World Vegetable Center

Studies on Control of Tomato Southern Blight

Wei-Jie, Syu
2022 summer intern/ Mycology Unit

Supervisor
Wallace Chen (Mycology Unit)
Ricardo Oliva (Plant Pathology)

ABSTRACT

Tomato southern blight disease caused by *Scelerotium rolfsii* is very common in tropical and subtropical regions in the world. Using chemical fungicides is not very successful in this plant disease management as the pathogen has not only wide host range but also the ability to produce sclerotia which are able to survive for many years in the soil. As an alternative eco-friendly approach to control the disease, a biocontrol agent (BCA) *Trichoderma* species and a plant defense inducer were used in this study. Four different treatments were applied in the greenhouse environment. Results from this study show neutralized phosphorous acid salts (NPS) treatment and the tomato accession are the main factors to affect the tolerance against the pathogen. Moreover, two trials for testing the effect of BCA show the application time might play an important factor to raise the survival ratio of tomato seedlings. Overall results from the present study may be concluded that choose a tolerant host plant, adding NPS or *Trichoderma* spp. 3 days before the infection could be some useful strategies for tomato southern blight management.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is belonged to the Solanaceae family that includes a large number of economically essential vegetables. It is grown worldwide and consumed in diverse ways, such as dishes, salads, sauces and drinks. Tomato fruit is an abundant source of vitamins, minerals, fibers and various chemicals that provide health and immunity benefits against various human disorders [8, 18, 20]. According to the Food and Agriculture Organization (FAO), tomato is one of the eight main vegetable species accounted for 16 percent of the total vegetable production between 2000 and 2019 [6]. In 2021, 98,340 tonnes of tomatoes was produced by Taiwan, and the top five of tomato producer regions were Chiayi, Kaohsiung, Nantou, Tainan and Yunlin County. The farm prices of production areas was 34.72 NT$ per kilogram [2].

Cultivation of tomato is highly susceptible for many phytopathogens, which resulted in the major yield loss and a notable decrease in fruit quality. Major fungal phytopathogens in tomato are causing Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Verticillium wilt (*Verticillium dahliae*), early blight (*Alternaria solani*), late blights (*Phytophthora infestans*) and southern blight disease (*Scelerotium rolfsii*) [5]. Among all the fungal diseases, southern blight is one of the most devastating disease commonly found in subtropical and tropical regions of the world. It caused by a soil born pathogen *S. rolfsii* infecting over 500 different plant species, including tomato, carrot, cotton, wheat, potato and maize [17]. The fungus persists for many years as sclerotia in soil or hyphae on infected crop debris. Tomato seedling infected by this pathogen shows damping-off symptom, while during the reproduction stage, the infected plant shows stem rot or wilting symptoms [10]. The infection usually occurs at the lower stem near the soil surface and starts with a small, water-soaked lesion. The rapidly developing lesions girdle the stem, resulting in a sudden and permanent wilting symptom within 2-4 days after the infection [14] White mycelium and abundant sclerotia develop on rotting tissues. Environmental conditions that favor the disease development are high temperatures (27 to 35˚C), humid conditions and acidic soil [9].

Today, this pathogen continues to cause considerable economic loss. Control efforts have often met with limited success due to the wide host range and the ability to produce large numbers of sclerotia that may persist in soil for several years. Synthetic chemical fungicides have long been used to reduce the incidence of plant diseases. However, they are costly, can have negative effects on the environment and health, and may induce pathogen resistance. Moreover, it is very difficult to protect tomato plants by fungicides application when plant foliage is expanded and dense and covers the crown zone where the infection usually begins [4]. Therefore, in order to reduce energy costs in farming and develop more eco-friendly control methods, research aimed at finding new and effective methods for controlling *S. rolfsii* are necessary.

Consequently, biological control, including the use of microorganisms or their antibiotics, offers an alternative or supplement to pesticides for the management of plant diseases without the negative impact of chemical control [21]. *Trichoderma* spp. are one of the most important fungi common in soil and plant roots and can be used as an effective biocontrol agents for soil borne fungal plant pathogens and some species are also known for their abilities to control plant diseases [7]. Darvin et al. [3] recorded 56.25 percent mycelial growth inhibition of *S. rolfsii* by *T. harzianum* through dual culture technique and also recorded complete inhibition of *S. rolfsii* by *T. viride* isolate through poisoned food technique. Besides, there are several chemical and biological compounds, known as resistance inducers, can induce plant defense response. Salts of phosphorous acid, phosphite (Phi), are generally applied as a pesticide, fertilizer, and biostimulator to boost nutrient absorption and assimilation as well as increase biotic and abiotic stress tolerance[1]. It can also directly inhibit oxidative phosphorylation of pathogen metabolism [12, 15]. Phi are less harmful to the environment and can be applied to prevent plant pathogens caused by oomycetes, particularly *Peronospora*, *Plasmopara*, *Phytophthora*, and *Pythium* genus [13].

The objective of this report is to determine what kind of treatment could be applied in different tomato species. Meanwhile, the use of isolated strategies may have low impact, for that reason, it is necessary to evaluate the effectiveness of integrated management strategies for controlling tomato damping-off disease. The result of this study could be useful to suggest a broader hypothesis for planning future agricultural practice in greenhouse environment.

MATERIALS AND METHODS

Tested plants preparation

Seven tomato cultivars were used for the experiment (Table 1). Each germinating seed was planted in a plastic pot containing Stender peat substrate (Stender AG., Germany) (**Fig. 1**). Every cultivar of the tomato seedlings were divided into 4 groups consisted of 72 pots and three repetitions for each treatment were used. The first group of pots received both biocontrol agent and phosphorous acid treatments, the second group received biocontrol agent treatment only, and the third group received phosphorous acid treatment only. The fourth group of pots was left untreated and served as control. All treatments were arranged on the greenhouse bench in a randomized complete block design. Plant materials were maintained by standard cultural practice. Fourteen-days-old tomato seedlings were used for in vivo bioassay experiment.

Pathogen used and preparation of inocula

Inoculum of *Sclerotium rolfsii* (Sr-92, **Fig. 2**) was prepared by taking 30 g rye grain with 25 ml sterile water in a 250 ml conical flask. After covering the mouth with filter paper and aluminum foil, the conical flasks were autoclaved at 121°C and 1.5 kg/cm^2 for 30 min and allowed to cool on a clean bench. The grains were inoculated with 10 mycelial disks (8 mm diameter) obtained from the actively growing margin of 5-day-old potato-dextrose-agar (PDA; 0.04 % potato infusion, 2 % dextrose, 2 % agar) cultures of Sr-92. The inoculated flasks were incubated at 28°C with alternating 12 h period of darkness and white light for 1 week and shaken daily to allow for consistent growth of *S. rolfsii*. The culture continued until the fungus thoroughly colonized the surface of rye grains. Artificial infestation was accomplished by adding 3 rye grains into soil of each pot and placed 5 mm apart from each 2-week-old tomato seedling.

Effect of biocontrol agents on *S. rolfsii*

Based on the results of previous tests in greenhouse (un-published data), one promising *Trichoderma* isolate (Tm-212, **Fig. 3**) were selected for the present study. The Tm-212 isolate was first cultured on PDA and incubated at 28˚C for 5 days. The fungal spores were harvested from the culture surfaces by flooding with sterile distilled water and gently scraping the colony surface with a sterile loop. The density of the spore suspension (10^7 spores/ml) were made with sterilized distilled water and calculated by the hemocytometer. Ten milliliters of the suspension was placed into the soil of each pot. This experiment was replicated two times, the suspension was added respectively 3 days before and 3 hours after the Sr-92 inoculation.

Efficacy of phosphorous acid against tomato southern blight

Special grade phosphorous acid (≥ 97.5 % H_3PO_3, white crystal, Kanto Chemical Co., Japan) and special grade potassium hydroxide (≥ 86 % KOH, white, thin piece, Kanto Chemical Co., Japan) were used for reagent preparation. To prepare a neutralized phosphorous acid solution (NPA), an equal weight of potassium hydroxide was added to the phosphorous acid water solution to produce the end concentration of phosphorous ion at 1000 mg/L. Because potassium hydroxide releases heat in the dissolving process whereas phosphorous acid absorbs heat, for the safe reason in further use, the phosphorous acid was first dissolved in water before directly adding equal weights of potassium hydroxide to the phosphorous acid solution. The NPA solution was applied to the tested plants within few hours to avoid oxidation of phosphorous ion in the air. Ten milliliters of the NPA solution was added into the soil of each pot 3 days before the Sr-92 inoculation.

Statistical analysis

Disease incidence was recorded daily for up to 7 days by counting the numbers of wilted and dead seedlings after the plant disease pathogen inoculation. The damping-off incidence on tomato seedlings in each treatment were calculated by following formula.

$$\textit{Disease incidence (\%)} = \frac{Amount of infected plant \in treatment}{Total amount of plant \in treatment} x\ 100$$

Data were analyzed by analysis of variance (ANOVA), and treatments means were compared by least significant difference test (LSD) at $P = 0.05$.

RESULTS

Disease development

Figure 4 displays the disease development within 3 days after inoculating Sr-92 in soil. The rye grain was used as the inocula and transferred from conical flasks to soil, it had its brown color appearance at first (**Fig. 4A**). After 24 hours later, white mycelia began to grow from the inoculation site (**Fig. 4B**), and the damping-off symptom only appeared on a few seedlings (**Fig. 4C**). After 48 hours later, more and more seedlings started showing the wilting symptoms (**Fig. 4D**). After 72 hours later, most susceptible host plant showed severe disease symptoms in the greenhouse (**Fig. 4E**). Moreover, the diameter of the basal part of infected plants usually became smaller compared to the normal stem (**Fig. 4F**). And after a whole week later, healthy plants and infected plants could be distinguished easily via their appearance (**Fig. 5A**), the healthy plants usually had more true leaves, larger leaf area, and were taller than the infected plants. Also, putting plants into a glass of water could find that healthy plant had larger root system (**Fig. 5B-C**).

First trial

Results of the first trials are presented in Figure 6. These line graphs show the changing of survival ratio (%) in each kind of accession for one week after the pathogen inoculation. About the pots only treated by BCA (green lines) and the pots which were untreated (purple lines), the survival ratio usually drop rapidly from day 1 to day 3, then became stable during the other 4 days. This development tendency could be seen on the other two kinds of treatment, too. Hence, the symptoms often showed in 4 days after the inoculation. Also, it is clear that the pot which was added both BCA and NPS (red lines), and the pot that was added only NPS (blue lines) always showed higher survival ratio than the other two kinds of treatments.

Second trial

The only difference between the first trial and the second trial was the time adding the BCA treatments. The green lines in Figure 7 shows the effect of adding biocontrol agent into soil 3 days before the pathogen inoculation, and it had a significant increase compared to the green lines in Figure 6. Besides, all of the observations mentioned in the last paragraph could also be found in the second trial.

DISCUSSION

Integrated pest management has been used for plant disease control in recent years, the combination of different kinds of strategies might lead to a greater effect resisting the biotic stress in agriculture. The findings, however, go against this original hypothesis. The treatments which includes NPS might be more effective than the other treatment without adding NPS. Some researchers [(11)] found that NPS can directly inhibit fungal mycelium growth, and invoke the plant defense system at the same time, so it has both direct and indirect pathway to raise the plant disease resistance. In this study, if the pot had already added NPS, adding extra Tm-212 suspension showed barely increase on the seedling survival ratio.

Another implication of the results from this study is that Trichoderma spp. should be added before the infection happens, based on the observation from the second trial. One possible explanation is that the effective pathogen control may require 10^5-10^6 conidia in one gram of the soil [(19)], so adding this biocontrol agent 3 or 4 days before the pathogen inoculation could ensure Trichoderma spp. have enough time to colonize in rhizosphere of the host plants.

The results of the first trial and the second trail indicate that adding different kinds of treatment leads to different increase level of the seedling survival ratio. Meanwhile, the effect of the same treatment also had an obvious difference among all tomato accessions. These results can be explained by assuming that treatment factor might interact with the accession factor in this study. However, it cannot explain the reason that made the untreated plant show little consistency between the first trial and the second trial. Checking the temperature and the humidity record of the greenhouse during the experimental process might gain some useful information. Increase the sample size or the total number of repetitions might be another way to make sure the difference between two trials is not because of the technical error made by experiment operators.

The study reported in the present paper demonstrate some important findings about making tomato seedling show more tolerance to the tomato southern blight pathogen. *Sclerotium rolfsii* has the ability to produce oxalic acid, polygalacturonase and cellulase to degrade plant cell wall [(16)], future research may try to find more promising combinations of integrated strategies for disease control based on the interaction between the host plant and the pathogen.

ACKNOWLEDGMENT

This study is supported by the World Vegetable Center. The author thank Ricardo Oliva and Wallace Chen for reviewing the manuscript, Ya-Ping Lin for assistance of statistical analysis, and the mycology lab as well as greenhouse of WorldVeg for providing the necessary facilities during the investigation.

REFERENCE

1. Ávila F. W., Faquin V., Da Silva, Lobato A. K., Ávila P. A., Marques D. J., et al. (2013). Effect of phosphite supply in nutrient solution on yield, phosphorus nutrition and enzymatic behavior in common bean (*Phaseolus vulgaris* L.) plants. Aust. J. Crop Sci. 7 713–722.
2. Agriculture and Food Agency, COA, Executive Yuan. AG. STATISTICS YEARBOOK 2021.
3. Darvin, G., Venkatesh, I. and Reddy, N. G. (2013). Evaluation of *Trichoderma* spp. against *Sclerotium rolfsii* in vitro. International Journalof Applied Biology and Pharmaceutical Technology. 4(4): 268-272.
4. De Curtis, F., Lima, G., Vitullo, D., & De Cicco, V. (2010). Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. Crop Protection, 29(7), 663-670.
5. Dixit, R., Agrawal, L., Gupta, S., Kumar, M., Yadav, S., Chauhan, P. S., & Nautiyal, C. S. (2016). Southern blight disease of tomato control by 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing *Paenibacillus lentimorbus* B-30488. Plant signaling & behavior, 11(2), e1113363.
6. FAO. 2021. World Food and Agriculture-Statistical Yearbook 2021. Rome.
7. Gajera, H.P.; Savaliya D.D.; Patel, S.V. and Goalkiya, B.A. (2015). *Trichoderma viride* induces pathogenesis related defense response against rot pathogen infection in groundnut (*Arachis hypogaea* L.). Infect Genet Evol. 34: 314-325.
8. Hwang ES, Bowen PE. (2002). Can the consumption of tomatoes or lycopene reduce cancer risk? Integr Cancer Ther1:121-132.
9. Kwon JH, Chi TTP, Park CS. (2009) Occurrence of fruit rot of melon caused by *Sclerotium rolfsii* in Korea. Mycobiol, 37:158-9.
10. Kwon, J. H., & Park, C. S. (2002). Stem rot of tomato caused by *Sclerotium rolfsii* in Korea. Mycobiology, 30(4), 244-246.
11. Lin, C. Y., Chen, K. S., Hwang, Y. S., Lo, H. L., & Hong, C. F. (2016). Effect of neutralized phosphorus acid on powdery mildew and fruit quality of muskmelon (Cucumis melo L.) in greenhouse. Taiwan agricultural research, 65(3), 261-268.
12. Lobato M., Olivieri F., Altamiranda E. G., Wolski E., Daleo G., Caldiz D., et al. (2008). Phosphite compounds reduce disease severity in potato seed tubers and foliage. Eur. J. Plant Pathol. 122 349–358.
13. Mohammadi, M. A., Cheng, Y., Aslam, M., Jakada, B. H., Wai, M. H., Ye, K & Qin, Y. (2021). ROS and oxidative response systems in plants under biotic and abiotic stresses: revisiting the crucial role of phosphite triggered plants defense response. Frontiers in Microbiology, 12.
14. Mullen J. (2001) Southern blight, Southern stem blight, White mold. The Plant Health Instructor
15. Norton G. W., Swinton S. M. (2018). “Precision agriculture: global prospects and environmental implications,” in Proceedings of the Twenty-fouth International Conference of Agricultural Economists: Incentives, Institutions, Infrastructure and Innovations.
16. Punja, Z. K., Huang, J. S., & Jenkins, S. F. (1985). Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence in Sclerotium rolfsii. Canadian Journal of plant pathology, 7(2), 109-117.
17. Rangarani, A., Rajan, C. P. D., Harathi, P. N., Bhaskar, B., & Sandhya, Y. (2017). Evaluation of fungicides and heribicides on *Sclerotium rolfsii*, incitant of stem rot diseases in groundnut (*Arachis hypogeal* L.). International Journal of Pure & Applied Bioscience, 5(3), 92–97.
18. Silaste ML, Alfthan G, Aro A. (2007). Tomato juice decreases LDL cholesterol levels and increases LDL resis#tance to oxidation. Br J Nutr; 98:1251-1258.
19. Singh, R., Maurya, S., & Upadhyay, R. S. (2016). The improvement of competitive saprophytic capabilities of Trichoderma species through the use of chemical mutagens. brazilian journal of microbiology, 47, 10-17.

20. Sultana, F., & Hossain, M. M. (2022). Assessing the potentials of bacterial antagonists for plant growth promotion, nutrient acquisition, and biological control of Southern blight disease in tomato. PloS one, 17(6), e0267253.
21. Wang SL, Yieh TC, Shih IL. (1999). Production of antifungal compounds by *Pseudomonas aeruginosa* K-187 using shrimp and crab shell powder as a carbon source. Enzyme Microbiology Technology; 25:142-148.

APPENDIX

Table 1. The list of the tested tomato plants

Scientific name	Vegetable introduction number	Place of origin
S. pennellii	VI030801	Mexico
S. chilense	VI031800	Chile
S. lycopersicum	Ts19	-
	VI009462	Mexico
	VI009488	Philippines
	VI030369	Brazil
	VI030379	Mauritius

Figure 1. Host plants preparation. (A) The environment of the greenhouse. (B) Sow the tomato seeds into soil, the number of tomato seeds in each pot depends on the germination rate of each accession. (C) Two-week-old seedlings will be used as the tested plant materials.

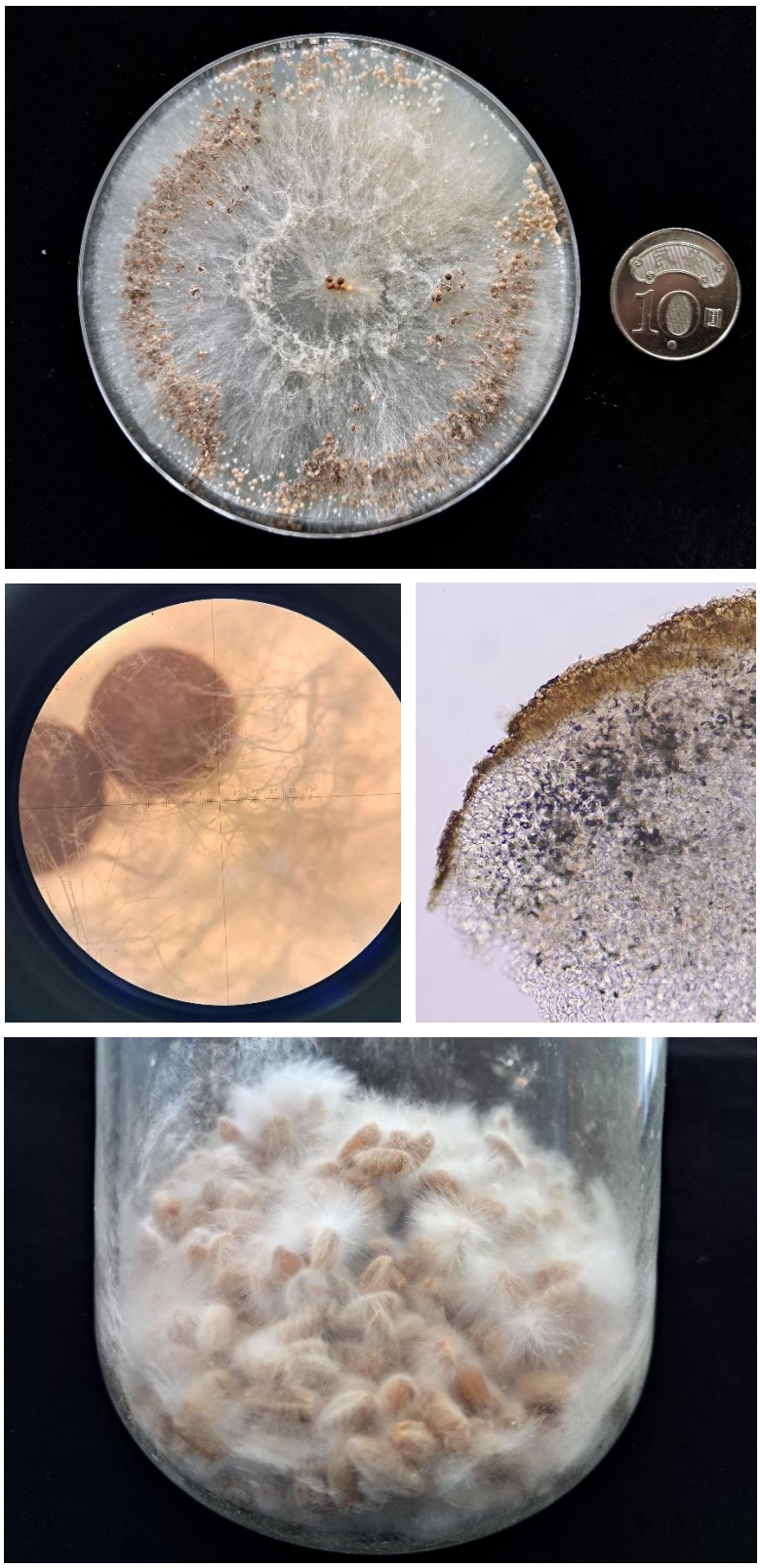

Figure 2. Pathogen inoculum preparation. (A) The colony of *Sclerotium rolfsii* (PDA, 5d). (B) The fungal mycelium colonized, forming sclerotia on the PDA medium. (C) Light microscope photograph of sclerotia which is composed of the rind layer and the medulla. (D) Seven day-old rye grain medium could be used as the inoculum material.

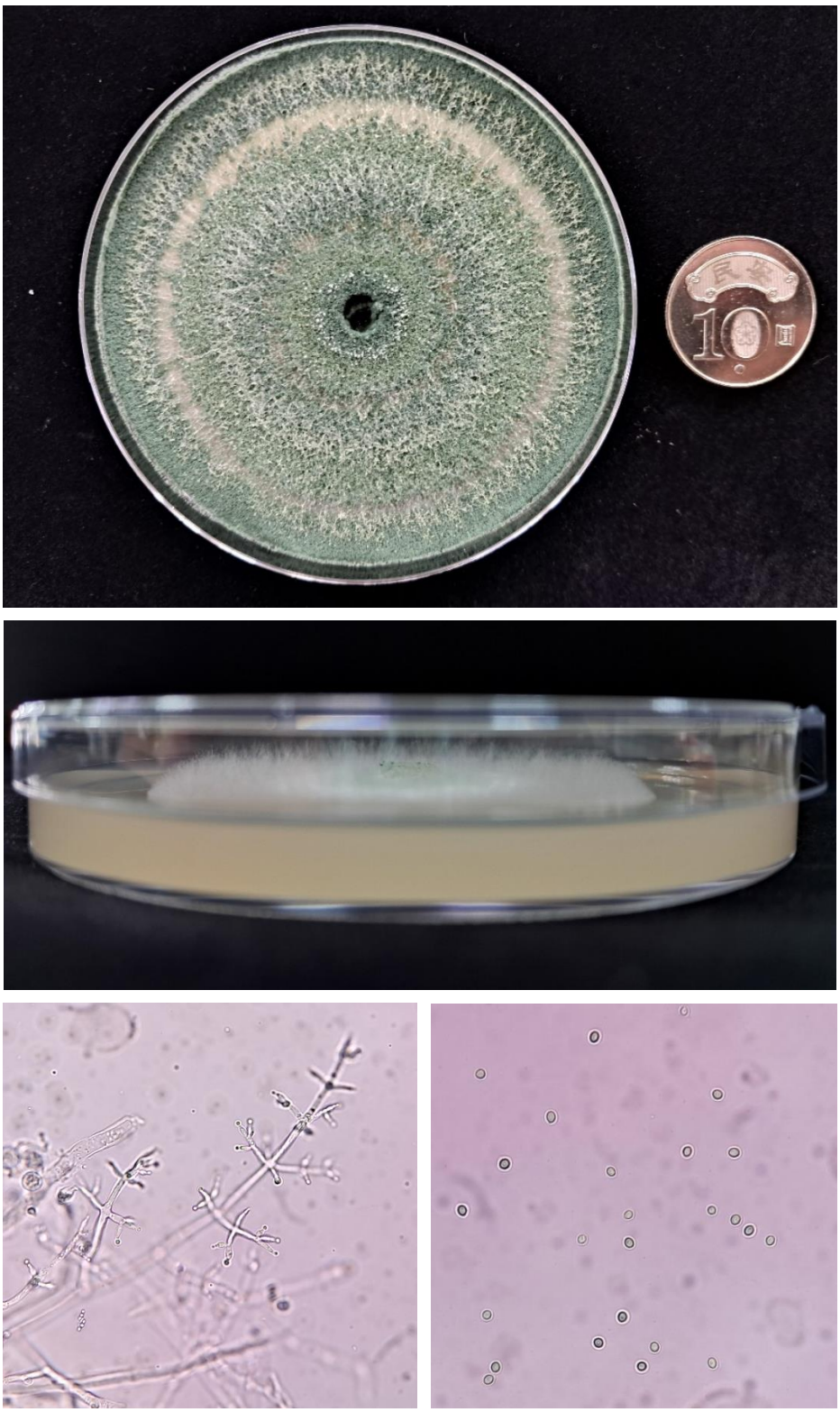

Figure 3. Biocontrol agent preparation. (A) The colony of *Trichoderma* spp. (PDA, 5d). (B) Aerial mycelium of Tm-212 (PDA, 4d). (C) Conidiophores and phialides. (D) Conidia.

Figure 4. Disease development. (A) Rye grain maintained its brown color appearance (0 dpi). (B) White mycelium grew on soil surface (1 dpi). (C) A few seedlings began to show damping-off symptoms (1 dpi). (D) More and more infected plants showed wilting symptoms (2 dpi). (E) Many susceptible plants showed severe symptoms. (F) The basal part of the infected plant became thinner compared to the normal stem near the soil surface.

Figure 5. The comparison between the healthy plants and the infected plants. (A) The whole plant height of the healthy plant (left) and the infected plant (right). (B) The root part of the healthy plant. (C) The root part of the infected plant.

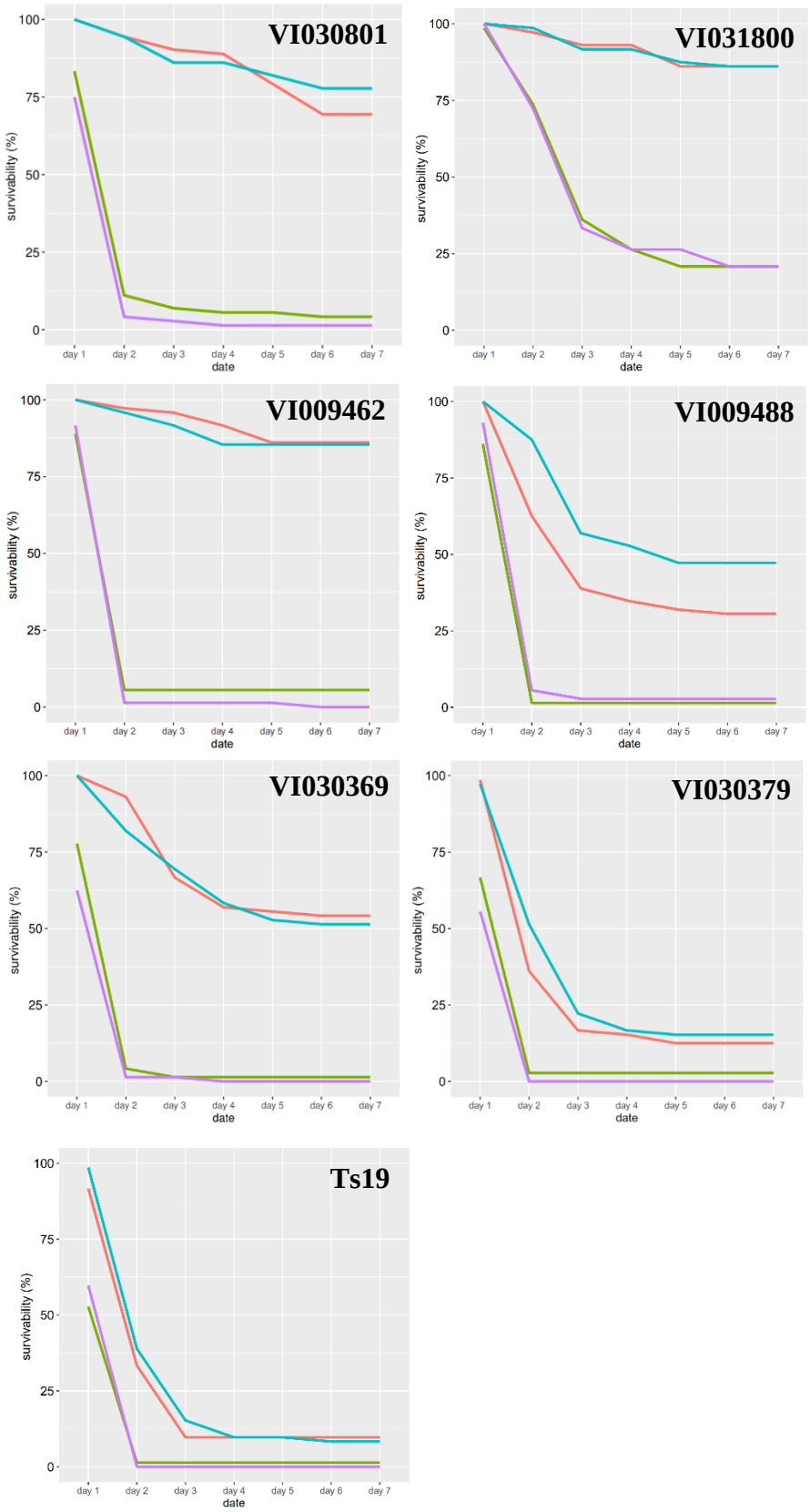

Figure 6. The change of host plant survival ratio (%) in one week after the pathogen inoculation (first trial). Red, green, blue and purple lines are represented to Tm-212 with NPS, only Tm-212, only NPS and untreated (control), respectively.

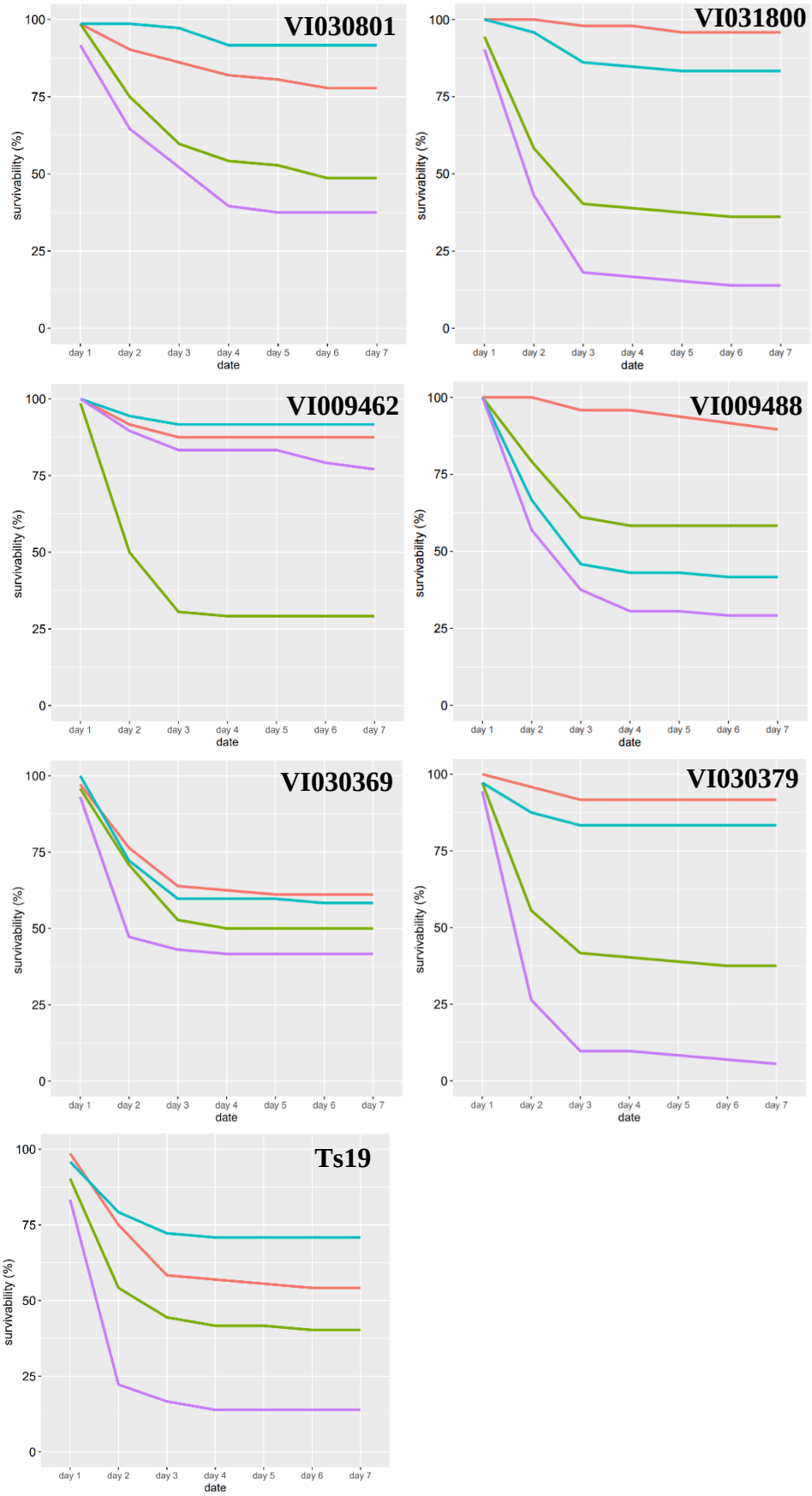

Figure 7. The change of host plant survival ratio (%) in one week after the pathogen inoculation (second trial). Red, green, blue and purple lines are represented to Tm-212 with NPS, only Tm-212, only NPS and untreated (control), respectively.