

***In vitro* and field responses of various active ingredients to *Fusarium solani*, which causes stem and root rot disease in balloon flower (*Platycodon grandiflorus*)**

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Abstract

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The balloon flower (*Platycodon grandiflorus*) production faces significant challenges due to stem and root rot disease (SRRD) caused by *Fusarium solani*. This study aims to evaluate the efficacy of fungicides on *F. solani* *in vitro* conditions, determine the effectiveness of soil treatment with bio-products before planting, and investigate their efficacies in controlling SRRD in field conditions. When bio-products were applied for soil treatment before planting, Biobac 50WP and Mocabi SL resulted in preventive effectiveness of 60.84% and 53.36%, respectively, and higher than other bio-products, which only ranging from 14.95% (Biogreen 4.5SL) to 41.14% (Ketomium). When used to control SRRD, their efficacies ranged from 34.94% to 50.84% and from 24.10% to 60.00% with Biogreen 4.5SL and Biobac 50WP two weeks and four weeks after treatment, respectively. Interestingly, Biobac 50WP sustained the highest efficacy compared with other bio-products. These results suggested that Biobac 50WP is a promising bio-product for soil treatment before planting and for controlling SRRD. *In vitro* conditions, tested fungicides demonstrated different efficacies on the mycelium growth of *F. solani* CCBHVN. Among them, mancozeb, fosetyl-aluminum, hexaconazole, and mancozeb + metalaxyl exhibited over 70% inhibition efficacy at a concentration of 1.000 ppm three days after treatment (DAT), decreased after 7 DAT. Notably, fosetyl-aluminum maintained high efficacy, exceeding 80% at 5 DAT, and sustained over 75% efficacy after 7 DAT. When they were applied to spray in the field condition, their efficacies ranged from 53.55% (metalaxyl) to 78.51% (fosetyl-aluminum) two weeks after the second treatment and from 39.27% (ningnanmycin) to 72.43% (fosetyl-aluminum) four weeks after the second treatment. These results indicated that fosetyl-aluminum is the best fungicide controlling SRRD in field conditions. Taken together, soil treatment before planting with Biobac 50WP and spraying Biobac 50WP or fosetyl-aluminum are effective measures for sustainable SRRD management in balloon flower cultivation.

Keywords: Balloon flower; *Fusarium solani*; *Platycodon grandiflorus*; bio-products; chemical fungicides

Introduction

The balloon flower (*Platycodon grandiflorus*) belongs to the bellflower (Campanulaceae) and is native to East Asia (China, Korea, Japan, Russian Far East, and East Siberia). However, there have been many challenges, with signifi-

cant damage caused by diseases. Stem and root rot disease, caused by *Fusarium solani*, has emerged as the most economical factor that caused severe damage, with incidence ranging from 30–50%; in some locations, it has been up to 80%. This disease caused seedling death, loss of plant density, and reduced yield and quality of tubers (Hieu et al.,

2023a). In addition, anthracnose disease (caused by *Colletotrichum incanum*) causes stem and branch death, leading to lower yield and quality of harvesting products (Hieu et al., 2023b).

Chemical fungicides have significantly suppressed disease, but the overuse and continuous use of the same group of chemical fungicides is the cause of the development of pathogen resistance, harmful effects on humans, and environmental pollution. Especially for medicinal plants like balloon flower in this study that are directly applied to humans. Therefore, bio-pesticides are promising due to their cost-effectiveness and environmentally friendly measures, especially human safety (Nazir et al., 2022). Antagonistic microorganisms are vital in controlling various plant diseases (Haas & Défago, 2005; Siddiqui, 2006) and promoting plant growth (Kloepper et al., 1980). In addition, cultivation techniques may help to improve the quality of soil health and to induce inappropriate environments for the development of diseases. However, in cases in which biocontrol and cultivation measures have not been applied from the beginning of the planting season and under the high pressure of diseases, chemical fungicides are necessary to significantly eliminate the inoculum in the field and protect the plants against diseases. In the case of balloon flower in our study, it takes 18 months from planting to harvesting; therefore, using chemical fungicides is acceptable because we have enough time to completely degrade the chemical fungicides in plants and soils.

Following the results of that research, we conducted several experiments to prevent pathogens *in vitro* conditions and against diseases in field conditions. The objectives of this study were to manage stem and root rot disease of balloon flower in Vietnam.

Materials and Methods

The antifungal efficacy of different fungicides on F. solani

The study assessed the antifungal efficacy of the mycelial growth of *F. solani* CCBHVN isolate using the PDA plate method of 6 fungicides with five replications; one Petri dish was used as a replication. They were Daiman 80WP (Active ingredient (a.i): Mancozeb (min 85%), Truong Thinh Co., Ltd.), Aliette 80WP (a.i: Fosetyl-aluminum (min 95%), Bayer Vietnam co., Ltd (BVL), Anvil 5SC (a.i: Hexaconazole (min 85%), Syngenta Viet Nam Co., Ltd), Vilaxyl 35WP (a.i: Metalaxyl (min 95%), Vietnam Antiseptic Joint Stock Company), Ridomil Gold 68WG (a.i: Mancozeb 640 g/kg + Metalaxyl-M 40 g/kg, Syngenta Viet Nam Co., Ltd), Bonny 4SL (a.i: Ningnanmycin, HAI Agro-

chemical Joint Stock Company), Treatment No.7: Control (distilled water).

The concentrations of each product were employed at 100, 500, and 1.000 ppm. For each fungicide concentration, it was added to PDA medium plates at temperatures ranging from 45-50°C. Once solidified, a 5-mm mycelial disc from *F. solani* CCBHVN isolate, which was reported to be the causal pathogen of the stem and root rot disease of balloon flower (Hieu et al., 2023a), cultivated for five days, was placed at the center of the Petri dish, ensuring direct contact with the medium supplemented with the respective fungicide. The Petri dishes were incubated at 28°C until the control dish reached maximum growth. The percentage of inhibition was calculated using the formula:

$$I(\%) = \frac{c - t}{c},$$

where: $I(\%)$ is the percentage of reduction in fungal growth; c is the diameter growth of the colony (cm) in control; and t is the diameter growth of the colony (cm) in the treatment.

The effects of soil treatment with bio-products on stem and root rot disease in the field conditions

The experiment consisted of 6 bio-products with three replications, 30 m² per plot, arranged in a complete randomized block design. They were Promot Plus WP (a.i: *Trichoderma* spp., Saigon Plant Protection Joint Stock Company), Biobac 50WP (a.i: *Bacillus subtilis*, Bion Tech Inc), Ketomium 1.5×10⁶ c.f.u/g power (a.i: *Chaetomium cupreum*), Actinovate 1 SP (a.i: *Streptomyces lydicus* WYEC 108, Biotechnology Application Co. Ltd), Biogreen 4.5SL (a.i: Chitosan, Thai Nam Viet Technology – Biochemistry Co., Ltd) and Mocabi SL (a.i: *Chaetomium* sp. 1.5×10⁶ CFU/ml + *Trichoderma* sp. 1.2×10⁴ CFU/ml, Nong Sinh Co., Ltd.) at the concentration 0.1%. The control was applied the same way with fresh water. Balloon flower were planted 15 days after treatment at a density of 250.000 plants/ha with 2 t of biofertilizers + 270 kg N + 200 kg P₂O₅ + 120 kg K₂O/ha. The disease severity was recorded on 0 to 9 visual scales in which 0 was no symptoms, level 1 leaves turned yellow and fell lower than 10%, level 3 leaves turned yellow and fell from 10% to 25%, level 5 leaves turned yellow and fall from 25% to 50%, level 7 leaves turn yellow and fall higher 50%, level 9 plants dry and die.

$$\text{Disease severity (\%)} = \frac{\text{Sum of all ratings}}{\text{Total number of ratings} \times 9} \times 100$$

The efficacy was calculated based on disease severity using the Abbott formula.

$$\text{Efficacy (\%)} = \left(1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment}}\right) \times 100$$

where: n = disease severity (%), T = treated, Co = control.

Efficacy of bio-products on stem and root rot disease in the field conditions

The experiment was conducted in the Bac Ha District of Lao Cai Province. The experiment consisted of seven treatments: No bio-product treatment (control) and six bio-products as indicated in the soil treatment experiment. Each treatment consisted of three plots as three replications, totaling 21 plots. Each plot size was 30 m² (Plot's dimensions: 5 × 6 m). The balloon flower plant was planted at a density of 250,000 plants/ha with 2 t of biofertilizers + 270 kg N + 200 kg P₂O₅ + 120 kg K₂O/ha. The experiment was conducted when stem and root rot appeared with a disease incidence of about 5%.

The bio-products were prepared in fresh water and applied directly to the soil surrounding the plant twice with seven days intervals at the 0.1% concentration when disease symptoms appeared in about 5% of the plants under natural infection. Disease severity (%) was recorded before, 2, and 4 weeks after treatment. The efficacy was calculated based on disease severity using the Henderson-Tilton formula.

$$\text{Efficacy (\%)} = \left(1 - \frac{n \text{ in } Co \text{ before treatment} \times n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment} \times n \text{ in } T \text{ before treatment}}\right) \times 100$$

where: n = disease severity (%), T = treated, Co = control.

Efficacy of chemical fungicides on stem and root rot disease in the field conditions

This experiment was similar to the bio-products experiment, except that all the bio-products were replaced with chemical fungicides used in the *in vitro* analysis. Disease severity (%) was recorded before, 2, and 4 weeks after treatment. The efficacy was calculated based on disease severity using the Henderson-Tilton formula.

Data analysis

All optimization studies were carried out, and the data was analyzed using single-factor analysis of variance (ANOVA). The data were all summarized as mean mean±SD (standard deviation). Mean separation was performed using the Duncan test at $P = 0.05$ whenever a significant ANOVA ($P < 0.05$) result occurred.

Results and Discussion

Efficacy of chemical fungicides on *F. solani* CCBHVN mycelial growth in vitro conditions

In vitro conditions, all a.i. inhibited the growth of fungal mycelium with differences between a.i. and concentrations

used. Among the tested a.i., only mancozeb, fosetyl-aluminum, hexaconazole, and mancozeb+metalaxyl have an inhibition efficacy of over 70% at a concentration of 1,000 ppm three days after treatment (DAT). The efficacy of these active ingredients (a.i.) gradually decreased after 7 DAT, except the fosetyl-aluminum reached a maximum of 5 DAT and maintained a high efficacy of over 75% after 7 DAT (Figure 1).

Effect of soil treatment with bio-products before planting on stem and root rot disease in the field conditions

Among the treatments, Biobac 50WP (*Bacillus subtilis*) and Mocabi SL (*Chaetomium* sp. + *Trichoderma* sp.) exhibited the most promising results in reducing the severity of stem and root rot disease compared with control (non-treatment). They showed disease severity of 11.63% and 13.85%, with corresponding effectiveness of 60.84% and 53.36% eight months after treatment, respectively. Other bio-products showed lower effectiveness. The disease severity ranged from 17.48% (with Ketomium (*Chaetomium cupreum*)) to 25.26% (with (with Biogreen 4.5SL (Chitosan)), resulting in effectiveness of 41.14% and 14.95%, respectively. Meanwhile, the disease severity in the control (water) was 29.70%.

This indicates the importance of applying soil treatments with Biobac 50WP (*Bacillus subtilis*) or Mocabi SL (*Chaetomium* sp. + *Trichoderma* sp.) 15 days before planting to prevent *F. solani* inoculum in the soil, protect young plants, and effectively reduce stem and root rot disease in balloon flower (Table 1).

Efficacy of bio-products on stem and root rot disease in the field conditions

Among the treatments, Biobac 50WP (*Bacillus subtilis*) exhibited the highest efficacy with disease severities of 3.11% and 3.63% with corresponding efficacies of 50.84% and 60.00% at two weeks and four weeks after the second treatment, respectively. Promot Plus WP (*Trichoderma* spp.), Ketomium (*Chaetomium cupreum*), Actinovate 1SP (*Streptomyces lydicus* WYEC 108), and Biogreen 4.5SL (Chitosan) also showed lower efficacy in reducing disease severity (Table 2).

Overall, Biobac 50WP (*Bacillus subtilis*) demonstrated consistent and effective control of stem and root rot disease in the field condition, making it a promising option for disease management in balloon flower cultivation.

Efficacy of chemical fungicides on stem and root rot disease in the field conditions

Among the tested fungicides, Aliette 80WP (fosetyl-aluminum) exhibited the highest efficacy with disease severities of 2.07% and 4.15% with corresponding efficacies of 78.51% and 72.43% at two weeks and four weeks after the second

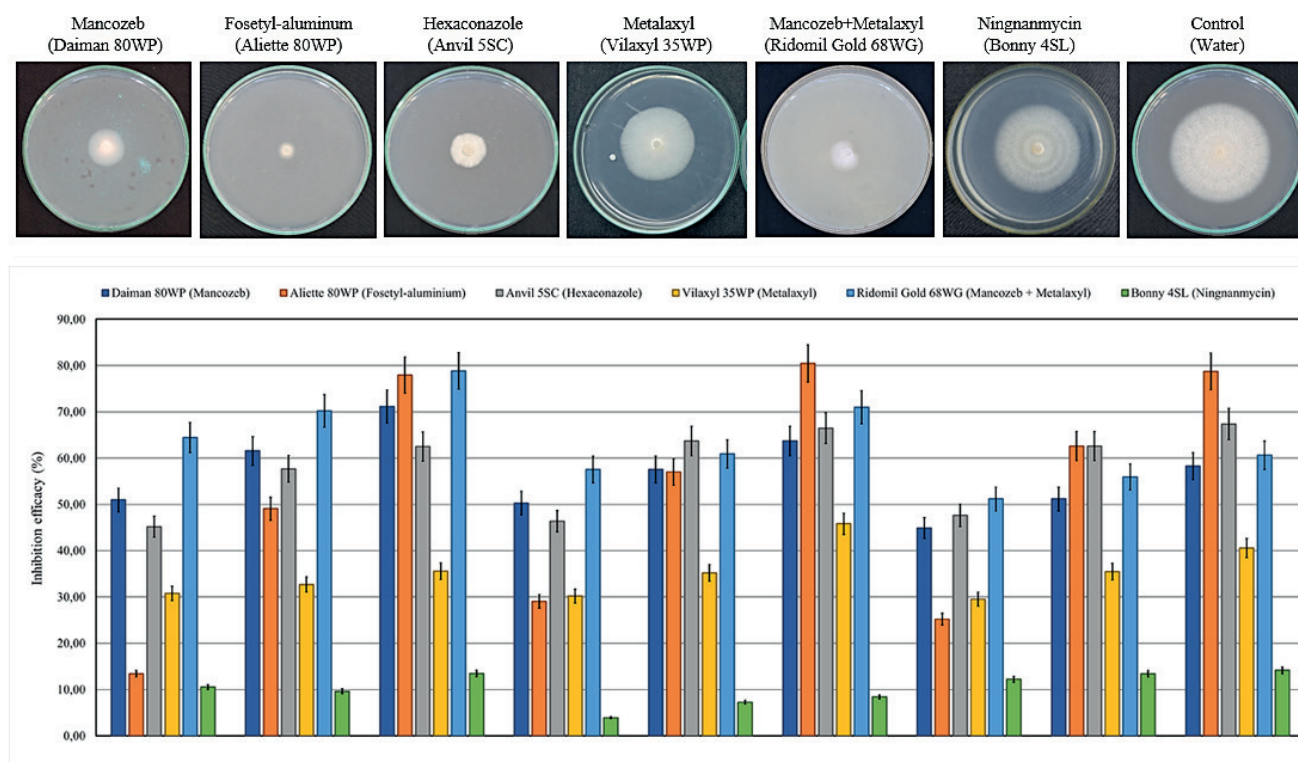


Fig. 1. Inhibition efficacy of chemical fungicides on the growth of *F. solani* CCBHVN mycelium under *in vitro* conditions

Table 1. Effects of soil treatment with bio-products before planting on stem and root rot disease eight months after treatment

Treatment	Disease severity, %	Effectiveness, %
Promot Plus WP (<i>Trichoderma</i> spp.)	22.81 ^{bc}	23.18 ^{dc}
Biobac 50WP (<i>Bacillus subtilis</i>)	11.63 ^f	60.84 ^a
Ketomium (<i>Chaetomium cupreum</i>)	17.48 ^{dc}	41.14 ^{bc}
Actinovate 1SP (<i>Streptomyces lydicus</i> WYEC 108)	20.44 ^{cd}	31.16 ^{cd}
Biogreen 4.5SL (Chitosan)	25.26 ^b	14.95 ^c
Mocabi SL (<i>Chaetomium</i> sp. + <i>Trichoderma</i> sp.)	13.85 ^{ef}	53.36 ^{ab}
Control (water)	29.70 ^a	—
CV(%)	10.61	19.90
LSD0.05	3.81	13.60
P	< 0.05	< 0.05

Note: Mean $\pm$ standard derivations followed by the same letter in the same column are not significantly different at $P < 0.05$ using Duncan's test

treatment, respectively. Anvil 5SC (hexaconazole) and Ridomil Gold 68WG (mancozeb + metalaxyl) also demonstrated efficacy in reducing disease severity, with 62.52% and 57.94% at two weeks after the second treatment, reducing after that to 62.52% and 46.19% four weeks after the second treatment, respectively. Vilaxy 35WP (metalaxyl) and Bonny 4SL (ningnanmycin) showed lower efficacies of 53.55%, 56.47% two weeks after the second treatment and 56.27%, 52.45% four weeks after the second treatment, respectively. Bonny 4SL (ningnanmycin) exhibited the lowest efficacy on stem and root rot disease. The control group showed the highest disease severity, highlighting the importance of chemical fungicide treatments in controlling stem and root rot disease in balloon flower cultivation. Overall, Aliette 80WP (fosetyl-aluminum) can control disease in field conditions (Table 3).

Under field conditions, the growth and development of pathogenic *Fusarium* fungi are considerably affected by environmental factors such as temperature, humidity, soil pH, soil fertility, and physiology of the host plants by geographical location and cultural practices (Brock et al., 1994; Theron & Holx, 1990; Tivoli et al., 1986). Our previous studies indicated that stem and root rot disease has become the most critical limiting factor for balloon flower production, and the

Table 2. Efficacy of bio-products on stem and root rot disease in the field conditions

Treatments	2 weeks after the second treatment		4 weeks after the second treatment	
	Disease severity, %	Efficacy, %	Disease severity, %	Efficacy, %
Promot Plus WP (<i>Trichoderma</i> spp.)	6.81 ^a	38.11 ^a	11.48 ^a	33.26 ^b
Biobac 50WP (<i>Bacillus subtilis</i>)	3.11 ^c	50.84 ^a	3.63 ^c	60.00 ^a
Ketomium (<i>Chaetomium cupreum</i>)	5.78 ^{ab}	38.88 ^a	7.78 ^b	44.61 ^{ab}
Actinovate 1SP (<i>Streptomyces lydicus</i> WYEC 108)	4.89 ^{bc}	38.30 ^a	7.70 ^b	31.15 ^b
Biogreen 4.5SL (Chitosan)	6.44 ^{ab}	34.94 ^a	10.74 ^a	24.10 ^b
Mocabi SL (<i>Chaetomium</i> sp. + <i>Trichoderma</i> sp.)	3.78 ^c	39.77 ^a	5.04 ^c	46.40 ^{ab}
Control	6.07 ^{ab}	—	8.74 ^b	—
CV(%)	19.52	35.86	11.47	35.16
LSD _{0.05}	1.83	26.18	1.61	25.51
P	< 0.05	> 0.05	< 0.05	< 0.05

Note: Mean ± standard derivations followed by the same letter in the same column are not significantly different at P < 0.05 using Duncan's test

infested areas are increasing yearly in Vietnam. We have accurately identified *F. solani* as the causal pathogen and its biological and ecological characteristics, essential to determining effective management strategies (Hieu et al., 2023a,b). Our previous studies have indicated that planting density and fertilization do not significantly affect the disease (data not shown). This suggests the need to figure out effective bio-products and chemical fungicides to control the disease. Therefore, our previous and current studies provided essential background information for developing integrated stem and root rot disease management.

Many other biocontrol agents have been used to manage different Fusarium diseases. Among the tested biological agents, *Trichoderma harzianum* showed high efficacy in inhibiting the mycelial growth of *Fusarium solani* f. sp. *pisi* (the causal pathogen of the root rot disease on pea (*Pisum sativum* L.)) (Nazir et al., 2022). *Bacillus* spp., for instance, has been employed to suppress tomato fusarium crown and root rot disease effectively (Hibar et al., 2006), significant inhibition of *F. verticillioides* growth and fumonisin B1 ac-

cumulation, alongside reducing rhizoplane and endorhizosphere colonization by the pathogen (Cavaglieri et al., 2005). Moreover, *Bacillus* spp. has shown efficacy in suppressing *F. oxysporum* growth in the cucumber rhizosphere and protecting the host plant from the pathogen (Cao et al., 2011). *B. subtilis* produces a variety of extra-cellular proteolytic enzymes, siderophores, and lipopeptides and induces plant disease resistance (Junqing et al., 2017; Liu et al., 2012). *Bacillus subtilis* PTS-394 suppresses tomato soil-borne diseases caused by *F. oxysporum* and *Ralstonia solanacearum* (Liu et al., 2012). It was reported that *B. subtilis* PTS-394 wettable powder showed high efficacy against pepper root rot caused by *F. solani* (Qiao et al., 2023). It was indicated that *B. subtilis* V26 effectively inhibited *F. oxysporum*, *F. solani*, *F. graminearum*, and *F. sambucinum* growth and promotes plant growth of potatoes (Khedher et al., 2021). Application of a consortium of *P. fluorescens*, *B. subtilis*, *Streptomyces* spp., *L. casei*, *R. palustris*, *S. cerevisiae*, *Azotobacter* sp. significantly reduced the disease severity and *F. proliferatum* population in roots and increased the pop-

Table 3. Efficacy of chemical fungicides on stem and root rot disease in the field conditions

Treatment	2 weeks after the second treatment		4 weeks after the second treatment	
	Disease severity, %	Efficacy, %	Disease severity, %	Efficacy, %
Daiman 80WP (mancozeb)	3.63 ^{bc}	56.47 ^c	5.41 ^c	52.45 ^{bcd}
Aliette 80WP (fosetyl-aluminum)	2.07 ^c	78.51 ^a	4.15 ^c	72.43 ^a
Anvil 5SC (hexaconazole)	2.67 ^{bc}	66.13 ^b	4.30 ^c	62.52 ^{ab}
Vilaxyl 35WP (metalaxyl)	4.89 ^b	53.55 ^c	7.11 ^{bc}	56.27 ^{bc}
Ridomil Gold 68WG (mancozeb + metalaxyl)	4.44 ^{bc}	57.94 ^{bc}	8.59 ^{ab}	46.19 ^{cd}
Bonny 4SL (ningnanmycin)	5.19 ^{ab}	53.90 ^c	10.44 ^a	39.27 ^d
Control (water)	7.63 ^a	—	11.56 ^a	—
CV(%)	34.75	7.49	23.68	15.34
LSD _{0.05}	2.70	8.33	3.10	15.31
P	< 0.05	< 0.05	< 0.05	< 0.05

Note: Mean ± standard derivations followed by the same letter in the same column are not significantly different at P < 0.05 using Duncan's test

ulation of plant beneficial microbes in the soil (Nguyen et al., 2019 a, b). The present study used bio-products for soil treatment to suppress *F. solani* CCBHVN before planting. Among the tested products, Biobac 50WP (*Bacillus subtilis*) and Mocabi SL (*Chaetomium* sp. + *Trichoderma* sp.) reduced the growth and development of stem and root rot diseases in field conditions (Table 1). In addition, when applied in the field conditions, only Biobac 50WP (*Bacillus subtilis*) had high efficacy against stem and root rot disease (Table 2). Therefore, Biobac 50WP (*Bacillus subtilis*) is recommended to treat soil before planting balloon flower plants. It can also be used after this disease appears in field conditions. However, further studies on using Biobac 50WP (*Bacillus subtilis*) in the planting season are necessary.

Many studies have been conducted to test the efficacy of different active ingredients in the diseases caused by *Fusarium* spp. Mancozeb is classified as a mode-of-action group M, a multi-site fungicide. It interferes with enzymes containing sulphhydryl groups, disrupting several biochemical processes within the fungal cell cytoplasm and mitochondria. Mancozeb has the best performance among non-systemic fungicides for the growth inhibition of *F. solani* (Singh et al., 2000; Allen et al., 2004). Metalaxyl inhibits endogenous RNA polymerase activity, which inhibits RNA synthesis. On the 7th day of incubation, a study found that mancozeb exhibited high inhibition of mycelium growth, followed by metalaxyl + mancozeb at the exact dosage (Nazir et al., 2022). Hexaconazole, a sterol biosynthesis inhibitor, has been reported to effectively control different *Fusarium* diseases (Chennakesavulu et al., 2013; Dibya Bharati, 2018; Khilari et al., 2019). Our previous study examined the effectiveness of various fungicides against *F. proliferatum*, the causal agent of the *Fusarium* root rot disease of the Indian mulberry plant. Among the tested active ingredients, prochloraz and metconazole exhibited the highest efficacy in inhibiting the growth of *F. proliferatum* under *in vitro* conditions, and they significantly reduced the incidence of *Fusarium* root rot disease in both pot and field conditions (Nguyen et al., 2019c). However, in another experiment, we also conducted *in vitro* assays, and the results indicated that they were ineffective against *F. solani* CCBHVN (data not shown). In the present study, fosetyl-aluminum, a systemic fungicide blocking mycelial growth and spore production, showed high efficacy against *F. solani* CCBHVN *in vitro* conditions and suppressed stem and root rot disease of balloon flower in field conditions.

Conclusion

Biobac 50WP (*Bacillus subtilis*) is an effective bio-product for soil treatment before planting to eliminate the disease

inoculum in the soil, prevent the development of stem and root rot disease, and protect balloon flowers in field conditions. Once the disease appears, this bio-product also can be used to control the disease. In addition to this bio-product, Aliette 80WP (fosetyl-aluminum) demonstrated the highest efficacy against stem and root rot disease, indicating its potential for controlling the disease in field conditions when necessary.

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