Antifungal activity of camphor against four phytopathogens of Fusarium

Huanran Huo
Northwest Normal University
Yan Gu
Northwest Normal University
Yueqin Cao
Northwest Normal University
Na Liu
Northwest Normal University
Pengfei Jia
Yuzhou Tianyuan Biotechnology Co. LTD
Weibao Kong (kwbao@163.com)
Northwest Normal University https://orcid.org/0000-0001-6151-5962

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Abstract

Fusarium, one of the main fungal pathogens, can infect field crops and cause great economic loss. This paper concerns a research on the antifungal activity of camphor. In our study, an assessment was made on the antifungal activity of camphor against four common phytopathogens: *Fusarium oxysporum* G5, *F. solani* G9, *F. verticillioide,* and *F. graminearum.* The method adopted was mycelial growth inhibition. The minimum inhibitory concentrations (MIC) of camphor against the four tested fungi were 4.0, 4.0, 4.0, and 2.0 mg/mL, and the half maximal inhibitory concentrations (IC_{50}) were 2.0, 2.0, 2.0, and 1.0 mg/mL, respectively. The paper proper also involves an investigation the, fungicidal mechanisms via cell membrane permeability, proteins and nucleic acids leakage and scanning electron microscopy. The results of preliminary antifungal mechanism revealed that camphor can cause cytomembrane destruction, enhancing the permeability of cytomembrane and releasing intracellular macromolecules, such as nucleic acids and proteins. Supposedly, the results suggested that the cytomembrane may be the target of camphor. In addition, these outcomes indicated that camphor can exhibit pronounced fungicidal activities against the four tested fungi and could be a promising alternative for the control of phytopathogenic *Fusarium*.

Highlights

- Camphor can significantly inhibit the growth of phytopathogenic *Fusarium*.
- Camphor can disrupt the permeability and integrity of *Fusarium* cytomembrane.
- Camphor may inhibit the growth of *Fusarium* at several different levels.
- Camphor can be used as an alternative to against phytopathogenic *Fusarium*.

Introduction

Fusarium is one of the dominant phytopathogens causing serious crop wilt, stem rot, root rot and other soil-borne diseases (Bodah 2017). Many kinds of crops, such as corn, wheat and other cereals, are extremely susceptible to F*usarium* species. As grain contaminants, they have a wide distribution and may cause farmers to sustain significant economic losses (Munkvold 2003; Kazan et al., 2012). Meanwhile, during their growth, they can metabolize some mycotoxins seriously harming the health of animals and humans, such as deoxynivalenol (DON) and zearalenone (ZEN) (Matny 2015). *F. oxysporum*, *F. solani*, *F. verticillioide* and *F. graminearum* are common phytopathogen species belonging in *Fusarium* genus, and they can cause many crop diseases. In particular, *F. oxysporum*, with a worldwide distribution of soil-borne fungal pathogen, can infect and cause diseases to over 120 different plant species including tomatoes, bananas and cotton (Fravel et al. 2003). *F. graminearum* is the key pathogen causing head blight and crown rot (Liu et al. 2015; Goswami and Kistler 2004), while *F. verticillioide* is the main cause of maize ear rot (Chulze et al. 2000).

In the production of industrial crops, the approach to control *Fusarium* diseases is to choose *Fusarium*-resistant cultivars or to apply chemical pesticides (Ferrigo et al. 2016). However, excessive and long-term application of chemically synthesized fungicides will not only cause resistance of phytopathogen, but also lead to soil and environmental pollution. Worse is that they may pose potential safety issues of food raw materials (Lee et al. 2014; Yang et al. 2018). In recent years, the discovery of efficient, green and safe natural fungicides from plants has attracted extensive attention, among which the volatile substances and alkaloids (such as matrine and oxymatrine) from plants are the hotspots (Yang and Zhao 2006; Andrade et al. 2014; Hu et al. 2014; Harkat-Madouri et al. 2015; Moss et al. 2017). Citrus essential oils consisting mainly of monoterpene hydrocarbons are widely used as fungicides in foodstuff and pharmaceutical industries (Jing et al. 2014).

Camphor ($C_{10}H_{16}O$, 1,7,7-trimethylbicyclo[2.2.1]-2-heptanone), a kind of bicyclic monoterpenoids, widely exists in some aromatic plants, such as *Cinnamomum camphora*, *Eucalyptus globulus* and *Artemisia annua.* It is the main component in the majority of plant essential oils (Green 1990). Previous investigations reported that camphor has been in use in medicine and cosmetics (Xiong et al. 2009). The insecticidal and insect-expelling efficacy of camphor has been widely confirmed (Moss et al. 2017; Guo et al. 2016). However, as a main component of some essential oils, whether it has promising antimicrobial activity needs further exploitation. To our knowledge, there are limited studies on the antifungal activity of camphor against the common and dominant phytopathogenic *Fusarium*. Therefore, the aim of this work is to evaluate the effects of camphor on the phytopathogenic fungi species of *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum* on the bases of the in vitro growth capacity of fungal mycelium, changes of cytomembrane permeability, leakage of intracellular compounds, and the morphology of hypha.

Materials And Methods

Fungal strains, culture media and conditions

Four plant pathogenic fungi species, *F. oxysporum* G5, *F. solani* G9 and *F. verticillioide* were obtained from the Laboratory of Microbial Resources and Technology, College of Life Sciences, Northwest Normal University. *F. graminearum* CICC 2697 was purchased from the China Center of Industrial Culture Collection. The fungi were cultured on potato dextrose agar (PDA) medium (200 g/L potato, 20 g/L dextrose and 15 g/L agar in distilled water) in a 90 mm diameter Petri dish at 28 ℃ for approximate 8 days in an incubator.

Chemicals

Camphor (analytical grade) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Tween-80 was supplied from Yantai Shuangshuang Chemical Co. (Yantai, China). Dimethyl sulfoxide (DMSO) was purchased from Shanghai Zhongqin Chemical Reagent Co. (Shanghai, China). All other reagents used in this work are all analytical pure except for special instructions.

Measurement of antifungal activity

The antifungal activity of camphor was detected by the method of mycelial growth inhibitory with some modifications (Irzykowska et al. 2013). Briefly, the appropriate volume of the stock solution (sterilized by a 0.22 μm organic filter) of camphor dissolved in dimethylsulfoxide (DMSO) was thoroughly mixed with a certain amount of unsolidified and sterilized PDA medium, and then it was poured into a Petri dish to prepare a series of gradient concentration plates (0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 mg/mL). Equal amount of DMSO mixed with PDA was adopted as the control medium. A 7-mm diameter mycelial disc of the four phytopathogenic fungi (*F. oxysporum* G5, *F. solani* G9 and *F. verticillioide* and *F. graminearum* CICC 2697) was punched from PDA culture and inoculated onto the centre of the Petri dish, respectively. The inoculum was cultivated in a light-free incubator at 28 ℃ for 8 days. The mycelial growth momentum of the four phytopathogenic fungi was evaluated according to the cross section method once every 24 h until the 8^{th} day. The growth curve of the fungi was represented by a line graph. Each set of the experiments was in triplicate.

Determination of cell membrane permeability

The changes of membrane permeability of the fungal mycelium treated with camphor were detected by the method reported previously with slight modifications and expressed as relative electric conductivity (REC) (Zhang et al. 2016). To be brief, the vigorous mycelial disks (7 mm) of the four *Fusarium* strains were inoculated in 100 mL of potato dextrose (PD) liquid medium and they were kept shaking for 3 days at 200 rpm and 28 ° C, respectively. After incubation, the cultures were filtered with filter paper to obtain hypha samples under aseptic condition. The samples were thoroughly rinsed with sterile distilled water, and then the samples were prepared after being filtered again. The fresh mycelial sample (1.0 g) was added into 100 mL aqueous solution containing 1.0 and 2.0 mg/mL of camphor, and 0.2% Tween-80, respectively. The electric conductivity of the mixture was marked as L_1. The mixture without addition of camphor was used as a control. The electric conductivity was assayed with a conductivity meter (AZ-8362, Taiwan, China) respectively at 1, 2, 4, 8, 12, 24, and 48 h and marked as L_2. In addition, the electric conductivity of the control prepared with boiled water for 30 min was remarked as L_0. The permeability of fungal cytomembrane was calculated and expressed as the following equation (1):

Relative electric conductivity (REC, %) = 100% × $(L_2\text{-}L_1)/L_0$ (1)

Detection of intracellular macromolecules leakage

As described in section 2.4, the leakage of intracellular macromolecules (nucleic acid and protein) from the fungal mycelium was detected according to Ma et al. (2017). After the mixtures were incubated at 28 ℃ for 48 h, 10 mL of the culture was collected and centrifuged at 5000 rpm for 10 min and the absorbances of nucleic acids and proteins in supernatant were determined by a UV-3600 spectrophotometer (Shimadzu, Japan) at 260 nm and 280 nm, respectively (Luo et al. 2014).

Scanning electron microscopic observation of mycelia morphology

After treatment with camphor for 48h, the hyphae were immobilized with 2.5% glutaraldehyde buffer, and refrigerated overnight at 4 ℃. They were then rinsed with 0.1mol/L PBS for three times, and the samples were dehydrated and replaced in anhydrous ethanol solution with concentrations of 30%, 50%, 70%, 80%, 90%, 100% in turn. The dehydration time was 10min and 15min each time. Then it was solidified overnight in a freezer. Finally, the samples were sprayed with gold and their morphology was observed using a JSM-5600LV scanning electron microscope (SEM, JEOL, Japan).

Statistical analysis

All cultivation and determination were performed in triplicate. The results from each experimental group were expressed as mean ± standard deviation. SPSS software (17.0, USA) was employed to analyze the significant difference between the groups at the level of 0.05.

Results

Efficacy of antifungal activity

The antifungal activities of camphor against the four plant pathogens *Fusarium* are shown in Table 1, Fig. 1 and Fig. 2. As can be seen from Fig. 1, significant differences begin to appear among the groups from the third day. With the extension of culture time and the increase of camphor concentration, the inhibitory effect of camphor on the growth of four tested fungi species gradually increases. When the concentration of camphor added was 2 mg/mL, none of the four tested *Fusarium* strains grow normally. Moreover, the sensitivity of different strains to camphor is different. For example, as can be seen from the growth graphs, *F. graminearum* shows strongest sensitivity to camphor. The results show that camphor has a strong inhibitory effect on the growth of the four tested plant pathogens, and the effect is concentration-dependent.

Fig. 2 shows the growth status of the four plant pathogens *Fusarium* after 8 days of cultivation. *F. oxysporum* G5、*F. solani* G9 and *F. verticillioide* do not show growth on PDA + 4 mg/mL camphor. *F. graminearum fails to* grow normally when the camphor dosage is 2 mg/mL. Therefore, the minimum inhibitory concentration (MIC) values of camphor against *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum are* determined to be 4.0, 4.0, 4.0, and 2.0 mg/mL, respectively. The half maximal inhibitory concentration (IC_{50}) is calculated as 2.0, 2.0, 2.0, and 1.0 mg/mL, respectively. These results conform to those of the previous study reported by Gazdağlı et al. (2018). They found that the MIC and the IC_{50} of camphor against on *F. culmorum* 9F and *F. graminearum* H11 were 2 mg/mL and 1 mg/mL, respectively.

Table 1 shows the absolute inhibition rate of camphor in vivo against the tested fungi. According to the Table 1, when the concentration of camphor is 1.00 mg/mL, the absolute inhibition rate of *F. graminearum* reaches 89.41%, much higher than those of other groups. In general, camphor has the strongest inhibitory effect on *F. graminearum*. When the concentration of camphor is 2.00 mg/mL, the absolute inhibition rates of *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum* reach

83.65%, 91.98%, 82.61%, and 95.84%, respectively, suggesting that camphor exhibit pronounced fungicidal activities against the four tested fungi.

Analysis of antifungal mechanism

Relative electric conductivity (REC) was detected to reflect the variation of fungal cytomembrane permeability. As shown in Fig. 3, the REC of camphor-treated hyphae suspension increases with dosage and time compared with that of untreated hyphae. After 8h of culture, the electric conductivity of camphor-treated *F. oxysporum* G5 began to increase rapidly. After incubation for more than 12 hours, the relative conductivity of *F. oxysporum* G5 in the treatment groups (1.00 and 2.00 mg/mL) began to exceed that in the control (Fig. 3A). 48 h after being treated with camphor of different concentrations :1.00 and 2.00 mg/mL, the relative conductivity of *F. solani* G9 reached 28.9% and 67.5%, respectively, much higher than that of the control group (Fig. 3B). Fig. 3C reflects that the relative conductivity of mycelium suspension (*F. verticillioide*) increases with the increase of camphor concentration and the prolongation of treatment time, showing a positive correlation. 48 h after the treatment, the relative conductivity of the control, 1 mg/mL and 2 mg/mL groups (*F. verticillioide*) became 16.9%, 24.9% and 35.1%, respectively. The results in Fig. 3D indict that during the incubation process, the relative conductivity of the control (*F. graminearum*) obviously remains unchanged, but with the addition of camphor, the relative conductivity increases significantly.

To explore the mechanism of camphor inhibiting *Fusarium* growth, observation was conducted on the morphology of the four *Fusarium* strains by SEM (Fig. 4). As shown in Fig. 4 A, C, E, G, without the treatment of camphor, the mycelium surfaces of *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum* untreated with camphor are relative smooth and complete. Whereas, 48 h after treatment with camphor, the morphology of mycelia changes in different degrees mainly in that the mycelia becomes folded or fractured (Fig. 4 B, D, F and H), indicating that camphor seriously interfers with cell wall synthesis and suppresses the growth of mycelia.

In order to investigate the effect of camphor on the membrane permeability of the *Fusarium* strains, determination was performed on the contents of nucleic acids and proteins in mixtures treated with camphor for 48 h (Fig. 5). Compared with the control group, after the treatment with 1 and 2 mg/mL of camphor, there is great increase in the absorption values at the characteristic wavelength of 260 nm (nucleic acid characteristic absorption peak) and 280 nm (protein characteristic absorption peak). This indicates that camphor has increased the permeability of cells, resulting in the leak of a large amount of intracellular nucleic acids and proteins. It was also found that the release degree of biomolecules varies with different strains. When the concentration of camphor was 2 mg/mL, *F. graminearum* showed the largest release of nucleic acids and proteins.

As to nucleic acid release(Fig. 5A), *F. solani* G9 ranks the first,, followed by *F. verticillioide* and *F. oxysporum* G5, and finally *F. graminearum*. In the matter of protein leakage(Fig. 5B), the first place goes to *F. solani* G9 *with* a significantly higher leakage than the control, followed by *F. verticillioide* and *F. graminearum*, and ultimately *F. oxysporum* G5. Compared with the control, at 260 nm (nucleic acids) and

280 nm (proteins), the absorbance values of *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum* in suspensions treated with camphor at 1.0 mg/mL increase 1.43, 2.25, 1.92, 1.25 folds and 1.44, 3.71, 2.61, 1.25 folds respectively, and at 2.0 mg/mL level, they increase 2.01, 6.34, 2.54, 1.71 folds and 2.00, 7.94, 3.91, 2.10 folds, respectively. The results obviously imply that camphor disruptes the intact cytomembrane structure of *F. solani* G9, while slightly affecting *F. oxysporum* G5 and *F. graminearum* affected slightly, so it can be concluded that the impact of camphor on the permeability and structure of *Fusarium* cytomembrane also varies with species.

To sum up, camphor can inhibit the growth of *Fusarium* through various ways at different levels. It is speculated that the antifungal mechanism of camphor to *Fusarium* mainly involves its interference with the normal gene expression and protein synthesis in fungal cells. In this way, it can cause damage to the structural integrity of the fungal cells and cytomembrane permeability, and make mycelium fold up or break at morphology level, as well as help the release of intracellular substances and the increase of REC at physiological level. Our study also suggests that camphor, as the main active ingredient of natural plant essential oils, has the potential to be developed as a fungicide for plant protection and industrial crop production.

Discussion

It is known that essential oils extracted from aromatic plants are widely used as fungicides (Arif et al. 2009; Gazdağlı et al. 2018; Yörük 2018). Camphor is the main active compound in essential oil. It is found in many aromatic plants, such as *Cinnamonum camphora*, *Piper capense*, *Salvia officinalis*, *Eucalyptus globulus* and *Artemisia annua* (Guo et al. 2014; Fu et al. 2015; Soidrou et al. 2013; Wijesundara and Rupasinghe 2018; Marinas et al. 2015; Harkat-Madouri et al. 2015). According to the previous reports and our findings, camphor is one of the main bioactive components in plant essential oils and an important effective antimicrobial substance, playing an important role in inhibiting pathogenic microorganisms including fungi. Therefore, it is speculated that the strong antimicrobial activity of some essential oil extracted from aromatic plants is related to its camphor content.

Generally, REC is adopted to evaluate the changes in cytomembrane permeability of microorganisms and other types of cells. In previous work, it was reported that monocaprin affects the REC of *Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium citrinum* and that the permeability of *P. citrinum* may be more easily disturbed (Ma et al. 2018). The results imply that the sensitivity of fungi strains to fungistat varies with species and genus. When the camphor concentration is set as 1.00 and 2.00 mg/mL, the final REC (48h after treatment) of *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum* reaches 15.8%, 28.9%, 24.9%, 82.2; and 18.9%, 67.5%, 35.1%, 93.0%, respectively. *F. graminearum* displays the highest rise in REC among the four tested *Fusarium* strains. These data suggest that the permeability of fungal cytomembrane may be more easily disturbed by camphor, resulting in the release of intracellular ions and charged biomolecules (Molatová et al. 2010; Ma et al. 2018).

The natural compounds extracted from plants seem to increase the permeability of microbial cell membranes, resulting in a leakage of cellular content (Burt 2004). Excessive leakage of intracellular ions and macromolecules, caused by increased membrane permeability, can result in cell death (Labbe and Saleh 2008). Storia et al. (2011) proposed that microbial cell wall and membrane are the targets of the antimicrobial activities of many natural plant compounds, such as carvacrol. The treatment of foodborne contaminated microorganisms with carvacrol could change the cell morphology and structure of some G^+ and G^- food-related bacteria. Previous study has also revealed that *β*-carboline oxadiazole derivatives could change the normal cell activities of *Rhizoctonia solani*, the dominant pathogenic fungus causing rice sheath blight (Zhang et al. 2018). Such adverse effects mainly embrace the decrease of mitochondrial membrane potential, the accumulation of reactive oxygen species, the blocked DNA synthesis and the destruction to cell structure. Thus, there could be a similar action mechanism of camphor against the four tested phytopathogenic *Fusarium* strains.

Cell membranes play an important role in maintaining the normal physiological and metabolic activities of cells. Many fungicides inhibit fungal growth by interfering with and destroying the formation and integrity of cell membranes (Avis 2007). When the cell membrane is destroyed, macromolecules are left out (Chavan and Tupe 2014). In the previous study, it was found that the *Mentha piperita* essential oil (MPE) changes the surface properties and permeability of *Fusarium sporotrichioides* hyphae (Rachitha et al. 2017). The increase of the concentration of MPE can trigger corresponding changes to cells, such as intracellular contents leakage, mycelia distortion, pH change, etc. The leakage of nucleic acid and protein manifests that camphor treatment can disturb the normal metabolism of *Fusarium*, and destroy the cell structure, thus inhibiting the growth of mycelia. As an important volatile component in natural plant extract, the potential antifungal mechanism of camphor could damage the fungus cell and disturb the cellular metabolism (Marilena et al. 2001).

The findings from Gazdağlı et al. (2018) revealed the antifungal mechanism of camphor on *F. graminearum* and *F. culmorum* through gene expression level. The analysis of qPCR shows that camphor treatment down-regulates the *tri5* (deoxynivalenol production) expression, while up-regulates the expression of some genes related to essential cellular activity directly determining the fungal life cycle, such as *hog1*, *mst20*, *CAT*, *POD*, *mgv1*, and *stuA* genes. The similar findings from Yörük (2018) show that tetraconazole (TCZ, an important antifungal agent) could fight against *F. graminearum* at genomic, epigenetics, transcriptomics and apoptotic levels. Increasing TCZ concentration could enhance the expression of genes related to apoptosis (*Hog1*) and oxidative stress (POD), whereas down-regulating the expression of *tri5*.

This study shows that camphor has strong antifungal activity against *F. oxysporum* G5, *F. solani* G9, *F. verticillioide* and *F. graminearum*, and that the absolute inhibition rate of the four phytopathogenic fungi could be increased by more than 80% by adding 2 mg/mL camphor in PDA media. The preliminary study on the mechanism exhibits that camphor could participate in and obstruct the formation of cell wall and cytomembrane of the phytopathogens. The involvement of camphor makes fungi release intracellular ions, nucleic acids and proteins necessary for normal cell activity, ultimately inhibiting the growth of

fungi. In addition, the essential oils extracted from some plant with strong antimicrobial activity may be related to their camphor content. Camphor may serve as a potential alternative fungicide for its friendliness to environment and humans. In the future, further studies will be conducted on the molecular regulation mechanism and transcriptomics of camphor inhibiting the growth of important plant pathogens.

Declarations

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Author's contribution

Quite a number of friends and colleages have made their contributions to the research and the paper proper. The experiments were conceived and designed by Weibao Kong, Na Liu and Huanran Huo. The experimental collection and the data assembly were in the charge of Huanran Huo, Yan Gu, and Yueqin Cao. Huanran Huo and Weibao Kong were responsible for data analysis and interpretation. Pengfei Jia volunteered to offer reagents and materials. Huanran Huo and Weibao Kong wrote the paper. Deepened discussions and paper revision were performed by Huanran Huo, Yueqin Cao, Yan Gu, Na Liu and Weibao Kong.

Availability of data and materials

This is an original publication and hence additional data not available.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. All the authors read and approved the final manuscript.

References

Andrade BFMT, Barbosa LN, Probst IS, Júnior AF (2014) Antimicrobial activity of essential oils. J Essent Oil Res 26(1):34–40

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R (2009) Natural products–antifungal agents derived from plants. J Asian Nat Prod Res 11(7):621–638

Avis TJ (2007) Antifungal compounds that target fungal membranes: applications in plant disease control. Can J Plant Pathol 29(4):323–329

Bodah ET (2017) Root rot diseases in plants: a review of common causal agents and management strategies. Agr Res Tech 5(3):555661

Burt SA (2004) Essential oils: their antibacterial properties and potential applications in foods–a review. Int J Food Microbiol 94:223–253

Chavan PS, Tupe SG (2014) Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. Food Control 46:115–120

Chulze SN, Ramirez ML, Torres A, Leslie JF (2000) Genetic variation in *Fusarium* section liseola from no-till maize in Argentina. Appl Environ Microb 66(12): 5312–5315

Endo EH, Cortez DAG, Ueda-Nakamura T, Nakamura CV, Dias-Filho BP (2010) Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*. Res Microb 161(7):534–540

Ferrigo D, Raiola A, Causin R (2016) *Fusarium* toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. Molecules 21:1–35

Fravel D, Olivain C, Alabouvette C (2003) Research review *Fusarium oxysporum* and its biocontrol. New Phytologist 157(3):493–502

Fu JT, Tang L, Li WS, Wang K, Cheng DM, Zhang ZX (2015) Fumigant toxicity and repellence activity of camphor essential oil from *Cinnamonum camphora* siebold against *Solenopsis invicta* workers (Hymenoptera: formicidae). J Insect Sci 15(1):845–849

Gazdağlı A, Sefer Ö, Yörük E, Varol Gİ, Teker T, Albayrak G (2018) Investigation of camphor effects on *Fusarium graminearum* and *F. culmorum* at different molecular levels. Pathogens 7:90

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5(6):515–525

Green BG (1990) Sensory characteristics of camphor. J Invest Dermatol 94(5): 662–666

Guo S, Geng Z, Zhang W, Liang J, Wang C, Deng Z, Du S (2016) The chemical composition of essential oils from *Cinnamomum camphora* and their insecticidal activity against the stored product pests. Int J Mol Sci 17(11):1836

Harkat-Madouri L, Asma B, Madani K, Said ZBOS, Rigou P, Grenier D, Allalou H, Remini H, Adjaoud A, Boulekbache-Makhlouf L (2015) Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. Ind Crops Prod 78:148–153

Hu J, Shi X, Chen J, Mao X, Zhu L, Yu L, Shi J (2014) Alkaloids from *Toddalia asiatica* and their cytotoxic, antimicrobial and antifungal activities. Food Chem 148:437–444

Irzykowska L, Bocianowski J, Baturo-Cieśniewska A (2013) Association of mating-type with mycelium growth rate and genetic variability of *Fusarium culmorum*. Cent Eur J Biol 8:701–711

Jing L, Lei Z, Li L, Xie R, Xi W, Guan Y, Sumner LW, Zhou Z (2014) Antifungal activity of citrus essential oils. J Agr Food Chem 62(14):3011–3033

Kazan K, Gardiner DM, Manners JM (2012) On the trail of a cereal killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance. Mol Plant Pathol 13:399–413

Labbe K, Saleh M (2008) Cell death in the host response to infection. Cell Death Differ 15(9):1339–1349

Lee TVD, Zhang H, Diepeningen AV, Waalwijk C (2014) Biogeography of *Fusarium graminearum* species complex and chemotypes: A review. Food Addit Contam A 32 (4):453–460

Liu C, Ogbonnaya FC, Bürstmayr H (2015) Resistance to *Fusarium* crown rot in wheat and barley: a review. Plant Breeding 134(4):365–372

Luo C, Zeng Z, Gong D, Zhao C, Liang Q, Zeng C (2014) Evaluation of monolaurin from camphor tree seeds for controlling food spoilage fungi. Food Control 46:488–494

Ma M, Wen X, Xie Y, Guo Z, Zhao R, Yu P, Gong D, Deng S, Zeng Z (2018) Antifungal activity and mechanism of monocaprin against food spoilage fungi. Food Control 84:561–568

Marilena M, Bersani C, Comi G (2001) Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int J Food Microbiol 67(3):187–195

Marinas IC, Oprea E, Chifiriuc MC, Badea IA, Buleandra M, Lazar V (2015) Chemical composition and antipathogenic activity of *Artemisia annua* essential oil from Romania. Chem Biodivers 12(10):1554–1564

Matny ON (2015) *Fusarium* head blight and crown rot on wheat & barley: losses and health risks. Adv Plants Agr Res 2:38–43

Molatová Z, Skivanová E, Macias B, Mcewan NR, Bezina P, Marounek M (2010) Susceptibility of campylobacter jejuni to organic acids and monoacylglycerols. Folia Microbiol 55(3):215–220

Moss ATH, Abdelfatta NAH, Mohafrash SMM (2017) Nanoemulsion of camphor (*Eucalyptus globulus*) essential oil, formulation, characterization and insecticidal activity against wheat weevil, *Sitophilus granarius*. Asian J Crop Sci 9(3):50–62

Munkvold G.P (2003) Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur J Plant Pathol 109:705–713

Rachitha P, Krupashree K, Jayashree GV, Gopalan N, Khanum, F (2017) Growth inhibition and morphological alteration of *Fusarium sporotrichioides* by *Mentha piperita* essential oil. Pharmacognosy

Res 9(1):74–79

Soidrou SH, Farah A, Satrani B, Ghanmi M, Jennan S, Hassane SOS, Lachkar M, Abed SE, Koraichi SI, Bousta D (2013) Fungicidal activity of four essential oils from *Piper capense*, *Piper borbonense* and *Vetiveria zizanoides* growing in Comoros against fungi decay wood. J Essent Oil Res 25(3):216–223

Storia AL, Ercolini D, Marinello F, Pasqua RD, Villani F, Mauriello G (2011) Atomic force microscopy analysis shows surface structure changes in carvacrol-treated bacterial cells. Res Microbiol 162:164–172

Wijesundara NM, Rupasinghe HPV (2018) Essential oils from *Origanum vulgare* and *Salvia officinalis* exhibit antibacterial and anti-biofilm activities against Streptococcus pyogenes. Microb Pathog 117:118–127

Xiong Y, Wu XR, Tu XM, Han QM (2009) Progress in pharmaceutical research of camphor. Lab Med Clin 6(12):999–1001

Yang XY, Zhao BG (2006) Antifungal activities of matrine and oxymatrine and their synergetic effects with chlorothalonil. J Forestry Res 17:323–325

Yang Y, Li M, Duan Y, Li T, Shi Y, Zhao D, Zhou M (2017) A new point mutation in β 2-tubulin confers resistance to carbendazim in *Fusarium asiaticum*. Pestic Biochem Phys 145:15–21

Yörük E (2018) Tetraconazole leads to alterations in *Fusarium graminearum* at different molecular levels. Appl Ecol Environ 16:6155–6167

Yorük E, Tunali B, Kansu B, Olmez F, Uz G, Zümrüt IM, Sarıkaya A, Meyva G (2016) Characterization of high-level deoxynivalenol producer *Fusarium graminearum* and *F. culmorum* isolates caused head blight and crown rot diseases in Turkey. J Plant Dis Prot 123(4):177–186

Zhang Y, Liu X, Wang Y, Jiang P, Quek SY (2016) Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Food Control 59:282–289

Zhang Z, Jiang Z, Zhu Q, Zhong G (2018) Discovery of *β*-carboline oxadiazole derivatives as fungicidal agents against rice sheath blight. J Agric Food Chem 66(37):9598–9607

Tables

Table 1 The inhibition of camphor in vivo against different species of *Fusarium*.

Camphor contents (mg/mL)	Absolute inhibition rate (%)			
	F. oxysporum G5	*F. solani* G9	*F. verticillioide*	*F. graminearum*
0.125	3.80 ± 1.43 a	14.55 ± 4.70 a	9.36 ± 1.34 a	7.37 ± 4.78 a
0.25	11.18 ± 1.22 ab	13.60 ± 2.53 a	15.88 ± 2.29 b	33.46 ± 7.80 b
0.50	23.61 ± 4.72 b	15.64 ± 0.52 a	23.99 ± 1.78 c	45.79 ± 3.95 b
1.00	54.63 ± 9.76 c	34.59 ± 4.98 b	54.36 ± 1.34 d	89.41 ± 5.17 c
2.00	83.65 ± 2.37 d	91.98 ± 3.51 c	82.61 ± 3.29 e	95.84 ± 0.13 c
4.00	100.00 e	100.00 c	94.60 ± 0.11 f	100.00 c

Note: Different lowercase letters in a same column indicate a significant difference at $p < 0.05$.

Figures

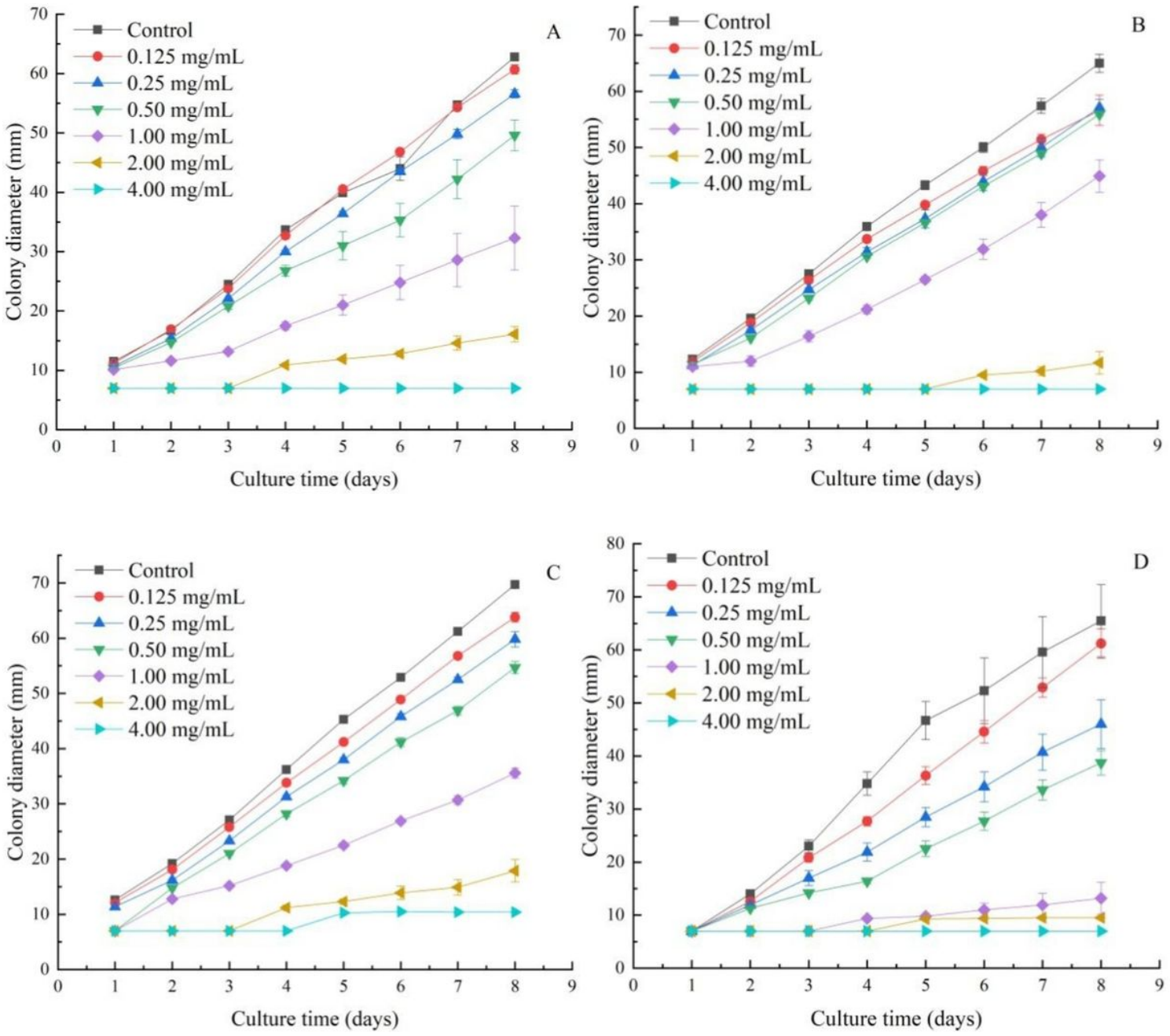

Figure 1

The growth curves of F. oxysporum G5 (A), F. solani G9 (B), F. verticillioide (C) and F. graminearum (D) at various camphor concentrations.

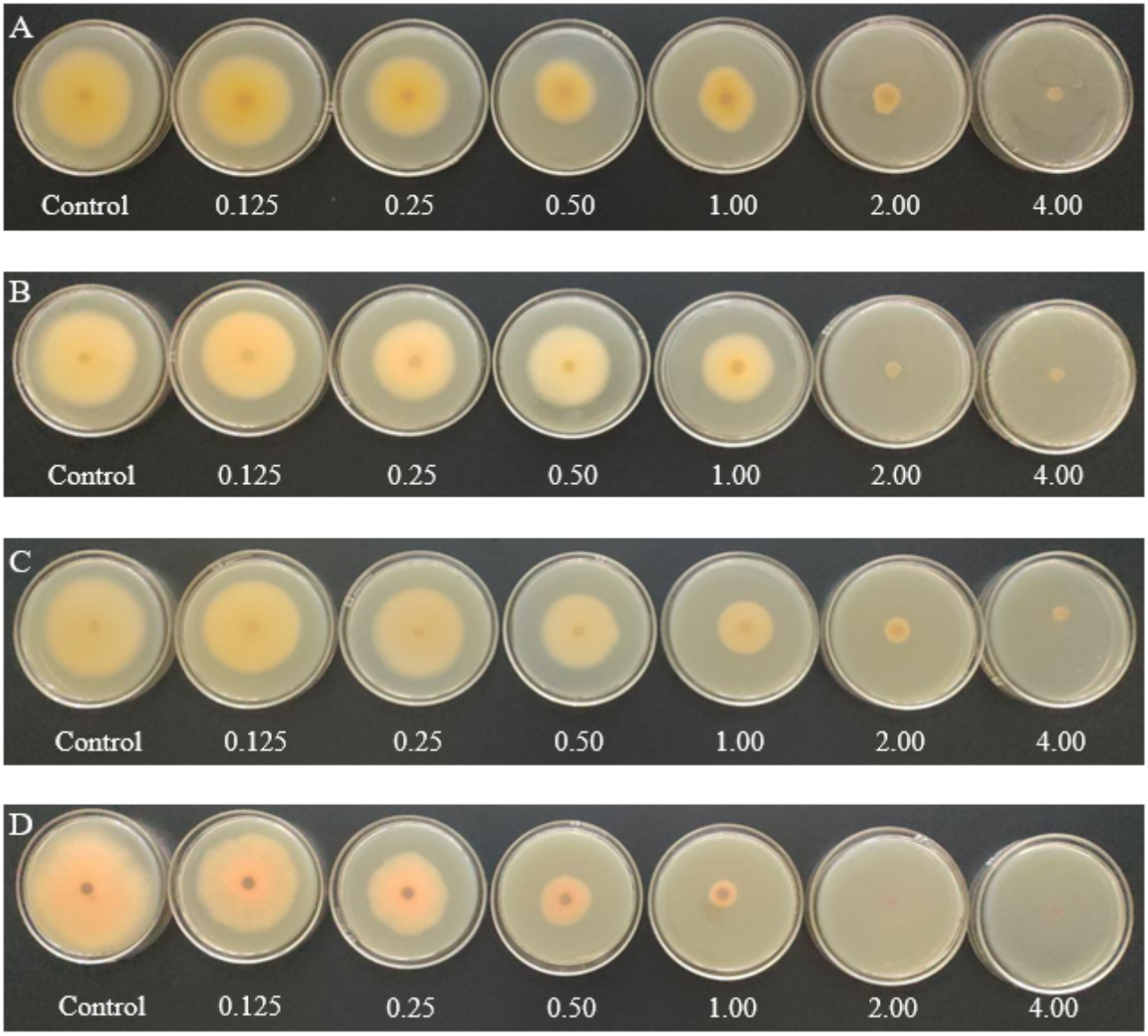

Figure 2

Inhibitory effects of camphor on the growth of F. oxysporum G5 (A), F. solani G9 (B), F. verticillioide (C) and F. graminearum (D) under different concentrations (mg/mL).

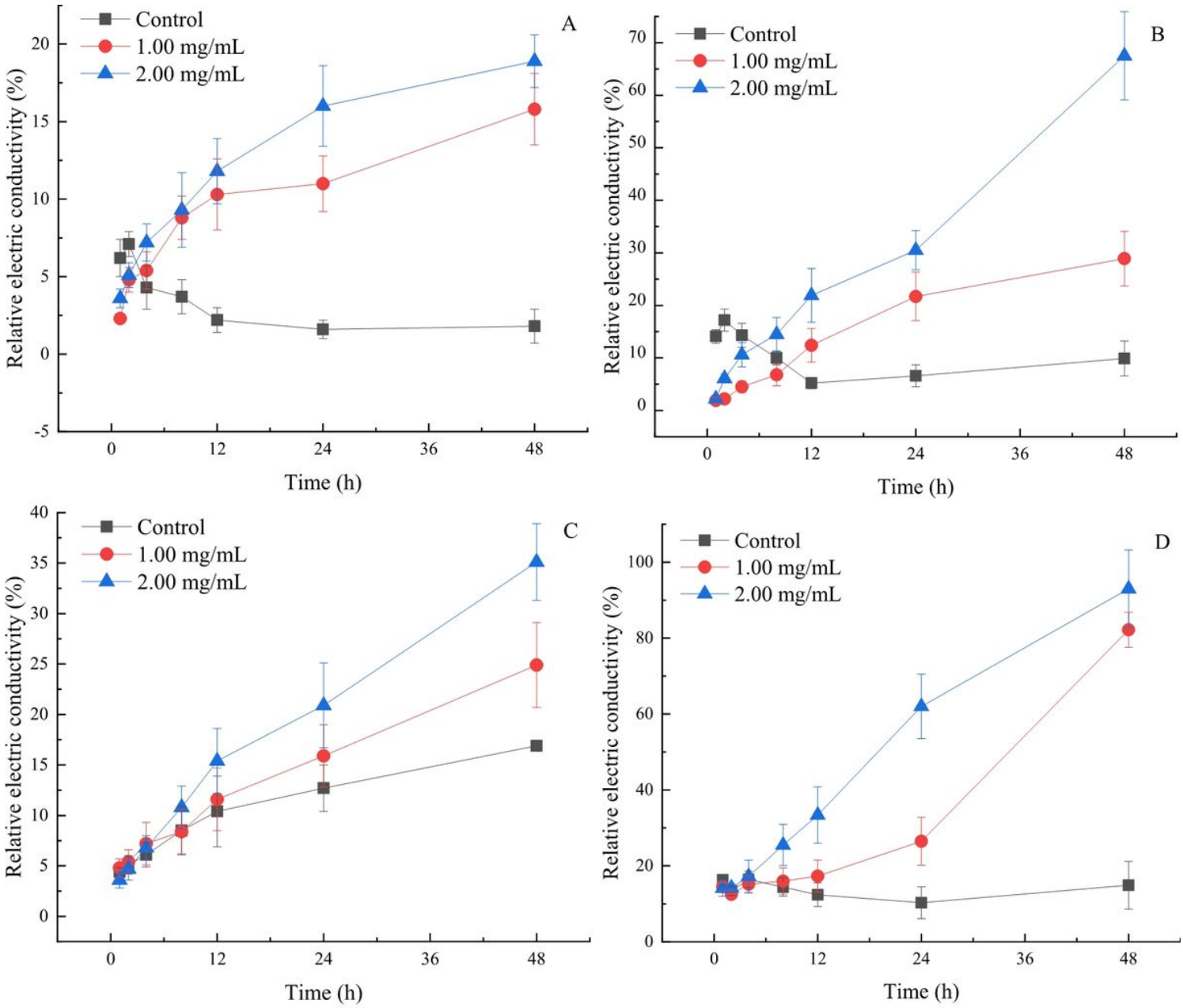

Figure 3

Effects of camphor treatment on the cytomembrane permeability of F. oxysporum G5 (A), F. solani G9 (B), F. verticillioide (C) and F. graminearum (D).

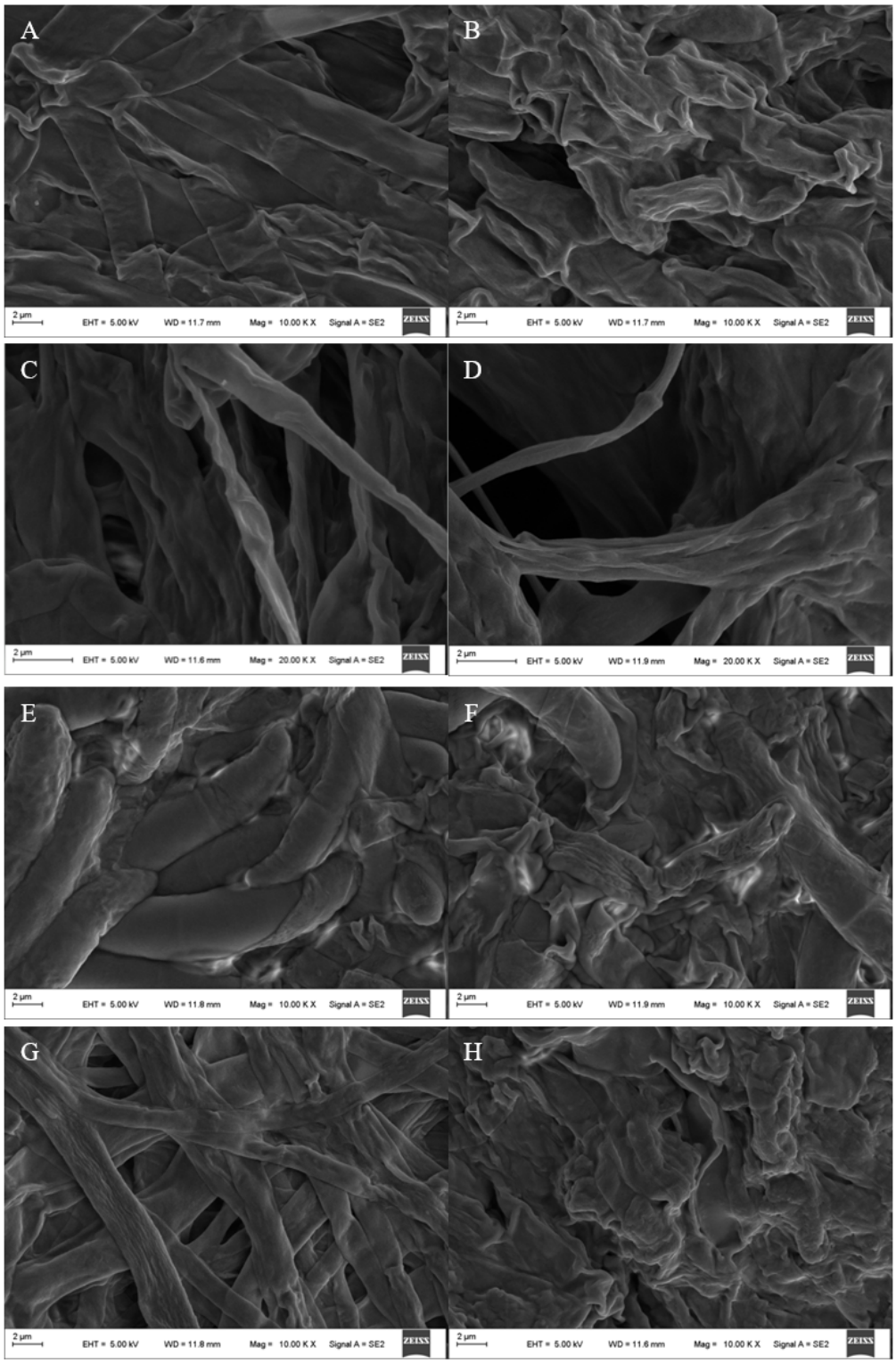

Figure 4

Scanning electron micrographs of F. oxysporum G5, F. solani G9, F. verticillioide and F. graminearum treated with camphor. A, C, E and G are F. oxysporum G5, F. solani G9, F. verticillioide and F. graminearum untreated with camphor, respectively; B, D, F and H are F. oxysporum G5, F. solani G9, F. verticillioide and F. graminearum treated with camphor at 2.00 mg/mL level, respectively.

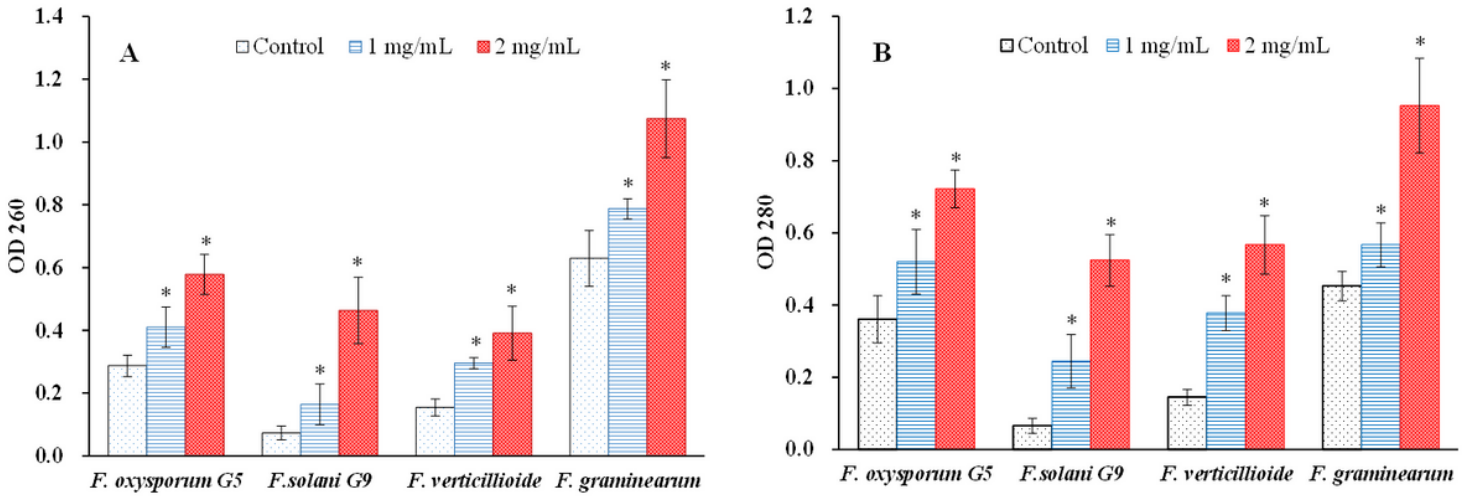

Figure 5

Release of nucleic acids (260 nm, A) and proteins (280 nm, B) of F. oxysporum G5, F. solani G9, F. verticillioide and F. graminearum treated with camphor at different concentrations (0, 1.00, and 2.00 mg/mL, *p < 0.05).

Supplementary Files

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