

I. INTRODUCTION

1.1. Background

Shallot (*Allium cepa* L. var. *aggregatum*) are one of Indonesia's leading horticultural commodities, playing a strategic role in the national economy due to their significant contribution to inflation and farmers' incomes in various production centers (Rosliani, 2022). In addition to being used as a seasoning in cooking, shallot also serve as raw materials for the food industry and traditional medicine due to their content of secondary metabolites such as allicin, which has antimicrobial properties, and quercetin, which acts as a potent antioxidant (Lanzotti, 2006; Ly *et al.*, 2005). Data from the Central Statistics Agency (BPS) indicate that shallot consumption continues to rise in tandem with Indonesia's population growth; however, productivity often declines due to technical challenges in the field, particularly pest and disease outbreaks. According to a BPS report (2023), national shallot production in 2022 was recorded at 1.98 million metric tons; however, this figure is heavily influenced by weather fluctuations and outbreaks of pests and diseases, which often lead to shortages and price hikes in the domestic market.

The most critical biotic challenge in shallot cultivation is *purple blotch* disease, caused by the pathogenic fungus *Alternaria porri*. This pathogen infects plants through stomata or wounds on the leaf surface, producing symptoms of curved, grayish-white spots that later develop into dark purple patches with concentric rings. The development of this disease is heavily influenced by the presence of dew and prolonged periods of leaf wetness, during which the fungal conidia require free water to germinate and penetrate plant tissues (Suheri & Price, 2000). In addition to causing mechanical damage, *A. porri* also releases host-specific toxins that disrupt cellular metabolism, leading to widespread chlorosis that ultimately results in tissue necrosis. In severe infections, shallot leaves turn yellow, wilt, and break prematurely, which directly inhibits photosynthesis and the translocation of nutrients to the bulb. Indonesia's tropical climate, with relative humidity above 80%, is highly conducive to the sporulation of this fungus, resulting

in potential yield losses of 50% to 80% during the rainy season (Udiarto *et al.*, 2005).

For years, the control of *A. porri* has relied heavily on synthetic fungicides containing active ingredients such as mancozeb or chlorothalonil. This excessive reliance on chemical pesticides has triggered various serious problems. In addition to increasing production costs, the indiscriminate application of chemicals has led to pathogen resistance, degradation of soil microflora, and residue contamination in products that can threaten consumer health (Ahmad *et al.*, 2024). Therefore, there is a need to raise awareness and understanding toward safer and more sustainable biological control through the use of antagonistic microorganisms. Bacteria of the genus *Bacillus* sp. are globally recognized as superior biological control agents (BCAs) due to their ability to form endospores that are resistant to extreme environmental conditions. The mechanism of *Bacillus* sp. antagonism against pathogenic fungi is highly complex, involving competition for space and nutrients, induction of systemic resistance (ISR), and, most importantly, the production of secondary metabolites. These secondary metabolites include cyclic lipopeptides such as surfactin, iturin, and fengycin, which act by penetrating the lipid bilayer of the fungal cell membrane, causing cytoplasmic leakage and the death of pathogenic cells (Hashem *et al.*, 2019; Gharsallah *et al.*, 2025). In addition to producing lipopeptide metabolites, *Bacillus* sp. also produces lytic enzymes such as chitinase, which can degrade the main components of the fungal cell wall.

Bacillus sp. is known to have a wide range of potential applications; however, the use of whole cells (cell suspensions) in the field often faces challenges due to the bacteria's low survival rate when exposed to UV light and extreme temperature fluctuations. Furthermore, ecologically speaking, *Bacillus* is a bacterium whose natural habitat is the rhizosphere; consequently, its ability to colonize and survive stably on the phyllosphere (leaves) is relatively low due to high abiotic environmental stress (Mazzola & Freilich, 2017; Ongena & Jacques, 2008). As a solution, the utilization of secondary metabolites offers a promising alternative to bridge this ecological niche gap. By extracting the active compounds metabolites from these soil-dwelling bacteria, they can be applied directly to leaves to inhibit the growth of *A. porri*, an airborne fungus, without relying on the viability

of bacterial cells on the leaf surface. Supernatant is defined as the initial separation product or crude product obtained from an extraction process using a centrifuge, which still contains a complex mixture of various active metabolites without undergoing further purification. The use of this supernatant is considered more effective than a single pure compound due to *the synergistic effect* among the various multicomponent compounds in simultaneously inhibiting pathogens. Analysis of the chemical components in secondary metabolites can be performed using *Gas Chromatography-Mass Spectrometry* (GC-MS) to identify the bioactive compounds contained within.

Based on the procedure conducted by Ningsih *et al.* (2025), compound identification was performed by extracting the liquid culture supernatant using methanol as a solvent, which was then analyzed using an *Agilent Technologies GC-MS* system with helium as the carrier gas and an HP-5ms *Ultra Inert* capillary column. The mass fragmentation patterns obtained from the mass spectrometer detector were then compared with the *National Institute of Standards and Technology* (NIST) database and the Wiley library to determine the types of compounds based on retention time and area percentage. The use of the supernatant also ensures that the active compounds are immediately available to disrupt the structure of pathogenic cells without having to wait for the bacteria to colonize and enter the growth phase in plant tissues (Dihazi *et al.*, 2012).

The *Bacillus* sp. bacteria used in this study were isolate D28, obtained from an exploration by Moekti *et al.* (2025) on *Avicennia* sp. mangrove leaves, which was tested *in vitro* against *Alternaria* sp. The results of *in vitro* antagonistic testing using *the dual-culture* method showed that isolate D28 had the best ability to inhibit the growth of *Alternaria* sp. compared to the other 34 isolates. Isolate D28 produced an average inhibition zone diameter of 12.5 mm on the 8th day of incubation, which was classified as “strong” according to the standards of David and Stout (1971). This pathogen’s inhibitory ability is driven by two main mechanisms: antibiosis and spatial competition. Through antibiosis, isolate D28 produces secondary metabolites in the form of antifungal compounds, as evidenced by the formation of a distinct clear zone around the pathogen’s colony. In addition, isolate D28 has a very rapid growth rate, enabling it to dominate the growth space and nutrients

within the petri dish, which effectively halts the expansion of the pathogenic fungal mycelium. It is this performance that makes isolate D28 a promising candidate for a biological agent capable of inhibiting the growth of *Alternaria* sp. in the field. Research on the efficacy of secondary metabolites from *Bacillus* has been extensively conducted on various soil-borne pathogens; however, specific testing using crude extraction methods to control *A. porri* on shallot still requires more detailed data, particularly regarding comparisons of efficacy *in vitro* (in the laboratory) and *in vivo* (on living plants). This is crucial to ensure that the inhibitory activity observed in petri dishes translates into actual protection in greenhouses or in the field. Therefore, the integration of these two testing methods is expected to provide a comprehensive picture of the biopesticide's potential to effectively and consistently suppress the rate of purple blotch infection.

1.2. Research Question

1. Which nonpolar compounds present in the *Bacillus* sp. D28 isolate are capable of suppressing the severity of purple blotch disease?
2. Can the secondary metabolites of *Bacillus* sp. act as growth promoters for shallot plants?
3. What is the effective concentration of *Bacillus* sp. secondary metabolites for suppressing the severity of purple blotch disease *in vivo*?

1.3. Research Objectives

1. To identify nonpolar compounds in the *Bacillus* sp. D28 isolate capable of suppressing the severity of purple blotch disease.
2. To measure the ability of *Bacillus* sp. secondary metabolites to stimulate shallot plant growth
3. To determine the optimal concentration of *Bacillus* sp. secondary metabolites for suppressing the severity of purple blotch disease in shallot.

1.4. Research Benefits

This research is expected to provide a scientific contribution in the form of an in-depth understanding of the antibiosis mechanism of *Bacillus* sp. secondary metabolites in inducing morphological damage to airborne pathogens. Practically, the results of this study will benefit farmers by providing an effective, economical,

and environmentally friendly strategy for controlling purple blotch disease, thereby reducing dependence on chemical fungicides.