KeAi
CHINESE ROOTS
GLOBAL IMPACT

International Journal of Innovation Studies

# MODELING OF SEMI-SOLID FERMENTATION MEDIUM FOR HIGH-YIELD CONIDIA PRODUCTION OF NOMURAEA RILEYI (FARLOW) SAMSON FOR LEPIDOPTERAN PEST CONTROL

**Trinh Thi Xuan [1,4*], Lam Thi Xuan Mai[1,5], Truong Thanh Xuan Lien[2,6], Nguyen Ly Thanh Duy[2,7], and Truong Thanh Quynh Dao[3,8], Son Pham Kim[1,9*]**

[1]: Plant Protection Department, College of Agriculture, Can Tho University, Can Tho City, Vietnam
[2]: Southern Plant Protection Centre, Tien Giang province, Vietnam
[3]: Cooperative Union of An Giang Province, Long Xuyen city, Vietnam
[4]: trinhthixuan@ctu.edu.vn; https://orcid.org/0009-0002-0513-7922
[5]: ltxmai@ctu.edu.vn; https://orcid.org/0009-0007-5973-469X
[6]: trươngthanhxuanlien@gmail.com
[7]: nltduy1996@gmail.com
[9]: pkson@ctu.edu.vn; https://orcid.org/0009-0004-9194-5719
* Correspondence: Trinh Thi Xuan, Tel: +84-91-9636915, Email: *trinhthixuan@ctu.edu.vn*

***Abstract***

The systematic literature review investigates Nomuraea rileyi's semi-solid fermentative process, which functions as an entomopathogenic fungus in Lepidopteran pest biocontrol applications. This review combines research conducted from 2020 to 2025 that focuses on maximizing conidia production for commercial use. A systematic review was conducted following PRISMA guidelines, through which researchers retrieved articles using Boolean operators within Scopus, Google Scholar, PubMed, and Web of Science databases. Studies using semi-solid fermentation, which reported quantitative data about conidia yield and viability along with controlled laboratory conditions, met all inclusion criteria. An evaluation process using the Newcastle-Ottawa Scale evaluated the studies to counteract potential biases. The production of conidia reaches its highest levels when scientists use rice materials as their substrate, especially when they work with broken rice and rice bran. The addition of yeast extract boosts conidial production, but implementation faces significant economic barriers due to supplement costs. The optimal conditions for obtaining viable conidial production involved maintaining temperatures between 25-30°C while keeping above 70% relative humidity. The review examines difficulties in large-scale conidial production because of expensive substrates and proposes implementing agricultural waste products to decrease manufacturing expenses. The study further advocates the research work into sustainable fermentation systems by utilizing various substrates, thus developing *N. rileyi* production at industrial scales for integrated pest management (IPM) applications.

**Keywords**: *N. rileyi*, entomopathogenic fungus, biocontrol, Lepidopteran pests, *Helicoverpa armigera*, *Spodoptera litura*, conidia production

Introduction

Biological insecticides and Biopesticides comprise natural organisms, including microorganisms, botanic extract, and certain natural enimies. These agents offer many advantages, such as low toxicity to humans and non-target organisms, biodegradability, and minimal environmental impact (Chaudhary et al., 2024). In biopesticides, the entomopathogenic fungi, including *Nomuraea rileyi* (*N. rileyi*) (Farlow) Samson, are perceived to be useful in controlling pests that affect agriculture. It is pathogenic to Lepidopteran larvae, especially *Helicoverpa armigera, Spodoptera exigua* and *Spodoptera litura* (Irsad et al., 2023). *N. rileyi* infects its hosts through conidia germinating on the insect cuticle's surface and penetrating, causing death through mycosis. (Lovett & Leger, 2017). Using conidia is the key factor in the biocontrol mechanism since conidia can spread throughout the field and infect the target pest.

Although *N. rileyi* is considered a biopesticide for controlling pests, its conidia production for commercial purposes presents a challenge. Conidia production usually occurs on solid substrates using traditional techniques, which may not be efficient, mainly due to batch production and high cost. (Rajamani & Negi, 2021). However, developing high-yield conidia remains critical to determining the best conditions to make this biocontrol agent cost-effective on a small scale. The high costs and lack of efficient fermentation media to support the growth of *N. rileyi* are the key challenges to its large-scale use in the industry. Thus, this study aims to assess various semi-solid media for conidia production of *N. rileyi* in laboratory settings. Therefore, the goal is to identify a cost-effective substrate that will provide a high yield of conidia and adequate viability so that *N. rileyi* can be commercialized for pest control.

## 2. Methodology

### Search Strategy

This study adheres to the PRISMA guidelines used to minimize biases and make the study more transparent and reproducible. (Moher et al., 2009). According to the PRISMA statement, the review methodology is comprehensive, and the results can be reproduced. Articles were retrieved from Google Scholar, Scopus, PubMed, and Web of Science using Boolean operators to combine the keywords 'semi-solid fermentation,' 'conidia production,' and '*Nomuraea riley*' (e.g., "semi-solid fermentation" AND "conidia production" AND "*Nomuraea riley*", "semi-solid fermentation" AND "conidia production", "semi-solid fermentation" AND "*Nomuraea riley*", "conidia production" AND "*Nomuraea rileyi*"). The research focused only on the articles published during 2022-2025 to provide updated information.

### Inclusion Criteria

- Studies focus on *N. rileyi* or related entomopathogenic fungi.
- Use of semi-solid fermentation techniques.
- Quantitative data on conidia yield or viability (e.g., conidia density or germination rates).
- Studies conducted under laboratory or controlled conditions.
- Studies published between 2020-2025

### Exclusion Criteria

- Studies focusing only on liquid fermentation methods.
- Studies lacking quantitative data on conidial yield or viability.
- Studies without transparent methodology or conducted outside controlled conditions.
- Non-peer-reviewed articles, theses, or reports.

- Studies published before 2020

Data Collection and Analysis

Data on the substrates employed in the process included rice, wheat, sorghum, and corn. In contrast, fermentation techniques included media preparation, nutrient supplements, sterilization and inoculation, and incubation conditions such as temperature, relative humidity, and incubation time. Conidia production was usually expressed on a substrate per gram, and conidia viability was tested using germination assays and bioassays. Studies that provided both conidia yield and viability data were prioritized. Data synthesis was based on thematic analysis that involved integrating data on the fermentation media, techniques, results, challenges, and gaps.

Quality Assessment

The quality of the included studies was assessed based on the Newcastle-Ottawa Scale, which considers aspects of the selection, comparability, and outcome (Table 1). This scale is essential for ensuring that only studies with minimal bias and robust methodology are included in the review (Luchini et al., 2017). It assists in assessing the reliability of participant selection and the control of confounding variables as well as the measurement of validity outcomes that ensure strong evidence to optimise *N. rileyi* production.

| **Study** | **Selection Representativeness** | **Selection exposed cohort** | **Ascertainment** | **Comparability Results not present at the start of the study** | **Comparability for confounders** | **Outcome** | **Outcome Assessment of outcome** | **Follow-up duration** | **Adequacy of follow-up** | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|
| Sowmya et al. (2022) | * | * | * | * | * | * | * | * | * | 9 |
| Ahirwar & Singh (2023) | * | * | * | * | * | * | * | * | * | 9 |
| Gómez-Valderrama et al. (2022) | * | * | * | * | * | * | * | * | * | 9 |
| Ranadev et | * | * | * | * | * | * | * | * | * | 9 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| al. (2024) | | | | | | | | | | |
| Sutthisa et al. (2024) | * | * | * | * | * | * | * | * | * | 9 |
| Villamizar et al. (2021) | * | * | * | * | * | * | * | * | * | 9 |

***Table 1:*** *Quality Assessment of Studies*

3. Results

Study Selection

The PRISMA process for this study began with identifying 1000 records through database searches. After removing 800 duplicate records, 200 studies remained for screening. No records were marked as ineligible by automation tools, nor were any removed for other reasons. These 200 records were screened based on their titles and abstracts, leading to the exclusion of 150 records that were deemed irrelevant. 50 full-text articles were assessed for eligibility, while 44 articles were excluded based on inclusion/exclusion criteria, particularly regarding *N. rileyi* soluble tablet formulations. Finally, six studies met all eligibility requirements and were included in the final review (Figure 1). This systematic process ensured that only the most relevant and high-quality studies were incorporated into the analysis.

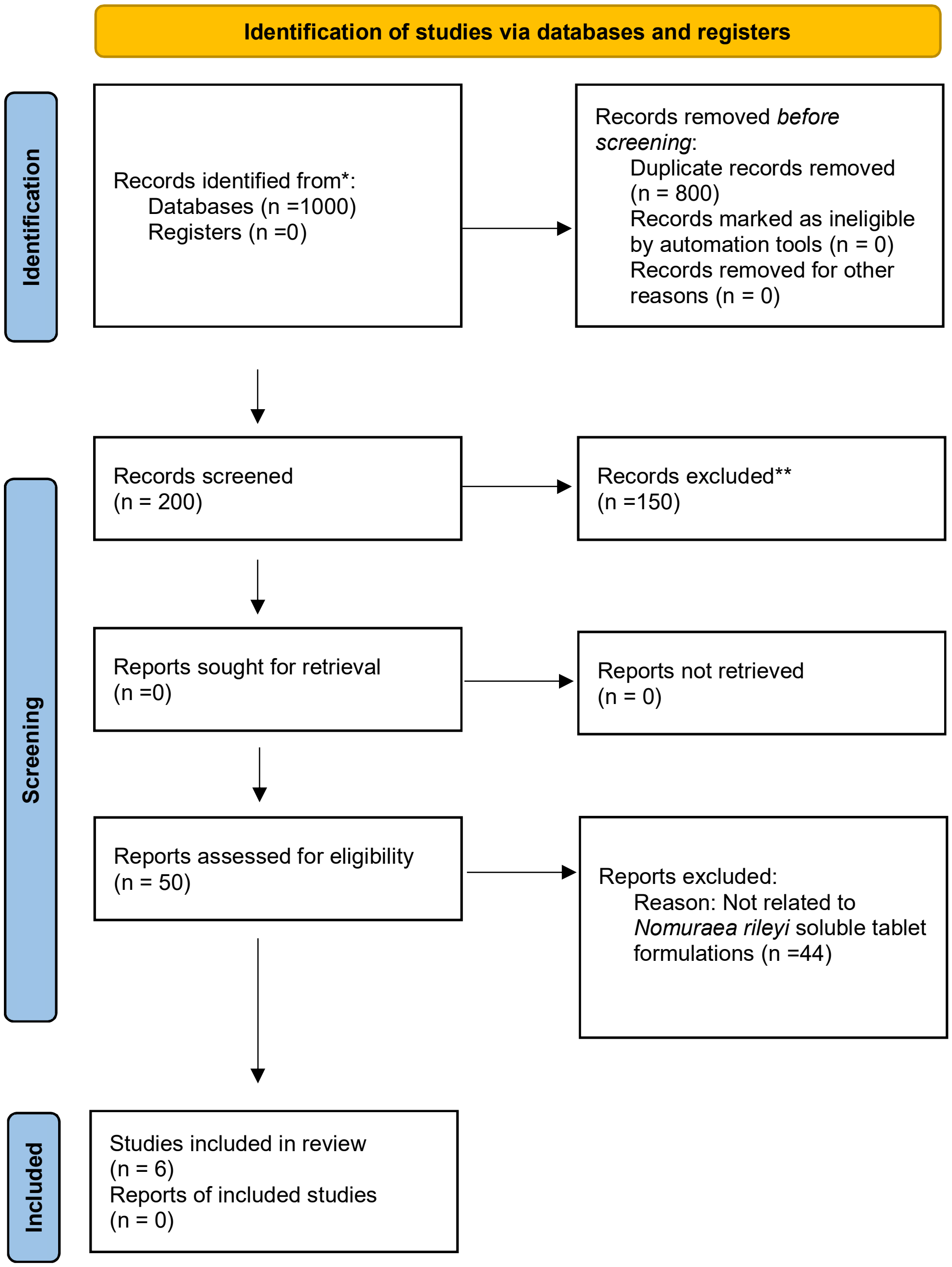


***Figure 1:*** *PRISMA Framework*

Study Overview Table

Table 2 summarises the selected studies, their objectives, the substrates applied, the fermentation methods used, incubation conditions, and the themes regarding *N. rileyi* conidia found in such studies.

***Table 2:*** *Summary of the selected studies*

| Study | Substrates Used | Fermentation Techniques | Incubation Conditions | Key Findings | Themes | Conidia Yield (spores/g) |
|---|---|---|---|---|---|---|
| Sowmya et al. (2023) | Rice, Wheat | Semi-solid fermentation with nutrient supplementation | Substrates were incubated post-inoculation (solid substrates evaluated at 15 days; liquid media monitored at 4–10 days) | Rice and wheat supported high conidial yields. Yeast extract supplementation improved conidia production. | Substrate Selection, Nutrient Supplementation, Incubation Conditions | High yield |
| Ahirwar and Singh (2023) | Not specified in the review | Not specified | Not specified | Entomopathogenic fungi (e.g., *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Nomuraea rileyi*) are effective bioinsecticides that are safe for non-target organisms and the environment, offering sustainable alternatives to chemical pesticides. | Safety, Efficacy, Insecticidal Toxin Production, Environmental Impact | Not Specified |
| Gómez-Valderrama et al. (2022) | Rice (150 g) combined with protein hydrolysate (150 mL of an 8% solution) | Solid-state fermentation using metallic trays, inoculated with 20 mL of a conidia suspension ($1 \times 10^6$ conidia/mL) | 10 days at 25 °C | The produced conidia of *M. rileyi,* when combined with SfMNPV, produced an additive effect that enhanced control | Integrated pest management, combined use of fungal and viral entomopathogens | Not Specified |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | of fall armyworm larvae in maize | | |
| Ranadev et al. (2024) | Sugarcane bagasse, paddy husk, post-mushroom substrate (PMS), and sorghum grains | Solid-state fermentation performed in 500 mL Erlenmeyer flasks; 100 g of processed substrate is used, with moisture adjusted to 60% | Incubation at 25 °C; flasks are manually mixed every 2 days for oxygenation; fermentation progress is monitored at 7, 14, and 21 days after inoculation. | Sorghum grains fortified with 10% molasses (treatment T8) produced the highest mycelial growth and spore yield, with PMS plus molasses also showing strong potential as an alternative substrate. | Substrate Selection, Fermentation Techniques, Incubation Conditions | The best treatment (T8) achieved spore counts on the order of $10^9$ conidia per gram at 21 days. |
| Sutthisa et al. (2024) | Talcum, corn starch, and tapioca starch | Powder formulation of Trichoderma asperellum MSU007 | Dual culture assays at 28°C; durian leaves inoculated with Colletotrichum sp. incubated at room temperature for 24 h before treatment. | Trichoderma asperellum MSU007 showed significant inhibition of Colletotrichum sp. in vitro, with formulation 3 having the highest effectiveness in reducing anthracnose severity on durian. | Trichoderma formulation, Biocontrol, Disease control | Formulation 3 maintained $4.33\times10^8$ cfu/g after 90 days of storage |
| Villamizar et al. (2021) | Oats, Rice | Semi-solid fermentation, conidia production on oat-based medium | Inoculated trays were incubated in a growing room under 24-hour artificial light at 23 ± 2°C. | Oat-based medium in semi-solid fermentation produced conidia with a yield of 7.41 x 108 conidia/g. | Substrate Selection, Incubation Conditions, Conidia Production | 7.41 x 108 conidia/g (oat-based medium) |

Thematic Analysis

The thematic analysis of the studies presented several key findings on the production of *N. rileyi* conidia.

1. Substrate Selection for Conidia Production

The choice of substrate contributes to determining the yield and quality of conidia of *N. rileyi* during semi-solid fermentation. The substrate is selected considering its nutrient content, surface area, and ability to support the growth of fungi. Some substrates examined in various studies included rice, wheat, sorghum, corn, and rice bran.

Rice was used as the substrate for producing the highest conidia yields in all the reviewed literature. This is probably because of the nutritional value of rice and the size of its particles, where *N. rileyi* obtained high-yield conidia. The parboiled rice provides the highest number of conidial counts of 9.76 × 10^8 spores/g after fermentation of 15 days. This is relevant to the study conducted by Ranadev et al. (2024), which explained that the highest conidial density and viability were evident in the rice culture medium.
Sowmya et al. (2023) also included rice bran as an ingredient that could be used to supplement the broken rice. This highlighted that supplemented broken rice or rice bran potentially affected the conidial yield while rice bran provided major nutrients to the substrate, helping fungi to grow while affecting increased conidia. However, the tested substrates in which wheat and sorghum encouraged development and germination of fungi, whereas conidial production was considerably reduced as compared to rice. The wheat-defined ones showed lower conidial production than rice among several fungi species used in various studies, as identifed by Ranadev et al. (2024). Ranadev et al. (2024) reported that solid fermentation on cooked rice produced $1.68\times10^{9}$ conidia/g at 20 days—approximately 2.27-fold higher than the yield from an oat-based semi-solid medium ($7.41\times10^{8}$ conidia/g). Villamizar et al. (2021) confirmed that rice-based media are particularly effective for high conidial production, while oat-based systems yield moderate results. Although other substrates such as wheat and sorghum have been examined in various studies, the evidence strongly favors rice as the superior substrate for enhancing conidia yield.
2. Impact of Nutrient Supplementation on Conidia Yield
The supplementation of yeast extract, vitamins, and sugars in the substrates improves conidia formation (Gómez-Valderrama et al., 2022; Sowmya et al., 2023). Moreover, various studies have presented how nutrient supplementation influences the formation of conidia along with the growth of mycelial that significantly enhances viability. In these studies, supplementation of yeast extract increased germination as well as conidial yield that recommends sustainable fungal growth. Sowmya et al. (2022) also validated that vitamins and sugars such as glucose and sucrose increased conidia production using rice and wheat substrates when included in the fermentation media. However, a potential challenge of supplementing nutrients' cost in large-scale production remains to be addressed. Supplementation helps enhance yields, while using high-quality nutrients such as yeast extract causes an increase in cost, limiting industrial uses. Gómez-Valderrama et al. (2022) also mentioned that although nutrient supplements augment fungal growth in large-scale production, they can be expensive due to the costs of yeast extract and other supplements. The sugars, including glucose and sucrose, were identified as enhancers of conidial production on different substrates, the extent of which depended on the substrate. Wheat-based media performs better in the presence of sugars, while rice-based media require comparatively less sugar addition for optimum yield. This means that various substrates may require diverse nutrients, which have not been explored to enhance fermentation. Sutthisa et al. (2024), although not focused on *N. rileyi*, found similar trends with Trichoderma asperellum MSU007 in terms of nutrient supplementation for improved biocontrol activity. This reinforces the broader applicability of nutrient-rich formulations for enhancing conidial production in fungi.

3. Incubation Conditions and Their Effect on Conidia Production

Temperature, Relative Humidity (RH), and incubation time were also noted to impact conidia production in all the reviewed studies. Sowmya et al. (2023) revealed a temperature of 30°C in conidia yield, while the RH must be at 70 -80% for appropriate fungal growth. Temperature and RH are considered correlated factors because both are concerned with the regulation of the metabolism rates of the fungi, mycelial growth, and production of conidia.

The incubation period is another critical component as highlighted by various studies reporting that the increasing incubation period from 7 to 21 days led to the higher production of conidia. Ahirwar and Singh (2023) stated that after 15 days, the yield of conidia was not very high, and the further incubation period contributed to higher production of conidia. Moreover, hand-shaking or rotating cultures are the important inoculation methods for improving the supply of oxygen to increase mycelial growth as well as conidia formation. According to Ranadev et al. (2024), the shaking or rotating culture vessels prove better for fungal growth since oxygen transfer is important for mycelial formation and sporulation. Therefore, relative humidity, temperature, and incubation time affect conidia yield while proper aeration can lead to high yields in a semi-solid fermentation system.

4. Challenges in Large-Scale Production

Several factors may prove problematic in producing large quantities of conidia of *N. rileyi* for commercial purposes. They include prices that can be regarded as a primary concern when working with large amounts as it may hinder the utilization of costly and limited products such as parboiled rice.

Ranadev et al. (2024) also mentioned that the efficiency of the substrates, such as sugarcane bagasse, is high, but the batch fermentation used in many studies is unsuitable for industrial levels. Batch fermentation, despite its success in laboratory-scale production, is inefficient for industrial-scale production, for it requires constant inoculation, monitoring, and harvesting, which is inefficient and reduces the economy of scale production. Sutthisa et al. (2024) identified that while certain substrates can enhance biocontrol efficacy, their cost can limit industrial use.

Another limitation is how best to handle the conidia after the harvest. The application of conidia in biocontrol is a sensitive process where drying and packaging can affect viability and efficacy. (Ahirwar & Singh, 2023; Gómez-Valderrama et al., 2022). Inadequate drying procedures affect the infectivity of the conidia, and inadequate packing exposes them to conditions that cause their deterioration over time. Further research should be conducted to optimize the drying and packaging techniques to enhance the usability of *N. rileyi* as a bioinsecticide.

5. Identification of Knowledge Gaps

The thematic analysis helped establish some of the significant gaps in knowledge. Thus, findings indicate cost-effective substitutes for parboiled rice for industrial use are a significant concern. Rice bran and other locally available materials may be feasible, but their availability and cost-effectiveness may be limited. Consequently, large-scale production requires optimization of conidia production using continuous fermentation systems. These limitations make the current batch fermentation method less automated and make it difficult to scalability, indicating the need for new technologies or innovations in the process.

There is still a need for research on techniques for aeration and light exposure. Although hand-shaking and rotating cultures have demonstrated some advantages, the effects of continuous aeration systems on conidia production remain unclear. The impact of light on the germination of conidia and mycelial growth needs further exploration, as some fungi require a specific light environment for the growth to occur. Hence, future research is required to optimize the large-scale production of *N. rileyi* with a particular emphasis on cost-effectiveness and conidial production yield and viability.

6. Discussion

This study aims to identify the optimum semi-solid fermentation media for producing *Nomuraea rileyi* conidia at the industrial level in controlled conditions. The supplementation of nutrients and conditions supporting the incubation considering the conidial yields and viability are also considered. *N. rileyi* is a mycological agent widely accepted in crops' biocontrol of lepidopteran pests. Due to the adverse environmental and health effects of chemical pesticides, there has been a shift to biological control. Thus, enhancement of the conidia yield of *N. rileyi* is vital for its commercialization and incorporation into IPM systems. This study identified the most suitable substrate(s) for the production of conidia, tested nutrient supplements, and assessed the impact of incubation parameters on fungal fermentation success. This study also shows a reduced availability of cost-effective and sustainable means of producing biological control agents such as *N. rileyi* to reduce chemical pesticide use in agriculture. (Faria & Wraight, 2007).

*N. rileyi*'s production is best performed as highlighted by this study by using substrates based on rice. Among the substrates, rice was considered the most suitable product but brown rice as well as parboiled rice showed the highest rate of conidial yield as compared to corn and wheat. It is under the findings of Ranadev et al. (2024), who isolated conidia in the different entomopathogenic fungi species of *Metarhizium anisopliae* and *Beauveria bassiana* on the basal media and rice. This is because the surface area of rice grains is high, and the nutrient content on the surface is dense, which is much suited for the growth and development of the fungal mycelia.

The substrate area will need aeration and nutrient uptake by fungi like *N. rileyi* to produce conidia. Rice, particularly broken rice, has a larger surface area that allows for an adequate supply of oxygen, which is required by the fungi in their metabolic processes and the generation of spores. (Abeer, 2023). Carbohydrates, proteins, and lipids in rice increase the efficiency of Mycetes and sporulation and *Metarhizium and Beauveria* species in terms of growth, as proven by certain studies. (Pham et al., 2009; Ramanujam et al., 2014)

The main challenge affecting parboiled rice production is the elevated prices of rice. In a study by Ramanujam et al. (2014), they placed high cost as the reason why the use of parboiled rice in large-scale biocontrol production is financially unfeasible. However, rice bran and broken rice are the readily available products that were also favorable in this paper. This means that cost-effective sources of rice, such as rice bran, could be cost-effective sources for the commercial production of conidia. (Sowmya et al., 2023). This highlights that the fungal growth is highly supported by wheat and sorghum but within a limit as compared to rice, also implying that these substrates cannot be ideal for producing high conidia. Moreover, this indicates that the substrates wheat and sorghum support fungal growths but to a limited level compared to rice, implying that these substrates are not ideal for high conidia production. This may coincide with the information about the metabolism of entomopathogenic fungi that have stated that wheat is unfavorable for protein content and its variation. (Ranadev et al., 2024).

Moreover, while sorghum was the most cost-efficient, it produced fewer conidia; therefore, choosing a substrate on cost reduces conidia production (Barra-Bucarei et al., 2016).

The impact of yeast extract, vitamins, and sugars was monitored on the count's level of *N. rileyi* conidia, which correlated with increased nutrient content. This study confirmed that the conidial production and viability increased in rice and wheat media supplemented with yeast extract. These findings are in agreement with Jaronski (2023) On the effects of nitrogen supplementation on the growth and spore production of fungi. Yeast extract also contains amino acids and vitamins, which are necessary for sporulation and mycelial growth, besides other factors (Aravinthraju et al., 2024; Mejia et al., 2024).

The increase in conidial yield as a result of nutrient supplementation to improve nutrition yield helps in conidial yield and mycelial development and sporulation in *N. rileyi* and other entomopathogenic fungi. (Mejia et al., 2024). Yeast extract supplementation further enhanced the conidia production by a moderate measure compared to rice supplementation which suggests the extent of increase is supported by the methods used in feeding the basal medium with the nutrients.

Sowmya et al. (2023) and Ranadev et al. (2024) noted that when it is performed commercially, the cost implication of supplementing nutrients and commercial supplements like yeast extracts is high. This also indicated that some unsupplemented substances in broken rice can increase forming conidia but at a slower rate. This study is one of the most important research areas that concerns large-scale biocontrol production by using supplements that can enhance conidial yield but at the cost of supplements (Barra-Bucarei et al., 2016).

There is a further need to research on responding to these potential challenges such as seeking effetive nutrient sources that could replace commercial supplements. Other substrates, such as molasses, corn steep liquor, and soybean meal, can cultivate other fungal species, as stated by Sowmya et al. (2023) and Mejia et al. (2024). They could also contribute to reducing cost to the extent that they do not suppress conidial yield and viability, enhancing the economic aspects of production.

This study evaluates the capability of various substrates in wheat, sorghum, rice, and corn in the growth of *N. rileyi* conidia on a semi-solid fermentation system. Viability, sustainability, and cost often select these substrates while promoting the growth of fungi. However, this research also validates the recent studies on entomopathogenic fungi endorsing fungi culture on rice-based media to produce conidia. Conversely, the previous research on entomopathogenic fungi, rice bran, and broken rice was not investigated to develop *N. rileyi* conidia.

Chaudhary et al. (2024) Showed that rice-based substrates promote fungal growth and conidia. They observed that rice was relatively better than another substrate for the conidia's large-scale production of entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana*. All the nutritional requirements clinically proven to be essential in the growth and reproduction of fungi, including carbohydrates, proteins, and lipids, are present in rice. Since broken rice has a larger surface area for the grain, oxygen diffusion is encouraged, and the mycelium and the conidia are nurtured for growth. Rice bran, which results from milling raw rice, has also been found to be ideal for fungal growth because of its fiber content and nutrients; therefore, rice bran can be more beneficial than other forms of rice. (Jaronski, 2023; Sowmya et al., 2023).

This study also revealed that broken rice supplemented with yeast extract supported the highest conidial production among all the tested media with 9.76 x 10^8 spores/g. This supports the studies of Jaronski (2023), revealing that rice is most suitable for the growth of *N. rileyi* conidia. The nutrient value of rice provides the means through which the mycelia and spores, as viable conidia, can grow in large quantities. Moreover, supplementation of nutrients with yeast extract also observed more growth of fungal concerns. Thus, substrate quality and nutrient medium are significant factors for the large-scale production of conidia.

Wheat, corn, and sorghum yielded fewer conidia in the current study, as reported and observed in the previous research. While wheat has been used as a substrate in other studies of entomopathogenic fungi, it produced less conidia than rice. Mascarin et al. (2024) Reported that rice was less effective in yielding *Metarhizium anisopliae* conidia, similar to this study where *N. rileyi* was used. Several factors can explain why wheat is less effective in this context than it used to be earlier in the year. More protein and fats are needed, but not as many carbohydrates are in rice. Simple sugars are beneficial in promoting the growth and sporulation of fungi, perhaps because rice-based media have been shown to produce more conidia than wheat. Similarly, the bioassay of sorghum and corn demonstrated undesirable results when field-tested for several entomopathogenic fungi, including *N. rileyi,* in this study. *Metarhizium anisopliae* sclerotia production was moderate to low in sorghum, according to Barra-Bucarei et al. (2016), while corn was identified as less suitable according to Sain et al. (2018). Sorghum and corn also produced less conidia than rice, meaning that substrate nutrient content and surface area also determine the extent of fungal production. Sorghum and corn contain fewer carbohydrates than rice, and their structure might not allow for as much mycelial growth and development.

Despite the efficacy of rice in conidia production, as seen in this study, the production cost of using rice media remains a concern and may not be commercially sustainable. In the earlier study, it was seen that parboiled rice is costly; therefore, it does not suit large-scale biocontrol production. (Ramanujam et al., 2014). Consequently, studying new, cost-effective materials is crucial in this context. Rice bran has also been observed to be a more economical substrate, and conidia yield has also been reported to be high, making it a possible candidate for large-scale production. Rice bran is usually considered a waste product, but due to its nutritional value, it can be employed in fungal fermentation. (Ranadev et al., 2024). Making conidia from agro-industrial waste such as rice bran reduces the cost of production and covers a significant constraint to the commercialization of fungal biocontrol agents.

Various research studies have focused on using agro-industrial residues for low-cost rice substitutes for fungal culturing. Despite the availability of numerous substrates, sugarcane bagasse has been proven ideal because of its low cost, especially for the mass production of conidia, especially when using *Beauveria bassiana* (Ranadev et al., 2024). They contain cellulose and hemicellulose, which are significant for fungal growth. *B. bassiana* and *M. anisopliae* have established before to grow and sporulate on these substrates effectively. However, sugarcane bagasse may be regarded as an industrially suitable medium for producing the conidia of *N. rileyi* on an industrial scale, but more research is needed. When conidia are grown on agro-industrial waste substrates, it is necessary to supplement nitrogen sources and vitamins (Aravinthraju et al., 2024).

To support fungal development and industrial enzyme production, a variety of nutrient-rich agro-industrial residues can be used as low-cost substrates. These include sugarcane bagasse, orange peel, wheat bran, corn straw, barley, rice straw, corn cob, husk, soy bran, and coffee husk. These residues are valuable because they contain proteins, vitamins, minerals, and sugars that are essential for fungal growth and metabolism, making them suitable for fermentation processes, according to Abeer (2023). Soybean powder have been used to cultivate several fungi, including *Metarhizium anisopliae* performed. Mejia et al. (2024). These agricultural by-products could be used to replace expensive products such as yeast extract, thus extending the implications of cost-savings from this study to future large-scale production of *N. rileyi* conidia. This study also emphasizes the need for another suitable, cost-effective substrate to be investigated to be used for the large-scale production of conidia of *N. rileyi*. Rice-based media are found to be greatly efficient in this study, but the cost of preparation makes it unsuitable for commercial production. Fungal biocontrol agents can be formulated from locally available low-priced raw materials such as rice bran, sugarcane bagasse, or corn steep liquor.

Future studies are also needed on utilizing wastes from other industries, particularly wood processing, to grow conidia substrates. Therefore, supplementing the nutrients to the agro-industrial wastes could enhance fungi growth and conidia production. Similarly, to increase the amount of conidia produced in agro-industrial waste substrates, studies of the optimality of specific parameters, such as aeration and incubation, should be done.

## Implications for Biocontrol

*N. rileyi* effectiveness can significantly contribute to the mycoinsecticide in controlling Lepidopteran larvae. By generating a significant number of conidia on the rice-based substrates, especially when nutrients augment it, then *N. rileyi* stands as a strong candidate for developing biological control in agriculture. It has been established that *N. rileyi* is safe in the environment, the pests attacked are targeted, and there is a low propensity for pests to develop resistance against the biocontrol agent (Faria & Wraight, 2007). However, it may also be used in IPM systems to contain toxic chemicals and improve agricultural practices.

The effectiveness as a biocontrol agent would depend on these factors because the virulence of *N. rileyi* depends on the ability of the conidia to be viable and germinate effectively. From this research study, nutrient improvement, particularly yeast, influences the efficaciousness of conidia, which is presumed to be important in field use. In bioinsecticide application, they must also know the amount of conidia and, most importantly, the number of viable and infectious spores. This study shows that controlling incubation conditions and supplementing nutrients positively impact the production of enhanced conidia to enhance the killing of pest larvae. However, certain limitations are associated with using *N. rileyi* as a biocontrol agent. The costs associated with rice and nutrient supplementation will limit large-scale production. According to Ramanujam et al. (2014), parboiling of rice and commercial nutrients increases the costs of production, and the use of chemical pesticides outweighs that of bio-pesticides in cost. Thus, there is a need to search for cheaper substrates and other nutrients to cultivate the fungus and have cost-effective yields for the conidia.

## Conclusion

In conclusion, in a semi-solid fermentation medium, large quantities of viable spores could be produced when *N. rileyi* was cultured using rice-based substrates, particularly broken rice and rice bran. Yeast extract was an effective supplement that improved conidial production but was a source of extra cost. Further studies should be aimed at the use of other cost-effective substrates, such as agro-industrial wastes, such as bagasse and corn steep liquor, among others, to improve yields while reducing costs. Moreover, improving fermentation conditions, such as continuous fermentation and aeration techniques, could be more useful for large-scale industries. Since *N. rileyi* is considered a mycoinsecticide, information on its cost-effective production is relevant. As for future research, the previous recommendations should be reconsidered and enhanced, including nutrient optimization, and investigations of other production systems that would make *N. rileyi* a more profitable solution for IPM.

References

Abeer, A. A. E. A. (2023). Valorization of agro-industrial wastes using fungi for industrial enzyme production. *International Journal of Frontline Research in Science and Technology*, *2*(2), 001-023. https://doi.org/10.56355/ijfrst.2023.2.2.0055

Ahirwar, N. K., & Singh, R. (2023). Assessing the safety and efficiency of entomopathogenic fungi as bioinsecticides: a brief review. *Journal of Earth & Environmental Waste Management*, *1*, 1-6.

Aravinthraju, K., Shanthi, M., Murugan, M., Srinivasan, R., Maxwell, L. A., Manikanda Boopathi, N., & Anandham, R. (2024). Endophytic Entomopathogenic Fungi: Their Role in Enhancing Plant Resistance, Managing Insect Pests, and Synergy with Management Routines. *J Fungi (Basel)*, *10*(12). https://doi.org/10.3390/jof10120865

Barra-Bucarei, L., Vergara, P., & Cortes, A. (2016). Conditions to optimize mass production of *Metarhizium anisopliae* (Metschn.) Sorokin 1883 in different substrates. *Chilean journal of agricultural research*, *76*(4), 448-454. https://doi.org/10.4067/s0718-58392016000400008

Chaudhary, R., Nawaz, A., Khattak, Z., Butt, M. A., Fouillaud, M., Dufossé, L., Munir, M., ul Haq, I., & Mukhtar, H. (2024). Microbial bio-control agents: A comprehensive analysis on sustainable pest management in agriculture. *Journal of Agriculture and Food Research*, p.101421.

Faria, M. R. D., & Wraight, S. P. (2007). Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, *43*(3), 237-256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Gómez-Valderrama, J., Cuartas-Otálora, P., Espinel-Correal, C., Barrera-Cubillos, G., & Villamizar-Rivero, L. (2022). Fungal and viral entomopathogens as a combined strategy for the biological control of fall armyworm larvae in maize. *CABI Agriculture and Bioscience*, *3*(1). https://doi.org/10.1186/s43170-022-00094-7

Irsad, Shahid, M., Haq, E., Mohamed, A., Rizvi, P. Q., Kolanthasamy, E., & (2023). Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. *Frontiers in Microbiology*, *14*, p.1208237.

Jaronski, S. T. (2023). Mass Production of Entomopathogenic Fungi: State of the Art. In *Mass Production of Beneficial Organisms* (pp. 357-413). https://doi.org/10.1016/b978-0-12-391453-8.00011-x

Lovett, B., & Leger, R. J. S. (2017). The insect pathogens. *The fungal kingdom*(923-943).

Luchini, C., Stubbs, B., Solmi, M., & Veronese, N. (2017). Assessing the quality of studies in meta-analyses: Advantages and limitations of the Newcastle Ottawa Scale. *World Journal of Meta-Analysis*, *5*(4), 88-84.

Mascarin, G. M., Golo, P. S., de Souza Ribeiro-Silva, C., Muniz, E. R., de Oliveira Franco, A., Kobori, N. N., & Fernandes, E. K. K. (2024). Advances in submerged liquid fermentation and formulation of entomopathogenic fungi. *Appl Microbiol Biotechnol*, *108*(1), 451. https://doi.org/10.1007/s00253-024-13287-z

Mejia, C., Rocha, J., Sanabria, J., Gomez-Alvarez, M. I., & Quiroga-Cubides, G. (2024). Performance of *Metarhizium rileyi* Nm017: nutritional supplementation to improve production and quality conidia. *3 Biotech*, *14*(3), 89. https://doi.org/10.1007/s13205-023-03911-6

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & Group, P. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Pham, T. A., Kim, J. J., Mm, S. G., & Kim, K. (2009). Production of blastospore of entomopathogenic B*eauveria bassiana* in submerged batch culture. *Mycobiology*, *37*(3), 218-224.

Rajamani, M., & Negi, A. (2021). *Biopesticides for pest management.*

Ramanujam, B., Rangeshwaran, R., Sivakumar, G., Mohan, M., & Yandigeri, M. S. (2014). Management of Insect Pests by Microorganisms. *Proceedings of the Indian National Science Academy*, *80*(2). https://doi.org/10.16943/ptinsa/2014/v80i2/3

Ranadev, P., Nagaraju, K., & Vasanth Kumari, R. (2024). From Waste to Utilization: Assessing the Feasibility of Using Post-Mushroom Substrate and Other Agro-Wastes for the Mass Production of Entomopathogenic Fungi. *Journal of Agricultural Science and Technology*, *10*.

Sain, S. K., Sathyanarayan, N., & Jeyakumar, P. (2018). Evaluation of biodegradable agricultural substrates for mass production of entomopathogenic fungi. *Indian J Pl Protec*, *46*(1), 78-83.

Sowmya, E., Hosamani, A. K., & Mamatha, M. (2023). Mass production of insect pathogenic hypocreale fungi, Metarhizium anisopliae on solid substrates and liquid media. *Journal of Biological Control*, 169-174. https://doi.org/10.18311/jbc/2022/30970

Sutthisa, W., Popranom, A., Taddeetrakool, A., & Khankhum, S. (2024). Development of Trichoderma formulation and application to control durian anthracnose disease. *Trends in Sciences*, *21*(1), 7276-7276.

Villamizar, L. F., Barrera, G., Hurst, M., & Glare, T. R. (2021). Characterization of a new strain of *Metarhizium novozealandicum* with the potential to be developed as a biopesticide. *Mycology*, *12*(4), 261-278.