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Efficacy of Fungicides and Biocontrol Agents in Managing *Sclerotinia sclerotiorum*-Induced White Mold in Marigold Varieties

Rumana Momotaz [1]* ✉, Md. Morshedul Islam [2], Khondoker Mohammad Alam [1], Md. Mahfuz Alam [3], Most. Arifunnahar [1], Salma Sarker [4], Md. Sabuj Ali [5], Dipta Majumder [5], Shaikh Sharmin Siddique [6]

[1] Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh
[2] Department of Agriculture, Teesta University, Rangpur, Bangladesh
[3] Training and Manpower Unit, Bangladesh Agricultural Research Council, Dhaka, Bangladesh
[4] Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh
[5] Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
[6] Department of Plant Pathology, Gazipur Agricultural University, Gazipur, Bangladesh

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Correspondence
Rumana Momotaz
✉: rumanamomotaz35@gmail.com

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ABSTRACT

White mold of marigold, induced by *Sclerotinia sclerotiorum*, is a destructive fungal infection that results in significant crop losses annually. This experiment assessed the effectiveness of several chemical fungicides and biocontrol agents in mitigating white mold disease. Laboratory, net house, and field experiments were conducted to assess the fungicides and biological agents. Under in vitro conditions, all fungicides demonstrated a significantly greater efficacy than the control in suppressing pathogen development. Score 250EC, Faja 70WP, Secure 600WG, Folicur 250EC, and Tilt 250EC were the most efficacious, as they entirely suppressed (100%) the pathogen's growth. Under field conditions, Score 250EC and Faja 70WP exhibited superior performance in the number of uninfected flowers/plants (In high-yielding varieties (HYV), 15.00 and 14.53; in local variety, 26.93 and 26.50, respectively) and the minimal count of infected flowers/plants throughout both varieties. The lowest disease incidence (10.55% in HYV and 24.40% in local variety), severity (22.83% in HYV, while local variety exhibited 14.02%), and reduction over control were seen with Score 250EC and Faja 70WP fungicides in both varieties. In the pot and field trial, neem oil cake was identified as an efficient biocontrol agent, increasing the number of uninfected flowers/plants and decreasing the number of infected flowers/plants in both varieties. In a pot experiment, neem oil cake resulted in 14.53 uninfected flowers/plants in HYV, whereas in a local variety, it was 19.87. Furthermore, in the field trial, the number of uninfected flowers/plants in the HYV was 15.39, whereas in the local variety it was 19.87. The incidence, severity, and decrease of disease in neem oil cake-treated plants were quite low compared to the control condition. Neem oil cake was identified as the second most effective substance in suppressing pathogen growth.

1. Introduction

Marigold (*Tagetes erecta* L.) is a member of the Asteraceae family and is planted globally as an ornamental plant. It serves various applications, including aesthetic uses with cut and loose flowers, essential oils, and carotenoid pigments for nutraceutical and pharmacological purposes, and is the most abundant source of xanthophylls (Gupta et al. 2022; Sowbhagya et al. 2013). The plant originates from Mexico and Guatemala, and is used in traditional Mexican medicine (Sowbhagya et al. 2004). The cultivation of marigolds is progressively rising in Bangladesh. In 2021-2022, the total marigold production was 2881.41 metric tons from 1124.92 acres, yielding an average of around 2.56 metric tons per acre (BBS 2023). In Bangladesh, 95% of farmers in the Jashore and Jhenaidah districts engage in the commercial cultivation of marigolds (Haque et al. 2013). Diseases significantly restrict marigold cultivation. Various pathogenic microorganisms, including fungi, viruses, and bacteria, adversely influence the plant, leading to diseases that result in yield loss (Gurjar et al. 2019). In Bangladesh, numerous plant diseases emerge because of the rapid expansion of commercial marigold cultivation. Fungal diseases are the most detrimental to crops, particularly in the past decade (Abdel-Wahed 2020). White mold, produced by Sclerotinia sclerotiorum, is one of the most destructive fungal diseases affecting various crops, legumes, sunflowers, tobacco, and many other flowering plants (Nahar et al. 2019; Rahman et al. 2020).

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In all cases, the pathogen's white mycelium covered the infected region, and dark-colored sclerotia developed on the affected tissue (Hansda et al. 2014). The pathogen induces blight or decay in any aerial or underground plant part. Infected plants are randomly scattered around the flower bed or garden (Grabowski et al. 2017).

Marigold white mold is a newly emerging disease in our country. So, an effective management approach is crucial at this moment. Various management strategies, including cultural, biological, and chemical methods, are implemented in our country. Fungicides inhibit spore germination by targeting fungi's cellular structure or growth (Willbur et al. 2019). Cultural practices and fungicides are insufficient due to the pathogen's soil-borne characteristics, the prolonged viability of sclerotia in the soil, the generation of airborne ascospores, and the pathogen's extensive host range (Smolińska et al. 2018). Organic amendments and biological agents, like neem oil, sawdust ash, Trichocompost, and poultry refuse, may serve as additional control methods (Mello et al. 2005). Therefore, combining chemical and biological approaches to attain efficient, sustainable, and ecologically responsible disease management. Chemical fungicides offer fast and reliable control over pathogens, particularly in conditions of excessive disease pressure, whereas biological agents like Trichocompost or neem-based products facilitate prolonged suppression via mechanisms such as antagonism, competition, and the induction of plant defense responses. This combination mitigates excessive reliance on pesticides, thereby diminishing the possibility of fungicide resistance, reducing environmental contamination, and enhancing soil health (Hamim et al. 2024). Utilizing agricultural residues as soil amendments might effectively inhibit the germination of sclerotia of *S. sclerotiorum*, hence diminishing the development of ascospores (Huang et al. 2002). Neem oil significantly suppresses fungal development (Mahmoud et al. 2011). Sawdust ash and Trichocompost possess the capability to mitigate disease infections induced by *S. sclerotiorum* (Rajput et al. 2019; Egbontan et al. 2022). In this study, we assessed the potential effectiveness of various chemical fungicides and bio-agents to mitigate the marigold white mold disease induced by *S. sclerotiorum* under both in vitro and in vivo conditions.

The fungicides and bioagents employed in this trial were chosen for their diverse modes of action, proven efficacy in controlling fungal infections in related crops, and wide availability in Bangladesh. The fungicides encompass both systemic and contact categories, providing a comparative assessment of their effectiveness and resistance management ability. Moreover, these items are frequently utilized by local farmers, making the outcomes practical and applicable. The bioagents were incorporated as environmentally friendly, cost-effective options to promote sustainable disease management. The selection aimed to fill the research gap in marigold disease management under local conditions and identify effective and practical control strategies.

2. Materials and Methods

2.1. Experimental site and planting material

The experiment was carried out at the laboratory, net house, and research field of the Plant Pathology division at the Bangladesh Agricultural Research Institute (BARI), Gazipur. This experiment assessed chemical and biological treatments against white mold disease in marigolds. Eight chemical fungicides were employed under both laboratory and field conditions. In addition, four biological agents were applied as biological treatments in net house and field conditions. The marigold varieties Inca (hybrid) and China gada (local) were used as planting material.

2.2. Preparation of chemical fungicides

Several types of chemical fungicides from distinct functional groups were chosen for in vitro and in vivo analysis. Eight fungicides were used in this experiment. Tilt 250EC at 0.5 ml L^{-1} (Propiconazole group), Autostine at 2 g L^{-1} (Carbendazim group), Secure 600WG at 1 g L^{-1} (Fenamidone + Mancozeb group), Score 250EC at 2 ml L^{-1} (Difenoconazole group), Folicur 250EC at 2 ml L^{-1} (Tebuconazole group), Theropy 80WP at 2 g L^{-1} (Mancozeb group), Mancoforce Plus 72WP at 2 g L^{-1} (Mancozeb 64% + Metalaxyl 8% group), and Faja 70WP at 2 g L^{-1} (Mancozeb 45% + Fosetyl AL 25% group), along with a control were used as a field rates. In the case of an in vitro experiment, 100 ppm concentrations of all fungicides were incorporated into the liquid potato dextrose agar (PDA) media. At first, appropriate amounts of each fungicide were dissolved to prepare a concentrated stock solution, which was then serially diluted with sterilized distilled water to obtain the desired concentration.

2.3. Evaluation of chemical fungicides against *Sclerotinia sclerotiorum* in the laboratory

The fungicides were evaluated for their efficacy against the radial mycelial development of Sclerotinia sclerotiorum using the poisoned food technique. All required fungicides were incorporated into melted potato dextrose agar (PDA) media to achieve a concentration of 100 ppm. The PDA media containing treatments was immediately poured into 90 mm sterile Petri plates at 20 ml per plate, following a completely randomized design (CRD) with five replications. Untreated PDA media served as a control. Each plate was inoculated with the previously isolated pathogen *S. sclerotiorum*. The inoculated plates were incubated at 25±1°C for 7 days, and radial mycelial growth was measured at 3, 5, and 7 days after incubation (DAI). The quantity of Sclerotinia per plate and the percentage of colony growth inhibition were documented using the following formula (Vincent 1947):

Percent inhibition (I) = (C - T/C) ×100

Where,

C= Average diameter of fungal colony in control
T= Average diameter of the fungal colony in the treatment group.

2.4. Evaluation of biological agents against *Sclerotinia sclerotiorum* in the net house

An artificially inoculated pot experiment was conducted in the net house. Soil was inoculated with *S. sclerotiorum* grown on barley grain colonized with @15 g pot^{-1} before 21 days of seedling transplanting. Four biological agents, such as Trichocompost @150 g pot^{-1}, Neem oil cake @120 g pot^{-1}, Sawdust ash @200 g pot^{-1}, and Poultry refuse @250 g pot^{-1} were incorporated with soil before 7 days of seedling transplanting. The pot experiment was

laid out in a complete randomized design (CRD) with five replications. Two seedlings were sown in each pot. The size of the pot was (60×30) cm^2, and each pot contained 15 kg of soil. Data were recorded on percent disease incidence and disease severity, disease reduction over control, number of infected flowers/plants, and number of uninfected flowers/plants.

2.5. Evaluation of chemical fungicides and biological agents against *Sclerotinia sclerotiorum* under natural epiphytotic conditions in the field

The field experiment was executed in the research area where natural infection by *S. sclerotiorum* developed in marigolds. The size of the unit plot was (2×1.5) m^2, and the line spacing was (40×40) cm^2. The randomized complete block design (RCBD) was employed with three replications. Each plot contained twenty seedlings. All chemical fungicides were administered at the recommended dosages following the onset of diseases at 7-day intervals. For biological management, Trichocompost at 2.5 tons/ha, Neem oil cake at 2 t ha^{-1}, Sawdust ash at 6 tons/ha, and Poultry refuse at 10 t ha^{-1} were incorporated into the soil at the appropriate rates before seedling transplantation, 7 days in advance. The plots were routinely examined to monitor indications of white mold disease, including the presence of white mycelium and sclerotia. Intercultural operations were conducted as required. Five plants per plot were randomly selected for sample collection. Data were collected on disease incidence, disease severity, disease reduction over control, number of infected flowers per plant, and number of uninfected flowers per plant.

2.6. Disease assessment

The percentage of disease incidence under net house and field conditions was calculated using the formula provided by James (1974).

$$\text{Percent disease incidence} = \frac{No.of\ infected\ leaves}{Total\ no.of\ leaves\ assessed} \times 100$$

Assessment of disease severity for white mold diseases was recorded 7 days after the last spray and calculated by using the following formula, which was provided by Suryadi et al., (2013).:

$$\text{Disease severity (\%)} = (\Sigma\ n \times v / N \times V) \times 100$$

Where, n= Number of infected flowers, v= Numerical value assigned to each category of infection based on the 0-5 visual scale, N= Total number of observed flowers, V= Maximum scale value

The disease intensity was determined by a 0-5 visual scale (Rahman and Rahid 2008). Here, 0= non-infected, 1= <1% flower area infected, 2= 1-10% flower area infected, 3= 11-20% flower area infected, 4= 21-50% flower area infected, 5= >50% flower area infected.

The percentage of disease reduction over control was calculated using the following formula:

$$\text{Percent of disease reduction over control} = \frac{x-y}{y} \times 100$$

Where, x = Mean value of each treatment (Tn, n = 1-8) and y = Mean under control (T_9)

2.7. Data analyses

Data were analyzed for ANOVA and Duncan's Multiple Range Test (DMRT) using the MSTAT-C program to compare the means for all phases of the Experiment.

3. Results

3.1. Effect of selected fungicides on radial mycelial growth and number of Sclerotia of *Sclerotinia sclerotiorum* on PDA media under in vitro conditions

The recorded data on the impact of selected fungicides on the radial mycelial growth of *Sclerotinia sclerotiorum* in PDA media under in vitro conditions indicated mycelial growth measurements of 0-39.54 mm, 0-55.44 mm, and 0-81.33 mm at 3, 5, and 7 days after inoculation (DAI), respectively (Table 1). The treatments exhibited significant variation from one another. In the evaluated treatments, complete inhibition of colony growth was observed in T_1 (Tilt 250EC), T3 (Secure 600WG), T_4 (Score 250EC), T_5 (Folicur 250EC), and T8 (Faja 70WP) at 3, 5, and 7 DAI. The highest radial mycelial growth of *S. sclerotiorum* was recorded in T_9 (control) across all observations. The highest count of Sclerotia per plate (51) was recorded in treatment T_9, while treatments T_1, T_3, T_4, T_5, and T_8 exhibited no Sclerotia at 7 DAI.

3.2. Effect of selected fungicides on uninfected and infected flowers/plants in field conditions

The quantity of uninfected and infected flowers per plant was positively influenced by different chemical treatments (Table 2). In both varieties, the highest number of uninfected flowers per plant (V_1= 15 and V_2= 26.93) of marigold was recorded in treatment T_4 (Score 250EC), which was statistically similar to T8 (Faja 70WP), while the lowest number of uninfected flowers was noted in treatment T_9 (Control). In the case of V_1, the highest number of infected flowers per marigold plant (6.99) was recorded in T_9 (Control), whereas the lowest number of infected flowers per plant (1.77) was noted in treatment T_4 (Score 250EC), which was statistically similar to treatment T_8 (Faja 70WP). In V_2, the highest number of infected flowers per plant (17.28) was recorded in T_9, which was statistically identical to treatments T_1 and T_3. Conversely, the lowest number of infected flowers per plant (8.69) was noted in treatment T_4, which was statistically similar to treatment T_8.

3.3. Effect of selected fungicides on white mold disease incidence (%) and disease severity (%) in field conditions

The impact of different fungicides on marigold white mold disease incidence ranged from 10.55% to 40.88% in V_1 (Inca-hybrid variety) and 24.40% to 47.92% in V_2 (Local variety) (Fig. 1a). T_9 (Control) had the highest disease incidence, while T_4 (Score 250EC) had the lowest. In contrast, all treatments significantly reduced disease compared to the control. T_6 (Theropy 80WP) had the lowest disease reduction of 23.63% in field conditions, while T_4 (Score 250EC) had the highest at 74.19%. The V_2 showed that T_4 (Score 250EC) reduced disease the most (49.08%), while T_3 (Secure 600WG) reduced minimum compared to the control. Disease severity ranged from 8.71% to 22.83% in V_1 and 14.02% to 26.44% in V_2 (Fig. 1b).

Table 1. Effect of selected fungicides on radial mycelial growth at 3, 5, and 7 DAI and number of Sclerotinia of *Sclerotinia sclerotiorum* on PDA media in the in vitro condition.

Treatments	Mycelia growth (mm)						No. of Sclerotinia per plate
	3 DAI		5 DAI		7 DAI		
	Radial colony growth (mm)	Inhibition of colony growth (%)	Radial colony growth (mm)	Inhibition of colony growth (%)	Radial colony growth (mm)	Inhibition of colony growth (%)	
T_1	0.00 d	100	0.00 d	100	0.00 d	100	0
T_2	8.13 c	79.43	11.07 c	80.03	16.08 c	80.22	28
T_3	0.00 d	100	0.00 d	100	0.00 d	100	0
T_4	0.00 d	100	0.00 d	100	0.00 d	100	0
T_5	0.00 d	100	0.00 d	100	0.00 d	100	0
T_6	21.47 b	45.70	26.23 b	52.68	30.97 b	61.92	33
T_7	6.43 c	83.73	7.08 c	87.22	12.03 c	85.20	26
T_8	0.00 d	100	0.00 d	100	0.00 d	100	0
T_9	39.54 a	-	55.44 a	-	81.33 a	-	51
Level of sig.	**	-	**	-	**	-	-
CV (%)	4.55		3.75		2.41		

Here, **= 1% level of significance, CV= Coefficient of variation, DAI= Days after incubation. T_1=Tilt 250EC, T_2=Autostine, T_3= Secure 600WG, T_4= Score 250EC, T_5= Folicur 250EC, T_6= Theropy 80WP, T_7= Mancoforce Plus 72 WP, T_8= Faja 70 WP, T_9= Control

Table 2. Effect of selected fungicides on the flower characters of white mold disease of Marigold in the field conditions.

Treatments	V_1 (HYV)		V_2 (LV)	
	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}
T_1	10.76 c	4.11 ab	19.98 c	14.14 ab
T_2	10.78 c	2.82 b	20.89 b	13.61 b
T_3	11.55 bc	4.32 ab	18.38 c	15.06 a
T_4	15.00 a	1.77 c	26.93 a	8.69 d
T_5	13.55 b	2.94 b	21.28 bc	11.28 c
T_6	11.26 bc	5.11 ab	22.0 bc	11.89 c
T_7	12.89 bc	3.54 b	19.17 c	11.83 c
T_8	14.53 ab	2.10 c	26.50 a	9.14 d
T_9	10.11 c	6.99 a	18.78 c	17.28 a
Level of sig.	**	**	**	**
CV (%)	12.44	12.05	14.19	15.41

Here, **= 1% level of significance; CV= Coefficient of variation; DAI= Days after incubation; V_1= Inca (hybrid) and V_2= China gada (local). T_1=Tilt 250EC, T_2=Autostine, T_3= Secure 600WG, T_4= Score 250EC, T_5= Folicur 250EC, T_6= Theropy 80WP, T_7= Mancoforce Plus 72 WP, T_8= Faja 70 WP, T_9= Control

The most severe disease was in T_9 for both kinds, whereas the least severe was in T_4. All treatments reduced diseases significantly compared to the control group. V_1 disease reduction was highest at 61.84% with T_4, and lowest at 23.62% with T_3. In V_2, T_4 had the highest disease decrease (46.97%), whereas T_2 (Autostine) had the lowest compared to the field trial control.

3.4. Effect of biological treatments on uninfected and infected flowers/plants in the net house

Various biological treatments had a significant impact on the number of uninfected and infected flowers per plant (Table 3). The highest number of uninfected flowers (14.53) was recorded in the T_2 treatment for V_1, while the lowest (6.80) was observed in the T_5 (Control) treatment, which was statistically similar to T_3. The V_2 indicated that the highest number of uninfected flowers per plant (19.87) occurred in the T_2 treatment, while the lowest number (8.84) was recorded in the T_5 (Control) treatment under pot conditions. The control (T_5) exhibited the highest numbers of infected flowers per plant, with values of 10.50 in V_1 and 16.95 in V_2 in both varieties. The T_2 treatment exhibited the lowest number of infected flowers per plant, with values of 3.28 for the V_1 variety and 6.25 for the V_2 variety.

3.5. Effect of selected bioagents on white mold disease incidence (%) and disease severity (%) in the net house.

The effect of selected biologic treatments on marigold white mold disease incidence varied from 18.42 to 60.69 % in V_1 (hybrid variety) and 23.93 to 65.72 % in V_2 (Local

variety Fig. 2a). Both kinds had the highest disease incidence (60.69%) and (65.72%) from T_5 treatment (Control) and the lowest (18.42%) and (23.93%) from T_2 treatment. However, all treatments reduced the disease significantly over the control. V_1 disease reduction was highest (69.66%) from T_2 (Neem oil cake) and lowest (10.94%) from T_3 (Sawdust ash).

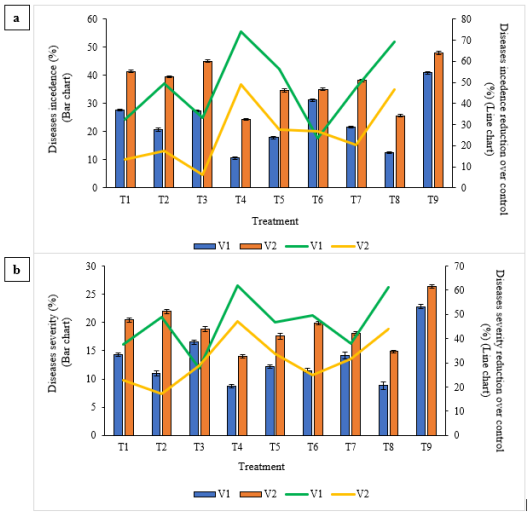

Figure 1. Effect of selected fungicides on white mold disease under two Marigold varieties in the field. (a) Disease incidence and disease incidence reduction over control; (b) Disease severity and disease severity reduction over control. Here, V_1= Inca (hybrid) and V_2= China gada (local); T_1=Tilt 250EC, T_2=Autostine, T_3= Secure 600WG, T_4= Score 250EC, T_5= Folicur 250EC, T_6= Theropy 80WP, T_7=Mancoforce Plus 72 WP, T_8= Faja 70 WP, T_9= Control.

Table 3. Effect of biological treatments on the flower characters of white mold diseases of Marigold in the net house.

Treatments	V_1 (HYV)		V_2 (LV)	
	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}
T_1	11.93 b	5.73 c	15.33 b	10.66 c
T_2	14.53 a	3.28 d	19.87 a	6.25 d
T_3	7.93 c	9.33 ab	10.16 c	15.71 b
T_4	9.50 bc	8.10 b	13.23 bc	12.43 bc
T_5	6.80 c	10.50 a	8.84 d	16.95 a
Level of significance	**	**	**	**
CV (%)	14.18	13.05	12.63	12.47

Here, **= 1% level of significance; CV= Coefficient of variation; DAI= Days after incubation; V_1= Inca (hybrid) and V_2= China gada (local). T_1= Trichocompost @150 g pot^{-1}, T_2= Neem oil cake @120 g pot^{-1}, T_3= Saw dust ash @200 g pot^{-1}, T_4= Poultry refuse @250 g pot^{-1}, T_5= Control.

The V_2 (local variants) revealed that T_2 (Neem oil cake) reduced disease by 63.60%, while T_3 (Sawdust ash) reduced disease by 7.60% over the control. V_1 (hybrid variety) had 20.77–30.62% disease severity and V_2 (local variety) 21.18–32.83 % (Fig. 2b). The highest disease severity was in T5 (Control) treatment in both varieties, whereas the lowest was in T_2. However, all treatments reduced the disease significantly over the control. Neem oil cake (32.17%) and sawdust ash (17.80%) reduced diseases the most and least in V_1. The V_2 (local variety) showed the best disease decrease (35.49%) in T_2 (Neem oil cake) and the lowest in T_3 (Sawdust ash) over T_9 (control) (Fig. 2b).

3.6. Effect of biological treatments on uninfected and infected flowers/plants in the field conditions

Several biological treatments significantly influenced the quantity of both uninfected and infected flowers per plant (Table 4). The T_2 treatment for V_1 recorded the highest number of uninfected flowers at 15.39, whereas the lowest count of 6.49 was observed in the T_5 (Control) treatment, which was statistically comparable to T_3. The V_2 results showed that the T_2 treatment had the highest number of uninfected flowers per plant (19.87), whereas the T_5 (Control) treatment recorded the lowest number (8.58) under pot conditions. The control (T_5) demonstrated the highest incidence of infected flowers per plant, recording values of 10.67 in V_1 and 16.95 in V_2 across both varieties. The T_2 treatment demonstrated the lowest incidence of infected flowers per plant, recording values of 2.15 for the V_1 variety and 6.78 for the V_2 variety.

Table 4. Effect of biological treatments on the flower characters of white mold diseases of Marigold in field condition.

Treatments	V_1 (HYV)		V_2 (LV)	
	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}	No. of uninfected flowers plants^{-1}	No. of infected flowers plants^{-1}
T_1	13.10 ab	4.37 c	15.11 b	10.94 c
T_2	15.39 a	2.15 d	19.87 a	6.78 d
T_3	7.94 c	9.12 ab	10.85 bc	15.17 a
T_4	9.34 b	7.89 b	13.16 b	12.67 bc
T_5	6.49 c	10.67 a	8.58 c	16.95 a
Level of sig.	**	**	**	**
CV (%)	17.29	16.84	15.66	17.80

Here, **= 1% level of significance; CV= Coefficient of variation; DAI= Days after incubation; V_1= Inca (hybrid) and V_2= China gada (local). T_1= Trichocompost @150 g pot^{-1}, T_2= Neem oil cake @120 g pot^{-1}, T_3= Saw dust ash @200 g pot^{-1}, T_4= Poultry refuse @250 g pot^{-1}, T_5= Control.

3.7. Effect of selected bioagents on white mold disease incidence (%) and disease severity (%) in field conditions

The impact of multiple biologic treatments on marigold white mold disease incidence was 12.26% to 62.18% for V_1 (hybrid variety) and 24.88% to 66.39% for V_2 (local variety) (Fig. 3a). The highest disease incidence was 62.18% and 66.39% for T_5 (Control), while the lowest was 12.26% and 24.88% for T_2 (Neem oil cake). Compared to the control group, all treatments significantly reduced disease. T_2 (Neem oil cake) reduced V_1 disease by 80.29%, whereas T3 (Sawdust ash) reduced it by 14.03%.

The T_2 (Neem oil cake) treatment reduced disease by 62.53% in the V_2 (local variety), whereas the T_3 (Saw dust ash) treatment reduced disease by 12.19% compared to the control (Fig. 3a). Disease severity ranged from 21.20% to 32.71% in V_1 (hybrid) and 23.83% to 34.82% in V_2 (local) (Fig. 3b). T_5 (Control) had the most severe disease for both types, whereas T_2 had the least. Compared to the control group, all treatments significantly reduced disease. Neem oil cake (T_2) reduced disease by 35.19% in V_1, while sawdust ash (T_3) reduced it by 17.95%. V_2 (local variety) had the maximum disease reduction of 31.56% in T_2 (Neem oil cake), while T_3 (Sawdust ash) had the lowest compared to T_9 (control) (Fig. 3b).

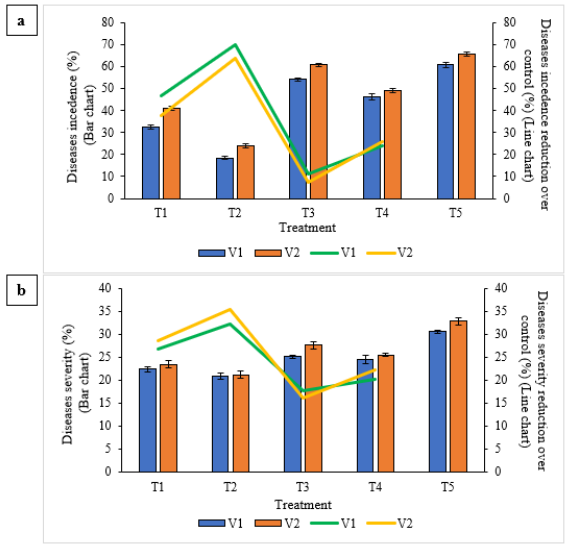

Figure 2. Effect of biological treatments on white mold disease under two Marigold varieties in the net house. (a) Disease incidence and disease incidence reduction over control; (b) Disease severity and disease severity reduction over control. Here, V_1= Inca (hybrid) and V_2= China gada (local); T_1= Trichocompost @150 g pot^{-1}, T_2= Neem oil cake @120 g pot^{-1}, T_3= Saw dust ash @200 g pot^{-1}, T_4= Poultry refuse @250 g pot^{-1}, T_5= Control.

4. Discussion

Marigold is infected by *Sclerotinia sclerotiorum*, resulting in white mold disease, which has presented a significant issue in Bangladesh during the past decade. Synthetic fungicides serve as the primary protection against *S. sclerotiorum* (Elsheshtawi et al. 2017; Mueller et al. 2002). Nevertheless, their utilization is significantly constrained globally due to environmental and health risks, in addition to consumer preferences for safe, natural alternatives (Hewedy et al. 2020). The in vitro assessment of Tilt 250EC at 0.5 ml L^{-1} (Propiconazole), Secure 600WG at 1 g/L (Fenamidone + Mancozeb), Score 250EC at 2 ml L^{-1} (Difenoconazole), Folicur 250EC at 2 ml L^{-1} (Tebuconazole), and Faja 70WP at 2 g L^{-1} (Mancozeb 45% + Fosetyl AL 25%) against the mycelial growth of *S. sclerotiorum* in marigold demonstrated that the evaluated concentrations effectively inhibit pathogen growth. This finding was validated by Rakesh et al. (2016). Score 250 EC (Difenoconazole) inhibits *S. sclerotiorum*, which causes stem rot in mustard (Roy et al. 2021), while Tabuconazole is effective on rapeseed mustard (Zamani-Noor, 2021), agreeing with the current findings.

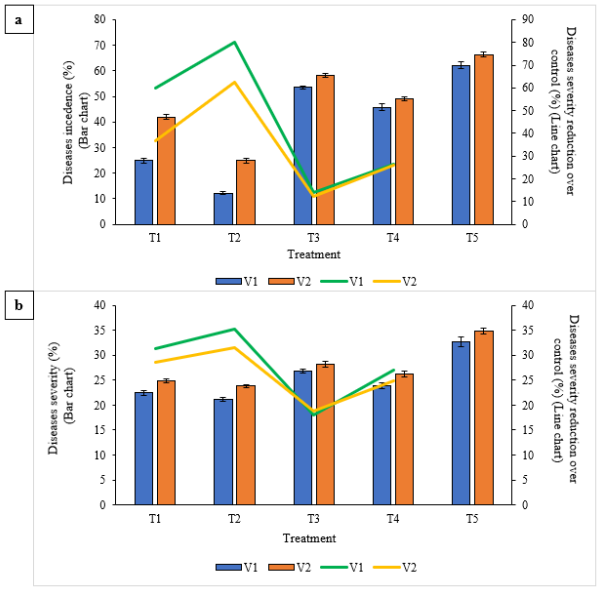

Figure 3. Effect of biological treatments on white mold disease under two Marigold varieties in the field. (a) Disease incidence and disease incidence reduction over control; (b) Disease severity and disease severity reduction over control. Here, V_1= Inca (hybrid) and V_2= China gada (local); T_1= Trichocompost @2.5 t ha^{-1}, T_2= Neem oil cake @2 tons/ha, T_3= Saw dust ash @6 t ha^{-1}, T_4= Poultry refuse @10 t ha^{-1}, and T_5= Control.

In the field assessment, fungicides had a substantial effect compared to the control condition. Field investigations indicate that various fungicides effectively manage *S. sclerotiorum*, although they do not achieve total control. Score 250EC at 2ml L^{-1} (Difenoconazole group) and Faja 70WP at 2g/L (Mancozeb 45% + Fosetyl AL 25% group) exhibited the lowest incidence of disease. Difenoconazole, a triazole fungicide, functions as a systemic demethylation inhibitor (DMI) by inhibiting ergosterol formation through the inhibition of the enzyme C14-demethylase. Ergosterol is a vital constituent of fungal cell membranes; its disruption results in decreased membrane integrity, suppression of mycelial proliferation, and diminished spore generation. This systemic action enables the fungicide to penetrate plant tissues, offering both protective and curative treatments against various fungal diseases. (Lucas et al., 2015). Sharma et al. (2017) similarly observed results for the carbendazim and mancozeb treatment on indian mustard. Chemical fungicides also mitigate flower infections caused by white mould fungus. Maximum uninfected flowers per plant were recorded from the specimens treated with Score 250 EC and Faja 70WP. The pot experiments demonstrated that biological amendments combined with soil effectively controlled *S. sclerotiorum*. The study's outcome reveals that Neem oil cake diminished both the incidence and severity of sclerotium disease in Marigold. The efficacy of neem oil cake may inhibit disease progression due to its antifungal ingredients' effects against *S. sclerotiorum* (Adusei and Azupio 2022). Neem-based products include rich bioactive chemicals, including azadirachtin, nimbin, salannin, and other limonoids, which exhibit potent antifungal effects. These chemicals can disrupt fungal cell membrane integrity, restrict spore germination, and obstruct fungal growth and development (Adusei and

Azupio 2022; Abrahim et al. 2025). Biological control exhibited comparable results in field conditions for both varieties as observed in pot experiments. Neem oil cake suppressed flower infections and produced the maximum yield of uninfected flowers. Neem oil reduced disease incidence by 80.29% in the hybrid marigold variety and by 62.53% in the local variety. Disease severity was found at 21.2% and 23.83% for the hybrid and local varieties, respectively, following the use of neem oil. Synthetic chemical fungicides pose health and environmental risks; conversely, neem oil appears to be an effective alternative for controlling marigold white mould disease. Moslem and El-Kholie (2009) reported that around 14 prevalent fungal species exhibit sensitivity to neem formulations. Various plant diseases, including anthracnose, downy mildew, rust, and black spot, have been reported to be treatable with neem extracts (Brahmachari 2004).

5. Conclusion

Among the evaluated fungicides, Score 250EC and Faja 70WP exhibited the most significant inhibition of mycelial growth and reduction of sclerotia production of *Sclerotinia sclerotiorum* in both laboratory and field conditions throughout both varieties. The biocontrol agent neem oil cake showed the most significant disease suppression compared to the control in both pot house and field conditions. Nonetheless, additional trials are required to evaluate the effectiveness of fungicides and biocontrol agents against *S. sclerotiorum* across various agroecological zones.

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