I. INTRODUCTION

1.1. Background

Chili (*Capsicum frutescens* L.) is one of the horticultural crops with great potential to be cultivated as a main crop because it has the potential to generate large profits. According to BPS (2024), chili production in East Java in 2023 decreased by 562.816 tons from 646.740 tons in 2022. One of the obstacles to reducing chili production is that the seeds used by farmers are of low quality and susceptible to disease (Sukapiring and Nurliana, 2020). The presence of pathogens carried by seeds, whether located on the surface or inside, can inhibit the germination process because these pathogens can transmit disease from the seeds to the plants. Therefore, it is important to treat seeds with special care to eliminate pathogens so that the seeds are guaranteed to be free from seed-borne fungal infections (Zahara, 2018).

Sukapiring and Nurliana (2020) reported several seed-borne pathogenic fungi in chili, such as *Fusarium solani, Alternaria alternata, A. Fumigatus, F. oxysporum, Aspergillus flavus Penicillium* sp., *A. Niger* dan *Colletotrichum* sp. According to Masniawati (2013), fungal infections in seeds have the potential to become a major source of inoculum that transmits disease to plants. Fungal infections carried by seeds can inhibit the activity of enzymes that play a role in the germination process, reducing viability, causing total damage to the seeds, and leading to embryo death (Dharmaputra, 2013).

Colletotrichum sp. fungi are one of the major pathogens affecting chili crops, causing losses of 50-65% (Rumahlewang et al., 2024). *Colletotrichum* sp. fungi are seed-borne pathogens that can reduce germination rates and infect seedlings, potentially causing chili seedlings to die (Naznin et al., 2016). Ranathunge et al., (2012) stated that chili seedlings germinated from seeds infected with *Colletotrichum* sp. fungus showed symptoms of necrosis in the cotyledon to hypocotyl parts. In severe attacks, this would cause the plants to die. According to Choudhary et al., (2013) *Colletotrichum* sp. fungi that infect seeds will experience pre-emergence and post-emergence damping-off in the sprouting phase. Akhtar et al., (2017) infection caused by *Colletotrichum* sp. fungus in seeds is indicated by damage and decreased seed viability. Welideniya et al., (2019) *Colletotrichum* sp. fungal infection shows the presence of aservulus on the surface of the seed coat, which affects germination and causes the seeds to fail to grow. Therefore, it is necessary to control the seed-borne pathogen *Colletotrichum* sp.

The treatment often used to control the seed pathogen *Colletotrichum* sp. is chemical fungicide. This is done because it is considered more effective and has a quick response on chili plants (Sila and Sopialena, 2016). However, long-term use causes resistance in target pathogens and has a negative impact on the environment and humans. Control using biological agents such as microorganisms is an environmentally friendly alternative control method. This control method is capable of inhibiting the growth of pathogens that cause plant diseases (Sudewi et al., 2022). Biological control agents can be bacteria and fungi. One bacterium that has the potential to be used as an environmentally friendly biological control agent is *Bacillus* sp. Bth-22.

Bacillus sp. Bth-22 is an endophytic bacterium obtained through the isolation of healthy eggplant stem tissue collected from Jombang (Purnawati and Nirwanto, 2021). The endophytic bacterium *Bacillus* sp. Bth-22 can suppress the intensity of seed-borne pathogen attacks and improve seed germination and plant growth (Fachrezzy, 2022; Setyowati, 2023; Sayekti, 2024). Fachrezzy's (2022) research shows that the application of *Bacillus* sp. Bth-22 endophytic bacteria suspension with a colony density of 10^8 was able to suppress the growth of pathogenic fungi carried by corn seeds by 51% when tested on PDA medium. Setyowati (2023) stated that soaking corn seeds with 15% and 25% concentrations of *Bacillus* sp. Bth-22 at concentrations of 15% and 25% can suppress the infection rate of pathogens carried by corn seeds by 42.8% and 50% in the blotter test method and 50% and 58.3% in the growing on test method. Setyowati (2023) also added that soaking corn seeds using 15% and 25% concentrations of *Bacillus* sp. Bth-22 secondary metabolites was able to increase germination rates by 73.3% and 86.7% in the blotter test method and 80% and 93.3% in the growing on test method. Sayekti (2024) stated that the application of *Bacillus* sp. Bth-22 suspension at a dose of 20 ml/plant was able to suppress the intensity of bacterial leaf blight disease by 23.5% at 30 DPI (Days Post Inoculation). In addition, the average plant length with *Bacillus* sp. Bth-

22 treatment at a dose of 20 ml/plant was 58.5 cm at 10 weeks after planting (WAP). According to Akrom et al., (2024) the application of *Bacillus* sp. Bth-22 beads with a sodium alginate concentration of 3% was able to reduce the intensity of fusarium stem rot disease by 41% at 28 DPI (Days Post Inoculation).

Directly applied biological agent suspensions have several drawbacks, such as the need to consider environmental conditions for microorganism growth, storage issues, and a relatively short shelf life. In addition, the protection provided by directly applied biological agent suspensions is limited to the area around the roots due to their ability to compete with other microbes (Soesanto, 2017). Soesanto (2017) also adds that the direct use of biological agents is still unable to overcome new attacks by plant pests. Therefore, the use of secondary metabolites from *Bacillus* sp. bacteria is expected to provide more effective control than direct application of suspensions.

Utami's (2019) research shows that the use of microbial secondary metabolites is more effective than direct suspension, because microbes need time to produce secondary metabolites that are used to inhibit pathogens. Soesanto (2017) also explains that the use of secondary metabolites has several advantages, such as being easy to apply in various ways, environmentally friendly, and more affordable. The application of secondary metabolites from the beginning of planting can improve the plant's defense system so that plants are able to fight off attacks from plant pests.

Microorganisms can produce secondary metabolites that play a role in supporting their survival in interaction with the surrounding environment. Soesanto (2014) explains that secondary metabolites have a high ability to control pests in the field. *Bacillus* sp. bacteria can produce antibiotic compounds such as surfactin, bacillomycin-D, fengycin, bacillibactin, bacilysin, macrolactin, bacillaene, difficidin, and putative peptides with unknown functions (Rabbee et al., 2020). Li et al., (2014) explain that the fengycin compound produced by *Bacillus velezensis* SQR9 shows antagonistic activity against *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora parasitica*, and *Verticillium dahliae* Kleb.

According to Hanif et al., (2016) seed soaking using 10% and 20% concentrations of endophytic bacterial secondary metabolites can suppress the growth of *Fusarium* sp. carried by corn seeds by 31.2% and 32.5% in vitro. According to Setyowati (2023) seed soaking with endophytic bacterial secondary metabolites at concentrations of 15% and 25% can reduce the infection rate of pathogens carried by corn seeds by 42.8% and 50% in the blotter test method and by 50% and 58.3% in the growing on test method. The results of Zahara's (2018) study showed that endophytic bacterial secondary metabolites at a concentration of 30% were able to suppress *Aspergillus flavus* carried by peanut seeds by 46.68% in vitro. Meanwhile, in vivo, treating peanut seeds with 30% concentration of endophytic bacterial secondary metabolites was able to suppress the infection rate by 52.17% in the blot test and 66.67% in the growing on test.

Based on several studies related to secondary metabolites produced by *Bacillus* sp. endophytic bacteria that can be used to control seed-borne fungi, further research should be conducted on the production of *Bacillus* sp. Bth-22 secondary metabolites and their application in controlling *Colletotrichum* sp. fungi in chili seeds. It is hoped that the secondary metabolites of *Bacillus* sp. Bth-22 applied to chili seeds will be more effective and efficient than using biological agents directly.

1.2. Problem Formulation

Based on the background, the research questions in this study are as follows:

1. Can the concentration level of secondary metabolites of the endophytic bacterium *Bacillus* sp. Bth-22 suppress the infection rate of the fungus *Colletotrichum* sp. in chili seeds?
2. Does the concentration level of secondary metabolites of the endophytic bacterium *Bacillus* sp. Bth-22 increase the germination rate and growth of chili plants?
3. What is the effective concentration level of secondary metabolites of the endophytic bacterium *Bacillus* sp. Bth-22 in suppressing the infection rate of *Colletotrichum* sp. fungi on chili seeds using the blotter test and growing on test methods?

1.3. Research Objectives

Based on the problem formulation, the objectives of this study are:

1. Determining the ability of various concentrations of secondary metabolites of *Bacillus* sp. Bth-22 bacteria to suppress the level of *Colletotrichum* sp. fungal infection in chili seeds.
2. Determining the ability of various concentrations of secondary metabolites of the endophytic bacterium *Bacillus* sp. Bth-22 to improve the germination and growth of chili plants.
3. Determining the concentration level of secondary metabolites of endophytic bacteria *Bacillus* sp. Bth-22 that are effective in suppressing the level of *Colletotrichum* fungal infection in chili seeds using the blotter test and growing on test methods.

1.4. Benefit of Research

The benefits obtained from this study are expected to provide information on the effect of the appropriate concentration level of secondary metabolites of the endophytic bacterium *Bacillus* sp. Bth-22 in suppressing the growth of *Colletotrichum* sp. fungi on chili seeds. The results of this study can also be used as an alternative to control diseases caused by *Colletotrichum* sp. fungi on chili seeds effectively, efficiently, and in an environmentally friendly manner.