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Cordyceps militaris Residue Extract Exhibits Potent Antiviral and Plant Growth-Promoting Effects

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Abstract

Cordyceps militaris is an important medicinal and edible fungus that contains a wide range of bioactive ingredients, including cordycepin, polysaccharides, ergosterol, mannitol, proteins, and carotenoids, which collectively confer tonic, anti-fatigue, immunopotentiating, antioxidant, anti-inflammatory, and metabolic-regulating properties. Notably, the culture residue of *C. militaris*, which remains rich in bioactive compounds, is mostly discarded during production, resulting in resource waste and potential environmental pollution. In this study, *C. militaris* culture residue extract (CME) was prepared by ultrasonic extraction, and its antiviral activity was evaluated using *Nicotiana benthamiana* via foliar spraying. The results showed that CME treatment significantly upregulated the expression of defense-related genes *PR1*, *PR2*, and *ICS1*, with *PR1* showing the most pronounced induction (13.20-fold before and 11.89-fold after TMV inoculation), thereby conferring strong antiviral activity. In addition, root irrigation with 10 mg/mL CME significantly increased plant height, stem diameter, dry weight, fresh weight, chlorophyll content, and carotenoid content in tomato plants. Taken together, these findings indicate that CME functions as a plant immune inducer capable of effectively suppressing tobacco viral diseases while promoting plant growth. This study not only provides a new strategy for the value-added reutilization of *C. militaris* culture residues but also offers a scientific basis for the green control of tobacco mosaic disease.

Keywords: *Cordyceps militaris*; residue extract; TMV; growth promotion

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1. Introduction

Tobacco mosaic virus (TMV) is a positive-sense single-stranded RNA virus belonging to the *Tobamovirus* genus. It infects a broad range of hosts [1,2]. Infection caused by TMV severely reduces crop yield and quality, as infected plants exhibit symptoms such as tissue yellowing and necrosis, followed by gradual deformation, stunted growth, and, in extreme cases, even death [3–5]. TMV particles are extremely resilient to environmental stresses, capable of surviving in external environments for extended periods while also maintaining their infectious activity for years or even decades [6,7]. At present, the primary strategies for managing TMV involve cultivating resistant varieties and applying certain chemical or biological pesticides. However, the development of resistant varieties is time-consuming, and their effectiveness is often reduced by the rapid evolution of viral

strains. In addition, chemical antiviral agents, such as ribavirin, may cause environmental concerns and generally provide only moderate and inconsistent control of TMV under field conditions [8,9]. These limitations highlight the urgent need for alternative and more sustainable strategies to control TMV infection.

As a new practice in plant protection, natural immune inducers have gained considerable attention due to their low toxicity to humans and animals, high environmental compatibility, a broad spectrum of action, and no risk of inducing drug resistance in pathogens [10]. Natural immune inducers can be derived from various sources, including animals, plants, microorganisms, or their metabolites, or produced during the interaction between plants and microorganisms as active molecules [11]. Currently, primarily five categories of plant immune inducers have been developed and used: inorganic compounds, organic acids, benzothiadiazoles, oligosaccharides, and protein polypeptides; in addition, immune-inducing bacteria are also widely applied [12–17].

Importantly, multiple studies have demonstrated that fungal-derived immune inducers can effectively suppress TMV infection by activating host defense pathways, underscoring their potential as alternative antiviral agents. For instance, the fungal serine protease AsES enhances TMV resistance in Arabidopsis and *Nicotiana benthamiana* plants by inducing salicylic acid-dependent defense responses, including callose deposition and upregulation of defense-related genes [18]. Proteins from the edible mushroom *Flammulina velutipes* significantly inhibit TMV accumulation via activating the salicylic acid (SA) signaling pathway, thereby strengthening plant antiviral immunity [19]. Recent studies have shown that the main active polysaccharide component, LW-1, from *Omphalia lapidescens*, exhibits a significant anti-TMV effect by triggering the MAPK signaling pathway, enhancing the plant immune system, and accelerating the synthesis and accumulation of plant defensins [20]. These findings suggest that fungi represent a valuable and underexplored resource for the development of novel plant immune inducers.

Cordyceps militaris is a well-known medicinal and edible fungus that has been extensively cultivated at an industrial scale in recent years [21,22]. According to a market analysis report, the global market for *Ophiocordyceps sinensis* and *C. militaris* was valued at USD 473.4 million in 2018 and is projected to grow at a CAGR of 10.4% through 2026. This rapid industry expansion inevitably generates large amounts of discarded culture residues [23]. The mushroom residue of *C. militaris* primarily consists of mycelium, residual substrate, and fermentation metabolites, and is rich in bioactive compounds such as cordycepin, polysaccharides, amino acids, and fungal proteins, rendering it a renewable biological resource [24]. Previous studies have explored the utilization of *C. militaris* residues in areas such as animal feed and cosmetics, demonstrating their feasibility for value-added applications [23,25,26]. Nevertheless, the majority of these residues are still discarded as waste, leading to environmental pollution and inefficient resource utilization [27]. More importantly, there has been no research on the preparation of plant immune inducers or antiviral agents using *C. militaris* culture residues. Based on the above considerations, the objective of this study was, therefore, to evaluate the antiviral activity of *C. militaris* culture residue extract (CME) against TMV using *N. benthamiana* as a model system, and to explore its potential effects on the expression of plant defense genes. In addition, the plant growth-promoting effects of CME were assessed in tomato plants. This study aims to provide a scientific basis for the agricultural reutilization of *C. militaris* culture residues and to explore their potential application in green and sustainable strategies for the control of plant viral diseases.

2. Materials and Methods

2.1. Experimental Materials and Instruments

Denatured proteins were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels (Epizyme Biomedical Technology Co., Ltd., Shanghai, China), transferred to nitrocellulose membranes (GE Healthcare, Chicago, IL, USA), blocked with a blocking solution (Epizyme Biomedical Technology Co., Ltd., Shanghai, China), and hybridized with an anti-green fluorescent protein (GFP) antibody and a Horseradish Peroxidase-conjugated goat anti-rabbit IgG (Sangon, Shanghai, China). Target protein bands were visualized using a chemiluminescence kit (Epizyme Biomedical Technology Co., Ltd., Shanghai, China) and imaged using a FluorChem E Imaging System (ProteinSimple, San Jose, CA, USA). RNA extraction kits, PrimeScript RT reverse transcription kits, and Taq Pro Universal SYBR qPCR Master Mix were purchased from Nanjing Vazyme Biotech Co., Ltd. (Nanjing, China). RT-qPCR was performed using a CFX Connect real-time fluorescent quantitative PCR instrument (Bio-Rad, Hercules, CA, USA).

The tested *C. militaris* strain JB01 was obtained via tissue isolation (Meiao Bioengineering Co., Ltd., Dongying, China) and preserved in the Culture Collection Center of Ludong University. The tomato variety used was "Fenguan No. 2" (Shouhe Seed Industry Co., Ltd., Weifang, China).

This study was conducted at the School of Horticulture, Ludong University, from January 2024 to December 2025.

2.2. Cultivation of C. militaris and Disposal of Residual Medium

After activation on potato dextrose agar (PDA) solid medium, a 0.5 cm^2 mycelial block of strain JB01 was inoculated into 150 mL liquid medium and cultured in the dark in a constant temperature shaker at 22 °C and 140 rpm for 5–6 days to prepare liquid spawn. An oat medium (30 g oats + 50 mL pure water) was dispensed into 500 mL tissue-culture bottles and sterilized at 121 °C for 25 min. After cooling, each bottle was inoculated with 5 mL of liquid spawn and subsequently cultured in the dark at 18 °C for 8–9 days. Scratching was performed to induce fruiting body formation once the mycelium had colonized two-thirds of the bottom of the bottle. The bottles were then transferred to a culture room maintained at 20 °C and 80–90% relative humidity for light incubation. The fruiting bodies were harvested after 50 days. The residual culture substrate was dried at 60 °C to a constant weight, pulverized using a grinder, and the powder was passed through an 80-mesh sieve for subsequent use.

2.3. CME Extraction

One gram of the *C. militaris* residue powder was accurately measured (to 0.001 g precision) and transferred into a 250 mL conical flask, followed by the addition of 80 mL ultrapure water. Ultrasonic extraction was conducted at 80 W and 25 °C for 3 h, after which the solution was made up to a final volume of 100 mL using ultrapure water. After treatment at 8000 rpm for 15 min, the precipitate was discarded to prepare the CME solution. Subsequently, 3 mL of the CME was passed through a 0.22 μm filter.

2.4. Optimization of CME Safe Concentration

The concentrations of CME selected in this study were determined on the basis of preliminary experiments. In preliminary foliar application assays, foliar spraying of 50 mg/mL CME did not induce any significant phenotypic alterations in tobacco seedlings. Accordingly, a 10 mg/mL CME solution was concentrated to 20, 50, 100 and 200 mg/mL using a rotary evaporator (Gongyi Yuhua Instrument Co., Ltd., Zhengzhou, China). Spanning from a clearly safe level to potentially phytotoxic levels, to define the optimal and safe

concentration range for subsequent antiviral assays. Then, five aqueous solutions of *CME* at different concentrations were evenly applied to the leaves of *Nicotiana benthamiana*. Three replicate plants were used for each treatment group. Tobacco seedlings were sprayed every 24 h for five consecutive treatments and then transferred to a greenhouse held at 24 °C for further cultivation. Plant growth was monitored throughout the treatment period. Twenty-four hours after the final treatment, plant height, plant width, leaf width, dry weight, fresh weight, and chlorophyll content were measured.

2.5. Antiviral Effects and Induced Resistance of Different Compounds

Tobacco seedlings at the 5–6 leaf stage with uniform growth and healthy appearance were selected for TMV resistance assays using CME. The treatment solutions were evenly sprayed onto the leaves, with deionized water serving as the negative control. Seedlings were treated once daily for five consecutive days and cultured in a greenhouse set at 24 °C under a 16 h light and 8 h dark cycle. At 24 h after the final treatment, the TMV-GFP vector was infiltrated into *N. benthamiana* plants [28]. At 3 days post-inoculation (dpi), whole-plant viral proliferation was assessed by observing fluorescence diffusion under ultraviolet (UV) light in the inoculated leaves. Images were taken for documentation. Subsequently, 0.1 g of inoculated leaves per seedling was collected and stored at −80 °C for subsequent Western blot and qRT-PCR analyses.

2.6. Western Blot Assay

The inoculated leaves subjected to different treatments were pulverized into a fine powder using liquid nitrogen. The powder was homogenized in extraction buffer, and the mixture was heated at 100 °C for 10 min before being centrifuged at 12,000 rpm for 10 min [29]. Electrophoretic separation of the samples was performed on a 12.5% SDS-PAGE gel, followed by their transfer to a nitrocellulose membrane. Subsequently, the membrane was sealed with blocking buffer and hybridized with the corresponding antibodies. Chemiluminescence kit-based visualization was used to detect antibody–antigen interactions, which were then imaged via the FluorChem E Imaging System.

2.7. Real-Time Fluorescence qPCR

RNA was isolated from two groups of leaf samples: those treated with reagent sprays but not inoculated with TMV, and those collected 3 days after TMV inoculation. The expression levels of resistance-related genes in the leaves were detected using real-time fluorescent qPCR. Total RNA was extracted following the RNA extraction kit protocols. Reverse transcription was completed using the PrimeScript RT reverse transcription kits. The relative expression levels of target genes were detected using the CFX Connect real-time fluorescent quantitative PCR instrument combined with Taq Pro Universal SYBR qPCR Master Mix. Three biological replicates were set for each sample. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table 1.

Table 1. Primers used in the study.

Primer Name	Sequence (5′-3′)
PR1-F	TTAGCAGCCGTCATGAAATCGT
PR1-R	GGCGTAGAACCTTTAACCTGGGA
PR2-F	GCAGCAGACGATGTAATGATGG
PR2-R	TCCACAAGCCTAGTGAGCCTC
ICS1-F	TCATCACTCGTGAAATGGTCG
ICS1-R	GAGGCTGGGAGTTAACCAAGT
Actin-F	AGGCTGTTCTTTCCCTCTATGC
Actin-R	CAACTTCTCCTTCACATCCCTAAC

2.8. Plant Growth Measurement

The tomato variety "Fenguan No. 2" was selected as the experimental material. Seedlings with uniform growth performance were chosen and root-irrigated with 50 mL of CME solutions at concentrations of 1, 5, and 10 mg/mL every 5 days. Water was used as the negative control. Treatments were conducted continuously for 25 days. The seedlings were cultured in a greenhouse maintained at 24 °C under a 16 h light and 8 h dark photoperiod. Three days after the last treatment, various growth and physiological indices of the tomato plants were measured to evaluate the effects of different concentrations of CME on tomato growth.

N. benthamiana seedlings with uniform growth were selected and root-irrigated with 20 mL of 10 mg/mL CME solution every 48 h. Clear water was used as the negative control. Since the CME was derived from the residual medium of *C. militaris* cultured on an oat substrate, it contains components from oat extract. An additional treatment group treated with oat extract alone was included to further verify the role of metabolites produced during *C. militaris* growth. The seedlings were treated continuously for 2 weeks and cultured in a greenhouse at 24 °C under a 16 h light and 8 h dark photoperiod. After 14 days, plant height, plant width, leaf width, fresh weight, and dry weight of *N. benthamiana* were measured to evaluate the growth-promoting effect of CME on the plants.

2.9. Statistical Analysis

All experiments were conducted with at least three independent biological replicates. Data are expressed as mean ± standard deviation (SD). Statistical analyses and graphical visualization were performed using GraphPad Prism version 8.0 and SPSS version 20.0. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc tests was applied for multiple comparisons, while independent-samples *t*-tests were used for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results

3.1. Safe Concentration Range of CME

To establish a basis for evaluating the potential antiviral activity of CME, it was first prepared by ultrasonic disruption, and then the effects of foliar spraying of CME were assessed on the growth of *N. benthamiana*. Tobacco seedlings at the four-to-six leaf stage with uniform growth and robust vigor were sprayed with CME at different concentrations for five consecutive days, using deionized water as the control. After 24 h of the final treatment, the growth phenotypes of the plants were photographed, and relevant growth parameters were measured. The results showed that plants treated with 10–50 mg/mL CME exhibited normal growth, with no significant difference compared with the control (Figure 1A). By comparison, when the concentration of CME reached 100 or 200 mg/mL, plant growth was significantly inhibited, exhibiting stunted growth and leaf chlorosis (Figure 1A). Furthermore, these high-concentration CME-treated plants exhibited significantly lower plant width, leaf width, and chlorophyll content compared with the control group (Figure 1B). In conclusion, the safe concentration range of CME for foliar application on *N. benthamiana* was found to be 10–50 mg/mL.

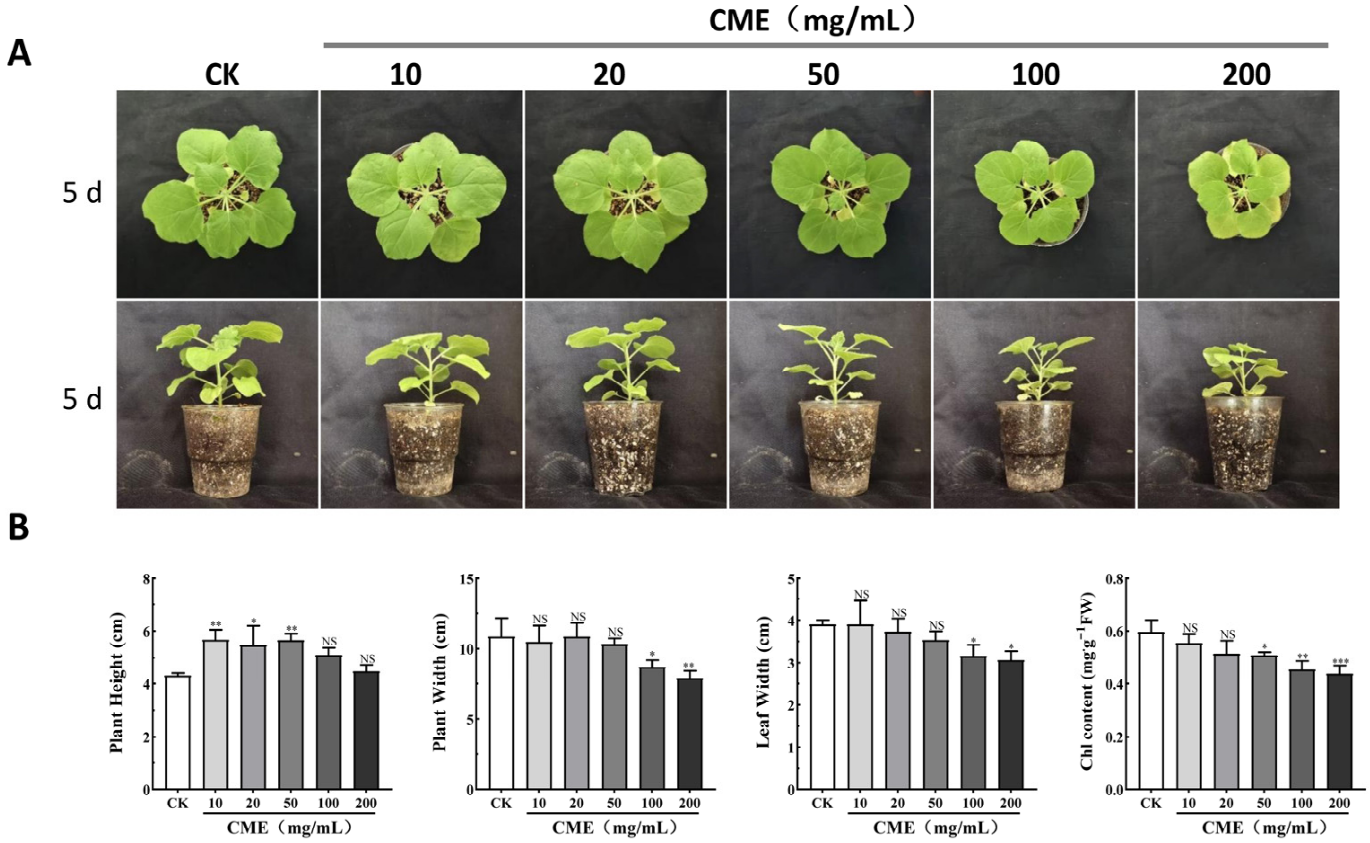

Figure 1. Growth effects of CME spraying on *N. benthamiana*. (**A**) Phenotypes of *N. benthamiana* plants at day 5 following CME treatment. (**B**) Plant height, plant width, leaf width, and chlorophyll content analysis after treatment with the CME for 5 days. "CK" stands for water treatment. Values are given as mean ± standard error (SE) from three biological replicates, with statistical evaluation by one-way analysis followed by LSD test at $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS, not significant).

3.2. CME Exhibited Excellent Antiviral Activity Against TMV

An infection assay using Tobacco mosaic virus expressing green fluorescent protein (TMV-GFP) was conducted in *N. benthamiana* to evaluate the antiviral potential of CME. The CME at a concentration of 50 mg/mL was specifically used for resistance assays. After *N. benthamiana* plants were treated with CME for 5 consecutive days, TMV-GFP was inoculated onto the leaves. The level of TMV-GFP infection was compared at 3 and 7 days after infection (Figure 2A). As shown in Figure 2B, the GFP fluorescence signals in CME-treated inoculated leaves were significantly lower than those in the negative controls at 3 days post-inoculation (dpi). At 7 dpi, significant GFP fluorescence was observed in the upper leaves of the control group, whereas only slightly expanded GFP signals were detected in a few upper leaves of the CME group. Furthermore, accumulation of TMV-GFP was measured using Western blot analysis. Relative to the control treatment, the accumulation of TMV-GFP in CME-treated plants dropped sharply, which was consistent with the fluorescence signals observed under UV light (Figure 2C). These results suggested that the CME provides significant protection against TMV infection in the plants.

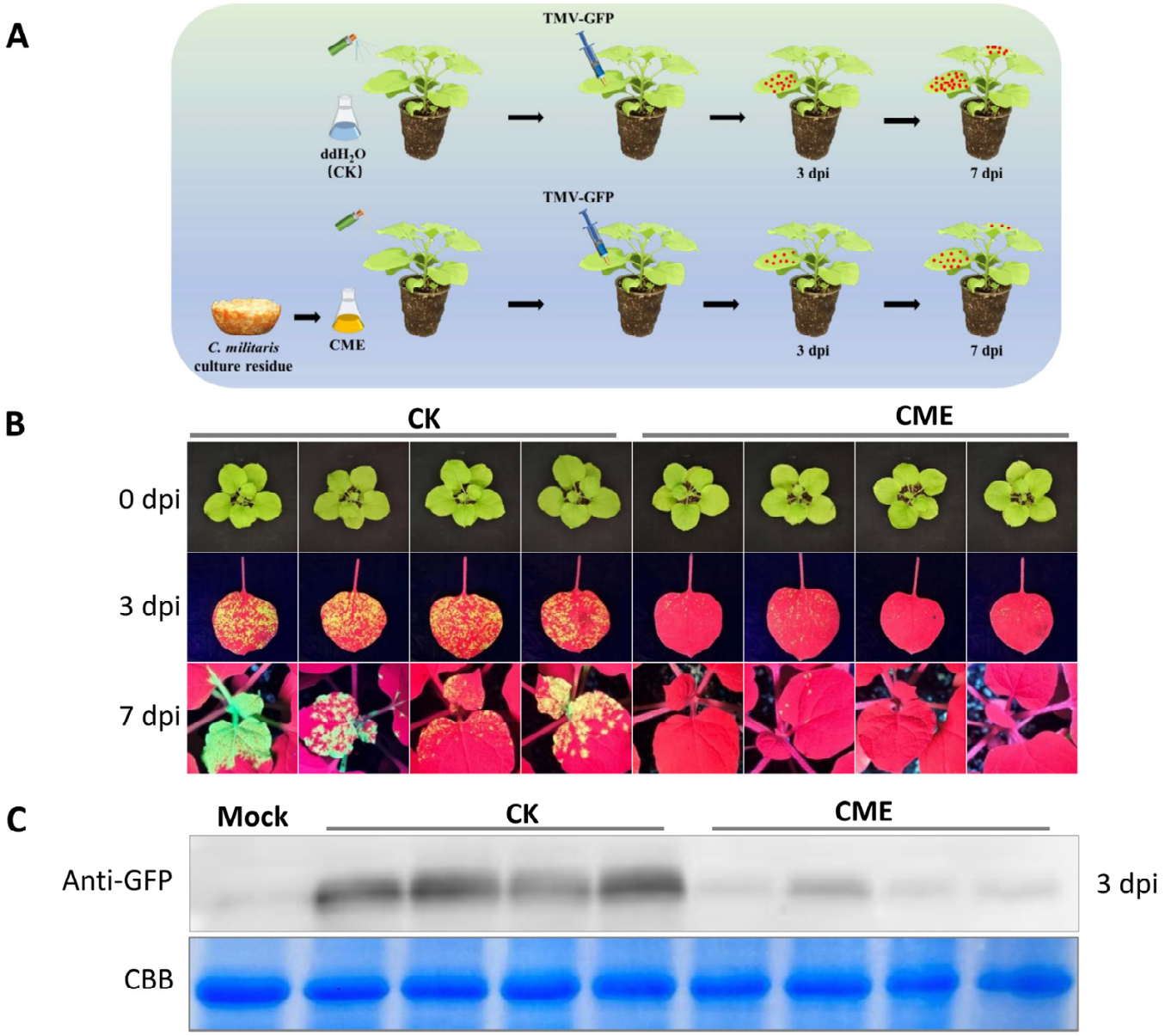

Figure 2. CME treatments inhibit the accumulation and systemic spread of TMV-GFP. (**A**) Detailed workflow for evaluating the preventive effect of CME spraying against TMV-GFP. Red dots indicate the simulated viral infection. (**B**) The infection of TMV-GFP in *N. benthamiana* leaves after 50 mg/mL CME treatment at 3 and 7 dpi. After a 5-day treatment with water and 50 mg/mL CME, *N. benthamiana* leaves were inoculated with TMV-GFP. (**C**) Western blot detection of the TMV-GFP accumulation in the inoculated leaves at 3 dpi. "CK" stands for water treatment. Coomassie brilliant blue (CBB) staining confirmed equal protein loading across all lanes.

3.3. Effects of CME on the Expression of Resistance Genes

Studies on abiotic-induced plant disease resistance have demonstrated that salicylic acid (SA) signaling is crucial for defense responses [30,31]. To test whether CME elicits hormone pathways, the expression levels of SA-responsive genes *PR1* and *PR2*, as well as the SA biosynthesis gene *ICS1*, were quantified through qRT-PCR in CME-treated *N. benthamiana*. The results showed that CME treatment consistently induced the expression of defense-related genes. At both 0 dpi and 3 dpi, the expression levels of *PR1*, *PR2*, and *ICS1* were significantly upregulated in CME-treated leaves compared with the control group, with the most pronounced upregulation observed in *PR1* (13.20-fold before and

11.89-fold after TMV inoculation) (Figure 3). Notably, compared with the pre-inoculation stage, the induction of *ICS1* by CME treatment was markedly enhanced at 3 dpi, indicating that CME-induced *ICS1* expression was substantially amplified under TMV infection conditions. These results suggested that CME can continuously activate the SA signaling pathway mediated by *PR1*, *PR2*, and *ICS1*, thereby enhancing the disease resistance of plants (Figure 3B).

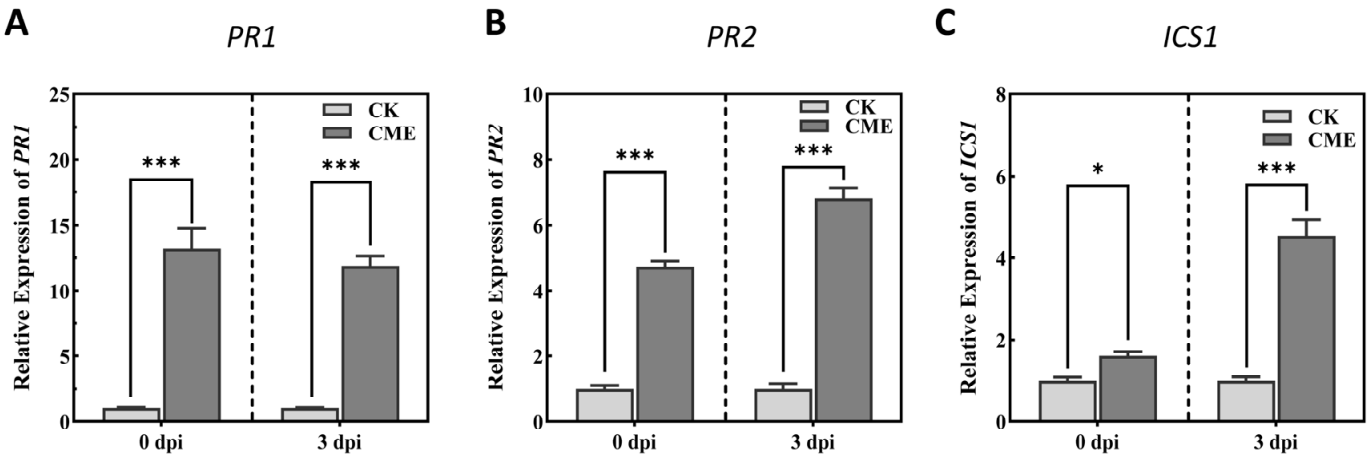

Figure 3. Effects of CME on expression of resistance genes in *N. benthamiana* plants. qRT-PCR analysis of the expression levels of *PR1* (**A**), *PR2* (**B**), and *ICS1* (**C**) in CME-treated *N. benthamiana* leaves with or without TMV-GFP infection. Transcript levels are shown as fold-change values normalized against the actin reference gene. "CK" indicates samples treated with water. Values are given as mean ± standard error (SE) from three biological replicates; statistical significance was determined using the independent-samples *t* test (compared with CK group): * $p < 0.05$, *** $p < 0.001$.

3.4. Growth-Promoting Effect of CME

The plant height, stem diameter, dry weight, fresh weight, chlorophyll content, and carotenoid content of tomato seedlings were measured following five root-irrigation treatments with different concentrations of CME to evaluate whether the CME can stimulate plant growth. The results showed that tomato seedlings under irrigation treatment with 1 or 5 mg/mL CME concentrations exhibited no significant differences from the control group in terms of growth. However, the tomato plants treated with 10 mg/mL CME exhibited significant increases in plant height, stem diameter, dry weight, fresh weight, chlorophyll content, and carotenoid content compared with the control group plants (Figure 4A,B). These findings suggested that 10 mg/mL CME has a growth-promoting effect on tomato plants.

As the raw material of CME contains some oats, an oat extract (OE) treatment group was simultaneously set up, and the effect of 10 mg/mL CME on the growth of *N. benthamiana* was tested through root drenching to determine whether its growth-promoting effects are primarily attributed to metabolites produced during *C. militaris* growth. Water and nutrient solution served as negative and positive controls, respectively. The results revealed that relative to the negative control, CME treatment significantly increased the plant width, leaf width, dry weight, and fresh weight of *N. benthamiana*. However, no significant differences were observed in the growth indices of OE-treated plants compared with the negative control group (Figure 4C,D). The aforementioned results indicated that 10 mg/mL CME has a significant growth-promoting effect on plants, which may primarily be attributed to the metabolites produced during the growth of *C. militaris*.

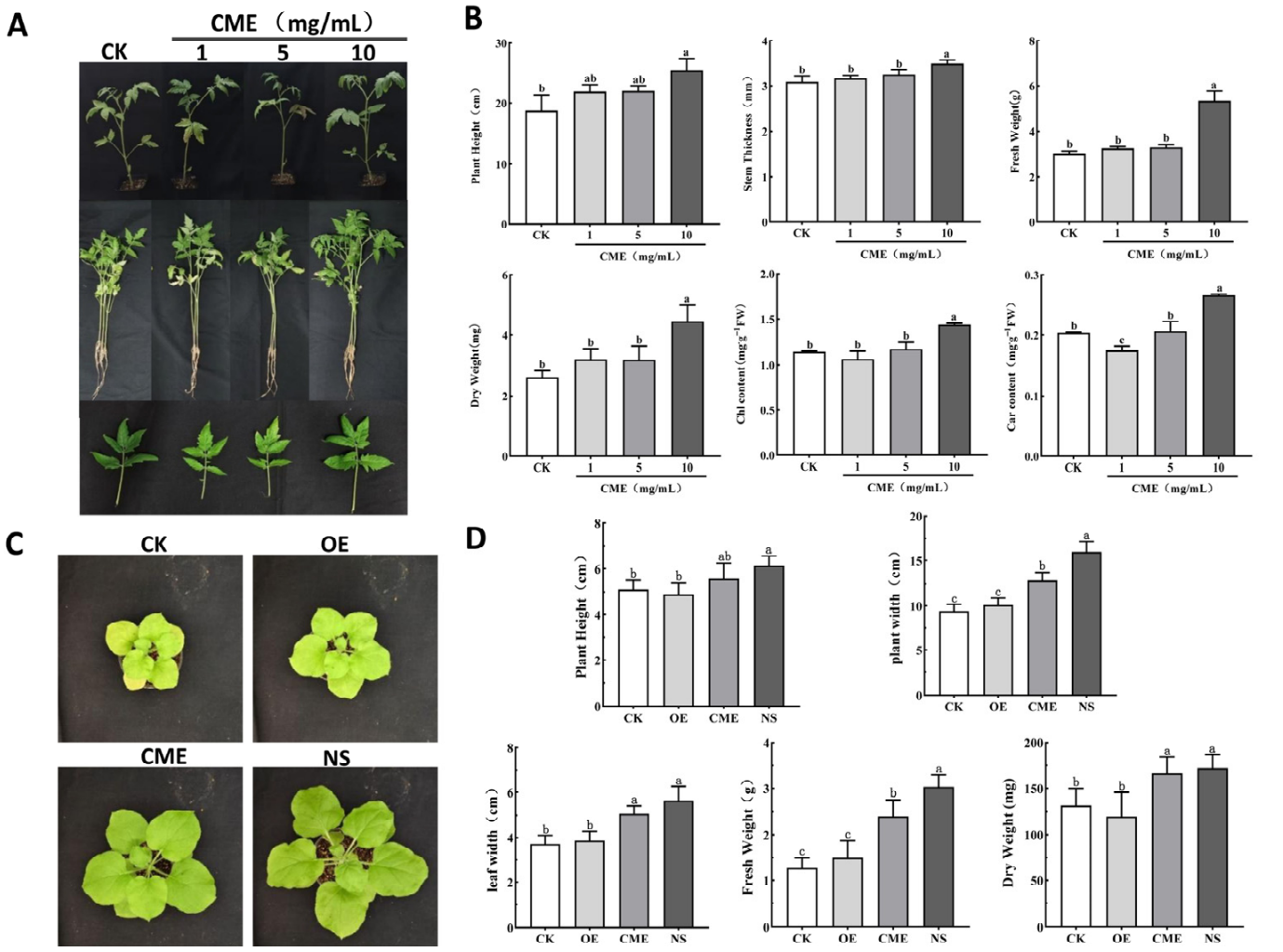

Figure 4. Growth effects of CME root irrigation in tomato and *N. benthamiana*. (**A**) Effects of CME root irrigation on tomato phenotype and growth. (**B**) Plant height, plant thickness, dry weight, fresh weight, chlorophyll content, and carotenoid content analyses after five applications of CME treatment. (**C**) Effects of CME root irrigation on *N. benthamiana* phenotype and growth. (**D**) Plant height, plant width, leaf width, fresh weight, and dry weight analyses after five applications of CME treatment. "CK" stands for water treatment. Values are given as mean ± standard error (SE) from three biological replicates. Different letters indicate statistically significant differences among treatments according to one-way analysis of variance (ANOVA) followed by LSD test at $p < 0.05$.

4. Discussion

Tobacco mosaic disease induced by TMV is a globally significant plant disease that severely constrains crop production [32]. Conventional strategies for managing TMV primarily involve the breeding of disease-resistant cultivars and the application of chemical agents [33]. However, the application of disease-resistant varieties is often limited by the emergence of diverse virus strains, whereas chemical control poses severe environmental pollution issues [34,35]. Consequently, the development of natural immune inducers has emerged as a research focus. In studies on fungus-derived immune inducers, culture filtrates from the endophytic fungus *Epicoccum* spp. were reported to confer resistance to TMV in *N. benthamiana*. Pre-inoculation application significantly inhibited TMV accumulation and reduced infection [36]. Our results showed that spraying *N. benthamiana* with a *C. militaris* cultivation residue extract (CME) at a concentration of 50 mg/mL significantly inhibited viral replication and systemic movement, demonstrating a strong preventive effect against TMV.

Salicylic acid (SA), a plant hormone, plays a crucial role in plant growth and development, abiotic stress resistance, and defense responses [37,38]. The *ICS* pathway is involved in SA biosynthesis, with its contribution varying among plant species. In *Arabidopsis*, 90% of defense-related SA production occurs through the *ICS* pathway [39,40]. The expression levels of *PR1* and *PR2* genes, which are markers of the SA signaling, serve as crucial molecular indicators of multiple genes linked to SAR and are involved in defense responses against various pathogens [41]. The *PR1* and *PR2* were significantly upregulated when plants faced biotic challenges, which are usually accompanied by enhanced resistance of plants to pathogens [42,43]. Recent studies have shown that phytochemicals like berberine and indole derivatives induce antiviral resistance by activating PR proteins and upregulating SA biosynthesis genes such as *PR1* and *ICS* [44,45]. Here, we show that the expression levels of *ICS1*, *PR1*, and *PR2* were markedly elevated in CME-treated tobacco relative to controls, both before and after TMV inoculation. The mechanism by which the CME induces tobacco disease resistance may involve the upregulation of the SA biosynthesis-related gene *ICS1*, which subsequently activates defense responses through *PR1* and *PR2*, to resist pathogen invasion.

Certain plant immune inducers exhibit growth-promoting effects. For instance, nanoparticles such as Chloroinconazide by Alginate-based Nanogel (CHI@ALGNP) and alginate-based ZhiNengCong (ZNC@Alg) not only show significant anti-TMV efficacy but also act as fertilizers to stimulate plant growth [46,47]. Here, we demonstrate that the growth of *N. benthamiana* and tomato seedlings could be promoted by continuous root irrigation with a 10 mg/mL CME solution. Specifically, treatment with the CME significantly increased plant height, fresh weight, dry weight, chlorophyll content, and carotenoid content of tomato seedlings. When oat extract (OE) was set as the control group for root irrigation in *N. benthamiana*, the growth-promoting effect of CME on plants was found to be primarily induced by metabolites produced during the growth of *C. militaris*. These findings establish CME as a promising antiviral agent that simultaneously boosts disease resistance and plant growth.

The anti-TMV activity observed in this study reflects the overall effect of CME as a crude extract derived from *C. militaris* cultivation residues. As a crude extract, the CME is rich in bioactive constituents such as cordycepin, polysaccharides, amino acids, and fungal proteins [27]. Previous studies have demonstrated that diverse fungal polysaccharides and proteins possess significant antiviral activity against TMV [48–50]. Recently, overexpression of the cordycepin biosynthesis genes *Cmcns1/cns2* in tobacco has been shown to enhance resistance to TMV [51]. We have preliminarily determined the concentrations of cordycepin (0.052 mg/mL) and polysaccharides (0.803 mg/mL) in the CME (50 mg/mL). Future work will focus on purifying these active compounds and evaluating the antiviral efficacy of individual components through bioactivity-guided fractionation. Furthermore, as these experiments were limited to controlled environments, the performance of CME under complex field conditions requires further verification. Future research will involve a comprehensive field-based evaluation to determine the practical applicability and environmental fate of CME in agricultural settings.

5. Conclusions

This study demonstrated that CME exhibited excellent antiviral activity, which could prevent tobacco mosaic virus (TMV) infection. The CME may achieve disease resistance by persistently upregulating the expression of resistance genes. These findings indicate the CME as a plant immune inducer that effectively suppresses tobacco viral diseases, thereby providing a scientific basis for field-scale control strategies against tobacco mosaic disease.

Author Contributions: Conceptualization, X.Z. and X.C.; methodology, G.S., F.Z., X.Z. and X.C.; validation, W.L.; formal analysis, F.Z.; investigation, G.S., F.Z., F.S., W.L. and Y.W.; data curation, G.S., F.Z. and X.Z.; writing—original draft preparation, G.S. and F.Z.; writing—review and editing, X.Z. and X.C.; funding acquisition, X.Z. and X.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

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