

**POTENTIAL OF SECONDARY METABOLITES OF ENDOPHYTIC  
BACTERIA *Bacillus* sp. BTH-22 AS AN ANTIFUNGAL AGENT AGAINST  
*Colletotrichum* sp. IN CHILI SEEDS (*Capsicum frutescens* L.)**

**THESIS**



**Written by:**

**NURUL AFIDA**  
**NPM: 21025010003**

**AGROTECHNOLOGY STUDY PROGRAM  
FACULTY OF AGRICULTURE  
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR  
SURABAYA  
2025**

**POTENTIAL OF SECONDARY METABOLITES OF ENDOPHYTIC  
BACTERIA *Bacillus* sp. BTH-22 AS AN ANTIFUNGAL AGENT AGAINST  
*Colletotrichum* sp. IN CHILI SEEDS (*Capsicum frutescens* L.)**

**THESIS**

Submitted to Fulfill the Requirements for  
a Bachelor's Degree in Agriculture  
Agrotechnology Study Program



**Written by :**

**NURUL AFIDA**  
**NPM: 21025010003**

**AGROTECHNOLOGY STUDY PROGRAM  
FACULTY OF AGRICULTURE  
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR  
SURABAYA  
2025**



APPROVAL SHEET

POTENTIAL OF SECONDARY METABOLITES OF ENDOPHYTIC  
BACTERIA *Bacillus* sp. BTH-22 AS AN ANTIFUNGAL AGENT AGAINST  
*Colletotrichum* sp. IN CHILI SEEDS (*Capsicum frutescens* L.)

Written by:

**NURUL AFIDA**  
NPM: 21025010003

Submitted on:  
16 September 2025

This thesis is accepted as one of the requirements for obtaining  
a Bachelor of Agriculture degree  
Universitas Pembangunan Nasional "Veteran" Jawa Timur

Approved by,

Main Supervisor

Second Supervisor

  
**Dr. Ir. Arika Purnawati, M.P.**  
NIP. 19650422 199003 2001

  
**Dr. Ir. Tri Mujoko, M.P.**  
NIP. 19660509 199203 1001

Known by,

Dean Faculty of Agriculture

Coordinator of the Agrotechnology  
Study Program

  
**Prof. Dr. Ir. Wanti Mindari, M.P.**  
NIP. 19631208 199003 2001

  
**Dr. Ir. Tri Mujoko, M.P.**  
NIP. 19660509 199203 1001



APPROVAL SHEET

POTENTIAL OF SECONDARY METABOLITES OF ENDOPHYTIC  
BACTERIA *Bacillus* sp. BTH-22 AS AN ANTIFUNGAL AGENT AGAINST  
*Colletotrichum* sp. IN CHILI SEEDS (*Capsicum frutescens* L.)

Written by:

**NURULAFIDA**  
NPM. 21025010003

Has been repaired on:

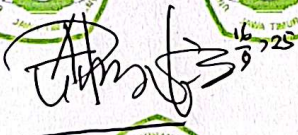
16 September 2025

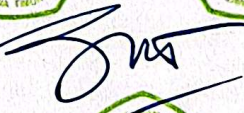
This thesis is accepted as one of the requirements for obtaining  
a Bachelor of Agriculture degree  
Universitas Pembangunan Nasional "Veteran" Jawa Timur

Approved by,

Main Supervisor

Second Supervisor

  
Dr. Ir. Arika Purnawati, M.P.  
NIP. 19650422 199003 2001

  
Dr. Ir. Tri Mujoko, M.P.  
NIP. 19660509 199203 1001



## PLAGIARISM STATEMENT

I, the undersigned:

Name : Nurul Afida  
Student ID : 21025010003  
Program : Bachelor (S1)  
Study Program : Agrotechnology  
Faculty : Agriculture  
Thesis Title : Potential of Secondary Metabolites of Endophytic Bacteria  
*Bacillus* sp. Bth-22 as an Antifungal Agent Against  
*Colletotrichum* sp. on Chili Seeds (*Capsicum frutescens* L.)

Hereby declare that this Thesis document does not contain any part of other scientific works previously submitted to obtain an academic degree at any Higher Education Institution, nor does it contain works or opinions previously written or published by others, except those that are properly cited in this document and fully listed in the references.

I further declare that this scientific document is free from plagiarism. Should any indications of plagiarism be found in the Thesis in the future, I am willing to accept sanctions in accordance with the prevailing laws and regulations.

This declaration is made truthfully, without any coercion from any party, and shall be used as intended.

Surabaya, 16 September 2025

Declarant



Nurul Afida

NPM. 21025010003

**POTENSI METABOLIT SEKUNDER BAKTERI ENDOFIT *Bacillus* sp.  
BTH-22 SEBAGAI ANTIFUNGI *Colletotrichum* sp. PADA BENIH  
CABAI RAWIT (*Capsicum frutescens* L.)**

**Nurul Afida<sup>1</sup>, Arika Purnawati<sup>1\*</sup>, Tri Mujoko<sup>1</sup>**

<sup>1</sup>Program Studi Agroteknologi, Fakultas Pertanian, Universitas Pembangunan Nasional “Veteran” Jawa Timur  
Jln. Rungkut Madya, Gn. Anyar, Kec. Gn. Anyar, Surabaya, Jawa Timur

\*Penulis korespondensi: [arika\\_p@upnjatim.ac.id](mailto:arika_p@upnjatim.ac.id)

**ABSTRAK**

Cabai rawit (*Capsicum frutescens* L.) merupakan komoditas hortikultura yang memiliki nilai ekonomi yang tinggi. Salah satu kendala produksi cabai rawit yaitu adanya patogen terbawa benih cabai yang disebabkan oleh jamur *Colletotrichum* sp. Pengendalian alternatif yang dapat digunakan yaitu dengan memanfaatkan metabolit sekunder *Bacillus* sp. karena memiliki kemampuan untuk memproduksi metabolit sekunder yang dapat digunakan sebagai antifungi. Penelitian ini bertujuan untuk mengetahui kemampuan taraf konsentrasi metabolit sekunder *Bacillus* sp. Bth-22 yang efektif dalam menekan infeksi jamur *Colletotrichum* sp. pada benih cabai rawit, meningkatkan daya kecambah dan pertumbuhan tanaman cabai rawit menggunakan metode *blotter test* dan *growing on test*. Penelitian ini menggunakan rancangan acak lengkap (RAL) satu faktor berupa taraf konsentrasi (10%, 15%, 20%, 25% dan 30%), kontrol negatif (suspensi patogen) dan kontrol positif (fungisida kimia propineb). Hasil penelitian menunjukkan bahwa konsentrasi terbaik metabolit sekunder *Bacillus* sp. yaitu konsentrasi 30% yang mampu menekan tingkat infeksi jamur *Colletotrichum* sp. pada benih cabai sebesar 47.6% pada metode *blotter test* dan 91.6% pada metode *growing on test*. Selain itu, metabolit sekunder *Bacillus* sp. konsentrasi 30% juga mampu meningkatkan persentase daya kecambah sebesar 64% pada metode *blotter test* dan 94.3% pada metode *growing on test*. Metabolit sekunder *Bacillus* sp. konsentrasi 30% mampu meningkatkan pertumbuhan tinggi tanaman sebesar 133% terhadap kontrol negatif dan 7% terhadap kontrol positif dan mampu meningkatkan pertumbuhan panjang akar sebesar 140% terhadap kontrol negatif dan 13% terhadap kontrol positif.

**Kata kunci:** Benih cabai rawit, perlakuan benih, *Bacillus* sp., *Colletotrichum* sp., metabolit sekunder

**ABSTRACT**

*Chili (Capsicum frutescens L.) is a horticultural commodity with high economic value. One of the major constraints in its production is seed-borne pathogens, particularly those caused by the fungus Colletotrichum sp. An alternative control strategy that can be applied is the utilization of secondary metabolites from Bacillus sp., as they possess the ability to produce antifungal compounds. This study aimed to determine the effective concentration levels of secondary metabolites of Bacillus sp. Bth-22 in suppressing Colletotrichum sp. infection on chili seeds, as well as to evaluate their effects on seed germination and*

*plant growth using blotter test and growing-on test methods. The experiment was arranged in a completely randomized design (CRD) with a single factor consisting of various concentrations (10%, 15%, 20%, 25%, and 30%), a negative control (pathogen suspension), and a positive control (chemical fungicide propineb). The results indicated that the optimal concentration of Bacillus sp. secondary metabolites was 30%, which suppressed Colletotrichum sp. infection on seeds by 47.6% in the blotter test and 91.6% in the growing-on test. Furthermore, the 30% concentration also enhanced germination by 64% in the blotter test and 94.3% in the growing-on test. In addition, the 30% concentration increased plant height by 133% compared to the negative control and 7% compared to the positive control, and improved root length by 140% compared to the negative control and 13% compared to the positive control.*

**Keywords:** *Chili seed, seed treatment, Bacillus sp., Colletotrichum sp., secondary metabolites*

## PREFACE

Praise and gratitude are hereby rendered to Allah SWT, the Almighty, for all the knowledge, guidance, and strength bestowed upon the author, enabling the completion of this thesis entitled “Potential of Secondary Metabolites of Endophytic Bacteria *Bacillus* sp. Bth-22 as an Antifungal Agent Against *Colletotrichum* sp. on Chili Seeds (*Capsicum frutescens* L.)”. This thesis is prepared as a partial fulfillment of the requirements for obtaining a Bachelor’s degree (S1) in the Agrotechnology Study Program, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur.

The author acknowledges that this thesis is far from perfect; therefore, constructive criticism and suggestions are highly appreciated for its future improvement. It is hoped that this thesis can contribute to the advancement of knowledge, particularly in the field of plant pathology and the application of endophytic bacteria as a biological control agent.

Surabaya, September 2025

Writer



## ACKNOWLEDGEMENTS

The author acknowledges that many parties have contributed to the completion of this thesis. On this occasion, the author would like to express sincere gratitude to:

1. Dr. Ir. Arika Purnawati, M.P., as the main supervisor, and Dr. Ir. Tri Mujoko, M.P., as the second supervisor, for her guidance, knowledge, direction, and motivation throughout the preparation of this thesis.
2. Dr. Ir. Yenny Wuryandari, M.P. and Dr. Ir. Herry Nirwanto, M.P., as examiners, for their knowledge, constructive criticism, and suggestions to improve the thesis.
3. Dr. Ir. Tri Mujoko, M.P., as the Coordinator of the Agrotechnology Study Program, Faculty of Agriculture, UPN “Veteran” Jawa Timur.
4. Prof. Dr. Ir. Wanti Mindari, M.P., as the Dean of the Faculty of Agriculture, UPN “Veteran” Jawa Timur.
5. Fathul Mukaromah, S.P., M.P., as field supervisor, and the entire staff of BBPPTP Surabaya, for their guidance and assistance during the research implementation.
6. Beloved family, especially my father and mother, who have always provided prayers, motivation, affection, and sincere love, serving as a source of strength for every step of the author, enabling the completion of this thesis. May Allah SWT always protect them and reward all the sacrifices they have made for me.
7. Ahmad Adibul Akrom, for always accompanying the author, providing unwavering support, dedicating time, energy, and thought, and patiently dealing with the author’s traits and behavior. Thank you for being by the author’s side until now.
8. Friends who have accompanied and assisted the author throughout the research and academic journey, namely: Denia, Revanda, Larasita, Farha, Yulia, Zerlinda, Sailatun, and Arman.
9. All other parties who have provided physical or moral support for the smooth completion of this thesis but cannot be mentioned individually in this limited space.

## TABLE OF CONTENTS

|                                                                             | Page |
|-----------------------------------------------------------------------------|------|
| PREFACE .....                                                               | i    |
| ACKNOWLEDGEMENTS .....                                                      | ii   |
| TABLE OF CONTENTS .....                                                     | iii  |
| LIST OF TABLES.....                                                         | vi   |
| LIST OF FIGURES.....                                                        | vii  |
| I. INTRODUCTION .....                                                       | 1    |
| 1.1. Background.....                                                        | 1    |
| 1.2. Problem Formulation .....                                              | 4    |
| 1.3. Research Objectives.....                                               | 5    |
| 1.4. Benefit of Research.....                                               | 5    |
| II. LITERATURE REVIEW .....                                                 | 6    |
| 2.1. Chili Seed.....                                                        | 6    |
| 2.1.1. Classification of Chili .....                                        | 6    |
| 2.1.2. Structure of Chili Seeds.....                                        | 6    |
| 2.1.3. Chili Seed Germination .....                                         | 7    |
| 2.2. Chili Production and Consumption.....                                  | 7    |
| 2.3. Constraints on Chili Plants.....                                       | 7    |
| 2.3.1. Pathogen Position in Seeds.....                                      | 8    |
| 2.3.2. Causing Seed Infection.....                                          | 8    |
| 2.4. Damping-off disease .....                                              | 8    |
| 2.4.1. Significance .....                                                   | 8    |
| 2.4.2. Symptoms of <i>Colletotrichum</i> sp. on Chili Seeds.....            | 9    |
| 2.4.3. Morphology of <i>Colletotrichum</i> sp. ....                         | 9    |
| 2.4.4. Bioecology of <i>Colletotrichum</i> sp.....                          | 10   |
| 2.4.5. Life Cycle of <i>Colletotrichum</i> sp. ....                         | 10   |
| 2.5. Biological Control.....                                                | 11   |
| 2.5.1. Endophytic Bacteria .....                                            | 11   |
| 2.5.2. <i>Bacillus</i> sp. Bth-22 Bacteria .....                            | 12   |
| 2.5.3. Utilization of <i>Bacillus</i> sp. Bth-22 as a Biological Agent..... | 13   |
| 2.5.4. Secondary Metabolite Compounds of <i>Bacillus</i> sp. Bacteria ..... | 14   |



|                                                                                                                                                  |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 2.6. Seed Treatment.....                                                                                                                         | 15 |
| 2.6.1. Seed Quality .....                                                                                                                        | 15 |
| 2.6.2. Health Testing of Seeds .....                                                                                                             | 16 |
| 2.6.3. Non-Incubation Method .....                                                                                                               | 16 |
| 2.6.4. Blotter test Method .....                                                                                                                 | 17 |
| 2.6.5. Growing on test Method .....                                                                                                              | 17 |
| 2.7. Hypothesis.....                                                                                                                             | 18 |
| III. RESEARCH METHODS .....                                                                                                                      | 19 |
| 3.1. Time and Place of Research.....                                                                                                             | 19 |
| 3.2. Tools and Materials.....                                                                                                                    | 19 |
| 3.3. Experimental Design.....                                                                                                                    | 19 |
| 3.4. Research Implementation.....                                                                                                                | 21 |
| 3.4.1. Preparation.....                                                                                                                          | 21 |
| 3.4.2. Isolation and Identification of <i>Colletotrichum</i> sp. Fungi .....                                                                     | 23 |
| 3.4.3. Koch's Postulates Test .....                                                                                                              | 23 |
| 3.4.4. Pathogenicity Test.....                                                                                                                   | 24 |
| 3.4.5. Rejuvenation of Endophytic Bacteria <i>Bacillus</i> sp. Bth-22.....                                                                       | 24 |
| 3.4.6. Production and Extraction of Secondary Metabolites from<br>Endophytic Bacteria <i>Bacillus</i> sp. Bth-22.....                            | 25 |
| 3.4.7. Seed Treatment with Secondary Metabolites from Endophytic<br>Bacteria <i>Bacillus</i> sp. Bth-22 using the Blotter Test Method .....      | 25 |
| 3.4.8. Seed Treatment with Secondary Metabolites from Endophytic<br>Bacteria <i>Bacillus</i> sp. Bth-22 using the Growing on Test<br>Method..... | 26 |
| 3.5. Data Analysis .....                                                                                                                         | 28 |
| IV. RESULTS AND DISCUSSION.....                                                                                                                  | 29 |
| 4.1. <i>Colletotrichum</i> sp. Fungi .....                                                                                                       | 29 |
| 4.2. Pathogenicity Results of <i>Colletotrichum</i> sp. Fungi .....                                                                              | 30 |
| 4.3. <i>Bacillus</i> sp. Bth-22 Bacteria .....                                                                                                   | 31 |
| 4.4. Production of Secondary Metabolites by <i>Bacillus</i> sp. Bth-22<br>Bacteria .....                                                         | 32 |
| 4.5. Results of <i>Colletotrichum</i> sp. Fungal Infection on Chili Seeds Using<br>the Blotter Test Method.....                                  | 33 |
| 4.5.1. Symptoms of <i>Colletotrichum</i> sp. Fungal Infection in Chili<br>Seeds Using the Blotter Test Method .....                              | 33 |

|                                                                                                                             |    |
|-----------------------------------------------------------------------------------------------------------------------------|----|
| 4.5.2. Infection Rate of <i>Colletotrichum</i> sp. Fungal Infection in Chili Seeds Using the Blotter Test Method .....      | 35 |
| 4.5.3. Infection Rate Suppression of <i>Colletotrichum</i> sp. Fungal in Chili Seeds Using the Blotter Test Method .....    | 37 |
| 4.6. Results of <i>Colletotrichum</i> sp. Fungal Infection on Chili Seeds Using the Growing on Test Method .....            | 40 |
| 4.6.1. Symptoms of <i>Colletotrichum</i> sp. Fungal Infection in Chili Seeds Using the Growing on Test Method .....         | 40 |
| 4.6.2. Infection Rate of <i>Colletotrichum</i> sp. Fungal Infection in Chili Seeds Using the Growing on Test Method .....   | 41 |
| 4.6.3. Infection Rate Suppression of <i>Colletotrichum</i> sp. Fungal in Chili Seeds Using the Growing on Test Method ..... | 43 |
| 4.7. Germination Rate of Chili Seeds .....                                                                                  | 45 |
| 4.8. Chili Plant Growth .....                                                                                               | 49 |
| V. CONCLUSION AND RECOMMENDATION .....                                                                                      | 53 |
| 5.1. Conclusion .....                                                                                                       | 53 |
| 5.2. Recommendation .....                                                                                                   | 53 |
| REFERENCES.....                                                                                                             | 54 |

## LIST OF TABLES

| No.             | Text                                                                                              | Page |
|-----------------|---------------------------------------------------------------------------------------------------|------|
| 4.1.            | Test of secondary metabolites of <i>Bacillus</i> sp. Bth-22 on plant height and root length ..... | 49   |
| <u>Appendix</u> |                                                                                                   |      |
|                 | Appendix 1. Analysis of Variance of Infection Rate in Blotter Test .....                          | 65   |
|                 | Appendix 2. Analysis of Variance of Infection Rate in Growing on Test .....                       | 65   |
|                 | Appendix 3. Analysis of Variance of Infection Rate Suppression Blotter Test .....                 | 65   |
|                 | Appendix 4. Analysis of Variance of Infection Rate Suppression Growing on Test .....              | 66   |
|                 | Appendix 5. Analysis of Variance of Germination Rate Blotter Test .....                           | 66   |
|                 | Appendix 6. Analysis of Variance of Germination Rate Growing on Test .....                        | 66   |
|                 | Appendix 7. Analysis of Plant Height Variation Using the Growing on Test Method .....             | 67   |
|                 | Appendix 8. Analysis of Root Length Variation Using the Growing on Test .....                     | 67   |



## LIST OF FIGURES

| No.   | Text                                                                                                                                                | Page |
|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 2.1.  | Structure of chili seeds.....                                                                                                                       | 6    |
| 2.2.  | Symptoms of <i>Colletotrichum</i> sp. fungal infection on chili seeds .....                                                                         | 9    |
| 2.3.  | Characteristics of <i>Colletotrichum</i> sp.....                                                                                                    | 10   |
| 2.4.  | Life cycle of <i>Colletotrichum</i> sp. ....                                                                                                        | 11   |
| 2.5.  | <i>Bacillus</i> sp. Bth-22 bacteria .....                                                                                                           | 13   |
| 3.1.  | Treatment layout of endophytic <i>Bacillus</i> sp. Bth-22 secondary metabolites using the blotter test method .....                                 | 20   |
| 3.2.  | Treatment layout of secondary metabolites of the endophytic bacterium <i>Bacillus</i> sp. Bth-22 using the growing-on test method.....              | 21   |
| 4.1.  | Colony morphology of <i>Colletotrichum</i> sp. fungi on PDA medium.....                                                                             | 29   |
| 4.2.  | Microscopic observation of <i>Colletotrichum</i> sp. fungus .....                                                                                   | 30   |
| 4.3.  | The appearance of <i>Colletotrichum</i> sp. symptoms on chili fruits.....                                                                           | 30   |
| 4.4.  | Characteristics of <i>Bacillus</i> sp. Bth-22 bacteria .....                                                                                        | 32   |
| 4.5.  | Production of secondary metabolites by <i>Bacillus</i> sp. Bth-22 bacteria....                                                                      | 32   |
| 4.6.  | Healthy seeds .....                                                                                                                                 | 33   |
| 4.7.  | Infected seeds .....                                                                                                                                | 34   |
| 4.8.  | The presence of black spots and mycelial growth on chili seeds .....                                                                                | 34   |
| 4.9.  | Infection rate of <i>Colletotrichum</i> sp. fungus on chili seeds using the blotter test method .....                                               | 35   |
| 4.10. | Infection rate suppression of <i>Colletotrichum</i> sp. fungus on chili seeds using the blotter test method.....                                    | 37   |
| 4.11. | Abnormal hyphae morphology due to treatment with secondary metabolites of <i>Bacillus</i> sp. Bth-22.....                                           | 39   |
| 4.12. | Symptoms of fungal infection using the growing on test method .....                                                                                 | 41   |
| 4.13. | Infection rate of <i>Colletotrichum</i> sp. fungus on chili seeds using the growing test method .....                                               | 42   |
| 4.14. | Infection rate suppression of <i>Colletotrichum</i> sp. fungus on chili seeds using the growing on test method.....                                 | 44   |
| 4.15. | Seed germination rate of chili at various concentrations of secondary metabolites of <i>Bacillus</i> sp. Bth-22 using the blotter test method ..... | 45   |

|                                                                                                                                                           |    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 4.16. Seed germination rate of chili at various concentrations of secondary metabolites from <i>Bacillus</i> sp. Bth-22 using growing on test method..... | 46 |
| 4.17. The effect of <i>Bacillus</i> sp. Bth-22 secondary metabolites on chili seed germination rate using the blotter test method .....                   | 47 |
| 4.18. The effect of <i>Bacillus</i> sp. Bth-22 secondary metabolites on chili seed germination rate using the growing-on test method .....                | 48 |
| 4.19. Plant height of chili under various treatments of secondary metabolite concentrations of <i>Bacillus</i> sp. Bth-22.....                            | 50 |
| 4.20. Root length of chili plants under various treatments of <i>Bacillus</i> sp. Bth-22 secondary metabolites.....                                       | 51 |