

Supplementary Materials: Quantitative Analysis of Fungal Contamination of Different Herbal Medicines in China

Gang Wang ^{1,2}, Mingyue Jiao ^{1,2}, Junqiang Hu ^{2,3}, Yiren Xun ^{1,2}, Longyun Chen ^{1,2}, Jianbo Qiu ², Fang Ji ², Yin-Won Lee ⁴, Jianrong Shi ² and Jianhong Xu ^{1,2,*}

¹ School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China;

wanggang2015@jaas.ac.cn (G.W.); jiaomy2021@163.com (M.J.);

xunyr2017@163.com (Y.X.); clyun99@163.com (L.C.)

² Jiangsu Key Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base, Ministry of Science and Technology/Key Laboratory for Agro-product Safety Risk Evaluation (Nanjing), Ministry of Agriculture and Rural Affairs/Key Laboratory for Control Technology and Standard for Agro-Product Safety and Quality, Ministry of Agriculture and Rural Affairs/Collaborative Innovation Center for Modern Grain Circulation and Safety/Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China;

2021216027@stu.njau.edu.cn (J.H.); 20120027@jaas.ac.cn (J.Q.);

jifang625@126.com (F.J.); jianrong63@126.com (J.S.)

³ Key Laboratory of Agricultural Environmental Microbiology, Ministry of Agriculture, College of Life

Sciences, Nanjing Agricultural University, Nanjing 210095, China

⁴ Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea; lee2443@snu.ac.kr

* Correspondence: xjh@jaas.ac.cn

Table S1. Sources of the herbal medicines used in this study

| Category | Samples (n) | Place of origin |
|-------------------|---|--|
| Fructus | <i>Crataegus pinnatifida</i> (6) | Yinan, Shandong / Nanyang, Henan |
| | <i>Gardenia flos</i> (6) | Fuding, Fujian / Jiujiang, Jiangxi |
| | <i>Semen euryales</i> (6) | Zhaoqing, Guangdong / Jining, Shandong |
| | <i>Semen sesami nigrum</i> (6) | Fuyang, Anhui / Zhoukou, Henan |
| | <i>Hovenia dulcis</i> (6) | Dushan, Guizhou / Ankang, Shanxi |
| | <i>Mulberry</i> (6) | Yancheng, Jiangsu / Guangyuan, Sichuan |
| | <i>Cannabis sativa</i> (6) | Taizhou, Jiangsu / Jincheng, Shanxi |
| | <i>Semen ziziphi spinosae</i> (6) | Taizhou, Jiangsu / Xingtai, Hebei |
| Radix and rhizome | <i>Cornus officinalis</i> (6) | Taizhou, Jiangsu / Luoyang, Henan |
| | <i>Pseudostellaria heterophylla</i> (6) | Taizhou, Jiangsu / Zherong, Fujian |
| | <i>Panax quinquefolium</i> (6) | Xinbin, Liaoning / Fusong, Jilin |
| | <i>Rhizoma phragmitis</i> (6) | Xuzhou, Jiangsu / Shangqiu, Henan |
| | <i>Glycyrrhiza uralensis</i> (6) | Taizhou, Jiangsu / Longxi, Gansu |
| | <i>Angelica sinensis</i> (6) | Longnan, Gansu / Yaan, Sichuan |
| | <i>Scutellaria baicalensis</i> (6) | Taizhou, Jiangsu / Yuncheng, Shanxi |
| | <i>Dioscorea opposita</i> (6) | Jiaozuo, Henan / Yulin, Guangxi |
| Whole herbs | <i>Pueraria lobata</i> (6) | Taizhou, Jiangsu / Tengxian, Guangxi |
| | <i>Taraxacum mongolicum</i> (6) | Taizhou, Jiangsu / Tianshui, Gansu |
| | <i>Epimedium brevicornum</i> Maxim. (6) | Taizhou, Jiangsu / Xihe, Gansu |
| Folium | <i>Folium mori</i> (6) | Yancheng, Jiangsu / Taizhou, Jiangsu |
| | <i>Nelumbinis folium</i> (6) | Fuzhou, Jiangxi / Caoxian, Shandong |
| Blossom | <i>Lonicera japonica</i> (6) | Linyi, Shandong / Suiyang, Guizhou |
| Vine | <i>Dendrobium officinale</i> (6) | Dehong, Yunnan / Huoshan, Anhui |

Table S2. Frequency of fungal contamination from different herbal medicines

| Herbs | Counts | Frequency (%) |
|-------------------------------------|--------|---------------|
| <i>Taraxacum mongolicum</i> | 46 | 26.59 |
| <i>Lonicera japonica</i> | 11 | 6.36 |
| <i>Folium mori</i> | 7 | 4.05 |
| <i>Glycyrrhiza uralensis</i> | 7 | 4.05 |
| <i>Cannabis sativa</i> | 6 | 3.47 |
| <i>Semen ziziphi spinosae</i> | 5 | 2.89 |
| <i>Cornus officinalis</i> | 5 | 2.89 |
| <i>Epimedium brevicornum</i> Maxim. | 4 | 2.31 |
| <i>Nelumbinis folium</i> | 4 | 2.31 |
| <i>Pseudostellaria radix</i> | 10 | 5.78 |
| <i>Panax quinquefolium radix</i> | 7 | 4.05 |
| <i>Rhizoma phragmitis</i> | 7 | 4.05 |
| <i>Semen euryales</i> | 6 | 3.47 |
| <i>Angelica sinensis radix</i> | 4 | 2.31 |
| <i>Gardenia flos</i> | 8 | 4.62 |
| <i>Dendrobium officinale</i> | 4 | 2.31 |
| <i>Scutellaria baicalensis</i> | 3 | 1.73 |
| <i>Crataegus pinnatifida</i> | 2 | 1.16 |
| <i>Dioscorea opposita</i> | 10 | 5.78 |
| <i>Pueraria lobata</i> | 2 | 1.16 |
| <i>Semen sesami nigrum</i> | 4 | 2.31 |
| <i>Hovenia dulcis</i> | 7 | 4.05 |
| <i>Mulberry fructus</i> | 4 | 2.31 |
| Total | 173 | 100 |

Table S3. Frequency of fungal contamination from *Taraxacum mongolicum*

| Category | Place of origin | | | | | | | |
|-------------------------------|---------------------|--------------------|---------------------|---------------------|--------------------|-----------------------|-------------------|----------------|
| | Taizhou, Jiangsu | Tianshui, Gansu | Yuncheng, Shanxi | Zhengzhou, Henan | Linyi, Shandong | Hangzhou, Zhejiang | Yulin, Guangxi | Luan, Anhui |
| <i>Fusarium spp.</i> | 5 | 3 | 2 | 2 | 3 | 2 | 2 | 1 |
| <i>Alternaria spp.</i> | 2 | 4 | 1 | - | 2 | - | 3 | 3 |
| <i>Epicoccum spp.</i> | - | 1 | 1 | - | - | 1 | - | - |
| <i>Nigrospora spp.</i> | 1 | - | - | - | - | - | - | - |
| <i>Trichocladium spp.</i> | | - | 1 | - | - | - | - | - |
| <i>Aspergillus spp.</i> | 1 | - | - | 1 | - | - | - | - |
| <i>Microdochium spp.</i> | - | - | 1 | - | - | - | - | - |
| <i>Botrytis spp.</i> | - | - | - | - | 1 | - | | |
| <i>Penicilium spp.</i> | 1 | - | - | - | - | - | - | - |
| <i>Neopestalotiopsis spp.</i> | - | - | - | - | - | 1 | - | - |

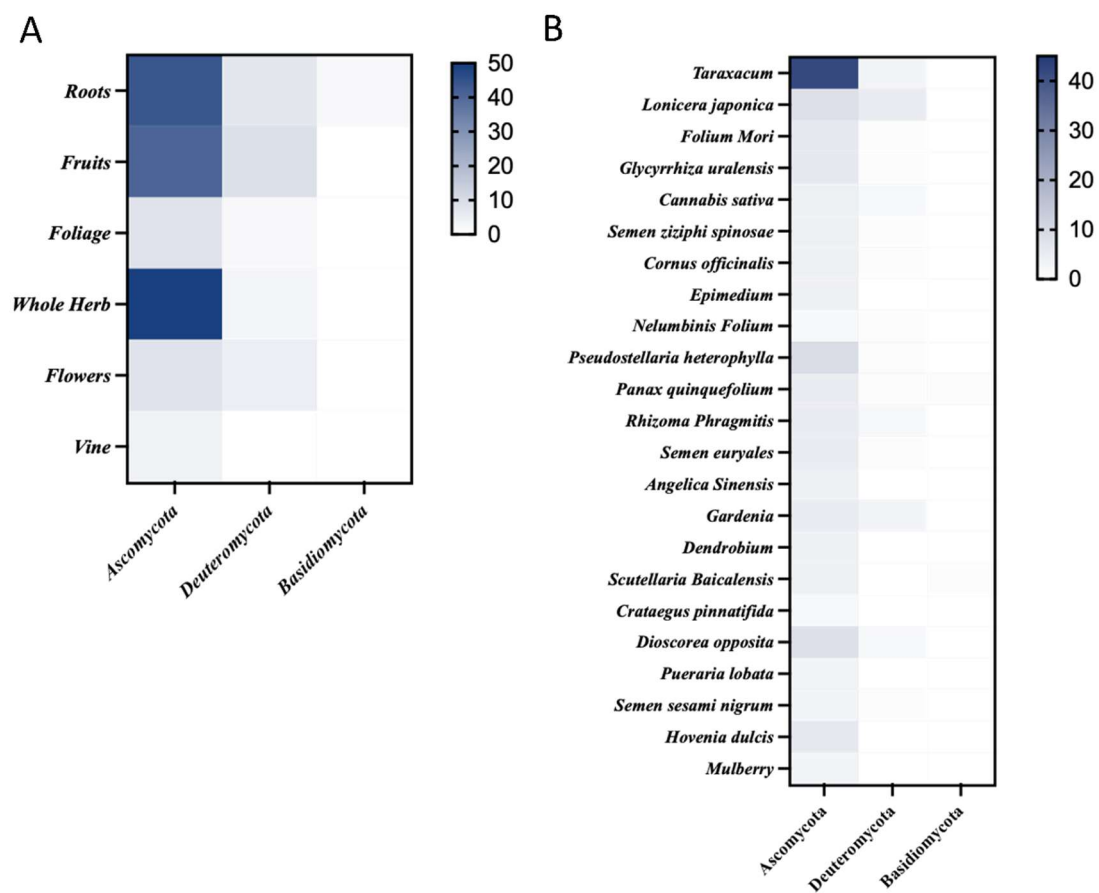
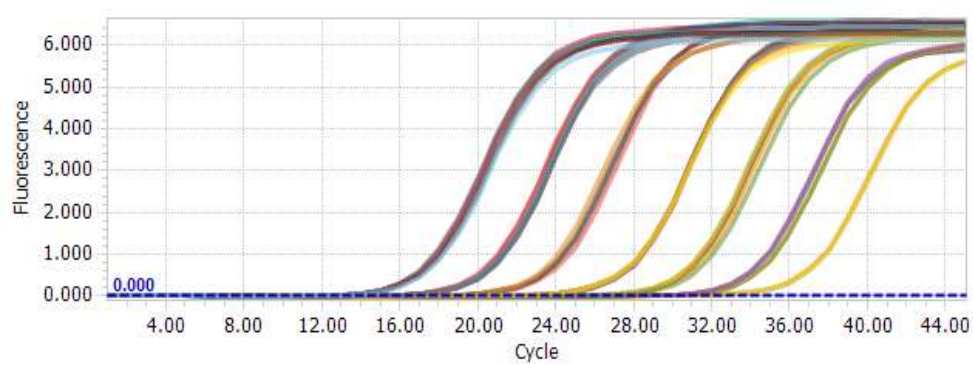
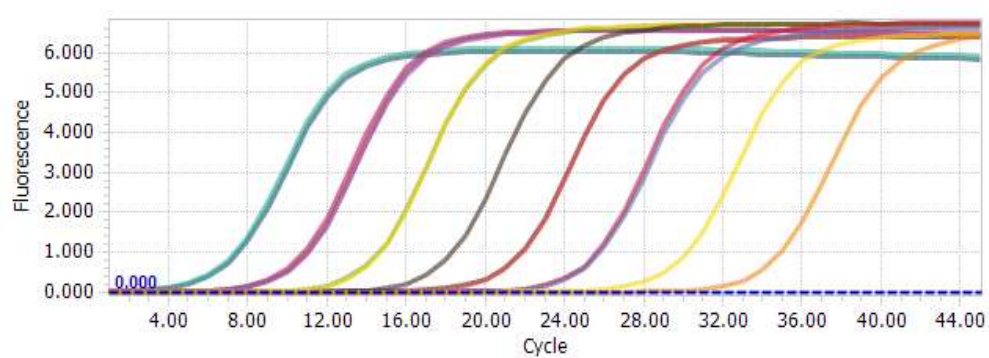


Figure S1. Contamination assessment of phytopathogenic fungi on different kinds of herbal medicines on a phylum level.



(A)



(B)

Figure S2. Sensitivity of the RT-qPCR assay for detection of *Fusarium* spp. (A) and *Alternaria* spp. (B)

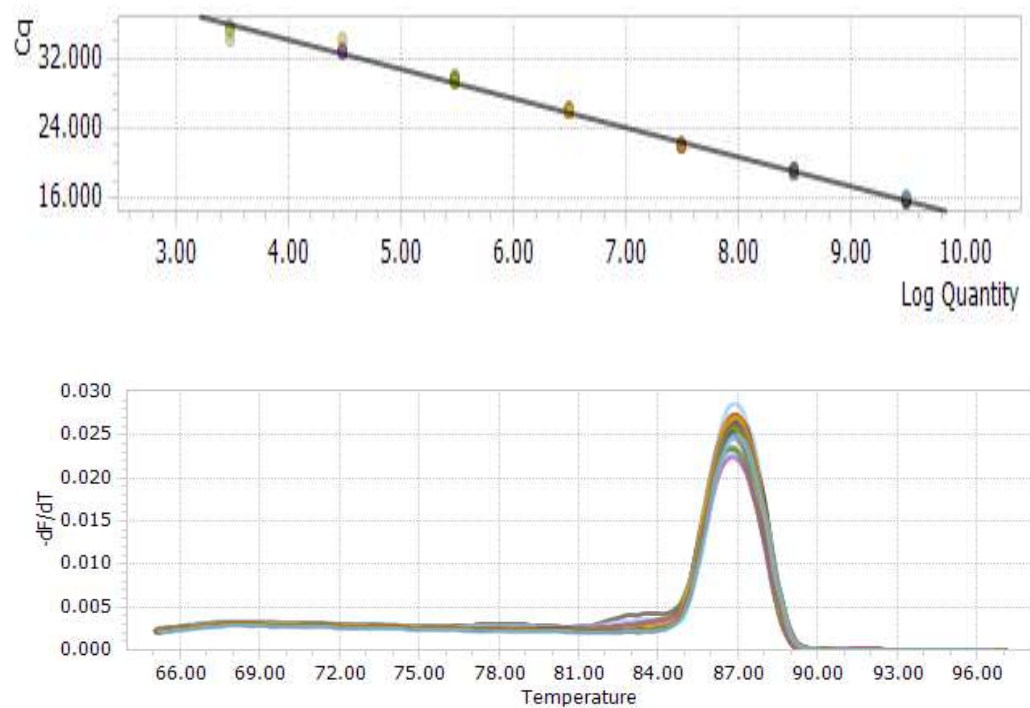


Figure S3. The calibration curve and dissociation curve of recombinant plasmids amplification products for *Fusarium EF-1 α* gene

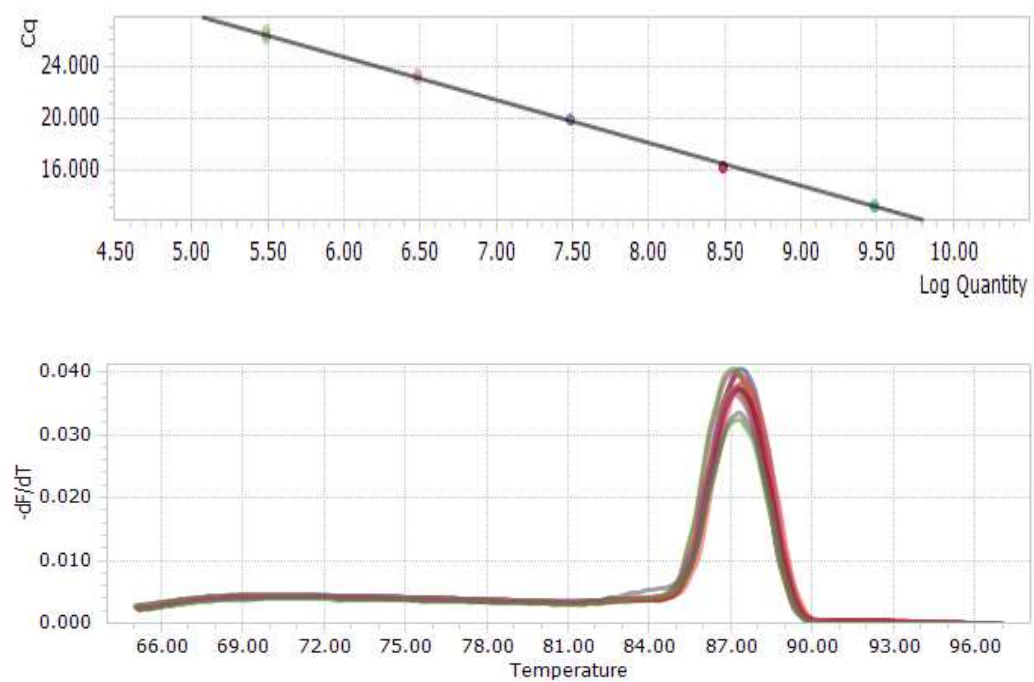


Figure S4. The external calibration curve and dissociation curve of recombinant plasmids amplification products for *Alternaria AQAltpks* gene



Trichocladium sp.



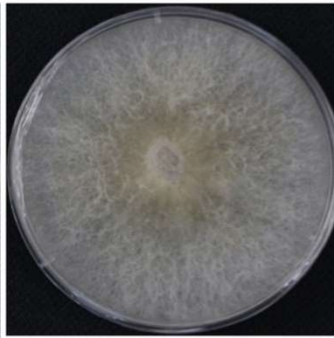
Epicoccum sp.



Nigrospora sp.



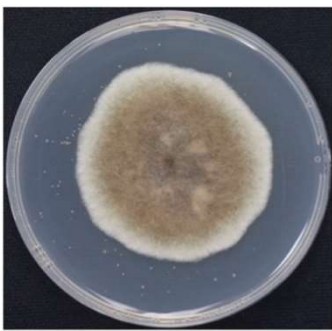
Aspergillus sp.



Botrytis sp.



Fusarium sp.



Alternaria sp.



Bipolaris sp.



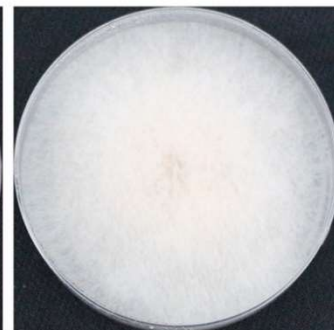
Penicillium sp.



Curvularia sp.



Cladosporium sp.



Apiospora sp.

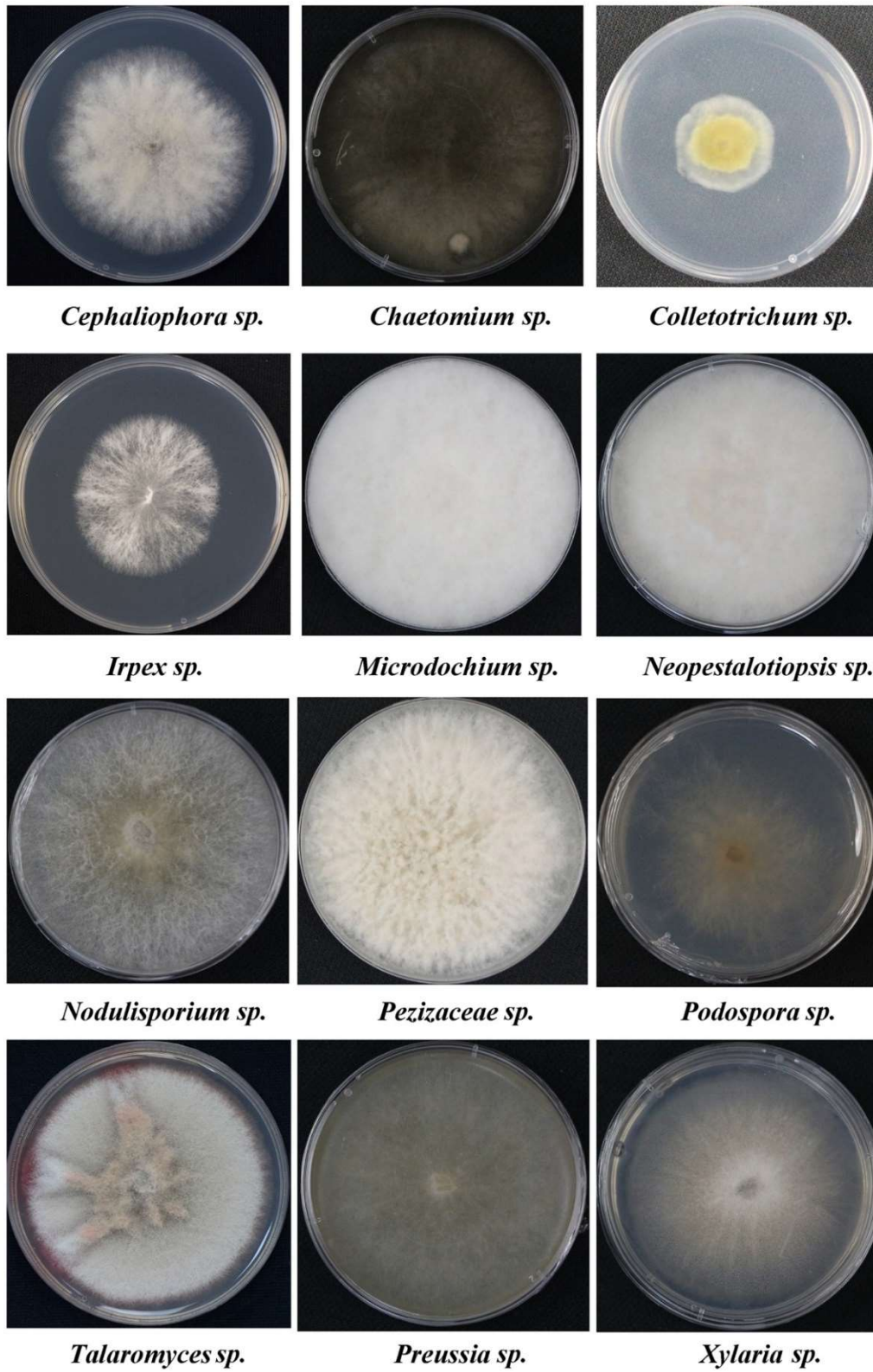


Figure S5. Colonial morphology of all the 24 genera of isolated fungi grown on PDA at 25 °C for 5 days