BAB IV

Septia Henik - Potential of indigenous entomopathogenic fungi diversity in the maize rhizosphere

Dosen-Dosen
Agribisnis
University of Muhammadiyah Malang

Document Details

Submission ID
trn:oid:::1:3096801023

Submission Date
Nov 30, 2024, 10:04 AM GMT+7

Download Date
Nov 30, 2024, 10:07 AM GMT+7

File Name
us_entomopathogenic_fungi_diversity_in_the_maize_rhizosphere.pdf

File Size
803.7 KB

11 Pages

4,328 Words

24,964 Characters

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text

Exclusions

- 1 Excluded Source

Match Groups

19 Not Cited or Quoted 7%
Matches with neither in-text citation nor quotation marks

2 Missing Quotations 0%
Matches that are still very similar to source material

0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation

0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

6% Internet sources
7% Publications
2% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

Match Groups

- **19** Not Cited or Quoted 7%
  Matches with neither in-text citation nor quotation marks
- **2** Missing Quotations 0%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

6% Internet sources
7% Publications
2% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	Publication	
Rahayu Relawati, Amalia Nur Milla, Muhammad Nur. "Concept of organic foods s...		3%
2	Internet	
www.bio-conferences.org		2%
3	Publication	
Henik Sukorini. "Shallot extension shelf life with fogging and the storage temper...		1%
4	Publication	
Ardahan Eski, Muhammed Murat Gezgin. "Susceptibility of different life stages of ...		0%
5	Publication	
Sengodan Karthi, Prabhakaran Vasantha-Srinivasan, Sengottayan Senthil-Nathan...		0%
6	Internet	
www.researchgate.net		0%
7	Publication	
Tarusikirwa, Vimbai Lisa. "Mechanisms of Population Establishment in Invasive S...		0%
8	Publication	
Siti Shilatul Najwa Sharuddin, Siti Rozaimah Sheikh Abdullah, Hassimi Abu Hasan,...		0%
9	Student papers	
Universitas Mercu Buana		0%
10	Internet	
arccjournals.com		0%

Potential of indigenous entomopathogenic fungi diversity in the maize rhizosphere

Erfan Dani Septia[1], *Henik* Sukorini[1*]
[1]Department of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, 65141 East Java, Indonesia

Abstract. Modern agriculture faces significant overreliance on chemical pesticides, leading to environmental pollution and pest resistance. As a promising alternative, entomopathogenic fungi (EPF) offer a biological approach to pest control. This study investigates the hypovirulence and virulence effects of EPF on corn and cucumber plants by measuring their Disease Severity Index (DSI). The treatment involves isolating and applying fungi to seeds to observe their pathogenic impact. The DSI, which measures disease intensity, is used to determine whether the fungi are virulent (DSI ≥ 2) or hypovirulent (DSI < 2). The virulence treatment involves exposing seedlings to fungal isolates and monitoring symptoms, such as brown hypocotyls, withered leaves, or seed death. Isolates like J5S1U2, J3S1U2, J2S2U1, and J2SU4 were found to be virulent towards cucumber, causing abnormal germination and severe stress. In contrast, hypovirulent isolates, such as J4S2U2, J3S2U1, and T5U1O, caused mild symptoms, including light brown patches under 0.5 cm on hypocotyls without significant wilting, making them safer for agricultural use. The treatment approach emphasizes selecting fungal isolates based on crop-specific virulence, aiming to optimize pest control strategies. Hypovirulent isolates, in particular, are identified as suitable candidates for integrated pest management, as they minimize plant stress while offering effective biological control.

1 Introduction

Modern agriculture faces growing challenges in managing pest populations sustainably and in an environmentally responsible manner. While initially effective, the overreliance on chemical pesticides has led to several serious concerns, including environmental pollution, adverse effects on human health, and, most critically, the development of pest resistance [1]. As pests evolve resistance to chemical treatments, the efficacy of these conventional methods diminishes, necessitating the exploration of alternative strategies. One promising approach is entomopathogenic fungi (EPF), naturally occurring microorganisms acting as biological control agents. EPF infects insect pests by penetrating their exoskeletons and increasing within the host, ultimately leading to the pest's death [2]. This biological control method

*Corresponding author: henik@umm.ac.id

offers an environmentally sustainable solution for pest management, avoiding the harmful consequences of chemical pesticides.

Several species of EPF, such as *Beauveria bassiana* and *Metarhizium anisopliae*, have been proven to be highly effective against various insect pests, including caterpillars, beetles, and aphids. For example, Beauveria bassiana has been successfully applied to control Helicoverpa armigera, a destructive caterpillar, by infecting its larvae and releasing toxins that lead to their death [3]. EPF as biocontrol agents minimizes the reliance on harmful chemical inputs and contributes to the broader objective of sustainable agriculture by promoting ecological balance and healthier ecosystems [4,5].

Indigenous strains of entomopathogenic fungi offer additional benefits due to their natural adaptation to local environmental conditions, such as soil composition, climate, and native plant species. These locally adapted fungi are often more stable and effective in their regions than non-indigenous strains [6]. A critical environment for EPF is the rhizosphere of maize *(Zea mays*), the thin layer of soil surrounding plant roots. This microbially rich zone supports the growth and activity of EPF, making it an ideal location for investigating fungal diversity. Given that maize is one of the most widely grown crops globally and plays a critical role in food security, understanding the diversity of EPF in its rhizosphere is essential for developing sustainable and effective pest control strategies on a larger scale [7].

The rhizosphere acts as a reservoir for beneficial microorganisms and as a habitat for EPF, contributing to plant health by serving as a biocontrol agent and promoting plant growth [8]. EPF can increase nutrient uptake and protect the root system from soil-borne pests. By isolating and studying these fungi from the maize rhizosphere, it is possible to harness biocontrol agents that are both highly effective and environmentally sustainable [9]. Maize is vulnerable to several pests, such as *Ostrinia nubilalis* (European corn borer) and *Spodoptera frugiperda* (fall armyworm), both of which pose significant threats to crop yields. While traditional pest control methods rely heavily on chemical pesticides, these approaches can harm the environment [10]. Indigenous EPF isolated from the maize rhizosphere provides an eco-friendly alternative for pest management. Having adapted to local environmental conditions, these fungi are more effective against region-specific pests [11]. For example, *Beauveria bassiana*, when isolated from the maize rhizosphere, has demonstrated effectiveness against *Spodoptera frugiperda* by penetrating its cuticle, proliferating within its body, and releasing enzymes that cause the pest's death. Similarly, *Metarhizium anisopliae* effectively targets soil-dwelling pests like *Diabrotica virgifera* (Western corn rootworm), one of the most damaging pests in maize cultivation.

The diversity of EPF is crucial for the resilience and effectiveness of biological pest control strategies. A diverse community of EPF enhances the possibility of targeting a broader range of pests, as different fungal species have specialized mechanisms for infecting various insect hosts. Moreover, maintaining a diverse population of EPF helps prevent the development of pest resistance, a common issue associated with the repeated use of chemical pesticides. For example, while *Metarhizium anisopliae* is particularly effective against soil pests, Beauveria bassiana controls foliage-dwelling insects, demonstrating the need for diverse fungal species in comprehensive pest management solutions [12]. The research aims to explore entomopathogenic diversity and evaluate the hypovirulence and virulence effects of indigenous entomopathogenic fungi (EPF) on corn and cucumber plants. By analyzing the

BIO Web of Conferences **143**, 01023 (2024) https://doi.org/10.1051/bioconf/202414301023
ICoN-BEAT 2024

Disease Severity Index (DSI), the study seeks to identify specific fungal isolates that are safe (hypovirulent) or harmful (virulent) to crops. The ultimate goal is to optimize agricultural pest control strategies by selecting EPF isolates that are both effective and crop-specific, thus promoting sustainable and integrated pest management solutions.

2 Material and Method

2.1 Soil Sampling

Soil samples were collected from two distinct agricultural areas. The first set of samples was taken from maize fields at the BALITKABI Research Station, located at Jl. Raya Kendalpayak No. 66, Segaran, Kendalpayak, Pakisaji District, Malang City, East Java. This location was selected for its use of chemical treatments. The second set of soil samples was collected from maize fields at Jl. Mulyo Dadi, Dau, Malang City, where organic treatments were applied. Soil samples were taken from five points within each field, with a minimum field size of 1/2 hectare. Sampling was conducted at a depth of 10-25 cm around the maize root zone, as this area is known to harbor microbial activity relevant to the study.

2.2 Entomopathogenic Fungi Baiting

After collecting soil samples, the soil was divided into 10 portions, with two plastic bottles containing soil samples per portion. The baiting process utilized *Tenebrio molitor* larvae (mealworms) as the bait for entomopathogenic fungi. In each sample portion, five larvae were placed into the soil, and the containers were covered with fabric or perforated bottle caps to prevent the larvae from escaping [13,14]. The baiting experiment was monitored over three weeks. As described by [14], this baiting method was conducted in a dark environment to encourage larval movement and increase the likelihood of contact with entomopathogenic fungi in the soil samples.

2.3 Preparation of Fungal Isolation Media

Potato Dextrose Agar (PDA) was used as the isolation medium for fungal growth [15]. To prepare 1 liter of PDA, the following ingredients were used: 500 grams of potatoes, 40 grams of granulated sugar, 36 grams of agar, and two capsules (500 mg each) of chloramphenicol as an antibacterial agent to prevent bacterial contamination [16].

2.4. Microorganism Exploration

2.4.1. Isolation of Entomopathogenic Fungi from Baiting Results

The isolation process involved transferring dead mealworms from the baiting experiment onto standard PDA plates [17]. The isolation procedure was conducted in a laminar airflow cabinet (LAF) to ensure a sterile environment. The first step involved washing the dead larvae using 70% ethanol and sterile distilled water. Four Petri dishes were prepared: two containing 70% ethanol and two containing sterile distilled water. The larvae were first immersed in ethanol for one minute and then in sterile water. After washing, the larvae were transferred to PDA plates, which were then wrapped and incubated for fungal growth.

2.4.2 Purification of Fungi

The purification aimed to separate fungal colonies based on their macroscopic morphology, including colony diameter, spore color, mycelium color, spore shape, and sporulation structures. Purification was performed by transferring a portion of the fungal colony from the initial isolation plate using a perforator and inoculating it onto a fresh PDA plate, placing the sample in the center of the dish. If morphological differences were still observed, further purification steps were undertaken until pure fungal cultures were obtained [18,19].

2.4.3 Microbial Identification

Microbial identification was performed both macroscopically and microscopically. Macroscopic characteristics included mycelium distribution patterns, colony color, the rate of mycelial growth covering the Petri dish's surface (expressed as daily growth rate), and colony texture. These observations were made over a 14-day period. The microscopic analysis focused on the structure, colour, and arrangement of conidia and conidiophores or sporangia, as described by [17,19]. The observed traits were then compared to reference data from scientific literature, including *Illustrated Genera of Imperfect Fungi* by Barnett and Hunter, for accurate identification of the fungi.

2.5 Hypovirulence Test

The hypovirulence test used corn seeds and cucumbers, the use of corn seeds because the study used corn samples as the main commodity of the field test to see whether fungi were virulent to corn. Cucumber seeds are used because this plant responds rapidly to pathogens and fungi attacks compared to other plants. The hypovirulence test, which is then virulent to corn and cucumber seedlings, causes the seedlings not to grow normally and even cause death due to the attack of virulent fungi. Cucumber and corn seeds were sterilized first using 70% alcohol for 1 minute. Then, the seeds were added with 2% Clorox for 30 seconds and then rinsed with sterile distilled water thrice (20). Then, the seeds were planted on PDA media, and microbial isolates were added for three days. One bottle was filled with three seeds. Cucumber seeds were incubated for 14 days, and observed growth of cucumber seeds every day. was then calculated with the Formula [15]:

$$DSI = \frac{\sum N}{Z}$$

Information: DSI= Disease Severity Index, N= The Severity value of each individual's disease, Z= Number of Individuals used

Disease Severity Index [15] :
0: Healthy plants are not infected
1: Normal growth, the leaves begin to show a yellowish color; there are light brown patches <0.25 cm,
2: Abnormal growth, light brown patches, brown patches of < 0.5 cm, and wetness areas <10% in hypocrites.
3: Abnormal growth of brown patches, chlorosis leaves, light brown to dark leaves > 1.0 cm and then joined by other patches and areas of wetness 10%<x<100% in hypocrites
4: Hypocots of black patches, withered leaves, and dead seedlings; infected seeds even die

The virulent Disease Severity Index value was ≥ 2, where the seeds are only abnormally capable, such as brown hypocots with withered leaves, and even the seeds die. Abnormal

germination of plants that are withered leaves, hypocots of black patches, and dead and still normal seedlings or hypovirulence patches of light brown < 0.5 cm and wet areas of <10% in hypocots, light brown to old patches > 1.0 cm and then joined by other patches and wet areas of 10%<x<100% in hypocots have not withered, and the hypocots are still firm and white [21,22].

3 Result and Discussion

3.1 Characterization of Entomopathogenic Fungi

The characterization of entomopathogenic fungi from the soil of the corn rhizosphere s obtained as many as 12 kinds of fungi, with the provision of codes J, S, and U (Corn Sample Caterpillars). The characteristics of the observed fungi include the pattern of mycelium distribution, the color of the colony without the front and back, the speed of growth of the mycelium meets the surface of the petri dish DAI (Days After Inoculation), the average accretion of colony growth (cm) mycelium meets the surface of the PDA medium and the texture of the colony. Microscopic observations were made over 14 days; the results of purification, characterization, and identification of fungi are presented in Figure 1.

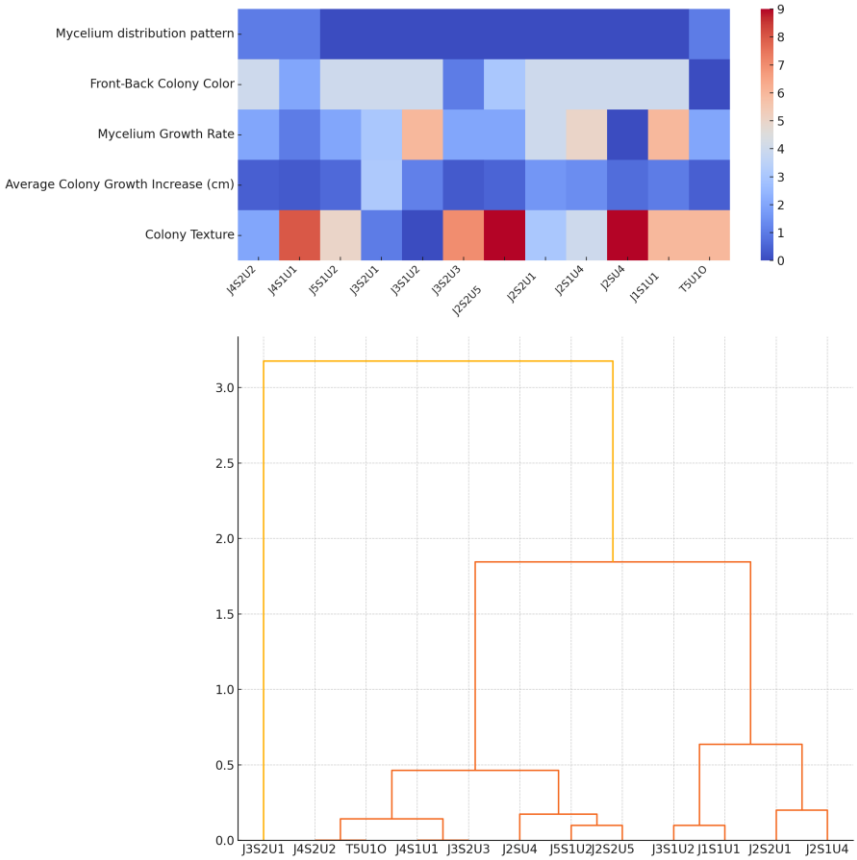

Fig 1. The morphological diversity and relationships among the isolates

The results of this study provide valuable insights into the morphological diversity and relationships among the isolates. The heatmap revealed variations in key traits such as

mycelium distribution, colony texture, colony color, and growth rate. Notably, isolates like J4S2U2, J3S2U1, and T5U1O exhibited significant differences in mycelial distribution patterns and colony texture compared to other isolates, indicating distinct growth behaviors. Similarly, isolates like J5S1U2 and J4S1U1 showed marked differences in colony color, reflecting greater diversity in pigmentation. The data on average colony growth rates revealed that J3S2U1 had the fastest growth, while isolates such as J2S2U1 and J1S1U1 exhibited slower growth rates. The dendrogram further illustrated the relationships between isolates by clustering them based on similarities across the measured variables. Isolates such as J2S1U4 and J2S2U1 were closely grouped, indicating a high degree of similarity in their characteristics. In contrast, isolates like J3S2U1 and J4S2U2 were placed on separate branches, suggesting they possess unique traits. Additionally, smaller subgroups, such as J5S1U2 with J2S2U5 and J1S1U1 with J3S1U2, demonstrated close relationships, reflecting similar morphological profiles across these groups.

3.2 Morphological Characterization of Entomopathogenic Fungi

These findings suggest that the observed morphological diversity among the isolates may have practical implications for their adaptability and potential applications. The distinct features of isolates like J3S2U1, particularly its rapid growth rate and unique colony characteristics, indicate that it may be more adaptable to specific environmental conditions or possess properties suited for targeted applications.

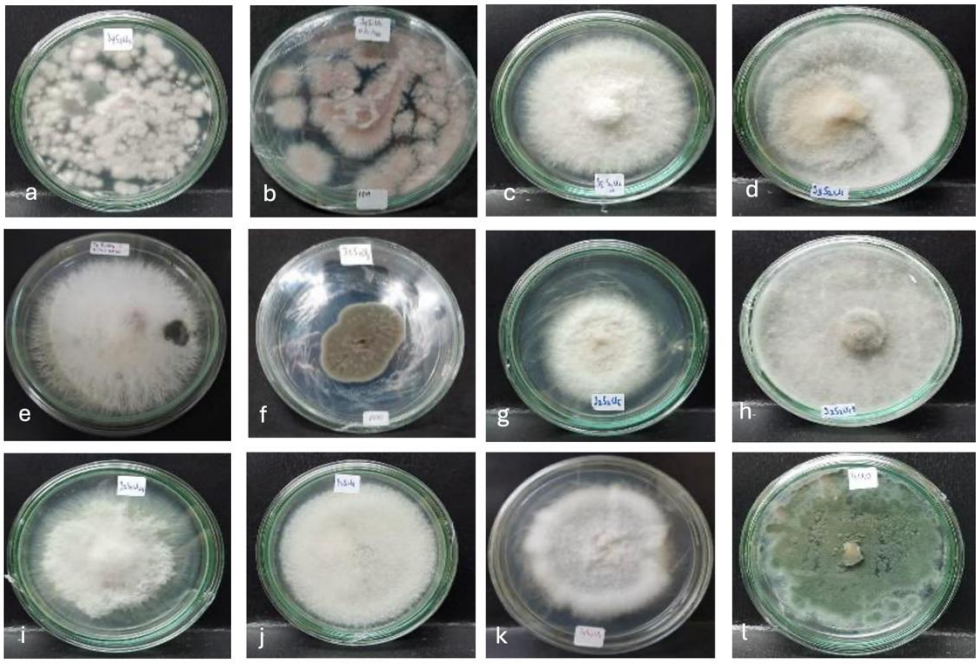

Fig. 2. Macroscopic Characterization of Entomopathogen Fungi, Note a. J4S2U2, b. J4S1U1, c. J5S1U2, d. J3S2U1, e. J3S1U2, f. J3S2U3, g. J2S2U5, h. J2S2U1, i. J2S1U4, j. J2SU4, k. J1S1U1, l. T5U1O. Where J= Fungi., S=Soil, U= replication.

On the other hand, the close similarities between isolates like J2S2U1 and J2S1U4 suggest that they share traits that could be valuable in similar contexts. The separation of isolates such as J4S2U2 and J3S2U1 in the dendrogram suggests that these isolates possess unique attributes, making them promising candidates for further exploration, particularly in the development of entomopathogenic fungi for pest control [23]. Colony colors have a wide

variety of colors but tend to be dominated by white. Figure 2. From macroscopic identification, J3S2U1 has the fastest growth of mycelium among fungi. J3S2U1, with a concentric distribution pattern of white-white front-back colony color, grew at the growth rate of mycelium for four days and filled the petri dish with an average growth of 3.1 cm, with a stringy rough colony texture. The number of fungi obtained can be influenced by the environmental conditions taken by the isolate.

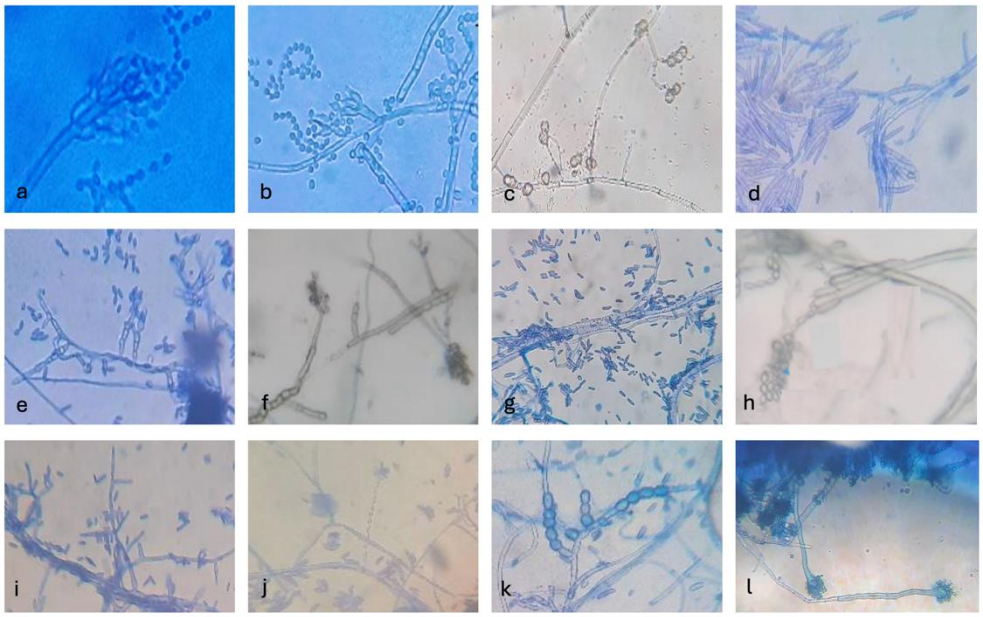

Fig. 3. Microscopic Characterization of Entomopathogen Fungi, Note a. J4S2U2, b. J4S1U1, c. J5S1U2, d. J3S2U1, e. J3S1U2, f. J3S2U3, g. J2S2U5, h. J2S2U1, i. J2S1U4, j. J2SU4, k. J1S1U1, l. T5U1O. Where J= Fungi., S=Soil, U= replication

The mycelium distribution pattern obtained two morphospecies: colonies that grow with concentric and radial patterns. For example, three isolates have a radial mycelium distribution pattern (such as the colony's radius spreading from the colony's centre towards the edge of the Petri dish). In comparison, nine isolates have concentric mycelium distribution patterns (neater pattern shapes and circles formed in a colony.

3.3 Entomopathogenic Fungi Hypovirulen Test

Based on the hypovirulence test, 12 fungi have the hypervirulent ability in corn and cucumber plants, as many as 8. The value of DSI = *Disease Severity Index,* which can be categorized as safe and harmless for plants to be applied fungi, namely <2. The corn plant commodity crosses Hypovirulen, which is safe to use because the corn plant is a plant that will be used for the next stage, namely seared. If the corn plant is safe from the hypovirulence test, then the plant can be used as presented in Figure 4.

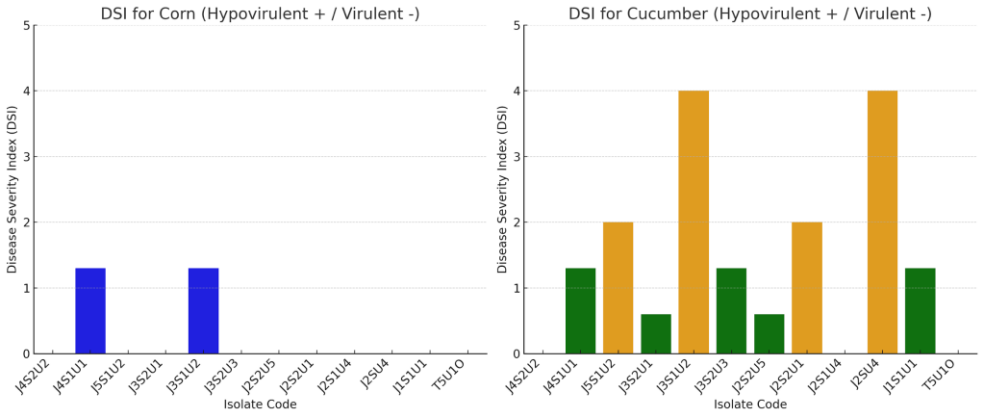

Fig. 4. The analysis of the Disease Severity Index (DSI) for both corn and cucumber

The Disease Severity Index (DSI) analysis results, based on the virulence threshold of DSI ≥ 2, offer critical insights into the effects of fungal isolates on corn and cucumber seedlings. A DSI value of 2 or higher indicates a severe impact on the seeds, where the plants exhibit symptoms such as brown hypocotyls, withered leaves, and, in worst cases, seed death. These virulent isolates cause abnormal germination, characterized by black patches on the hypocotyls, severely withered leaves, and seedling death, indicating significant plant stress. In contrast, isolates with DSI values below 2, particularly those that demonstrate hypovirulence, exhibit less severe symptoms [22]. For these isolates, seedlings remain healthy, with only minor effects such as small, light brown patches under 0.5 cm in diameter and wet areas covering less than 10% of the hypocotyls. In these cases, the seedlings do not show significant wilting, and the hypocotyls remain firm and white, indicating minimal damage and the plant's ability to continue normal growth.

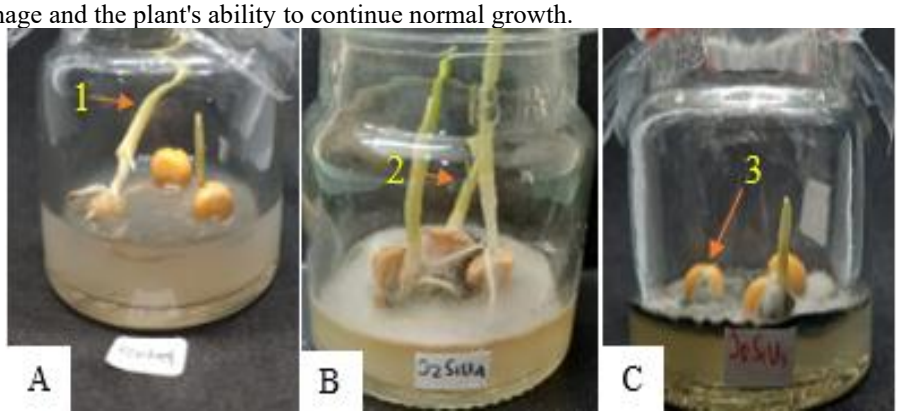

Fig. 5. Corn Seed Hypovirulence Test Results: Control; (A) Sprouts (1), J2S1U4Hypovirulence; (B) Sprouts (2), J5S1U1 Virulence; (C) No germination.

Isolates with intermediate DSI values, ranging between 1.0 and 2.0, cause more noticeable patches, which may extend beyond 1.0 cm and sometimes merge into larger areas of infection. However, even with these patches and wet areas affecting between 10% and 100% of the hypocotyl, the plants do not exhibit wilting, and the hypocotyls remain structurally sound. These symptoms suggest a level of fungal activity that does not yet threaten the overall health of the seedlings. In this study, isolates such as J5S1U2, J3S1U2, J2S2U1, and J2SU4 showed DSI values of 2.0 or higher in cucumber, indicating virulence and the potential for severe seedling damage or death. These isolates caused significant plant

stress, leading to abnormal germination and severe symptoms. On the other hand, isolates like J4S2U2, J3S2U1, and T5U1O consistently demonstrated low DSI values, representing hypovirulent behaviour. These isolates induced only minor, superficial symptoms, with the seedlings remaining healthy and viable [24].

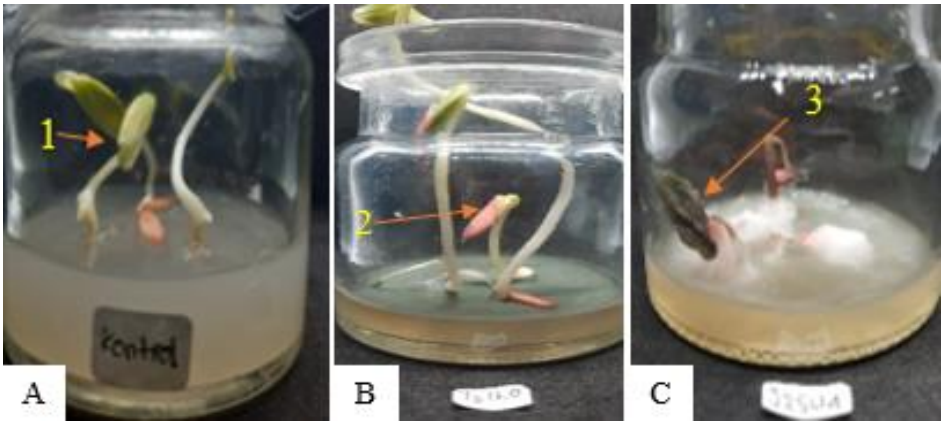

Fig. 6. Results of Cucumber Seed Hypovirulence Test: Control; (A) Sprouts (1), T5U1O Hypovirulence; (B) Sprouts (2), J2SU4 Virulence; (C) Dead Seeds.

The findings emphasize distinguishing between virulent and hypovirulent isolates when developing fungal biocontrol strategies or selecting fungal agents for agricultural applications [25]. Isolates with DSI values below 2, which cause only mild symptoms without compromising plant health, are more suitable for use in integrated pest management systems. In contrast, virulent isolates should be avoided or used with extreme caution to prevent significant crop damage.

4 Conclusion

The study's results clearly distinguish between virulent and hypovirulent entomopathogenic fungi in corn and cucumber plants. While all isolates were safe for use in corn, several isolates exhibited virulence in cucumber, with DSI values of 2.0 or higher. These virulent isolates, including J5S1U2, J3S1U2, J2S2U1, and J2SU4, caused severe damage to cucumber seedlings, leading to abnormal germination and increased mortality rates. On the other hand, isolates such as J4S2U2, J3S2U1, and T5U1O remained hypovirulent across both crops, causing only minimal symptoms, thus proving to be more suitable for broader agricultural use. The study emphasizes the importance of crop-specific testing in fungal biocontrol strategies to avoid potential crop damage while maximizing the benefits of fungal applications in sustainable agriculture.

Acknowledgements

Thank you to the University of Muhammadiyah Malang facilities and indofood research nugraha for the funding for Number SKE.20/CC/X/2020, which have been instrumental in supporting the completion of this research.

References

1. S. Samanta, A. Maji, M. Das, S. Banerjee, A. Bhattacharjee, N. Pal, An updated integrated pest management system: a footprint for modern-day sustainable agricultural practices. Uttar Pradesh Journal Of Zoology. **45**(8), 71–79 (2024).
2. S. Karthi, P. Vasantha-Srinivasan, S. Senthil-Nathan, Y.S. Han, M.S. Shivakumar, R.K. Murali-Baskaran, Entomopathogenic fungi promising biocontrol agents for managing lepidopteran pests: review of current knowledge. Biocatal Agric Biotechnol. **58**, 103146 (2024).
3. S. Karthi, P. Vasantha-Srinivasan, S. Senthil-Nathan, Y.S. Han, M.S. Shivakumar, R.K. Murali-Baskaran Rk, entomopathogenic fungi promising biocontrol agents for managing lepidopteran pests: review of current knowledge. Biocatal Agric Biotechnol. **58**, 103146 (2024).
4. M.D. Joshi, A.K. Srivastava, M. Ashaq, S. Jaggi, P. Gupta, W. Hasan, Biocontrol agents and plant protection. Uttar Pradesh Journal Of Zoology. **45** (16), 109–131 (2024).
5. M. Melkumyan, B. Babayan, A. Grigoryan, A. Yesayan, crops biological protection: phytopathogens growth inhibition by the entomopathogens. Bioactive Compounds In Health And Disease-Online Issn: 2574-0334; Print Issn: 2769-2426. **7**(8), 361–374 (2024).
6. L. Sui, H. Zhu, D. Wang, Z. Zhang, M.J. Bidochka, L. Barelli L, Tripartite interactions of an endophytic entomopathogenic fungus, Asian corn borer, and host maize under elevated carbon dioxide. Pest Manag Sci. **80**(9), 4575–4584 (2024).
7. Irsad, M. Shahid, E. Haq, A. Mohamed, P.Q. Rizvi, E. Kolanthasamy, Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. Front Microbiol. **26**, 14 (2023).
8. S. Mantzoukas, F. Kitsiou, D. Natsiopoulos, P.A. Eliopoulos, Entomopathogenic fungi: interactions and applications. Encyclopedia. **2**(2), 646–656 (2022).
9. A. Ikhwan, E.D. Septia, A. Sholihah, Characterization of rhizobacteria secondary metabolites on maize (*Zea mays*) in marginal land. in: iop conference series: earth and environmental science (Institute Of Physics, 2023).
10. J.M.P. Sari, S. Herlinda, S. Suwandi, E. Elfita, Effect of endophytic entomopathogenic fungal conidia and blastospores induced in maize plants by seed inoculation on spodoptera frugiperda immune response and mortality. Biodiversitas. **24**(10), (2023).
11. N. Thakur, P. Tomar, S. Kaur, S. Jhamta, R. Thakur, A.N. Yadav, Entomopathogenic soil microbes for sustainable crop protection. (529–571, 2021).
12. D. Zembrzuski, D.A. Woller, S. Jaronski, L.R. Black, K.C. Reuter, D. Grief, Understanding how diet and temperature affect survival and subsequent sporulation in a major rangeland grasshopper pest, melanoplus sanguinipes, infected with the entomopathogenic fungus, metarhizium robertsii. Biological Control. **183**, 105268 (2023).
13. M.N. Yakubu, M.A. Ladan, F.A. Deba, S. Isma'il, U.S. Haruna, H.U. Aliyu, Biodiversity and virulence characterization of entomopathogenic fungi isolated from soils in different regions of Nigeria. Egypt J Biol Pest Control. **32**(1), 93 (2022).
14. A. Eski, M.M. Gezgin, Susceptibility of different life stages of tenebrio molitor (Coleoptera: Tenebrionidae) to indigenous entomopathogenic fungi. J Stored Prod Res. **98**, 102008 (2022).
15. E.D. Septia, A. Zakia, A. Zainudinn, M. Maftuchah, A. Gofur, Y.E. Abbabiel, Quality and resistance optimization of banana (*Musa acuminata L.*) vegetative seeds through the addition of indole butyric acid with biological agents induction against fusarium wilt disease intensity. Kultivasi. **23**(2), (2024).
16. O.J. Oledibe, I.B. Enweani-Nwokelo, R.N. Okigbo, A.N. Achugbu, Formulation of fungal media from local plant materials. Advanced Gut & Microbiome Research. **2023**, 1–4 (2023).
17. E. Abd-Elazeem, M. Nada, A. Amer, R. Hussien, Isolation and identification of entomopathogenic fungi associated with the spiny bollworm (sbw), earias insulana, (boisd.)

(Lepidoptera: Nolidae) cadavers and evolution of their metabolites against insect's biological parameter. Egyptian Journal Of Agricultural Research. **0**(0):0–0 (2024).
18. S.S. Monica, P. Rajanikanth, P. Duraimurugan, S.A. Basha, D.S. Chary, Characterization of endophytic entomopathogenic fungal isolates: growth dynamics, viability, density, and morphological attributes. J Sci Res Rep. **30**(7), 74–81 (2024).
19. H. Mehrmoradi, S. Jamali, H.R. Pourian. Isolation of entomopathogenic fungi from cultivated and uncultivated soils and evaluation of virulence against callosobruchus maculatus (f.) (coleoptera:chrysomelidae). Journal Of Entomological Society Of Iran. **42**(1), 29–53 (2024).
20. K. Rathod, A. Patel, R. Mulani, Isolation, characterization and molecular identification of *Rhizobium Spp*. and *Trichoderma Spp*. from nodule and rhizospheric soil of *Arachis hypogaea*. Acta Scientific Microbiology. **1**, 47–56 (2023).
21. E.D. Septia, A. Zakia, A. Zainudinn, M. Maftuchah, A. Gofur, Y.E. Abbabiel, Quality and resistance optimization of banana (*Musa Acuminata L*.) vegetative seeds through the addition of indole butyric acid with biological agents induction against fusarium wilt disease intensity. Kultivasi. **23**(2), (2024).
22. O. Aguín, J.P. Mansilla, C. Salinero, M.J. Sainz, Biological control with transmissible hypovirulence: keys to success in chesnut blight. (147–63, 2020).
23. M.I. Russo, M.F. Vianna, A.C. Scorsetti, N. Ferreri, J.M. De Abajo, Entomopathogenic fungi as dual control agents against two phytopathogens and the lepidopteran pest rachiplusia nu in soybean (*Glycine max (L.) Merr*). Journal Of Fungi. **10**(2), 93 (2024).
24. A. Leonard, J.P. Egonyu, F. Khamis, C. Tanga, S. Ekesi, S. Kyamanywa, Identification and virulence screening of fungal and bacterial entomophathogens of the edible long-horned grasshopper ruspolia differens (Orthoptera: Tettigoniidae) from Uganda. African Entomology. **24**, 31 (2023).
25. W. Solomon, T. Janda, Z. Molnár, unveiling the significance of rhizosphere: implications for plant growth, stress response, and sustainable agriculture. Plant Physiology And Biochemistry. **206**, 108290 (2024).