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Antifungal activity of a methanolic extract of *Glycyrrhiza uralensis* Fisch. root against *Fusarium oxysporum* and *Phytophthora capsici*

Dang-Minh-Chanh Nguyen[1, 2*], Thi-Hoan Luong[3], Van-Viet Nguyen[2], Woo-Jin Jung[1]

[1]*Department of Agricultural Chemistry, Institute of Environmentally Friendly Agriculture (IEFA), College of Agriculture and Life Sciences, Chonnam National University, 77 Yongbong-ro, Buk-gu District, Gwangju City, Republic of Korea*

[2]*Research and Development Institute for Agriculture Nafoods, 162A Nguyen Tuan Street, Thanh Xuan Trung Ward, Thanh Xuan District, Hanoi, Vietnam*

[3]*National Institute of Medicinal Materials (NIMM), 3B Quang Trung Street, Trang Tien Ward, Hoan Kiem District, Hanoi, Vietnam*

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Abstract:

In the present study, we investigated the antifungal activity of a methanolic extract of *Glycyrrhiza uralensis* Fisch. root, which inhibited the growth of the plant pathogens *Fusarium oxysporum* and *Phytophthora capsici*. The EC_{50} values of the *G. uralensis* Fisch. extract three, six, and nine days after treatment were 113.4, 150.6, and 191.4 µg/ml for *F. oxysporum* and 84.7, 148.6, and 190.1 µg/ml for *P. capsici*, respectively, and the antifungal activity exhibited a dose-dependent response. The MIC value of *G. uralensis* Fisch. extract was found to be 100, 200, and 400 µg/ml at three, six, and nine days after treatment for *F. oxysporum*. In contrast, for *P. capsici*, the MIC value was 50, 100, and 100 µg/ml at three, six, and nine days after treatment. After three days of incubation with 400 µg/ml *G. uralensis* Fisch. extract, the hyphae of *F. oxysporum* exhibited abnormalities and atrophy, leading to mycelial collapse. In contrast, the hyphae of *P. capsici* displayed swelling with increased branching. These results furnish valuable insights into the antifungal metabolites present in *G. uralensis* Fisch. extract, contributing to the development of environmentally friendly products aimed at controlling the growth of pathogenic fungi.

Keywords: **antifungal activity, medicinal plant, mycelial morphology, pathogenic fungus.**

Classification numbers: **2.2, 3.1**

1. Introduction

Fusarium species are ubiquitous soil-borne pathogens in a wide range of horticultural and food crops, causing destructive vascular wilt, rot, and damping-off diseases [1], which curtail plant production and yield. *F. oxysporum*, in particular, results in the demise of both young and mature plants, leading to significant economic losses [2]. Various strategies have been implemented to mitigate the impact of this pathogen, encompassing the elimination of afflicted plants, sanitation measures, the utilisation of disease-free tissue-cultured plants, and tolerant cultivars [3]. *P. capsici* is a soilborne oomycete pathogen afflicting several cash crops including solanaceous, leguminous, and most cucurbitaceous species [4, 5]. This pathogen carries substantial economic and scientific significance, responsible for annual losses exceeding one billion dollars in global vegetable production [5, 6]. Moreover, due to its resistance to currently available compounds, there is an urgent need to identify novel promising agents for its management.

Chemical fungicides are widely employed worldwide for the control of pathogenic fungi in various crops. Nevertheless, these fungicides possess a broad spectrum of activity, affecting beneficial soil microorganisms and leading to a swift resurgence of soil-borne pathogens. Additionally, their usage is constrained due to the presence of toxic residues, causing substantial environmental harm [7, 8]. Although several effective fungicides are recommended for root rot disease, they are not regarded as long-term solutions [9]. Consequently, there is an imminent requirement for alternative methods of fungal control in agriculture that are both less detrimental to human health and the environment [10, 11].

Plant preparations offer potential alternatives to nematode control products since they often serve as

*Corresponding author: Email: ndmchanh75@gmail.com

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alternative sources of treatment chemicals, degrade into non-toxic by-products, and exert minimal detrimental effects on non-target organisms and the environment. Numerous plants possessing antifungal properties have been identified and can be harnessed for disease management [12]. Furthermore, natural products exhibit low mammalian toxicity, high target specificity, biodegradability, and contain numerous active ingredients, thus displaying biocidal activity against multiple pests and pathogens [13]. Extracts from specific plants have traditionally been employed as natural fungicides in small-scale farming systems where the economic feasibility of synthetic chemicals was limited [14, 15]. Many medicinal plants represent a rich source of antifungal agents, and these plants are employed medicinally in various countries as sources of potent and efficacious drugs [12]. Researchers have conducted studies aimed at identifying novel antifungal substances produced by plants in recent years. These investigations have focused on the discovery of compounds with broad-spectrum activity that could be valuable for the treatment of plant diseases, resulting in the isolation of numerous antifungal compounds [16, 17].

G. uralensis Fisch. is a perennial medicinal plant indigenous to China, Mongolia, Russia, and Korea [18]. It predominantly thrives in arid and semi-arid desert grasslands, desert margins, and loess hilly regions [19]. The plant thrives in dark, humid, and dry climates with prolonged sunshine and low temperatures [19]. Its root, measuring 1-3 cm in diameter, possesses brown skin and a light yellow interior, exhibiting a sweet taste. The plant boasts robust roots, an upright stem, multiple branches, a height ranging from 30-120 cm, spiny glands, and white or brown hairs [18]. Biologically, liquorice extract has demonstrated antioxidant, anti-inflammatory, antiviral, cytotoxic, and anti-diabetic activities, and is extensively employed in the treatment of hepatitis, bronchitis, and malaria [20-22]. The primary pharmacological components found in the root of *G. uralensis* Fisch. are glycyrrhizic acid and liquiritin [21, 22]. Despite numerous studies exploring the applications of *G. uralensis* Fisch. extracts for medicinal purposes [23], their antagonistic effects against plant pathogenic fungi have yet to be investigated. Thus, the objective of this study is to evaluate the antifungal activity of *G. uralensis* Fisch. extract.

2. Materials and methods

2.1. Plant materials and chemicals

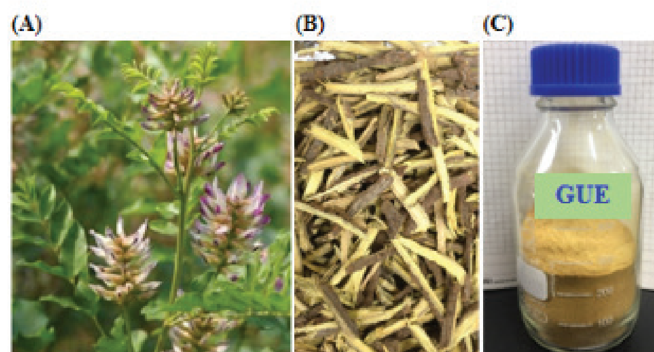

Fig. 1. Morphological characteristics of (A) the plant and (B) the stem, and (C) the crude powder obtained from methanolic extraction of the roots of *G. uralensis* Fisch. (GUE) (Sapa, Lao Cai province, Vietnam).

Samples of *G. uralensis* Fisch. were gathered from Sapa (Lao Cai province, Vietnam) in November 2022. The plant exhibits an upright growth with a stem measuring 0.5-1.5 m in height. Its leaves are feather-like, featuring 9-17 ovate leaflets, and its flowers are butterfly-shaped and pale purple (Fig. 1A). The stem displays a light yellow colour (Fig. 1B). The extraction process was adapted from the procedure outlined in [9]. In summary, dried *G. uralensis* Fisch. roots (1.0 kg) were cut into 3-5 cm segments and subjected to extraction using 80% methanol (5 l×3) with agitation at 150 rpm, maintained at 27±1°C for a duration of 72 hours. Subsequently, the methanolic extract was filtered, evaporated to dryness under vacuum conditions at 40±2°C (Eyela N-1000, Tokyo, Japan), and freeze-dried to obtain *G. uralensis* Fisch. extract (GUE) in the form of a yellow powder (Fig. 1C). The collected GUE was stored at 4°C until further use.

Fungal pathogens *F. oxysporum* f. sp *lycopesici* KACC 40032 and *P. capsici* KACC 40473 were obtained from the Korean Agricultural Culture Collection (Suwon, Republic of Korea). All chemicals and solvents utilised were of analytical grade.

2.2. Determination of the effect of GUE on mycelial growth

The toxicity of GUE against the tested fungi was assessed using a method that included the poison medium technique employing the PDA (potato dextrose agar) medium and the broth micro-dilution method [24]. In brief, mycelial culture blocks (6 mm in diameter) of the same age were placed in Petri dishes (90×15 mm)

containing 10 ml of PDA medium, resulting in final GUE concentrations of 0, 25, 50, 100, 200, or 400 µg/ml. An equal volume of methanol was used as a control. The samples were incubated in darkness at 25±1°C, and mycelial growth was assessed at 3, 6, and 9 days after treatment (day 3, day 6, and day 9, respectively). The effective concentration that inhibited mycelial growth was computed using the following formula [25]:

$$Inhibition\ (\%) = \left[\frac{C-T}{C} - 6\right] \times 100$$

where C and T are the hyphal extension in the control and plates treated with GUE, respectively. The experiments were performed twice, with three replicates for each treatment. The EC_{50} (half-maximal effective concentration) values of the GUE were calculated on day 3, day 6, and day 9.

2.3. Determination of minimum inhibitory concentration (MIC)

The MIC of GUE against the pathogens was determined using the microtiter broth dilution method with 2-fold serial dilutions, covering various GUE concentrations (0, 25, 50, 100, 200, and 400 µg/ml) [26]. The lowest GUE concentration that completely inhibited fungal growth, as observed visually, was defined as the MIC value.

2.4. Determination of the effect of GUE on mycelial morphology

The impact of GUE on the hyphal morphology of *F. oxysporum* and *P. capsici* was assessed following the procedures detailed in [27] and [8]. On day 3, fungal blocks were sectioned into 3 mm fragments and subsequently stained with lactophenol blue. To eliminate excess lactophenol blue solution, the samples underwent three washes with sterile water. The mycelial morphology of both the control and GUE-treated *F. oxysporum* and *P. capsici* was examined under a light microscope (40X magnification). PDA fungal culture devoid of GUE served as the control. The experiments were replicated three times for each treatment.

2.5. Statistical analysis

All data were presented as the mean ± standard deviation, based on the number of observations. A one-way analysis of variance (ANOVA) followed by Tukey's test was conducted to evaluate the significance of differences among the treatment means, with a significance level set at p≤0.05. The Statistical Analysis System 9.4 (SAS Institute Inc., Cary, NC, USA) was employed for statistical analysis.

3. Results and discussion

3.1. The inhibitory effect of GUE on the mycelial growth of *F. oxysporum* and *P. capsici*

Several extracts of *G. uralensis* Fisch. have been documented for their antibacterial, antifungal, and antiviral properties, encompassing crude alcoholic root extracts or its predominant purified compounds against human pathogens (*Arthrinium sacchari*, *Chaetonmium funicola*, and *Candida albicans*) [28-30]. However, to the best of our knowledge, there have been limited reports on the antifungal activity of a *G. uralensis* Fisch. extract against plant pathogenic fungi. In our investigation, we observed that GUE exhibited antifungal activity against both *F. oxysporum* and *P. capsici*, as depicted in Figs. 2 and 3, respectively. It is evident that GUE

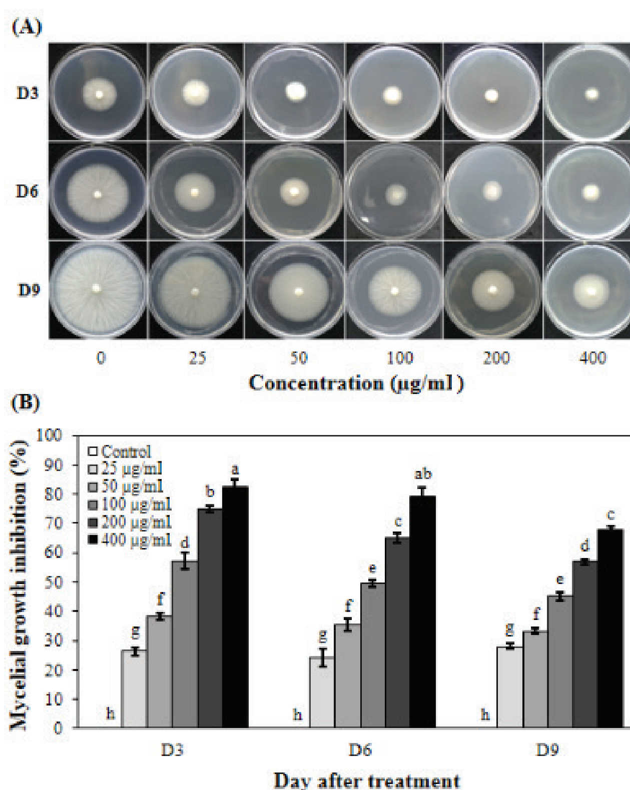

Fig. 2. Mycelial growth inhibition of *F. oxysporum* by GUE at various concentrations and treatment times. (A) Petri dishes of *F. oxysporum* incubated on PDA medium at 25°C in the dark with a final concentration of *G. uralensis* Fisch. extract (GUE) of 0, 25, 50, 100, 200, or 400 µg/ml (0 µg/ml is the methanol control); **(B)** Graphical representation of mycelial growth inhibition by GUE. The mean values followed by different letters in each column represent statistically significant differences based on Tukey's HSD test (p≤0.05). The error bars indicate the standard deviation of the mean (n=3). D3, D6, and D9 correspond to 3, 6, and 7 days after treatment, respectively.

significantly impeded the mycelial growth of both species, with inhibition ranges of 26.3-82.6, 24.1-79.2, and 28.1-67.8% for *F. oxysporum* and 27.4-83.7, 33.1-78.5, and 20.0-70.1% for *P. capsici*, observed three, six, and nine days after treatment with 25-400 µg/ml of GUE. Consequently, the EC_{50} values for GUE mycelial inhibition were calculated as 113.4, 150.6, and 191.4 µg/ml for *F. oxysporum* and 84.7, 148.6, and 190.1 µg/ml for *P. capsici*, respectively (Table 1). As portrayed in Figs. 2 and 3, the antifungal activities of GUE were highly pronounced in inhibiting the mycelial growth of *F. oxysporum* and *P. capsici* utilising the paper disc method. Specifically for *F. oxysporum,* the MIC value of GUE was found to be 100, 200, and 400 µg/ml at 3, 6, and 9 days post-treatment. In contrast, for *P. capsici*, the MIC value was 50, 100, and 100 µg/ml at 3, 6, and 9 days post-treatment.

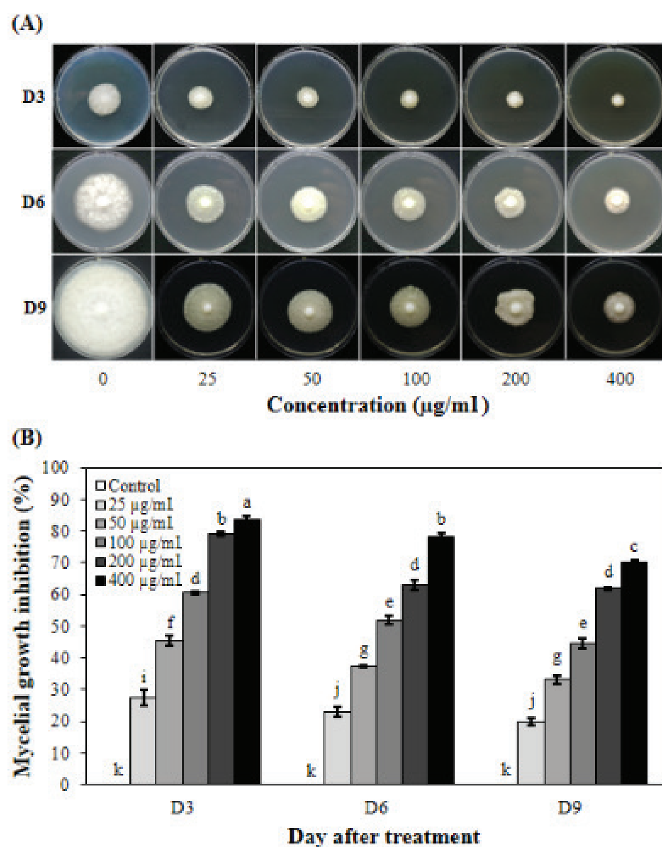

Fig. 3. Mycelial growth inhibition of *P. capsici* by GUE at various concentrations and treatment times. (A) Petri dishes of *P. capsici* incubated on PDA medium at 25°C in the dark with a final concentration of *G. uralensis* Fisch. extract (GUE) of 0, 25, 50, 100, 200, or 400 µg/ml (0 µg/ml is the methanol control); **(B)** Graphical representation of mycelial growth inhibition by GUE. The mean values followed by different letters in each column represent statistically significant differences based on Tukey's HSD test ($p \leq 0.05$). The error bars indicate the SD of the mean (n=3). D3, D6, and D9 correspond to 3, 6, and 9 days after treatment, respectively.

Table 1. The EC_{50} values for inhibiting the mycelial growth of *F. oxysporum* and *P. capsici* by the methanolic extract of the roots of *G. uralensis* Fisch. after various treatment times.

Pathogenic fungus	EC_{50} (µg/ml)		
	Day 3	*Day 6*	*Day 9*
F. oxysporum	113.4±1.8[c]	150.6±2.2[b]	191.4±1.1[a]
P. capsici	84.7±1.6[d]	148.6±2.3[b]	190.1±2.5[a]

Each value is the mean ± standard deviation from 2 experiments with three replicates. The mean values followed by different letters in each column represent statistically significant differences based on Tukey's HSD test ($p \leq 0.05$).

In separate studies, numerous compounds present in Chinese liquorice roots and rhizomes have exhibited anti-H1N1 virus activity, with inhibitory rates ranging from 47 to 82% at concentrations of 100 µM, corresponding to IC_{50} values within the range of 39.6-49.1 µM. These compounds include glycyrrhizin, β-acetoxyglycyrrhizin, and liquorice saponins A3, E2, and G2 [20]. Aqueous and ethanolic extracts from Chinese liquorice have also demonstrated a broad inhibitory spectrum against Gram-positive and -negative bacteria, yeast, and fungi. Moreover, *G. glabra* extract at concentrations of 10 to 100 mg/ml has displayed significant anti-yeast activity against *C. parapsilosis* [31]. The majority of antimicrobial effects attributed to Chinese liquorice extract can be attributed to isoflavonoid components, particularly hispaglabridin, β,4'-O-methylglabridin, glabridin, glabriol, and 3-hydroxyglabrol [31-33]. Dodecamethyl cyclohexasiloxane extracted from *Argemone ochroleuca* latex has demonstrated antifungal activity against *Candida* species and *Drechslera halodes* [34]. S. Hermann, et al. (2022) [23] reported that *G. glabra* leaf extract effectively controls a variety of bacterial plant pathogens and the oomycete *P. infestans*, not only *in vitro* or on the model plant *Arabidopsis*, but also on a crop plant (tomatoes). Furthermore, *Pseudomonas syringae* was eradicated by a 0.2% *G. glabra* leaf extract *in vitro*.

3.2. The effect of GUE on the mycelial morphology of F. oxysporum and P. capsici

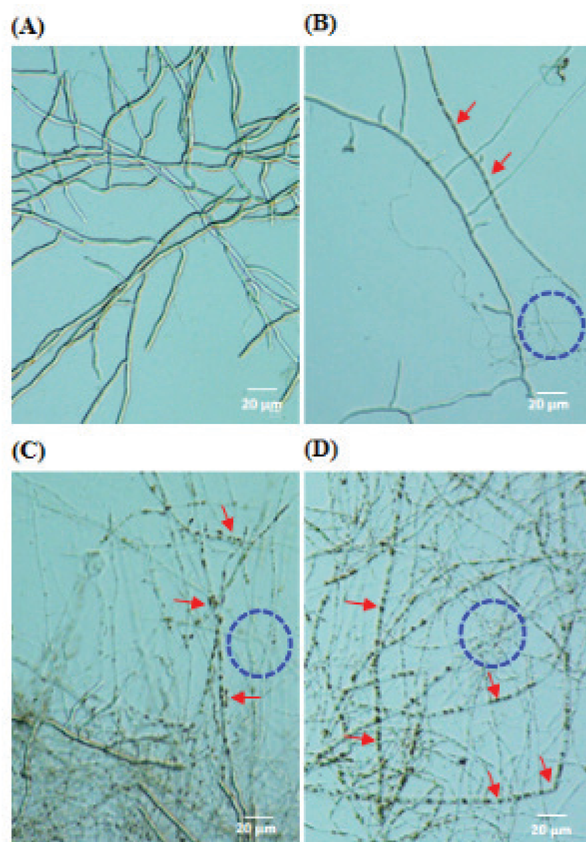

Fig. 4. Light microscope images (40X magnification) of the mycelial morphology of *F. oxysporum* after 3 days with and without treatment with *G. uralensis* Fisch. extract.

The mycelial morphology of *F. oxysporum* on day 3, with and without treatment with GUE, is presented in Fig. 4. In the case of *F. oxysporum*, Fig. 4A illustrates the growth of healthy mycelia on the control plates, while Fig. 4B reveals their slight impairment, featuring transparent cytoplasm, when exposed to 100 µg/ml GUE. Further treatment with 200 µg/ml GUE resulted in atrophied and fragmented mycelia, as indicated by the arrow in Fig. 4C. Subsequently, treatment with 400 µg/ml GUE resulted in abnormal, atrophied, and collapsed mycelia, as shown by the arrow and circle in Fig. 4D.

Figure 5 displays the mycelial morphology of *P. capsici* on day 3, with and without treatment with GUE. In the case of *P. capsici*, Fig. 5A demonstrates the growth of healthy mycelia on the control plates, while Fig. 5B reveals their mild perturbation, featuring transparent cytoplasm, when exposed to 100 µg/ml of GUE. Furthermore, Fig. 5C exhibits several swollen and

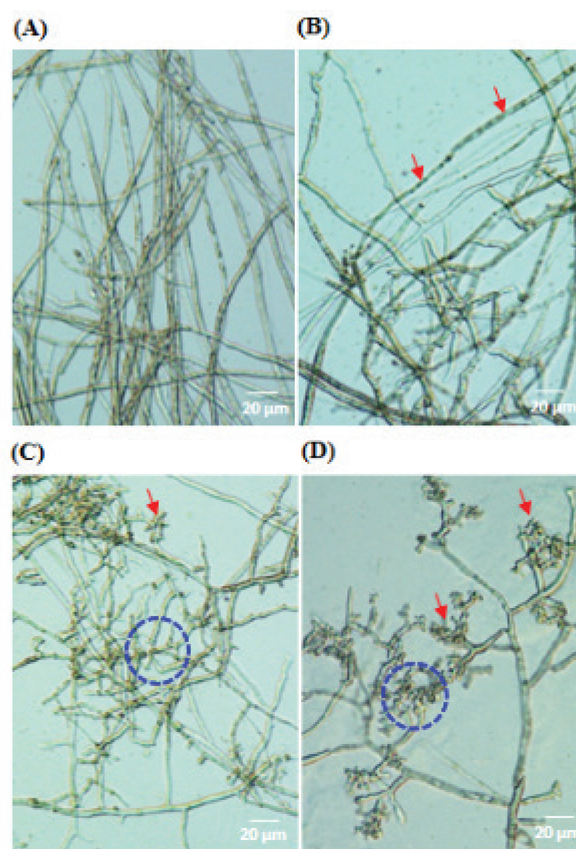

Fig. 5. Light microscope images (40X magnification) of the mycelial morphology of *P. capsici* after 3 days with and without treatment with *G. uralensis* Fisch. extract.

aberrant mycelium tips following treatment with 200 µg/ml of GUE, as indicated by the arrow and circle. Finally, when treated with 400 µg/ml of GUE, Fig. 5D shows a majority of swollen mycelium tips with increased branching, again illustrated by the arrow and circle. These results are in line with existing literature, suggesting that essential oils can exert versatile effects on cell walls, cell membranes, and cytoplasmic contents [35-38].

Mechanisms of action for crude extracts or compounds from *G. uralensis* Fisch. on fungi and oomycetes have been scarcely investigated. One plausible mechanism underlying their antifungal activity involves the degradation of fungal cell walls by inhibiting chitin synthesis [39]. Additionally, Q. OuYang, et al. (2019) [40] reported the dissolution of the cell wall of *Geotrichum citri-aurantii*, along with a reduction in chitin content, concluding that active compounds can hinder mycelial growth by compromising the integrity of fungal cell walls. In a similar vein, D.M.C. Nguyen, et al. (2013) [9] observed the collapse and shrinkage of *F. solani* hyphae

following incubation with gallic acid (500 µg/ml) purified from *Terminalia nigrovenulosa* for 24 hours. Likewise, Y. Wang, et al. (2021) [8] demonstrated the distortion and shrivelling of *P. capsici* mycelium 3 days after treatment with 140 mg/l cinnamaldehyde.

4. Conclusions

In this study, we explored the antifungal potential of the methanolic extract of *G. uralensis* Fisch.. It exhibited significant promise as an alternative to synthetic fungicides for combating plant pathogens. Future investigations should aim to identify the chemical structure of the active compounds present in *G. uralensis* Fisch. extract. Once these structures are elucidated, efforts should be directed towards developing their fungicidal potency and stability, with the goal of reducing costs and rendering them practical for field applications.

CRediT author statement

Dang-Minh-Chanh Nguyen: Methodology, Data analysis, Writing, Editing; Thi-Hoan Luong: Methodology, Data analysis, Writing; Van-Viet Nguyen: Methodology, Data analysis, Writing; Woo-Jin Jung: Methodology, Data analysis, Writing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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