



Antifungal activity of dimethyl trisulfide and potential biocontrol against *Alternaria alternata* on postharvest *Lycium barbarum*

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Abstract *Lycium barbarum*, is a medicine-food homology plant, so sustainable control of the post-harvest Goji berry black mold caused by *Alternaria alternata* is particularly critical. In this paper, the impact of dimethyl trisulfide (DMTS) on *A. alternata* was studied, as well as the effects of DMTS on hypha ultrastructure, membrane permeability, reactive oxygen production, and essential enzymes of the cellular defense system. DMTS was shown to significantly inhibit the spore germination and hyphal growth of *A. alternata* in a concentration-dependent manner. The results of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed that *A. alternata* cells were separated between cytoplasm

and cell wall, organelles such as mitochondria were vacuolate or even disappeared, and the cytoplasm was fused. PI staining, conductivity measurements, and intracellular nucleic acid and protein content measurements showed that the permeability of the cell membrane changed after DMTS treatment, leading to the leakage of intracellular nucleic acid and protein. DMTS could also cause the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA), thus causing membrane oxidative damage. Through directly destroying the fungal cell and indirectly inducing membrane damage, DMTS can control the postharvest Goji berry black mold, and is a potential application prospect in the control of postharvest diseases.

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Abbreviations

DMTS	Dimethyl trisulfide
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
PI	Propidium iodide
SOD	Superoxide dismutase
CAT	Catalase
EC ₅₀	The half inhibitory concentration
POD	Peroxidase
PDA	Potato dextrose agar medium
MDA	Malondialdehyde

H ₂ O ₂	Hydrogen peroxide
ROS	Reactive oxygen species

Introduction

Lycium barbarum, a medicine-food homolog plant from the Solanaceae family, is distributed mainly in Northwest China, such as Ningxia, Qinghai, and Gansu (Muatasim et al., 2018). Seven species of *L. barbarum* have been reported in China, of which *L. barbarum* is considered to have the best medicinal value. As one of the pillar industries of the Ningxia economy, *L. barbarum* plays an important role in the increase of local farmers' income (Zhang et al., 2022). Meanwhile, due to its high nutritional value and medicinal value, *L. barbarum* is desired by consumers at home and abroad (Huang et al., 2022). For a long time, the dried fruit of *L. barbarum* was the primary commodity in circulation. With the growing interest in natural and fresh fruits, there is an increased need for market share of fresh fruit of *L. barbarum*, especially in Central and Northern Europe, where the nutritional value and sensory acceptance of fresh berries are highly valued (Costa et al., 2019; Schiassi et al., 2020; Zhao et al., 2015).

As berry fruits, *L. barbarum* is high in sugar content and juicy, so it is difficult to store and transport due to decay. Research has shown that the primary pathogenic fungi of decay in postharvest *L. barbarum* is *Alternaria alternata* (Liu et al., 2017). This fungus includes latent infection and the ability to infect at low temperatures, which can limit the development of postharvest storage and cold chain logistics of fresh fruit (Belbahi et al., 2016). At the same time, after infection with *A. alternata*, *L. barbarum* fruit produced alternariol, alternariol monomethyl ether, alternanene, tengxin and other toxins, which are harmful to the human body and threaten the health of consumers (Xing et al., 2020). Therefore, the effective control of *A. alternata* is key to the development of the *L. barbarum* industry. For a long time, traditional physical methods such as refrigerated atmosphere storage and ionizing radiation etc., have been used to control postharvest decay. However, none of these methods can effect complete control (Ali et al., 2019; Strano et al., 2022; Zhao et al., 2020). On the other hand, chemical fungicides, including imazalil and kresoxim-methyl, have been shown

to have substantial effects on postharvest diseases (Lahlali et al., 2022). However, the extensive use of these fungicides causes many problems, such as the production of fungicide residues, pathogen resistance, and environmental pollution (Luo et al., 2023; Saito et al., 2019). Consequently, there is an urgent need to develop novel agents to satisfy the strong requirements of public acceptance and environmental safety. Recently, the use of biological control agents (BCAs) seems to be a promising approach, which involves natural plant essential oils or antifungal organic compounds, and is considered an effective, safe, and environmentally friendly approach against pathogens (Dukare et al., 2019; Rahman et al., 2023; Zhao et al., 2022).

Garlic is famous for its good antimicrobial activity, and garlic oil and ethylcin have successfully been used to control agricultural fungal and bacterial diseases (Nakamoto et al., 2020; Tavares et al., 2021). With further research, other plants of the *Alliaceae* family, such as *Allium cepa* and *Allium tuberosum*, have also been shown to significantly inhibit many kinds of microorganisms (El-Sayed et al., 2017; Li et al., 2016; Vukovic et al., 2023). The essential oil and aqueous extract of *Allium mongolicum*, a characteristic xerophytic plant of *Allium*, has also showed broadspectrum antimicrobial activity.

Dimethyl trisulfide (DMTS), a new type of antifungal compound, can be produced from the volatile oil of *Allium* species, such as leeks and onions, as well as from broccoli, cabbage, limburger cheese, and some bacteria and fungi (Bastaki et al., 2021; Li et al., 2014). DMTS has shown inhibitory effects on plant pathogenic fungi using a fumigation method, such as *Penicillium italicum*, *Fusarium oxysporum*, and *Colletotrichum gloeosporioides* on mango (Sheoran et al., 2015; Sun et al., 2022). Compared with traditional fungicides, the mode of action of DMTS without direct contact with the control object is more suitable for the disease control of postharvest fruits. Therefore, the effect of DMTS on the control of Goji berry black mold is in accordance with the concept of green biological control. Our previous experiments also found that DMTS has an excellent inhibitory effect on the mycelial growth of *A. alternata* on *L. barbarum* *in vitro*. The *in vivo* inhibitory effect of DMTS on *A. alternata* on *L. barbarum* and the inhibition mechanism still need to be explored. Therefore, the present study aims to systematically evaluate

the antifungal effects of DMTS on *A. alternata* on *L. barbarum* and attempt to explore the underlying mechanism involved in the DMTS inhibitory effect. This work could provide an adequate basis for developing biopreservation agents to control *A. alternata* efficiently. In addition, it could broaden the biological protection pathways of postharvest fruits.

Materials and methods

Materials

DMTS was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd., which was pure with a quality fraction of 98%. The strain of *A. alternata* was obtained from the Key Laboratory of Storage and Processing of Plant-Based Agriculture Products and conserved in our laboratory before the experiment. All reagents were analytical grade.

Antifungal activity of DMTS against *A. alternata*

Screening of DMTS against *A. alternata* in vitro

Using the fumigation method (Rangsuwan et al., 2021), the effect of DMTS on the mycelial growth of *A. alternata* was determined in the laboratory. DMTS was dissolved in 5% tween 80 to form different concentrations of compounds, and every sterile filter paper (diameter 7 cm) contained 1 mL of the solution in a 9 cm petri dish, the base of which contained PDA, with *A. alternata* inoculated onto the base. The cap of the petri dishes were placed with filter paper to achieve inner reagent concentrations of 0.001 $\mu\text{L mL}^{-1}$,

0.0015 $\mu\text{L mL}^{-1}$, 0.002 $\mu\text{L mL}^{-1}$, 0.0025 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, 0.006 $\mu\text{L mL}^{-1}$, 0.0125 $\mu\text{L mL}^{-1}$, 0.025 $\mu\text{L mL}^{-1}$, 0.05 $\mu\text{L mL}^{-1}$, and 0.1 $\mu\text{L mL}^{-1}$. For control treatments, the cap of the petri dishes without DMTS was treated similarly. The petri dishes were sealed with paraffin film and cultured at 28 ± 1 °C. The experiments were repeated three times. The radial growth diameter of the tested *A. alternata* were measured, and the inhibition rate was calculated as described in Eq. (1). The half inhibitory concentration (EC_{50}) was defined as the concentration of dimethyl trisulfide corresponding to a 50% inhibition rate.

$$\text{Inhibition rate (\%)} = (C - T) / (C - 4) \times 100\% \quad (1)$$

where C represents the radial growth of pathogen on untreated PDA, and T represents the radial growth of pathogen on treated PDA.

Viability of conidia in vitro

The experimental method was designed according to the literature (Rangsuwan et al., 2021). To test the viability of conidia of *A. alternata*, 10 μL conidium suspensions (1.5×10^6 spore/ml) of *A. alternata* were spread on the surface of the center of a concave slide containing water agar medium. The concave slide was then placed inside petri dishes (diameter 9 cm) with different concentrations (0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$ and 0.006 $\mu\text{L mL}^{-1}$) of DMTS, then cultured for 2, 4, 6, and 8 h at 28 °C, separately, as well as a control group without DMTS. The separate experiments were carried out in triplicate. Spore germination of *A. alternata* was observed and recorded under the microscope, and the conidial germination rate was calculated using Eq. (2).

$$\text{Conidial germination rate (\%)} = \text{Number of germinated spores} / \text{Total spores} \times 100\% \quad (2)$$

Inhibitory effect of DMTS on *A. alternata* of Goji berry fruit

The Goji berry fruits were surface-sterilized with 75% alcohol and dried naturally on an ultra-clean bench. Each culture plate had 8 Goji berry fruits place on them, which were then punctured with a vaccination needle in equator of each fruit (1 mm $\times$ 1 mm) and inoculated with 2 μL 1.5×10^6 spore/ml suspension.

The Goji berry fruits inoculated with pathogens were then fumigated with 0 $\mu\text{L mL}^{-1}$, 0.0015 $\mu\text{L mL}^{-1}$, 0.002 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, 0.004 $\mu\text{L mL}^{-1}$, 0.005 $\mu\text{L mL}^{-1}$, and 0.006 $\mu\text{L mL}^{-1}$ DMTS respectively at room temperature for 24 h, after which the culture plates were balanced in the ultra-clean bench for 6 h and cultured in a 28 °C incubator. Each treatment was repeated 6 times, the diameter of the

pathogen was observed and measured, and the inhibition rate was calculated according to Eq. (3).

$$\text{Inhibition rate (\%)} = (C - T)/C \times 100\% \quad (3)$$

where C represents the radial growth of the pathogen on the Goji berry fruit, and T represents the radial growth of the pathogen on treated Goji berry fruit.

Ultrastructural observation of mycelia by TEM and SEM

Before treatment with DMTS, the hyphae were cultured for 48 h in a PDA medium. The hyphae samples were treated with DMTS, at concentrations of 0 $\mu\text{L mL}^{-1}$, 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, and 0.006 $\mu\text{L mL}^{-1}$, respectively (Wang et al., 2021). After treatment for 4 days, the mycelium was sampled and prepared for observation by scanning electron microscope (SEM, Hitachi SU8100) and transmission electron microscope (TEM, Hitachi HT7800), respectively.

Disruption of *A. alternata* cell membrane structures by DMTS

PI staining experiment

The method described in Liu et al. (2010) was slightly modified for this experiment. Propidium iodide (PI) was used in this study to determine the membrane integrity of hypha of *A. alternata*. PI can only cross the membranes of dead cells, which exhibit red fluorescence when stained with PI. After incubation for 2 d on PDA medium, the *A. alternata* was treated with DMTS of different concentrations 0 $\mu\text{L mL}^{-1}$ (control group), 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$ and 0.006 $\mu\text{L mL}^{-1}$ for 3 d. The mycelium was then collected and suspended in PI with a concentration of 2.5 mg. mL^{-1} , after which the mixtures were maintained at 28 °C for 10 min in the dark, centrifuged at 8000 $\times g$ for 2 min at 4 °C and the precipitates were washed several times with phosphate buffered saline (PBS) to ensure that residual dye was removed. Samples were then resuspended again in PBS and these suspensions were placed on slides. Unstained mycelium suspensions were used as controls. Samples were observed and photographed under a fluorescence microscope. (LEICA DM3000, Germany).

Cytoplasmic leakage assays of *A. alternata*

The experiment was designed according to the methods in Wang et al. (2020) with minor modifications. The 0.5 mL *A. alternata* spore suspensions were added to 100 mL PDB medium at 28 °C on a rotary shaker. After 2 d of incubation, mycelia were harvested by centrifugation (8000 $\times g$, 10 min) and washed with sterile distilled water. Then 1 g mycelia were resuspended in different concentrations of DMTS and cultured at 28 °C on a rotary shaker for 0 min, 30 min, 60 min, 90 min, and 120 min. A clear supernatant of the cultures was gained by centrifugation at 8000 $\times g$ for 5 min and used for cell membrane permeability determination through an electric conductivity meter. A sample prepared as described above but without *A. alternata* was used as an appropriate blank. The absorbance of nucleic acids and protein at 260 nm (OD_{260}) and 280 nm (OD_{280}) were measured using enzyme calibration (Thermo Varioskan LUX, Finland). All experiments were performed in triplicate.

Effect of DMTS on MDA and H_2O_2 content in mycelia of *A. alternata*

The relative substance content of *A. alternata* mycelia treated with different concentrations of DMTS was determined. The content of MDA and H_2O_2 of mycelium was determined by the TBA method according to MDA kit (A003-1) and OFR kit (S0038) respectively, which were all produced by Suzhou Comin Biotechnology Co., Ltd. Each treatment was replicated thrice.

Effect of DMTS on POD, SOD, and CAT contents in mycelia of *A. alternata*

The activities of SOD and CAT in mycelium were determined by the SOD kit (SOD-1-W) and CAT kit (CAT-1-Y) of Suzhou Comin Biotechnology Co., Ltd., respectively. Peroxidase (POD) activity was determined by the POD (BC0090) kit of Beijing Solarbio Science & Technology Co., Ltd. Each treatment was replicated thrice.

Statistical analysis

The data were analyzed using Microsoft Excel 2013 and SPSS 22.0. The standard deviations were calculated as well as Duncan’s multiple difference significance analysis, and the difference is significant when $p < 0.05$.

Results and discussion

Results

The antifungal activity of DMTS against A. alternata

Hyphae inhibition of DMTS on *A. alternata* in vitro The experimental results are shown in Fig. 1. The inhibition rate against *A. alternata* was positively correlated with the DMTS concentration. DMTS at $0.006 \mu\text{L mL}^{-1}$ could wholly inhibit mycelial growth,

Fig. 1 Inhibitory effect of different DMTS concentrations on mycelium of *Alternaria alternata*. *Note:* According to Duncan’s multiple comparisons, different letters indicate significant differences ($P < 0.05$)

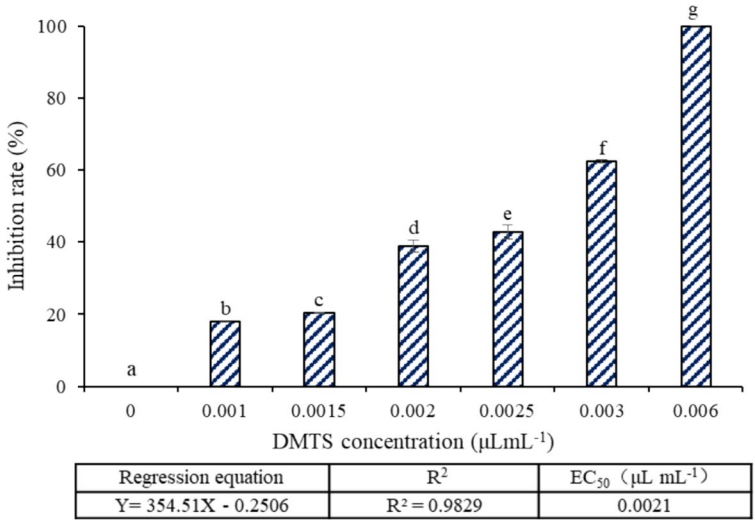
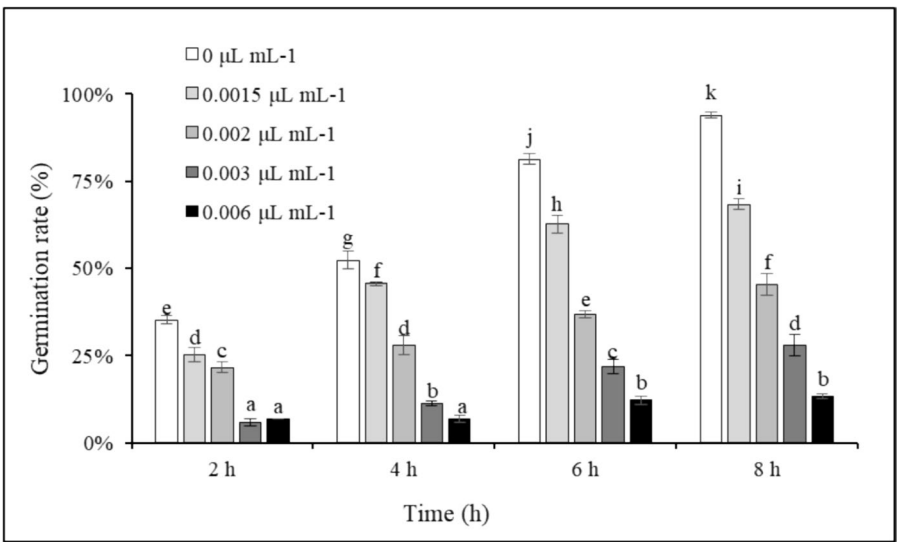


Fig. 2 The variation of spore germination rate of *Alternaria alternata* affected by different DMTS concentrations. *Note:* h-hour; According to Duncan’s multiple comparisons, different letters indicate significant differences ($P < 0.05$)



and the EC_{50} of DMTS against *A. alternata* was $0.0021 \mu\text{L mL}^{-1}$.

Viability testing of conidia of *in vitro* As shown in Fig. 2, the germination rate of the spores gradually increased, which was related to the duration of treatment, but the spore germination rate was significantly lower than that of the control in each period. After 8 h treatment with $0.006 \mu\text{L mL}^{-1}$ DMTS, survival rates

of spore reduced to 15%. In contrast, the survival rate in the control group was 94%, significantly different than the treatment group ($P < 0.05$). These results demonstrated that DMTS could cause serious damage to the viability of conidia.

The inhibition effect of DMTS against *A. alternata in vivo* After fumigation with different concentrations of DMTS for 24 h, the inhibition effect on *A.*

Fig. 3 Inhibitory rate of different DMTS concentrations on *Alternaria alternata* of “Ningqi 1” Goji berry fruit with time *in vivo*. Note: d-day; According to Duncan’s multiple comparisons, different letters indicate significant differences ($P < 0.05$)

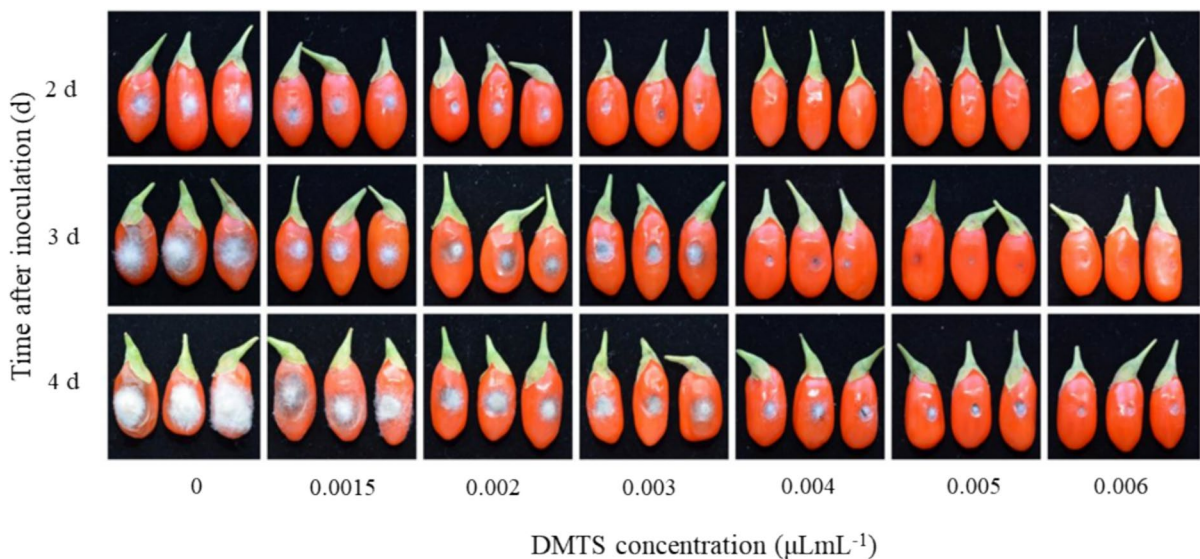
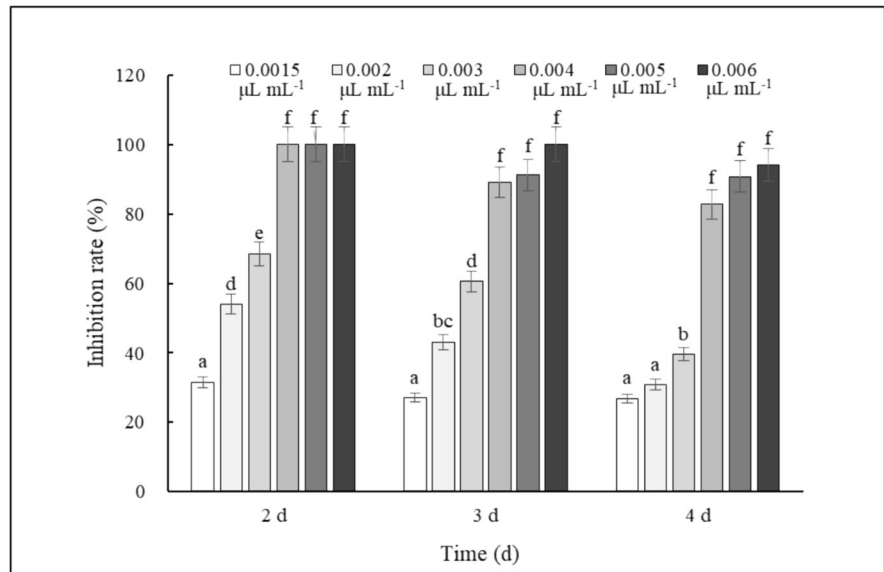


Fig. 4 Inhibitory effect of different DMTS concentrations on *Alternaria alternata* of “Ningqi 1” Goji berry fruit with time *in vivo*. Note: d-day

alternata of "Ningqi 1" Goji berry fruit depended on the concentration of DMTS (Fig. 3 and Fig. 4). On the 2nd day, treatment with 0.004~0.006 $\mu\text{L mL}^{-1}$ DMTS could completely inhibit the growth of the pathogen on Goji berry fruit. On the 4th day, the Goji berry fruits in the control group were almost completely covered with vigorous mycelium of the fungus. However, in the treatment group subjected

to 0.006 $\mu\text{L mL}^{-1}$ DMTS, only small black spots appeared at the inoculation place, and there was almost no hypha expansion (Fig. 4). On the 4th day after inoculation, after being treated with 0.0015 $\mu\text{L mL}^{-1}$, 0.002 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, 0.004 $\mu\text{L mL}^{-1}$, 0.005 $\mu\text{L mL}^{-1}$, and 0.006 $\mu\text{L mL}^{-1}$ DMTS, the inhibition rates of *A. alternata* on Goji berry fruits were 26.86%, 30.76%, 39.6%, 82.74%, 90.75%

Fig. 5 Inhibitory rate of different DMTS concentrations on *Alternaria alternata* of "Ningnongqi 15" Goji berry fruit with time *in vivo*. Note: d-day; According to Duncan's multiple comparisons, different letters indicate significant differences ($P < 0.05$)

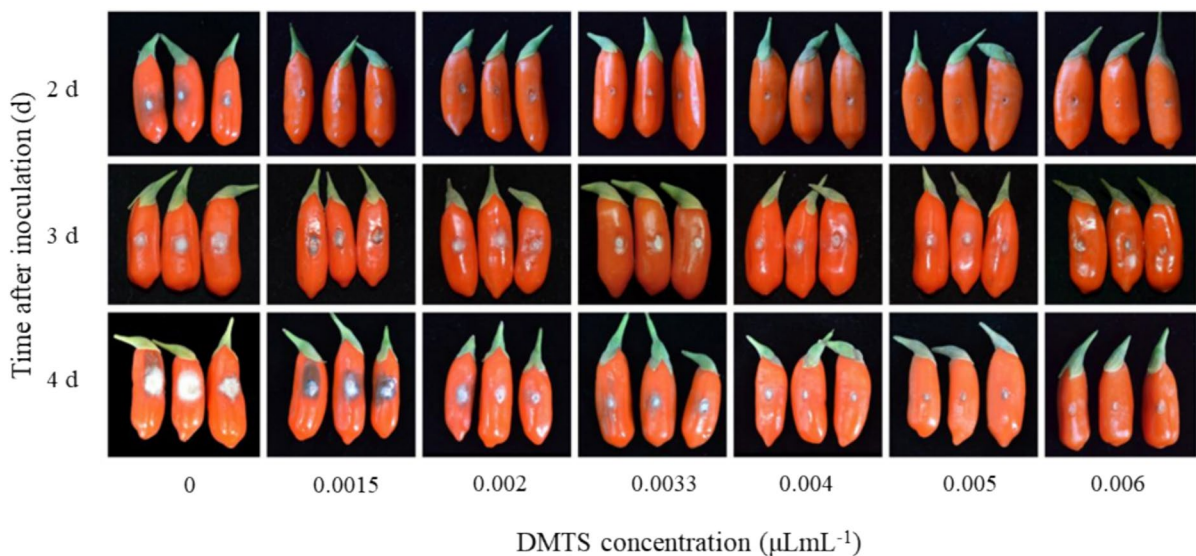
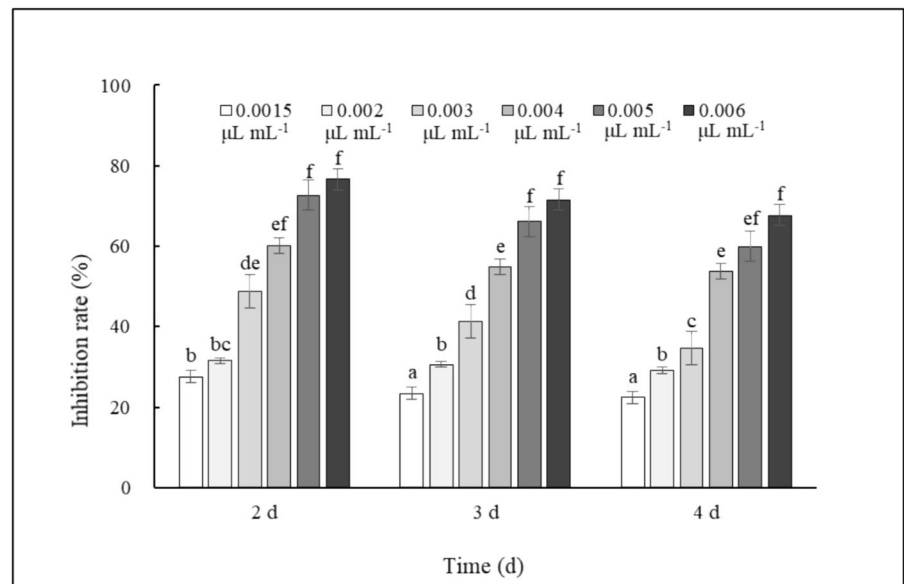


Fig. 6 Inhibitory effect of different DMTS concentrations on *Alternaria alternata* of "Ningnongqi 15" Goji berry fruit with time *in vivo*. Note: d-day

and 94.08%, respectively compared with the control group (Fig. 3).

The inhibitory effect of DMTS on *A. alternata* of “Ningnongqi 15” Goji berry fruit was as follows (Fig. 5). The diameter of the pathogen in the treatment group was significantly smaller than that of the control group, and the high concentration treated with 0.004–0.006 $\mu\text{L mL}^{-1}$ DMTS only showed a small disease spot at the inoculation site (Fig. 6). After being treated with 0.0015 $\mu\text{L mL}^{-1}$, 0.002 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, 0.004 $\mu\text{L mL}^{-1}$, 0.005 $\mu\text{L mL}^{-1}$ and 0.006 $\mu\text{L mL}^{-1}$ DMTS, the inhibition rates of *A. alternata* on Goji berry fruit were 22.43%, 29.15%, 34.62%, 53.76%, 59.97% and 72.4%, respectively, compared with the control group (Fig. 5).

Ultrastructural observation of mycelia by TEM and SEM

The morphological photos of *A. alternata* hyphae observed by SEM are shown in Fig. 7. The hyphae before treatment (Fig. 7, A1) presented a normal and intact tubular shape with a smooth cell wall. The tubular hyphae were substantial and well-rounded. However, the hyphae showed some damage after being treated with 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$,

and 0.006 $\mu\text{L mL}^{-1}$ DMTS for 4 d (Fig. 7, A2, A3, A4). As the treatment concentration increased, the hyphae gradually wrinkled, collapsed, and folded. The cytoplasmic loss occurred in the hyphae treated with DMTS.

The photographs of the internal structure of DMTS-treated *A. alternata* hyphae observed by TEM are shown in Fig. 7. The hyphae before treatment (Fig. 7, B1) exhibited a well-preserved ultrastructure, including uniform cell walls, plasma membranes, mitochondria, and vacuoles. In contrast, after treatment with different concentrations of DMTS, the *A. alternata* hyphae were destroyed to various degrees. Gaps were observed between the cell walls and the plasma membranes, which allowed for protoplasm leakage and promotion of vacuolar formation in some sections of hyphae. Mitochondria were distributed in the cytoplasm, and seemed to be intact and normal for the control group but vacuolated in the treatment group (Fig. 7, B2, B3). Particularly in those treated with 0.006 $\mu\text{L mL}^{-1}$ DMTS, the organelles such as mitochondria became unclear or even disappeared and black round particles appeared (Fig. 7, B4).

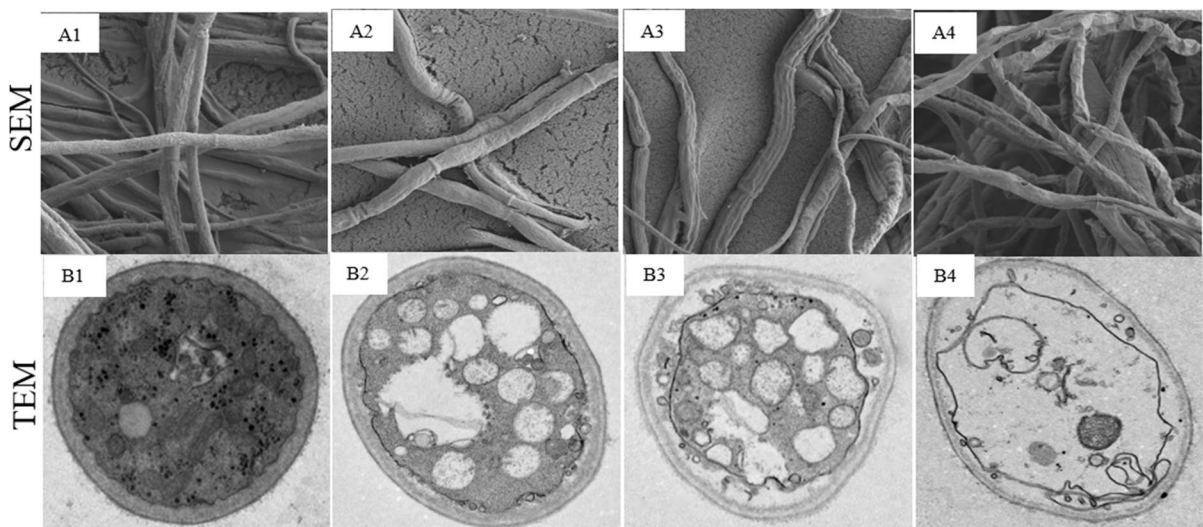


Fig. 7 Effects of different DMTS concentrations on hyphae morphology of *Alternaria alternata*. (up: $\times 1500$, down: $\times 4000$; the concentrations of DMTS from left to right were 0 $\mu\text{L mL}^{-1}$, 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$ and 0.006 $\mu\text{L mL}^{-1}$.)

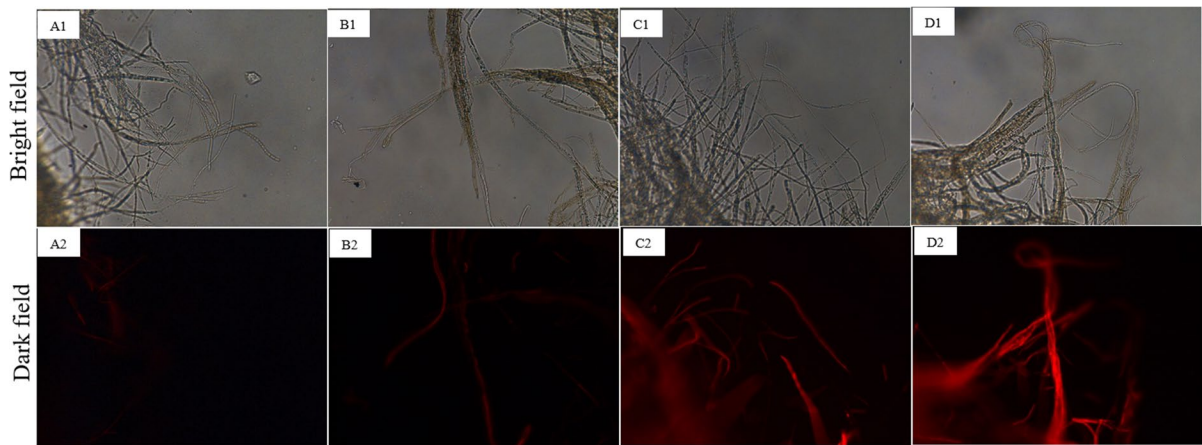


Fig. 8 Effects of different DMTS concentrations on hyphae membrane integrity of *Alternaria alternata* mycelium ($\times 40$) through fluorescence microscopy. (The concentrations of

DMTS from left to right were $0 \mu\text{L mL}^{-1}$, $0.0015 \mu\text{L mL}^{-1}$, $0.003 \mu\text{L mL}^{-1}$ and $0.006 \mu\text{L mL}^{-1}$.)

Disruption of *A. alternata* cell membrane structures by DMTS

Propidium iodide staining experiment for determination of membrane integrity After being treated with different concentrations of DMTS, the hyphae were stained with PI, and the results are shown in Fig. 8. PI could hardly enter *A. alternata* by penetrating the integrated cell membrane and binding to nucleic acid, so very few of the *A. alternata* cells in the control group were stained. In contrast, as shown in Fig. 8, the proportion of *A. alternata* cells stained increased with increasing concentrations of DMTS, in a dose-dependent manner. These results indicate that DMTS takes an action on the plasma membrane structure and disrupts cell membrane integrity, causing PI to flow into cells and bind to DNA, increasing the red fluorescence of the hyphae (Trilisenko et al., 2021).

Cytoplasmic leakage assays of *A. alternata* To further study the damage to the cell membrane of *A. alternata*, the conductivity, protein (OD_{280}) content, and nucleic acid (OD_{260}) of the medium was measured after DMTS treatment. Compared with the control group, after DMTS treatment, the permeability of

the mycelium membrane was increased with the treatment duration and DMTS concentration. After being treated with $0.006 \mu\text{L mL}^{-1}$ DMTS for 120 min, the conductivity of the culture was 4 times that of the control (Fig. 9A). The protein and nucleic acid and nucleic acid leakage stayed at a deficient level in mycelia incubated in a medium without DMTS during the experiment. In contrast, as shown in Fig. 9B and Fig. 9C, leakage of nucleic acid and protein in *A. alternata* mycelia was detected in treatments with DMTS, and the leakage increased with time and DMTS concentration. At the first 30 min, nucleic acid leaked markedly in the DMTS treatments, and the leakage increased slowly after 30 min. The leakage of protein exhibited a similar trend as nucleic acid.

Effect of DMTS on MDA and H_2O_2 content in mycelia of *A. alternata*

The relationship between the MDA content and the concentration of DMTS is shown in Fig. 10A. After the mycelia of *A. alternata* were treated with DMTS, the MDA content was positively correlated with the concentration of DMTS. After being treated with $0.0015 \mu\text{L mL}^{-1}$, $0.003 \mu\text{L mL}^{-1}$, and $0.006 \mu\text{L mL}^{-1}$ of DMTS, MDA content was

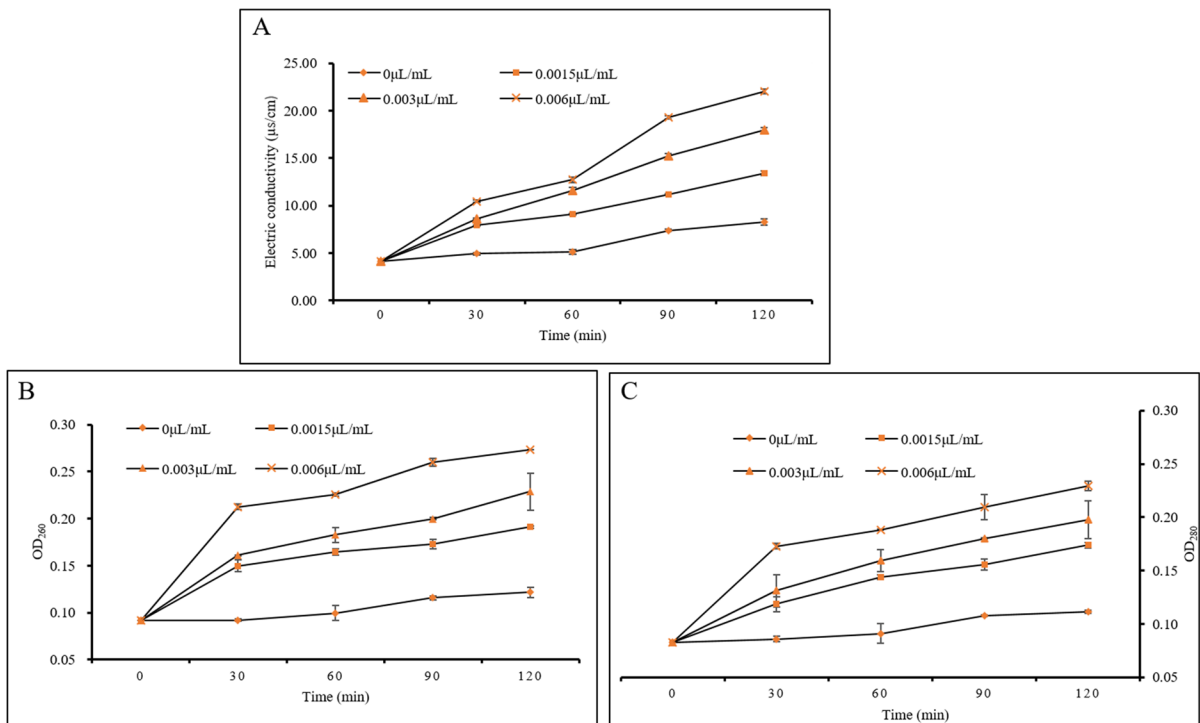


Fig. 9 The variation of electrical conductivity (A), nucleic acid leakage (B) and protein leakage (C) of *Alternaria alternata* affected by different DMTS concentrations with time. Note: standard deviation was showed on the curve as 'I'

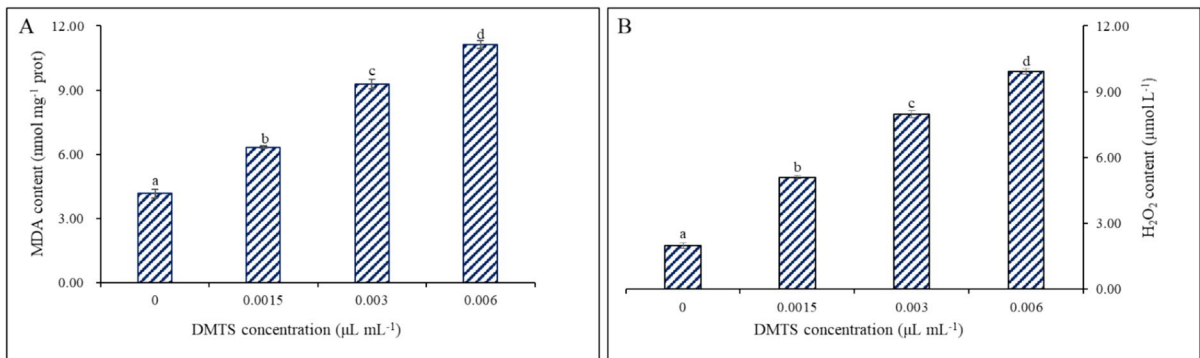


Fig. 10 Effect of different DMTS concentrations on MDA (A) and H_2O_2 (B) content in mycelia of *Alternaria alternata*. Note: According to Duncan's multiple comparisons, different letters indicate significant differences ($P < 0.05$)

2.17 nmol g^{-1} , 5.12 nmol g^{-1} and 6.97 nmol g^{-1} higher than that of the control, respectively, and there were significant differences among the different treatment groups ($P < 0.05$). As shown in Fig. 10B, after $0.0015 \sim 0.006 \mu\text{L mL}^{-1}$ DMTS treatment, H_2O_2 content in mycelia was significantly higher than that of the control group. A gradually

increasing trend with the increase of DMTS concentration was found. Compared with the control group, H_2O_2 content in mycelia was about 2.5 times higher after $0.006 \mu\text{L mL}^{-1}$ DMTS treatment. Based on the results above, the mycelia of *A. alternata* treated with DMTS could lead to the collection of H_2O_2 and MDA, accompanied by oxidative damage.

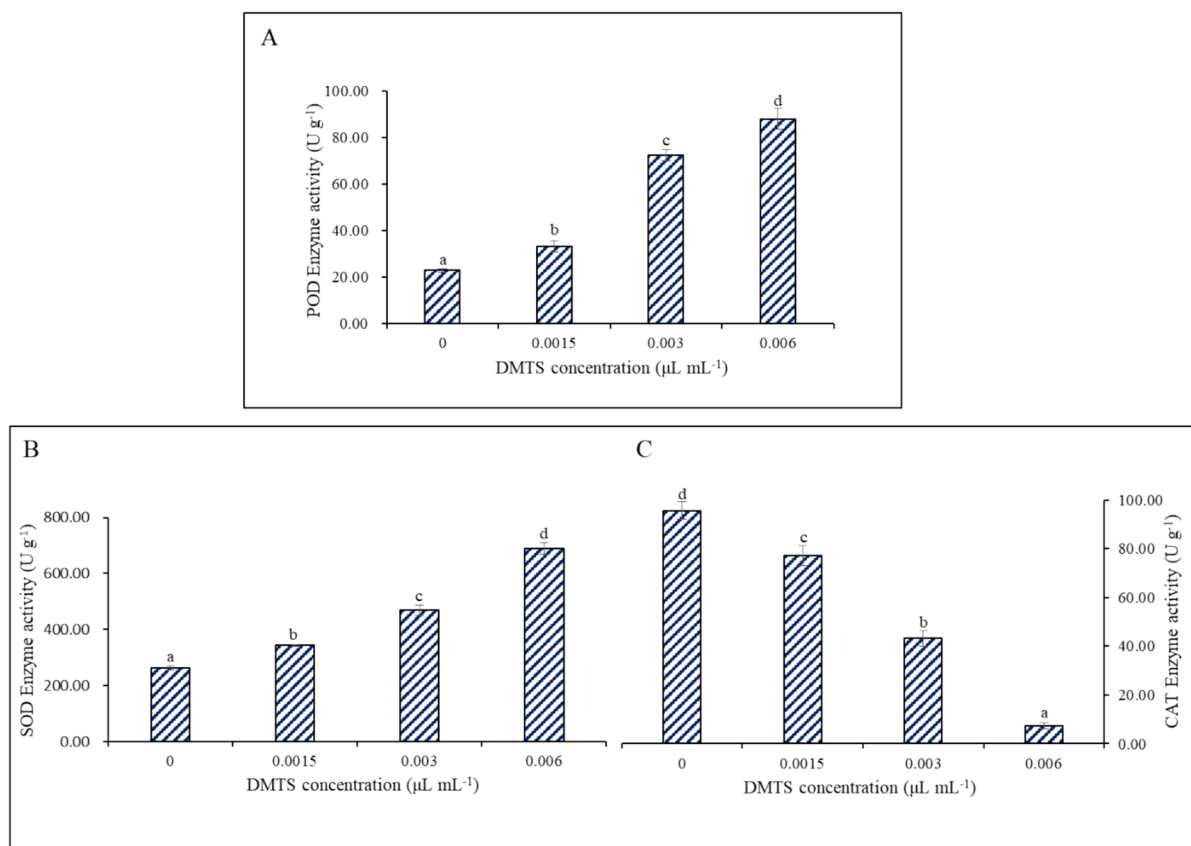


Fig. 11 Effect of different DMTS concentrations on POD (A), SOD (B) and CAT (C) content in mycelia of *Alternaria alternata*. Note: According to Duncan's multiple comparisons, different letters indicate significant differences ($P < 0.05$)

Effect of DMTS on POD, SOD and CAT content in mycelia of *A. alternata*

Figure 11A shows that POD activity exhibited an increasing trend after DMTS treatment and increased with the increase of DMTS concentration. The POD activity of mycelium treated with 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$, and 0.006 $\mu\text{L mL}^{-1}$ were 7.46 U g^{-1} , 44.91 U g^{-1} , and 69.28 U g^{-1} higher than that of the control group, respectively. The trend in the change of SOD after DMTS treatment was consistent with that of POD, and SOD activity was significantly increased, with significant differences among different concentrations ($p < 0.05$). The SOD activity treated with 0.0015 $\mu\text{L mL}^{-1}$, 0.003 $\mu\text{L mL}^{-1}$ and 0.006 $\mu\text{L mL}^{-1}$ DMTS were 79.52 U g^{-1} , 232.77 U g^{-1} , and 395.77 U g^{-1} higher than that of the control group, respectively (Fig. 11B). However, after DMTS treatment, CAT activity showed a decreasing trend,

especially when treated with a high concentration of 0.006 $\mu\text{L mL}^{-1}$, the CAT activity was 90.58 U g^{-1} lower than that of the control group (Fig. 11C).

Discussion

L. barbarum is a medicine-food homology plant that contains a variety of polysaccharides, phenols, organic acids, and so on. It is mainly distributed in China's Ningxia, Gansu, and other northwest regions (Teixeira et al., 2023). As one of the main economic crops in Ningxia, the healthy development of the *L. barbarum* industry is crucial to increasing farmers' income. Goji berry black mold can infect Goji berry fruits, especially during postharvest storage. When contaminated with black mold, the appearance and internal quality of *L. barbarum* is seriously affected. At the same time, the toxin produced by the black mold is harmful to the human body. Therefore, it is

of great significance to strengthen the control of Goji black mold to improve the quality of *L. barbarum* (Zhao et al., 2023).

Plant essential oil is a kind of secondary metabolite with substantial volatility in plants, offering crucial antifungal activity and low toxicity. It has been proven to be a potential safety measure for controlling postharvest diseases (Wu et al., 2023). Before this experiment, based on the reported good antifungal activity of volatile oils from *Allium* plants such as garlic and onion (Vukovic et al., 2023), we obtained 46 compounds through the analysis of volatile oils from *A. mongolicum*, in which sulfide accounted for a large proportion, and the highest content of DMTS was 15.25%. It was reported that DMTS has good antimicrobial activity against *Colletotrichum gloeosporioides* and *Botryosphaeria dothidea* (Tang et al., 2019), which provides a basis for DMTS application in preventing and controlling fungal diseases. Therefore, we conducted preliminary research on the inhibitory effects of DMTS on *F. solani*, *A. solani*, *V. mali*, *F. graminearum*, *C. cassiicola*, *A. alternata*, *F. oxysporum* and *F. sulphureum*, and the inhibition EC_{50} were 0.00123 $\mu\text{L mL}^{-1}$, 0.00182 $\mu\text{L mL}^{-1}$, 0.00240 $\mu\text{L mL}^{-1}$, 0.00094 $\mu\text{L mL}^{-1}$, 0.00042 $\mu\text{L mL}^{-1}$, 0.00212 $\mu\text{L mL}^{-1}$, 0.00143 $\mu\text{L mL}^{-1}$ and 0.00208 $\mu\text{L mL}^{-1}$, respectively, and DMTS exhibits good broad-spectrum antifungal activity.

Traditionally, Goji berries are mainly distributed in the market as dried fruits. With the change of consumption concept and the development of breeding technology, the market share of fresh Goji berry fruits has gradually increased in recent years (Ge et al., 2018). As a direct edible fruit, black mold of *L. barbarum* affects its quality, so control of black mold should choose a greener and more volatile antimicrobial agent, which is more in line with the requirements of safe production. This study found that the volatile compound DMTS can act against *A. alternata* by inhibiting mycelial growth and spore germination. DMTS exists naturally in *Allium* plants such as *A. mongolicum*, garlic, and chives, which have always been used as food (Sun et al., 2022). Therefore, using DMTS to control Goji berry black mold disease is safe for humans, animals, and the environment, and provides a possible new pathway to prevent Goji berry black mold. In this experiment, consequently, we selected “Ningqi 1” and “Ningnongqi 15” to verify the inhibitory effect of DMTS on Goji berry black

mold in postharvest fruit *in vivo*. The results showed that on the 4th day after inoculation with the pathogen, the surface of the fruit in the control group were completely infected with hypha, but 0.006 $\mu\text{L mL}^{-1}$ of DMTS can almost completely control *A. alternata* on Goji berry fruit. Therefore, it suggests that DMTS can be used as a potential alternative compound to control Goji berry black mold efficiently.

To further clarify the inhibitory effect of DMTS on *A. alternata*, the antifungal mechanism of DMTS was explored in this study. Ultrastructural observations on the hyphal cells of *A. alternata* demonstrated that DMTS caused damage to the mycelium of *A. alternata*, making it grow slowly or even die. DMTS also reduced the infection rate of *A. alternata* on fruits *in vivo* and effectively inhibited Goji berry black mold. Previous studies have shown that essential oils can penetrate the plasma membrane due to their lipophilicity (Feng et al., 2019; Wu et al., 2022). At the same time, essential oils can cause depolarization of organelles such as the mitochondrial membrane, and its permeability between the inner and outer membranes of mitochondria can also seriously be damaged, or even disappear, leading to cell death (Li et al., 2013; Yan et al., 2021). Through transmission electron microscopy and scanning electron microscopy, we found that with the increase of DMTS concentration the plasma membrane of the mycelium was indeed separated from the cell wall, and even part of the mitochondria was ablated. Previous studies have also shown that DMTS can inhibit fungi such as *Colletotrichum gloeosporioides* and bacteria such as *Escherichia coli* O157:H7 by destroying the cell wall, plasma membrane, mitochondria, and other subcellular structures (Tang et al., 2020). In the PI staining and conductivity measurement experiments, compared with the control group, the conductivity of the treatment group increased with the increase of DMTS concentration, and the PI staining was also aggravated, which was consistent with the results of the ultrastructural observations that DMTS destroyed the cell membrane of *A. alternata*. The damage to cell walls and membranes can lead to macromolecular leakage and fragmentation (Wu et al., 2022). Nucleic acids control the growth, development, and heredity of fungi (Ning et al., 2019). Proteins provide crucial functional components and indispensable nutrient substances for the growth and development of living fungi. In this experiment, the nucleic acid and

protein content in mycelial cells was determined, and the results showed that the content of nucleic acid and protein increased with the increase of DMTS concentration and treatment time.

ROS are a by-product of cell metabolism. The accumulation of large amounts of ROS in cells under stress is one of the leading causes of membrane oxidative damage. In addition, ROS can also attack biological macromolecules, leading to protein denaturation or DNA damage (Furukawa & Sakuda, 2019; Zhang et al., 2019). H_2O_2 is one of the stable sources of ROS in cells, so its content can reflect the degree of cell injury (Yin et al., 2021). As one of the products of lipid peroxidation decomposition in cell membranes, MDA is used to quantify the degree of lipid peroxidation and the damage of microorganisms under stressed conditions (Zheng et al., 2023). In the experiment, after being treated with 0.0015~0.006 $\mu\text{L mL}^{-1}$ DMTS, the H_2O_2 and MDA contents in mycelia were significantly higher than those in the control group and showed a gradual increasing trend with the increase in DMTS concentration. This indicates that DMTS, as an exogenous substance, has caused varying degrees of damage to the mycelial cells, furthering the cells' oxidative damage, affecting the normal metabolism of *A. alternata*.

Under normal environmental conditions, the production and removal of reactive oxygen in biological cells of microorganisms is in a dynamic equilibrium state. However, when it is adversely affected by external influences, the equilibrium state of intracellular ROS production and removal will be disturbed, thus triggering a series of oxidative stress reactions. SOD, CAT and POD are necessary intracellular antioxidant enzymes, which can clear ROS and reduce membrane lipid peroxidation (Wen et al., 2021), thus maintaining the normal function of cells. The research has shown that SOD is an essential protective enzyme that can remove toxic superoxide anion free radicals, leading to the production of H_2O_2 and O_2 in the decomposition process of superoxide free radicals, and is the first line of defense against ROS (Jaikua et al., 2016). The decomposition of H_2O_2 into O_2 and H_2O is catalyzed by CAT, which protects cells from excessive oxidative stress to a great extent (Gao et al., 2016). POD is also one of the main antioxidant enzymes, which can catalyze the decomposition of H_2O_2 and prevent cell death caused by excessive accumulation

of H_2O_2 (Ballester et al., 2010; Shi et al., 2022). Figure 11 showed that after the mycelia of *A. alternata* were treated with DMTS, the SOD and POD activities were significantly increased, which could regulate the membrane lipid peroxidation process and prevent oxidative damage of mitochondria through increased antioxidant activity. However, CAT activity showed a sharp decline, resulting in excessive accumulation of hydrogen peroxide and ROS, leading to membrane oxidative damage, and finally the death of microorganisms.

In conclusion, DMTS showed broad-spectrum antifungal activity and had good *in vitro* and *in vivo* inhibitory effects on the black mold of the medicine food homology *L. barbarum* postharvest fruit. As a volatile liquid, DMTS can inhibit the growth of the pathogen by directly destroying the cell membrane, destroying mitochondria, and affecting the activities of a variety of stress-resistant enzymes. The research laid the foundation for developing new antifungal agents against Goji black mold. Also, it provided the basis for studying the mechanism of DMTS inhibiting other types of pathogens. In the next step, molecular biology and omics technology will be used to identify the critical action target of DMTS to provide the basis for developing new antifungal agents.

Conclusion

Based on the significant inhibitory effect of DMTS on the hypha growth of *A. alternata* and the occurrence of Goji berry black mold *in vivo*, it may provide an efficient, safe, and environmentally friendly method for controlling Goji berry black mold disease in practice. Furthermore, the preliminary research into the antifungal mechanisms in this paper has laid a foundation for the further study of DMTS antifungal targets.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare that they have no conflict of interest.

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References

- Ali, S., Khan, A. S., Malik, A. U., Anjum, M. A., Nawaz, A., & Shah, H. M. S. (2019). Modified atmosphere packaging delays enzymatic browning and maintains quality of harvested litchi fruit during low temperature storage. *Scientia Horticulturae*, 254, 14–20. <https://doi.org/10.1016/j.scienta.2019.04.065>
- Ballester, A. R., Izquierdo, A., Lafuente, M. T., & González-Candelas, L. (2010). Biochemical and molecular characterization of induced resistance against *Penicillium digitatum* in citrus fruit. *Postharvest Biology and Technology*, 56(1), 31–38. <https://doi.org/10.1016/j.postharvbio.2009.10.002>
- Bastaki, S. M. A., Ojha, S., Kalasz, H., & Adeghate, E. (2021). Chemical constituents and medicinal properties of *Allium* species. *Molecular and Cellular Biochemistry*, 476(12), 4301–4321. <https://doi.org/10.1007/s11010-021-04213-2>
- Belbahi, A., Leguerinel, I., Méot, J. M., Loiseau, G., & Bohuon, P. (2016). Modelling the effect of temperature, water activity and carbon dioxide on the growth of *Aspergillus niger* and *Alternaria alternata* isolated from fresh date fruit. *Journal of Applied Microbiology*, 121(6), 1685–1698. <https://doi.org/10.1111/jam.13296>
- Costa, E. A., Sousa, P. H. M., Siqueira, A. C. P., et al. (2019). Fruit pastes with organic honey texturized with gellan gum: Bioaccessibility of antioxidant activity and sensory analysis fruit pastes with gellan and organic honey. *Food Science and Technology*, 39(3), 667–676. <https://doi.org/10.1590/fst.05518>
- Dukare, A. S., Paul, S., Nambi, V. E., et al. (2019). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: A review. *Critical Reviews in Food Science and Nutrition*, 59(9), 1498–1513. <https://doi.org/10.1080/10408398.2017.1417235>
- El-Sayed, H. S., Chizzola, R., Ramadan, A. A., & Edris, A. E. (2017). Chemical composition and antimicrobial activity of garlic essential oils evaluated in organic solvent, emulsifying, and self-microemulsifying water based delivery systems. *Food Chemistry*, 221, 196–204. <https://doi.org/10.1016/j.foodchem.2016.10.052>
- Feng, H. E., Jinhai, B., Shuxian, C., et al. (2019). Antifungal activity and mechanism of an essential oil from *Eremothecium ashbyii*. *Chemical Journal of Chinese Universities*, 40(2), 272–278. <https://doi.org/10.7503/cjcu20180477>
- Furukawa, T., & Sakuda, S. (2019). Inhibition of aflatoxin production by paraquat and external superoxide dismutase in *Aspergillus flavus*. *Toxins*, 11(2), 107. <https://doi.org/10.3390/toxins11020107>
- Gao, M. H., Ng, T. W., An, T. C., et al. (2016). The role of catalase and H₂O₂ in photocatalytic inactivation of *Escherichia coli*: Genetic and biochemical approaches. *Catalysis Today*, 266, 205–211. <https://doi.org/10.1016/j.cattod.2015.11.021>
- Ge, X. Z., Guo Z. L., Yang J., Zhang J. R., & Bi, Y. (2018). Effect of cool and room temperature on storability of fresh goji berries removed stem. *Food Ferment Science and Technology*, 54, 16–21. <https://doi.org/10.3969/j.issn.1674-506X.2018.06-004>
- Huang, T., Qin, K., Yan, Y. M., He, X. R., Dai, G. L., & Zhang, B. (2022). Correlation between the storability and fruit quality of fresh goji berries. *Food Science and Technology*, 42, e46120. <https://doi.org/10.1590/fst.46120>
- Jaikua, W., Kueakhai, P., Chaithirayanon, K., et al. (2016). Cytosolic superoxide dismutase can provide protection against *Fasciola gigantica*. *Acta Tropica*, 162, 75–82. <https://doi.org/10.1016/j.actatropica.2016.06.020>
- Lahlali, R., Ezrari, S., Esmael, Q., El-Hamss, H., Belabess, Z., & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. *Microorganisms*, 10(3), 596. <https://doi.org/10.3390/microorganisms10030596>
- Li, W. R., Shi, Q. S., Ouyang, Y. S., Chen, Y. B., & Duan, S. S. (2013). Antifungal effects of citronella oil against *Aspergillus niger* atcc 16404. *Applied Microbiology and Biotechnology*, 97(16), 7483–7492. <https://doi.org/10.1007/s00253-012-4460-y>
- Li, W. R., Shi, Q. S., Liang, Q., Huang, X. M., & Chen, Y. B. (2014). Antifungal effect and mechanism of garlic oil on *Penicillium funiculosum*. *Applied Microbiology and Biotechnology*, 98(19), 8337–8346. <https://doi.org/10.1007/s00253-014-5919-9>
- Li, W. R., Shi, Q. S., Dai, H. Q., Liang, Q., Xie, X. B., & Huang, X. M. (2016). Antifungal activity, kinetics and molecular mechanism of action of garlic oil against

- candida albicans. *Scientific Reports*, 6, 22805. <https://doi.org/10.1038/srep22805>
- Liu, J., Zong, Y., Qin, G., Li, B., & Tian, S. (2010). Plasma membrane damage contributes to antifungal activity of silicon against *Penicillium digitatum*. *Current Microbiology*, 61(4), 274–279. <https://doi.org/10.1007/s00284-010-9607-4>
- Liu, Y., Wang, H., Yan, D., et al. (2017). Isolation, identification and biological characteristics of pathogenic fungus from Chinese wolfberry fruit. *Transactions of the Chinese Society of Agricultural Engineering*, 33(z1), 374–380. <https://doi.org/10.11975/j.issn.1002-6819.2017.z1.056>
- Luo, Y. W., Chen, X., Che, J. X., Zhang, Y. H., Ouyang, Q. L., & Tao, N. G. (2023). (E)-2-Octenal suppresses the growth of a prochloraz-resistant *Penicillium italicum* strain and its potential antifungal mechanisms. *Postharvest Biology and Technology*, 205, 112515. <https://doi.org/10.1016/j.postharvbio.2023.112515>
- Muatasim, R., Ma, H., & Yang, X. (2018). Effect of multimode ultrasound assisted extraction on the yield of crude polysaccharides from *Lycium barbarum* (Goji). *Food Science and Technology*, 38, 160–166. <https://doi.org/10.1590/1678-457X.14417>
- Nakamoto, M., Kunimura, K., Suzuki, J. I., et al. (2020). Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinylidithiin, ajoene and diallyl polysulfides (Review). *Experimental and Therapeutic Medicine*, 19(2), 1550–1553. <https://doi.org/10.3892/etm.2019.8388>
- Ning, H. Q., Li, Y. Q., Wang, Z. S., & Mo, H. Z. (2019). The synergistic antibacterial properties of glycinin basic peptide against bacteria via membrane damage and inactivation of enzymes. *Food Biophysics*, 14(2), 132–141. <https://doi.org/10.1007/s11483-018-09564-w>
- Rahman, M. M., Wills, R. B. H., Bowyer, M. C., Golding, J. B., Kirkman, T., & Pristijono, P. (2023). Potential control of postharvest fungal decay of citrus fruits by crude or photochemically changed essential oils—a review. *Food Reviews International*. <https://doi.org/10.1080/87559129.2023.2204157>
- Rangsuwan, S., Rattanakreetakul, C., & Pongpisutta, R. (2021). Competency of clove and cinnamon essential oil fumigation against *Toxigenic* and *Atoxigenic Aspergillus flavus* Isolates. *Journal of Pure & Applied Microbiology*, 15(3), 1325–1337. <https://doi.org/10.22207/JPAM.15.3.23>
- Saito, S., Michailides, T. J., & Xiao, C. L. (2019). Fungicide-resistant phenotypes in *Botrytis cinerea* populations and their impact on control of gray mold on stored table grapes in California. *European Journal of Plant Pathology*, 154(2), 203–213. <https://doi.org/10.1007/s10658-018-01649-z>
- Schiassi, M. C. E. V., Carvalho, C. S., Lago, A. M. T., et al. (2020). Optimization for sensory and nutritional quality of a mixed berry fruit juice elaborated with coconut water. *Food Science and Technology*, 40(4), 985–992. <https://doi.org/10.1590/fst.28919>
- Sheoran, N., Valiya, N. A., Munjal, V., et al. (2015). Genetic analysis of plant endophytic *Pseudomonas putida* BP25 and chemo-profiling of its antimicrobial volatile organic compounds. *Microbiological Research*, 173, 66–78. <https://doi.org/10.1016/j.micres.2015.02.001>
- Shi, H., Zhou, X. Y., He, X. E., Wang, R. C., Zeng, E. L., & Zhou, W. H. (2022). Study on the antifungal mechanism of Chinese herbal extract on *Botryosphaeria dothidea*. *Journal of Food Processing and Preservation*, 46(5), e16631. <https://doi.org/10.1111/jfpp.16631>
- Strano, M. C., Altieri, G., Allegra, M., et al. (2022). Post-harvest technologies of fresh citrus fruit: Advances and recent developments for the loss reduction during handling and storage. *Horticulturae*, 8(7), 612. <https://doi.org/10.3390/horticulturae8070612>
- Sun, M., Duan, Y., Liu, J. P., Fu, J., & Huang, Y. (2022). Efficacy of Dimethyl trisulfide on the suppression of ring rot disease caused by *Botryosphaeria dothidea* and induction of defense-related genes on apple fruits. *Frontiers in Microbiology*, 13, 796167. <https://doi.org/10.3389/fmicb.2022.796167>
- Tang, L. H., Mo, J. Y., Guo, T. X., et al. (2019). Antifungal effects of dimethyl trisulfide against *Colletotrichum gloeosporioides* infection on mango. *Journal of Phytopathology*, 167(7–8), 445–450. <https://doi.org/10.1111/jph.12816>
- Tang, L. H., Mo, J. Y., Guo, T. X., et al. (2020). *In vitro* antifungal activity of dimethyl trisulfide against *Colletotrichum gloeosporioides* from mango. *World Journal of Microbiology & Biotechnology*, 36(1), 4. <https://doi.org/10.1007/s11274-019-2781-z>
- Tavares, L., Santos, L., & Zapata Norena, C. P. Z. (2021). Bioactive compounds of garlic: A comprehensive review of encapsulation technologies, characterization of the encapsulated garlic compounds and their industrial applicability. *Trends in Food Science & Technology*, 114, 232–244. <https://doi.org/10.1016/j.tifs.2021.05.019>
- Teixeira, F., Silva, A. M., Delerue-Matos, C., & Rodrigues, F. (2023). *Lycium barbarum* berries (Solanaceae) as source of bioactive compounds for healthy purposes: A review. *International Journal of Molecular Sciences*, 24(5), 4777. <https://doi.org/10.3390/ijms24054777>
- Trilisenko, L., Valiakhmetov, A., & Kulakovskaya, T. (2021). Phosphate efflux as a test of plasma membrane leakage in *Saccharomyces cerevisiae* cells. *Canadian Journal of Microbiology*, 67(3), 226–230. <https://doi.org/10.1139/cjm-2020-0040>
- Vukovic, S., Popovic-Djordjevic, J. B., Kostic, A. Z., et al. (2023). *Allium* Species in the Balkan region-major metabolites, antioxidant and antimicrobial properties. *Horticulturae*, 9(3), 408. <https://doi.org/10.3390/horticulturae9030408>
- Wang, T. L., Li, Y. C., Bi, Y., et al. (2020). Benzyl isothiocyanate fumigation inhibits growth, membrane integrity and mycotoxin production in *Alternaria alternata*. *RSC Advances*, 10(3), 1829–1837. <https://doi.org/10.1039/C9RA09225K>
- Wang, Z., Zhong, T., Chen, X., Yang, B., Du, M., & Wang, K. (2021). Potential of volatile organic compounds emitted by *Pseudomonas fluorescens* ZX as biological fumigants to control citrus green mold decay at postharvest. *Journal of Agricultural and Food Chemistry*, 69(7), 2087–2098. <https://doi.org/10.1021/acs.jafc.0c07375>
- Wen, L. R., Zhou, T., Jiang, Y. M., Gong, L., & Yang, B. (2021). Identification of prenylated phenolics in mulberry

- leaf and their neuroprotective activity. *Phytomedicine*, 90, 153641. <https://doi.org/10.1016/j.phymed.2021.153641>
- Wu, H., Zhao, F., Li, Q., et al. (2022). Antifungal mechanism of essential oil against foodborne fungi and its application in the preservation of baked food. *Critical Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2022.2124950>
- Wu, C. Y., Wang, C. Y., Sun, G. J., et al. (2023). Antibacterial characteristics of allyl methyl disulfide and dimethyl trisulfide of *Allium tenuissimum* flower essential oil against *Escherichia coli* O157: H7. *Industrial Crops and Products*, 202, 117058. <https://doi.org/10.1016/j.indcrop.2023.117058>
- Xing, L. J., Zou, L. J., Luo, R. F., & Wang, Y. (2020). Determination of five *Alternaria* toxins in wolfberry using modified QuEChERS and ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry*, 311, 125975. <https://doi.org/10.1016/j.foodchem.2019.125975>
- Yan, J. Q., Wu, H., Chen, K. Y., Feng, J. J., & Zhang, Y. S. (2021). Antifungal activities and mode of action of *Cymbopogon citratus*, *Thymus vulgaris*, and *Origanum heracleoticum* essential oil vapors against *Botrytis cinerea* and their potential application to control postharvest strawberry gray mold. *Foods*, 10(10), 2451. <https://doi.org/10.3390/foods10102451>
- Yin, F. M., Liu, Q. F., Zhang, B. J., et al. (2021). Microemulsion preparation of waltheria indica extracts and preliminary antifungal mechanism exploration. *Industrial Crops and Products*, 172, 114000. <https://doi.org/10.1016/j.indcrop.2021.114000>
- Zhang, J., Ma, S., Du, S., Chen, S., & Sun, H. (2019). Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. *Journal of Food Science and Technology*, 56(5), 2611–2620. <https://doi.org/10.1007/s13197-019-03747-0>
- Zhang, L., Qi, W., & So, K. F. (2022). Present and future perspective of bioactive substances from *Lycium barbarum*. *Chinese Science Bulletin*, 67(4–5), 329–331. <https://doi.org/10.1360/TB-2021-1362>
- Zhao, Q. S., Dong, B. T., & Chen, J. J. (2015). Effect of drying methods on physicochemical properties and antioxidant activities of wolfberry (*Lycium barbarum*) polysaccharide. *Carbohydrate Polymers*, 127, 176–181. <https://doi.org/10.1016/j.carbpol.2015.03.041>
- Zhao, B., Hu, S., Wang, D., Chen, H., & Huang, M. (2020). Inhibitory effect of gamma irradiation on *Penicillium digitatum* and its application in the preservation of ponkan fruit. *Scientia Horticulturae*, 272, 109598. <https://doi.org/10.1016/j.scienta.2020.109598>
- Zhao, X., Zhou, J., Tian, R., & Liu, Y. (2022). Microbial volatile organic compounds: Antifungal mechanisms, applications, and challenges. *Frontiers in Microbiology*, 13, 922450. <https://doi.org/10.3389/fmicb.2022.922450>
- Zhao, L. A. K., Wang, J. J., Zhang, H. Y., et al. (2023). Inhibitory effect of carvacrol against *Alternaria alternata* causing goji fruit rot by disrupting the integrity and composition of cell wall. *Frontiers in Microbiology*, 14, 1139749. <https://doi.org/10.3389/fmicb.2023.1139749>
- Zheng, L., Gu, X., Xiao, Y., et al. (2023). Antifungal activity of *Bacillus mojavensis* D50 against *Botrytis cinerea* causing postharvest gray mold of tomato. *Scientia Horticulturae*, 312, 111841–111841. <https://doi.org/10.1016/j.scienta.2023.111841>

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