International Journal of Environment, Agriculture and Biotechnology
Vol-10, Issue-4; Jul-Aug, 2025
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Optimizing Cattle Manure Incubation Method with *Trichoderma* sp. Starter for Contamination Control

Gustin Meynindra Sasa Dilla[1], Agus Purwanto[2], Antonius Budiawan[3]

[1]Biology Department, Universitas Katolik Widya Mandala Surabaya, Madiun City, East Java, Indonesia
biology.gustin.m.22@ukwms.ac.id
[2]Biology Department, Universitas Katolik Widya Mandala Surabaya, Madiun City, East Java, Indonesia
agus.purwanto@ukwms.ac.id
*Corresponding author: Agus Purwanto
[3]Pharmacy Diploma III Department, Universitas Katolik Widya Mandala Surabaya, Madiun City, East Java, Indonesia
antonius.budiawan@ukwms.ac.id

Received: 20 Jul 2025; Received in revised form: 19 Aug 2025; Accepted: 24 Aug 2025; Available online: 31 Aug 2025

Abstract— *The successful production of Trichoderma sp. starter cultures requires an appropriate medium and controlled environmental conditions to support mycelial growth and prevent contamination. This study evaluated the effect of sterilized sugar solution volume and lid type on the success rate of Trichoderma sp. starter incubation in a cattle manure medium. Our experimental design included two treatments: Starter A used 50 mL of sugar solution with an open container (no lid), while Starter B utilized 10 mL of sugar solution with a two-layer sterile gauze as a porous lid. Visual observations were conducted over 21 days, monitoring mycelial growth, medium color changes, and contamination. Results showed that Starter B exhibited mycelial colonization from day one, achieving green sporulation by day 14 without any signs of contamination. Conversely, Starter A showed indicators of anaerobic fermentation, including a pungent odor and larval infestation by day 5, which significantly inhibited Trichoderma sp. growth. These findings suggest that an incubation method with a lower sugar solution volume and a porous lid effectively maintains optimal moisture, aeration, and medium sterility.*

Keywords—***Incubation, cattle manure, contamination, sugar solution, Trichoderma***.

I. INTRODUCTION

The increasing global livestock population is significantly contributing to the accumulation of organic waste within the industry. Cattle manure represents a major component of this waste due to its substantial volume and high organic matter content. Rich in nitrogen (N), phosphorus (P), and potassium (K) compounds, cattle manure holds potential as a raw material for organic fertilizer. Effective management of this nutrient-rich waste is crucial to prevent land and water pollution, mitigate greenhouse gas emissions, and control pathogen spread [1].

Decomposition is a fundamental biological process for recycling organic matter, though its natural rate is often slow and contingent on the activity of indigenous microbe. The efficiency of this process can be enhanced through the application of a bioactivator, such as *Trichoderma* sp. This fungus is well-known for its production of lignocellulolytic enzymes, including cellulase, xylanase, and ligninase, which facilitate the breakdown of complex organic polymers into simpler, more plant-available forms [2]. Beyond its role in decomposition, *Trichoderma* sp. also exhibits antagonistic activity against various plant pathogens and possesses a high degree of environmental adaptability, making it an effective and natural biological agent [3]. The successful utilization of *Trichoderma* sp. in decomposition systems requires the production of an active inoculum. This starter culture necessitates a growth medium that provides a rich source of nutrients and maintains an optimal moisture level. Cattle manure

presents a promising and readily available alternative as a support medium, given its abundant organic nutrient composition [4][5].

A primary challenge in utilizing cattle manure as a starter medium for *Trichoderma* sp. inoculum is its inherently high moisture content and susceptibility to contamination by competing microorganisms and pests. Environmental conditions, including aeration, water activity, and carbon availability, are critical factors that significantly influence mycelial growth. Specifically, excessively high moisture levels or anaerobic conditions within the medium can severely inhibit the growth of the *Trichoderma* sp. inoculum [6].

Anaerobic fermentation within a cattle manure medium can lead to the production of toxic metabolites, such as ammonia and organic acids, which suppress the growth of *Trichoderma* sp. and attract unwanted insect pests, particularly flies [6][7]. The presence of these insects can lead to physical contamination, such as the introduction of larvae, which significantly compromises the quality and stability of the starter medium. The methodology described by Sutarman et al. (2020) did not specifically investigate the influence of lid material and the volume of a sugar solution on the incubation conditions of the cattle manure [8]. Therefore, based on these observations, a modification of the protocol is necessary to optimize these two variables, with the goal of producing a more sterile and robust *Trichoderma* sp. starter culture that is readily applicable for use by local farmers.

II. MATERIALS AND METHOD

2.1 Materials

The growth medium used in this study consisted of 100 g of fresh cattle manure, which was sterilized in a dry-heat oven at 105°C for one hour. The sugar solution was prepared by dissolving 1 g of sugar in distilled water, followed by sterilization using an autoclave. The *Trichoderma* sp. inoculum was obtained from a compact culture grown on a corn rice base that had been incubated for seven days.

2.2 Method

This experimental study was conducted at the Biology Laboratory, Faculty of Agricultural Technology, Universitas Katolik Widya Mandala Surabaya, Madiun Campus. The research modified the *Trichoderma* sp. starter production method described by Sutarman et al. (2020), utilizing cattle manure as the primary growth substrate. One hundred grams of fresh cattle manure was sterilized in a dry-heat oven at 105°C for one hour to reduce initial moisture content and inhibit potential contaminants. The sterilized manure was then cooled to room temperature. A sterile sugar solution was prepared by dissolving 1 gram of sucrose in a volume of distilled water corresponding to the specific treatment volume. This solution was sterilized by autoclaving at 121°C for 15 minutes. The *Trichoderma* sp. inoculum was sourced from a seven-day-old compact culture grown on a rice and corn medium. A 10-gram portion of this inoculum was aseptically homogenized with the cooled sugar solution before being poured onto the sterilized cattle manure substrate. The experiment was a two-treatment study, with each treatment involving different incubation conditions and sugar solution volumes: Treatment A: The medium was incubated without a lid and with the addition of 50 mL of sugar solution. Treatment B: The medium was covered with a two-layer sterile gauze lid and received 10 mL of sugar solution. All samples were incubated at room temperature (28–30°C) for 21 days. The substrate was stirred daily for the first three days, and sterile distilled water was misted onto the surface if it appeared dry to maintain optimal moisture levels. Observations were conducted over the 21-day incubation period. Parameters assessed included: Mycelial growth and density, changes in medium color and aroma, presence of contaminants, such as other fungal species or insect larvae. The successful production of the *Trichoderma* sp. starter culture was indicated by the characteristic green coloration of the mycelium.

2.3 Data Analysis

Data from this qualitative study were analyzed descriptively, with findings presented in tables and figures. A narrative approach was used to provide a comprehensive and in-depth description of the observed phenomena.

III. RESULTS AND DISCUSSION

Morphological observations revealed significant differences in colonization, sporulation, and contamination between Starter A and Starter B. Starter A, which was incubated without a lid and with 50 mL of sugar solution, exhibited signs of anaerobic fermentation from the initial day, as evidenced by gas production and a pungent, acidic odor. By day five, the presence of insect larvae indicated a clear failure of the process due to contamination (Table 1).

Starter A was monitored daily due to the immediate detection of fermentation symptoms from the initial day of incubation. In contrast, Starter B, which was incubated with a two-layer sterile gauze lid and 10 mL of sugar solution, maintained stable, sterile conditions with no significant visual changes. This allowed for periodic observation. The difference in monitoring intensity

between the two treatments directly corresponded to the biological fluctuations observed in each condition.

Anaerobic fermentation on starter A triggered by over moisture beyond substrate obsorption capacity and lead to microbe fermentatif activity and also toxin production such as ammonium and acid. The presence of insect larvae within the medium provides strong evidence of external contamination (Fig. 1), a finding that is consistent with the principles established by FransiscoRedi's experiment on open organic matter [7].

Table 1: Starter A observation result

Day	Starter A observation result
1	The medium showed signs of active fermentation, including gas formation and a pungent, acidic odor. The substrate was moist and had a brownish color
2	Gas production continued, accompanied by a more intense and foul odor
3	The pungent and unpleasant odor became significantly stronger
4	The strong odor began to dissipate, while the substrate remained moist
5	The presence of insect larvae was detected. This finding confirmed external contamination and indicated the failure of the starter culture production.

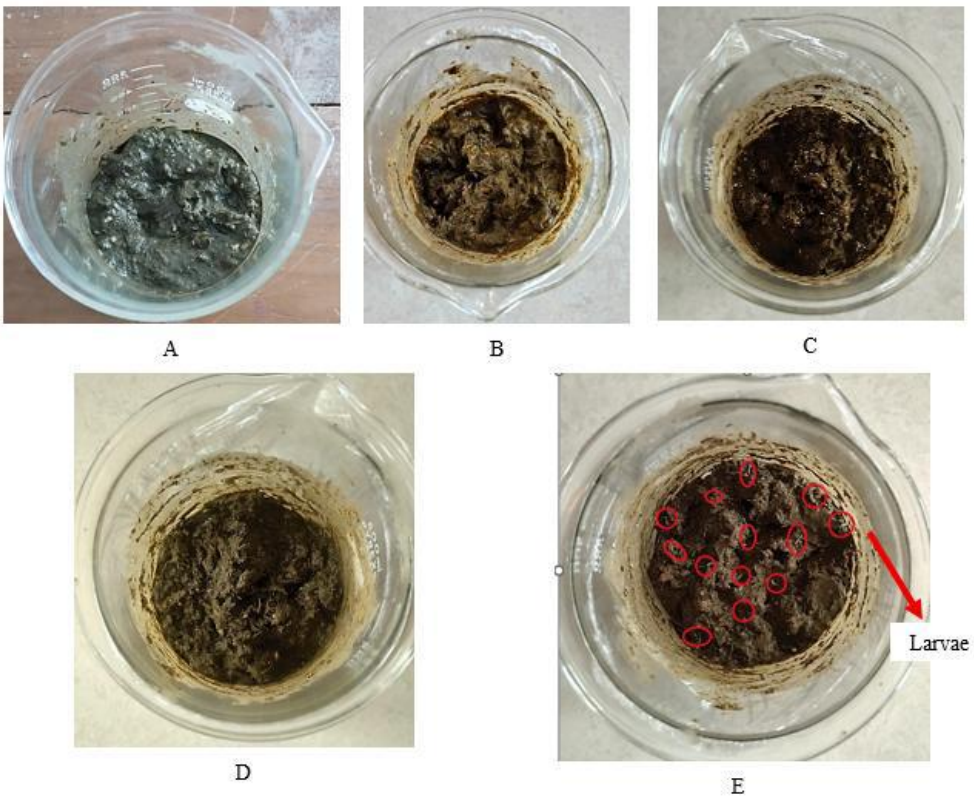

Fig. 1: Starter A Trichoderma sp. incubation on day 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). Larvae presence was detected on day 5 (E).

Starter B showed different results compared to Starter A, with mycelium spotted from the initial day of observation (Table 1). The medium remained neutral, and moisture conditions were stable. This early mycelium growth indicates that the *Trichoderma* sp. propagules colonized the sterile medium, which contained an even distribution of nutrients, in a rapid manner [2]. The two-layer sterile gauze maintained air circulation and prevented contamination without significant moisture loss. This aeration structure provided a competitive advantage to the *Trichoderma* sp., supporting its domination in the medium environment. Aerobic fungi, such as *Trichoderma* sp., grow optimally when oxygen circulation is sufficient [9]. The sterile gauze also prevented saprophytic eggs or

larvae-carrying insects from entering the medium. This condition aligns with Fransisco Redi's experiment, which highlighted the importance of physical protection on open media to prevent contamination from unwanted organisms [7].

Sporulation in Starter B began to appear on day 14, indicated by a color change to light green on the medium surface due to conidia accumulation (Fig. 2). The green color became darker and stable by day 21, confirming that the asexual reproduction process was completed optimally [10, 11]. This success demonstrates that the medium formula and incubation system used for Starter B were effective in supporting the entire life cycle of *Trichoderma* sp.

Table 2: Starter B observation result

Day	Starter B observation result
1	White mycelium appeared on the inoculation spot. The substrate was moist and brown, with a neutral aroma
7	The mycelium spread evenly, creating a compact texture. The culture had a distinct, earthy Trichoderma aroma, with no signs of contamination
14	The surface began to turn greenish due to sporulation. The characteristic Trichoderma aroma was strong, and the medium was loose and stable
21	The green color became darker and more stable. The medium appeared dry on the surface but remained moist internally. The starter was considered fully developed

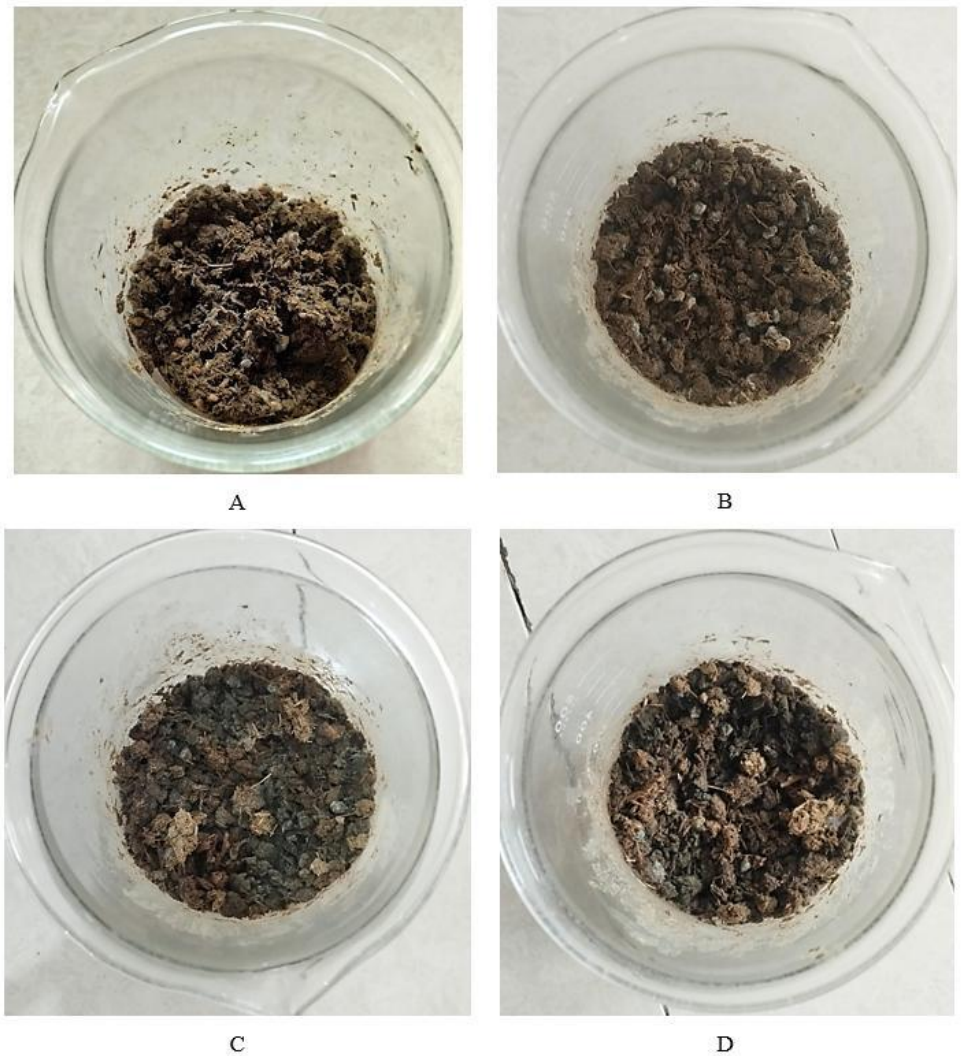

Fig. 2: Starter B Trichoderma sp. incubation on day 1 (A), 7 (B), 14 (C), and 21 (D). Starter B incubation was done on day 21 (D).

The medium formulation and closed-lid system used for Starter B supported the conversion of organic matter into a value-added natural product. Controlling moisture and aeration effectively prevented anaerobic fermentation and inhibited the growth of flies, which are attracted to volatile compounds such as ammonium and organic acids. Animal manure typically has a water content ranging from 27-86%, a condition that supports the growth of fly larvae. Meanwhile, an optimal range of 65-85% moisture is ideal for flies to lay eggs [6]. This finding is consistent with the conditions observed in Starter A, which was too moist and open, allowing flies to lay their eggs in the medium. In contrast, the controlled and enclosed system applied to Starter B successfully prevented such contamination. The composition of cattle manure plays an important role in mycelial colonization because it contains cellulose (25.2%), hemicellulose (18.6%), and lignin (20.2%), as well as sufficient levels of nitrogen, phosphorus, and potassium [12, 13]. A C/N ratio in the ideal range of 16.6-25% provides a suitable environment for decomposer microorganisms to thrive [14]. Trichoderma sp. utilizes this substrate through the synergistic production of cellulase, xylanase, and ligninase enzymes, which degrade the lignocellulosic material [10, 2].

The volume of the sugar solution significantly affected the medium's condition and initial microbial activity. The 50 mL solution used in Starter A created excessive moisture, which triggered anaerobic fermentation and inhibited mycelial growth. This condition arose because high moisture levels decrease oxygen availability and slow down the organic matter degradation process [15]. In contrast, Starter B, with its 10 mL sugar solution, maintained a stable moisture level, providing optimal growth conditions for the mycelium. The ideal moisture content for a medium is in the range of 40-60% [16]. The sugar, acting as a simple carbon source, activated the metabolism of *Trichoderma* sp. without excessively increasing osmotic pressure [16]. The stirring process from day 1 to day 3 helped distribute the propagules and oxygen, which is crucial for active mycelial formation [18]. The discontinuation of stirring after day 3 then supported the stable production of sporulative structures [19]. This allowed the transition from the vegetative to the reproductive phase to occur optimally without mechanical disturbance. The incubation temperature was maintained at 27-30°C throughout the study. This temperature range is optimal for the growth of *Trichoderma* sp., as well as for the production of cellulolytic enzymes and asexual reproduction [9]. The success of sporulation demonstrates that both temperature and medium moisture were within the ideal physical conditions that supported the fungus's biological activity.

The method of Sutarman et al. (2020) [8] was modified from using rice husk to cattle manure, demonstrating the effectiveness of an alternative substrate. As a cheap, local material rich in nutrients, cattle manure provided excellent support for efficient inoculum production. The combination of moisture control, sugar solution volume, and a closed-lid system yielded a stable and contamination-free *Trichoderma* sp. starter.

This starter has significant potential as a bioactivator for decomposing farm manure. The use of *Trichoderma* sp. has been proven to increase decomposition rates and enhance the quality of organic fertilizer [20, 11]. The method developed in this study provides a simple, cost-effective, and efficient process for producing a cattle manure-based starter. Applying this method can promote the conversion of farm waste into a value-added natural product, support an environmentally sustainable farming system, and encourage the efficient use of local resources.

IV. CONCLUSION

The volume of the sugar solution and the type of lid significantly affected the success of *Trichoderma* sp. Starter incubation in a cattle manure-based medium. A starter incubated with 10 mL of sugar solution and a two-layer sterile gauze lid showed active mycelial growth, optimal sporulation, and remained contamination-free for 21 days. This optimized method provides an applicable approach for producing a farm waste-based starter, which can serve as an environmentally friendly bioactivator for fertilizer.

REFERENCES

[1] Kevin, D., and Sari, D. A. (2025). Pemberdayaan masyarakat melalui pemanfaatan kotoran sapi menjadi biogas sebagai sumber energi terbaharukan. Jurnal Pengabdian dan Pemberdayaan Masyarakat, 7(1), 119–127.

[2] Jumadi, O. M., Junda, M., Caronge, M. W., and Syariffudin. (2021). Trichoderma dan Pemanfaatan. Makassar: Jurusan Biologi FMIPA UNM.

[3] Uruilal, C. A., Kalay, A. M., Kaya, E., and Siregar, A. S. (2018). Pemanfaatan kompos ela sagu, sekam dan dedak sebagai media perbanyakan agens hayati Trichoderma harzianum Rifai. Agrologia, 1(1), 21–30.

[4] Umbola, M. A., Lengkong, E., and Nangoi, R. (2020). Pemanfaatan agen hayati Tricho-kompos dan PGPR (plant growth promotion rhizobacteria) pada pertumbuhan vegetatif tanaman cabai keriting (Capsicum annuum L.). Cocos, 12(1), 1–15.

[5] Kakabouki, I. A., Folina, A., Efthimiadou, A., Karydogianni, S., Zisi, C., Kouneli, V., Kapsalis, N. C., Katsenios, N., and Travlos, I. (2021). Evaluation of processing tomato pomace after composting on soil properties, yield, and quality of processing tomato in Greece. Agronomy, 11(1), 1–15.

[6] Saidi, D. M., Maryana, and Widiarti, I. W. (2022). Pengelolaan Limbah Ternak Sapi. Yogyakarta: LPPM UPN "Veteran" Yogyakarta.

[7] Suryani, Y., and Taupiqurrahman, O. (2021). Mikrobiologi Dasar. Bandung: LP2M UIN SGD Bandung.

[8] Sutarman, A., Prihatiningrum, E., and Miftakhurohmat, A. (2020). Teknologi Tepat Guna Aplikasi Pupuk Hayati Plus pada Tanaman Hortikultur. Sidoarjo: Umsida Press.

[9] Sakiah, S., D. Arfianti, A. B. Silalahi, and I. Lesmana. 2024. "Pemanfaatan *Trichoderma* sp dan Aspergillus sp dalam Pengomposan Tandan Kosong Kelapa Sawit." Jurnal Pertanian Berkelanjutan 2 (1): 37-43.

[10] Mulyadi, E. S. Hayat, and S. Andayani. 2022. "Effect of Compost and *Trichoderma* on Onion Growth and Yield." Jurnal Inovasi Penelitian 3 (3): 5551-5560.

[11] Nazari, A. P. D., O. F. Kurniadinata, and Nuraliah. 2023. "Pengaruh Dosis Pupuk Kompos Campuran *Trichoderma* sp., Limbah Media Tanam Jamur, dan Kotoran Sapi Terhadap Pertumbuhan dan Hasil Tanaman Cabai Keriting (*Capsicum annuum* L.)." Jurnal Agroekoteknologi Tropika Lembab 6 (1): 7-14.

[12] Febriansyah, A. j. Alfarobi, K. A. Ridwan, L. Salamah, R. A. Putra, and S. I. A. Sari. 2023. "Pengaruh Penambahan Limbah Cair Tahu dan Kulit Pisang terhadap Biogas dari Kotoran Ternak sebagai Solusi Energi Alternatif Masa Depan." Jurnal Penelitian Sains 25 (2): 103-108.

[13] Indriyani, N., S. Heremba, I. Agustian, M. Salim, S. Ma'arif, I. Resky, and T. Panjaitan. 2022. "Pemanfaatan Kotoran Ternak Sebagai Biogas Dan Pupuk Organik Di Desa Klasmelek." Jurnal ABDIMASA Pengabdian Masyarakat 5 (1): 69-74.

[14] Arohaman, D. F., R. Priyadarshini, and S. B. Santoso. 2023. "Pengaruh Jenis Cacing dengan Komposisi Media Bahan Baku Batang Pisang, Kotoran Sapi dan Cocopeat terhadap Kandungan Unsur Kimia Vermikompos." Agricultural Journal 6 (3): 711-723.

[15] Indriyani, N., S. Heremba, I. Agustian, M. Salim, S. Ma'arif, I. Resky, and T. Panjaitan. 2022. "Pemanfaatan Kotoran Ternak Sebagai Biogas Dan Pupuk Organik Di Desa Klasmelek." Jurnal ABDIMASA Pengabdian Masyarakat 5 (1): 69-74.

[16] Sofa M., G. M. Hatta, and Y. F. Arifin. 2022. "Analisis Kompos Berbahan Dasar Sampah Organik di Lingkungan Kampus dengan Aktivator EM4, Kotoran Sapi, dan Kotoran Unggas dalam Upaya Mendukung Gerakan Kampus Hijau." Jurnal Hutan Tropis 10 (1): 70-80.

[17] Day, T. M. W., H. D. Beja, and J. Jeksen. 2022. "Teknik Perbanyakan Massal Jamur Trichoderma sp. pada Beberapa Media Tumbuh sebagai Agens Pengendali Hayati." JURNAL LOCUS Penelitian & Pengabdian 1 (2): 81-89.

[18] Fadlillah, L. N., R. Despita, and A. Rahmi. 2023. "Perbanyakan *Trichoderma*, Sp. Dengan Menggunakan Berbagai Media Cair." (Politeknik Pembangunan Pertanian Malang) 233-242.

[19] Sukmasari, M. D., and A. O. R. Harti. 2023. "Pelatihan Perbanyakan Trichoderma Sp. Lokal Sebagai Agen Hayati di Desa Palabuan Kecamatan Sukahaji Kabupaten Majalengka." Jurnal Pengabdian Kepada Masyarakat 4 (2): 1022-1027.

[20] Dewi, R. S. and R. Z. Ahmad. 2021. "Pemanfaatan *Trichoderma* spp. dan *Gliocladium virens* dalam Pembuatan Kompos." Jurnal Mikologi Indonesia 5 (1): 30-40.