ANTAGONISTIC EVALUATION OF *CHAETOMIUM* SP. FOR FUNGI CAUSING TUBER ROT DISEASE ON NGOC LINH GINSENG (*Panax vietnamensis* Ha et Grushv.)

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SUMMARY

Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv) is a rare and valuable medicinal herb endemic to Vietnam. However, diseases on ginseng over the years, particularly tuber rot, have severely impacted its yield and quality. This research aims to identify the cause of tuber rot disease in Ngoc Linh ginseng and explore potential biological control measures. Through isolation and morphological identification, *Fusarium solani* was identified as the pathogen responsible for tuber rot in ginseng. Pathogenicity test of isolated *F. solani* on the Ngoc Linh ginseng slices was performed using the artificial inoculation method under laboratory conditions. The results showed that the ginseng slices developed symptoms five days after re-inoculation with isolated *F. solani.* Further, the study evaluated the antagonistic activity of *Chaetomium* sp. in inhibiting the growth of *F. solani* using a dual – culture method on PGA medium. The antagonistic efficiency recorded after 11 days of culturing was 64.1% when *Chaetomium* sp. and *F. solani* wereinoculated on the same day and 71.8% when *Chaetomium* sp. was inoculated three days before *F. solani.* These results demonstrate that *Chaetomium* sp. has a high inhibitory effect on the growth of *F. solani* mycelium. The findings of this study provide a valuable foundation for developing disease prevention strategies for Ngoc Linh ginseng, contributing to the conservation and sustainable cultivation of this rare medicinal herb.

*Keywords***:** *Chaetomium*, dual - culture, *Fusarium solani*, Ngoc Linh ginseng, tuber rot.

INTRODUCTION

Ngoc Linh ginseng (*P.vietnamensis* Ha et Grushv.) is a rare and valuable medicinal herb endemic to Vietnam, first discovered in 1973 on Ngoc Linh mountain in Kon Tum province. Stems and tuber roots of ginseng contain up to 52 types of saponins, while the leaves contain 19 types of pamaran saponins (Duc *et al*.,1994). In addition, majonoside R2, a unique ocotillol-type saponin exclusive to Ngoc Linh ginseng, has garnered attention for its therapeutic potential, including neuroprotective, antioxidant, anti-inflammatory, antibacterial properties, and anticancer effects by enhancing apoptosis (Liu *et al.,* 2017; Thanh *et al*., 2023). Due to its rarity and medicinal benefits, Ngoc Linh ginseng commands a high market value, fetching between 1000 to 3000 USD per kilogram of fresh rhizomes (Lee *et al.,* 2018). However, its cultivation is challenging due to specific environmental requirements and lengthy growth periods. Besides, the problem of pests and diseases in Ngoc Linh ginseng is still ongoing, seriously affecting the quality and quantity of ginseng. In 2015, Xiaolin Jiao và đồng tác giả identified *F. solani* and *F. oxysporum* as the primary causes of root rot in *Panax Quinquefolius* in Beijing, China. Early symptoms include reddish-brown to orange-brown lesions on roots, leading to dry rot in advanced stages. Similarly, Zhang và đồng tác giả (2020) found a high incidence (33-44%) of *Fusarium*-induced root rot in these plants, with symptoms like soft, soggy, dark brown to black roots. Recently, Chen và đồng tác giả (2024) found *Pseudomonas* was the dominant bacterium, and *Fusarium*, particularly *F. oxysporum*, was the dominant fungus in *P. vietnamensis* plants with root rot. Given the high value of Vietnamese ginseng, it is crucial to prioritize the identification of effective biocontrol agents over chemical fungicides to sustainably manage root rot diseases in this plant. A study on *Chaetomium* spp demonstrated its antagonistic effects against various fungi, including *Fusarium* spp., which is known to cause root and stem rot and can severely reduce yield in different plants such as tomatoes, beans, peas, and potatoes (Seethapathy *et al.,* 2022). The study highlights *Chaetomium* spp.'s ability to inhibit these fungal pathogens through mechanisms such as mycoparasitism, where *Chaetomium* directly attacks and degrades the fungal hyphae, and the production of antifungal metabolites that suppress the growth of these pathogens. These findings suggest that *Chaetomium* could be a promising candidate for managing root rot diseases in *P. vietnamensis*, especially given its effectiveness against similar fungal pathogens in other plants. Further research specifically targeting *P. vietnamensis* would be beneficial to confirm its efficacy. Therefore, the research aims to identify the causative agent of tuber rot disease in Ngoc Linh ginseng and to provide essential scientific insights for developing targeted disease prevention measures.

MATERIALS AND METHODS

Materials

Ngoc Linh ginseng's tuber with symptoms of rot, wilting, dark brown, and wet lesions were collected from Ngoc Linh mountain, located at geographical coordinates 14°59'16" - 14°59'39" north latitude and 107°54'25" - 107°54'52" east longitude. Post-harvest samples were promptly transported to the laboratory for immediate analysis.

Research methods

Fungi Isolates

Tuber samples were cleaned with tap water and sectioned at the boundary between healthy and diseased tissue. The surface was sterilized with 70% ethanol followed by a rinse with distilled water. Samples were air-dried on sterile paper for 15 minutes before being cultured on Water Agar (WA) medium. After three days of incubation at room temperature (30-35°C), sections showing peak fungal mycelium growth were removed and transferred to Potato Glucose Agar (PGA) medium. The experiment was conducted in triplicate, with two dishes per trial, and incubated for 10 days (Burgess *et al*., 2009).

Morphology identification

After culturing on PGA medium for 5-7 days, monitor the mycelial growth and observe the fungus's coloration in the petri dish. Use a microscope with a 40X objective to examine and document specific characteristics of *Fusarium* spp., including macroconidia, microconidia, chlamydospores, and other relevant features. Referencing "The *Fusarium* Laboratory Manual" (Leslie & Summerell, 2006), classify fungal morphology based on identified characteristics. Although molecular identification methods offer precise classification, the morphological approach remains a reliable and widely accepted method for identifying *Fusarium* species. This method has been extensively validated in various studies (Leslie & Summerell, 2006; Burgess *et al.,* 1994; Nelson *et al.,* 1983), and has been shown to accurately differentiate *Fusarium* species based on distinctive morphological traits, ensuring dependable results when molecular techniques are not feasible. The molecular characterization results of 23 *Fusarium* spp. in the study by Trabelsi và đồng tác giả (2017) were consistent with their morphological analyses, further supporting that identifying *Fusarium* species based on morphological characteristics is a reliable method.

Pathogenicity test

After identification, the fungi responsible for tuber rot disease in Ngoc Linh ginseng were tested for pathogenicity using artificial inoculation under controlled laboratory conditions. Healthy Ngoc Linh ginseng slices, approximately 0.5 cm ± 0.1 thick, were used for pathogenicity test. The experiment consisted of two treatments: control slices and slices inoculated with fungal spore fluid at a density of 10^6 spores/ml. Each treatment was replicated three times, with two ginseng slices per replication. To initiate infection, wounds were created on the ginseng slices using a needle. 20 µL of fungal spore fluid was applied to the wound site using a micropipette and distilled water used for control. Ginseng slices were placed in a plastic box (20 x 11 x 5.5 cm) and covered with food wrap. The wrap was punctured with a needle in several places for ventilation. The box was then incubated under laboratory conditions at 30-35°C. All tools used were sterilized with 70% ethanol. The ginseng slices were monitored continuously until symptoms of the disease appeared, documenting the progression of infection (Burgess *et al*., 2009).

Evaluation of the antagonistic activity of *Chaetomium* sp.

The antagonistic potential of *Chaetomium* sp. against the isolated fungi was assessed via the dual-culture method on PGA medium. Two experiments were conducted: Experimental 1 involved simultaneous inoculation of *Chaetomium* sp. and the isolated fungi, while Experimental 2 included *Chaetomium* sp. inoculated three days prior to the isolated fungi. Each experiment comprised a control (sole culture of the isolated fungi) and an antagonistic treatment combining *Chaetomium* sp. with the isolated fungi. The study was replicated three times, with two 8 cm diameter petri dishes per replication. Incubation occurred under controlled laboratory conditions (30-35°C). Fungal growth on the petri dishes was monitored and measured at intervals of five, seven, nine, and eleven days. Antagonistic effectiveness was evaluated using Soytong's assessment scale (1988). Antifungal efficiency is calculated according to the formula:

$$PIMG = \frac{R1-R2}{R1} \times 100\ (\%)$$

In which: *PIMG:* Percent Inhibition of Mycelial Growth; R1: Radius of the isolated fungal mycelium in the control dish; R2: Radius of the isolated fungal mycelium in antagonistic interaction.

Antagonistic efficiency is classified as follows: *PIMG* > 75%: very high antagonistic ability; *PIMG* from 61 – 75%: high antagonistic; *PIMG* from 51 - 60%: has average antagonistic; *PIMG* < 50%: low antagonistic.

RESULTS AND DISCUSSION

Isolation and identification fungi cause tuber rot disease on Ngoc Linh ginseng

Based on observed morphological characteristics, the pathogenic fungus causing disease in ginseng was identified as *Fusarium solani*. *F. solani* grows quickly on PGA medium, filling the petri dish within six days after inoculation. The mycelium appeared white, and spread flat across the culture medium, with a cream-colored reverse side (Figure 1).

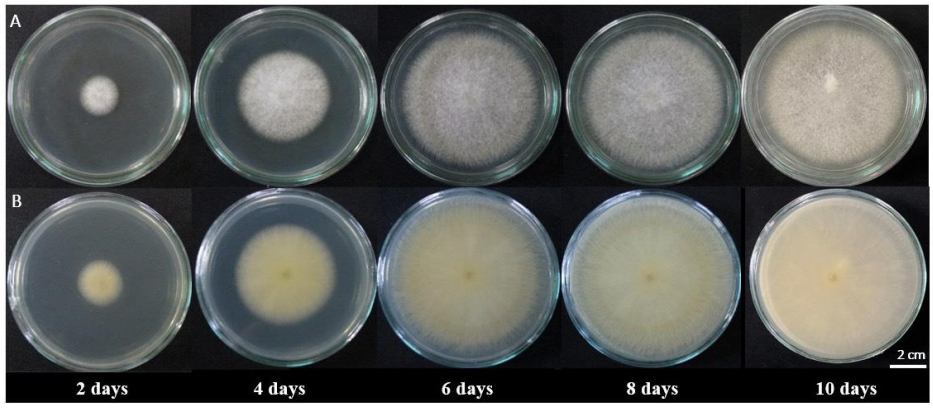

Figure 1. Characteristics of *F. solani* on the PGA medium

A: Front of the petri dish; B: Back of the petri dish

The morphology of macroconidia, microconidia, phialide and chlamydospore were observed using a 40X objective microscope (Figure 2). Macroconidia are relatively wide, slightly curved, stout, and robust, with apical cells exhibiting three shapes: papillate, blunt, and hooked. Basal cells may display a distinct foot shape, varying in development. Macroconidia typically features 5 to 7 septa, measuring approximately 29.07 – 34.45 µm x 5.31 – 5.70 µm (Figure 3).

Microconidia exhibit five basic shapes: oval, fusiform, reniform, allantoid, and obovoid (Figure 4), often lacking septa or occasionally having one, with dimensions averaging 10.89 – 13.98 µm x 5.44 – 5.91 µm. Chlamydospores are abundant in the mycelium, predominantly globose or subglobose, occurring singly, in clusters, or short chains, with an average diameter of about 6.23 µm (Figure 2G–H). Phialides of *F. solani* are monophialidic, subcylindrical or cylindrical, arising from conidiophores. Sporodochial conidiophores form on distinct collarettes at the tip, averaging approximately 87.36 µm in height (Figure 2E). The results of morphological characteristics of fungal mycelium and spores recorded above are consistent with the description of the *F. solani* of Gerlach and Nirenberg (1982), as well as of Nelson và đồng tác giả (1983).

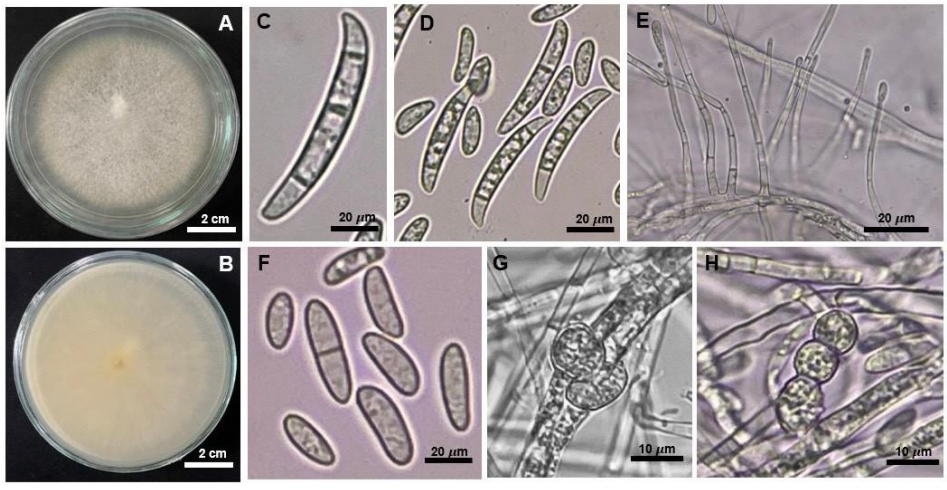

Figure 2. Characteristics of *F. solani's* spores under microscope

A: Front of the petri dish after ten days cultured; B: Back of the petri dish after ten days cultured; C: Macroconidia; D: Macroconidia and microconidia; E: Phialide; F: Microconidia; G – H: Chlamydospore.

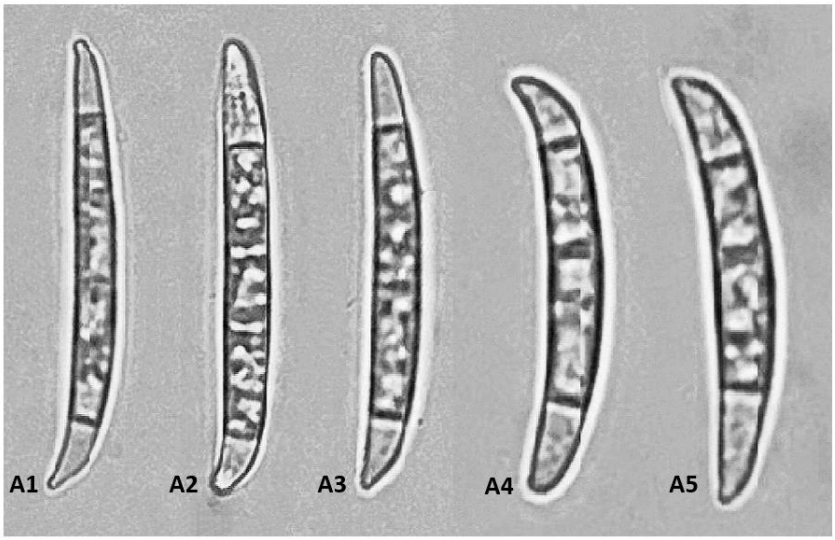

Figure 3. Morphological features of *F. solani's* macroconidia under the microscope 40X objective.

A1: Slender with no significant curvature; A2: Widest at the apical third, wedge – shaped; A3: Unequally curved; A4: Widest at the basal portion; A5: Widest at the middle portion.

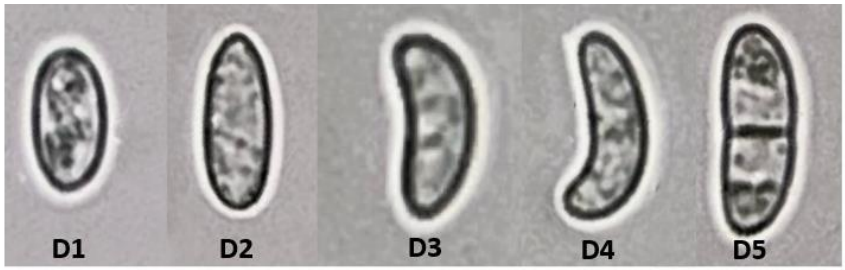

Figure 4. Basic morphological features of *F. solani's* microconidia under the microscope 40X objective

D1: Oval; D2: Fusiform; D3: Reniform; D4: Allantoid; D5: Obovoid.

Research worldwide has consistently identified *Fusarium* spp. as a prominent pathogen affecting various ginseng species such as *Panax ginseng* and *Panax quinquefolius*. Lee Soon-Gu's study (2014) conducted in Korea from 1982 to 1985, involving 115 samples including rotten roots and soil, identified 11 species, with *F. solani* being predominant in 55 out of 115 isolated samples. Similarly, Zamir and Punja (1997) collected leaf and root samples from *P. quinquefolius* gardens in British Columbia between 1992 and 1996, reporting that the *Fusarium* genus accounted for 30.5%, primarily comprising *F. solani, F. oxysporum, F. avenaceum,* and *F. equiseti*. In China, Guan và đồng tác giả (2019) isolated and identified 10 *Fusarium* species from *P. ginseng* seeds, including *F. solani* and *F. oxysporum* as the most common, consistent with their role as pathogens causing rot during the ginseng growth cycle. Their research indicated that *Fusarium* spp. in seeds can infect ginseng plants and 2-year-old tubers, adversely impacting yield and quality by attacking the roots and causing tuber rot. Moreover, Jiao và đồng tác giả (2015) investigated the effects of *F. solani* and *F. oxysporum* on ginsenoside metabolism *in P. quinquefolius* roots. Their study, which involved artificially infecting roots and assessing ginsenoside concentrations, showed significant reductions in ginsenoside levels at the infection sites compared to adjacent healthy areas. This underscores *Fusarium* spp.'s ability to diminish ginsenoside content in ginseng through metabolic processes. Despite these global studies, research on pests and diseases affecting Ngoc Linh ginseng in Vietnam remains limited, leading to ineffective disease prevention strategies.

Pathogenicity test of isolated fungal disease on the Ngoc Linh ginseng slices

Testing the pathogenicity of *F. solani* on Ngoc Linh ginseng slices under controlled laboratory conditions revealed distinct disease symptoms. Within three days of inoculation, white mycelium appeared at the inoculation site, while the ginseng slices remained moist. By the fifth day, the slices exhibited drying and shrinkage, accompanied by darkening and a slight brownish tint at the infection site, indicating pronounced decay. The disease rate reached 100% by the fifth day, mirroring initial symptoms observed in rotten Ngoc Linh ginseng samples.

Figure 5. Disease symptoms appeared on Ngoc Linh ginseng slices caused by *F. solani*

Evaluation of the antagonistic activity of *Chaetomium* sp. on *F. solani* by dual – culture method

Chaetomium sp. identified and stored at the microbiology laboratory, Research Institute for Biotechnology and Environment, Nong Lam University - Ho Chi Minh City was used to evaluate the ability to antagonism with *F. solani*. The antagonism activity against *F. solani* were conducted in two ways, including *Chaetomium* sp. and *F. solani* were inoculated concurrently and *Chaetomium* sp. inoculated three days prior *F. solani*.

Table 1. The inhibitory effect of the *F. solani* by the antagonist fungus *Chaetomium* sp. inoculated concurrently

The time recorded after cultured	Radius of the *F. solani* in the control treatment (cm ± SD)*	Radius of the *F. solani* in the antagonism treatment (cm ± SD)*	*PIMG* Values (% ± SD)**
5 days	2,80 ± 0,00^{a}	2,40 ± 0,10^{b}	14,28 ± 3,57^{c}
7 days	4,33 ± 0,49^{a}	2,60 ± 0,10^{b}	39,36 ± 8,58^{b}
9 days	5,50 ± 0,10^{a}	2,50± 0,00^{b}	54,54 ± 0,83^{a}
11 days	6,50± 0,00^{a}	2,30 ± 0,12^{b}	64,10 ± 1,78^{a}

*: Different letters in the same row indicated that values were significantly different ($p < 0,05$).

**: Different letters in the same column indicated that values were significantly different ($p < 0,05$).

Table 2. The inhibitory effect of the *F. solani* by the antagonist fungus *Chaetomium* sp. inoculated three days before

The time recorded after cultured	Radius of the *F. solani* in the control treatment (cm ± SD)*	Radius of the *F. solani* in the antagonism treatment (cm ± SD)*	*PIMG* Values (% ± SD)**
5 days	2,74 ± 0,05^{a}	2,12 ± 0,08^{b}	24,12 ± 2,38^{d}
7 days	3,90 ± 0,28^{a}	2,06 ± 0,05^{b}	50,79 ± 1,84^{c}
9 days	5,14 ± 0,31^{a}	2,00 ± 0,12^{b}	62,91 ± 2,50^{b}
11 days	6,50± 0,00	1,94 ± 0,17^{b}	71,80 ± 1,78^{a}

*: Different letters in the same row indicated that values were significantly different ($p < 0,05$).

**: Different letters in the same column indicated that values were significantly different ($p < 0,05$).

After 11 days of dual-culture, *Chaetomium* sp. demonstrated significant inhibition of *F. solani* mycelium growth, achieving 64.1% and 71.8% Pathogen Inhibition Growth Rate (PIMG) when inoculated simultaneously and three days prior, respectively (Table 1, Table 2). According to Soytong's (1988) assessment scale, these results indicate highly antagonistic behavior in both experiments (Figure 6, Figure 7). Comparatively, studies by Thu Huong và đồng tác giả (2021) identified *Chaetomium* sp. strains C1 and C2 with antagonistic efficiencies of 67.7% and 51.1% against *Fusarium* spp. in *Cucurbitaceae* plants. Additionally, Wahba và đồng tác giả (2016) found that *Chaetomium globosum* could reduce *F. solani* growth by 22.2% and 38.9% when inoculated concurrently and three days prior in tomato plants suffering from root rot. These findings collectively suggest that *Chaetomium* sp. exhibits medium to high antagonistic efficacy (51% - 75%) against *F. solani*. However, research on beneficial microorganism resistance to pathogens in Ngoc Linh ginseng remains limited. Thus, this study provides a foundational basis for future research aimed at developing effective strategies to prevent tuber and root rot diseases in ginseng.

Figure 6. Evaluation of the antagonistic activity of the fungus *Chaetomium* sp. against *F. solani* after 11 days (inoculated concurrently)

Figure 7. Evaluation of the antagonistic activity of the fungus *Chaetomium* sp. against *F. solani* after 11 days (inoculated *Chaetomium* sp three days before)

CONCLUSIONS

This study identifies *Fusarium solani* as the causative agent of tuber rot disease in Ngoc Linh ginseng, consistent with findings in other Panax species. Re-inoculation of *F. solani* on Ngoc Linh ginseng slices resulted in disease symptoms appearing within five days under laboratory conditions. Moreover, the study demonstrates that *Chaetomium* sp. effectively inhibits *F. solani* growth, achieving high antagonistic efficiency after 11 days of culture. These findings provide a foundational basis for future research on disease prevention strategies for Ngoc Linh ginseng, particularly the potential use of beneficial antagonistic microorganisms to mitigate fungal diseases.

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ĐÁNH GIÁ KHẢ NĂNG ĐỐI KHÁNG CỦA *CHAETOMIUM* SP. ĐỐI VỚI NẤM GÂY BỆNH THỐI CỦ TRÊN SÂM NGỌC LINH (*Panax Vietnamensis* Ha et Grushv.)

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TÓM TẮT

Sâm Ngọc Linh (*Panax vietnamensis* Ha et Grushv) là một loại dược liệu thân thảo quý hiếm, đặc hữu của Việt Nam. Tuy nhiên tình trạng bệnh hại trên sâm trong nhiều năm qua, đặc biệt là bệnh thối củ ảnh hưởng nghiêm trọng đến sản lượng và chất lượng sâm. Nghiên cứu này nhằm xác định nguyên nhân gây bệnh thối củ trên sâm Ngọc Linh và khảo sát khả năng sử dụng *Chaetoium* sp. như một biện pháp kiểm soát sinh học tiềm năng. Thông qua phương pháp phân lập và xác định hình thái, *Fusarium solani* đã được xác định là tác nhân gây bệnh thối củ trên sâm Ngọc Linh. Sau đó tiến hành thử nghiệm khả năng gây bệnh của *F. solani* trên lát sâm Ngọc Linh bằng phương pháp lây nhiễm nhân tạo ở điều kiện phòng thí nghiệm, kết quả lát sâm xuất hiện triệu chứng bệnh sau 5 ngày tiến hành lây nhiễm. Tiếp tục tiến hành đánh giá khả năng đối kháng của *Chaetomium* sp. trong việc ức chế sự phát triển của *F. solani* bằng phương pháp đồng nuôi cấy trên môi trường PGA. Hiệu suất đối kháng ghi nhận sau 11 ngày nuôi cấy lần lượt là 64.1% khi đồng nuôi cấy *Chaetomium* sp. và *F. solani* và 71.8% khi cấy *Chaetomium* sp. trước *F. solani* 3 ngày. Kết quả đạt được có thể làm tiền đề nghiên cứu về biện pháp phòng trừ bệnh trên củ sâm Ngọc Linh, góp phần bảo tồn loại dược liệu quý hiếm này.

Từ khóa: Chaetomium, đồng nuôi cấy, Sâm Ngọc Linh, *Fusarium solani,* bệnh thối củ.

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