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Optimizing Semi-Solid Fermentation Substrates for Enhanced Conidia Production of *Nomuraea rileyi* (Farlow) Samson as a Biopesticides for Insect Control

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ABSTRACT

Biological insecticides have gained importance in modern society because they cause minimal environmental harm and reduce pests' ability to develop resistance. *Nomuraea rileyi*, a facultative entomopathogenic fungus, is suitable for infecting lepidopteran pests, including *Spodoptera litura* and *Helicoverpa armigera*. This work aims to identify a suitable semi-solid fermentation medium to increase the conidia production of *N. rileyi* and enhance its biological control ability as mycoinsecticide. Research is carried out on broken rice, wheat, sorghum, and corn as substrates, both with and without nutrient addition. It also covers incubation under set conditions, colony harvesting, and conidial yield and germination determination using a haemocytometer. All the study was conducted using a completely randomized design (CRD) and a test of significance was set at $p < 0.05$ using analysis of variance (ANOVA). This work aimed to establish the effect of various substrates on growth and conidia formation in *N. rileyi*. The highest conidia count of 10×10^8 CFU/g on day 15. The result was obtained from BR + Nu followed by sorghum 7×10^8 CFU/g, corn 7×10^8 CFU/g and wheat 6.5×10^8 CFU/g. Thus, post-drying, the highest corresponding viability was observed in the product containing BR+Nu – 6.20×10^8

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CFU/g. Adding rice bran (BR+RB, 150 g each) still increases conidial yield up to 14 × 108 CFU /g at 15 DAI. These indings thus assert that broken rice and supplementation affect conidia yield and viability.

Keywords: Nomuraea Rileyi; Biopesticides; Semi-solid Fermentation; Entomopathogenic Fungi

1. Introduction

Biological insecticides are quite important in modern practices, particularly as they act as a green method of pest control, thus mitigating the adverse effects of synthesized insecticides on the ecosystem and the formation of pest resistance [1]. The biopesticides market is expected to reach USD 15.66 billion by 2029 from USD 6.92 billion in 2024 with a CAGR of 15.2% during the forecast period of 2024-2029, driven by an increase in consumers' preference for organic foods due to regulatory authorities restricting the use of chemical pesticides [2, 3]. One of the versatile entomopathogenic fungi is *Nomuraea rileyi* (Farlow) Samson, which has potential use for the biocontrol of major lepidopteran pests, including *Spodoptera litura* (armyworms) and *Helicoverpa armigera* (cotton bollworm) [4]. These fungi attach to the larvae and penetrate the cuticle layer with the help of enzymes such as chitinase and protease secreted by the fungi. They produce secondary metabolites within the insects that cause paralysis due to toxins, and the insect dies within 4–6 days [5]. Semi-solid media plays an important role in determining the efficiency of conidia production of *N. rileyi*. Jaronski's (2023) research has shown that crushing sorghum and adding 1% yeast extract is an effective method for yielding many viable conidia as it is economical for large-scale production [6]. Furthermore, supplementing the fermentation medium with agro-wastes such as rice bran and wheat bran enhances the biopesticide's conidial yields, thus making the process economical and sustainable [7]. The advanced development of the growth conditions of *N. rileyi*, including nutrient contents, moisture content, and incubation conditions, enhances the virulence and uniformity of the fungi [8].

Metarhizium rileyi (formerly *N. rileyi*) is a lepidopteran-specific entomopathogenic fungus that pathologically affects *Spodoptera frugiperda* and *Chrysodeixis*. Fungal conidia adhere to the insect's cuticle, beginning the infection [9]. When conditions are favourable, these conidia germinate and form germ tubes that penetrate the cuticle by secretion of hydrolytic enzymes such as chitinases and proteases [8]. The fungus undergoes a yeast-like phase transition in the hemocoel, and its cells proliferate through the budding formation of hyphal bodies. In this phase, the pathogen can evade the host's immune responses and rapidly reproduce in the nutrient-rich hemolymph [10]. As the infection progresses, mycelial bodies exceed some critical density and trigger quorum sensing, returning to the hyphal form. Coordination between host preference and the production of these secondary metabolites, including destruxins that suppress the insect's immune system, also correlates with the transition to host pathogenesis [11]. The mycelia grow to such an extent that the host is enveloped, and then fungal structures emerging from the cadaver form conidiophores and conidia, completing the infection cycle and aiding in transmission to subsequent hosts [12].

However, some challenges are involved in the mass production of *N. rileyi* conidia, which limit its use as a biocontrol agent. One major concern is that the fungus takes a relatively long time to grow and develop sporangia [13]. This may prove disadvantageous for wider operations, as it is slower than the other production methods. As for the growth conditions, to ensure that temperature, humidity, pH level, and available oxygen are controlled to yield the highest amount of conidia and preserve their functionality [8]. Another challenge is the high cost and limited availability of proper substrates for solid-state fermenters used in conidia production [14]. Using inexpensive agricultural by-products such as rice, sorghum, or beer barley can also be effective. Still, the quality and consistency of the substrate ingredients are important for the successful production of conidia [15]. Therefore, there is a need to work on harvesting and formulation techniques to ensure conidial viability during storage and application to be useful in using *N. rileyi* as a mycoinsecticide. This research aims to improve a semi-solid fermentation medium to increase the conidia of *N. rileyi*. This entomopathogenic fungus is used in mycoinsecticides to control *lepidopteran* larvae. The objective is to determine the composition of the substrate or nutrient medium; carbon and nitrogen sources are preferred to obtain the highest conidial yield and viability.

The second objective is to assess the effect of moisture levels, aeration, and incubation conditions on fungal growth and sporulation. The third objective is to test the virulence of conidia produced under these conditions to facilitate efficient biological control of insect pests.

2. Materials and Methods

2.1. Microorganism and Cultural Conditions

The *N. rileyi* CTU116 strain used in this research was isolated from the cadaver of armyworn in the vegetable field of Mekong Delta of Viet Nam and is maintained at the Plant protection Departments, College of Agricultural, Can Tho University. The original strain is kept under optimal conditions to ensure it remains virile and stable. It is preserved at -35°C in a cold Storage solution using a cryotube with a dehydrating agent, glycerol, which protects the fungi cells during storage. First, the strain was cultured on Sabouraud Maltose Agar Yeast (SMAY), a medium prepared with 40g maltose, 10g peptone, 10g yeast extract, and 20g agar per litre of distilled water to provide sufficient nutrients for the fungal strain's growth. This is because the fungus development for all the fungal structures peaks at a temperature of 25°C within 21 days. It was then incubated at 37°C for the desired time and then kept at 4°C until used again. These maintenance and preservation methods are useful for possibly using this strain in experiments and for other studies in the future[16].

2.2. Preparation of Liquid Culture

The conidia suspension was prepared from the conidia taken from the stock culture grown on Sabouraud Maltose Agar Yeast (SMAY) plates. The conidia were suspended in sterile distilled water, and the suspension was sonicated with 0.01% Tween 80, an antifoaming agent that disperses the conidia evenly. The conidia concentration was measured using a hemocytometer (Thomas, Japan), enabling an accurate count of the organisms[17]. It was then adjusted to a concentration of 1.0×10^7 conidia/mL with 0.02% Tween 80 to maintain uniformity in the experiments. For liquid culture preparation, 10 microliters of the conidia suspension were transferred into 250 ml of liquid media in a 500 ml Erlenmeyer flask. It was incubated at 25 ± 0.1°C in a shaker incubator at 180 rpm to enhance aeration and homogeneity of the cultures. This prepared fungal suspension was used as seed culture for soil fermentation to ensure optimum growth of fungi.

2.3. Semi-Solid Fermentation Substrates

The substrate media used in this study included corn, broken rice, crushed sorghum (soaked for 12 hours prior to use), and wheat. In this experiment, these substrates were tested under different circumstances to identify suitable ground for the fungi to grow. Each substrate was weighed (300 g), then prepared for watering, and finally put in polypropylene bags with dimensions of 10 × 35 cm × 4.5 cm[18]. The bags were tightly closed using PVC tubes plugged with cotton, each measuring 6 inches in length. The bags were sterilized at 121°C and 15 psi for 15 minutes to neutralize contamination and then left to cool down to room temperature (**Table 1**).

Table 1. Ingredients of Substrates Medium.

Substrates	Nutrition				
	MT (g)	P (g)	YE (g)	Vitacap (g)	Water (mL)
Broken Rice (BR+Nu)	3	0.5	0.3	0.005	210
Wheat (Wh+Nu)	3	0.5	0.3	0.005	180
Sorghum (Sg+Nu)	3	0.5	0.3	0.005	210
Corn (Co+Nu)	3	0.5	0.3	0.005	60
Broken Rice (BR)	-	-	-	-	210
Wheat (Wh)	-	-	-	-	180
Sorghum (Sg)	-	-	-	-	210
Corn (Co)	-	-	-	-	60

Note: Maltose = MT; Peptone = P; Yeast extract = YE.

2.4. Fermentation Process and Incubation Conditions

A complete randomization design (CRD) was used in the experiment due to the eight treatments, which are the

eight substrates used in this research. A 7-day-old SMAY medium broth culture was used for each substrate, and the bacterial clumps were dispersed to increase the surface area to volume ratio for fungal growth. The specimens were incubated under different temperature and relative humidity (RH) conditions in static culture, and the bags were manually agitated every 24 hours. After 7 days of incubation, they were transferred to 500 lux light conditions for a further 21 days for spore germination. At the end of the 21-day cultivation period, the fermented medium was collected, and conidial yield was evaluated both before and after drying the mycelial mat at 38°C for 24 hours [19]. To quantify the number of spores obtained from each fermented substrate, 1 g of the fermented sample was taken in a flask, and 5 ml of 0.01% Tween 80 was added to it, then mixed in a vortex for some time and made to count the number of spores present in the samples using a hemocytometer. All experiments were repeated in triplicate, and the conidial yield's arithmetic mean was determined to enhance precision (see **Table 1**).

2.5. Assessment of Conidia Production

The methods of conidia harvesting both before and after the drying process included the collection of spores from the growth medium. For each sample, 5 grams of the medium was to be mixed with 15 mL of 0.02% tween 80 in a 100 mL flask. The conidia release was carried out in a rotary shaker at 1000 revolutions per minute for 30 minutes. This suspension was further filtered through two layers of cheesecloth, which filtered it and ensured that the suspension prepared was pure conidial. After the incubation period, the above-cultivated medium was dried at 38°C for the next 24 hours, and then the medium was powdered very finely [16]. Conidia were collected from one gram of the powdered sample using 9 mL of 0.02% tween 80 before the conidia count and viability was determined. Conidia was counted using a haemocytometer by estimating the spore concentration in the haemocytometer (Thomas, Japan). Conidia load was evaluated based on the formula:

$$\textbf{Spores/g} = 4 \times 10^{6} \times \mathbf{a} \times \mathbf{b}$$

In which: a is the number of spores per smallest cell, and **b** is the dilution factor. This technique effectively determined the concentration of conidia in the suspensions before and after drying. To determine viable spore count, a disc rub method was used in which 5 µL of conidial suspension was spread on an MAYP agar plate and then incubated at 25°C for 5, 11, 15, and 20 days. The conidial suspension was diluted by a factor of 10 and 5 mL of the resulting 10^{-2} dilution spread on three MAYP plates using an L-shape spreader to further quantify the germination rates [20]. Plates were covered with a sterilized coverslip and sealed with parafilm before being incubated for 18-24 hours at an optimal temperature. After incubation, 5% germination of the spores was noted in all the treated and control samples to get the actual count of conidia viable in the substrate.

2.6. Statistical Analysis

The statistical analysis method employed formed part of the process of comparing yields of conidia across treatments. Analysis of variance (ANOVA) was performed to compare and test the significance of the means of the conidia yield on different substrates. It also enabled us to determine variability and to find out whether the results were statistically significant, thereby preventing conclusions based on coincidence [21]. Results were presented as mean ± standard deviation (SD), while comparison across the groups was made at a significance level of $p < 0.05$. All the tests were conducted using statistical software, enhancing the credibility of the findings regarding the effectiveness of different substrates in conidia production.

3. Results

3.1. Fungal Growth on Different Substrates

The findings reveal that various substrates promote different mycelial growth patterns of *N. rileyi* after 5, 11, 15, and 20 days of inoculation. Growth was evaluated qualitatively using "+" signs, where the number of "+" represents the extent of growth of the fungi. From the results, two of the nutrient-supplemented substrates showed extensive mycelial growth up to the 15th and 20th days (+ + +). These were broken rice (BR+Nu) and corn (Co+Nu), implying that they are suitable nutrient sources for the growth of the fungi. Sorghum (sg+Nu) and wheat (Wh+Nu) also possessed the capacity to support mycelial growth, though it was relatively

less compared to corn and broken rice and was recorded positive by days 11 and 15 and reached + + + by day 20. BR and Co demonstrated high mycelial growth (++ by day 15 and + + + at day 20), while WH and Sg showed moderate growth, with ++ by day 11 and 15, and + + + at day 20. The findings indicate that nutrient supplementation promotes the growth of the fungi, especially with the composite of grains such as wheat and sorghum, which had relatively weaker growth in the basic forms. Thus, it can be concluded that broken rice and corn were the most suitable substrates for colonization of *N. rileyi*, especially in the presence of added nutrients (see **Table 2**).

Table 2. Growth of *N. rileyi* Mycelium on Media With Different Substrates at 5, 11, 15, and 20 Days after Inoculation.

Substrates	The Mycelium of *N. rileyi* Development on the Surface of Different Mediums at Days after Inoculation			
	5	11	15	20
Broken Rice (BR+Nu)	+	+ +	+ + +	+ + +
Wheat (Wh+Nu)	+	+ +	+ +	+ + +
Sorghum (Sg+Nu)	+	+ +	+ +	+ + +
Corn (Co+Nu)	+	+ +	+ + +	+ + +
Broken Rice (BR)	+	+ +	+ ++	+ + +
Wheat (Wh)	+	++	++	+ + +
Sorghum (Sg)	+	++	+ +	+ + +
Corn (Co)	+	+ +	+ +	+ + +

Notes: (-): Mycelium not development, (+): Mycelium development 10-30% substrate surface area, (+ +): Mycelium development 30-50% substrate surface area, (+ + +): Mycelium development over 50% substrate surface area, T0C= 25±1; RH = 70%±3.

The images depict *N. rileyi* mycelial growth on the substrates from initial colonization to maturity (A–D). Image A harbours less colonization, implying that the substrate has a low nutrient value or that **Figure 1** was captured at an earlier stage. Compared to image A, image B shows moderate fungal distribution, which shows an advancement but still porous interweaving. Image C has the most significant fungal growth of the mycelium, which can be considered an ideal habitat for developing *N. rileyi*. In image D, there is a great amount of mycelial spread compared to image C but a slightly lower level of colonization compared to the most efficient substrate. These results concord with the previous research in which broken rice and corn, in the presence of nutrients, encouraged maximum mycelial growth. At the same time, wheat and sorghum depicted less colonization (**Figure 1**).

3.2. Effect of Different Substrates on Aerial Conidia Production

The graphs depict the conidial yield (x10^8 CFU/g) of *N. rileyi* in regards to substrate type—broken rice, wheat, sorghum, and corn—or in terms of incubation length at 5, 11, 15, or 20 days under either (+Nu) conditions or the control. In the first graph comparing the average conidia production at different time lapses concerning Broken Rice plus Nutrient (BR+Nu) and Broken Rice (BR), it was observed that the average conidial production in the samples of the former increased over time. On day 15, BR+Nu reached its maximum yield, equal to 10 times 10 to the power of 8 CFU/g, but by day 20, it reduced to 8 times 10 to the power of 8 of CFU/g. The non-supplemented BR yielded comparatively fewer spores that reached a maximum of ~7 × 10^{8}CFU/g on day 15 but declined slightly **(Figure 2**).

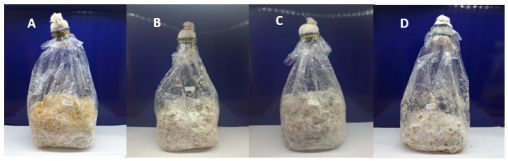

Figure 1. The mycelium of *N. rileyi* development on the different substrates at 20 days after inoculation: (a) broken rice, (b) wheat, (c) sorghum and (d) corn.

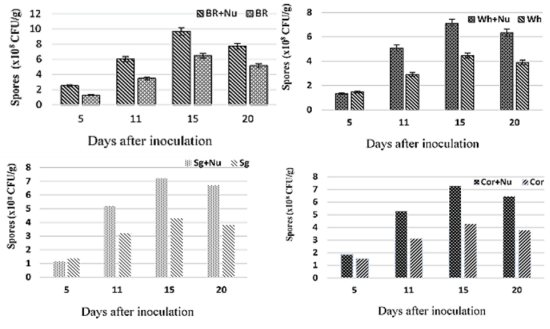

Figure 2. Effect of different substrates on aerial conidia.

The second graph (Wheat – WH +Nu vs. WH) shows that supplementation positively affected conidial production

at either early or later incubation stages. WH +Nu peaked at 6.5 x 10^8 CFU/g on day 15 and WH at 5 x 10^8 CFU/g on day 20. This reveals that spore germination was initiated in WH +Nu more rapidly than in the control when nutrients became available. However, the yield difference between WH +Nu and WH was reduced over the course of time, implying that wale is lagging. The third graph of the Sorghum shows that Sg+Nu had their powerful spore yield on the fifteenth day at 7 × 10^8 CFU/g while Sg reached its optimum at 5 × 10^8 CFU/g on the twentieth day. Supplementation increased growth in the initial days of incubation, while non-supplemented sorghum was also adequate to produce good conidia over a longer period.

The fourth graph (Corn - Cor+Nu vs. Cor) indicates that Cor+Nu was populated to ~7 × 10^8 CFU/g on day 16, whereas the population of Cor was only ~5 × 10^8 CFU/g after day 20. It was noticed that supplementation efficiently increases early-stage conidia formation. BR+Nu was the highest yielding (~10 × 10^8 CFU/g) followed by others in the order sorghum, corn, and wheat; all types of supplementation increased conidia formation to varying extents in all the media (see **Figure 2**).

3.3. Viability of Conidia after Drying

The assessment of spore viability post-drying reveals significant variation across different fermentation media. The supplementation of Broken rice with nutrient (BR+Nu) increased spore production (7.16 × 10^8 CFU/g after drying and viable spores (6.20 × 10^8 CFU/g), indicating that rice bran is beneficial for fungal growth and conidial survival. Considerable fungal development was also supported by corn (Co+Nu) spore yields of 5.32 x 10^8 CFU/g and viable spore counts of 4.32 × 10^8 CFU/g, as well as wheat (Wh+Nu) spore yields of 5.00 × 10^8 CFU/g and viable spore counts of 4.3 × 10^8 CFU/g. Broken rice alone (BR) was also very good among non-supplemented substrates, yielding 5.12 × 10^8 CFU/g after drying, 4.24 × 10^8 CFU/g viable spores, but was better than wheat (Wh), sorghum (Sg) and corn (Co), which had much lower BF yield. The two wheat samples showed the lowest viability (2.48 × 10^8 CFU/g live spores), then sorghum (2.96 × 10^8 CFU/g). These findings indicate that nutrient supplementation, specifically with broken rice, greatly increases aerial conidia production and spore survival (see **Table 3**).

3.4. Effect of Rice Bran Addition on Conidia Yield

The findings of aerial conidia yield before drying under different substrates are another factor fitted with the DAI levels 5, 11, 15, and 20 on the x-axis. The conidial yield is given in terms of the number of spores per gram with three substrate compositions of BR (300g), BR (210g) /RB (90g) and BR (150g) /RB (150g). The highest conidia yield was obtained when using BR (150g) + RB (150g), with a maximum of 14 x 10^8 spores/gr of 15 DAI and conidia yield remained near 13 x 10^8 spores/gr at the 20 DAI. BR+RB condition continuously gave intermediate yields ranging from 6 x10^8 peer-review spores per gram at 5 DAI to 12 x10^8 peer-review spores per gram at 20 DAI. The last yield was obtained for the BR at 300g/100 ml, producing a spore yield of about 4 x10^8 spores/gr at 5 DAI and rising to about 9 x10^8 spores/gr at 20 DAI. The findings indicate that combining BR and RB at 150g generates a high aerial conidia count, especially at 15 DAI (see **Figure 3**).

Table 3. Effect of Rice Bran with Broken Rice on Aerial Conidia Production.

Treatment	Spores (10^8 CFU/g)	
	After Drying	Live Spores
Broken Rice (BR+Nu)	7.16a	6.20a
Wheat (Wh+Nu)	5.00b	4.16b
Sorghum (Sg+Nu)	4.60c	3.60c
Corn (Co+Nu)	5.32b	4.32b
Broken Rice (BR)	5.12b	4.24b
Wheat (Wh)	3.24e	2.48e
Sorghum (Sg)	3.88d	2.96d
Corn (Co)	3.80d	3.08d

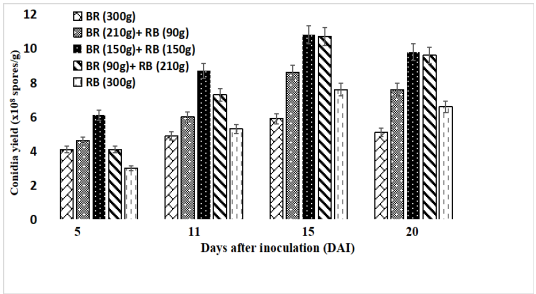

Figure 3. Effect of different substrates on aerial conidia before drying.

The results depict the number of conidia produced before and after drying with the five different substrate compositions: BR (300g), BR (210g) plus RB (90g), BR (150g) plus RB (150g), BR (90g) plus RB (210g), and RB (300g.

Two samples are the object of comparison for the initial supply of live spores and spores after drying. In this experiment, the highest conidial yield was obtained in the treatment of BR (150g) + RB (150g), producing approximately 7.5 x10^8 spores/gr before drying and about 7.0 x10^8 spores/gr after drying, which shows that drying had minimal impact on the yield. The characteristics of the BR (210g) + RB (90g) mixture were practically similar to that of the BR, around 7 x10^8 peer-review spores/gr before drying and slightly above 6 x10^8 peer-review spores/gr after drying. These findings indicate that combining BR and RB at different ratios significantly increases spore output and enhances stability during drying. The BR 150g + RB 150g yielded the best result (**Figure 4**).

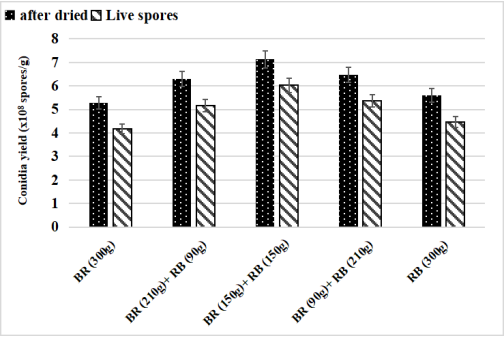

Figure 4. Conidial Yield (x10^8 spores/g) after Drying and Live Spores.

4. Discussion

The findings of this discussion enable the improvement of a semi-solid fermentation medium to boost conidia production of *Nomuraea rileyi* for use in the development of a mycoinsecticide to control lepidopteran larvae. The choice of a suitable substrate and fermentation conditions is essential to grow the fungi optimally, develop the sporulation process and enhance the viability of conidia. On the other hand, wheat and sorghum demonstrated only moderate growth, indicating that these plants are not ideal but remain feasible for fungal colonization. These findings align with Rämä and Quandt's (2021) observation that nutrient-rich substrate increased the sporulation and viability of fungi [22]. However, Uwineza et al. (2024) opined that fungal growth is promoted with rice-based substrates because they contain reasonable amounts of carbohydrates, which form an essential energy source in the breakdown of glucose by fungi [23]. Similarly, Shafiekhani et al. (2018) pointed out that moisture is a crucial factor supporting fungal growth in broken rice, which is stored in an environment that helps retain moisture [24].

In contrast, wheat and sorghum exhibited comparatively less mycological infestation. This finding is consistent with Wahab et al. (2023), who found that the presence of such nutrients improves the enzymatic function, which in turn promotes better fungal growth [25]. The findings establish that enhanced growth of *N. rileyi* results from nutrient supplements; among the media, broken rice and corn are ideal for producing large quantities of conidia from a mass production perspective.

The results prove that different substrates affect the conidial production of *N. rileyi*, and the highest conidia yield was obtained from broken rice (BR+Nu), while sorghum (Sg+Nu), corn (Co+Nu), and wheat (Wh+Nu). Of these, the substrate composition and nutrient supplementation are of utmost importance in sporulating fungi for enhanced yields in large-scale biopesticide production. BR+Nu was the most prolific producer of conidia, reaching a maximum on day 15 and declining on day 20. However, Etesami (2019) noted that broken rice has the most suitable nutrient balance necessary to support the fast growth of fungi that lead to the formation of spores [26]. The high carbohydrate content found in rice fosters the metabolism of pathogenic fungi, which established that mycelial growth and sporulation are improved when using a rice-based substrate [27]. Conclusively, according to Kimaru et al. (2020), crushed grains such as rice and sorghum enhance fungal growth and development, a conclusion supported by the present study [28]. Wheat (WH+Nu) exhibited moderate conidial production, though different from BR+Nu; it peaked on day 20. This indicates that although wheat can support the growth of fungi, it may not be as effective as rice medium for conidia production in terms of its efficiency. However, Capurso (2021) emphasised that wheat has lower carbohydrate values for growth than rice and should be supplemented [29].

Sorghum supplemented with Nutrient (Sg+Nu) recorded moderate conidial yield at day 15, and thereafter, the yields decreased while in treatment control; sorghum only (Sg) had slightly poor yields up to day 20 and was gradually increasing. As stated by Dias et al. (2018), the substratum of sorghums possesses a good nitrogen-to-carbon ratio that enhances the efficiency of enzymes in sporulating fungi [30]. However, Little and Perumal's (2019) findings indicated that the conidia yield of sorghum might take longer

to achieve its maximum yield than broken rice [31]. Similarly, Corn (Co+Nu) supplementation enhanced the frequency of conidia production compared to the control, but the value recorded was slightly lower than that of BR+Nu and Sg+Nu. This is in line with Iwanicki et al. (2020) who observed that corn-based substrates are good sources of nutrients, but the production of fungal spores can be achieved by supplementing the culture medium [32]. The study also noted that the remaining non-supplemented Co had a mean conidial yield lower than the supplemented corn; thus, other nutrients are required to grow the fungi on this substrate.

Evaluation of conidial germination potential after drying reflects differences in the fermentation medium, where broken rice with nutrients gives the best result out of all tested media. Nishimwe et al. (2020) discussed that carbohydrate-rich substrates like rice help enhance the sporulation potential by offering the best energy source and adequate humidity most conducive to conidia germination [33]. While broken rice aided conidial growth significantly, corn and wheat also showed similar effects to some extent in supporting conidial viability. This finding supports the study done by Mapuranga et al. (2022). Wheat-based media supports fungal growth, but the low value of carbohydrates hinders conidial generation and survival [34]. Sorghum had the lowest viability, as observed by Naeimi et al. (2020), who mentioned that sorghum is less viable than rice because the incubation period for the production of conidia in sorghum-based substrate is longer [35]. It was inferred from the findings that the proper selection of substrate and nutrient supply significantly affects the increase in conidial yield along with its sustainability. However, Wang et al. (2023) provided similar insights about the improvement of fungal development using supplements like rice bran and yeast extract in the fermentation media while at the same time controlling production cost [36]. These findings corroborate the need to precisely choose the kinds of substrates most favorable for conidial survival in how biopesticides should be formulated and applied on a large scale.

However, this study has some limitations that need to be addressed. The incubation period was set at 20 days, which may not effectively allow the maximum conidial yield of the preferred substrates like sorghum since this organism may take up to 30 days for maximum sporulation. The study was confined to only a few common substrates, namely broken rice, wheat, sorghum and corn and other agriculture byproducts, such as barley and millet, that could also support the growth of fungi, and their conidia were not researched. Key environmental factors such as pH and oxygen concentration, which are relevant to sporulation, were not stabilized as much as they should have been. Further studies should emphasise controlling environmental conditions to assess the beneficial conditions in enhancing conidial viability and, in turn, improving large-scale biopesticide production. Based on the results, the following are recommended towards improving the semi-solid conidia production in *Nomuraea rileyi.* First, the concentration of nutrients and the C/N ratio should be systematically varied to see which substrate is more effective in the growth and sporulation of fungi [32]. Moisture content and aeration conditions play an essential role because great attention is paid to fungal conidia's high humidity and lack of oxygen. Applying an aeration program can increase yield [37]. Rice bran or soybean meal byproducts produced in large quantities in the agro-industrial process could supplement the cost-effective substrates and improve fermentation efficiency [38]. Finally, variations can also be made on the incubation temperatures and pH, which enhance the fungal metabolism, hence higher conidial viability and field effectiveness.

5. Conclusion

In this research, the findings prove that the choice of a substrate base and nutritional enrichment significantly affects the conidial yield and germination of *Nomuraea rileyi*. BR+Nu yielded the highest conidia count and germination among the analysed samples, surpassing sorghum, corn, and wheat samples. The findings support that carbohydrate sources improve fungal metabolism, sporulation, and conidial stability, making carbohydrate substrates suitable for industrial-scale biopesticide production. It was also observed that nutrient supplementation increased conidial yield on all the types of media, confirming the efficiency of the fungal fermentation process. However, issues such as prolonged incubation periods, stabilization of pH, and aeration control need to be studied further. Further research should, therefore, consider other agro-waste materials that can effectively be utilized as substrates for fermentation. It brings essential information for furthering the knowledge of how operating conditions can be adjusted to enhance efficiency in the semi-solid fermentation of fungal-based biopesticides

for pest control, thus offering a relevant contribution to sustainable pest management in the agricultural field.

Author Contributions

X.T.T. wrote the first draft of the manuscript. X.T.T., M.L.T.X., X.L.T.N., S.P.K., and D.T.T.Q. participated in reviewing and editing the manuscript. All authors have read and agreed to the published version.

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Data Availability Statement

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Conflicts of Interest

The authors declare no conflict of interest.

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