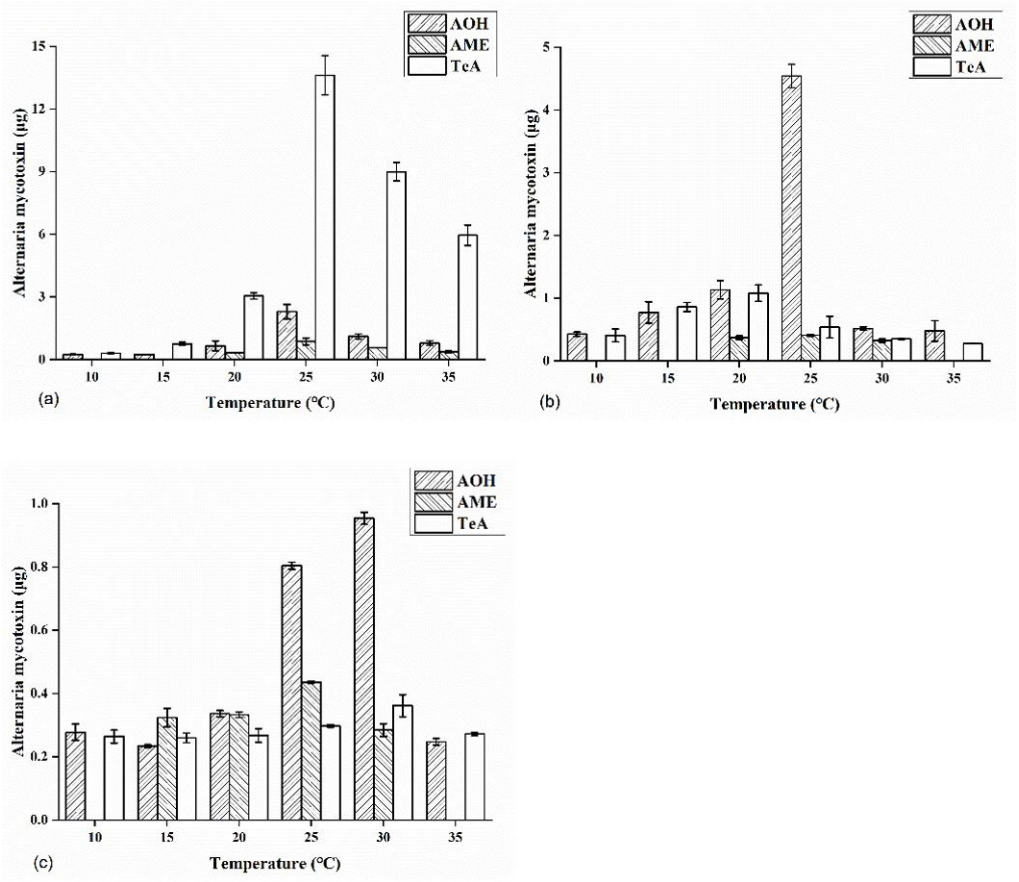

Figure 2. Effect of temperature on *Alternaria* mycotoxin content (a) 0.99 a_w PDA; (b) 0.90 a_w PDA; and (c) jujube agar medium (JAM).

Table 3. Accuracy factors (A_f) and bias factors (B_f) for each of the growth predictive models.

Growth models	A_f	B_f
Ratkowsky square-root model	1.164	1.035
Huang square-root model	1.164	1.034
Rosso cardinal model	1.165	1.036
General polynomial model	1.193	1.044

Table 4. Correlation among *Alternaria* mycotoxins and growth responses (Pearson coefficients).

Medium	Mycotoxins(μg/d)	Radius growth rate (mm/d)	Dry weight growth rate (mg/d)
PDA 0.99 a_w	AOH	0.9134	0.8834
	AME	0.8841	0.9834
	TeA	0.7570	0.8806
PDA 0.90 a_w	AOH	0.5035	0.5887
	AME	0.9997	0.9794
	TeA	0.5977	0.6563
JAM	AOH	0.8063	0.9938
	AME	0.4097	0.9375
	TeA	0.4355	0.7716

No significant delay in toxin production or fungal growth was observed under the optimum conditions.

3.6 Correlation between growth and mycotoxin

The colony diameter growth rate was significantly correlated with the dry weight growth rate of mycelia and daily *Alternaria* mycotoxin production (Table 4). *Alternaria* mycotoxin production was always correlated with the dry weight of mycelia when significant correlations were detected.

4 Discussion

The growth and toxin production probabilities of mixed and single inoculations are very similar under non-isothermal conditions, considering the interactions between the same strains and the temperature changes (Aldars-García et al., 2015). Growth models are used to predict growth boundaries and the models were applied to prevent toxin production. Polysporous inoculation resulted in a higher growth rate and a shorter lag period than monosporous inoculation, and the polysporous inoculation probability model is more accurate (Aldars-García et al., 2017). Polyspore inoculation was used in this experiment, and the hysteresis period was relatively short, which was greatly affected by the inoculation amount.

The germination time of *A. arborescens* on tomato medium was the lowest at 0.995 a_w, and 25 °C and 30 °C, and the maximum growth rate was 7.21 mm/d at 0.995 a_w and 30 °C, and 6.97 mm/d at 25 °C ($P > 0.05$). AOH, AME, and TeA greatly accumulated at 0.975 a_w and 30 °C, although a large number of toxins were detected at 25 °C (Vaquera et al., 2014, 2016). The optimum temperature for maximum toxin content and maximum growth rate was the same, but the a_w value was different. In this experiment, the growth rate of *A. alternata* on jujube agar medium was 11.25 mm/d at 25 °C. The total AOH, AME, and TeA contents accumulated at 25 °C, and a correlation was detected between the AOH, AME, and TeA contents and the maximum growth rate. Climate can also affect the growth and toxicity of *A. alternata* on tomato, including temperature and other factors (Van de Perre et al., 2015). High or low temperatures can inhibit the production of mycotoxins. When the temperature at harvest time is close to the optimum temperature for fungal toxicity, the toxin content will increase, leading to deteriorated crops. Therefore, it is important to consider the effects of temperature during crop growth, harvest and storage, and use models to predict the effects of temperature on fungal growth and the toxins produced to take preventive measures in advance.

The optimum temperature for *A. alternata* on Sabouraud dextrose agar (SDA) was 23.99 °C, the minimum temperature was −4.06 °C, and the maximum temperature was 34.99 °C. Growth rates verified on pear culture medium were almost the same at the optimum temperature but were significantly lower on pears than on SDA (Sardella et al., 2018). *A. alternata* has an optimum temperature of 25.18 °C, a minimum temperature of 0.46 °C, and a maximum temperature of 36.93 °C on PDA. The temperature range varies depending on the strain and medium. The boundaries of aflatoxin production and fungal growth on pistachios are the same, but the optimum growth temperature is different. The temperature at which the maximum toxin production occurs is earlier than the maximum fungal growth rate (Marín et al., 2012). Different from the results of this experiment, the maximum fungal growth rate was accompanied by maximum toxin accumulation, which may have been caused by differences between the strain and the substrate.

The independent black pepper experimental data were used to verify the established model, and the B_f (0.73-1.03) and A_f (0.97-1.36) showed that the model examined was a conservative prediction of the growth rates of *Aspergillus flavus* and *Aspergillus parasitica* (Yogendrarajah et al., 2016). *Botrytis cinerea* and *Penicillium expansum* tested in simulated grape juice medium and grape juice agar, the B_f is close to 1 indicates a safe prediction, the A_f (1.11-1.29) is a large deviation (Judet-Correia et al., 2010). In this study, *A. alternata* was verified on jujube agar medium, and the accuracy factor indicated a large deviation, while the bias factor indicated that the model had a safe prediction.

A. tenuissima and *A. arborescens* produce AOH and AME *in vitro* and on apple fruits (Ntasiou et al., 2015). However, the toxin-producing capacity of *Alternaria* strains between *in vitro* culture and its actual occurrence in food is not strongly correlated. Although AOH and AME are the toxins most commonly produced by isolates on medium, they are much less prevalent in pepper fruits. In contrast, TeA is produced *in vitro* by a smaller number of isolates, but more fruits are contaminated with this toxin (Masood et al., 2015). Therefore, convenient and rapid toxin detection methods unique to different foods are an important tool for control strategies and are not limited to toxin models to reduce toxin risk.

5 Conclusion

In this study, primary and secondary models were established through the effects of different temperatures on *A. alternata*, and the applicability of the model was confirmed in jujube agar medium. No significant delay was observed in the production of *Alternaria* mycotoxins or fungal growth under the optimum conditions, which provided a theoretical basis for the risk assessment. The colony diameter growth rate was significantly correlated with the dry weight growth rate of mycelia and daily *Alternaria* mycotoxin production. It may be possible to build a growth model for harmful fungi isolated from different food substrates, compare the same and different growth conditions of the same fungus, set different correction factors, and apply them to actual food to prevent fungal toxins.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Conceptualization, Die Hu and Hua Ji; sample analysis, Yawen Xue and Caihong Jiang; statistical analysis, Chunhui Shan and Xiaomeng Kou; writing-original draft preparation, Die Hu; writing-review and editing, Fengxian Tang and Hua Ji. All authors have read and agreed to the published version of the manuscript.

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