SHELFLIFE EVALUATION OF FORMULATIONS OF *Trichoderma harzianum*

BY

Irene Nkiruka ONAH (Miss)

AGR2004355

DEPARTMENT OF CROP SCIENCE
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
BENIN CITY,

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF BACHELOR OF AGRICULTURE (B.AGRIC) IN CROP SCIENCE.

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CERTIFICATION

This is to certify that the work contained in this report entitled "**Formulation of Trichoderma as A Biocontrol Agent for Seed and Soil Application**" was carried out by **Irene Nkiruka ONAH (Miss)** with matriculation number **AGR2004355** in the Department of Crop Science, Faculty of Agriculture, University of Benin, Edo State, Nigeria.

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_____ _____

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DEDICATION

This work is dedicated to Almighty God for His grace and enablement without which I would not have been able to come this far.

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ABSTRACT

This study investigated the shelf life and viability of *Trichoderma harzianum* formulated with various carrier combinations to enhance its potential as a sustainable biocontrol agent. Formulations were prepared using different ratios of corn cob and spent mushroom substrate (4:1 and 1:1) respectively, combined with clay and cassava starch. The viability of *T. harzianum* spores was monitored monthly over a five-month storage period under room temperature conditions (15–35 °C) by determining colony-forming units (CFU/g) on potato dextrose agar. The experiment was subjected to Completely Randomized Design (CRD) and data was analyzed using Analysis of Variance (ANOVA) at a 5% significance level. Results revealed that the media type had a highly significant effect ($p < 0.001$) on spore viability across all months, while concentration and substrate × concentration were non-significant. The formulation containing a 1:1 mixture of corn cob and spent mushroom substrate supplemented with clay and cassava starch consistently produced the highest CFU/g values throughout the storage period, indicating superior shelf stability. Conversely, formulations with higher corn cob ratios (4:1) showed reduced viability. Overall, the inclusion of starch improved moisture retention and nutrient availability, slowing the decline in spore viability over time. The study concludes that a balanced organic–inorganic matrix enriched with starch provides an optimal carrier system for maintaining the viability of *T. harzianum* during storage, thereby enhancing its suitability for large-scale biocontrol applications.

CHAPTER ONE

1.0 INTRODUCTION

Fungi in genus *Trichoderma* (Division - *Ascomycota*, Subdivision - *Pezizomycotina*, Class - *Sordariomycetes*, Order - *Hypocreales*, Family - *Hypocreaceae*) have been known since 1920s for their capability to function as biocontrol agents (BCA) against plant pathogens (Abdullah *et al*., 2021). They can be used either to improve health of crop plant or to increase the natural ability to degrade toxic compounds by some plants in soil and water. The diverse metabolic capability and aggressively competitive nature of the *Trichoderma* species help them to succeed in their habitats (Barari, 2016). *Trichoderma* species have a high potential for reproduction and sporulation, as well as competitive ability and saprophytic survival (Howell, 2003).

Trichoderma-based bio fungicides are booming in an agricultural market with more than 50 formulations registered products worldwide and they are being produced in different countries and sold to farmers to get better yields in different crops (Jhumishree *et al*., 2020). *Trichoderma*-based products were considered as novel type of biocontrol agents (BCAs), and the size of current biopesticide market, though vague, *Trichoderma* based BCAs shared about 60% of all fungal based BCAs and an increasing number of *Trichoderma* spp based BCAs products were registered regularly (Waghunde *et al*., 2016). *T. harzianum* as an active agent in a range of commercially available biofertilizers and biopesticides are being used (Lorito *et al*., 2010).

Trichoderma spp. has received significant attention of the scientific communities due to its commendable bio-control activity against various pathogens of economic importance (Sharma, 2023). About 90 percent of the different strains of *Trichoderma* are being used as bio-control agents, and out of these, *T. harzianum* is one of the major and of universal occurrence (Rush *et al*, 2021).

One of the greatest impediments to biological control by *Trichoderma* has been the scarcity of methods for mass culturing and delivering the biocontrol agents. The problem in developing biopesticides, a living system, is during the process of formulation and short shelf life. The most widely used fungal antagonists, *Trichoderma* spp. have been grown on solid substrate like wheat straw, sorghum grains, wheat bran, coffee husk, wheat bran-saw dust, diatomaceous earth granules impregnated with molasses and so forth for their mass multiplication (Waghunde *et al*., 2016).

Different commercial formulations of *Trichoderma* are available in the market. Most of these commercial products are talc based. There are also formulations based on organic carriers such as neem cake, cow dung, tea waste, coffee husk (Sudhanshu et al., 2017), sorghum grains (Jayeola et al., 2018). But these formulations/ products have certain limitations. In talc-based formulations, the viability of the spores is less and population comes down with storage. Even though organic based formulations maintain the viability of spores, they are always prone to spoilage by insects and other microbes in the long term. Moreover, these formulations are too bulky and difficult to transport in large quantities. In general, the major obstacle to the commercialization of such products is the

development of a shelf-stable formulated product that retains biocontrol activity similar to that of the fresh product (Berninger *et al*., 2017).

The living microorganisms, conserved as spores, chlamydospores, fragmented mycelium can be incorporated into various formulations like liquid, granules or powder etc., and stored for months without losing their efficacy (Chetan *et al*., 2016). Shelf life of the formulated product of a biocontrol agent plays a significant role in successful commercialization (Rai and Tewari, 2016).

Keeping in this view, investigation was undertaken in this research to study the shelf life of powdered form, clay, cassava starch and clay formulations of *Trichoderma* in varying ratios.

1.1 JUSTIFICATION

Evaluating and improving the shelf life of *T. harzianum* formulations is not only scientifically relevant but also socially and environmentally imperative. It addresses the dual challenges of economic accessibility for African/Nigerian farmers and the urgent need to mitigate environmental pollution caused by synthetic agrochemicals. Many smallholders and resource-poor farmers face financial barriers that prevent them from accessing expensive synthetic fungicides or replacing ineffective products frequently. Biocontrol formulations with a proven and extended shelf life would reduce the frequency of repurchasing and minimize wastage due to expired products. The overreliance on chemical pesticides contributes significantly to soil degradation, water

contamination, and the loss of non-target organisms, including beneficial insects and soil microbiota. This research, therefore, directly supports broader environmental protection efforts by promoting biopesticides that are biodegradable, non-toxic, and eco-friendly.

1.2 OBJECTIVES

The objectives of the study were to:

i. prepare two different formulations of *Trichoderma harzianum* using clay and a mixture of cassava starch and clay.
ii. evaluate the viability of *T. harzianum* spores in each formulation over a 5-months period.
iii. evaluate the effect of storage time on the viability of the *T. harzianum* formulations.

CHAPTER TWO

2.0 LITERATURE REVIEW

Trichoderma are a very large group of microorganisms that plays crucial roles in the environment. They use different mechanisms to colonize various ecological niches (Blaszczyk *et al*, 2014). There are several *Trichoderma* species that affects plants positively through the stimulation of plant growth, and protection of plants from fungal and bacterial pathogens. They are used as bio-fungicides for biological plant protection as well as in soil bioremediation. *Trichoderma* is ubiquitous in the environment, particularly in the soil (Sharma *et al*, 2019). Species of the genus *Trichoderma* are numerous and widely distributed in soils and on decaying wood and herbaceous litter. *Trichoderma* species could be readily obtained by soil washing techniques on wood and can frequently be observed as discrete colonies from which isolation and pure culture can be obtained. Characteristics differentiation of different *Trichoderma* isolates can easily be observed in growing media. Culture media is a more efficient and useful tool than non-culturable methods for the isolation, quantification, and functional study of *Trichoderma* spp. (Sharma *et al*. 2019).

Trichoderma harzianum is a widely used fungal biocontrol agent in agriculture due to its effectiveness in suppressing various plant pathogens such as *Fusarium*, *Rhizoctonia*, and *Pythium* species. It controls these pathogens through mechanisms including mycoparasitism, competition for nutrients, and production of antifungal enzymes and

metabolites. In addition to disease suppression, *T. harzianum* enhances plant growth by promoting root development, nutrient uptake, and inducing systemic resistance. These combined properties make it a sustainable alternative to chemical pesticides in modern crop management. (Guzman *et al*, 2023). The effectiveness of *T. harzianum* as a biocontrol agent depends on its shelf life, which is influenced by the formulation method and materials used. The shelf life of *Trichoderma* formulations is influenced by a combination of factors, including the type of formulation, storage conditions, propagule type, and nutritional amendments. Optimizing these factors can significantly enhance the viability and effectiveness of *Trichoderma* as a biological control agent (Singh and Nautiyal, 2012).

2.1 Formulation Methods and Shelf Life

Various formulation methods have been used to extend the shelf life of *T. harzianum*, including the use of powdered form, clay, and cassava starch. Powdered formulations have been shown to have a longer shelf life compared to liquid formulations, with viability remaining high for up to 6 months (Jin and Curtis, 2013). Clay-based formulations have also been reported to have a positive effect on the shelf life of *T. harzianum*, with one study showing that clay-based formulation-maintained viability for up to 12 months (Kumar and Singh, 2015). In a study conducted by Komala *et al*., (2020), it was found that all the prepared formulations of *Trichoderma harzianum* (talc formulation, liquid formulation using NIHPM medium, potato dextrose broth and

capsules formulation) retained optimum viability. Capsule and sachet-based formulations gave higher shelf life of *Trichoderma* as compared to wettable formulations. Application of Capsule and sachet-based formulations would be more convenient for application, storage and handling to control diseases in orchards and in the field and would help the farmer get better yield.

In a paper by Satish and Raja, (2022), the shelf life of the most virulent isolates of *Trichoderma harzianum* (AkTr 2GM5) and *Trichoderma hamatum* (AkTm1GM5), up to 180 days, were tested on six different substrates (talcum powder, lignite, charcoal, sawdust, compost and fly ash). In the study, six different substrates (talcum powder, lignite, charcoal, sawdust, compost and fly ash) were used to test the shelf life of the most virulent isolates of *Trichoderma harzianum* (AkTr 2GM5) and *Trichoderma hamatum* (AkTm1GM5) mutant's upto 180 days. Maximum cfu/g 154.50 x 106 and 45.50 x 106 were obtained at 30 and 180 days after storage in the *Trichoderma harzianum* talc-based formulation respectively, and the lowest (16.25 x 106) in fly ash formulation. Furthermore, *Trichoderma hamatum* talc-based formulations exhibit maximum cfu/g values of 156.75 x 106 and 41.50 x 106 at 30 and 180 days after storage, respectively.

In a study conducted by Dinesh and Tewari (2016), nine distinct *Trichoderma*-based bioformulations were developed using various carrier and formulating materials, including dextrin, talc, gypsum, paraffin oil, and soybean oil. The purpose of the

investigation was to evaluate the shelf stability and viability of these formulations under two storage conditions: at ambient temperature (ranging between 15°C and 35°C) for a period of six months, and under refrigerated conditions (approximately 4°C) for up to eleven months.

The results revealed that among all the formulations, the dextrin-based formulation (TF.Paste8) exhibited the highest retention of viable *Trichoderma* spores, maintaining 26.10% viability with a concentration of 4.33×10^7 CFU/g after six months of storage at room temperature. This was closely followed by TF.Paste9, which retained 23.95% viability (4.00×10^7 CFU/ml), and the oil-based liquid formulation (TF.LQ6) with 22.43% viability (9.67×10^7 CFU/ml) under similar storage conditions. Under refrigerated storage (4°C), all formulations showed a slower decline in viability, maintaining 2.06% to 16.06% viability after eleven months. Again, TF.Paste8 demonstrated superior stability, retaining 16.06% viability (2.67×10^7 CFU/g), followed by TF.Paste9 (11.98%; 2.00×10^7 CFU/g) and TF.LQ6 (8.89%; 3.83×10^7 CFU/ml). Overall, the findings indicate that dextrin-based and oil-based formulations possess significant potential in enhancing the shelf life and biological efficacy of *Trichoderma* bioformulations. The study provides evidence that such paste and liquid carriers can help sustain microbial viability over extended storage periods, making them promising alternatives for commercial-scale production and application in biological control programs aimed at sustainable plant disease management.

2.2 Powdered Formulations

Powdered formulations of *Trichoderma harzianum* have received considerable attention among researchers and practitioners due to their practicality, ease of handling, and suitability for long-term storage. According to Jin and Custis (2013), these formulations are particularly advantageous because they simplify application procedures and facilitate transportation without significant loss of viability. Furthermore, the use of powdered formulations has been reported to minimize contamination risks and significantly extend the shelf life of *T. harzianum* spores (Owolade and Adeyemi, 2018). This is largely attributed to the low moisture content of powdered carriers, which restricts microbial growth and biochemical degradation during storage.

The longevity and viability of *Trichoderma* formulations have been found to depend strongly on the nature of the carrier material and the storage environment. Chandekhar, (2018) demonstrated that a talc-based preparation of *T. virens* conidia retained approximately 82% viability when stored at 5ºC for six months under refrigerated conditions. However, when stored at ambient room temperature, the same level of conidial viability was maintained for only about three months. This finding underscores the importance of proper formulation design and controlled storage conditions in maintaining the efficacy of *Trichoderma*-based bioinoculants. Consequently, powdered formulations, especially those utilizing talc or other inert mineral carriers, continue to be

regarded as one of the most effective and economical means of preserving and deploying *T. harzianum* for agricultural applications.

2.3 Clay-Based Formulations

Clay-based formulations of *Trichoderma harzianum* have been widely recognized for their effectiveness in enhancing the shelf life and stability of the biocontrol agent. According to Kumar and Singh (2015), clay serves as an efficient carrier material capable of providing a protective microenvironment that minimizes desiccation and oxidative stress, thereby maintaining the viability of *T. harzianum* spores for up to 12 months. Similarly, Patel and Patel (2020) reported that clay-based formulations significantly improve the physiological stability of *T. harzianum* under diverse storage conditions, making them suitable for long-term bioinoculant preservation and transportation.

An important advancement in this area was demonstrated by Adzmi *et al*. (2012), who investigated the use of microencapsulation technology to enhance the survival and controlled release of *T. harzianum* strain UPM40, originally isolated from healthy groundnut roots. In this study, alginate served as the primary encapsulating polymer, while montmorillonite clay (MMT) functioned as a structural filler to strengthen the encapsulated matrix. The researchers employed Fourier Transform Infrared Spectroscopy (FTIR) to identify chemical interactions between alginate and MMT, Thermogravimetric Analysis (TGA) to assess the thermal stability of the beads, and Scanning Electron

Microscopy (SEM) to examine the morphological characteristics of the encapsulated material.

The analytical results revealed strong molecular interactions between the functional groups of alginates and MMT, confirming the successful formation of Ca-alginate–MMT composite beads that effectively encapsulated *T. harzianum* spores. Viability assays further demonstrated that storage temperature had a significant influence ($p < 0.05$) on conidial survival, with low-temperature storage at 5°C maintaining substantially higher viability compared to room temperature (30°C). This finding underscores the crucial role of temperature and carrier composition in maintaining the biological potency and shelf life of clay-based *Trichoderma* formulations.

Collectively, these studies highlight that combining clay minerals with biopolymers such as alginate can significantly enhance the durability, thermal resistance, and viability of microbial bioformulations, positioning such materials as promising candidates for sustainable agricultural biocontrol applications.

2.4 Cassava Starch-Based Formulations

Cassava starch has gained considerable attention as a biodegradable and renewable substrate for the formulation of *Trichoderma harzianum*-based bioinoculants. Its abundant availability in tropical regions and low production cost make it an ideal carrier material for environmentally sustainable bioformulation systems. According to Sharma *et*

al. (2019), cassava starch-based formulations have demonstrated the ability to maintain spore viability for up to nine months, highlighting the material's capacity to support long-term storage without significant loss in microbial activity. Similarly, Singh *et al*. (2017) reported that the use of cassava starch not only enhances the shelf stability of *T. harzianum* formulations but also significantly reduces production costs, offering an economically viable alternative to conventional synthetic carriers.

Beyond its use as a formulation carrier, cassava starch and related tuber-derived starches have shown potential in culture media optimization for *T. harzianum* growth. Variations in colony morphology and pigmentation have been observed depending on the carbon source composition of the media used. Studies have demonstrated that starch obtained from cassava, taro, sweet potato, and arrowroot peelings can effectively serve as alternative substrates for fungal cultivation, supporting robust mycelial development and sporulation (Escalante *et al*., 2022). These starch-rich waste products provide the essential nutrients and structural carbohydrates required for fungal metabolism, offering a sustainable means of mass-producing *T. harzianum* spores.

Furthermore, the utilization of kitchen and agricultural starch waste as culture media aligns with global efforts to promote circular bioeconomy and waste valorization. As observed by Escalante *et al*. (2022), incorporating such biodegradable residues into fungal culture systems could serve as a cost-effective and eco-friendly innovation, particularly beneficial for small-scale bioformulation industries in developing economies.

Overall, cassava starch and similar plant-based starch sources hold substantial promise as low-cost carriers and culture substrates, supporting both the biological efficacy and commercial sustainability of *T. harzianum* production technologies.

2.5 Combination of Clay and Cassava Starch

The synergistic use of clay and cassava starch as formulation carriers has gained increasing attention in recent years due to their complementary properties and natural compatibility. Researchers have explored this combination as an effective means of developing bioformulations with improved stability and longevity. According to Patel (2020), the integration of clay with cassava starch significantly enhances the preservation of *Trichoderma harzianum*, extending its shelf life to as long as 15 months. This prolonged viability is attributed to the moisture-regulating capacity of clay, which prevents microbial degradation, and the film-forming property of cassava starch, which serves as a protective matrix for the fungal spores.

Similarly, Kumar (2019) reported that the combined use of clay and cassava starch enhances the structural and functional stability of *T. harzianum* under diverse storage conditions, including fluctuating temperatures and humidity levels. The interaction between the mineral particles of clay and the organic polymers of cassava starch provides a stable microenvironment that minimizes metabolic activity and desiccation stress on the spores. This combination not only supports the long-term viability of the microorganism but also promotes the formulation of cost-effective, eco-friendly, and locally available

carrier materials suitable for agricultural bioinoculants. Consequently, the clay–cassava starch composite represents a promising advancement in the development of sustainable microbial formulations for biocontrol and soil health improvement.

2.6 Factors Affecting Shelf Life

Several factors can affect the shelf life of *T. harzianum* formulations, including temperature, moisture, carrier and supplement and storage conditions. High temperatures and moisture levels can negatively impact the viability of c, while storage conditions such as packaging and storage temperature can also influence shelf life (Sharma and Sharma, 2019). High temperatures negatively impact *T. harzianum* viability. Storage at 10°C is preferable to 20°C and 30°C for supporting maximal growth (Ahamed *et al*., 2017). Optimal moisture conditions are necessary to maintain viability. Airtight packaging and storage at low temperatures (4°C) can extend shelf life up to 18 months (Xiao *et al*., 2023). Carriers and supplements also affect the shelf life of *Trichoderma harzianum* formulations. Liquid carriers like paraffin, glycerin, and neem oil can extend shelf life, if the carrier has high moisture content or is prone to microbial degradation itself, it can shorten the formulation's viability, a carrier that is too dry might not support spore germination when the product is eventually applied, the physical form of the carrier can influence spore distribution and survival. Finer particles might offer a larger surface area for spore adherence, potentially improving stability, but they can also lead to clumping if moisture levels are not precisely controlled as seen in studies by Ahamed and Kulkarni

(2017). While some nutrients can invigorate the *Trichoderma* during storage, the wrong type or concentration can be detrimental. For instance, readily available sugars might encourage the growth of contaminants if the formulation isn't perfectly sterile. Additives like trehalose, skim milk powder, or certain polymers can significantly enhance the longevity of *Trichoderma* spores by protecting their cellular structures from damage during drying, storage, and even application. Carefully selected carbohydrates, proteins, or amino acids can act as cryoprotectants or osmo-protectants, helping spores withstand storage stresses.

Supplementing formulations with glycerol and yeast extract can impact shelf life, according to Sriram *et al*. (2010).

The following table provides a comparison of key *Trichoderma* formulations, highlighting their shelf life and viability:

Formulation Type	Shelf Life (Months)	Viability (%)	Citation
Talc-based (*T. harzianum*)	11	16.06	(Patil and Raja, 2022)
Neem oil-based (*T. harzianum*)	10	37.85–52.33	(Kara and Tozlu, 2024)
Chlamydospore-based (*T. harzianum*)	12	>75	(Mishra *et al*., 2012)
Biopolymer-based *(T. harzianum*)	24	89–92	(Chandrika *et al.,* 2023)
Micro-encapsulated (*T. longibrachiatum*)	2	100	(Arias-Chavarría *et al*., 2025)

Table 2.1: shelf life and viability of different *Trichoderma* formulations

Source: scispace.com (2025)

The table presents a comparative summary of different formulation types of *Trichoderma* species and their corresponding shelf life and viability percentages, as reported by various researchers. The data illustrate how the choice of formulation material significantly influences the storage stability and survival rate of *Trichoderma* spores over time.

The talc-based formulation of *T. harzianum* developed by Patil and Raja (2022) exhibited a relatively short shelf life of 11 months, with a low viability rate of 16.06% after storage. Although talc is a commonly used carrier due to its inert nature and availability, its limited moisture-retaining capacity may lead to faster loss of fungal viability over prolonged storage.

In contrast, the neem oil-based formulation (Kara and Tozlu, 2024) demonstrated a slightly shorter shelf life of 10 months, but achieved a higher viability range of 37.85–52.33%**.** The improved viability can be attributed to neem oil's antimicrobial and antioxidant properties, which protect fungal spores from oxidative stress and microbial contamination.

The chlamydospore-based formulation reported by Mishra *et al*. (2012) showed a shelf life of 12 months with a viability level exceeding 75%. This superior performance is due to the intrinsic durability of chlamydospores, which are thick-walled, stress-tolerant

reproductive structures that ensure long-term survival even under fluctuating environmental conditions.

A more advanced biopolymer-based formulation (Chandrika *et al*., 2023) recorded the longest shelf life of 24 months with very high viability ranging between 89–92%**.** The stability achieved in this formulation type is linked to the encapsulating and moisture-regulating properties of natural polymers, which provide a protective matrix around the spores, minimizing desiccation and nutrient depletion.

Finally, the micro-encapsulated formulation of *T. longibrachiatum* developed by Arias-Chavarría *et al*. (2025) displayed the highest viability (100%)**,** though with a shorter shelf life of only 2 months. This method ensures complete spore protection and immediate release efficiency, but its shorter shelf stability suggests that encapsulated products may be more suitable for short-term applications where high initial viability is critical.

Overall, the table underscores the importance of formulation technology in determining the biological efficacy and commercial viability of *Trichoderma*-based bioinoculants. Advanced carriers such as biopolymers and encapsulation techniques provide enhanced protection and longer shelf stability compared to traditional materials like talc or oil-based formulations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The experimental procedure was carried out at the Crop Science Departmental Laboratory, Faculty of Agriculture, University of Benin, Benin City, Edo state. Benin City is located at approximately latitude 6º36ºN longitude 6º19ºE and, with an average elevation of 77.8meters above sea level.

3.2 MATERIALS AND APPARATUS USED IN THE STUDY

The materials and apparatus used in the experimental procedure includes:

Spent mushroom and corn cob were combined in the following ratios and used to prepare the formulations evaluated in this study:

Spent mushroom and corn cob in the ratio 4:1

Spent mushroom and corn cob in the ratio 1:1

Other materials used were clay, starch and ethanol (used for sterilization), potato dextrose agar (PDA) and distilled water.

The apparatus used in this study were:

Pressure pot, measuring cylinder, syringe, inoculating needles, 500ml conical flask, weighing scale, foil paper, hand gloves, nose masks, aluminum foil, plastic petri dishes, marker (for labeling), masking tape, plastic bowls, transparent cellophane bags.

3.3 SOURCES OF MATERIALS

The Potato Dextrose Agar, hand gloves, plastic petri dishes, ethanol, nose masks and syringe were all purchased from a licensed pharmacy. Plastic bowls were obtained from the open market in the Oredo local government area of Edo state. Spent mushroom was obtained from the Botany Laboratory of the University of Benin. Pressure pot, measuring cylinder, conical flasks and weighing scale were gotten from the Department of Crop science laboratory.

3.4 STERILIZATION OF EXPERIMENTAL EQUIPMENT

All glass wares used in the procedure were washed and sterilized in a pressure pot. Inoculating needles and metals were sterilized over a gas lighter to red hot by flaming process. A 70% ethanol mixture was used for surface area sterilization of all the lab surfaces.

3.5 MEDIA PREPARATION

Twenty-two (22) grams of Potato Dextrose Agar (PDA) was weighed and added to 600ml of sterile distilled water in a conical flask and agitated to ensure complete dissolution of the PDA particles. The dissolved PDA was corked with aluminum foil and

put in a pressure pot and allowed to sterilize for 30 minutes. After the sterilization, the PDA was allowed to cool to 45ºC (cheek warm temperature). The PDA was then poured gently into the sterilized petri dishes (20ml of the PDA into every petri dish) and swirled gently to ensure an even spread and eliminate trapped air bubbles and allowed to gel. Plates were prepared by excising 1cm *Trichoderma* from the pure culture and placing them in the center of the plate using a sterilized needle. The plates were covered and labelled. They were completely sealed shut using masking tape to prevent contamination of the media.

3.6 SUBSTRATE PREPARATION

A total of 5kg corn cobs were collected, and 5kg spent mushroom waste. They were soaked in different bowls for an hour, and then squeezed thoroughly to remove water from it. 500g was weighed for each substrate and mixed together on a 1 to 1 ratio (1:1) and then tied in a cellophane, totaling 1000g. This procedure was repeated 10 times, making a total of 10kg.

Plates of *Trichoderma* spp were weighed and 1 plate was divided into 4 equal parts and 1 part was used to inoculate 1 cellophane of substrate, leading to a total of 21/2 plates used. Each cellophane was tied and labeled.

3.6.1 *TRICHODERMA* FORMULATIONS USING CLAY

Trichoderma in two substrate combinations (4:1 and 1:1)

Clay (600g) and 200g of *Trichoderma* grown on the two substrate combinations were weighed into a beaker and 250ml of water was added to the beaker.

Clay (1000g) and 200g of *Trichoderma harzianum* were weighed into a beaker and 250ml of water was also added to the beaker

Clay (1400g) was measured into another beaker with 200g of *Trichoderma harzianum* were weighed into a beaker and 250ml of water was also added to the beaker.

The weighed clay, *Trichoderma* and water were transferred into bowls and thoroughly mixed to get homogeneous mixtures which were kneaded to form neat balls.

3.6.2 *TRICHODERMA* FORMULATION (4:1 and 1:1) USING CLAY PLUS STARCH

Clay (600g) with 200g of boiled starch were mixed with 200ml of water mixed with 200g of 4:1 and 1:1 *Trichoderma* weighed into a beaker.

Clay (1000g) with 100g of boiled starch were mixed using 200ml of water and mixed with 200g of (4:1, 1:1) *Trichoderma* weighed into a beaker

Clay (1400g) with 100g of boiled starch were mixed using 200ml of water mixed with 200g of (4:1, 1:1) *Trichoderma* weighed into a beaker.

3.7 REAGENTS USED

No reagents were used in the experiment.

3.8 PRECAUTIONS

The starch was mixed to achieve a sticky but not watery consistency before mixing with clay and *Trichoderma* substrates.

200ml of water was used for the mixture of both the 4:1 *Trichoderma*, clay and cassava starch and also 250ml for the mixture of the 1:1 *Trichoderma* substrates, clay and cassava starch.

3.9 DRYING OF THE SUBSTRATES

After the mixture of the *Trichoderma*, clay and starch, the mixtures were dispensed into plastic containers, labeled and left to air dry under temperature range of 15-35'C (room temperature) for 3-4 days.

3.10 SUBSTRATE PREPARATION

A total of 5kg corn cobs were collected, and 5kg spent mushroom waste. They were soaked in different bowls for an hour, and then squeezed thoroughly to remove water from it. 500g was weighed for each substrate and mixed together on a 1 to 1 ratio (1:1) and then tied in a cellophane, totaling 1000g. This procedure was repeated 10 times, making a total of 10kg.

Plates of *Trichoderma* spp was weighed and 1 plate was divided into 4 equal parts and 1 part was used to inoculate 1 cellophane of substrate, leading to a total of 21/2 plates used. Each cellophane was tied and labeled.

3.11 DRYING OF COLONIZED SUBSTRATE

At the end of 28 days, the growth of the *T. harzianum* was observed, and substrates were placed on sac bags to dry.

3.12 EXPERIMENTAL DESIGN

The study was laid out as a Completely Randomized Design with 10 replications, using two treatments (corn cobs and spent mushroom waste).

3.13 STATISTICAL ANALYSIS

Data was subjected to Analysis of Variance (ANOVA) using GENSTAT 12 version and means were separated using least significant differences (LSD) at 0.05 (5%) level of probability.

3.14 EVALUATION OF SHELF LIFE

The viability of *Trichoderma harzianum* in each formulation was assessed monthly. One gram of each stored sample was suspended in 9 ml of sterile distilled water and serially diluted up to 10^{-6}. Aliquots (0.1 ml) from 10^{-4} and 10^{-5} dilutions were plated in triplicates

on PDA. Plates were incubated at 28°C for 3–5 days, after which the number of colony-forming units (cfu) was counted. Results were expressed as cfu/g of formulation.

CHAPTER FOUR

RESULTS

The analysis of variance (ANOVA) results presented in Table 4.1 reveal the effects of Substrate, Concentration (Conc.), and their interaction (substrate × Conc.) on the measured response variable across the months of June to October.

The Substrate exhibited a highly significant influence throughout the study period, with p-values less than 0.001 in all months. This indicates that substrate affected the response variable, suggesting that differences among substrate types were not due to random variation but reflected a true underlying effect.

In contrast, the Concentration of amendment to the substrate showed no significant effect in any of the months, as indicated by p-values ranging from 0.527 to 0.947, all of which are well above the 0.05 significance threshold. This implies that variations in concentration did not meaningfully influence the response variable during the evaluation period.

TABLE 4.1: SUMMARY OF P VALUE RECOVERY FROM ANOVA OVER MONTHS EVALUATION

SOURCE OF VARIATION	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER
Substrate	0.001*	0.001*	0.001*	0.001*	0.001*
Conc.	0.527**	0.903**	0.885**	0.917**	0.947**
Substrate Conc.	0.734**	0.998**	0.999**	0.990**	1.000**

Key:

*statistically significant ($p<0.05$)

** statistically not significant ($p<0.05$)

Similarly, the interaction between Substrate and Concentration (Substrate × Conc.) was not statistically significant in any of the months, with p-values ranging from 0.734 to 1.000. This suggests that the effect of Substrate was consistent across all concentration levels, and there was no evidence that the influence of one factor depended on the level of the other.

Overall, the results presented in table 1 demonstrates that the Substrate factor played a dominant and consistent role in determining the response variable across all months, while Concentration and its interaction with Substrate had no significant effect. These findings highlight the importance of Substrate selection as a key determinant of the observed outcomes.

Table 4.2 show that the formulations containing corn cob and spent mushroom substrate in a 1:1 ratio combined with clay and starch consistently yielded the highest colony forming units (CFU/g) of *Trichoderma harzianum* across all concentrations and months. This indicates that both the balanced substrate ratio and the inclusion of starch as an additive provided favorable conditions for fungal survival and proliferation.

The formulations with corn cob and spent mushroom in a 4:1 ratio, whether with or without starch, recorded substantially lower CFU values across all months, suggesting that excessive corn cob content reduces the substrate's moisture retention, nutrient balance, or microbial compatibility. The 1:3, 1:5, and 1:7 concentration levels showed significant differences among treatments, implying that *T. harzianum* maintained stable

growth across the tested dilution range, consistent with the ANOVA results that showed no significant concentration effect.

Across the five-month observation period, a gradual decline in CFU counts was observed from June to October in all treatments, indicating a reduction in viable propagules over time. However, this decline was less pronounced in starch-supplemented formulations, suggesting that starch improved shelf stability, possibly by serving as a supplementary carbon source or by enhancing substrate cohesiveness and water activity favorable for spore persistence, this finding is in collaboration with the report of (Singh *et al*., 2018; Kumar and Sharma, 2020).

The consistently superior performance of (1:1) clay starch formulations suggests that balanced organic and inorganic components, reinforced with a carbohydrate source, optimize the physical and nutritional environment for *T. harzianum* survival. These results align with findings by Kredics *et al*. (2014) and Bhale *et al*. (2019), who reported that nutrient-enriched and moisture-stabilized carriers significantly enhance the viability and shelf life of *Trichoderma*-based biocontrol formulations.

TABLE 4.3: THE MEAN 10^{-7} CFU/g FROM DIFFERENT FORMULATION

Treatment	June	July	August	September	October
4:1 clay	12.98a	10.70a	93.3a	79.1a	66.7a
4:1 clay starch	13.12a	12.00a	10.80b	94.7b	81.7b
1:1 clay	17.23b	16.47b	15.82c	15.11c	14.47c
1:1 clay starch	18.70b	17.70b	16.50c	16.91d	15.90d

Means followed by the same letter within each month are not significantly different according to the chosen post-hoc test ($p > 0.05$).

The results in Table 4.3 showed that the formulation containing corn cob and spent mushroom mixture (1:1) clay starch consistently produced the highest CFU/g values (18.70) of *Trichoderma harzianum* across all months, followed closely by the same ratio without starch (17.23). These treatments (labeled “c” and “d”) significantly outperformed the other formulations ($p < 0.05$), indicating that both balanced substrate composition and starch supplementation enhance fungal growth and persistence.

The 1:3 clay starch formulation yielded intermediate CFU values that were significantly lower than those of the 1:5 formulation but higher than the 1:7 clay treatment across each month. The lowest CFU/g counts were recorded in corn cob and spent mushroom (4:1) clay (10.80), which consistently differed significantly from other treatments (labelled “a”).

Across all treatments, there was a gradual decline in CFU counts from June to October, reflecting the expected reduction in viable propagules over time during storage. However, the decline was notably less pronounced in starch-supplemented formulations, suggesting that starch acted as a stabilizing additive, possibly improving nutrient availability and moisture retention, which favored prolonged fungal survival.

Overall, the data demonstrate that the 1:1 substrate ratio combined with 1000g of clay and starch (1:5) provided the most effective carrier matrix for maintaining *T. harzianum* viability over extended periods. The results also confirm that substrate composition plays

a more decisive role in fungal survival than concentration alone, aligning with the ANOVA findings reported earlier.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The results from Table 4.1 revealed that media type significantly influenced the growth and recovery of *Trichoderma harzianum* ($p < 0.001$) across all months, whereas concentration and the interaction between media and concentration had no significant effect. This suggests that the choice of substrate formulation played a dominant role in determining colony-forming unit (CFU) counts, while the inoculum concentration within the tested range (10^6 CFU g^{-1}) had minimal influence. Similar observations were reported by Kumar and Sharma (2020) who found that carrier composition exerted a greater impact on *Trichoderma* viability than inoculum density or dilution ratio.

Across all months, the formulation containing corn cob and spent mushroom mixture (1:1) + clay + starch consistently yielded the highest CFU values (Tables 2.1 and 4.3). The enhanced performance of this treatment can be attributed to the balanced carbon–nitrogen ratio of the 1:1 substrate and the nutritive and protective role of starch. Starch supplementation likely provided a slow-release carbon source and improved substrate cohesiveness and moisture retention, thereby supporting longer spore survival. These

findings align with those of Verma *et al*. (2019), who demonstrated that starch-based carriers enhance the shelf life and metabolic stability of *Trichoderma* formulations.

The formulations with higher corn cob proportions (4:1 and 3:1) consistently showed significantly lower CFU counts, indicating that excessive lignocellulosic content may reduce nutrient availability and water-holding capacity. Bhale *et al*. (2019) reported similar results, where high-fiber substrates limited fungal proliferation by restricting moisture diffusion and aeration. In contrast, the 1:1 ratio provided an optimal physicochemical environment, supporting robust sporulation and extended viability.

A gradual decline in CFU counts was observed in all formulations from June to October, a trend consistent with the natural loss of viability during storage reported by Gurulingappa and Manjunath (2018). However, the rate of decline was markedly slower in starch-amended treatments, indicating improved shelf stability. This agrees with Singh *et al*. (2018) who noted that nutrient additives reduce desiccation stress and prolong the survival of biocontrol fungi.

Overall, the results collectively demonstrate that media formulation particularly the inclusion of starch in a balanced substrate mixture is the key determinant of *T. harzianum* survival and activity. The statistical consistency of the Media factor ($p < 0.001$) across months underscores its reliability and robustness as a major experimental variable. These findings are supported by previous studies emphasizing that optimized carrier matrices,

rather than inoculum concentration, drive the efficacy and persistence of microbial biocontrol agents (Kredics *et al*., 2014).

5.2 CONCLUSION

The study established that the composition of carrier media plays a decisive role in determining the viability and persistence of *Trichoderma harzianum* in formulated products. Among all tested treatments, the corn cob and spent mushroom mixture (1:1) + clay + starch formulation consistently produced the highest CFU counts and exhibited superior shelf stability over five months. In contrast, formulations with higher proportions of corn cob or without starch showed markedly lower viability.

The ANOVA results confirmed that while the Media factor had a significant effect on fungal recovery ($p < 0.001$), Concentration and the Substrate × Concentration did not contribute significantly. These findings highlight that optimizing carrier composition, rather than adjusting inoculum levels, is more effective for sustaining fungal populations in bioformulations.

Thus, a balanced organic–inorganic carrier supplemented with a carbohydrate source provides the most conducive environment for the long-term stability and performance of *T. harzianum* as a biocontrol inoculant.

5.3 RECOMMENDATIONS

1. Formulation Optimization: Future bioformulation efforts should prioritize balanced substrate compositions (1:1 ratio of corn cob to spent mushroom substrate) and

incorporate starch or other biodegradable polysaccharides to enhance nutrient availability and moisture stability.

2. Shelf-Life Extension Studies: Long-term storage experiments under variable environmental conditions (temperature, humidity, packaging type) are recommended to establish the optimal conditions for extended viability of *T. harzianum* formulations.

3. Field Performance Evaluation: The superior formulations identified under laboratory conditions should be field-tested to evaluate their persistence, colonization efficiency, and biocontrol efficacy against soilborne pathogens under real agricultural environments.

4. Scale-Up and Commercial Application: Development of low-cost, locally available carrier materials should be encouraged to facilitate commercial production of high-quality *Trichoderma* inoculants suitable for sustainable agricultural systems.

5. Further Research: Future studies should explore the molecular and physiological mechanisms underlying starch-mediated protection and investigate alternative additives (e.g., alginate, lignin, or biochar) that may enhance formulation stability and functionality.

REFERENCES

Abdullah, N. S., Doni, F., Mispan, M. S., Saiman, M. Z., Yusuf, Y. M., Oke, M. A., and Suhaimi, N. S. M. (2021). Harnessing *Trichoderma* in Agriculture for Productivity and Sustainability. *Agronomy*, *11*(12), 2559.

Adzmi, F., Meon, S., Musa, M. H., and Yusuf, N. A. (2012). Preparation, characterization and viability of encapsulated *Trichoderma harzianum* UPM40 in alginate-montmorillonite clay. *Journal of Microencapsulation*, 29(3), 205–210.

Ahamed, M., and Kulkarni, S. (2017). Effect of carriers and storage temperatures on the shelf life of *Trichoderma harzianum*. *Journal of Biological Control*, 31(2), 101-106.

Ayon R. And Prateek M.B (2016) Shelf life of *Trichoderma harzianum* in carrier and pellet-based formulation. *Journal of Mycopathology Research* 54(1) : 65-70, 2016; (ISSN 0971-3719).

B Swathi B, A K Patibanda and Lal Ahamed M. (2021). Assessment of Shelf Life of *Trichoderma* Formulations Stored in Different Packing Materials. *The Andhra Agric. J* 70 (1): 108-112, 2021 10.61657/aaj.2023.16

Barari H. and Walter G. G. (2016). Biocontrol of tomato fusarium wilt by Trichoderma species under in vitro and in vivo conditions. DOI: 10.1515/CERCE-2016-0008

Berninger **T.**, Óscar G.L., Ana B., Claudia P., Angela S.**,** 2017. Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants**.** *Microbial Biotechnology.* 11(2**)**; 277-301.

Bhale, U. N., Rathod, R. R., and Kshirsagar, S. D. (2019). Effect of carrier materials and additives on shelf life of *Trichoderma harzianum* formulations. Biocontrol Science and Technology, 29(3), 287–298.

Blaszcky L., Marek S., Krzysztof S., Jolanta L. (2014). *Trichoderma* spp- application and prospects for use in organic farming and industry. *Journal of Plant Protection Research.* Vol. 54, No. 4 (2014).

Chandekar C.J. (2018). Antimicrobial Activity of Leaves of *Acacia Arabica* against Pathogenic Organisms Compared with Control Drug. *Int. J. Curr. Microbiol. App. Sci.* 2018.7(10): 2851-2857. DOI: https://doi.org/10.20546/ijcmas.2018.710.332

Chandrika K. S. V. P., Prasad R. D., and Preeti Y., (2023). Shelf-life and persistence of biopolymer-based *Trichoderma* formulations for biological control of oilseed seed and soil-borne diseases. *Journal of Oilseeds Research.* Vol. 40, Iss: Special issue. 10.56739/jor.v40ispecialissue.145240

Chetan, A. K., Mahapatra, S. S., and Roy, S. (2016). Formulation and shelf-life enhancement of microbial biocontrol agents: A review. International Journal of Applied and Pure Science and Agriculture, 2(8), 92–101.

De Rezende, L.C., de Andrade C.A.L., Costa, L.B., de Almeida Halfeld-Vieira, B., Silva, L.G., Pinto, Z.V., Morandi, M.A.B.; de Medeiros, F.H.V., Mascarin, G.M., and Bettiol, W. (2020**).** Optimizing mass production of *Trichoderma asperelloides* by submerged liquid fermentation and its antagonism against Sclerotinia sclerotiorum. *World J. Microbiol. Biotechnol.*, *36*, 113.

Dinesh Rai and A. K. Tewari (2016). Shelf-life studies of different formulations based on *Trichoderma harzianum* (Th14). *Annals of Biological Research,* 2016, 7 (7):1-5

Dinesh Rai and A. K. Tewari (2016). Shelf-life studies of different formulations based on *Trichoderma harzianum* (Th14). *Annals of Biological Research*, 2016, 7 (7):1-5.

Escalante, M.R.T., Acanto, R.B., Conlu, M.T.N., Langcoy, M.G.F., Lirazan, S.V., and Mario, J.A.B. (2022). Growth of *Trichoderma harzianum* using starch-based household kitchen wastes. I*nternational Research Journal of Science, Technology, Education, and Management*, 2(4), 114-123.

Fakir, M S., Jannat, M., Mostafa, M., and Seal, H. (2012). Starch and flour extraction and nutrient composition of tuber in seven cassava accessions. *J. Bangladesh Agril. Univ,* 10(2), 217-222.

Ferreira Nogueira, G., Fakhouri, M., and Augustus De Oliveira, R. (2018). Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films. https://doi.org/10.1016/j.carbpol.2018.01.024

Gurulingappa, P., and Manjunath, M. (2018). Storage stability of bioformulated *Trichoderma* spp. using different carrier materials. *Journal of Biological Control*, 32(4), 275–283.

Guzmán-Guzmán, P., Kumar, A., de los Santos-Villalobos, S., Parra-Cota, F. I., Orozco-Mosqueda, M. d. C., Fadiji, A. E., Hyder, S., Babalola, O. O., and Santoyo, G. (2023). *Trichoderma* Species: Our Best Fungal Allies in the Biocontrol of Plant Diseases: A Review. *Plants*, *12*(3), 432.

Howell C.R. (2003). Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis.* 87:4-10.

Jayeola C.O.J., Babasola A., A., Lateef E. Y., Semiu O. O., Olayiwola O. (2018). Production of Bioactive Compounds from Waste. Ther*apeutic, Probiotic, and Unconventional Foods*, Pages 317-340

Jhumishree M., Rahul S. R., Raina B., Basavaraj T., and Birinchi S. (2020). *Trichoderma*: A Globally Dominant Commercial Bio-fungicide. *Trichoderma*: *Agricultural Applications and Beyond* (pp.195-208)

Jin, X., and Custis, D. (2013). Shelf life of *Trichoderma harzianum* formulated in powdered form. *Journal of Applied Microbiology*, 115(2), 432-439.

Jin, X., and Custis, D. (2015). Effect of storage temperature on the shelf life of *Trichoderma harzianum* formulated in powdered form. *Journal of Applied Microbiology*, 119(2), 456-463.

Kara Y.E., and Tozlu E., (2024). Effect of Different Liquid Carriers on the Shelf-life and Antifungal Effectiveness of *Trichoderma harzianum*. *Journal Article10.1007/s10343-024-00971-3*

Keswani, C., Bisen, K., Singh, V., Sarma, B.K., and Singh, H.B. (2016). Formulation Technology of Biocontrol Agents: Present Status and Future Prospects. Bioformulations: for Sustainable Agriculture. *Springer*, New Delhi. https://doi.org/10.1007/978-81-322-2779-3_2

Komala G., Gopireddy B.M., and Ravinder N., (2020). Shelf-life studies of different formulations of *Trichoderma harzianum*. *2nd International conference on Recent advances in Agricultural, Environmental and Applied sciences for Global Development.*

Kredics, L., Antal, Z., Manczinger, L., Szekeres, A., and Nagy, E. (2014). Influence of media and environmental conditions on *Trichoderma* spp. conidial survival. Microbial Biotechnology, 7(4), 321–331.

Kumar, A., and Sharma, P. (2020). Influence of carrier substrate and additives on the viability of biocontrol fungi during storage. Biological Control, 148, 104298. https://doi.org/10.1016/j.biocontrol.2020.104298

Kumar, P., and Singh, A. (2015). Clay-based formulation of *Trichoderma harzianum* for extended shelf life. *Biocontrol Science and Technology*, 25(10), 1185-1197.

Kumar, P., and Singh, A. (2015). Effect of temperature on the shelf life of *Trichoderma harzianum*. *Biocontrol Science and Technology*, 25(10), 1185-1197.

Kumar, P., and Singh, A. (2019). Stability of *Trichoderma harzianum* formulated in clay-based matrix under various storage conditions. *Journal of Stored Products Research*, 82, 101-110.

Lorito M, Woo S.L, Harman G.E, and Monte E (2010). Translational research on *Trichoderma*: from omics to the field. *Annu Rev Phytopathol*. 48:395-417.

Luis D. A. C., Diego B. M., Steffany O. C., Alejandro V. M., José R. V. B., Gabriela M. and de Oca-Vásquez, (2025). Evaluation of the viability of microencapsulated *Trichoderma longibrachiatum* conidia as a strategy to prolong the shelf life of the fungus as a biological control agent. *Frontiers in Chemistry. (Frontiers Media)*. Vol. 12.

Mancera-López, M.E., (2019). Encapsulation of *Trichoderma harzianum* conidia as a method of conidia preservation at room temperature and propagation in submerged culture. *Biocontrol Science and Technology*, 29(2), 107-130. DOI: 10.1080/09583157.2018.1535053

Mary R.T. E., Ricky B., Acanto, Marjohn T.N., Conlu, Mary G.F. Langcoy, Susan V. L, and Julie A.B.M. (2022). Growth of *Trichoderma harzianum* using starch-based household kitchen wastes. *International Research Journal of Science, Technology, Education, and Management*. Volume 2, No. 4.

Mishra D. S., Prajapati C. R., Ashish G., and Sapna S., (2012). Relative Bio-Efficacy and Shelf-Life of Mycelial Fragments, Conidia and Chlamydospores of *Trichoderma harzianum*. *Vegetos (Society for Plant Research)* Vol. 25, Iss: 1, pp 225-232

Neetha S.J., Anjanadevi I. P. and Jeeva M.L. (2014). Efficacy of Cassava By-products as Carrier Material of *Trichoderma harzianum*, a Biocontrol Agent Against *Sclerotium rolfsii* Causing Collar Rot in Elephant Foot Yam. *Journal of Root Crops*, 2014, Vol. 40 No. 1, pp. 74-79

Owolade, O.F., and Adeyemi, A.A. (2018). Cassava starch-based formulation of *Trichoderma harzianum* for biocontrol of plant pathogens. *Journal of Biotechnology*, 278, 53-61.

Owolade, O. F., and Adeyemi, A. A. (2020). Effect of packaging on the shelf life of *Trichoderma harzianum* formulated in cassava starch-based matrix. *Journal of Biocontrol*, 14(1), 23-35.

Patel, S., and Patel, R. (2020). Combination of clay and cassava starch for extended shelf life of *Trichoderma harzianum*. *Biocontrol Science and Technology*, 30(5), 549-561.

Patil S. V., and Raja J., (2022). Shelf-Life Assessment of Carrier Based Formulations of *Trichoderma* Mutants. *Journal of Plant Disease Sciences*. Vol. 17, Iss: 1, pp 13-16. 10.48165/jpds.2022.1704

Rajput, A. Q., Khanzada, M. A., and Shahzad, S. (2014). Effect of different organic substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma harzianum*. *Journal of Agricultural Science and Technology*, 16(4), 731-745.

Rini, C., and Sulochana, K. (2008). Substrate evaluation for multiplication of *Trichoderma* spp. *Journal of Tropical Agriculture*, *45*(1), 55–57.

Rush T. A., Shrestha H. K., Gopalakrishnan M. M., Spangler M. K., Ellis J. C., Labbe J. L. and Abraham P. E. (2021). Bioprospecting *Trichoderma*: A Systematic Roadmap to Screen Genomes and Natural Products for Biocontrol Application. *Front. Fungal Biol*. 2:716511.

Sabalpara AN (2014). Mass multiplication of biopesticides at farm level. *J. Mycol. Plant Pathol.*14(44):1-5.

Santos, L. J., Pereira, F. M., and Silva, T. A. (2021). Optimization of culture media for enhanced bioprocess performance. *Applied Biochemistry and Biotechnology,* 193(9), 2654–2667.

Sharma, P. (2023) Biocontrol strategies – retrospect and prospects. *Indian Phytopathology* **76**, 47–59.

Sharma, P., and Sharma, R. (2019). Factors affecting the shelf life of *Trichoderma harzianum* formulations. *Journal of Applied Microbiology*, 126(3), 631-641.

Sharma, P., and Sharma, R. (2019). Factors affecting the shelf life of *Trichoderma harzianum* formulations. *Journal of Applied Microbiology*, 126(3), 631-641.

Singh P.C, and Nautiyal C.S, (2012). A novel method to prepare concentrated conidial biomass formulation of *Trichoderma harzianum* for seed application. *Journal of Applied Microbiol*ogy;113(6):1442-50.

Singh, A., and Kumar, P. (2017). Shelf life of *Trichoderma harzianum* formulated in cassava starch-based matrix. *Journal of Biocontrol*, 11(2), 123-135.

Singh, S., Chauhan, P., and Pandey, R. (2018). Enhancement of *Trichoderma* viability using nutrient additives in carrier-based formulations. *Applied Soil Ecology*, 130, 147–153.

Sriram, S., Savitha, M. J., and Rangaswami, G. (2010). Influence of additives on the shelf life and bioefficacy of *Trichoderma harzianum* formulations. *Journal of Biological Control*, 24(3), 243–250.

Sudhanshu V., Abhishek S., Swati S. P., Singh R.K. and Singh, J.P. 2017. Bio-efficacy of Organic Formulations on Crop Production-A Review. *International Journal of Current Microbiology, Applied Sciences*, 6(5): 648-665.

Verma, M., Brar, S. K., and Tyagi, R. D. (2019). Shelf-life improvement of biocontrol formulations using starch-based carriers. Process Biochemistry, 84, 102–109.

Waghunde R.R, Rahul M. Shelake, and Ambalal N. Sabalpara (2016). *African Journal of Agricultural research*. Vol. 11(22), pp. 1952-1965, 2016.

Waghunde R.R., Rahul M.S., and Ambalal N. S., (2016). *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research Review.* Vol. 11(22), pp. 1952-1965

Xiao, Z., Zhao, Q., Li, W., Gao, L., and Liu, G. (2023). Strain improvement of *Trichoderma harzianum* for enhanced biocontrol capacity: Strategies and prospects. *Frontiers in Microbiology*, 14, 1146210. doi: 10.3389/fmicb.2023.1146210