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Investigating the effect of some common fungicides against gray mold disease on greenhouse-grown cucumbers

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Abstract

Gray mold disease in cucumber plants is caused by the fungus *Botrytis cinerea*, which significantly affects various crops, including beans, lettuce, peas, tomatoes, and cucumbers. The fungus infects all aerial parts of the plant and typically enters through wounds. Spores of this pathogen manifest as gray mold under high relative humidity conditions. Given the severity of the damage caused by this fungus, chemical control measures are essential. Various fungicides exhibit different efficacies against *B. cinerea*; therefore, identifying the most effective options is crucial. In this study, the fungicides Signum (1.5 g/L), Rovral-TS WP (1 g/L), Luna Sensation 500 SC (0.4 g/L), Bellkute (500 g/ha), Nativo (160 g/ha), Daconil (2 L/ha), and Captan 72% WP (2.5 g/L), were evaluated for their effectiveness against the disease. These doses were applied according to the manufacturer's instructions. The fungicides were tested using a randomized complete block design with four replications, which included two control treatments: one with spraying and one without. All applied fungicides reduced the number of infected fruits and flowers compared to the untreated control. However, Signum and Luna demonstrated the most significant effects, reducing infected flowers by 47% and 17%, respectively. Moreover, these fungicides effectively lowered the amount of fruit shriveled and visible mycelium and spore coverage by 54% and 23%, respectively, compared to the control. Our results indicate that Signum and Luna effectively manage *B. cinerea* in cucumber greenhouse production.

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Introduction

Diseases are among the most significant limiting factors in greenhouse cucumber production, leading to increased production costs and a decrease in both the quantity and quality of the product (Berdugo et al., 2014). Cucumber gray mold is a particularly detrimental disease affecting greenhouse cucumbers, thriving under conditions of high humidity, low temperatures, low light, and inadequate ventilation. The causal agent of cucumber gray mold is *Botrytis cinerea*. The symptoms of cucumber gray mold include soft rot, shriveling, and a gray or brown discoloration that typically starts at the tip of the fruit and progresses toward the stem end. This disease primarily affects fruits and flowers; however, under conditions of high humidity and the presence of wounds, it can also attack other aerial parts of the plant (Azadvar, 2020). The *B. cinerea* has a significant potential to develop resistance to fungicides due to its genetic diversity, short life cycle, and prolific reproduction. While the application of effective fungicides can mitigate damage, the repeated use of a limited range of fungicides increases the likelihood of resistance development (Shao et al., 2021). The use of chemical control programs is one of the key methods for managing plant diseases both before and after harvest. Fungicides employed in significant quantities to control *B. cinerea* account for 10% of the global fungicide

market (Abbey et al., 2019). Various fungicides have been introduced worldwide for the management of gray mold (Ghayeb Zamharir et al., 2021). These fungicides exhibit a broad spectrum of activity and include newer and more specific compounds such as Fluazinam, Boscalid, Carbendazim, Iprodione, and Procymidone (Matheron and Porchas., 2004). Fluazinam is an important pyrimidine amine fungicide that has been widely used to control various fungal diseases in crops. It has a broad-spectrum antifungal activity and can inhibit the growth of several fungal pathogens. Fluazinam exerts its antifungal effect through a unique mode of action that involves multiple pathways. Specifically, it triggers oxidative stress in fungal cells by inducing reactive oxygen species (ROS) accumulation and caspase activation, leading to cellular damage and eventual cell death. Additionally, fluazinam causes fungal membrane permeabilization and protein carbonylation, which further exacerbate oxidative stress and hasten cell death (Wu et al., 2022). Boscalid acts by inhibiting the growth of fungi and disrupting their spore production, effectively reducing their ability to spread and cause damage. Succinate dehydrogenase inhibitors (SDHI) of this type act by binding at the quinone reduction site of the enzyme complex, preventing ubiquinone from doing so. As a consequence, the tricarboxylic acid cycle and electron transport chain cannot function. The chemical's mode of action is the inhibition of mitochondrial ATP production in fungal cells. Specifically, boscalid inhibits the succinate-ubiquinone oxidoreductase system in Complex II of the mitochondrial electron transfer chain (Aubee et al., 2010). Carbendazim is a systemic fungicide from the group of benzimidazoles, which is very effective against many fungal pathogens. Carbendazim inhibits the polymerisation of free tubulin molecules by binding an arginin residue of the β-tubulin subunit and acts by disrupting cell division through linking to the nuclear spindle, which inhibits fungal growth (IPCS, 1993). This fungicide are potent inhibitors of tubulin polymerization and exert their antifungal activities by targeting the β-tubulin subunit of the microtubules, which results in the arrest of microtubule formation and a failure in cell division, subsequently leading to cell death (Davidse., 1973). Iprodione's mode of action is that it inhibits DNA and RNA synthesis in the germinating fungal spore was well as inhibiting the enzyme NADH cytochrome c reductase, thereby preventing lipid and membrane synthesis and ultimately mycelium growth (Fernandez-Cornejo et al., 2014). Procymidone has antiandrogenic properties, and it competitively inhibits the binding of androgens to it is receptor, thereby preventing androgen function (APVMA., 2022). These fungicides can be categorized into five groups based on their mechanisms of action:
1- fungicides that affect fungal respiration, 2- anti-microtubule toxins, 3- compounds that impact osmotic regulation, 4- fungicides whose toxicity can be reversed by amino acids, and 5- inhibitors of sterol biosynthesis (Iwaniuk & Lozowicka, 2022; Leroux, 2007; Leroux et al., 2002). However, despite the availability of a wide range of botryticides with diverse modes of action, the emergence of resistant strains of *B. cinerea* is a significant concern. This pathogen can undergo mutations in its genome, enabling it to survive in various environments and resulting in substantial damage to crops globally (Harper et al., 2022). Additionally, the use of botryticides and pesticides, in general, poses environmental risks, leading to widespread pollution of soils, air, and water, which adversely affects biodiversity. Human health is also at risk, as exposure to these chemicals can lead to various diseases (Jacometti et al., 2010). This study investigates the efficacy of the fungicides Pyraclostrobin + Boscalid WG 33.4% (Signum), Trifloxystrobin + Fluopyram 500 SC (Luna Sensation 500 SC), Iprodione + Carbendazim 52.5% (Rovral-TS WP), Chlorothalonil 72% SC (Daconil), Captan 72% WP, Trifloxystrobin + Tebuconazole 75 WG (Nativo), and Iminoctadine tris 40% WP (Bellkute) in controlling gray mold disease on cucumber. The study also compares the effectiveness of these fungicides against each other.

Materials and Methods

In vivo conditions

This experiment was conducted using a randomized complete block design with nine treatments and four replications under greenhouse conditions (temperature: 18-32°c and humidity: 50-70 %) to evaluate the efficacy of the studied fungicides. The treatments are detailed in Table 1. The cucumber variety 485 Nickerson was used for the experiments, as it is available and commonly cultivated in the southern region of Kerman Province. Each experimental plot consisted of a single row of plants measuring 10 meters in length, with a spacing of 25 centimeters between plants and 50 centimeters between rows. Nutritional requirements and essential plant care were provided throughout the study.

Spraying

The application of experimental treatments commenced upon the first observation of disease symptoms, using a calibrated 20-liter sprayer. Treatments were administered at seven-day intervals over four

applications. The efficacy of the treatments was assessed by estimating disease incidence, which involved counting 100 randomly selected flowers and 100 fruits per plot. The total number of infected flowers and fruits was recorded before each foliar spray and again seven days after the final application. To evaluate the impact of the treatments on disease progression, the area under the disease progression curve (AUDPC) was calculated based on the average disease occurrence in successive evaluations, following the formula established by Campbell and Madden (1990).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right) 1t_{i+1} + t_i 1$$

In this formula, n is the number of evaluation times, i is the evaluation time, yi and ti are the average severity of the disease and time in the previous evaluation, yi + 1 and ti + 1 are the average severity of the disease and time in the current evaluation, respectively. The effectiveness of the treatments in reducing the disease was calculated using the following formula for the averages compared to the sprinkled control.

$$ef = 100 - (\frac{\bar{x}t}{\bar{x}c} \times 100)$$

Table 1. Experimental treatments.

Number	Treatment	Dosage
1	Daconil	2 liters per hectare
2	Luna Sensation	0.4 per thousand
3	Rovral-TS	1 per thousand
4	Captan	2.5 per thousand
5	Signum	1.5 per thousand
6	Nativo	160 grams per hectare
7	Bellkute	500 grams per hectare
8	Control by spraying	
9	Control without spraying	

In vitro conditions

Isolation and identification of the disease agent

Following the observation of disease symptoms, the pathogens were cultured, isolated, and identified. To isolate fungal pathogens, infected samples of leaves, stems, and fruits exhibiting characteristic symptoms were collected. These samples were washed and disinfected with a 5% sodium hypochlorite solution before being cultured on Potato Dextrose Agar (PDA) medium supplemented with lactic acid (1 ml/L). The obtained isolates were purified on 2% water-agar (WA) medium and subsequently cultured on PDA using the mycelium tip culture method. The isolates were identified on the basis of their phenotypic characteristics, such as appearance and coloration of the mycelium, as well as microscopic observations of conidiophores and conidia. The morphological features of the isolates were compared with previously reported descriptions of *Botrytis cinerea* (Crous et al. 2009; Ellis, 1971). All Petri dishes were incubated at an ambient temperature of 25°C.

Preparation of concentrations of fungicides

For all fungicides, a stock solution of 200 mg/liter of the active ingredient was prepared. Utilizing a serial dilution method, further concentrations of 1, 10, 100 and 1000 mg/liter were derived, taking into account the percentage of the active substance in each formulation. The effectiveness of each concentration was calculated, and ultimately, a range of active ingredient concentrations was tested for each of the Fungicides (Mavandadi et al., 2016).

Effect of fungicides on the germination percentage of *B. cinerea* conidia

To assess the germination percentage of *B. cinerea* conidia, a culture medium was prepared consisting of 10 gr of glucose, 2 gr of KH_2PO_4, 2 gr of K_2HPO_4, and 12.5 gr of agar per liter of distilled water. Each 90 mm Petri dish was filled with 20 ml of the appropriate fungicide concentration mixed with the culture medium

and allowed to cool completely. To prepare the conidial suspension, distilled water was added to a 15-day-old fungal culture, followed by 1 ml of Tween 80. The Petri dish was shaken vigorously to disperse the conidia. A drop of the suspension was placed under a microscope slide for counting, and the concentration was adjusted to 2 x 10^5 conidia per milliliter under sterile conditions. Subsequently, 350 µl of this suspension was added to the Petri dishes containing culture medium with varying fungicides concentrations, alongside a control dish containing only distilled water. After 24 hours, 100 conidia were examined under a microscope using a 40x objective lens. Conidia were considered germinated if the length of the germ tube exceeded the conidial length. The percentage of ungerminated conidia was calculated in comparison to the control after 24 hours (Mavandadi et al., 2016).

Effect of fungicides on mycelial growth of *B. cinerea*

In this study, we employed potato-dextrose-agar (PDA) medium to assess the mycelial growth of *B. cinerea*. Fungicide solutions were prepared according to the previously described methodology and incorporated into the culture medium, with distilled water was used for the control treatment. Under completely sterile conditions, a 5 mm diameter and 3 mm thick disc was extracted from the growing edge of a 7-day-old *B. cinerea* colony, maintained at 25 °C. This disc was then positioned upside down at the center of the PDA medium and incubated at 25 °C. After three days, the colony diameter was measured. Three replicates were conducted for each treatment and control. The inhibition percentage of each fungicide on *B. cinerea* mycelial growth was calculated using the formula I=(C-T/C)* 100 (Meng et al., 2007), where I represent the diameter of the treatment colony and C denotes the diameter of the control colony. The inhibition percentage of each fungicide on the mycelial growth of *B. cinerea* was calculated (Mavandadi et al., 2016).

Statistical Analysis

This study was designed and conducted based on randomized complete block design .Analysis of variance (ANOVA) for the observations and means comparison was performed using Duncan's multiple range test with SAS version (9.4) software.

Results

Effect of fungicides on the germination percentage of *B. cinerea* conidia

The morphological characteristics of the causative fungus included the colony growth on the PDA culture medium, which initially appeared colorless and later turned gray-brown. Conidia were oval, transparent, and colorless to brown, measuring 6.8–16.5 x 5–10 micrometers. The conidiophore was long and elongated, measuring 1–1.5 micrometers in length and 5–10 micrometers in diameter, often branching with a flat base that narrowed slightly at the branching point. Thick threads with a diameter of 10–12.5 micrometers were also observed (Fig.1).

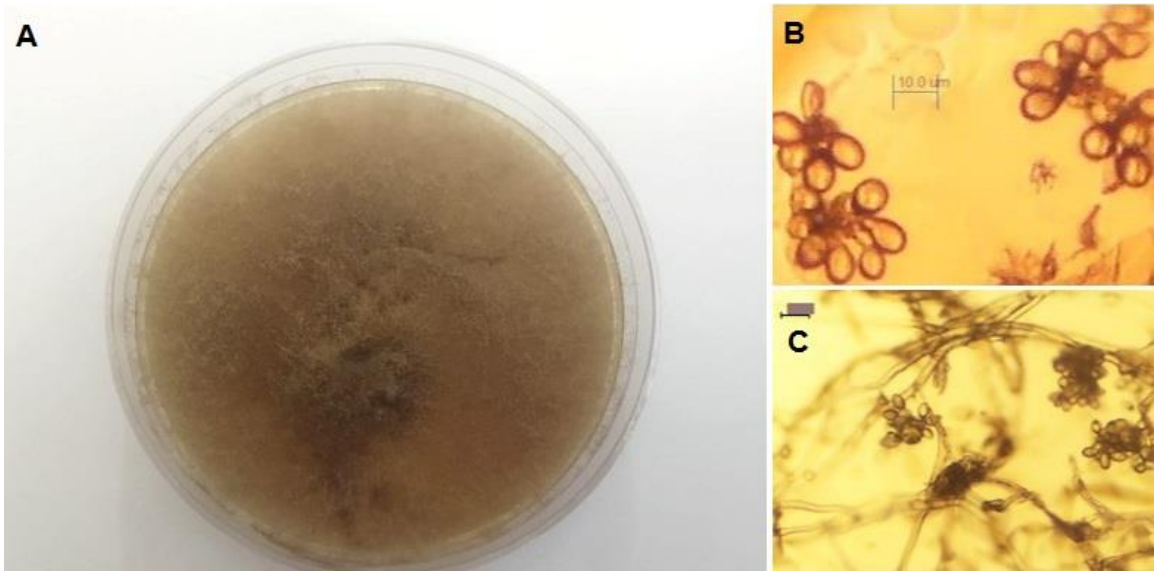

Fig. 1. *Botrytis cinerea*: A. Colony on PDA after 7 days at 25 °C. B. Conidia (scale bar: 10 µm). C. Conidiophores with conidia (scale bar: 20 µm).

The analysis of variance regarding the impact of fungicides and their concentrations on controlling the germination of *B. cinerea in vitro* revealed a statistically significant effect of fungicide application compared to the control, with a significance level of 1%. Furthermore, significant differences were observed among the seven fungicides utilized concerning their efficacy in inhibiting fungal germination, also at the 1% level. There was a statistically significant difference between various concentrations of fungicides at the 1% probability level, and the interaction effect between fungicides and their concentrations on the percentage of germination control was significant as well (Table 2). Given the significant interaction effect, the individual effects of each fungicide at different concentrations on controlling fungal germination were analyzed separately According to the results (Table 3), the application of Captan at a concentration of 1000 ppm led to 100% control of fungal germination compared to the control. Other tested concentrations also significantly affected fungal germination control, although the efficacy decreased with lower concentrations. The highest percentage of germination among the Captan treatments was observed at 1 ppm, where fungal germination was inhibited by 66.24%. Based on the findings (Table 3), the application of Signum fungicide at a concentration of 1000 ppm achieved complete control over fungal germination compared to the control. Other concentrations also notably affected fungal germination control; however, less efficiency was observed with decreasing concentrations. The highest percentage of fungal germination among Signum treatments was noted at 1 ppm, where the germination percentage decreased to 73.22%. The results on the average effects of various Daconil concentrations on the percentage of fungal germination control (Table 3) indicated that the application of this fungicide at 1000 ppm led to complete inhibition of germination. No significant differences were detected among the other concentrations in this regard. Our data (Table 3) demonstrated that the application of Nativo fungicide at concentrations of both 100 and 1000 ppm resulted in 100% control of fungal germination compared to the control. Other concentrations also exhibited significant effects on controlling fungal germination, with no significant difference observed between the 1 and 10 ppm treatments concerning the inhibition of fungal rejuvenation under laboratory conditions. Results comparing the average effects of different Bellkute fungicide concentrations on the control of *B. cinerea* germination indicated that the application of this fungicide at concentrations of 100 and 1000 ppm resulted in 100% control of fungal germination under laboratory conditions. The concentrations of 10 and 1 ppm exhibited 88.83% and 85.51% inhibition of fungal germination, respectively. No significant differences were noted between the 100 and 1000 ppm concentrations, nor between 10 and 1 ppm concentrations, in terms of their inhibitory effects on fungal germination (Table 3). The evaluation of different concentrations of Luna Sensation and Rovral-TS fungicides indicated that all four concentrations tested were effective in achieving 100% control of *B. cinerea* germination compared to the control.

Table 2. ANOVA of fungicide and concentration on control of *Botrytis cinerea* spores germination.

SOV	df	MS
Control vs treatment	1	24396.89**
Fungicide	6	3237.20**
Con	3	3367.68**
Fungicide×Con	18	2020.68**
Error	58	240.83
CV (%)	-	2.29

**: Significant at 0.01 probability level.

Table 3. Mean Comparison of concentration effect for seven fungicides on control of *Botrytis cinerea* spores germination.

Concentration	Captan	Signum	Daconil	Nativo	Bellkute	Lona	Rovral-TS
Control	0e±0	0d±0	0c±0	0c±0	0c±0	0b±0	0b±0
1	66.24d±1.14	73.22c±4.47	80.47b±1.39	83.83b±1.67	85.51b±1.40	100a±0	100a±0
10	74.51c±1.46	77.78c±0.57	81.80b±0.54	86.58b±1.05	88.83b±0.38	100a±0	100a±0
100	94.73b±0.83	89.55b±0.21	88.39b±1.13	100a±0	100a±0	100a±0	100a±0
1000	100a±0	100a±0	100a±0	100a±0	100a±0	100a±0	100a±0

The means with common letters in each column are not significantly different from each other based on Duncan's test.

Effect of fungicides on mycelial growth of *B. cinerea*

The results of the analysis of variance regarding the impact of fungicides and their concentrations on inhibiting fungal colony growth (Table 4) demonstrated a significant difference in colony growth between the control and the fungicides treatments at the 1% probability level. Additionally, the effectiveness of the seven fungicides in inhibiting fungal growth varied significantly (1% probability level). There was also a significant difference among the applied concentrations and their interaction with the fungicide in controlling fungal growth, with a significance level of 1%.

Efficacy of different fungicides in inhibiting fungal growth

The Rovral-TS fungicide was the most effective in inhibiting fungal growth, achieving complete inhibition (100%) across all tested concentrations (Table 5). While there was no statistically significant difference between the control of fungal growth during germination of Luna Sensation and Rovral-TS fungicides, an investigation into their effects on colony growth revealed that Luna Sensation effectively prevented fungal growth at concentrations of 10, 100, and 1000 ppm (Table 4). However, when the concentration was reduced to 1 ppm, fungal growth occurred in 20% of samples (Table 5).

The Signum fungicide exhibited similar efficacy to Luna Sensation, controlling fungal growth by 91%. Nonetheless, Signum achieved 100% inhibition of fungal growth only at concentrations of 100 and 1000 ppm (Table 5). At the lowest concentration of 1 ppm, Signum inhibited fungal growth by up to 70% (Table 5). Similarly, Nativo fungicide also achieved 100% growth control at concentrations of 100 and 1000 ppm. However, at the lowest concentration of 1 ppm, it only inhibited fungal growth by 20% (Table 5).

Table 4. ANOVA of fungicide and concentration on the inhibition percentage of *Botrytis cinerea* colonies growth.

SOV	df	MS
Control vs treatment	1	41648.85**
Fungicide	6	37447.75**
Con	3	14992.83**
Fungicide×Con	18	2782.93**
Error	58	140.13**
CV (%)	-	5.63

**: Significant at 0.01 probability level.

Table 5. Mean Comparison of concentration effect for seven fungicides on the inhibition percentage of *Botrytis cinerea* colonies growth.

Concentration	Captan	Signum	Daconil	Nativo	Bellkute	Lona	Rovral-TS
Control	0e±0	0d±0	0e±0	0c±0	0e±0	0c±0	0b±0
1	0.66d±0.33	77.91c±2.91	4.16d±4.16	20.41b±0.41	58.19d±0.41	80b±1.9	100a±0
10	9.08c±0.58	87.08b±0.41	18.75c±2.60	85.41b±0.83	75.43c±7.53	100a±0	100a±0
100	20.41b±2.31	100a±0	24.16b±3.97	100a±0	84.17b±0.42	100a±0	100a±0
1000	53.75a±1.91	100a±0	35.83a±1.10	100a±0	100a±0	100a±0	100a±0

The means with common letters in each column are not significantly different from each other based on Duncan's test.

Performance of Bellkute, Daconil and Captan fungicides

Bellkute fungicide completely inhibited fungal growth only at a concentration of 1000 ppm. Conversely, both Daconil and Captan fungicides reduced fungal growth compared to the control, but neither achieved complete inhibition at any concentration (Table 5). The highest control percentages for these two these fungicides were observed at the 1000 ppm concentration, achieving 35% and 53% inhibition, respectively (Table 5).

Area under the disease progression curve for cucumber flowers and fruits infected by *B. cinerea* in greenhouse conditions

The analysis of variance results (Table 6) indicated that the effects of different treatments (control and fungicide) on the area under the disease progression curve for the number of cucumber flowers and fruits infected with *B. cinerea* in greenhouse conditions were significant at the 1% probability level. A comparison of means revealed that the application of Signum fungicide

reduced the area under the disease progression curve by approximately 50%, notably decreasing the number of infected flowers and fruits compared to both treated and untreated control groups (Table 7). Meanwhile, the fungicides Luna Sensation and Rovral-TS demonstrated the highest efficacy in controlling fungal germination and growth under laboratory conditions. Although both fungicides significantly reduced the number of infected fruits and flowers compared to the control, their effectiveness was lower under greenhouse conditions than in laboratory settings (Table 7).

Table 6. ANOVA of AUPDC for cucumber flower and fruit.

SOV	df	MS	
		Flower	Fruit
Block	3	0.10**	0.40**
Treatment	8	5.40**	5.21**
Error	24	0.01	0.03
CV (%)	-	1.62	3.08

**: Significant at 0.01 probability level.

Table 7. Mean comparison of seven fungicides and controls on AUPDC for cucumber flower and fruit

Fungicide	Control-WS	Control-without WS	Captan	Signum	Daconil	Nativo	Bellkute	Lona	Rovral-TS
Flower	8.32a±0.05	8.28a±0.08	7.48c±0.05	4.39f±0.10	7.25d±0.10	7.69b±0.09	7.28d±0.05	6.84e±0.05	7.39cd±0.08
Fruit	7.12a±0.10	7.01a±0.22	6.31b±0.07	3.22f±0.12	5.79d±0.08	6.12bc±0.14	5.87cd±0.17	5.43e±0.07	6.18b±0.15

The means with common letters in each row are not significantly different from each other based on Duncan's test.

Discussion

Despite the comparable effectiveness of Luna Sensation and Rovral-TS fungicides, their preharvest interval (PHI) must be considered when selecting a treatment. Luna Sensation has a PHI of less than three days, while Rovral-TS maintains an effective shelf life of more than 20 days. Both fungicides yield similar effects in promoting healthy crop production. Ghayeb Zamharir et al. (2021) reported that concentrations of 0.5 g/L and 0.4 g/L of Luna Sensation, along with 1.5 g/L and 1.25 g/L of Signum, reduced the number of infected flowers by 95%, 89.8%, and 95%, respectively. Additionally, reductions in infected fruits were reported at rates of 95.5%, 90.4%, and 90.4% (Ghayeb Zamharir et al., 2021). Although there was no significant difference between Luna Sensation and Rovral-TS regarding fungal germination control, an evaluation of their effects on plant growth indicated that the application of Luna Sensation at concentrations of 10, 100, and 1000 ppm completely inhibited fungal growth. However, reducing the concentration to 1 ppm resulted in a 20% chance of fungal growth.

The results revealed clear differences in the effectiveness of the seven tested fungicides, in both laboratory and greenhouse conditions. Rovral-TS and Luna Sensation demonstrated 100% control of *B. cinerea* germination across all tested concentrations. In terms of fungal growth inhibition, Rovral-TS maintained complete control at all concentrations, while Luna Sensation achieved complete inhibition at concentrations of 10, 100, and 1000 ppm. Although the fungicides Captan and Daconil demonstrated significant efficacy in controlling fungal growth under laboratory conditions compared to the control treatment, they exhibited lower effectiveness than Luna sensation and Rovral-TS in inhibiting both spore germination and mycelial growth. In greenhouse trials, all fungicides tested reduced the number of fruits and flowers infected by *B. cinerea*, with Signum being the most effective, followed by Luna Sensation. Signum reduced the number of infected flowers and fruits by 46.98% and 39.17%, respectively, compared to the untreated control. According to some researchers, the effectiveness of fungicides can be influenced by various biological and environmental factors that directly impact fungal cells metabolism (Ijaz et al., 2015; Peerzada et al., 2020; Reinprecht, 2010).

Signum fungicide possesses strong protective properties due to its active ingredients, pyraclostrobin and Boscalid, which can move systemically and locally within plant tissues. Pyraclostrobin disrupts energy production in fungal cells by interfering with the electron transport chain at cytochrome complex III, leading to a cessation of ATP production. Similarly, Boscalid disrupts the function of complex II in the

electron transfer chain, further impairing fungal metabolism and halting energy production. The synergistic effect of these two components enhances the overall efficacy of disease control (Hauke et al., 2004). Signum also provides additional benefits beyond simple disease management, promoting positive effects on plant health. Its formulation minimizes the risk of resistance development. Furthermore, it is effective against powdery mildew (Hauke et al., 2004). Ayoub et al. (2018) tested the inhibitory effects of the commercial fungicides Switch (SWITCH) and Signum on the mycelial growth and spore germination of *B. cinerea*. They reported that complete inhibition of fungal growth was achieved with 16.77 µg/ml of Signum and 14.47 µg/ml of Switch under laboratory conditions. In greenhouse tests, Signum at a concentration of 125 g/L resulted in 66.7% inhibition of gray mold. This study also indicated that combining Signum with organic peroxyacetic acid increased the fungicide's efficacy while reducing the required application rates (Ayoub et al., 2018). Additionally, Sapieha-Waszkiewicz et al. (2011) reported the effectiveness of Signum in controlling *B. cinerea* on strawberry. Our findings reveal significant variation in the effectiveness of standard fungicide treatments against cucumber gray mold (*B. cinerea*). Choosing the right fungicide should be based on this effect. It is also very important to consider the phi period and the risk of resistance in the *B. cinerea* population.

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Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

M. Afshari: Field and laboratory works & writing original draft preparation. **H. R. Alizadeh, A. Abbasi & S. M. Alavi:** Supervision, methodology, writing, reviewing & editing.

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