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In vitro Efficacy of Five Modern Fungicides Compared with Mancozeb against *Botrytis fabae* Sardi, the Causal Agent of Chocolate Spot of Faba Bean

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Abstract

Chocolate spot of faba bean, caused by the fungus Botrytis fabae Sardi, is one of the most destructive fungal diseases constraining faba bean (Vicia faba L.) productivity and inflicting severe economic losses on farmers in Syria and the surrounding region. The present study aimed to evaluate the in vitro efficacy of five modern fungicides belonging to different chemical groups (STROBY EXPERT, CAPRIO TOP, PROCYMIDON STAR, MATADOR EXPERT and MENOL) in comparison with the conventional contact fungicide MANCOZEB, in inhibiting the mycelial growth of B. fabae, and to identify the most effective fungicide and concentration. The poisoned food technique on Potato Dextrose Agar (PDA) was applied at three concentrations per fungicide - the full recommended dose, half dose and quarter dose - together with an untreated control (sterile distilled water). Mycelial colony diameter was measured after seven days of incubation and the percentage inhibition was calculated.

STROBY EXPERT achieved the highest inhibition (83.26%) at the full dose, followed by PROCYMIDON STAR (82.05%) and MATADOR EXPERT (81.80%), with highly significant differences from MANCOZEB, which did not exceed 66.52% at the full dose and collapsed to only 5.96% at the quarter dose, indicating that the pathogen has acquired resistance to this conventional multi-site fungicide. The study further demonstrated a sharp decline in the efficacy of all tested fungicides as the concentration decreased, confirming the importance of strict adherence to recommended doses and of fungicide rotation. The adoption of modern single-site fungicides with potent active ingredients in integrated management programs, together with continuous monitoring of resistance development, is recommended in order to sustain chemical efficacy against B. fabae.

Keywords: *Botrytis fabae*; fungicide efficacy; poisoned PDA; chocolate spot

1. Introduction

1.1 Importance of faba bean and cultivated area

Faba bean (Vicia faba L.) is one of the most important cool-season grain legumes cultivated worldwide. It is rich in proteins, dietary fibre, vitamins and minerals, and therefore occupies a central place in the food-security systems of many developing countries, where it is consumed as a staple traditional dish. According to the Food and Agriculture Organization (FAO, 2020), faba bean is grown on approximately 25 million hectares worldwide, producing around 25 million tonnes annually, with China, India and Myanmar leading global production. In Syria, faba bean is considered a successful cash crop cultivated on roughly 200 000 ha to produce about 300 000 tonnes per year, supporting both the food and the rural-employment sectors, in spite of persistent constraints such as water scarcity and rising production costs (FAO, 2020).

1.2 Chocolate spot of faba bean

Chocolate spot of faba bean is caused by the fungus Botrytis fabae Sardi. The disease is one of the most damaging fungal disorders affecting faba bean at all growth stages, from the seedling to the mature plant, and may cause additional losses during storage. Symptoms appear as grey or brown lesions on pods and seeds that progressively enlarge until the fungus covers the affected tissue with a sporulating mat (Elad et al., 2016). Damage ranges from seed deterioration and loss of germination to secondary infections, and yield losses may reach 3050% when no effective control measure is implemented (Singh et al., 2019).

1.3 Current control practices

Control of chocolate spot relies mainly on fungicidal applications that limit the spread of B. fabae, the most widely used products being fungicides containing carbendazim and myclobutanil applied at regular intervals before flowering and through the growing season, with a 15-day interval between sprays. Good agricultural practices such as improved canopy aeration, reduction of relative humidity, removal of crop residues, crop rotation and the use of resistant or tolerant cultivars are also recommended, in order to reduce the dependence on chemical inputs and to safeguard environmental and human health (Abd El-Aziz et al., 2018).

Recent global reports confirm that Botrytis spp. populations are increasingly resistant to several single-site and multi-site fungicide classes, including methyl-benzimidazole carbamates (MBCs), dicarboximides, quinone-outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs) (Abbey et al., 2024; Liu et al., 2024; Wu et al., 2024; Wu et al., 2025). The emergence of multidrug-resistant (MDR) phenotypes, particularly in B. cinerea, the sister species of B. fabae, raises serious concerns about the long-term sustainability of chemical control and highlights the need to continuously screen new active ingredients against local pathogen populations (Derbalah et al., 2026; Jang et al., 2026). The present work contributes to this effort by comparing the in vitro efficacy of five modern fungicides with that of the conventional mancozeb against a Syrian isolate of B. fabae.

2. Literature Review

2.1 Faba bean as a strategic crop

Faba bean is one of the most globally important crops, particularly in regions with temperate climates, as a key source of plant protein and energy for both humans and livestock. According to the American Society of Agronomy (2022), faba bean is classified among the staple food

crops due to its richness in protein, amino acids, vitamins and minerals (Smith et al., 2022), and is also used as a high-quality forage crop in areas requiring inexpensive sources of protein (Jones & Lee, 2023). Within the framework of sustainable agriculture, faba bean improves soil fertility through biological nitrogen fixation, thereby reducing the need for synthetic fertilizers, supports crop diversification, and enhances the resilience of cropping systems to diseases and pests (Johnson & Patel, 2022).

2.2 Chocolate spot and its economic impact

Chocolate spot of faba bean caused by B. fabae is among the most destructive fungal diseases affecting a wide range of hosts including faba bean, grapevine, tomato, strawberry and ornamental plants (Elad et al., 2016). Studies conducted in Syria, Lebanon and Jordan have shown that B. fabae can cause yield losses of 40-60% in faba bean, accompanied by deterioration of grain quality and a reduction in viability for both seeding and storage (AlAhmad et al., 2022). In Iraq, losses were estimated at approximately 50% of total production, particularly under rainy and humid conditions, with a negative impact on farm income and product quality (Abouelenein et al., 2022). Miller and Roberts (2023) reported losses of 3050% in infected areas as a result of tissue and grain deterioration. Recent work has further shown that the disease damages stems and leaves, reducing photosynthesis and weakening plant growth and productivity , thereby underscoring the need for effective control strategies and a precise definition of effective fungicide rates in order to reduce economic and environmental damage (Li & Zhang, 2024).

2.3 Taxonomy and species complex of Botrytis fabae

Botrytis fabae is classified within the phylum Ascomycota, family Sclerotiniaceae, and is characterised by a high dispersal and adaptation capacity, with strains differing in their sensitivity to fungicides and in their fitness under varying environmental conditions (Williamson et al., 2007). Strains are differentiated on the basis of genetic variation, environmental response and pathogenicity traits, and this differentiation has produced cryptic taxa such as B. fabae sensu stricto and B. pseudofaba, in addition to other lineages with enhanced ability to withstand fungicide pressure (López et al., 2021).

2.4 Infection mechanism and disease cycle

Infection usually begins when damaged or senescent plant tissues are colonised by Botrytis mycelium, which grows both inter- and intracellularly. The fungus releases conidia that are dispersed by wind, water or agricultural tools to healthy tissues, particularly under humid and moderately warm conditions. Once attached, the conidia germinate and produce germ tubes that invade host tissues and subsequently yield new conidial masses that cover the lesion with a grey-brown sporulating layer (Elad et al., 2016). Disease development is rapid under high relative humidity and temperatures of 15-25 C; the conidia produced perpetuate the secondary cycle within the same plant and spread to neighbouring fields (Williamson et al., 2007).

2.5 Laboratory and field evaluation of Botrytis control

Fungal resistance to fungicides is one of the most prominent challenges facing agricultural pest management worldwide, and the genus Botrytis is among the plant pathogens most prone to developing resistance to conventional fungicides. Loss of efficacy undermines productivity across many crops and causes substantial economic damage (Fernández-Ortuño et al., 2023). Laboratory studies on fungicide-amended media are therefore essential for selecting the appropriate active ingredient and for reducing the dependence on conventional products that may accelerate the build-up of resistance (Elad & Pertot, 2014).

Sabbour and Abdel-Rahim (2020) demonstrated that PDA media amended with fungicides at 50-200 ppm allow a reliable estimation of the ability of a given active ingredient to inhibit colony growth. In their study, stroby expert produced complete inhibition at 200 ppm, with a colony diameter of 0 mm versus 85 mm in the untreated control, whereas carbendazim achieved 89.5% inhibition with an 8.9 mm colony, and mancozeb did not exceed 72.3% with a 23.5 mm colony, indicating that the efficacy of some conventional fungicides has already declined (Fernández-Ortuño et al., 2023). The SDHI class has recently emerged as a key alternative, achieving complete inhibition at low concentrations (Zhang et al., 2021), while other studies have emphasised the necessity of defining optimal application rates before field deployment in order to ensure efficacy and minimise environmental impact (El Husein & Rashed, 2018), with continuous resistance monitoring to safeguard sustainability (Kato & Takahashi, 2015; Malik & Malik, 2019).

At the field scale, El-Kholy (2007) evaluated ten fungicides including tebuconazole, topsinM, maxim, copperoxychloride and difolatan, and found that topsin-M gave the highest reduction in disease incidence (80%) compared with the control. In a trial by Smith and Doe (2023), the fungicide maxim at 750 mL ha-1 was the most effective, reducing disease by 85%, followed by tebuconazole (78%). Ahmed et al. (2024) reported that early and repeated fungicide application improved disease control by 87%, while Nguyen and Tran (2022) showed that the chemical topsin-M was superior to biological control with Trichoderma sp., reducing disease by 87% and improving yield and economic return. Most recently, Derbalah et al. (2026) demonstrated that, in field trials over two seasons, the recommended fungicide (mancozeb) provided the highest control of chocolate spot of faba bean, while the monoterpenes linalool, carvone and cineole - tested as eco-friendly alternatives - significantly reduced disease severity and induced defence enzymes, suggesting their potential as sustainable complements to chemical control.

With respect to resistance and the impact of reduced rates, Leroux et al. (2020) reported high variability in the sensitivity of B. fabae and B. cinerea populations to single- and multi-site fungicides at sub-lethal concentrations, while Hahn and Hausladen (2021) demonstrated that the dose-response relationship of modern fungicides against Botrytis spp. carries important implications for resistance management. Amiri et al. (2021) confirmed that low doses of single-site systemic fungicides may select for resistant individuals in fungal populations, and Dean et al. (2022) emphasised the role of sub-lethal doses in the evolution of resistance in plant-pathogenic fungi. Wu et al. (2024) further showed that procymidone application contributes to the development of multidrug resistance in B. cinerea, and Wu et al. (2025) reviewed the efflux-mediated mechanisms that underpin multidrug resistance and the management strategies required to contain it. Liu et al. (2024) characterised the molecular basis of SDHI resistance in B. cinerea, while Jang et al. (2026) reported a high frequency of QoI-resistant genotypes in B. cinerea populations from Korean strawberry farms, and Abbey et al. (2024) confirmed widespread resistance to multiple fungicide classes in B. cinerea and related Botrytis spp. Weber and Hahn (2021) summarised the resistance mechanisms, fitness costs and management strategies in B. cinerea.

3. Objectives

The present study aims to evaluate the in vitro efficacy of five modern fungicides belonging to different chemical groups, alongside the conventional contact fungicide MANCOZEB, in inhibiting the mycelial growth of Botrytis fabae Sardi - the causal agent of chocolate spot of faba bean - and to identify the most effective fungicide and concentration. The work is intended

to contribute to the development of more effective and sustainable chemical control strategies against this damaging pathogen.

4. Materials and Methods

4.1 Fungicides and pathogen isolate

Six fungicidal formulations were evaluated: five modern products belonging to different chemical groups (STROBY EXPERT, CAPRIO TOP, PROCYMIDON STAR, MATADOR EXPERT and MENOL), and the conventional contact fungicide MANCOZEB, included as a comparative reference toward which the test pathogen has already shown reduced sensitivity. An untreated control consisting of sterile distilled water only was also included. The commercial name, active ingredient, manufacturer, recommended field dose and the three invitro concentrations used in this study are summarised in Table 1. A single-spore isolate of B. fabae was obtained from the Plant Pathology Laboratory of the Biological Control Directorate at Hama, Syria, and maintained on PDA at 22 +/- 2 C.

Table 1. Fungicides tested in vitro, their active ingredients and the three concentrations used in the poisoned PDA assay.

No.	Commercial name	Active ingredient	Field dose	Full dose (lab)	1/2 dose	1/4 dose
1	STROBY EXPERT	Kresoxim-methyl 50% WDG	30-50 g/100 L water	0.30 g/L	0.15 g/L	0.075 g/L
2	CAPRIO TOP	Pyraclostrobin 5% + Metiram 55%	150-200 g/100 L water	0.15 g/L	0.075 g/L	0.04 g/L
3	PROCYMIDON STAR	Procymidone 50% WP	50-100 g/100 L water	0.5 g/L	0.25 g/L	0.125 g/L
4	MATADOR EXPERT	Myclobutanil 12.5% EC	50 mL/100 L water	0.5 mL/L	0.25 mL/L	0.13 mL/L
5	MENOL	Triadimenol 25% EC	100 mL/100 L water	0.1 mL/L	0.05 mL/L	0.025 mL/L
6	MANCOZEB	Mancozeb 500 g/kg WG	50-100 g/100 L water	0.5 g/L	0.25 g/L	0.13 g/L

4.2 Preparation of poisoned PDA medium

Fungicide stock solutions were prepared at three concentrations for each product (the full recommended dose shown on the label, half dose, and quarter dose) following the manufacturers' recommendations. All glassware and tools were sterilised by autoclaving at 121 C for 20 min to avoid contamination. The required volumes of each fungicide solution were aseptically added to molten sterile PDA (cooled to approximately 45 C), mixed thoroughly, and poured into 90 mm Petri dishes. Once solidified, each plate was inoculated at the centre with a 0.5-mm mycelial plug cut from the edge of an actively growing 7-day-old B. fabae colony. Inoculated plates were incubated at 22 +/- 2 C in the dark. The colony diameter (mm) was measured after seven days of incubation using a transparent ruler; in the untreated control, the fungal colony had covered the entire plate surface by that time.

4.3 Experimental design and statistical analysis

The experiment was arranged in a Completely Randomised Design (CRD) with three replicates per treatment. Data were analysed using the Genstat 12 statistical package. Analysis of variance (ANOVA) was performed and treatment means were separated using the Least Significant Difference (LSD) test at the 0.01 probability level to compare fungicide efficacy and to identify the most effective treatment against B. fabae. The percentage inhibition of mycelial growth was calculated according to the formula:

$$\textbf{Inhibition (\%)} = [(C - T) / C] \times 100$$

where C is the mean colony diameter (mm) in the untreated control and T is the mean colony diameter (mm) in the treated plate after seven days of incubation.

Results (Revised with Field Verification and Microscopic Figures)

5. Results

The infection by Botrytis fabae Sardi was verified on faba bean plants growing in farmers' fields of Idlib Governorate, in the Kafr Yahmoul and Ma'artamisrayn areas. Diseased leaves showing the typical chocolate-spot lesions were collected during the 2023–2024 growing season and brought to the laboratory for isolation. The pathogen was re-isolated on Potato Dextrose Agar (PDA), purified by single-spore transfer, and the identity of the resulting isolate was confirmed on the basis of cultural and microscopic features (Figures 1 and 2). The verified isolate was subsequently used as the test pathogen in all poisoned-PDA assays reported below.

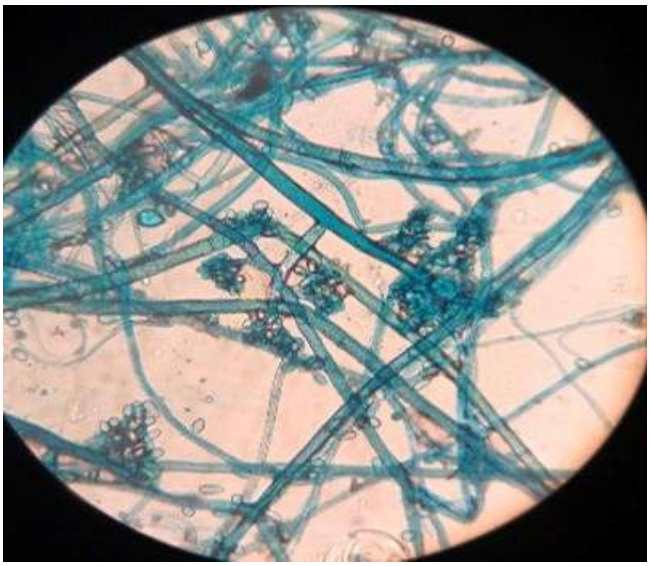

Figure 1. Microscopic morphology of Botrytis fabae mycelium stained with lacto-phenol cotton blue, showing the septate, branched hyphae and the typical dense mycelial network of the isolate recovered from infected faba bean leaves collected in the Kafr Yahmoul area of Idlib Governorate (magnification 400x).



Figure 2. Characteristic chocolate-spot symptoms on faba bean leaves naturally infected with Botrytis fabae in farmers' fields at Ma'artamisrayn (Idlib Governorate). Lesions appear as dark-brown to blackish circular to irregular spots of varying size that coalesce into larger necrotic patches as the disease progresses.

5.1 Inhibition at the full recommended dose

At the full recommended dose, STROBY EXPERT achieved the highest inhibition of B. fabae colony growth, reaching 83.26% with a colony diameter of 11.50 mm, followed by PROCYMIDON STAR (82.05%, 12.33 mm) and MATADOR EXPERT (81.80%, 12.50 mm), then CAPRIO TOP (79.39%, 14.16 mm) and MENOL (72.83%, 18.66 mm). The conventional contact fungicide MANCOZEB was the weakest, with only 66.52% inhibition and a colony diameter of 23.00 mm. Highly significant differences (LSD0.01 = 2.30 mm for colony diameter and 3.20% for inhibition) separated the modern fungicides from MANCOZEB (Table 2).

Table 2. In vitro inhibitory effect of the poisoned PDA media on the mycelial growth of Botrytis fabae at the full recommended dose.

Fungicide	Mean colony diameter (mm)	Inhibition (%)
STROBY EXPERT	11.50	83.26
CAPRIO TOP	14.16	79.39
PROCYMIDON STAR	12.33	82.05
MATADOR EXPERT	12.50	81.80
MENOL	18.66	72.83
MANCOZEB	23.00	66.52
Control (sterile distilled water)	69.16	0.00
LSD0.01 (without control)	2.30	3.20

5.2 Inhibition at half dose

When the concentration was reduced to half of the recommended dose, the inhibition percentages declined generally. STROBY EXPERT still achieved the highest inhibition (69.20%), followed by MATADOR EXPERT (67.17%), PROCYMIDON STAR (66.69%) and CAPRIO TOP (62.29%). MENOL and MANCOZEB declined markedly, to 54.16% and

40.75%, respectively, with clear significant differences (LSD0.01 = 3.20 mm and 5.19%) separating the modern fungicides from the conventional one (Table 3).

Table 3. In vitro inhibitory effect of the poisoned PDA media on the mycelial growth of Botrytis fabae at half of the recommended dose.

Fungicide	Mean colony diameter (mm)	Inhibition (%)
STROBY EXPERT	21.47	69.20
CAPRIO TOP	26.28	62.29
PROCYMIDON STAR	23.22	66.69
MATADOR EXPERT	22.88	67.17
MENOL	31.95	54.16
MANCOZEB	41.30	40.75
Control (sterile distilled water)	69.17	0.00
LSD0.01 (without control)	3.20	5.19

5.3 Inhibition at quarter dose

Reducing the concentration to a quarter of the recommended dose caused a marked collapse in the efficacy of most fungicides. STROBY EXPERT declined to 49.43%, while PROCYMIDON STAR achieved the best result at this low concentration (53.64%), followed by MATADOR EXPERT (46.63%) and CAPRIO TOP (38.21%). MENOL collapsed to
27.00% and MANCOZEB to only 5.96%, indicating that it had practically lost its ability to inhibit B. fabae at this low concentration. Significant differences were recorded (LSD0.01 = 0.34 mm and 9.20%).

Table 4. In vitro inhibitory effect of the poisoned PDA media on the mycelial growth of Botrytis fabae at a quarter of the recommended dose.

Fungicide	Mean colony diameter (mm)	Inhibition (%)
STROBY EXPERT	34.74	49.43
CAPRIO TOP	42.45	38.21
PROCYMIDON STAR	31.85	53.64
MATADOR EXPERT	36.67	46.63
MENOL	50.15	27.00
MANCOZEB	64.60	5.96
Control (sterile distilled water)	68.17	0.00
LSD0.01 (without control)	0.34	9.20

5.4 Identification of the most effective fungicide and concentration

The fungicide STROBY EXPERT was the most effective in inhibiting the mycelial growth of B. fabae, with an inhibition of 83.26% at the full dose (colony diameter 11.50 mm), declining to 69.20% at half dose (21.47 mm) and to 49.43% at quarter dose (34.74 mm). It is therefore evident that, as the dose decreased, colony growth increased and the fungicide effect weakened, while high rates were able to inhibit mycelium and spore production effectively. The results

also show that B. fabae has developed resistance to the conventional MANCOZEB, particularly at low concentrations, and that, although the modern fungicides performed well at full dose, they may lose their protective activity when the dose is reduced below the recommended level.

7. Conclusion

The present in vitro study shows that the modern fungicides STROBY EXPERT (kresoximmethyl), PROCYMIDON STAR (procymidone) and MATADOR EXPERT (myclobutanil) provide highly significant inhibition of the mycelial growth of Botrytis fabae in comparison with the conventional contact fungicide MANCOZEB. STROBY EXPERT was the most effective treatment overall, achieving 83.26% inhibition at the full recommended dose, while MANCOZEB collapsed to 5.96% at the quarter dose, indicating that the Syrian isolate tested has already acquired resistance to this multi-site inhibitor. The sharp decline in efficacy observed for all fungicides at reduced concentrations confirms the importance of strict adherence to the recommended doses and of fungicide rotation within an integrated diseasemanagement framework. Given the recent global reports of multidrug resistance in B. cinerea and B. fabae populations, continuous monitoring of resistance evolution in local pathogen populations, the rotation of single-site fungicides with different modes of action, and the integration of chemical control with cultural practices and biocontrol agents are recommended to ensure the sustainability of chemical control of chocolate spot of faba bean.

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Author contributions

Mohammed Al-Saeed: Research concept and design; performance of laboratory experiments; data collection and analysis; writing of the first draft. Ahmed Al-Murrah: Scientific supervision; critical revision of the manuscript; final approval of the submitted version. Both authors read and approved the final manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval: This laboratory study did not involve experiments on humans or vertebrate animals and did not require institutional ethics approval. Conflict of interest: The authors declare that they have

no financial or personal relationships with any organization or individual that could inappropriately influence the work reported in this paper.

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