Influence of ventilation on airborne fungi in greenhouses: A case study of tomato greenhouses

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Abstract. With the rapid development of greenhouses, the indoor air quality, particularly airborne microorganisms, is closely related to the health of farmers and needs more attention. In this study, the concentrations of airborne fungi at seedling, fruiting and harvesting stages in typical tomatoes greenhouses were tested. Temperature, relative humidity and the microbial concentrations were analysed. It was found that the dominant fungal genera are *Aspergillus* and *Cladosporium*, no matter it was in which growth stage. Ventilation is an effective way to reduce the concentrations of airborne fungi through dilution and decrease the relative humidity.

1 Introduction

The enclosed structure of greenhouses makes the internal environment obtain the characteristics of high temperature, high humidity and poor ventilation [1]. It is incredibly conducive to the growth of airborne fungi [2]. Thus, working in greenhouses is easily exposed to high levels of airborne fungi [3], which are associated with respiratory symptoms [4], including rhinitis [5], allergy [6], and asthma [7].

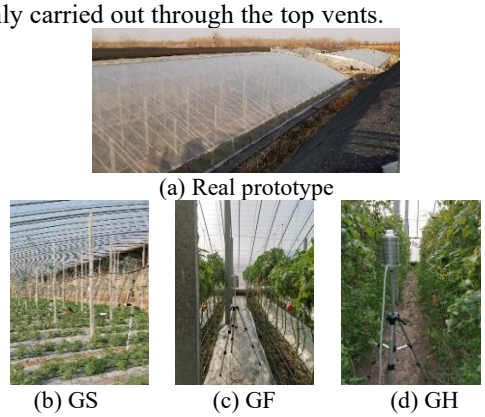
Due to the high exposure levels and elevated risk of respiratory disease, it is urgent to reduce fungal exposure in greenhouses. Ventilation is a universal way to decrease air contaminants in various occasions. In greenhouses, researches on the effect of ventilation were primarily focused on the control of indoor temperature and humidity. Ventilation was an important mean to prevent excess heat and humid in greenhouses [8]. Revathi [9] emphasized the importance of the ventilation rate according to thermal stratification and spatial temperature distribution studies of the experimental greenhouse structure. The installation of an innovative ventilation concept could improve crop growing conditions due to a better control of the greenhouse climate [10].

Limited research focuses on the influence of ventilation on airborne microorganisms in greenhouses. In view of this, a case study in three greenhouses with different growth stages of tomato plants was conducted to estimate the influence of ventilation on airborne fungi. It is expected to provide first-hand information for related occupational exposure researches.

2 Materials and methods

2.1 The tested greenhouses

The tested greenhouses were located in Weifang, which is known as a primary vegetable-producing region in China [45]. Three greenhouses with tomato plants in seedling stage (GS), fruiting stage (GF) and harvesting stage (GH) were selected as study objects, shown in Fig. 1. The three tomato greenhouses had the similar structure which constructed by three walls located east, west, and north, and a curved transparent plastic film in the south [11]. The heights of tomato plants were 0.35 m, 1.5 m and 1.95 m in the GS, GF and GH greenhouse, respectively. During sampling, natural ventilation was mainly carried out through the top vents.



(a) Real prototype
(b) GS (c) GF (d) GH

Fig. 1. Scenes of the greenhouses.

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2.2 Sampling, culturing and counting of airborne microorganisms

Field tests of airborne fungi were performed on three consecutive days in December 2020 in GS, GF and GH greenhouse. Two indoor sampling points (P_{I-1} and P_{I-2}) and one outdoor sampling point (Po) were set up in each greenhouse, shown in Fig. 2. At the length direction, P_{I-1} and P_{I-2} were located at 1/3 L and 2/3 L, respectively. At the span direction, they were located at 1/2 S. Considering the human breathing zone, the sampling height was kept at 1.5 m above ground [12]. Airborne fungi in each greenhouse were sampled in the morning before ventilation. The sampling was repeated after 40 min of ventilation because farmers would generally enter greenhouses for working almost within 40 minutes. The indoor microbial concentration after 40 minutes of ventilation is instructive to evaluate the microbial exposure of farmers. Po was located at the open and well-ventilated place, 10 m away from the greenhouses. Three replicates were conducted for each sampling.

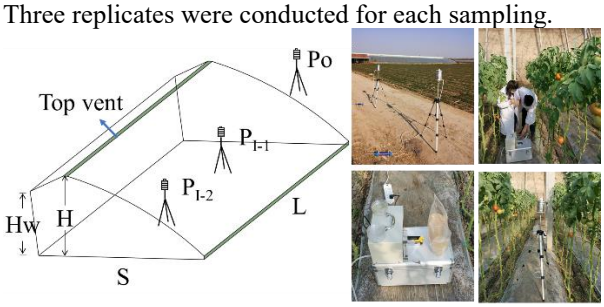

Fig. 2. Schematic of the greenhouses.

Airborne microorganisms were collected using a six-stage Andersen sampler (FA-3, China; I: > 7.0 μm, II: 4.7~7.0 μm, III: 3.3~4.7 μm, IV: 2.1~3.3 μm, V: 1.1~2.1 μm, VI: 0.65~1.1 μm) from the three greenhouses with an airflow rate of 28.3 L/min for 5 min. Potato dextrose agar (PDA) was used as the culture media for the sampled fungi. After sampling, the plate was sealed and transferred to the laboratory and placed in a constant temperature incubator. Fungal colonies were incubated at 28 ºC for 3~5 days. After completing the culture, the number of colonies was counted immediately and adjusted by a positive-hole correction table [13]. The airborne fungal concentrations were calculated by Eq. (1):

$$C=(Pr\times1000)/(t\times Q) \quad (1)$$

Where, C is the airborne fungi aerosol concentration (CFU/m^3), Pr is the corrected colony, Q is the sampling airflow rate (L/min) and t is the sampling time (min).

2.3 DNA extraction and polymerase chain reaction amplification

After counting, fungal colonies were isolated from petri plates. The three replicate plates from the same source were gathered together as one sample. DNA-based analysis was used to identify taxonomic composition of airborne fungi by Novogene Co., LTD [13].

2.4 Environmental factor measurements

During airborne microorganisms sampling, key environmental parameters were measured, including temperature (T), relative humidity (RH). Temperature and relative humidity were measured by automatic recorders with an interval of 5 minutes.

3 Results and discussion

3.1 Airborne fungal concentrations among the three greenhouses

Fig. 3 illustrates airborne fungal concentrations in the three greenhouses. The airborne fungal concentration in GS greenhouse before ventilation (C_{BV-S}) was 24187.3 CFU/m^3. After 40 minutes of ventilation, fungal concentration (C_{V-S}) decreased to 12517.7 CFU/m^3. The reduction ratio was 48.2%. The outdoor fungal concentration (C_{O-S}) was 2221.7 CFU/m^3, which was highly lower than indoor fungal concentrations. In GF greenhouse, C_{BV-F}, C_{V-F} and C_{O-F} were 2609.5 CFU/m^3, 3037.1 CFU/m^3, 911.7 CFU/m^3, respectively. The indoor concentration after 40 min of ventilation was slightly larger than it before ventilation. In GH greenhouse, it was 32756.2 CFU/m^3, 12395.8 CFU/m^3 and 3257.9 CFU/m^3 of C_{BV-H}, C_{V-H} and C_{O-H}. The reduction ratio of ventilation was 62.2%.

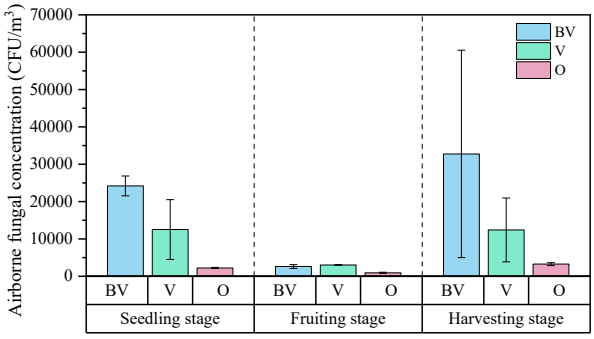

Fig. 3 Airborne fungal concentration in the three greenhouses. BV=Before ventilation, V=Ventilation for 40 minutes, O=Outdoor.

The same downtrend was found in the three greenhouses. It can be seen that indoor airborne fungal concentration were higher than outdoor airborne fungal concentration, which is similar to the results observed by Li et al. [14] . The airborne fungal concentration under ventilated conditions is lower than that before ventilation, indicating that ventilation has a direct effect on reducing the air microbial concentration in the greenhouse.

According to the measurement results, after 40 minutes of ventilation, the airborne fungal concentration in the greenhouse is still much higher than the outdoor concentration, so necessary protective measures need to be taken. Although ventilation is very effective in reducing indoor pollutants, the time and volume of ventilation need to be evaluated according to the actual situation.

3.2 Taxonomic composition of airborne fungi

Fig. 4 displays the taxonomic composition of airborne fungal genus of the three greenhouses. In GS greenhouse, the total counts of airborne fungal genus before ventilation, after ventilation for 40 min and in outdoor air were 14, 14 and 13. The common genera was 13, which was *Aspergillus*, *Cladosporium*, *Filobasidium*, *Fusarium*, *Gibberella*, *Hannaella*, *Monographella*, *Papiliotrema*, *Penicillium*, *Sarocladium*, *Sporobolomyces*, *Talaromyces* and *Vishniacozyma*. The abundant airborne fungal genus was *Aspergillus*, followed by *Cladosporium* and *Penicillium*. Concentrations of *Aspergillus* before ventilation, after ventilation for 40 min and in outdoor air were 22054.2 CFU/m^3, 10420.8 CFU/m^3 and 105.0 CFU/m^3 respectively. For *Cladosporium*, they were 1441.3 CFU/m^3, 746.9 CFU/m^3 and 1092.4 CFU/m^3. They were 497.1 CFU/m^3, 358.7 CFU/m^3 and 108.6 CFU/m^3 for *Penicillium*.

In GF greenhouse, the total counts of airborne fungal genus before ventilation, after ventilation for 40 min and in outdoor air were 15, 17 and 16. The common genera was 15, which was *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Cryptococcus*, *Filobasidium*, *Gibberella*, *Microascus*, *Monographella*, Naganishia, *Penicillium*, *Sarocladium*, *Stemphylium*, *Talaromyces* and *Trichoderma*. The abundant airborne fungal genus was *Aspergillus*, followed by *Cladosporium* and *Penicillium*. Concentrations of *Aspergillus* before ventilation, after ventilation for 40 min and in outdoor air were 1774.8 CFU/m^3, 1103.1 CFU/m^3 and 242.1 CFU/m^3 respectively. For *Cladosporium*, they were 585.4 CFU/m^3, 1102.5 CFU/m^3 and 1092.4 CFU/m^3. They were 47.4 CFU/m^3, 178.8 CFU/m^3 and 55.7 CFU/m^3 for *Penicillium*.

In GH greenhouse, the total counts of airborne fungal genus before ventilation, after ventilation for 40 min and in outdoor air were 12, 12 and 11. The common genera was 10, which was *Acremonium*, *Aspergillus, Botrytis*, *Cladosporium*, *Gibberella*, *Microascus*, *Naganishia*, *Papiliotrema*, *Penicillium and Talaromyces*. *Aspergillus*, *Cladosporium* and *Penicillium* were still the top three fungal genus. Concentrations of *Aspergillus* before ventilation, after ventilation for 40 min and in outdoor air were 6173.5 CFU/m^3, 4088.0 CFU/m^3 and 848.5 CFU/m^3 respectively. For *Cladosporium*, they were 20758.6 CFU/m^3, 5110.0 CFU/m^3 and 1280.4 CFU/m^3. They were 1528.3 CFU/m^3, 127.2 CFU/m^3 and 109.4 CFU/m^3 for *Penicillium*.

Aspergillus, *Cladosporium* and *Penicillium* were the dominant fungal genera both in indoor and outdoor air of the three greenhouses, in accordance with findings from other greenhouses. The predominant fungus taxa recovered in a Spanish greenhouses were species of *Cladosporium* and *Botrytis* [15]. In five Danish greenhouses which produced tomatoes and cucumbers, *Penicillium* was the most common genus, followed by *Cladosporium* [16]. *Aspergillus/Penicillium*-like and *Cladosporium* were belong to the top 5 fungi in two greenhouses with ornamental plants [14].

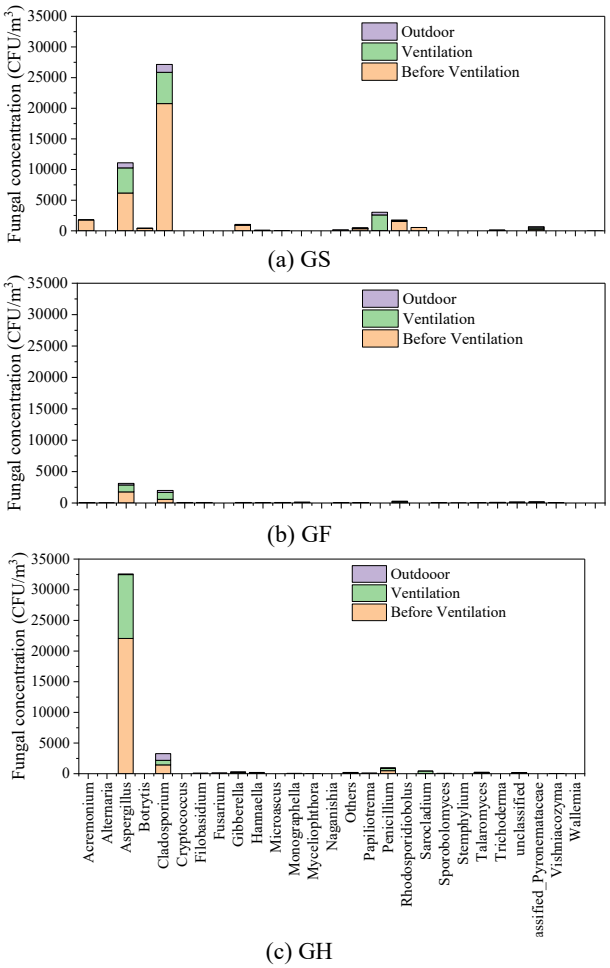

Fig. 4 Taxonomic composition of airborne fungal genus in the three greenhouses.

Aspergillus spp. belong to Risk Group 2 according to Ref. [17]. Diseases caused by *Aspergillus spp.* include clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, Farmers's lung), superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), and invasive pulmonary and extrapulmonary infections [14]. *Cladosporium* is a type of outdoor particles, predominated inside greenhouses, probably originating from the outside air [5]. Workers in tomato houses are liable to become allergic to the spores of *Cladosporium fulvum* [18]. *Penicillium* species are reported to produce different types of mycotoxins which exhibit a variety of biological activities [19]. Therefore, it is important to take actions to decrease the concentrations of airborne fungal in greenhouses. Ventilation is an effective measure from abovementioned results of counting.

3.3 Environmental factors

Fig. 3 illustrates indoor and outdoor temperature and relative humidity of the three greenhouses. It can be seen that there was a strong correlation between indoor and outdoor temperature and relative humidity. Due to frequent heat and mass exchange after ventilation, indoor temperature and relative humidity change trend

was consistent with outdoor, but indoor relative humidity and temperature were still higher than outdoor. As the solar radiation gradually increased, indoor and outdoor temperature gradually rose while relative humidity decreased.

During the sampling period, in GS greenhouse, the indoor relative humidity decreased from 73% to 37%. Air temperature increased from 19.88℃ to 27.06℃. The indoor relative humidity in GF greenhouse decreased from 96.0% to 60.0%. The indoor temperature was increased from 14.44℃ to 32.81℃. The indoor relative humidity in GH greenhouse decreased from 93.4% to 73.1%. The fluctuation of temperature was small. The highest temperature was 17.1℃, while the lowest was 12.6℃. The temperature difference was 4.5℃.

The reduction of relative humidity is beneficial for farmers. Humidity more than 65% may cause the incidence of upper respiratory problems which might increase and can have adverse effects on people suffering from asthma and allergies [20]. *Aspergillus*, *Penicillium* and *Cladosporium* were positively correlated with temperature, relative humidity, light and negatively with air movement and air pressure [14]. In the three greenhouses, ventilation not only reduced the concentration of airborne fungi by dilution, but also inhibited the growth of airborne fungi by reducing relative humidity.

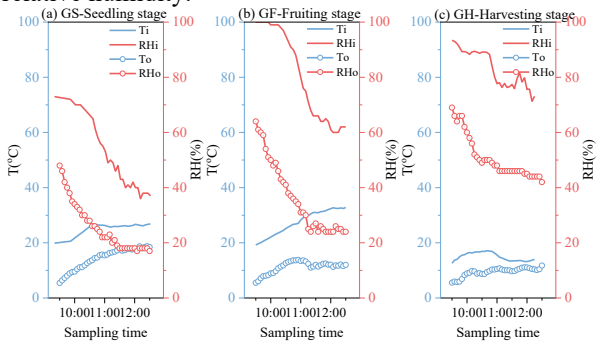

Fig. 5 Indoor and outdoor temperature and relative humidity in greenhouses during sampling time.

4 Conclusions

The concentrations of airborne fungi at seedling, fruiting and harvesting stages in typical tomatoes greenhouses were tested. The dominant genera of airborne fungi were *Aspergillus*, *Cladosporium* and *Penicillium* before or after ventilation. Ventilation has a certain effect on reducing indoor fungal concentration. The reduction of airborne fungi in GS and GH greenhouses were 48.2% and 62.2%.

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