MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INSECT PESTS AND FUNGAL PATHOGENS OF GINGER (*Zingiber officinale* Roscoe) OF MIZORAM

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CERTIFICATE

I certify that the thesis entitled **"Morphological and molecular characterization of insect pests and fungal pathogens of ginger (*Zingiber officinale* roscoe) of Mizoram."** submitted to the Mizoram University for the award of the degree of Doctor of Philosophy in Horticulture, aromatic and Medicinal Plants by Albana L. Chawngthu is a record of the research work carried out during the period 2019 - 2025 under my supervision and guidance and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this University or any other University or institution of higher learning.

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DECLARATION

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April 2025

I **Albana. L. Chawngthu**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants (HAMP).

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Abbreviations

ANOVA	**Analysis of variance**
BOD	**Biochemical Oxygen Demand**
BLAST	**Basic Local Alignment Search Tool**
cm	**centimeter**
COI	**Cytochrome c oxidase subunit I gene**
DNA	**Deoxyribonucleic Acid**
FI	**Field Infestation**
IPM	**Integrated pest management**
ITS	**Internal Transcribed Spacer**
MEGA	**Molecular Evolutionary Genetics Analysis**
mg	**milligram**
mm	**millimeter**
ML	**Maximum likelihood**
NA	**Nutirent agar**
NCBI	**National Center for Biotechnology Information**
NJ	**Neighbour joining**
PCR	**Polymerase Chain Reaction**
PDA	**Potato Dextrose Agar**
PI	**Pathogenecity Index**
RH	**Relative humidity**
SD	**Standard Deviation**
Sp.	**Species (singular)**
Spp.	**Species (plural)**
WA	**Water Agar**
WD	**Weight of diseased rhizome**
WH	**Weight of healthy rhizome**

CHAPTER I

Introduction

1.1 Overview

India is distinguished by an impressive and extensive diversity that encompasses both its flora and fauna, reflecting a unique ecological tapestry. Often referred to as the "magical land of spices," this vast country takes pride in its extraordinarily rich and varied assortment of spice types, which play an integral role in its cultural and culinary heritage. Among these, the earliest recognized oriental spice is ginger, which is scientifically classified under the nomenclature *Zingiber officinale* Roscoe and is a proud member of the *Zingiberaceae* family. The entire plant exudes a refreshingly aromatic fragrance that captivates the senses and enhances the atmosphere of the environments in which it is found. Nevertheless, it is primarily the subterranean rhizomes of this remarkable species that are held in the highest regard for their exceptional spice value and culinary applications. This particular spice has earned its place as one of the most commonly utilized culinary condiments across the globe, and it is also renowned for its significant medicinal properties and notable anti-bacterial characteristics. The ginger rhizome, with its multifaceted applications, stands out as one of the most essential spices on an international scale, serving not only as a fundamental ingredient in the food industry but also playing a vital role in pharmaceuticals and cosmetics, thereby demonstrating its versatility and importance in various sectors. **(Malu *et al*., 2009)**.

1.2 Global scenario of ginger production:

India has persistently maintained its status as the preeminent global producer of ginger, accounting for over 40% of worldwide output. As reported by the Indian Ministry of Agriculture and Farmers Welfare **(2023)**, the volume of ginger produced in India surged from approximately 760,000 metric tons in 2015 to in excess of 2.4 million metric tons by 2023. The predominant geographical areas engaged in the agricultural production of ginger, a highly sought-after spice known for its culinary and medicinal properties, include the Indian states of Mizoram, Kerala, Assam, Meghalaya, Karnataka, and Sikkim, each of which possesses unique climatic and soil

conditions that are conducive to the successful cultivation of this rhizomatous herbaceous perennial. Nonetheless, challenges such as rhizome rot and marketing limitations continue to impede productivity and financial viability **(Chattopadhyay *et al*., 2017).**

Nigeria occupies the second position globally, generating more than 800,000 metric tons annually. Despite its considerable production capacity, the majority of Nigeria's ginger is utilized domestically, with only a minor fraction reaching international markets **(FAO, 2022)**. Nevertheless, as interest in non-oil exports escalates, ginger has assumed strategic significance within Nigeria's agricultural diversification initiatives. The potential for growth remains substantial, although it is hampered by inadequate mechanization, inconsistent planting materials, and post-harvest losses **(Olaniyi *et al*., 2021).**

China, with production figures approaching 670,000 metric tons, is a crucial participant in both ginger cultivation and exportation. Chinese ginger is extensively supplied to Europe, North America, and the Middle East. Its predominance in global markets is supported by efficient supply chains, robust processing infrastructures, and stringent quality assurance protocols **(Wang *et al*., 2019)**. The ginger industry in China is characterized by high levels of mechanization, alongside significant investments in research and development, particularly concerning storage techniques and disease management.

Additional noteworthy ginger-producing nations include Indonesia, Nepal, Thailand, Bangladesh, and Peru. For instance, Nepal produced over 328,000 metric tons in 2023, primarily for internal consumption and limited exports to India and Bangladesh **(FAOSTAT, 2023)**. Indonesia and Thailand also play critical regional roles, profiting from favorable agroclimatic conditions and traditional agricultural knowledge. Although Peru is a smaller producer, it has rapidly gained recognition in the export market due to its organic ginger, particularly targeting the U.S. and European Union markets.

The global landscape of ginger production is influenced by a confluence of agronomic, economic, and climatic elements. Climate change introduces both

challenges and opportunities, as altered precipitation patterns and increasing temperatures may impact rhizome development and pest interactions. Furthermore, the global spice market is increasingly shaped by sustainability considerations, leading to a transition towards organic certification and traceable supply chains **(Ghosh *et al*., 2021).**

Over the past decade, global ginger production has experienced consistent growth, with India, Nigeria, and China at the forefront. Ongoing research focusing on disease management, the development of high-yield cultivars, and advancements in post-harvest technologies, coupled with supportive policy frameworks, will be vital in sustaining this growth trajectory and addressing the escalating global demand.

1.3 Status of ginger production in India

India ranks among the foremost cultivators of ginger on a global scale, with this particular plant occupying the largest area and yielding the highest output **(Meenu *et al.*, 2017)**. The annual worldwide trade in ginger is estimated to possess a valuation of approximately US$190 million. It is estimated that around 60–70% of ginger is produced on a global basis. Of this total, approximately 30% undergoes processing into dry ginger, 50% is utilized for the production of green ginger, and the remaining 20% is converted into seed **(Parveen *et al*., 2014)**. The Indian regions of Odisha, Arunachal Pradesh, Meghalaya, Gujarat, Kerala, Karnataka, and Assam act as major cultivators of ginger, collectively accounting for approximately 65% of the country's overall production. **(Jayashree *et al*., 2015)**.

Ginger possesses considerable economic and cultural significance within the vast geographic and demographic landscape of India. This nation proudly holds the title of the largest producer of ginger on a global scale, accounting for an impressive estimated 40% of the entire world's ginger production, with a remarkable output reaching approximately 2.1 million tonnes during the agricultural year spanning from 2022 to 2023. The primary regions contributing to this noteworthy production include the northeastern states such as Assam, Meghalaya, Arunachal Pradesh, and Mizoram, as well as the southern states of Kerala and Karnataka, all of which play a vital role in India's ginger cultivation and harvesting endeavors.

In terms of consumption patterns, India primarily utilizes the majority of its ginger production for domestic purposes, whereas China has established itself as a dominant force in international markets, especially when it comes to the export of processed ginger products, as indicated by the **OECD/FAO in 2022**. Ginger flourishes in warm and humid climatic conditions, characterized by well-drained soil, which renders tropical and subtropical regions as the most suitable environments for its cultivation. The surge in global demand for ginger can be attributed to a growing interest in natural remedies and functional foods, as well as the increasing recognition of ginger's pharmacological properties, which include its notable anti-inflammatory, antioxidant, and antimicrobial effects, as documented by **Ali *et al*. in 2008**.

Therefore, it can be conclusively stated that ginger constitutes a vital economic crop, playing an essential role in contributing significantly to agricultural exports and supporting the livelihoods of rural communities across various regions, particularly in Asia and Africa.

1.4 Status of ginger production in North-East India

In the north-eastern region of India, the total agricultural land dedicated to the cultivation of various spices encompasses a substantial area of approximately 140,000 hectares, which translates into an impressive production yield of around 436,800 tonnes, resulting in an average productivity rate of 3.12 tonnes per hectare, as reported in an anonymous source from 2003. Within this expansive landscape, the specific area allocated for ginger cultivation is recorded to be approximately 33,200 hectares, leading to a notable total production output of about 191,000 tonnes, which corresponds to an average yield rate of 5.8 tonnes per hectare, significantly surpassing the national average productivity rate of 3.5 tonnes per hectare, as indicated in the Basic Statistics of the Northeast Region published in 2002. Among the states within this geographical region, Meghalaya stands out as the preeminent producer of ginger, additionally attaining the notable status of being the second-largest producer in the nation as a whole contributing a substantial share approximately 20% to the national ginger production, which is only slightly less than Kerala, the leading state that accounts for 23.08% of the total production in India. Within the broader context of ginger cultivation in the northeastern region of India, the state of Meghalaya stands

out as the leading producer, demonstrating exceptional levels of ginger output, while the adjacent states of Mizoram and Arunachal Pradesh also play a crucial role in contributing to the cumulative ginger production in this area, thereby underscoring the interconnectedness of agricultural practices and economic vitality among these neighboring states; however, it is crucial to note that Arunachal Pradesh exhibits the highest productivity rates among these states, as illustrated in Table 1. Furthermore, Meghalaya enjoys a higher per capita annual availability of ginger in comparison to the national average availability, which serves as an indicator of the farmers' growing interest and enthusiasm for cultivating this lucrative crop, as the soil quality, climatic conditions, and various ecological factors in the region are exceedingly conducive to the growth and development of ginger.

Table 1.1: Statewise status of ginger productivity and production in the North East India as per **FAOSTAT data 2024**

State	Area (Ha)	Productivity (metric tonnes)	Productivity (t/ha)
Meghalaya	**10.855**	46.59	5.55
Mizoram	4.53	**100.00**	**22.08**
Arunachal Pradesh	4.62	38.03	8.26
Assam	4.21	32.11	7.65
Manipur	1.29	12.55	9.87
Nagaland	1.36	12.39	9.06
Sikkim	5.12	24.01	4.73
Tripura	1.37	5.47	4.10
Northeast Total	**33.35**	**271.15**	**71.3**

1.5 Status of ginger production in Mizoram

In the broader context of India, ginger occupies an estimated area of 0.17 million hectares, yielding a considerable production volume of approximately 1.84 million tonnes, which indicates a commendable productivity rate of 10.72 tonnes per hectare, according to the **FAOSTAT report from 2020**. The state of Assam is recognized as the leading producer of ginger in the country, closely followed by the states of Maharashtra and West Bengal, while Mizoram holds the seventh position in the overall production rankings of ginger in India, as noted in the **NHB** report for the years **2017-2018.** The productivity of ginger in Mizoram during the agricultural year of 2018-19 has been recorded at an impressive rate of 7.03 tonnes per hectare, which positions it among the highest productivity levels for ginger cultivation in the nation, as indicated in the **Economic Survey for the fiscal year 2019-2020**.

In the state of Mizoram, indigenous ginger cultivars such as Thinglaidum, Thingpui and Thingria, have been reported to dominate the ginger cultivation landscape, as documented by **Rahman *et al*. in 2008**. Specifically, the 'Thinglaidum' cultivar is prevalent in terms of area coverage, characterized by its small size, extreme pungency, and minimal fiber content, along with a distinctive blackish ring, and it is primarily utilized in the processing of dried ginger, which has a wide array of industrial applications. The 'Thingpui' cultivar occupies a significant second position in terms of area, known for its bold and light-yellow rhizome, which possesses a milder pungency, making it suitable for table consumption and domestic culinary uses. It is important to highlight that the state of Mizoram has garnered significant acknowledgment in the agricultural domain by securing a prestigious geographical indication (GI) tag specifically for these two distinct cultivars of ginger, which are collectively identified under the appellation of "Mizo Ginger," thereby enhancing their reputation and marketability on both national and international platforms. As the interest among farmers in the region for ginger cultivation continues to grow, it is largely due to the favorable climate, soil conditions, and other ecological factors that enhance the quality of ginger production. In Mizoram, there exists considerable potential for improving yield per unit area, which in turn would contribute to an increase in the overall ginger production levels. Additionally, farmers are engaged in the cultivation of black ginger, a unique variety characterized by its bluish-black

rhizomes, which is produced both for commercial sale and for personal consumption, and this particular variety commands a premium price in the market owing to its recognized therapeutic and medicinal properties.

The production of ginger within the geographic confines of Mizoram has experienced a remarkably dynamic evolution over the years, characterized by a complex interplay of both significant growth trajectories and formidable challenges that have emerged in the agricultural landscape. Mizoram has positioned itself as a pivotal contributor to the overall ginger production within the Northeast region of India, thereby underscoring its importance in the agricultural sector. In the agricultural year spanning 2014 to 2015, Mizoram recorded an impressive output of approximately 28,390 metric tons of ginger; however, in the subsequent years, the production trajectory exhibited a steady and marked improvement, ultimately achieving a notable peak of 61,001 metric tons in the year 2020. Although there was a slight decline in ginger production in the following years, the output remained relatively stable, with figures indicating 60,291 metric tons in 2024 and an even marginally higher figure of 60,337 metric tons in 2025, thereby underscoring the inherent resilience and adaptability of the ginger sector in Mizoram as highlighted by **CEIC Data in 2025**.

In spite of the presence of these favorable natural conditions that ostensibly support ginger cultivation, the farmers engaged in ginger production within Mizoram have encountered a myriad of persistent agronomic challenges that have significantly impeded their agricultural endeavors. Among the most pressing issues plaguing these farmers is the rampant prevalence of various pests and diseases, with particular emphasis on the destructive soft rot caused by *Pythium* spp., in addition to the detrimental infestations of rhizome and shoot borers that have further complicated the agricultural landscape. These agronomic challenges reached a critical point in the year 2024, affecting a staggering number of over 6,500 farming families distributed across a total of 263 villages, thereby highlighting the widespread nature of the issue. In a proactive response to this alarming situation, the state government of Mizoram initiated a comprehensive campaign known as **"Operation Ginger-2024 Fehchhuah Runpui,"** which was an intensive effort aimed at assessing the full extent of the pest damage inflicted upon the ginger crops and providing the necessary technical support

and assistance to the farmers who were adversely affected, as reported by **UNI India in 2024.**

Furthermore, the issues of marketing and price realization emerged as critical concerns for ginger growers who were striving to navigate the complexities of the agricultural market. Despite the Mizoram government's efforts to enact agricultural marketing regulations as early as 1996, with subsequent amendments made in 2008, the enforcement of these regulations remained notably weak, leaving a significant number of farmers vulnerable to exploitation at the hands of unscrupulous middlemen who sought to profit from the farmers' labor. To effectively address this pressing issue, the Mizoram government introduced an initiative known as the **'Bana Kaih'** (Hand holding) program in the year 2025. Under the auspices of this scheme, the government undertook the direct procurement of ginger from farmers at a guaranteed minimum price of ₹50 per kilogram, facilitated through a robust network comprising 675 primary farmer societies and 54 secondary collection centers, which aimed to stabilize market prices and ensure that farmers received fair and just compensation for their produce.

Moreover, the government also recognized the imperative need for substantial value addition and infrastructure development aimed at enhancing the overall economic viability of ginger cultivation within the region. In light of this recognition, strategic plans were formulated to establish state-of-the-art facilities dedicated to the sorting, grading, drying, and processing of ginger into various value-added products, including but not limited to ginger paste, powder, and ale. These concerted efforts are anticipated to significantly enhance the shelf life, marketability, and overall appeal of Mizoram ginger, thereby expanding its reach and acceptance in both domestic and international markets.

In addition to these initiatives, research and development activities played an instrumental and pivotal role during this transformative period. Rigorous studies conducted by esteemed agricultural institutions were instrumental in the identification of high-yielding ginger genotypes, including varieties such as Bold Nadia, PGS 102, Bhaise, and Gurubathani, which have been empirically demonstrated to be particularly suitable for large-scale cultivation under the unique conditions prevalent in Mizoram. These identified varieties exhibited promising and favorable results in terms of

rhizome yield, inherent resistance to diseases, and overall economic returns that are of paramount importance to the farmers engaged in ginger cultivation, as documented in the **Environmental Conservation Journal in 2022**.

In conclusion, the decade spanning from 2015 to 2025 has undeniably marked a transformative and pivotal phase in the ginger sector of Mizoram, characterized by a series of both challenges and advancements. While the journey through this period has been fraught with setbacks related to pest infestations and marketing constraints, the implementation of proactive policy measures, the introduction of scientific interventions, and the growing awareness among farmers have collectively laid a robust groundwork for the establishment of a more sustainable and economically viable ginger industry within the state, thereby offering a glimpse of promise for the future of ginger production in Mizoram.

1.6 Biochemical compounds of ginger

Since ginger is classified as one of the most prominent species within the *Zingiberaceae* family and possesses noteworthy medicinal, nutritional, and ethnomedicinal properties, it has garnered extensive utilization as a spice, culinary component, and traditional remedy across various cultures worldwide **(Dhanik *et al*., 2017)**. In relation to its medicinal attributes, ginger exhibits a range of beneficial properties including anti-inflammatory, anti-carcinogenic, antipyretic, antioxidant, anti-diabetic, hepatoprotective, antibacterial, renal, and effects **(Kumar *et al*., 2017)**. Zingiber officinale Roscoe comprises of approximately194 distinct types of volatile oils, 85 varieties of gingerol, and 28 categories of diarylheptanoid compounds, which may facilitate the exploration of further applications of *Zingiber officinale* Roscoe.

The primary bioactive constituents found within the rhizome of ginger can be broadly classified into non-volatile and volatile phenolic compounds. It is widely recognized that the compounds belonging to these classifications, particularly the non-volatile pungent phenolics, are integral to the pharmacological effects associated with the ginger rhizome. The principal varieties of gingerols and their corresponding derivative compounds, known as gingerdiols, are classified as phenolic alkanones that are discerned within viable rhizomes. **(Magdy *et al*., 2020; Tchombé *et al*., 2012;**

Semwal *et al*., 2015). The predominant components of the volatile oil comprise mono- and sesquiterpenes, which include terpenes, camphene, curcumene, beta-phellandrene, cineole, terpineol, geranyl acetate, borneol, linalool, geraniol, limonene, and alpha-zingiberene (30–70%), in addition to beta-sesquiphellandrene **(Zadeh *et al.*, 2014).** The key aroma-producing compound, zingiberol, along with zingiberene, diarylheptanoids, vitamins, gingediol, and phytosterols have also been detected within the oleoresin **(Zadeh *et al*., 2014).**

1.7 Insect Fauna and Fungal pathogens associated with Ginger and their Control Strategies

Despite the considerable advantages associated with ginger, which is recognized for its dual role as both a culinary spice and a medicinal agent, its cultivation faces substantial challenges that arise from a multitude of biotic and abiotic factors that can severely hinder its growth and productivity. Among the biotic factors are a diverse array of pathogens that include various viruses, bacteria, fungi, and nematodes, all of which pose significant threats to ginger crops. On the other hand, the abiotic factors that influence ginger cultivation encompass adverse environmental conditions like sunburn, which can arise from excessive exposure to high light intensity, as well as lime-induced chlorosis, a condition that occurs when there is an over-application of lime in the soil, adversely affecting the health of ginger plants **(Paret *et al*., 2010)**. The primary diseases caused by fungal pathogens, as part of the biotic determinants, include various forms of rot such as rhizome rot, soft rot, Sclerotium rot, and a condition known as yellows disease, which can all significantly diminish crop yields. Specifically, *Fusarium oxysporum* f.sp. *zingiberi* E.E. Trujilo, which has been identified in multiple geographical locations including Hawaii, Australia, and Korea **(Trujilo, 1964; Stirling, 2004; Farr and Rossman, 2010)**, is regarded as the leading causal agent that contributes to the deterioration of ginger rhizomes, a condition that is often associated with a vascular wilt phenomenon commonly referred to in agricultural literature as "fusarium yellows." In addition to this, various species belonging to the genera *Pythium* and *Fusarium* have been linked to the occurrence of the aforementioned "soft rot," with notable examples including *Pythium myriotylum* Drechsler, *Fusarium oxysporum*, and *Fusarium solani* **(Wang *et***

***al*., 2003)**, alongside *Pythium aphanidermatum* (Edson) Fitzpatrick **(Kavita and Thomas, 2008)**, all of which have been reported in several regions such as Taiwan, Malaysia, the United States, Japan **(Farr and Rossman, 2010)**, India **(Ravindran and Nirmal Babu, 2005)**, and Australia **(Stirling, 2004)**.

Within the geographical confines of the Indian subcontinent, the cultivation of ginger, scientifically designated as *Zingiber officinale* Roscoe, stands as one of the most widely cultivated and commercially vital agricultural products, which is particularly significant for smallholder and marginal farmers who engage in its production across an array of states that include, but are not limited to, Himachal Pradesh, Assam, Kerala, Meghalaya, Karnataka, Sikkim, Orissa, and the northeastern region of the country, with a notable emphasis on Mizoram. Despite the economic importance and cultural significance of ginger cultivation, the overall yield and quality of this crop are profoundly hindered by the detrimental effects of various insect pests and fungal pathogens, which present substantial and formidable challenges that farmers must confront in numerous ginger-producing areas around the globe **(Kavitha *et al*., 2008; Stirling, 2009)**. A multitude of fungal genera, which includes, but is not limited to, *Fusarium, Pythium, Penicillium, Aspergillus, Mucor, Eurotium,* and *Rhizopus*, have been identified as the primary etiological agents that are responsible for the occurrence of rhizome rot in ginger, thereby compromising the integrity and viability of the crop **(Belay *et al*., 2012)**. Within this expansive spectrum of pathogenic species, *F. oxysporum* f.sp. *zingiberi* (Foz), along with *F. solani, F. equiseti, F. semitectum, P. aphanidermatum,* and *P. myriotylum*, have emerged as the most prevalent and impactful species that exert a negative influence on ginger rhizomes, consequently leading to a reduction in overall crop yield and quality **(Pegg, 1974; Levesque, 2004; Ramteke, 2011; Senapati, 2005; Moreira,2013)**.

Ginger, is unfortunately subject to a multitude of more than twenty distinct diseases that can be traced back to a variety of etiological agents including insects, fungi, bacteria, and viruses **(Dake, 1995**). Among the plethora of diseases that ginger can suffer from, the soft rot or rhizome rot stands out as the most catastrophic, exhibiting an alarming prevalence on a global scale particularly in regions where intensive ginger cultivation practices are employed. It has been conclusively

established through various studies that multiple species of the genus Pythium are implicated in the etiology of rhizome rot affecting ginger plants. The species known to contribute to this destructive disease include *Pythium aphanidermatum* (Edson) Fitz., *Pythium butleri* Subram, *Pythium deliense* Meurs, *Pythium myriotylum* Drechsler, *Pythium pleroticum*, and *Pythium ultimum* **(Sharma *et al*., 1995; Thomas, 1938; Haware 1974; Sahare, 1962; Dohroo,1987).** Moreover, it is worth noting that additional pathogenic factors, specifically various species of the genus *Fusarium*, such as *F. oxysporum* and *F. solani*, along with several unidentified *Fusarium* species, have also been identified as contributing to the yellowing disease that ginger plants may experience.

In addition to the aforementioned fungal pathogens, another crucial category of antagonistic agents that significantly impacts ginger production is composed of various insect pests. A comprehensive examination of the insect pests that adversely affect ginger cultivation has been extensively documented in the context of agricultural studies conducted in India. Among the most prominent insect pests that pose a serious threat to ginger plants are the rhizome scale, scientifically designated as *Aspidiella hartii* Ckll., the shoot borer known as *Conogethes punctiferalis* Guen., the white grub classified as *Holotrichia setticolis*, and the leaf roller referred to as *Udaspes folus*, all of which can potentially inflict extensive and severe damage to the host plant, particularly when conditions of substantial infestation prevail **(Devasahayam *et al*., 2005; Nair, 2013)**. These pests, through their feeding habits and reproductive strategies, can undermine the health and productivity of ginger crops, leading to significant economic losses for farmers and impacting overall agricultural sustainability in regions reliant on this vital spice.

Insect pests pose a significant and multifaceted threat to the cultivation of ginger (*Zingiber officinale*), which is not only a crop of immense economic importance but also one that is widely cultivated across various regions for its notable therapeutic properties and culinary applications that are deeply ingrained in numerous cultures. The presence and proliferation of these insect pests can lead to a substantial decline in both the quantity of the yield and the overall quality of ginger, which in turn can have far-reaching implications for the economic viability of ginger cultivation as well as the stability and integrity of the entire supply chain that relies on this valuable crop

(Chattopadhyay *et al.*, 2010). Among the insect pests that are recognized as particularly detrimental to the successful cultivation of ginger, some of the most harmful include shoot borers (*Conogethes punctiferalis*), rhizome flies (*Mimegralla coeruleifrons*), and aphids (*Aphis gossypii*), each of which exhibits a preference for targeting different anatomical structures of the ginger plant, ultimately leading to adverse effects on the overall health and vitality of the crop **(Kumar *et al.*, 2011).** In particular, it has been observed that shoot borers can cause extensive damage to the vegetative parts of the plant, frequently resulting in substantial reductions in yield, while rhizome flies pose a direct threat by compromising the integrity of the rhizomes, thereby leading to both decreased yields and a decline in quality that can affect marketability **(Sharma *et al.*, 2015)**. The adoption and implementation of effective pest management strategies, which should encompass a combination of both biological and chemical control measures, is absolutely essential to ensure the continued health and productivity of ginger crops, thereby safeguarding the interests of farmers and the agricultural economy at large **(Singh *et al.*, 2018)**. In addition, ongoing research focused on understanding the complex ecological interactions and life cycles of these insect pests is of paramount importance, as it is crucial for developing sustainable and integrated pest management strategies that are specifically designed to protect ginger crops from the detrimental effects posed by these harmful insect pests.

Most pathogens affecting ginger are predominantly found in soil, rendering it exceedingly challenging to mitigate exposure without commencing with highly sterile soil. Various plant extracts, including those from *Azadirachta indica*, *Agave americana*, *Cassia fistula*, *Eucalyptus terticornis* and *Vitex negundo*, have been employed in the management of diseases induced by *Fusarium* and *Pythium* in ginger, achieving varying levels of efficacy **(Sharma, 1998).** The limonoids derived from neem have been reported to exhibit effectiveness in the management of plant diseases of various types **(Dohroo, 1995).** The integration of cultural, biological, and chemical strategies for disease management alongside soil solarization has garnered considerable scholarly interest to date **(Davis, 1991; Indresenan, 1981; Ram *et al.*, 1998; Jayasekhar, 2000; Rajan, 2002).** The fungi belonging to the genus *Trichoderma* represent a vast assemblage of microorganisms that significantly

contribute to ecological balance. They employ a myriad of mechanisms to establish dominance across diverse ecological niches. Numerous species of *Trichoderma* have been observed to enhance plant vitality by promoting growth and safeguarding against fungal and bacterial pathogens **(Błaszczyk, 2014; Mudyiwa, 2016; Selvakumar, 2016)**. Notably, *Trichoderma harzianum* and *Trichoderma viride* are among the most frequently utilized species in the realm of biological plant protection, serving as bio-fungicides as well as agents for bio-remediation **(Rathore, 1992; Das et al., 2019; Asad, 2014).**

1.8 Molecular characterization of Insect pest and fungal pathogens

The detailed molecular characterization of fungal pathogens and insect pests is of utmost importance for the successful implementation of pest management strategies, precise disease diagnosis, and the formulation of sustainable agricultural practices that can withstand the challenges posed by these organisms. Historically, conventional methodologies for the identification of such pathogens have predominantly relied on morphological characteristics, which, despite their usefulness, often yield ambiguous results and can be exceedingly time-consuming, thereby hindering timely decision-making in agricultural contexts. The advent of polymerase chain reaction (PCR)-based techniques, particularly those that facilitate the amplification of the Internal Transcribed Spacer (ITS) region for fungal entities and the Cytochrome c Oxidase Subunit I (COI) gene for insect populations, has undeniably revolutionized the domain of molecular diagnostics, leading to significant advancements in phylogenetic studies as well. These innovative techniques enable researchers and practitioners to achieve a level of precision in identification that was previously unattainable, while simultaneously deepening our understanding of the genetic diversity exhibited by pathogens and pests on both local and global scales, which is crucial for effective management.

1.8.1 Molecular Characterization of Fungi Utilizing the ITS Region

The Internal Transcribed Spacer (ITS) region has emerged as the most prevalent molecular marker employed for the identification of fungal species, due to its remarkable degree of variability and its inherent capability to effectively distinguish

between species that reside within the same genus, which is an essential aspect of mycological research. This particular region is strategically located between the 18S and 28S ribosomal RNA (rRNA) genes, which are well-conserved across various fungal taxa, thus facilitating the design of primers that can amplify a broad spectrum of fungal species. The ITS region exhibits sufficient variability in its nucleotide sequences, rendering it particularly effective for species-level identification, especially when dealing with fungi that possess similar morphological characteristics— a challenge that many mycologists encounter in their research endeavors **(Lücking *et al*., 2018).**

The application of ITS sequencing presents numerous advantages over traditional morphological identification methods, which can often be labor-intensive, subjective, and fraught with challenges, particularly in cases involving fungi that exhibit similar or overlapping morphological traits. Moreover, the capability of the ITS region to facilitate identification from small or degraded samples, including environmental DNA, significantly enhances its applicability in biodiversity assessments and disease diagnostics, thereby making it an invaluable tool in contemporary mycology **(Voigt *et al*., 2012)**. The ability of this region to amplify fungal DNA, combined with its broad accessibility through databases such as GenBank, has established it as a standard reference point for molecular phylogenetic studies and has contributed to the ongoing development of comprehensive fungal taxonomy **(Schmidt *et al*., 2013)**. Furthermore, the ITS region is instrumental in tracing evolutionary relationships among diverse fungal species and plays a critical role in monitoring the dissemination of pathogenic species within both agricultural and natural ecosystems.

This particular molecular approach holds even greater significance in regions such as Mizoram, which is characterized by a high degree of fungal diversity attributable to the varied climatic conditions that favor the proliferation of multiple fungal species that adversely affect economically important crops such as ginger **(Banerjee *et al*., 2020).**

In the specific context of Mizoram, where various fungal pathogens including *Pythium* and *Fusarium* pose serious threats to ginger production, the utilization of

PCR-based ITS amplification techniques can significantly enhance the accuracy of pathogen detection. Empirical studies have demonstrated that these fungal pathogens can result in considerable rhizome rot, which is a primary contributor to crop losses and economic detriment **(Lal *et al.*, 2019)**. By employing advanced molecular techniques, the identification of fungal species can be performed swiftly, facilitating the implementation of targeted control measures that are essential for maintaining crop health and productivity. Moreover, this molecular approach is also pivotal in tracing the spread of disease-causing fungi across various regions, thereby allowing researchers and agricultural practitioners to correlate outbreaks with specific environmental factors or farming practices, thus promoting more informed and effective agricultural management strategies **(Morais *et al.*, 2021)**.

1.8.2 Molecular Characterization of Insect Pests Utilizing the COI Gene

The Cytochrome c Oxidase Subunit 1 (COI) gene has gained widespread recognition and acceptance in the scientific community as a highly effective tool for the identification of various insect species, primarily due to its highly conserved sequences that remain consistent across different species, while simultaneously exhibiting sufficient variability that allows for the distinction of individual species from one another, thereby establishing it as an exemplary molecular marker for taxonomic purposes. Situated within the mitochondrial genome, the COI gene plays a critical role in the electron transport chain; its relatively slow mutation rate facilitates reliable and consistent sequence-based comparisons across an array of insect taxa, as evidenced by the foundational work conducted by Hebert et al. in the year 2003. In addition to its inherent biological significance, COI sequences have been meticulously cataloged in comprehensive global databases such as the Barcode of Life, which provides an extensive reference library that researchers can utilize for the precise identification of insect species.

The application of COI-based DNA barcoding offers numerous advantages when integrated with traditional morphological identification methods, which can often be subjective and present significant challenges, particularly for cryptic species or life stages that may be difficult to differentiate based solely on morphological characteristics. The polymerase chain reaction (PCR) amplification of the COI gene

enables the precise and rapid identification of insect species extracted from a range of samples, including those that may be damaged or consist of immature specimens (Bik *et al*.,2012). This capability proves to be especially valuable within the field of applied entomology, particularly in the realm of pest management, where timely and accurate identification of insect species is of paramount importance. Moreover, the sequencing of COI not only aids in species identification but also supports phylogenetic studies that contribute to our understanding of evolutionary relationships among species and their distribution, both of which are critically important for the management of invasive species and the monitoring of biodiversity **(Hebert *et al*.,2003; Jadhav *et al*., 2020).**

The molecular identification of insect pests through the analysis of the COI gene further facilitates the exploration of pest population structure, their migration patterns, and the dynamics surrounding the spread of resistant pest strains. This understanding of pest dynamics is indispensable in the context of integrated pest management (IPM) strategies, which aim to control pest populations effectively. In the unique geographic context of Mizoram, where the topography and climatic conditions exhibit significant variability from lowland to highland regions, the identification of pest populations and the examination of their genetic variations across these diverse zones become imperative for the development of localized and effective pest management strategies **(Singh *et al*.,2017).**

1.9 Constructing Phylogeny: Perspectives on a Global and Local Scale

The integration of advanced molecular techniques for the characterization of both fungal pathogens and insect pests allows researchers to construct robust phylogenetic trees, which are essential for elucidating the evolutionary relationships that exist among these organisms. Comprehensive global studies that utilize Internal Transcribed Spacer (ITS) and COI gene sequences have yielded valuable insights into the phylogenetic relationships of key agricultural pests and pathogens, thereby linking them to their host plants, geographic dispersal, and various environmental factors that may influence their prevalence, as reported by **Schneider *et al*. in 2020**. By systematically comparing local populations in Mizoram with extensive global databases, researchers are afforded the opportunity to identify both endemic and

invasive species while also assessing their genetic diversity, thus enhancing our understanding of local and global ecological dynamics.

1.10 Challenges and Constraints in Mizoram

In the region of Mizoram, where agricultural practices are characterized by their diversity and often heavily rely on traditional knowledge systems, the employment of molecular tools in the characterization of pests and pathogens presents a unique opportunity to merge contemporary scientific approaches with local farming methodologies. This convergence enables researchers to formulate control measures that are specific to the region and to inform relevant policy decisions regarding pest management strategies, thus fostering a more sustainable approach to agricultural practices in the face of ongoing environmental changes and challenges.

The cultivation of ginger holds a paramount significance in the agricultural economy of Mizoram, a region located in the northeastern part of India, whereby this particular crop not only enhances the livelihoods of local farmers but also plays an essential role in ensuring food security for the communities dependent upon it. The unique agro-climatic conditions that characterize Mizoram, which include an abundance of rainfall, rich and fertile soil, and a climate that maintains moderate temperatures throughout the year, create an environment that is exceptionally conducive to the flourishing of ginger cultivation. The ginger produced in this state is especially esteemed for its elevated gingerol content, its pronounced pungency, and its distinctive aroma; these qualities have not only earned it recognition on a global scale but have also culminated in the granting of a Geographical Indication (GI) tag specifically for “Mizo Ginger,” thereby enhancing its prestige and marketability **(HS Lalduhsangi, 2023)**

Mizoram has established itself as one of the foremost states in India in terms of ginger production, making a considerable contribution to the overall national output of this essential agricultural commodity. The state's agricultural landscape primarily revolves around both subsistence and commercial farming practices related to ginger, with the districts of Aizawl, Champhai, and Kolasib emerging as the principal hubs for ginger production within the state. In recent years, there has been a noticeable upsurge

in the cultivation of ginger, a trend that can largely be attributed to the escalating demand for this spice in both domestic and international markets, thereby providing farmers with lucrative opportunities. Traditionally, farmers have relied on age-old agricultural techniques, such as Jhum cultivation, which is a form of shifting cultivation prevalent in the region, although there are ongoing initiatives aimed at modernizing these farming practices to increase efficiency and yield.

The geographical features and landscape of Mizoram are endowed with an exceptionally favorable climate that facilitates the cultivation of a wide array of vegetables, as well as an extensive assortment of spices, which notably includes varieties such as chili, ginger, turmeric, tejpat, cinnamon, coriander, fennel, ajwain, dill, fenugreek, and garlic. Among the numerous spices that are cultivated within this region, ginger (*Zingiber officinale*), which belongs to the family *Zingiberacea*e, emerges as a predominant cash crop that is particularly cultivated in jhum lands, thereby significantly contributing to the livelihood and financial stability of numerous ginger farmers throughout the state. The implementation of geographic information systems (GIS) has proven to be an invaluable tool in the assessment of land suitability for ginger cultivation, leading to the identification of four Indian states—namely Mizoram, West Bengal, Orissa, and Kerala—as the most favorable locations for this particular agricultural endeavor **(Parthasarathy *et al*., 2008)**. The entirety of Mizoram has been recognized for its exceptional suitability for the cultivation of ginger **(Utpala *et al*., 2006)**, and it has gained notoriety for producing ginger that is characterized by its notably low fiber content. In addition to its low fiber, ginger cultivated in Mizoram possesses several distinctive attributes, including a rich aroma, a pronounced pungency, a high gingerol content, and an unmistakable taste that sets it apart from ginger grown in other regions. These remarkable and unique characteristics of the indigenous ginger varieties found in Mizoram can be attributed to the specific agro-climatic conditions that prevail in this region, which create an ideal environment for cultivation. Studies have indicated that ginger cultivated at higher elevations yields a greater concentration of oleoresin and achieves higher oil recovery rates **(Ngachan and Deka, 2008)**. When comparing the ginger produced in Mizoram to that from other regions of India, it has been documented that Mizoram ginger exhibits significantly

higher oil recovery rates (ranging from 1.6 to 2.5 percent as opposed to the 1.5 to 2.0 percent found elsewhere) and oleoresin content (between 5.9 and 8.56 percent in contrast to the 5 to 8 percent typical of other areas) **(Spice Board, 2007)**.

In the region of Mizoram, India the cultivation of ginger has been recognized as a crop of significant economic importance, largely attributable to its elevated market value, which has rendered it a vital component of the local agricultural economy. Nevertheless, it has been observed that in recent years, the extensive production of this high-value crop within the state has encountered substantial limitations, primarily due to various disease-related challenges that arise from the infestation of insect pests and the prevalence of pathogenic fungi. Among the agricultural community in Mizoram, there exists a notable deficiency in comprehensive knowledge regarding the specific causative agents responsible for the diseases that afflict ginger crops, which can significantly hinder effective disease management. As a direct consequence of this limited understanding, farmers have resorted to the indiscriminate application of highly toxic chemicals, including insecticides and herbicides, in an attempt to mitigate the impact of the pathogens that cause ginger diseases, often without adhering to recommended dosages or receiving proper guidance. This hazardous practice poses a substantial risk to both the health and overall quality of the ginger crop, as well as the soil health, leading to a marked reduction in rhizome yield, and ultimately results in a failure to effectively combat the ongoing disease issues. In light of these pressing concerns, it becomes critically important for farmers to enhance their awareness and understanding of the various pathogens that affect ginger crops, particularly those related to insect pests and fungal agents, as this knowledge is essential for the formulation of improved disease management strategies and consequently, for the augmentation of crop yield. However, it is noteworthy that there exists a significant paucity of information pertaining to the specific causative agents of ginger diseases within Mizoram, with the exception of a few reports detailing soft-rot pathogenic fungi that have been identified in the Aizawl district **(Rosangkima, 2018)**.

The process of cultivating ginger, is profoundly affected by various insect pests and fungal pathogens, which present considerable obstacles to ginger production in a multitude of regions around the world, including the northeastern Indian state of

Mizoram. In spite of the robust production capabilities of ginger within this state, the extensive cultivation efforts are hindered by a plethora of disease-related challenges that are directly linked to both the aforementioned insect pests and fungal organisms. Due to the farmers' insufficient comprehension of the specific etiological agents that are responsible for these debilitating diseases, there has been a widespread and indiscriminate use of harmful chemicals, such as insecticides and herbicides, employed in an attempt to eradicate the pathogens, often applied without proper regard for recommended dosages or sufficient expert advice. This hazardous practice carries the potential to negatively affect not only the health of the crops in question and their overall quality, but it also poses risks to the soil health, ultimately resulting in reduced yields of the rhizomes and failing to effectively address the disease problems at hand.

It is of utmost importance that farmers receive comprehensive education regarding the various disease-causing pathogens that impact ginger crops, with a particular focus on the insect pests and fungi, in order to improve their disease management strategies and, consequently, to boost crop productivity significantly. In response to these pressing challenges, the present research initiative has been proposed to conduct both morphological and molecular characterization of the insect pests and fungal pathogens that are implicated in the diseases affecting ginger crops in the Champhai and Serchhip districts of Mizoram, India. To achieve this, specimens of the relevant pests, as well as plant material exhibiting signs of disease, will be systematically collected from a variety of ginger farms located within the defined geographic areas. The scope of the proposed study encompasses a multitude of parameters, which include the morphological identification of the pathogens involved, the polymerase chain reaction (PCR) amplification of select genes, and the rigorous testing of pathogenicity. The anticipated outcomes of this investigation are expected to make a substantial contribution to the development of effective disease management strategies, which will, in turn, enhance the quality of the soil, improve environmental conditions, and ultimately lead to an increase in the yields of ginger crops.

The process of identifying significant potential diseases that could adversely affect ginger cultivation, along with their respective pathogenic organisms and the varying degrees of severity associated with these diseases, has profound implications

for the development of effective and sustainable disease management strategies tailored to this crop. However, the establishment of appropriate protocols for the post-harvest management of ginger rhizomes within the ginger cultivation regions of Mizoram has emerged as a particularly formidable challenge; this difficulty is largely due to the insufficient understanding and knowledge regarding the principal etiological agents that are intricately linked to the occurrence of soft rot. The primary objective of this research endeavor was to conduct an exhaustive and comprehensive survey aimed at identifying the various etiological agents and fungal species that are responsible for causing soft rot in ginger rhizomes specifically within the primary production region located in Mizoram, India. In addition to this, the investigation sought to pinpoint and catalog other potential diseases that might pose a significant threat to ginger production, along with a detailed assessment of their respective levels of intensity and prevalence across various districts in Mizoram, India.

Given the considerable economic significance of ginger for the agricultural community in Mizoram, as well as the severity of damage inflicted by both insect pests and soil-borne pathogens, coupled with the conspicuous absence of safe and effective management strategies within these regions, the present study is deemed both timely and necessary. The results and findings derived from this study are anticipated to offer an accurate and up-to-date regional assessment regarding the occurrences of insect pests and fungal pathogens that affect ginger, which will prove invaluable in the formulation and design of sustainable, eco-friendly, and effective pest and disease management strategies tailored to the specific needs of these regions. By establishing a solid foundation of knowledge on the causative agents of plant disease among farmers, this initiative will serve as an effective tool for the control and reduction of the application of toxic chemicals, which have often been utilized without proper dosage and guidance. The replacement of such hazardous practices with the adoption of eco-friendly alternatives, such as *Trichoderma* isolates, in conjunction with improved cultural practices, is expected to significantly enhance the quality of soil and environmental conditions, ultimately leading to an increase in crop yield and overall agricultural productivity.

Therefore, in the context of the proposed research studies, it is suggested that a comprehensive investigation be undertaken to conduct both morphological and molecular characterizations of the causative agents associated with common ginger diseases, with a particular focus on identifying insect pests and various fungal species from a range of ginger farms located within the different districts of Mizoram. Additionally, it is planned that in vitro pathogenicity tests will be executed in order to meticulously assess and quantify the extent of damage that is inflicted by these isolated fungal pathogens on ginger crops, thereby providing valuable insights that could facilitate better management practices. The examination of insect and fungal pathogens that afflict ginger crops in the state of Mizoram is of paramount importance, particularly when considering the substantial economic and cultural implications that this crop holds within the context of the region. Ginger stands as one of the primary cash crops for a significant number of farmers in Mizoram, where the prevailing agro-climatic conditions—which are notably defined by high levels of rainfall, elevated humidity, and soils rich in organic matter—foster an environment that is not only conducive to the successful cultivation of ginger but also facilitates the rapid proliferation of various pests and pathogens that pose a threat to the crop. In recent years, there have been alarming occurrences of severe outbreaks of soft rot instigated by *Pythium* species and Fusarium wilt, which have collectively resulted in substantial yield losses that jeopardize the livelihoods of farmers and pose a serious risk to the overall production of spices within the region.

Moreover, insect pests including the notorious shoot borer, scientifically referred to as *Conogethes punctiferalis*, alongside the rhizome fly known as *Mimegralla coeruleifrons*, have been frequently documented as causing considerable damage both in the cultivated fields and during the critical post-harvest storage period, thereby exacerbating the challenges faced by ginger producers. Despite the significant impact these pests and diseases have on the ginger industry, there remains a notable dearth of region-specific research aimed at comprehensively understanding the biological characteristics, seasonal patterns of occurrence, and effective management strategies for these threats within the unique hilly landscape that defines Mizoram.

In light of these pressing challenges, there is an urgent need for thorough and comprehensive studies that will facilitate the identification of the diversity, distribution, and pathogenic potential of both insect and fungal threats that confront ginger cultivation in this region. Such investigations will play a pivotal role in the formulation of sustainable and environmentally friendly pest and disease management strategies that are specifically tailored to align with the local agrarian context, ultimately bolstering crop resilience, enhancing food security, and improving rural incomes for the farming communities in Mizoram.

1.11: Objectives

The following objectives are set forth to be carried out during the study:-

1. Collection and morphological characterization of insect pest of ginger.
2. Isolation and morphological characterization of fungal pathogen of ginger
3. Molecular identification and phylogeny of insect pests and isolated fungi from ginger.

CHAPTER – 2

Review of Literature

A multitude of countries across the globe, including but not limited to the likes of China, Japan, India, Nigeria, Taiwan, Sri Lanka, Fiji, Hawaii, Australia, and Korea, engage diligently in the agricultural endeavor of ginger cultivation, an herb that is classified as tropical and is renowned for its wide-ranging applications that span culinary, medicinal, and therapeutic domains, thereby illustrating its remarkable versatility and significance across diverse cultural landscapes. Among these nations, India occupies a particularly notable and esteemed position as a leading producer of ginger, a status attributed to the fact that the cultivation of this particular crop necessitates substantial land resources, and it is further distinguished by its ability to achieve the highest yield levels when compared to other ginger-producing countries **(Meenu, 2017; Anisha, 2017)**. It is particularly significant to acknowledge that nearly 65% of India's total ginger production is derived from its key ginger-producing states, which include Gujarat, Meghalaya, Arunachal Pradesh, Karnataka, Orissa, Kerala, and Assam, thereby underscoring the geographical concentration and regional specialization of this vital agricultural practice **(Jayashree, 2007)**. Moreover, ginger is not only widely acknowledged but is also extensively employed as both a seasoning agent and a culinary component; it simultaneously serves as an integral element of traditional medicinal practices in a myriad of cultures across the globe, a reality that can be attributed to its classification as one of the most important species within the *Zingiberaceae* family, which is endowed with essential therapeutic, nutritional, and indigenous medicinal properties **(Kumara M *et al*., 2017)**.

Ginger, scientifically designated as *Zingiber officinale*, is extensively acknowledged as one of the most significant and widely utilized spices across the globe, serving not only as a culinary delight but also as a vital component in various traditional medicine systems. This remarkable perennial plant exhibits a distinctive creeping growth pattern, which is characterized by its elongated, lanceolate leaves, striking yellow-green inflorescences, and a stout, tuberous rhizome that serves multiple purposes within the plant’s lifecycle. The rhizome, in particular, plays an essential role as a storage organ, and it is particularly notable for its unique and pungent flavor profile that enhances a plethora of culinary dishes and beverages. Throughout

history, ginger has been esteemed as one of the most versatile medicinal plants, possessing a vast array of biological activities that contribute to its status in both popular and traditional medicine. Since ancient times, ginger has been employed in the realms of Ayurvedic and Traditional Chinese Medicine to address a multitude of health concerns, including but not limited to cardiovascular disorders, irregularities in menstrual cycles, foodborne diseases, osteoarthritis, epileptic conditions, nausea, inflammatory diseases, respiratory issues, motion sickness, dysmenorrhea, and various forms of cancer, along with countless other medical conditions that impact human well-being. In addition to its medicinal uses, ginger has been recognized for its significant antimicrobial and antioxidant properties, which further enhance its value in health-related applications. The extensive medicinal attributes of ginger, combined with the wealth of knowledge that has been accumulated over centuries regarding its therapeutic applications, provide a robust foundation for contemporary researchers aiming to conduct further investigations that seek to protect and improve human health in the face of a myriad of diseases. Such research endeavors are particularly crucial in an era marked by the emergence of antibiotic-resistant pathogens and the increasing prevalence of chronic diseases, thereby highlighting the importance of revisiting traditional herbal remedies like ginger. The ongoing exploration of ginger's biochemical mechanisms and its potential therapeutic effects could pave the way for new interventions and preventive strategies in medicine. Consequently, the rich history and modern relevance of ginger underscore its critical role in both culinary and medicinal contexts, thereby warranting continued scholarly attention and investigation. **(Bhatt *et al*., 2013).**

2.1 Taxonomic position

The genus Zingiber belongs to the family *Zingiberaceae* under the order *Zingiberales* and the tribe *Zingibereae* **(Holtum, 1950)**. *Zingiberaceae* includes three other tribes: *Hedychieae, Alpinieae* and *Globbeae* **(Petersen, 1889; Burtt and Smith, 1972).** The tribe *Zingibereae* has seven other genera: *Boesenbergia, Camptandra, Roscoea, Kaempferia, Amomum, Hedychium* and *Curcuma* **(Kress *et al*., 2002)**. Jatoi et al. (2006) using rice microsatellite markers assessed the genetic variability among three genera of the family *Zingiberaceae* : *Zingiber, Alpinia* and *Curcuma* and found

the origin of the genera diverse covering eight Asian countries. *Zingiber* contains 150 species and four sections distributed throughout tropical Asia, China, Japan and tropical Australia besides the subspecies (varieties): *Z. officinale* var. *rubra* and *Z.officinale* var. *rubrum* **(Muda et al., 2004)**. *Z. officinale* is included in the section II, *Lampuzium* **(Sabu, 2003; Larsen and Larsen, 2006)**. The family *Zingiberaceae* is represented by about 46 genera, distributed through the tropics and subtropics. The type genus of this family is Zingiber. The plant is an aromatic herb and its taxonomic position is as follows:

Domain : Eukaryota
Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Monocotyledonae
Order : Zingiberales
Family : Zingiberaceae
Genus : *Zingiber*
Species : *officinale*

Ginger, a widely cultivated crop, is known to be afflicted by an astonishing array of over twenty distinct diseases that have their origins in various biological factors including insects, fungi, bacteria, and viruses **(Dake, 1995)**. Among the plethora of diseases that can impact ginger, the soft rot or rhizome rot stands out as the most devastating and destructive, demonstrating a widespread prevalence across the globe in regions where ginger is cultivated at an intensive scale. Numerous studies have reported that several species belonging to the genus *Pythium* are implicated in the onset and progression of rhizome rot in ginger crops. These species include, but are not limited to, *Pythium aphanidermatum* (Edson) Fitz., P. *Butleri* Subram, *P. Deliense* Meurs, *P. Myriotylum* Drechsler, *P. pleroticum* and *P. ultimum* **(Sahare *et al*., 1962, Dohroo *et al*., 1987, Nair *et al*., 2013, Sharma *et al*., 1998).** In addition to these *Pythium* species, the fungal pathogens *Fusarium oxysporum, F. solani*, and several unidentified species within the *Fusarium* genus have also been reported to be associated with the yellowing disease that affects ginger plants. Under conditions characterized by warmth and high humidity, the soft rot disease of ginger can escalate

to alarming levels, leading to significant economic losses for farmers and producers alike **(Dohroo *et al.* 1995)**. This disease is not confined to a specific region but is instead prevalent in almost all areas around the world where ginger is cultivated. The initial documentation of this disease can be traced back to the year 1907 when it was first recorded in Surat, Gujrat, India **(Butler, 1907).** Furthermore, it is important to highlight that another significant category of disease-causing agents includes various insect pests that can contribute to the deterioration of ginger crops.

The Internal Transcribed Spacer (ITS) region, which encompasses the ITS1 forward primer and the ITS4 reverse primer, is frequently employed as a molecular marker gene for the identification of fungi due to its substantial variability among distinct fungal species. The marker region amplifies the highly variable ITS1 and ITS2 sequences surrounding the 5.8S-coding sequence and situated between the small subunit-coding sequence (SSU) and the large subunit-coding sequence (LSU) of the ribosomal operon **(White *et al.,* 1990).** DNA barcoding has been proposed as a uniform molecular methodology for the identification of insect species as well as fungal species across all developmental stages **(Senapati and Ghose, 2005).** In order to accurately determine and establish the identity of the pathogen using advanced molecular methodologies, an innovated booster polymerase chain reaction (PCR) technique specifically designed for the reliable detection of fungal pathogens employing a specialized primer that was derived from the ribosomal DNA internal transcribed spacer 1 (rDNA ITS1) region in combination with the universal primer ITS2 (**Wang *et al*., 2003)**.

A multitude of distinct species belonging to the genus *Pythium* and *Fusarium,* have been thoroughly documented and recognized as significant causative agents responsible for the manifestation of soft rot disease in a wide array of agricultural settings across various regions of the globe. These *Pythium* species are classified as fungal-like microorganisms that are organized within the *Pythiaceae* family, which itself is situated within the *Peronosporales* order of the *Oomycota* phylum, thus categorizing these organisms as members of the broader kingdom *Stramenopila* **(Webster and Weber, 2007).**

This sophisticated method was successfully implemented for the identification of a variety of fungal pathjogens within naturally infected ginger rhizomes,

demonstrating its efficacy, although it unfortunately yielded no positive results when DNA was extracted from ginger rhizomes obtained from agricultural fields that were devoid of the target fungal pathogen. Furthermore, a straightforward and effective methodology has been thoroughly elucidated for the cultivation of oospores of *P. myriotylum*, which is known to induce soft rot disease specifically in ginger crops, thereby contributing to the understanding of the life cycle and pathogenicity of this organism in agricultural contexts (**Yella *et al*., 2006)**.

The primary etiological factors that contribute to the degeneration and subsequent decline of ginger rhizomes predominantly include the fungal pathogen *Fusarium oxysporum* f.sp. zingiberi E.E. Trujilo, which has been extensively recorded in various geographic locales such as Hawaii, Australia, and Korea, as indicated by the scholarly works of **Trujilo in 1964, Stirling in 2004, and Farr and Rossman in 2010**; this specific pathogen is also closely associated with vascular wilt conditions that are commonly referred to under the collective nomenclature of "." In addition *fusarium yellows* to this, a multitude of *Pythium* species play a pivotal role in the onset of a condition colloquially known as "soft rot," with particular emphasis on *Pythium myriotylum* Drechsler, as documented by **Wang *et al*. in 2003**, and *Pythium aphanidermatum* (Edson) Fitzpatrick, which has been studied by **Kavita and Thomas in 2008**; both of these organisms have been observed in an array of countries, encompassing Taiwan, Malaysia, the United States of America, Japan as noted by **Farr and Rossman in 2010**, as well as India according to the research of **Ravindran and Nirmal Babu in 200**5, and Australia, as highlighted by **Stirling in 2004**.

Another critical factor contributing to the decomposition of ginger rhizomes is the fungal species *Sclerotium rolfsii* Saccardo, which instigates a pathological condition commonly referred to as "cotton rot" in ginger crops, as revealed in the findings presented by **Stirling in 200**4; this particular pathogen has been documented across diverse geographic regions including Australia, the United States, South Africa, and Venezuela, as corroborated by **Farr and Rossman in 2010**. Moreover, the bacterium *Dickeya chrysanthemi* **(Brenner et al., 2005)** Samson et al., emerges as the preeminent causative agent of "soft rot" specifically within the Australian context **(Stirling, 2002; 2004)**, and this pathogen has also been recorded in Brazil **(Malavolta Jr. and Almeida, 1998)**.

Lastly, the bacterium *Enterobacter cloacae* (Jordan) Hormaeche and Edwards has been identified as the causative agent of a decay phenomenon described as "water-soaked" or "wet rot," this particular bacterial pathogen has been documented in ginger cultivation settings situated within Hawaii, illustrating the widespread impact of these pathogens on ginger production and health **(Nishijima *et al*., 2004)**.

The affliction commonly referred to as yellow disease represents a significant and pressing concern for the cultivation of ginger, particularly because its prevalence has markedly increased in regions characterized by warm and humid climatic conditions, which are conducive to the spread of such plant-pathogenic maladies. The initial documentation detailing the characteristics and impact of this disease was provided by the researcher **Simmonds in the year 1955**, specifically with reference to its occurrence in Queensland, Australia. The etiology of yellow disease can be attributed to the fungal organism known as *Fusarium oxysporum* Schlechtend ex Fr. f.sp. zingiberi Truzillo **(Yang et al., 1988)**. In addition to the primary pathogen, there have also been various other species within the *Fusarium* genus, including *F. solani* (Mart.) Sacc. and *F. equiseti* (Corda) Sacc., along with several unidentified *Fusarium* species, which have also been documented as having associations with the rhizomes of ginger plants. A study conducted by **Sharma and Dohroo in 1990** specifically identified *F. oxysporum* as the predominant pathogen responsible for the manifestation of yellow disease in the agricultural context of Himachal Pradesh. Furthermore, the second most frequently isolated fungal species contributing to this disease was identified as *F. solani*, **(Dohroo, 1987; Chauhan and Patel, 1990)** however, it is noteworthy that other species, such as *F. moniliforme* Sheld., *F. graminearum* Achwabe, and *F. equiseti*, have also been reported to be associated with the pathologically affected ginger plants, as indicated by the findings of **Sharma and Dohroo in 1980**, **Bhardwaj *et al*. in 1988b**, and the aforementioned **Dohroo in 1987**. Importantly, research conducted by **Dohroo and Sharma in 1992**b revealed that isolates of *F. oxysporum* f. sp. zingiberi exhibit variability in terms of their aggressiveness, highlighting the complexity of disease dynamics and the potential for differential impacts on ginger crops depending on the specific isolate involved.

A multitude of fungal species has been meticulously documented as significant contributors to the most substantial production losses encountered in agricultural

practices, particularly when considered alongside various other microbial pathogens that manifest at different developmental stages under natural environmental conditions **(Robert et al., 2017; Trigiano and Ownley, 2017).** In particular, the highly destructive and remarkably adaptable pathogens belonging to the *Aspergillus* genus, as well as those from the *Penicillium* genus, are frequently identified as common pathogens and represent the most prevalent fungal species found in both the cultivation and storage conditions of ginger **(Meenu and Kaushal, 2017)**. Numerous scientific investigations have consistently demonstrated that the diseases afflicting ginger plants are often closely associated with the presence of *Pythium* species, as well as *Fusarium oxysporum* and *Pratylenchus coffeae*, thereby highlighting the complex interplay of pathogens affecting this crop **(Rahman et al., 2009)**. Furthermore, there exists documented evidence of the emergence of ginger bacterial wilt disease, which is caused by the pathogen *Ralstonia solanacearum*. **(Hunduma et al., 2016)**. According to the findings presented by **Rahman et al. (2009)**, the prevalence of rhizome diseases has adversely affected ginger cultivation across numerous states in India over the past few years, leading to a significant decline in rhizome yield; thus, the issues of wilt and soft rot affecting ginger rhizomes have emerged as major limiting factors that hinder the successful cultivation of this economically important crop.

Phyllosticta leaf spot disease has been gaining prominence across numerous states owing to the significant leaf decay and blight it induces. The initial documentation of this disease was made in the Godavari district of Andhra Pradesh and the Malabar region of Kerala, which was formerly known as Madras state **(Ramakrishnan, 1942)**. The etiology of Phyllosticta leaf spot is attributed to the pathogen *Phyllosticta zingiberi* T.S. Ramakr.

Ginger represents a significant cash crop that is cultivated extensively throughout the region of Mizoram, where farmers predominantly engage in organic practices along with relying on rainfed irrigation systems for its growth. Nonetheless, the productivity of ginger within this state is frequently compromised by a multitude of diseases, with fungal infections being the most pronounced and detrimental among them. These infections, which are primarily caused by various fungal pathogens, not only lead to a marked decrease in yield but also pose a substantial threat to the long-term viability and sustainability of ginger farming practices within the region. Over

the years, a plethora of research studies have been undertaken with the aim of identifying and characterizing the principal fungal pathogens that adversely affect ginger crops in Mizoram.

The most devastating fungal disease affecting ginger, as documented in various reports from Mizoram, is soft rot, which is predominantly instigated by the pathogens *Pythium aphanidermatum* and *Pythium myriotylum*. Although the *Pythium* species are scientifically classified as oomycetes, they are frequently categorized alongside traditional fungal pathogens due to their analogous pathogenic characteristics and behavior. A comprehensive survey conducted by **Lallianzuala *et al*. (2016**) focused on the incidence of soft rot in the Aizawl and Lunglei districts, revealing a significant correlation between the severity of the disease and the presence of poorly drained soils combined with elevated humidity levels in the environment. The manifestation of soft rot results in the development of water-soaked lesions on the rhizomes of ginger plants, which ultimately leads to significant decay and the production of unpleasant odors. This particular disease demonstrates its highest prevalence during the monsoon season, a time when soil moisture levels are markedly elevated.

In addition to the afflictions caused by soft rot, another major fungal disease that has been reported in Mizoram is Fusarium yellows, which is instigated by the *pathogen Fusarium oxysporum* f. sp. zingiberi. As outlined in the research conducted by **Lalramchuanga *et al*. (2018)**, this disease is characterized by the yellowing of leaves, stunted growth patterns, and a gradual wilting of the plant, which is often misinterpreted as a sign of nutrient deficiency among farmers and agricultural practitioners. This specific pathogen is capable of surviving in the soil as well as in infected plant debris, which complicates effective management strategies. Moreover, their study underscored the potential effectiveness of biocontrol agents such as *Trichoderma harzianum*, advocating for their use as a viable alternative to conventional chemical fungicides.

Furthermore, leaf spot diseases, particularly those induced by the pathogens *Phyllosticta zingiberi* and various species of Alternaria, have also been comprehensively documented in the state of Mizoram. A notable study conducted by **Zothanmawia and Vanlalruata (2019)** observed a pervasive presence of leaf spot symptoms in the Champhai and Serchhip districts, especially within fields that were

densely planted with ginger. The infections caused by these pathogens lead to the formation of necrotic lesions on the foliage, which tend to coalesce, subsequently resulting in premature defoliation and, as a consequence, negatively impacting the overall development of the rhizomes.

Emerging fungal pathogens, such as *Colletotrichum gloeosporioides*, have also been isolated from ginger plants exhibiting symptomatic signs in the region of Mizoram. **Lalrinzuali *et al*. (2021)** documented the occurrence of anthracnose-like symptoms, which are characterized by dark, sunken lesions appearing on both the leaves and pseudostems of the plants, across multiple organic ginger farms. Their molecular characterization efforts ultimately confirmed the pathogen as *C. gloeosporioides*, which had previously been underreported in this particular geographic region.

Despite the formidable threat that fungal pathogens present to ginger cultivation, it is noteworthy that disease management strategies in Mizoram continue to be predominantly preventive and rooted in traditional practices. Recent investigative work carried out by **Lalthazuali *et al*. (2022)** emphasizes the urgent necessity for the development of integrated disease management strategies, which should ideally combine the utilization of resistant cultivars, the practice of soil solarization, the implementation of biological control measures, and the adoption of crop rotation techniques. However, it is important to recognize that scientific evaluations of indigenous agricultural practices remain strikingly limited in scope and depth.

Fungal diseases exert a profound influence on the global cultivation of ginger, scientifically known as Zingiber officinale Rosc., particularly within tropical locales such as India, where environmental conditions are conducive to the proliferation of various pathogenic fungi. The prompt and accurate identification of these fungal pathogens is of paramount importance in the realm of effective disease management strategies aimed at mitigating the adverse effects these pathogens have on ginger crops. Over the years, a multifaceted approach has been employed to identify fungi associated with diseases affecting ginger, utilizing a blend of morphological characteristics, cultural attributes, and advanced molecular techniques to achieve a comprehensive understanding of the pathogens involved.

Historically, the identification of fungi has predominantly depended on morphological and cultural traits, which encompass a range of characteristics including the visual appearance of fungal colonies, the morphology of spores, and the structural features of hyphae when cultivated on selective growth media, such as Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA). For instance, the pathogen *Fusarium oxysporum* f. sp. zingiberi, responsible for causing Fusarium yellows in ginger, can be recognized by its distinctive sickle-shaped macroconidia and microconidia that are produced in characteristic false heads, as evidenced by the work of **Meena *et al.* (2013)**. In a similar vein, *Pythium aphanidermatum*, which is a significant contributor to the phenomenon of soft rot in ginger plants, is identified through the examination of its coenocytic hyphae and the presence of spherical sporangia, as documented by **Lalramliana *et al*. (2016).**

Nevertheless, these traditional methodologies are often plagued by a lack of precision, particularly when it comes to distinguishing between species that exhibit morphological similarities or when dealing with cryptic fungal species that may not be readily identifiable through conventional means. As a result, there has been a marked increase in the adoption of molecular techniques that serve to complement and enhance the traditional identification methods. The polymerase chain reaction (PCR) has emerged as the most prevalent molecular technique, specifically targeting distinct genomic regions for identification purposes. Among these regions, the internal transcribed spacer (ITS) region of ribosomal DNA has gained widespread acceptance as a universal barcode for identifying various fungal species, as highlighted in the seminal work of **White *et al*. (1990)**. For example, **Singh *et al*. (2015)** successfully utilized ITS1 and ITS4 primers to amplify the ITS region for the identification of *Colletotrichum gloeosporioides* and various Fusarium species present in ginger rhizomes afflicted by disease in the Northeastern region of India.

In the state of Mizoram, the study conducted by **Lalrinzuali *et al.* (2021)** employed molecular techniques to accurately identify *Colletotrichum gloeosporioides* from ginger exhibiting symptoms consistent with anthracnose. The amplification of the ITS region was followed by sequencing and subsequent comparisons with data available in the NCBI GenBank database using BLAST analysis, which ultimately confirmed the species-level identity of the fungus with a remarkable similarity of over

99%. This particular research endeavor underscored the critical role that molecular diagnostics play in the early detection of emerging pathogens that threaten ginger cultivation.

Moreover, methodologies such as Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers have been incorporated into some investigations to evaluate the genetic variability present among various fungal isolates. While these techniques are not as commonly utilized in routine diagnostic practices, they nonetheless provide invaluable insights into the epidemiological dynamics and population structure of pathogenic fungi, as noted by **Gupta *et al*. (2017).**

Furthermore, recent advancements in the field have introduced the application of quantitative real-time PCR (qPCR), which facilitates the early detection and quantification of specific fungal DNA within plant tissues and soil samples, thereby enabling timely interventions to mitigate disease progression. Although the application of this technique remains somewhat limited in the context of ginger cultivation in India, it undeniably holds substantial promise for enhancing field-level diagnostics in the future.

Despite the strides made in molecular identification techniques, certain limitations persist that must be acknowledged. For instance, relying solely on ITS sequences may not provide sufficient resolution for species-level identification in some genera, particularly with respect to Fusarium and Colletotrichum. Consequently, experts have recommended the adoption of multilocus sequencing approaches, which involve the examination of additional genes such as β-tubulin, TEF1-α (translation elongation factor), and GAPDH to achieve more accurate species identification, as suggested by Crous et al. (2006).

While morphological and cultural methods continue to serve as foundational elements in the identification of fungi affecting ginger crops, the integration of molecular approaches—especially those centered on ITS-based sequencing—has markedly improved both the accuracy and efficiency of identification processes. The synergistic application of these diverse methodologies is essential for the effective diagnosis and management of ginger diseases, particularly in regions such as Mizoram, where the agricultural economy is heavily reliant on this vital crop.

Insect pests undoubtedly constitute a considerable and formidable obstacle to the effective cultivation and agricultural productivity of ginger **(Awal *et al*., in 2003; Firake *et al*., 2015)**. Furthermore, beyond the immediate and direct physical harm that these insect pests impose on the ginger plants, it is essential to emphasize that certain insect species also contribute to the dissemination of a variety of diseases, which can exacerbate and complicate the overall health status of the crop in question. Extensive research has successfully identified approximately twenty distinct species of insect pests that are recognized to impact ginger crops throughout the various developmental and growth stages of the plant in the Indian subcontinent **(Devashayam and Koya, 2005)**. Among the myriad of these identified pest species, several stand out as particularly significant, including the white grub, scientifically classified as *Holotrichia* spp., a type of scale insect referred to as *Aspidiella hartii*, the rhizome fly known as *Mimegralla coeruleifrons*, and the shoot borer, which is scientifically designated as *Dichocrocis punctiferalis*; these species have been subject to comprehensive examination by Jacob in his pivotal research conducted in 1980, which thoroughly analyzed their adverse effects on ginger cultivation **(Jacob, 1980)**.

The distinctive and unique climatic conditions that prevail in the northeastern part of India give rise to a complex of pests that manifests considerable variances and discrepancies when compared to those encountered in other geographical areas throughout the country **(Azad Thakur *et al*., 2012; Firake *et al*., 2016)**. In addition to this, the northeastern region of India is notably characterized by an extraordinary diversity of natural enemies that are specifically adapted to target and control crop pests, which has been extensively documented in the studies conducted by **Firake et al. in 2012** and in the years that followed. Nevertheless, in spite of the critical and undeniable importance of acquiring a comprehensive understanding of these intricate dynamics and interactions, there persists a substantial and significant gap in the extensive body of information that pertains to the pest complex specifically associated with ginger, thereby underscoring the urgent necessity for further and more detailed investigation.

An extensive and varied collection of insect pests that significantly impact the growth and production of ginger has been thoroughly documented across the vast geographical expanse of India, which clearly points to a considerable ecological

dilemma that poses serious challenges not only for farmers but also for agricultural researchers and scientists engaged in the field. Among the insect pests that are most notably recognized as posing a dire threat to the health and viability of ginger plants are the rhizome scale, which is scientifically identified as *Aspidiella hartii* Ckll., the shoot borer known as *Conogethes punctiferalis* Guen., the white grub categorized as *Holotrichia setticolis*, and the leaf roller referred to scientifically as *Udaspes folus*, all of which are capable of causing extensive and grievous harm to the host ginger plant, particularly when the populations of these pests escalate to alarmingly high and unsustainable levels **(Davis; 1991; Indresena *et al*., 1981)**. A substantial number of the pathogens that adversely affect ginger crops are intrinsically soil-borne in nature, which serves to complicate the preventive measures aimed at averting exposure to these harmful agents, thereby making it imperative to establish and maintain highly sterile soil conditions right from the initiation of cultivation practices to mitigate potential risks. In the pursuit of developing effective management strategies against diseases caused by various pathogens, including but not limited to *Fusarium* and *Pythium* in ginger crops, an array of plant extracts has been utilized, which encompasses a diverse range of botanicals such as *Azadirachta indica*, *Agave americana*, *Cassia fistula*, *Eucalyptus terticorn*is, and *Vitex negundo*, and these have been tested with varying degrees of success and effectiveness **(Ram *et al*., 1999)**.

The amalgamation of diverse methodologies encompassing cultural, biological, and chemical approaches for the management of diseases, particularly when these methodologies are synergistically combined with the innovative technique known as soil solarization, has notably risen to prominence as a significant area of inquiry and practical implementation in the realm of modern agricultural practices **(Rajan *et al*..2002).** The fungi that are classified within the extensive genus Trichoderma represent an incredibly broad and varied assembly of microorganisms that play a crucial and indispensable role in sustaining various ecosystems, thereby underscoring their considerable ecological significance within those systems. These remarkable fungi have developed and employ a multitude of sophisticated mechanisms that facilitate their effective colonization of a wide array of ecological niches, which in turn greatly contributes to their overall success and proliferation as a taxonomic group. A plethora of species that belong to the *Trichoderma* genus have been

documented to exert numerous beneficial influences on the overall health of plants, as they not only stimulate growth and development but also provide a level of protection against a wide range of fungal and bacterial pathogens that pose a significant threat to the vitality and wellbeing of plants **(Asad *et al*., 2014).**

The North Eastern (NE) region of India, distinguished by its abundant biodiversity and conducive agro-climatic conditions, represents a significant area for the cultivation of ginger (*Zingiber officinale*). Nonetheless, the productivity of ginger within this region is considerably compromised by insect pests and fungal pathogens. Over the years, numerous investigations have been conducted across the NE states—particularly in Mizoram, Meghalaya, Assam, Nagaland, and Arunachal Pradesh—illuminating the diversity, intensity, and repercussions of these biotic stresses on ginger cultivation.

Regarding insect pests, the shoot borer (*Conogethes punctiferalis*) has been documented as the most detrimental pest in the region. Investigations conducted in Mizoram and Meghalaya revealed a significant occurrence of shoot borer infestations during the monsoon season, resulting in "dead heart" symptoms and considerable yield reductions **(Sailo *et al*., 2017; Marbaniang *et al*., 2015).** Rhizome scale (*Aspidiella hartii*) and white grubs (*Holotrichia* spp.) were also documented as prevalent in ginger cultivation areas of Assam and Nagaland **(Bordoloi *et al*., 2018)**, impacting both field and storage phases. White grubs were particularly noted for their destructive feeding behaviour on roots and rhizomes, culminating in plant wilting and inadequate rhizome development.

Concurrently, fungal diseases, especially soft rot induced by *Pythium myriotylum*, have been rigorously examined due to their devastating implications. As per **Das *et al*. (2016)**, soft rot disease has been recognised as the most economically detrimental disease in Assam, with the potential to devastate entire fields under conditions of high humidity. Similarly, in Meghalaya and Arunachal Pradesh, *Fusarium oxysporum f.* sp. *zingiberi* were frequently isolated from wilted plants displaying yellowing and vascular browning symptoms **(Lal *et al*., 2019)**. These studies also observed that the persistent monocropping of ginger in the region has resulted in an accumulation of inoculum in the soil, thereby escalating disease occurrence over time.

Comprehensive surveys conducted in NE India **(ICAR-RC NEH, 2020)** documented not only the presence of *Pythium* and *Fusarium* species but also emerging pathogens such as *Sclerotium rolfsii*, which is responsible for collar rot and rhizome rot. In Mizoram, recent molecular investigations have endeavoured to ascertain the genetic diversity of *Fusarium* and *Pythium* isolates, aiding in the comprehension of their pathogenic variability **(Lalramliana *et al*., 2021)**. These initiatives have generated insights into disease dynamics and are facilitating the formulation of region-specific disease management strategies.

Molecular methodologies, particularly those based on polymerase chain reaction (PCR), have emerged as invaluable instruments for the precise identification at the species level and for conducting phylogenetic assessments of insect pests **(Hebert *et al*., 2003; Ratnasingham & Hebert, 2007).**

The process of molecular identification generally entails the amplification of conserved genetic regions, including mitochondrial cytochrome c oxidase subunit I (COI), 16S rRNA, and nuclear ribosomal internal transcribed spacer (ITS) regions. Notably, the COI gene has gained prominence as a "DNA barcode" owing to its considerable interspecific variability and the presence of relatively conserved primer-binding sites **(Folmer *et al.,* 1994)**. This methodology allows for swift and accurate identification, even from incomplete or juvenile specimens, which often poses challenges within morphological taxonomy.

Studies employing PCR techniques have utilized universal primers, such as LCO1490 and HCO2198, for the amplification of COI gene regions **(Folmer *et al*., 1994)**. These primers have demonstrated efficacy across a wide array of insect taxa, encompassing species within Lepidoptera and Diptera that impact ginger crops. In the investigation conducted by **Kumar *et al*. (2020)**, COI sequences derived from *Conogethes punctiferalis* larvae gathered from ginger fields were successfully correlated with existing GenBank records, thereby confirming species identification and underscoring genetic diversity within populations. Likewise, primers targeting the 16S rRNA gene have been employed to identify dipteran pests, such as rhizome maggots, with primers 16Sar and 16Sbr **(Simon *et al*., 1994)** exhibiting high rates of amplification success.

Sophisticated PCR methodologies, including quantitative PCR (qPCR) and multiplex PCR, are increasingly recognized for their capacity to facilitate the simultaneous detection of multiple pest species and for quantifying pest populations **(Chaudhary *et al*., 2015)**. Furthermore, high-throughput sequencing (HTS) technologies have augmented conventional PCR by enabling metabarcoding techniques, which can identify complex pest communities present in environmental samples, such as soil and plant debris **(Taberlet *et al*., 2012)**.

Notwithstanding the efficacy of these molecular approaches, certain challenges persist. DNA degradation in specimens collected from the field, issues related to primer specificity, and the necessity for curated sequence databases for under-researched insect taxa may impede accuracy. To mitigate these concerns, region-specific DNA barcode libraries are being established to enhance local pest identification initiatives **(Shashank *et al*., 2014)**.

The application of molecular identification through PCR-based techniques has markedly advanced the research and management of insect pests affecting ginger. The utilization of conserved genetic regions such as COI and 16S rRNA, in conjunction with universal primers like LCO1490-HCO2198 and 16Sar-16Sbr, has proven to be robust across diverse pest taxa. As sequencing technologies continue to evolve, the integration of these methods with traditional morphological approaches will further refine pest monitoring and integrated pest management strategies.

The biodiversity-rich area of Northeast India, particularly the state of Mizoram, has garnered increasing scholarly interest due to its distinctive and predominantly undocumented insect fauna. The interplay of intricate topographical features, substantial precipitation, and diverse vegetation has facilitated the emergence of numerous endemic and economically pertinent insect species. Morphological classification has historically constituted the cornerstone for cataloging insect biodiversity within this region. Recent initiatives have integrated traditional taxonomy with molecular methodologies to improve species delineation and attain a more nuanced understanding of pest dynamics.

In Mizoram, several pivotal morphological investigations have concentrated on agriculturally significant insect pests, especially those detrimental to ginger, turmeric, and various horticultural crops. **Lalramliana *et al*. (2015)** cataloged pest

insects associated with ginger in the districts of Aizawl and Kolasib, identifying shoot borers (*Conogethes punctiferalis*), rhizome flies (*Formosina flavipes*), and aphid species predicated on external morphology and genital characteristics. In a similar vein, **Tawnenga and associates (2016)** conducted a survey of white grub species (*Scarabaeidae*) infesting farmland in Mizoram, employing larval and adult morphological keys to differentiate between *Holotrichia* spp. and *Anomala* spp., which are prominent soil pests within the locale.

Although morphological investigations have established a foundational basis, constraints such as cryptic speciation and phenotypic plasticity have prompted researchers to embrace molecular methodologies. Techniques such as DNA barcoding and PCR-based identification have seen increased application throughout Northeast India. **Shashank *et al.* (2014)** compiled one of the initial barcode databases for Lepidoptera pests from the Northeast, incorporating specimens from Mizoram. This investigation utilized COI gene sequences amplified using universal primers LCO1490 and HCO2198 **(Folmer *et al.*, 1994)** to authenticate the identity of pest moths and document intraspecific variability.

In a further notable endeavor, **Lalhmingliani *et al.* (2019)** employed both morphological and molecular techniques to identify and confirm species of *Dacus* and *Bactrocera* (*Tephritidae*) fruit flies in Mizoram. The markers COI and ITS2 were utilized for species differentiation, yielding insights into overlapping morphotypes among *Bactrocera dorsali*s and closely related species. The amalgamation of morphometric analysis with molecular phylogenetics provided a more comprehensive identification framework.

Regional institutions, including Mizoram University and the ICAR Research Complex for the NEH Region, have pioneered molecular investigations to bolster pest management strategies. For instance, **Singh *et al.* (2020)** applied COI sequencing to ascertain incursions of *Spodoptera frugiperda* (Fall armyworm) in Northeast India, including early detections within Mizoram. These investigations highlight the increasingly pivotal role of molecular diagnostics in the surveillance of invasive species.

The insect biodiversity of Mizoram and Northeast India has been partially elucidated through traditional morphological studies; however, molecular

methodologies have afforded a heightened resolution of species boundaries, facilitating the identification of cryptic pests and invasive species. Subsequent research is poised to benefit from integrative taxonomic frameworks that amalgamate morphological characteristics, molecular markers (COI, 16S rRNA, ITS2), and ecological data, thereby enriching our comprehension of insect pest diversity in this relatively uncharted region.

In addition to pathogen documentation, various studies have appraised biological control agents. For instance, *Trichoderma harzianum* and *Pseudomonas fluorescens* have demonstrated encouraging outcomes in mitigating soft rot and *Fusarium* wilt occurrences in field trials conducted in Assam and Meghalaya **(Gogoi *et al.*, 2017)**. Likewise, neem-based formulations and pheromone traps have been advocated for the management of shoot borers within organic ginger farming systems being promoted in the region.

In summary, research from NE India has substantially enhanced the understanding of the intricate interactions between ginger and its principal insect and fungal adversaries. Nevertheless, there persists a necessity for further location-specific inquiries, particularly concerning resistant varieties, eco-friendly control strategies, and the impacts of climate change on pest and pathogen prevalence.

CHAPTER 3

Collection and Morphological characterization of Insect pest of Ginger

3.1 General Introduction:

Insect pests are a significant threat to ginger (*Zingiber officinale* Roscoe), a high-value spice and medicinal crop cultivated widely in tropical and subtropical regions. Ginger is prized not only for its culinary uses but also for its medicinal properties, which have been recognized for centuries. However, the productivity and quality of ginger are severely hampered by various insect pests, which lead to direct damage and increase the plant's susceptibility to secondary infections by fungal and bacterial pathogens. This introduction provides an overview of the major insect pests affecting ginger, focusing on their distribution, biology, damage patterns, and management practices. Furthermore, it highlights recent research in India and globally on managing these pests to ensure sustainable ginger cultivation.

3.2 Major Insect Pests of Ginger:

3.2.1. Shoot Borer (*Conogethes punctiferalis* Guenée)

The shoot borer, scientifically classified as *C. punctiferalis* and belonging to the family *Pyralidae*, represents the most significant and detrimental insect pest affecting the cultivation of ginger and turmeric within the geographical boundaries of India. The larvae of this particular pest exhibit a behavioral tendency to bore into the pseudostems of the plants, where they engage in feeding on the internal shoot structures, ultimately leading to observable symptoms such as yellowing and desiccation of the infected pseudostems. The identification of this pest infestation can typically be established through the presence of a borehole located on the pseudostem, which serves as an exit point for the frass that is expelled, in conjunction with the wilting of the central shoot, both of which are considered hallmark indicators of the detrimental effects caused by the pest **(Devasahayam and Koya 2005)**. In terms of their physical characteristics, the adult moths are categorized as medium-sized, possessing a wingspan that ranges from 18 to 24 millimeters; their wings and bodies exhibit a pale straw yellow coloration adorned with minute black spots that contribute to their distinctive appearance. The life cycle of this pest includes five distinct larval

instars; the fully mature larvae are characterized by a light brown hue, sparse hair covering, and an overall length that measures between 16 and 26 millimeters. During their reproductive phase, adult female moths are capable of laying between 30 and 60 eggs throughout their lifespan, and it has been observed that the pest can complete approximately six to seven generations within a single crop season in agricultural fields. The presence of this pest is continuous and can be monitored throughout the entirety of the crop season, with a notable increase in population density occurring specifically during the months of September and October in the state of Kerala, India. Furthermore, it is important to note that the shoot borer exhibits a high degree of polyphagy, as evidenced by its documented occurrence on over 35 different host plants, many of which hold significant economic value within the agricultural landscape of India **(Devasahayam and Koya 2005; Jacob 1981)**.

3.2.2. Rhizome Fly (*Mimegralla coeruleifrons* Macquart)

The rhizome fly is another major pest in ginger-producing regions, especially in India. The adult fly lays eggs on the ginger plant's rhizome, and upon hatching, the larvae feed on the rhizome tissues, leading to significant yield loss and degradation of rhizome quality. According to a study by **Gupta et al. (2015)**, rhizome fly infestations can lead to 20%-30% yield reduction. Research has shown that good cultural practices, such as crop rotation and destruction of infested rhizomes, are critical in minimizing the damage caused by the rhizome fly. Chemical control has been found to be partially effective, although insecticide resistance is a concern **(Rani *et al.*, 2020).**

3.2.3. White Grub (*Holotrichia serrata* F.)

White grubs are larvae of beetles belonging to the *Scarabaeidae* family, primarily *Holotrichia serrata*, which have emerged as a serious pest in India. These larvae feed on the roots of ginger plants, causing wilting, stunted growth, and, in severe cases, death. White grubs are challenging to manage due to their subterranean habits and extensive feeding period **(Thakur *et al*., 2019)**. Entomopathogenic nematodes and fungi, such as *Beauveria bassiana*, have shown potential for white grub control **(Saha *et al*., 2018)**. Additionally, practices like deep summer plowing, crop rotation, and organic amendments reduce white grub populations and improve soil health.

The larvae of the white grubs, scientifically classified under the genus *Holotrichia* and belonging to the subfamily *Melolonthinae*, frequently inflict substantial harm upon ginger cultivation in specific locales within northeastern India. These voracious grubs engage in the consumption of the root systems and newly developed rhizomes of the ginger plants, causing significant physiological stress to the affected flora. As a direct consequence of such pest infestations, one often observes a pronounced yellowing of the foliage, which serves as a visible indicator of distress, and in instances of extreme infestation severity, it becomes imperative to excise the pseudostem at its base to mitigate the spread of the infestation further. In plantations that are severely compromised by this pest, the potential exists for total crop loss, which can have devastating economic repercussions for local farmers. The adult forms of Holotrichia spp., which are frequently encountered in the region of Sikkim in India, are characterized by their dark brown coloration and exhibit a size range of approximately 2.5 cm to 3.5 cm, making them easily recognizable. In their larval stage, these grubs present a creamy white appearance and can typically be found residing in the soil, where they carry out their destructive feeding activities. The emergence of the adult beetles occurs in substantial numbers coinciding with the onset of the summer monsoon rains, particularly during the months of April and May, which is a crucial period for their population dynamics **(Varadarasan *et al*., 2000).** This seasonal pattern of emergence is of significant interest to entomologists and agricultural scientists alike, as it directly influences the timing of pest management strategies that must be employed by ginger farmers.

Understanding the life cycle and habits of *Holotrichia* spp. is essential for the development of effective control measures aimed at preserving ginger crops from the ravages of these pests. Consequently, ongoing research efforts are required to explore and implement integrated pest management techniques that can mitigate the impact of these grubs on ginger cultivation in affected regions. The combination of ecological knowledge and practical agricultural strategies will be paramount in ensuring the sustainability of ginger production in northeastern India, thereby safeguarding the livelihoods of those who depend on this vital crop for their economic stability. Ultimately, addressing the challenges posed by white grubs is not merely an

agricultural concern, but one that intersects with broader issues of food security and rural development in the region.

3.2.4. Aphids (*Myzus persicae* Sulzer and *Aphis gossypii* Glover)

Aphids are among the most common pests of many crop species, including ginger. These small, sap-sucking insects weaken plants by draining essential nutrients and act as vectors for several viral diseases. In ginger, Myzus persicae and Aphis gossypii are notable aphid species, particularly in India, where they thrive in humid environments. Aphids infestations in ginger fields can lead to severe reductions in crop quality and market value **(Kumar *et al.,* 2016).** Aphid populations can be controlled through biological agents, such as ladybird beetles and parasitoids, as well as neem-based biopesticides **(Patil *et al*., 2018)**.

3.2.5. Thrips (*Panchaetothrips indicus* Bagnall)

Thrips, especially *Panchaetothrips indicus*, are another significant pest of ginger in tropical regions. These small insects feed on plant sap, causing leaf curling, discoloration, and reduced photosynthetic ability. In India, studies have shown that thrips infestations are more severe during dry spells **(Prakash *et al*., 2021)**. Biological control, including the use of predatory mites and entomopathogenic fungi, has shown promise in managing thrips populations **(Bhagwati *et al*., 2019)**.

3.3 Global and Regional Research on Management Practices

Insect pest management in ginger involves an array of practices, including cultural, biological, and chemical controls. However, in recent years, the focus has shifted towards sustainable and eco-friendly methods due to the adverse effects of chemical insecticides, such as resistance development, environmental contamination, and adverse effects on non-target species.

3.4 Integrated Pest Management (IPM)

Several studies emphasize the importance of IPM for managing ginger pests. IPM strategies involve monitoring pest populations, using biological controls, and applying insecticides only when necessary. Integrating pheromone traps for the shoot

borer with the release of natural predators reduced shoot borer infestations by up to 60% without adverse effects on the crop ecosystem **(Hegde *et al*., 2017)**. IPM has proven especially beneficial in India, where diverse cropping systems and climatic conditions can influence pest populations.

3.5 Biological Control

Biological control methods have gained traction as environmentally sustainable pest control options. For instance, entomopathogenic fungi like *Beauveria bassiana* have been effective against white grubs **(Saha *et al*., 2018)**. Similarly, research in China and Thailand has shown that natural predators, such as predatory beetles and parasitoid wasps, significantly control aphid populations in **ginger (Cheng *et al*., 2019)**. These biological agents are specific to the pest, do not harm beneficial insects, and reduce the reliance on chemical insecticides.

3.6 Botanical Insecticides

Botanical insecticides, derived from plant extracts, offer an alternative to synthetic chemicals. Neem-based insecticides have shown effectiveness against a variety of ginger pests, particularly aphids and shoot borers. Studies in India and Nepal have shown that neem oil and neem cake are effective in reducing pest populations while also promoting plant health **(Yadav *et al*., 2020)**. Other plant extracts, such as garlic and chili, have also demonstrated repellent properties against rhizome fly and aphid infestations.

3.7 Chemical Control and Resistance Issues

Chemical insecticides are commonly used for ginger pest management, but their effectiveness is diminishing due to resistance issues. Repeated applications of common insecticides against the rhizome fly led to reduced efficacy, likely due to resistance development **(Rani *et al.,* 2020)**. This resistance is a growing concern globally, as it necessitates higher doses of chemicals, thereby increasing production costs and environmental risks.

Insect pests pose significant challenges to ginger cultivation worldwide, particularly in tropical regions where warm and humid climates favor pest

proliferation. Studies in India and globally emphasize the need for effective pest management strategies to minimize yield losses and maintain crop quality. The shift towards integrated pest management and biological control methods reflects a growing awareness of sustainable agricultural practices, especially in light of chemical resistance issues. Ongoing research is essential to refine these management strategies, particularly in specific agro-climatic contexts like Mizoram, where local pest dynamics and farmer practices influence ginger cultivation.

3.8 Materials and Methods

3.8.1. Sampling and Preservation:

The adults and larvae of various insect species that are known to infest the ginger plant, were meticulously gathered through a combination of methods including handpicking, the use of insect nets, light traps, and pitfalls, which were strategically employed during the early morning hours, throughout the afternoon, into the evening, and even during the night, from a total of 117 different ginger farms located across various districts within the state of Mizoram, India. The sampling efforts were specifically concentrated in areas where observable symptoms indicative of rotting or damage caused by insect activity were present, particularly in regions rich in organic material that exhibited signs of rot **(Hennig in 1935)** along with instances of wilt **(Karmawati *et al.*, 1990)**.

In total, a carefully curated collection of 30 ginger samples per farm that displayed symptoms consistent with insect-induced rotting was amassed from each of the 117 ginger farms, culminating in an extensive total of 3,510 infected ginger plants that were subjected to thorough examination and analysis. The sampling process was diligently carried out over the span of one year, specifically from October 2021 until October 2022, a period which coincided with the onset of the rainy season, a time during which the *Zingiberaceae* family of plants flourishes and thrives remarkably well in response to the climatic conditions. The occurrence of heavy rainfall has been identified as an optimal condition conducive to the vigorous growth and proliferation of various microorganisms, which can significantly impact the health and integrity of ginger crops. The adult and larval specimens that were collected during these sampling efforts were subsequently utilized for both morphological and molecular identification

purposes, thereby facilitating a comprehensive understanding of the insect populations present within the sampled ginger plants. For the sake of preserving the integrity of the samples used for morphological as well as molecular identification, these specimens were meticulously placed into a solution of 70% alcohol, which serves to effectively halt any further biological activity and allows for accurate identification in subsequent analyses. The samples were then transported to Research and Instrumentation Center, Department of Zoology, Pachhunga University College, Aizawl, Mizoram for molecular as well as morphological identification.

3.8.2 Morphological Identification

Morphological identification of insect pests involves examining key physical characteristics that help distinguish one species from another. Accurate identification is crucial for effective pest management, as each pest species may require a different control strategy. Based on the following features the collected insect specimens were identified using Leica S9i Microscopes on the whole part of body to describe shape, size, color, and genitalia parts as specific characters of species. The entirety of the gathered pest species was systematically identified in accordance with their morphological traits as delineated by **Atwal (1993**) and **Hashmi (1994)**, utilizing taxonomic keys and a variety of internet databases. Based on the key identification points insects collected were identified morphologically using the following characteristic features:

Body Segmentation and Size

Insects are divided into three main body segments: the head, thorax, and abdomen. The size and proportion of these segments can vary significantly across insect families and orders. For instance, the shoot borer (*Conogethes punctiferalis*) has a distinct, elongated thorax compared to other lepidopterans **(Patel *et al*., 2016)**. Additionally, body size is often specific to species and varies with environmental conditions, such as climate and diet, making it a valuable morphological identifier in field assessments **(Singh *et al*., 2017).**

Antennae Structure

Antennae play a critical role in the morphological identification of insect species. Different insect orders exhibit unique antennae structures, such as the clubbed antennae seen in butterflies (*Lepidoptera*) or the filamentous antennae in flies (*Diptera*). For example, aphids (*Aphididae*), common ginger pests, have elongated, thin antennae segmented into five parts, which help distinguish them from other small sap-sucking insects **(Blackman & Eastop, 2000)**. The presence or absence of specific antennal features, such as a scape, pedicel, and flagellum, assists in differentiating insect species and families **(Chapman, 2013).**

Wing Morphology and Venation

Wing characteristics, including shape, size, venation, and texture, are fundamental to insect identification, especially in groups like *Diptera, Lepidoptera,* and *Hymenoptera*. For instance, thrips (*Thysanoptera*), which infest ginger crops, have slender, fringed wings with few or no veins, unlike other common pests such as whiteflies (Hemiptera) **(Mound & Kibby, 1998)**. Wing venation patterns are particularly significant in distinguishing between closely related species, as demonstrated in studies by **Jarzembowski *et al.* (2014)** that highlight specific vein configurations as key differentiators in insect taxonomy.

Leg Structure and Modifications

Leg structure varies significantly across insect orders and often indicates the insect's behavior or habitat. For example, white grubs (*Scarabaeidae*) are characterized by their robust, fossorial (digging-adapted) legs, which are essential for their subterranean lifestyle **(Ritcher, 1966)**. In contrast, aphids have elongated legs adapted for plant movement but lack adaptations for burrowing, making this characteristic useful for distinguishing them from soil-dwelling pests **(Blackman & Eastop, 2006)**.

Head Capsule and Mouthparts

The structure of the head capsule and mouthparts is highly specialized among insect species, aiding in identification. Herbivorous insects, such as rhizome flies (*Diptera*), typically have piercing-sucking or sponging mouthparts, adapted for

feeding on plant juices **(McAlpine, 1981)**. Chewing mouthparts, found in beetles like white grubs, are adapted for gnawing through plant tissue, making this feature valuable in distinguishing between pest species in ginger fields **(Lawrence *et al.*, 2011)**.

Abdominal Segmentation and External Genitalia

The abdomen's segmentation and external genitalia structures are crucial for distinguishing between closely related insect species. In many Lepidoptera, for example, the shape and structure of the genitalia are often the only reliable features for species-level identification **(Klots, 1970)**. Detailed examination of genitalia is a common practice in entomological studies, as it is highly species-specific and allows precise identification even among cryptic species **(Huber & Norrbom, 1999)**.

3.9 Calculation of percentage incidence

The metric of percentage incidence serves as an essential and critical indicator within the realm of agricultural pest management, as it provides invaluable insight into the overall health and viability of crops. This particular metric enables researchers and practitioners to accurately quantify the proportion of plants that have been adversely affected by a specific pest in relation to the total number of plants that have been observed and assessed within a given area or field. In the context of ginger cultivation, which is a crop that requires careful monitoring and management, an in-depth understanding of percentage incidence becomes absolutely crucial for making informed decisions regarding pest control strategies and interventions, thereby ensuring the sustainability and productivity of the crop. Through the application of this metric, agricultural professionals are empowered to identify trends, assess the severity of pest infestations, and implement effective management practices that are grounded in empirical data.

A representative area or plot within the expansive ginger field, ensuring that the chosen site accurately reflects the overall characteristics and conditions of the entire cultivation area was judiciously selected. Following the selection of the designated plot, the next critical step involves meticulously counting the total number

of ginger plants present within the sampled area, denoted as (T), which serves as an essential quantitative measure for subsequent analyses and comparisons.

Assessment was conducted to identify and meticulously count the specific number of ginger plants that exhibit visible symptoms indicative of pest infestation, labeled as (I), in order to ascertain the extent of the problem and implement effective management strategies.

In addition to identifying the affected plants, the particular type of pests present, the specific symptoms displayed by the infested plants, and the prevailing environmental conditions at the time of observation was document.

From the observations and assessment made Percentage incidence of specific insect pest was calculated using the formula:

$$\textbf{Percentage Incidence} = \frac{T}{I} \times 100$$

Where T = *Total no of infected plants*

I = *Total no of plants assessed*

3.10 Results and discussion

Survey of the insect pest and disease of ginger

A diverse array of insect species was meticulously collected utilizing a light trap, pitfall and insect net methodology; however, it is noteworthy that only three distinct species of insects were recorded and identified for the purpose of monitoring and analyzing the population dynamics and trends over time as these insects were considered to be specific pest of ginger. The specific insect species cataloged include *Mimegralla coeruleifrons*, *Congethes punctiferalis*, *Holotrichia* spp., all of which have been documented in order to better understand their ecological significance and population fluctuations within the studied environment. It is significant to note that a comprehensive total of nine species of insects were identified during this research

endeavor, as illustrated in Table 1.1, which provides detailed insights into the biodiversity present in the area under investigation.

Table 1.1: List of collected and identified insect specimens from infested ginger fields of Mizoram

Common Name	Scientific Name	Order	Family
Rhizome fly	***Mimegralla Coerulifrons***	**Diptera**	**Micropezidae**
Shoot borer	***Congethes punctiferalis***	**Lepidoptera**	**Pyralidae**
White grub	***Holotrichia spp.***	**Coleoptera**	**Melelonthidae**
Green Stink Bug	*Nezara viridula*	Pentatomidae	Hetroptera
Beetle	*Xylotrupes gideon*	Coleoptera	Scarabaeidae
Long horned beetle	*Rosalia ttenua*	Cerambicidae	Coleoptera
Moth	*Callidula ttenuate Moore*	Cerambicidae	Coleoptera
Cutworm	*Agrotis segetum Hampson*	Noctuidae	Lepidoptera
Cicada	*Pacna repanda*	Cicadellidae	Homoptera

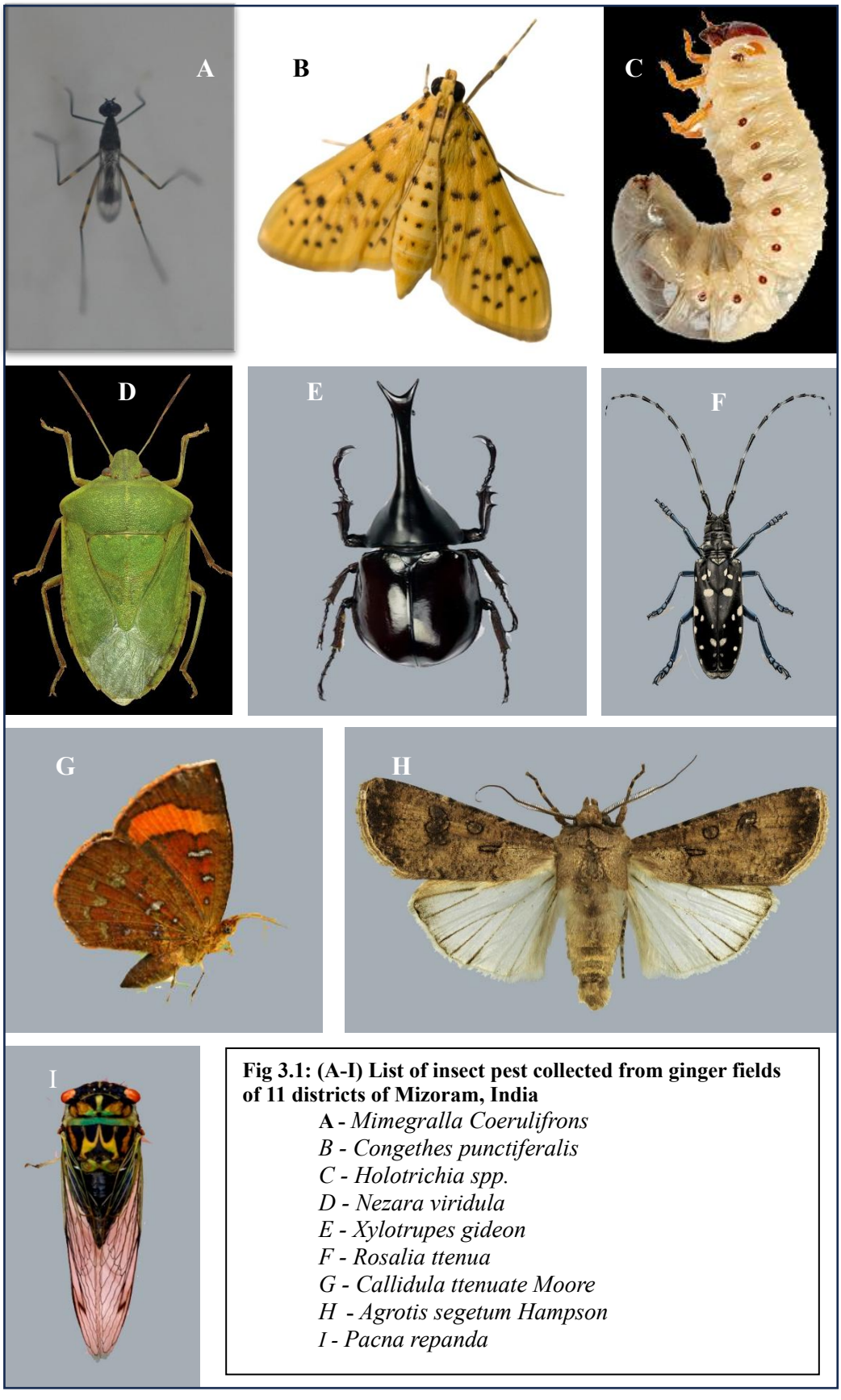


Fig 3.1: (A-I) List of insect pest collected from ginger fields of 11 districts of Mizoram, India

A - *Mimegralla Coerulifrons*
B - Congethes punctiferalis
C - Holotrichia spp.
D - Nezara viridula
E - Xylotrupes gideon
F - Rosalia ttenua
G - Callidula ttenuate Moore
H - Agrotis segetum Hampson
I - Pacna repanda

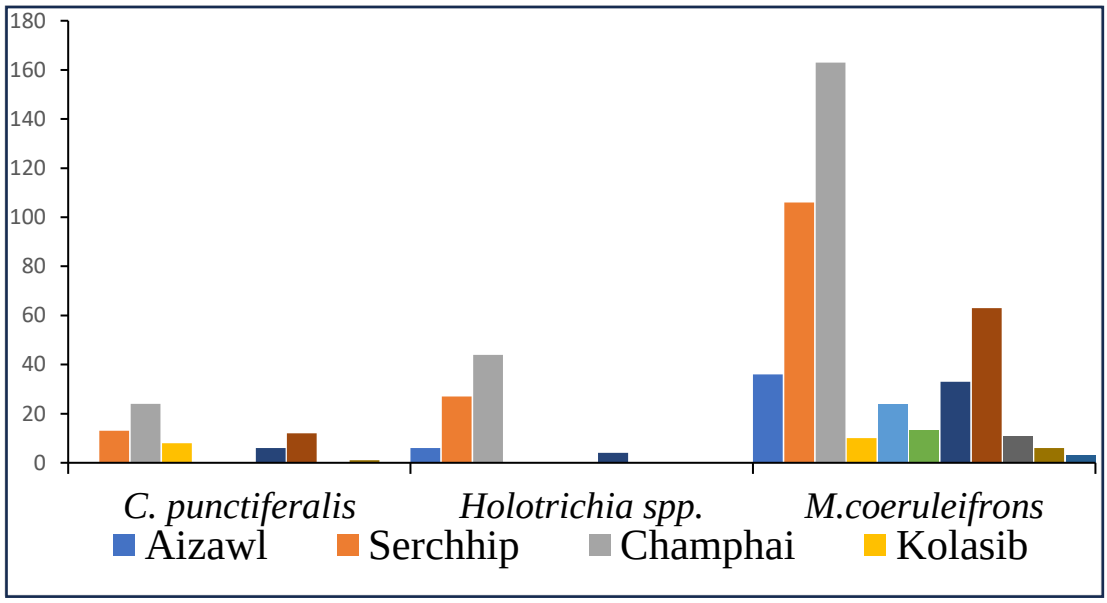
The insect known as the rhizome fly or the stilt legged fly, which is scientifically classified as *Mimegralla coeruleifrons (Fig 1.1 A)* and the shoot borer, specifically identified as *Conogethes punctiferalis (Fig 1.1 B)*, poses a significant threat to ginger cultivation and has been identified as a critical pest impacting the health and productivity of ginger crops. Moreover, the presence of the white grub, classified under the genus *Holotrichia* spp., contributes to the array of challenges faced by ginger farmers, as these pests collectively inflict substantial damage on the plants.

It is essential to understand that the interaction of these various pests can lead to a compounded negative effect on the overall yield and quality of ginger, making integrated pest management strategies crucial for effective control. Therefore, researchers and agricultural specialists must prioritize the study of these pests and their behaviors to develop advanced methods for mitigating their impact on ginger production. In conclusion, the identification and characterization of these pest species are vital for informing agricultural practices and ensuring the sustainability of ginger farming in affected regions.

In the context of the field survey that was conducted, it became evident that ginger crops are subject to a multitude of threats, specifically from various insect pests as well as the deleterious effects of rhizome rot, which presents a considerable challenge under prevailing field conditions. Alarmingly, it was observed that nearly all ginger fields surveyed exhibited signs of infestation by both rhizome rot and the rhizome fly, indicating a pervasive issue affecting crop health and yield. Farmers across the region reported that rhizome rot constituted the most significant problem encountered in the field, highlighting the urgent need for effective management strategies. The relationship between rhizome rot and the rhizome fly was particularly pronounced, with recorded field infestation (FI) rates reaching a staggering 46.11% for rhizome rot and 22.62% for rhizome fly in specific locations such as Champhai districts of Mizoram thereby underscoring the critical nature of these pests as illustrated in table 1.2. Rhizome rot reported by most of farmers was the major problem in the field condition. The rhizome rot and rhizome fly was found to be associated with each other with the maximum field infestation of 22.63%

Moreover, the polyphagous white grub emerged as an additional and troubling issue in nearly all the districts surveyed, with the highest percentage of field infestation recorded at an alarming 6.11% in Champhai District which further complicates the agricultural landscape and necessitates comprehensive pest management approaches.

Fig 3.2 Graphical representation of the insect pest collected form 11 districts of Mizoram during the year June 2021- May 2022



Rhizome rot reported by most of farmers was the major problem in the field condition. The rhizome rot and rhizome fly was found to be associated with each other with the maximum field infestation of 22.63%

Table 1.2 : Prevalence of insect pests and disease in different ginger growing areas of Mizoram (June 2021 – May 2022)

Prevalance of Insect Pest in different ginger growing areas of Mizoram											
District	**No. of field surveyed**	**No of plants studied per district**	**No of plants studied per field**	**Insect pest**						**Disease**	
				White grub		**Rhizome fly**		**Shoot borer**		**Rhizome Rot**	
				A	**B (%)**	**A**	**B (%)**	**A**	**B (%)**	**A**	**B (%)**
Aizawl	13	390	30	6	1.53	36	9.23	-	-	89	22.82
Serchhip	16	480	30	27	5.62	103	21.45	13	2.7	174	36.25
Champhai	24	720	30	44	**6.11**	163	**22.63**	24	**3.33**	332	**46.11**
Kolasib	11	330	30	-	-	10	3.03	8	2.42	87	26.36
Mamit	9	270	30	-	-	24	8.88	-	-	32	11.85
Lunglei	6	180	30	-	-	13	7.22	-	-	31	17.22
Saitual	10	300	30	12	4	33	11	6	2	133	44.33
Khawzawl	12	360	30	-	-	63	17.5	12	**3.33**	156	43.33
Hnahthial	6	180	30	-	-	11	6.1	-	-	12	6.66
Lawngtlai	5	150	30	-	-	6	4	1	0.66	9	6
Siaha	5	150	30	-	-	3	2	-	-	6	4

3.11 Biology and seasonal incidence

***Mimegralla coeruleifrons* (Rhizome fly or Stilt legged fly):**

The species known as the rhizome fly or stilt legged fly, scientifically referred to as *Mimegralla coeruleifrons*, exhibits a reproductive behavior that is characterized by the deposition of eggs predominantly located at the base of various plants, with a notable concentration of these eggs being situated adjacent to rhizomes that are afflicted by disease; the primary period during which these eggs are laid occurs between the months of August and September, indicating a specific temporal ecological pattern. The incubation duration for these eggs is observed to vary significantly, with a range of approximately two to three days, suggesting environmental factors may influence this developmental stage. Upon emerging from the eggs, the newly hatched maggots display a remarkable transparency, are distinctly segmented, and possess a pale white coloration; shortly after their hatching, these larvae begin to burrow into the rhizomes, subsequently consuming the internal contents entirely. Of particular significance is the association of this fly species with the occurrence of soft rot disease, which appears to play a critical role in its life cycle. It is noteworthy that although the adult female flies exhibit a strong preference for laying their eggs in proximity to diseased or decaying rhizomes, instances have been recorded where the maggots also target adjacent healthy rhizomes subsequent to their feeding on the already compromised rhizomes.

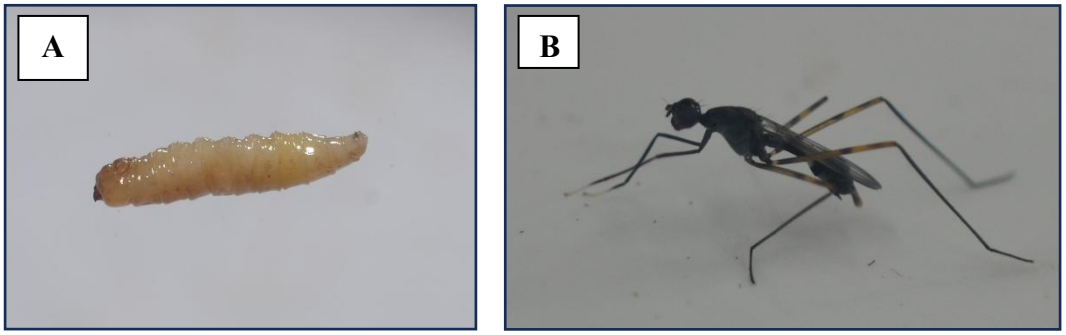


Fig 3.3 : Larvae (A) and adult (B) stages of *Mimegralla coeruleifrons*

The fully matured maggot reaches an elongate form, measuring an average length of 10.03 ± 0.15 mm and a width of 1.80 ± 0.03 mm, with both ends tapering gradually, highlighting the unique morphological characteristics of this larval stage. The duration of the larval period, during which the maggots develop, has been

documented to last approximately 14 ± 1 days, a timeframe that reflects the metabolic and developmental processes inherent to this species. Furthermore, the fully developed maggots have been observed to undergo pupation within the tunnels that they create in the infested rhizomes, demonstrating a clear behavioral adaptation to their environment. It is also important to note that plants that have been infested by this species may be effortlessly uprooted by hand, indicating the extent of the damage incurred.

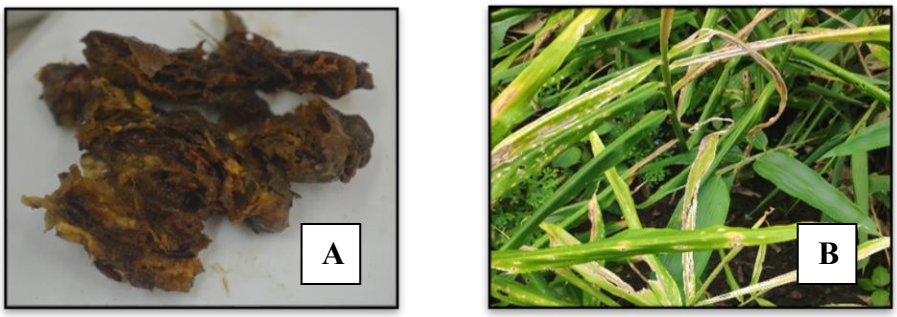


Fig 3.4 : Damage symptoms induced by *Mimegralla coeruleifrons* on rhizome of ginger (A) and leaves of ginger plant (B).

The puparium, which serves as the transitional stage between larva and adult, exhibits an elongate shape measuring approximately 6.8 ± 0.1 mm by 1.23 ± 0.02 mm, which is considerably smaller than the adult form; this structure begins as a brownish hue and undergoes a transformation to a darker brown or black coloration as it matures, with the duration of the pupal phase averaging around 7 ± 1 days. The adult fly itself is characterized by a black coloration, elongated legs, a slim body, and wings that are marked or spotted, particularly around the anal cells; it is worth noting that female individuals are generally larger than their male counterparts, with males measuring approximately 16.5 ± 2.5 mm in length and females averaging 21 ± 2 mm. The longevity of the adult flies has been recorded to be around 8.5 ± 1.5 days, while the entire lifecycle of this species is completed within a period of approximately 30 ± 3 days, thus illustrating the rapid turnover of generations.

Fig 3.5 : Life cycle of *Mimegralla coeruleifrons*

The onset of rhizome fly infestation has been documented to commence during the early days of July; this infestation subsequently escalates by the end of August, followed by a notable decline that is observed from October onward, culminating in the lowest levels of infestation at the end of November. The rhizome fly, scientifically designated as *Mimegralla coeruleifrons*, is classified as a species complex that presents significant challenges in terms of identification when relying solely on external morphological characteristics, which complicates the understanding of its ecological role. **Sontakke (2000)** provided a comprehensive analysis indicating that the rhizome fly, specifically the *Mimegralla* spp., exhibits heightened activity and inflicts considerable damage primarily during the August to September timeframe under prevailing field conditions in the region of Odisha. Further supporting this observation, **Ghorpade *et al*. (1988)** noted that the peak of infestation occurs between mid-August and mid-October, a period that is characterized by intermittent rainfall, overcast weather conditions, lower temperatures, and elevated levels of relative humidity, all of which contribute to the life cycle dynamics of this pest species.

Conogethes punctiferalis (Shoot Borer)

The eggs belonging to this particular species were discovered meticulously positioned on the surfaces of leaves or the delicate stems of plants, and it was noted through careful observation that the incubation period for these eggs was approximately 7 days, with a slight variability of ±1 day. Following this incubation phase, the duration of the larval stage was recorded to extend over a period of 18 days, with a margin of variability of ±2 days, during which the larvae engaged in a behavior of remaining hidden or concealed beneath a protective layer composed of silk and frass, which is essentially their excrement, eventually leading to the formation of a pupa within this protective enclosure.

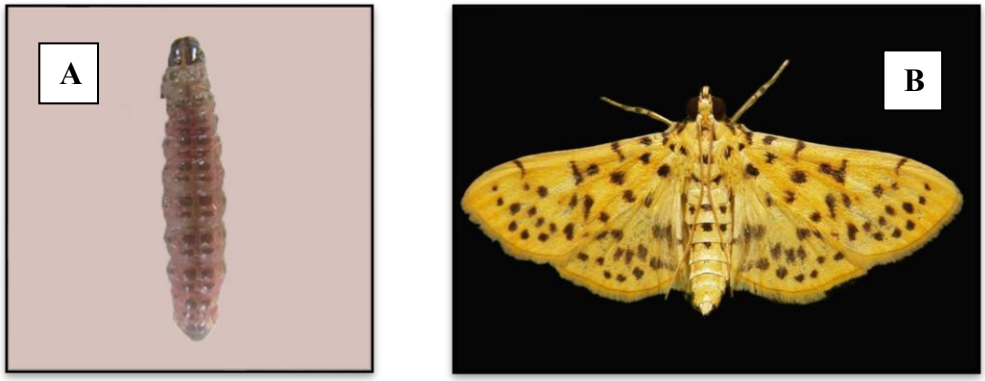


Fig 3.6 : Larvae (A) and adult (B) stages of *Congethes punctiferalis*

Furthermore, the duration of the pupal stage was determined to last for about 7 days, with the same variability of ±1 day, indicating a consistent pattern across the life cycle stages. After careful observation and analysis, it was concluded that the entire life cycle of this organism was completed in a total time frame of approximately 38 days, with a margin of error of ±2 days.

The initial damage caused by the shoot borer was first observed at the beginning of July, with an infestation rate of 3.7%; however, it is noteworthy that the maximum level of infestation was recorded during the middle and latter part of August, reaching a notably high percentage of 25.9%. This infestation showed a statistically significant positive correlation with various environmental factors, including the minimum temperature (r= 0.337), evening relative humidity (r= 0.410), and rainfall (r= 0.238). The findings discussed above are corroborated by the research conducted

by **Nybe in 2001**, who similarly noted that the incidence of infestation was notably higher during the months of August through October. Additionally, the work of Shylesha *et al*. in 2006 further supports these conclusions, suggesting a consistent trend in the life cycle and behavior of this pest in relation to environmental conditions.

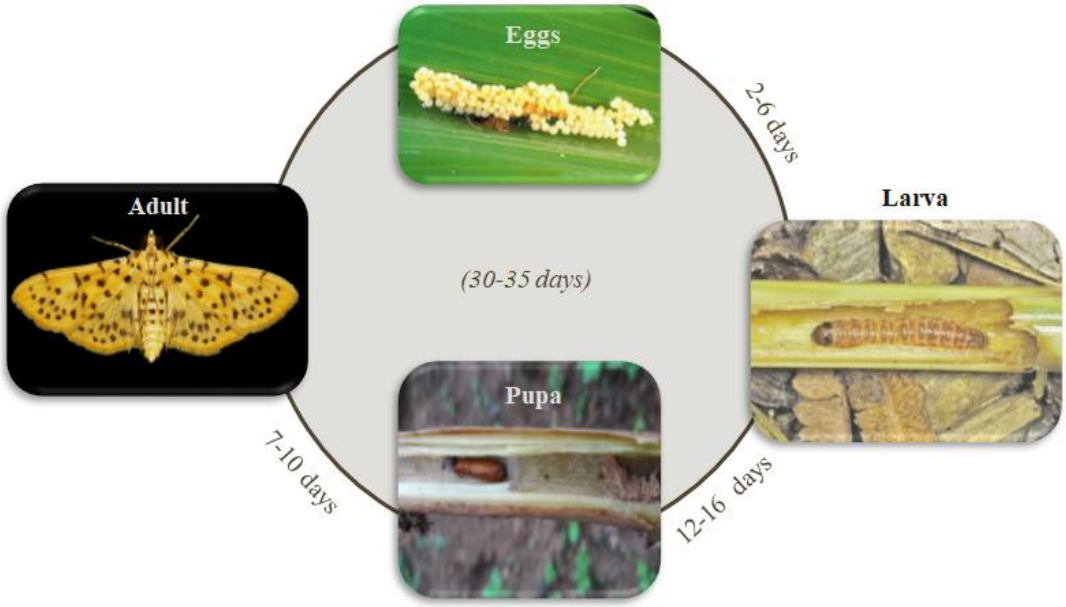


Fig 3.7 : Life cycle of *Congethes Punctiferalis*

Fig 3.8 : (A) Damaged induced by *C. punctiferalis* larvae on ginger rhizome.
(B) Frass extrudation of shoots by *C. punctiferalis.*

Holotrichia spp. (White Grubbs)

White grubs, scientifically classified as members of the genus *Holotrichia* within the subfamily *Melolonthinae*, are notorious for inflicting significant and detrimental damage to ginger plants, particularly in specific regions of northeastern India where their prevalence is notably high. These grubs exhibit a voracious feeding behavior, targeting not only the delicate roots of the ginger plants but also the newly formed rhizomes, which are crucial for the plant's growth and development. The infestation by these pests results in a conspicuous yellowing of the leaves, which serves as a visual indicator of the underlying damage being inflicted upon the plant, and in cases of severe infestation, it becomes necessary to implement drastic measures, such as cutting the pseudostem at its base, in order to halt the further spread of these harmful pests.

In plantations that are heavily infested, there exists a substantial risk of losing the entire crop, which poses a significant threat to the livelihoods of farmers and agricultural stakeholders dependent on ginger production. The adult forms of Holotrichia spp., which are frequently observed in the Sikkim region of India, are characterized by their dark brown coloration and exhibit a size range of approximately 2.5 cm to 3.5 cm, thereby making them relatively conspicuous within their habitat. In contrast, the grubs themselves are creamy white in appearance and are predominantly found residing within the soil, where they continue their destructive feeding habits. During the warmer months of April and May, coinciding with the onset of the summer showers, the adults of this species emerge in substantial numbers, contributing to the rapid increase in the population of these pests, as documented by Varadarasan *et al*. in the year 2000.

These grubs particularly target the base of the pseudostem, the roots, and the developing rhizomes, which are essential for the overall health and productivity of the ginger plants. The infestation inflicted by these pests not only leads to a noticeable yellowing of the leaves, which is an alarming sign for farmers, but it also results in the formation of large holes within the rhizome, consequently diminishing its market value and economic viability. In cases of severe infestation, the loss of the entire crop becomes a very real possibility, which can have devastating economic repercussions

for the affected plantations. The adult beetles of this species are distinguished by their dark brown exoskeleton and typically measure approximately 2.5 mm in width and 1.5 mm in length, making them relatively small yet impactful in terms of their destructive capabilities. The grubs, which are creamy white in color, find their habitat within the soil, where they can remain hidden while continuing their feeding activities.

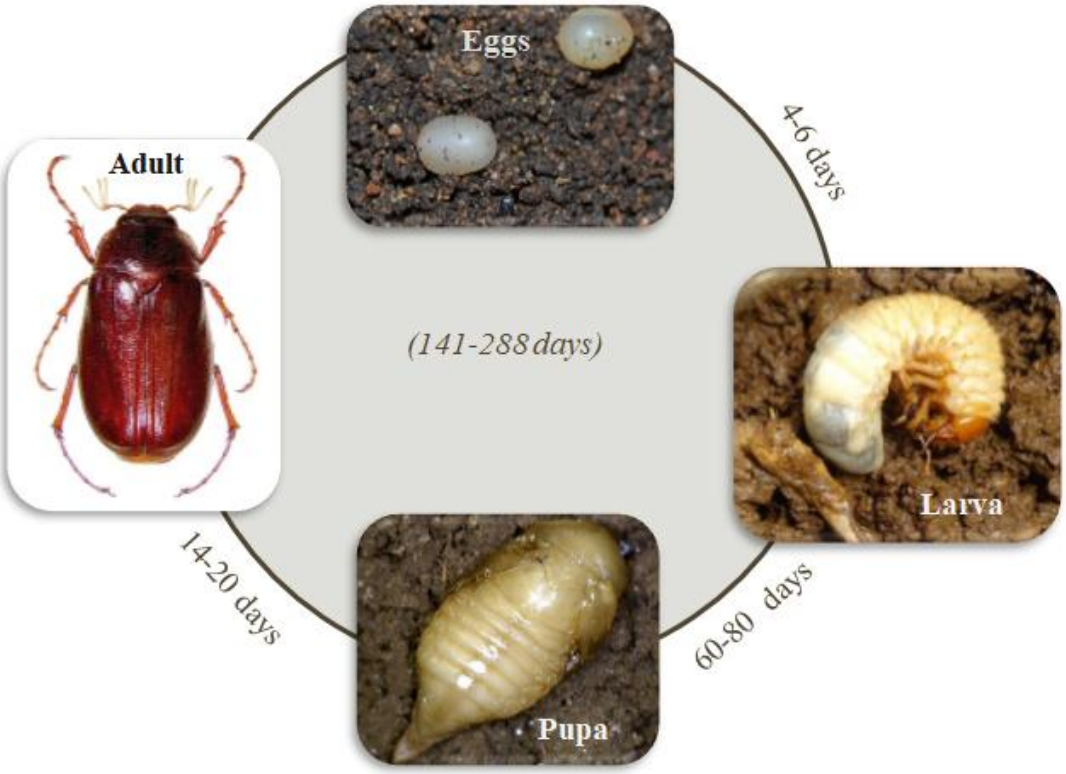


Fig 3.9 : Life cycle of *Holotrichia* spp. (White grubs)

Fig 3.10: Larvae of white grub infecting ginger.

Fig 3.11: Damage symptoms induced by Holotrichia spp. on ginger rhizome.

Fig 3.12: Wilting of shoots and leaves induced by white grubs on shoot and leaves of ginger plant

3.12 Summary and Conclusion

A total of 117 distinct agricultural fields dedicated to the cultivation of ginger were meticulously visited and examined in order to systematically observe and analyze the incidence of various insect pests that were affecting the standing crop.

The identification of the insect pests, as well as the associated diseases, was carried out through a comprehensive assessment that considered multiple factors such as the observable symptoms presented by the plants, the morphological characteristics of the pests themselves, the nature of the damage inflicted upon the crops, and other distinguishing features that could aid in accurate identification.

From the collection efforts utilizing light traps, nets, and pitfall traps, a total of 9 different species of insects were documented; however, after thorough examination, it was determined that only 3 species were specifically associated with ginger crops, namely *Congethes punctiferalis, Mimegralla coerulifrons*, and *Holotrichia* spp., which are particularly noteworthy for their relevance to ginger cultivation.

The highest recorded incidences of the aforementioned insect species were observed in the Champhai District, where the frequency of *C. punctiferalis* was measured at 3.33%, *Holotrichia spp.* exhibited a significant incidence rate of 22.63%, and *M. coeruleifrons* was noted at 6.11%; this data correlates with a concerning rhizome rot percentage that reached 46.11%.

It was alarming to find that nearly all of the ginger fields surveyed exhibited signs of infestation related to both rhizome rot and the presence of the ginger rhizome fly, indicating a widespread agricultural issue that could have serious implications for ginger production in the region.

The rhizome rot, which was reported by a substantial number of farmers as the predominant problem affecting their crops, was observed to be particularly severe under the prevailing field conditions, highlighting the urgent need for effective management strategies.

Moreover, it was noted that the occurrence of rhizome rot was found to be closely associated with the infestation of rhizome fly, with the two issues seemingly

exacerbating one another, leading to a maximum field infestation rate that reached a concerning level of 22.63%.

This relationship between rhizome rot and the rhizome fly infestation underscores the complexity of pest management in ginger cultivation, where multiple factors must be considered to develop an effective control strategy.

Consequently, the findings from this extensive field study emphasize the critical importance of ongoing monitoring and research to understand the dynamics of pest populations and their impact on ginger crops, thereby ensuring sustainable agricultural practices.

Ultimately, addressing the dual challenges posed by both rhizome rot and the associated insect pests will be essential for safeguarding the livelihoods of ginger farmers and maintaining the viability of ginger as a valuable agricultural commodity in the region.

The effective management of plant diseases is a crucial and indispensable aspect of commercial vegetable farming that requires diligent attention and strategic planning. Empirical evidence suggests that the implementation of preventive measures, which include the utilization of botanical pesticides alongside various cultural practices within the framework of organic farming, has demonstrably resulted in a significantly reduced incidence of disease problems when compared to the challenges faced by conventional farming practices. Nevertheless, the situation in Mizoram is quite concerning, as the absence of viable organic disease curative methods has unfortunately culminated in a notable increase in crop yield losses for those engaged in organic agricultural practices. This alarming reality has, in turn, compelled many farmers to reduce their reliance on organic farming methods, and in some instances, to compromise the integrity of established organic farming standards in pursuit of greater productivity.

The widespread availability of inexpensive chemical pesticides in the marketplace has further exacerbated this dilemma, leading conventional farmers to exhibit a pronounced reluctance to adopt preventive cultural methods that could enhance the sustainability of their farming operations. Consequently, the promotion

and accessibility of biological pesticides as effective curative strategies for managing plant diseases hold substantial promise, as they are likely to provide organic farmers with the necessary tools and incentives to improve their crop yields. By fostering a culture of innovation and encouraging the integration of these biological solutions, the agricultural sector can potentially mitigate the adverse effects of plant diseases, thereby enhancing overall productivity and sustainability.

Ultimately, a concerted effort to support organic farmers through the development and dissemination of biological pesticides could significantly bolster the resilience of the organic farming sector, ensuring its viability in the face of mounting agricultural challenges. In summation, the interplay between disease management strategies and farming practices is critical, and addressing this issue with a focus on sustainable solutions is imperative for the future of agriculture in Mizoram.

Organic agriculture, which is strongly advocated and supported within the state of Mizoram, encounters significant challenges when it comes to the effective management of insect pest populations that threaten crop yields. The restricted availability of potent biopesticides, combined with a pervasive lack of awareness regarding the potential benefits of biological control strategies, as well as the sluggish pace at which integrated pest management (IPM) practices are being adopted, collectively serve to undermine the efforts aimed at significantly reducing pest infestations that can devastate crops. In addition to these challenges, the stringent organic certification standards that are often imposed frequently limit the permissible use of even the most minimally synthetic pest control inputs, thus leaving farmers with an increasingly narrow range of options for achieving timely and effective pest control measures.

Although organic farming is recognized for its potential to provide substantial long-term ecological benefits, the inherent reliance on natural inputs and the application of traditional agricultural methods can often prove inadequate when confronted with the high levels of pest pressure that are characteristic of the region. Furthermore, the situation is exacerbated by the presence of inadequate pest surveillance systems, a deficiency in comprehensive extension services, and a stark

shortage of focused research into pest biology and control strategies that are tailored to the distinctive agro-ecological conditions present in Mizoram, which complicates pest management efforts even further.

In light of these pressing challenges, there exists a critical need for more extensive and comprehensive research initiatives that are specifically designed to deepen our understanding of the life cycles, ecological preferences, and dynamic behaviors of the insect pests that significantly impact ginger cultivation. Targeted studies that investigate the intricate interactions between ginger pests and their natural enemies, assess the effectiveness of locally sourced biopesticides, and develop region-specific IPM strategies will create a robust foundation upon which sustainable pest management practices can be established. There is an equally urgent necessity for participatory approaches that actively involve farmers in the process, thereby facilitating the integration of traditional agricultural knowledge with cutting-edge scientific innovations.

By systematically addressing these identified gaps, Mizoram stands to significantly enhance the productivity and resilience of its ginger farming sector, all while steadfastly maintaining its commitment to the principles of organic agriculture. Strengthening the existing research and extension frameworks, fostering collaboration among diverse stakeholders, and ensuring that farmers have reliable access to organic pest control solutions will be pivotal in overcoming the current constraints that hinder agricultural progress and securing the future viability of ginger cultivation in this region.

CHAPTER 4

Isolation and Morphological characterization of Fungal Pathogens of Ginger

4.1 General Introduction

The production levels and overall productivity rates of ginger have experienced a considerable decline, reducing them to notably minimal figures, and this unfortunate trend can be attributed to a multitude of factors that have been extensively documented in the academic literature **(Zakir *et al*., 2018; Shimelis, 2021)**. Numerous scholarly investigations have indicated that the primary culprits behind the diminished production of ginger include the complex nature of diseases affecting the crop, the unavailability of clean and disease-free planting materials, the reduced productivity linked to the repetitive usage of planting stock, and the phenomenon of degeneration over time, all of which contribute significantly to the overall low yield of this important spice **(Senapati and Ghose, 2005)**.

Specific species belonging to the genera *Aspergillus* and *Penicillium* have been identified as responsible for both leaf spot diseases as well as spoilage of ginger rhizomes, highlighting the detrimental impact these pathogens have on the crop's health and viability **(Berza *et al*., 2012; Meenu and Kaushal, 2017; Mekuria and Alemu, 2020)**. In addition to their effect on ginger, these fungal species have also been documented as causative agents of diseases in a variety of other plant species, thereby underscoring their pervasive nature and adaptability **(Photita *et al*., 2005; Louw and Korsten, 2014; Kazi et al., 2019)**. Furthermore, the alarming case of ginger bacterial wilt, which has been reported, represents yet another significant factor contributing to the decline in ginger production, emphasizing the urgent need for effective management strategies **(Kifelew *et al*., 2015; Hunduma *et al*., 2016**).

A multitude of fungal species has been identified as leading contributors to the substantial loss in ginger production, often in conjunction with various other microbial pathogens that adversely affect the crop at multiple stages of its growth and post-harvest handling under natural conditions **(Robert *et al*., 2017; Trigiano and Ownley, 2017)**. Notably, the highly destructive and versatile pathogens belonging to the

Aspergillus species and *Penicillium* genera are among the most common pathogens found in ginger farming and during the storage processes, demonstrating their abundance and prevalence in both field and post-harvest environments **(Meenu and Kaushal, 2017)**. Numerous research endeavors have illustrated that the diseases affecting ginger are frequently associated with other pathogens such as *Pythium* species, the notorious *Fusarium oxysporum* (*F. oxysporum*), and *Pratylenchus coffeae*, indicating a complex interplay of factors that contribute to the overall health of ginger crops **(Rahman *et a*l., 2009)**.

A detailed study conducted by **Berza *et al.* (2012)** identified six distinct genera of fungi present in spoiled ginger samples collected from southern Ethiopia, including *Fusarium, Penicillium, Aspergillus, Rhizopus, Eurotium*, and *Muco*r, thereby elucidating the diversity of fungal pathogens that threaten ginger crops. According to the findings of **Rahman *et al.*, 2009**, rhizome diseases have increasingly affected ginger crops across various states in India over recent years, leading to a notable decline in rhizome yield; thus, wilt and soft rot of ginger rhizomes have emerged as critical limiting factors hindering successful ginger cultivation.

In the northeastern regions of the country, the livelihoods of countless impoverished farmers have become significantly reliant on ginger production, as it serves not only as a vital source of sustenance but also as a major income-generating crop for the local economy, with a substantial proportion of the nation's ginger production originating from this area. However, in recent times, the cultivation of ginger has increasingly become fraught with challenges stemming from the emergence of unknown diseases, which have notably complicated the farming process and diminished the overall yield. As a direct consequence of these disease outbreaks, farmers have suffered significant financial losses, resulting in a detrimental impact on their additional income derived from ginger plantations, which have traditionally been a cornerstone of their economic stability.

Furthermore, this issue transcends individual farmers, representing a national crisis that not only affects local income but also results in substantial losses in foreign currency earnings and the overall performance of the national market. Although there

have been scholarly investigations into the incidence of bacterial wilt disease and postharvest fungal diseases affecting it, it is crucial to note that the incidence of such diseases has not yet been thoroughly examined in other regions of the country, including Mizoram. Additionally, there exists a conspicuous gap in the research landscape, as no comprehensive studies have been conducted in Mizoram focusing on the identification of fungal diseases affecting ginger through the application of advanced molecular techniques, indicating an urgent need for further academic inquiry and exploration in this field.

The establishment of appropriate guidelines for the management of ginger production in Mizoram has proven to be a formidable challenge, primarily attributable to the insufficient knowledge regarding the principal causative agents implicated in ginger disease. Consequently, the precise identification of fungal pathogens at the species level is of paramount importance for the implementation of necessary intervention strategies. Moreover, a comprehensive understanding of fungal diversity and distribution is essential for the formulation of effective management practices. In light of these considerations, the objective of this study was to identify the predominant pathogenic fungi at the species level through the examination of morphological characteristics and the application of DNA sequencing of the internal transcribed spacer (ITS).

4.2 Materials and Methods

4.2.1 Study area

The study was conducted at Research and Instrumentation Centre, Department of Zoology, Pachhunga University College, Aizawl, Mizoram during the month of September 2021– October 2023.

4.2.2 Sample collection

Foliar symptoms of soft rot disease appear as light yellowing of the tips of lower leaves which gradually spreads to the leaf blades. In early stages, the middle portion of the leaves remain green while the margins become yellow. The yellowing spreads to all leaves of the plant from lower region upwards and is followed by

drooping, withering and drying of pseudo stems. Based on these criteria, rhizomes, roots leaves and pseudostems of diseased ginger are collected for direct isolation and identification of fungi. Samples were collected from the month of June 2020 where symptoms of soft rot disease started appearing.

From each farm, a minimum of 20 diseased ginger plant i.e 5 shoots, 5 leaves and 10 rhizomes from each ginger fields were collected from a total of 117 ginger farms: Aizawl District 13, Serchhip District 16, Champhai district 24, Kolasib District 11, Mamit district 9, Lunglei district 6, Saitual District 10, Khawzawl 12, Hnahthial 6, Lawngtlai 5, Siaha District 5). The samples were then transported to Research and Instrumentation Center, Department of Zoology, Pachhunga University College, Aizawl, Mizoram for direct isolation, morphological and molecular identification.

4.2.3 Isolation of fungi from samples

The isolation of fungal organisms was performed directly from diseased ginger exhibiting symptoms indicative of soft rot, as elucidated by **Le *et al*. (2014).** The affected segments of the rhizome were meticulously rinsed with continuous tap water, subjected to disinfection using a 1% sodium hypochlorite solution for a duration of five minutes, and subsequently washed multiple times with sterile distilled water to eliminate any residual disinfectant before being sectioned into cubes measuring 2-3 mm utilizing a sterilized surgical scalpel. The isolation of fungal pathogens was accomplished through the application of a tissue-transplanting methodology. Both diseased and healthy ginger tissues were introduced onto 1.5% water agar (WA) plates augmented with 0.3 g/l of ampicillin and gentamicin (Himedia). All inoculated plates were maintained within an incubator (Zeistech LS-116 BOD Incubator) at a controlled temperature of 25 ± 1°C. The cultures were systematically monitored, and the resultant isolated single mycelial hyphae were transferred to potato dextrose agar (PDA, Himedia) medium using the hyphal tip transfer technique for the purpose of purification, as outlined by Leslie et al. (2006). The plates were subsequently incubated again at 25 ± 1°C within a BOD incubator to facilitate the development of pure cultures **(Rosangkima, 2018).**

4.2.4 Identification through morphological identification

The methodological framework employed in this study adheres to a systematic general-to-specific paradigm, which has been meticulously applied to facilitate the identification of fungal isolates, advancing from the broader taxonomic classification of genus down to the more precise determination at the species level. This analytical journey commenced with an examination of cultural characteristics, subsequently transitioning to an intricate analysis of microscopic features that are critical for accurate identification. In a controlled laboratory environment, the fungal isolates were cultivated on Potato Dextrose Agar (PDA) plates, maintained at a stable temperature of 26±1°C for a duration ranging from four to seven days, thereby ensuring optimal growth conditions for the organisms under study. The morphological characteristics that were central to this investigation primarily encompassed the examination of both spore and colony characteristics, which serve as vital indicators of fungal identity and classification, as corroborated by the findings of **Leslie et al. (2006)** and **Gerlach (1982).**

The macroscopic observations conducted during the study were informed by a thorough assessment of various colony attributes, including but not limited to colony color, pigmentation patterns, and the overall growth rate of the fungal isolates. To quantify the growth of the colonies, the diameter was meticulously recorded on a daily basis over a ten-day period, with three replicates conducted for each measurement to ensure statistical reliability and validity. Following this period, the average mean daily growth, expressed in millimeters per day, was meticulously calculated to ascertain the growth rate of the fungal isolates under the specified conditions. Additionally, pigmentation variations and the specific color of the colonies were taken into account, recognizing their significance in the morphological characterization process. In order to facilitate detailed microscopic observations, the size and shape of both macroconidia and microconidia were carefully examined, with particular attention paid to their distinct structural features. To prepare for these microscopic evaluations, slide cultures of the fungal isolates were systematically created, as described in the methodology outlined by **Aneja *et al*. (2005).**

In the course of the species identification process, reference was made to established illustrative literature that provides a foundation for morphological species identification, as documented in the works of **Leslie (2006), Gerlach (1982)**, and **Seifert (1996)**. The meticulous observations regarding the shapes of microconidia and macroconidia, along with the assessment of septation patterns, were carried out using a compound microscope set to a magnification of 100X, specifically utilizing the Olympus CX21iLEDFS1 model, which allows for high-resolution imaging of fungal structures. This comprehensive and methodologically rigorous approach not only underscores the importance of both macroscopic and microscopic characteristics in fungal taxonomy but also highlights the intricate processes involved in the accurate identification of fungal species within the broader context of mycological research.

4.2.5 In-vitro pathogenicity test

The pathogenicity associated with the identified genera was systematically established through the rigorous methodologies delineated by **Salami and Akintokun, 2008**; **Yusuf and Okusanya, 2008**. The determination of pathogenicity involved meticulously testing the capacity of each genus to induce rot in rhizomes that were both fresh and healthy, thereby ensuring that the experimental conditions were controlled and that the results were as reliable as possible. Fresh rhizomes, specifically those within the age range of 9 to 12 months, were procured from the designated sample collection site ensuring that the material was appropriate for the study.

In preparation for the experiments, the rhizomes underwent a thorough washing process in sterile distilled water, followed by a surface sterilization procedure utilizing a 1% sodium hypochlorite solution, which was then succeeded by a series of five consecutive washes with sterile distilled water to eliminate any potential contaminants. To facilitate the introduction of the mycelial discs, holes with a depth of 1mm were carefully excavated in the rhizome using a sterile cork-borer with a diameter of 1 mm; the plugs that were extracted from these holes were subsequently replaced with 1 mm diameter mycelial discs obtained from potato dextrose agar (PDA), which were then positioned at the bottom of the excavated holes within the fresh rhizomes.

To ensure consistency and compensate for the added thickness of the mycelial discs, a small section of the rhizome plug was judiciously excised prior to the placement of the plug back into the hole. The plug was then carefully reinserted, and the wounded regions were meticulously sealed using sterile agar blocks of approximately 5 mm thickness, thereby effectively preventing any extraneous infections that could compromise the integrity of the experiment. The rhizomes were subsequently weighed to establish a baseline measurement and were then incubated for a period of 15 days at a controlled room temperature of 26 ºC ±1, ensuring optimal conditions for any potential pathogenic activity. For the purposes of the pathogenicity test, a total of three healthy rhizomes were utilized for each genus being tested, thus providing a robust dataset for analysis. A control experiment was executed concurrently, utilizing sterilized PDA discs of identical diameter which were similarly inserted into the holes of healthy rhizomes to serve as a comparative baseline.

Upon reaching the conclusion of the 15-day incubation period, the rhizomes were reweighed to ascertain the pathogenicity index for each genus in question, thus allowing for a quantitative assessment of the pathogenic effects observed. The determination of the pathogenicity index was carried out using the expression as specified by **Salami and Akintokun in the year 2008**, which provided a standardized method for interpreting the results obtained from the experimental trials conducted.

$$\text{Pathogenicity index (PI)} = 100\text{-}[\frac{WD}{WH} \text{ X } 100]$$

Wherein the variables WD=weight of the disesed rhizome and WH=weight of healthy rhizome respectively, it is essential to maintain precise distinctions between the two in order to facilitate a comprehensive understanding of the comparative analysis.

The rhizomes underwent a meticulous procedure whereby they were systematically severed along the designated plane of inoculation utilizing a scalpel blade that had been subjected to rigorous sterilization protocols, and subsequently, the particular type of rot that manifested as a result of this inoculation was thoroughly characterized to ascertain its specific attributes.

4.3 Results and Discussions

Table 4.1: Sample collection site from 11 districts of Mizoram

District	No. of field surveyed	No of samples collected per field	Part of plant collected		
			Shoot	Leaves	Rhizome
Aizawl	13	20	5	5	10
Serchhip	16	20	5	5	10
Champhai	24	20	5	5	10
Kolasib	11	20	5	5	10
Mamit	9	20	5	5	10
Lunglei	6	20	5	5	10
Saitual	10	20	5	5	10
Khawzawl	12	20	5	5	10
Hnahthial	6	20	5	5	10
Lawngtlai	5	20	5	5	10
Siaha	5	20	5	5	10

Samples of ginger were meticulously collected from a variety of geographical regions within the state of Mizoram, with a specific emphasis placed on the examination of the shoots, leaves, and rhizomes of plants that exhibited signs of infection. The visual symptoms that were systematically observed during the collection process encompassed a range of pathological manifestations, including but not limited to leaf blight, a pronounced yellowing of the foliage, wilting of the shoots, and rot affecting the rhizomes. The most frequently encountered symptoms comprised of the appearance of brown necrotic lesions on the leaves, the wilting phenomena observed in the shoots, as well as the soft rot that was prevalent in the rhizomes, all of which are indicative of a fungal infection. The samples that were gathered were meticulously categorized based on the severity of the infection present, and they were subsequently stored under sterile conditions to facilitate further morphological

examinations and molecular analyses that are essential for understanding the nature of the pathogens involved.

The methodical collection of samples from various parts of the ginger plant ensured that there was a comprehensive representation of the different sites affected by infection, thereby allowing for a more thorough understanding of the disease dynamics. The predominance of infections located in the rhizomes can likely be attributed to the presence of soil-borne fungal pathogens, such as Fusarium spp. and Pythium spp., which are known to thrive under the moist soil conditions that characterize the agricultural landscape of Mizoram.

Infections affecting the leaves and shoots, which are often considered to be secondary in nature, suggest a potential for systemic spread of the pathogen or could be indicative of surface contamination from the surrounding environment. This observation is consistent with findings from previous studies that underscore the rhizome as being the primary point of infection in ginger crops. The identification of symptomatic plants at the field level served to provide initial insights into the diversity of pathogens present, which will be further validated through rigorous laboratory analyses conducted in the future.

This study highlights the critical importance of employing a multi-part sampling approach, which is instrumental in tracing the progression of pathogens that affect ginger, and it serves as a foundational basis for the development of effective strategies aimed at managing diseases that threaten ginger crops.

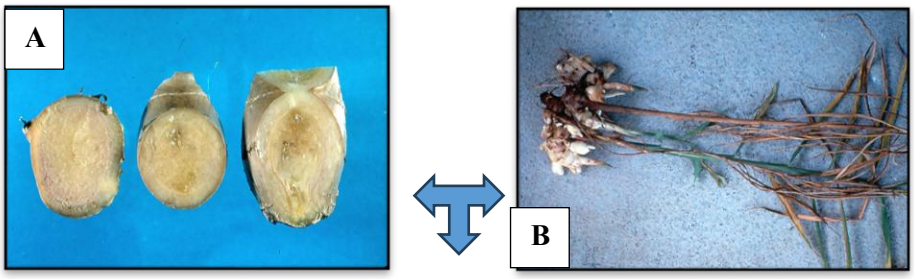


Fig 4.1: (A-B) Ginger rhizome exhibiting symptoms of rotting and wilting induced by fungal pathogens.

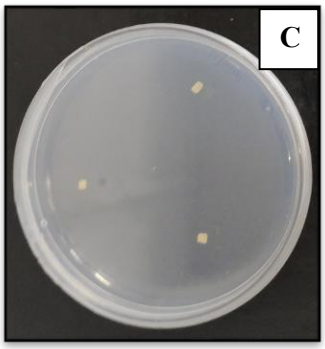


Fig 4.2: (C) Explant of ginger rhizome, leaves and shoots inoculated onto 1.5% water agar (WA).

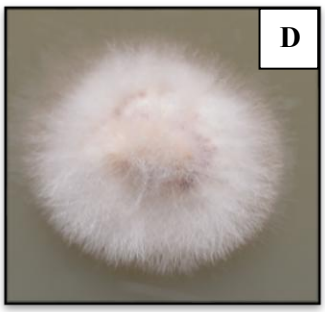


Fig 4.3: (D) Emergence of mycelial hyphae on water agar, transferred to Potato Dextrose Agar (PDA)

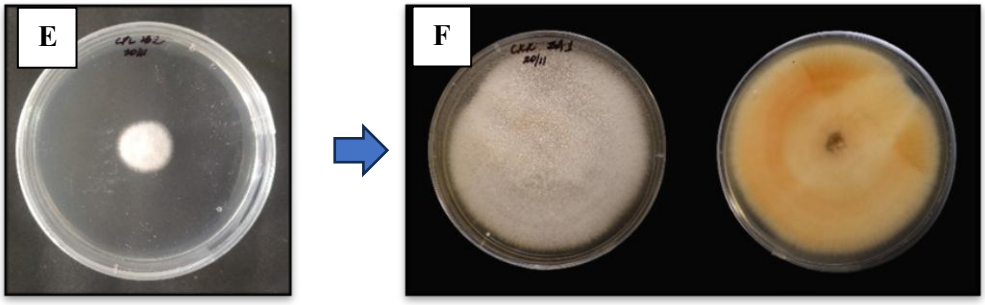
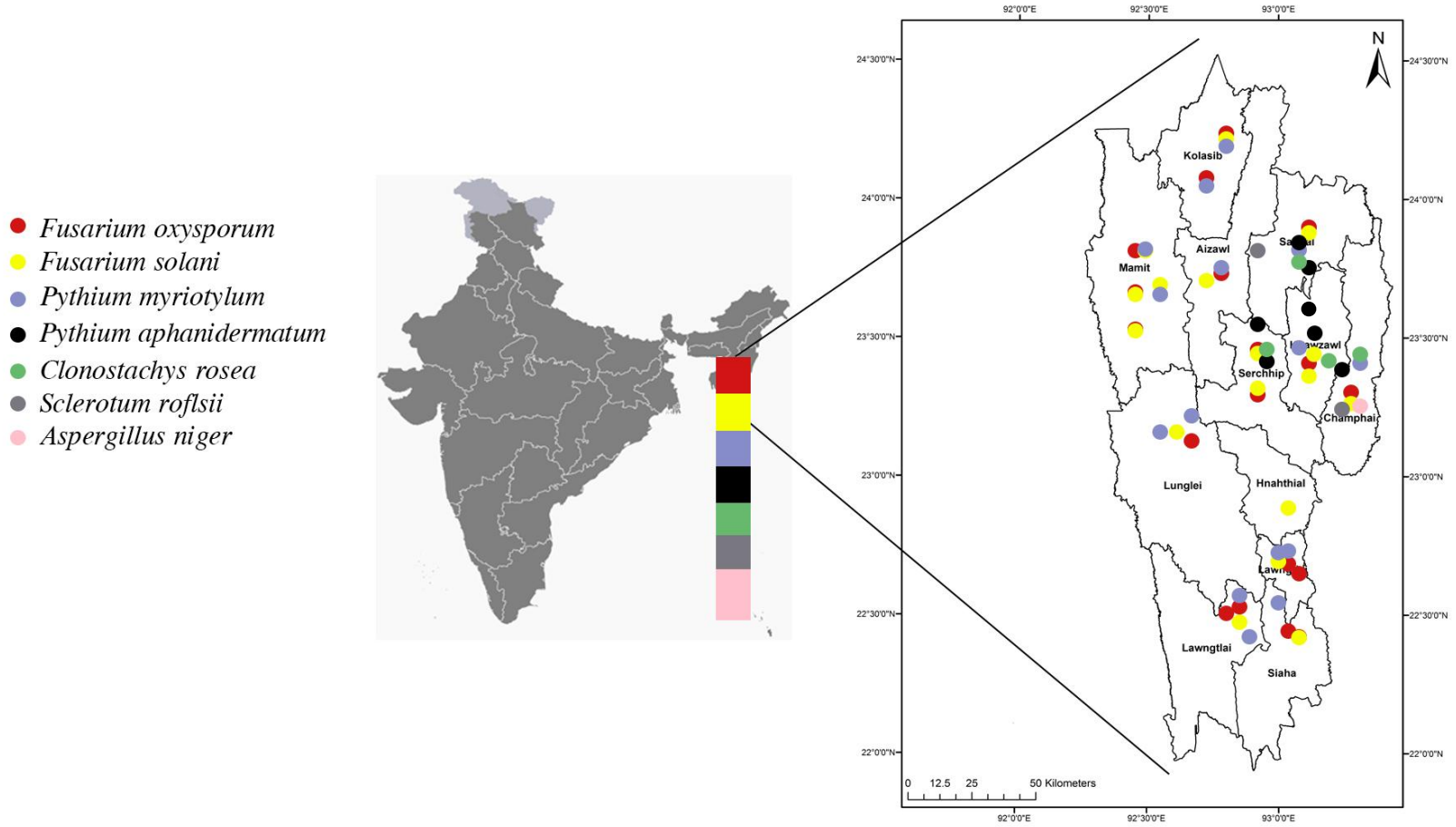


Fig 4.4: (E-F) Pure cultures of fungi isolated from diseased ginger incubated at 26ºC

Fig 4.5: Fungal distribution from different ginger growing districts of Mizoram.



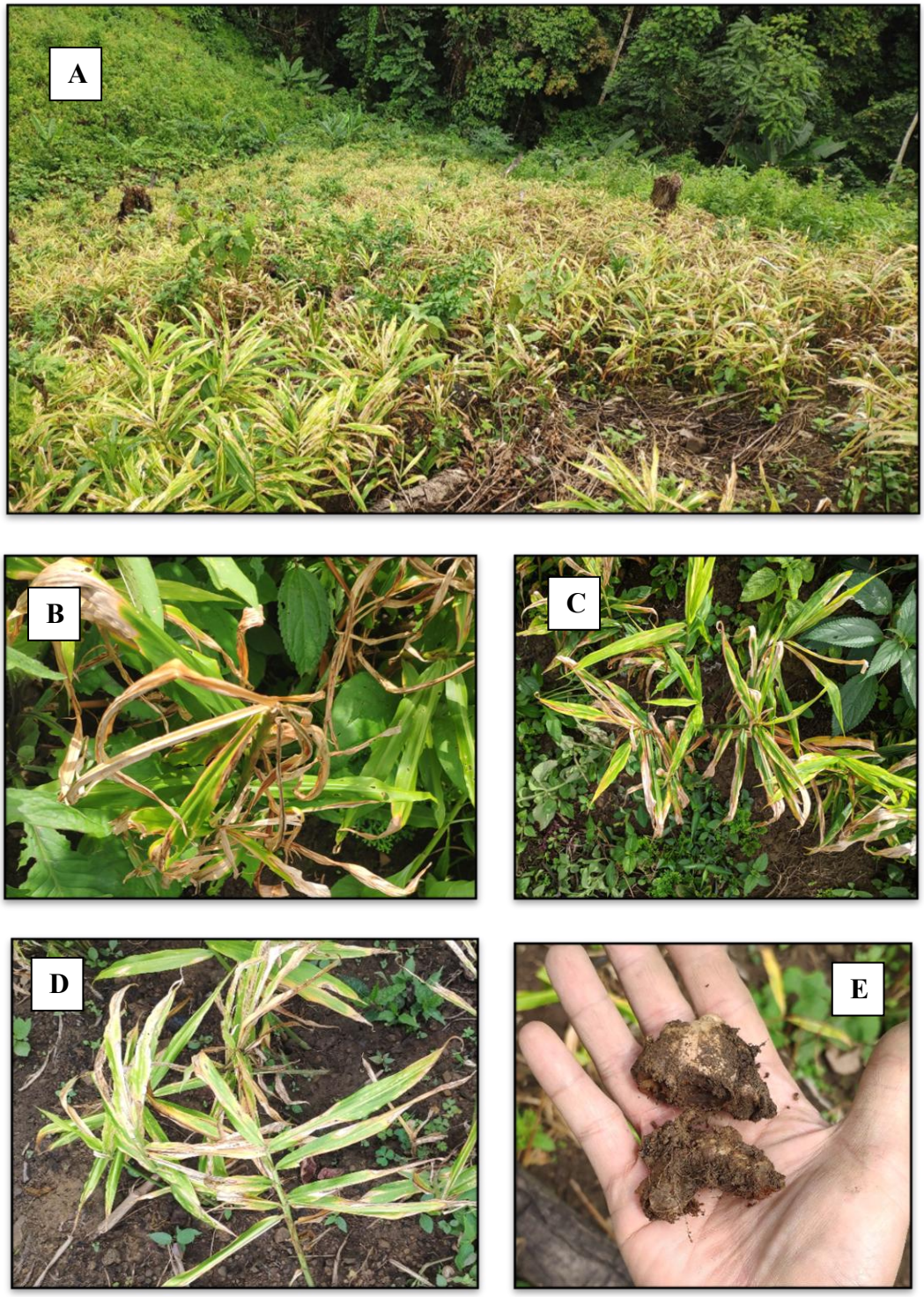


Fig 4.6: Ginger plantation exhibiting symptoms of wilting induced by fungal pathogens. (A, B, C, D); Soft rot of ginger rhizome induced by fungal pathogens. (E)

Table 4.2 : Microscopic and macroscopic stuidies of collected fungal isolates from ginger of Mizoram

Microscopic	*F.oxysporum*	*F.solani*	*P.myriotylum*	*P.aphanidermatum*	*C. rosea*	*N. intermedia*	*S.rolfsi*	*A.niger*
No. of isolates	*108*	*112*	*205*	*198*	*4*	*3*	*2*	*2*
Macroconidia-								
Length (µm)	3-septate = 27.4±1.2.	3-septate = 31.2±1.3.	1-septate = 8.2±1.8.	Non-septate 22-27±0.2	2.9±0.3	3-7 Septate 24.5±1.2	Septate, 5 to 12 µm.	3.5-5
Width (µm)	3-septate = 5.6±0.6.	3-septate = 5.4±0.5.	3.2±0.7		1.6±0.1	13±1	1.0-1.5	
Shape	Oval to kidney-shaped,, ellipsoidal	Curved with pointed basal cells, ellipsoidal	Elipsoidal, hyaline and,smooth	lobate sporangia, globose, smooth	smooth-walled, hyaline and globose to obliquely round in shape	hyaline, becoming yellow-brown, then dark brown and opaque at maturity	hyaline, Globose, smooth surfaced scleroti	globose to subglobose
Conidial septation	3-6 septate	3-7 septate	1-2 septate	Non-septate	Multiple septation	Multiple septation	Multiple septation	-
Common septation	3-septate (57%)	3-septate (61%)	1-septate (82%)	Non-septate	4-septate (72%)	5 septate (51%)	3 septate (51%)	-
Macroscopic								
Colony colour[a]	White to creamy white	White-creamy	White	White	White to light orange pink	orange to sordid buff	White	White to black
Pigmentation[b]	White with creamy whiite, yellow to orange zonation	White creamy with yellow to orange zonation	White to creamy white	White and irregular margins with radial growth pattern	Dull pale, yellow to salmon	yellow to orange zonation	white to buff white	white to pale black
Growth rates[c]	6.3±0.4 mm	4.3±0.3 mm	40±0.4mm	50±0.1	2.2±0.2mm	23±1.1	3.3±0.6	4.3±1.2

[a]Colony colour were determined by observing the upper surface of the colony . [c]Growth rates were taken after 3 days of incubation at 25 °C.
[b]Pigmentation were determined by observing the lower surface of the colony

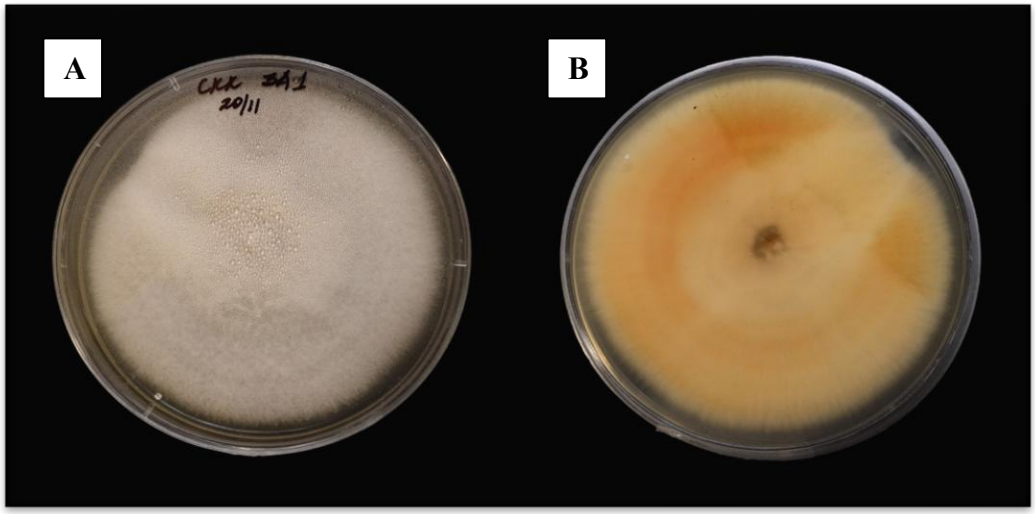


Fig 4.7: Colony picture-front (A) and back (B) of *Fusarium oxysporum*

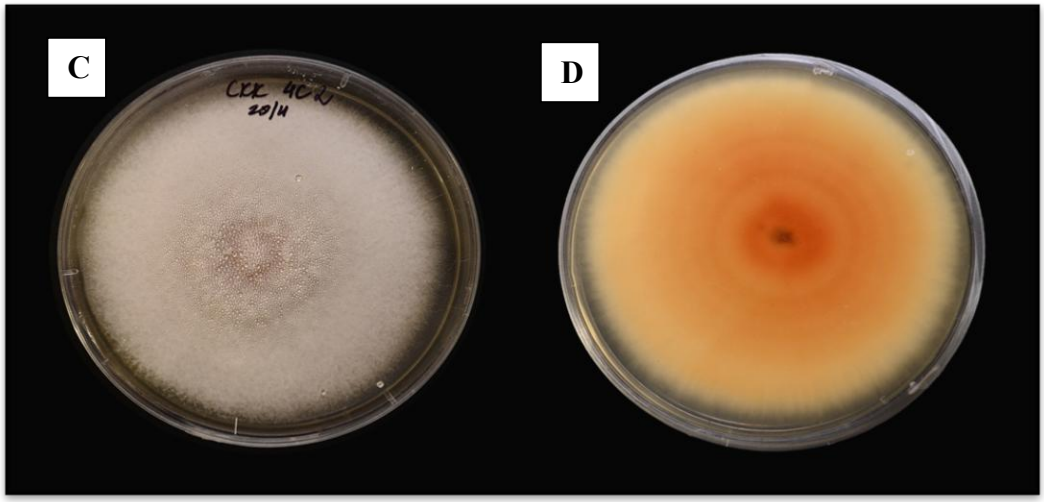


Fig 4.8: Colony picture-front (C) and back (D) of *Fusarium solani*

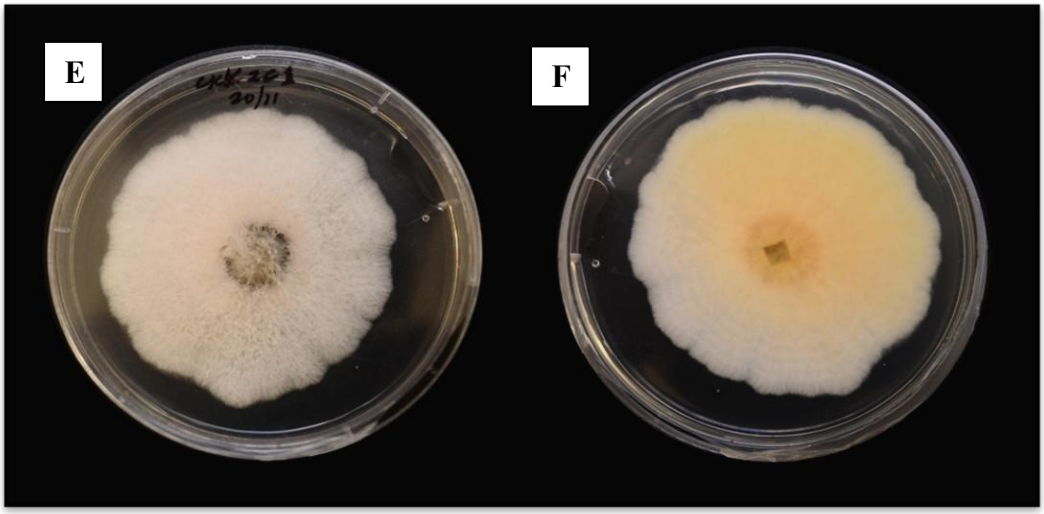


Fig 4.9: Colony picture-front (E) and back (F) of *Sclerotium rolfsii*

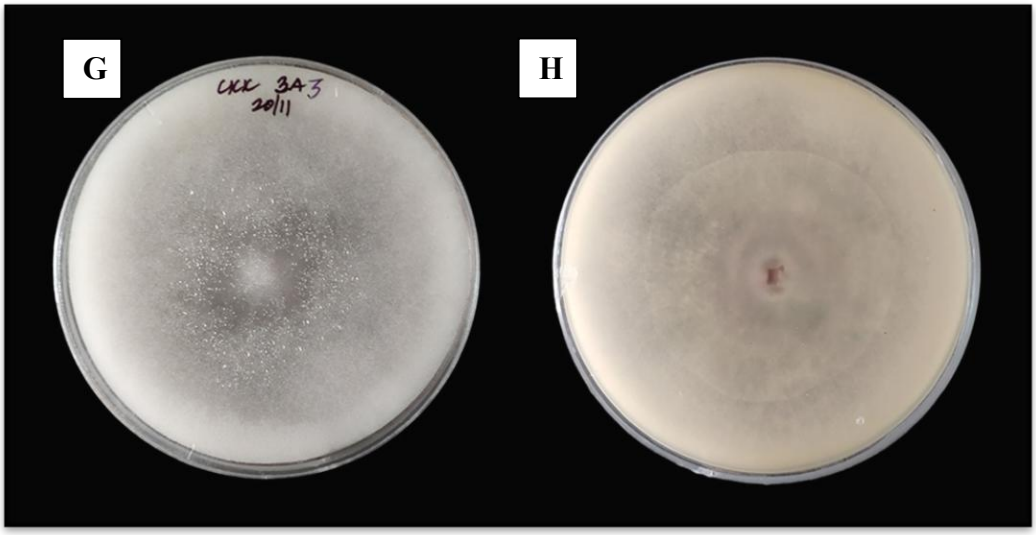


Fig 4.10: Colony picture-front (G) and back (H) of *Pythium myriotylum*

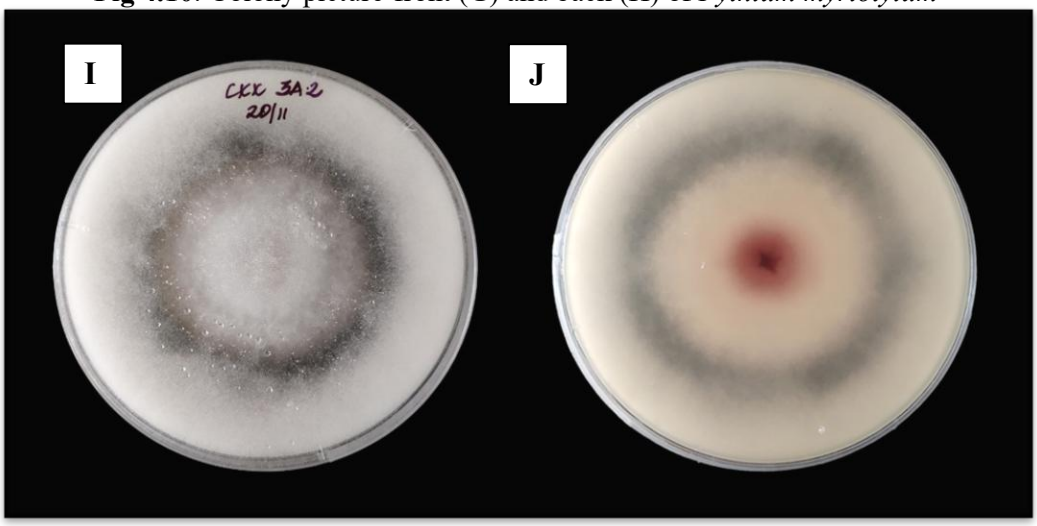


Fig 4.11: Colony picture-front (I) and back (J) of *Pythium aphanidermatum*

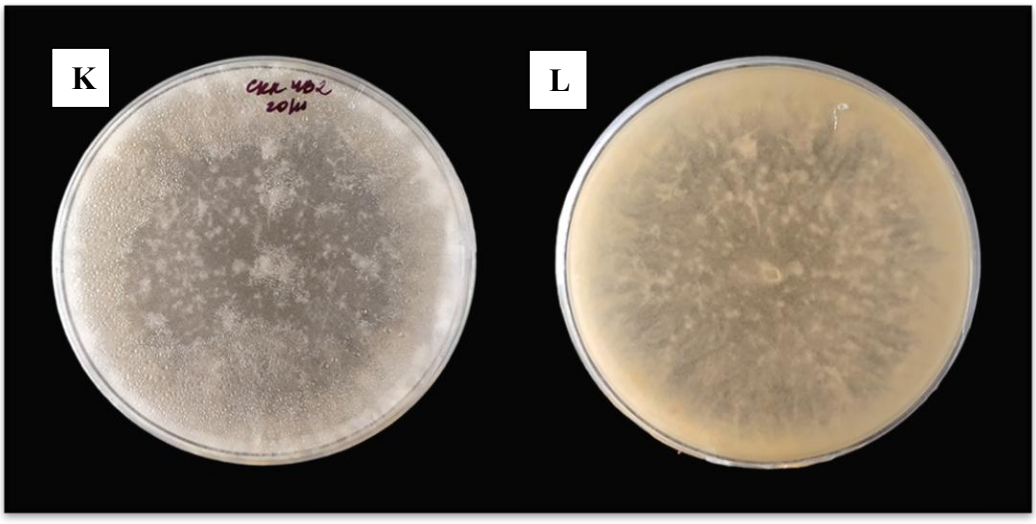


Fig 4.12: Colony picture-front (K) and back (L) of *Neurospora intermedia*

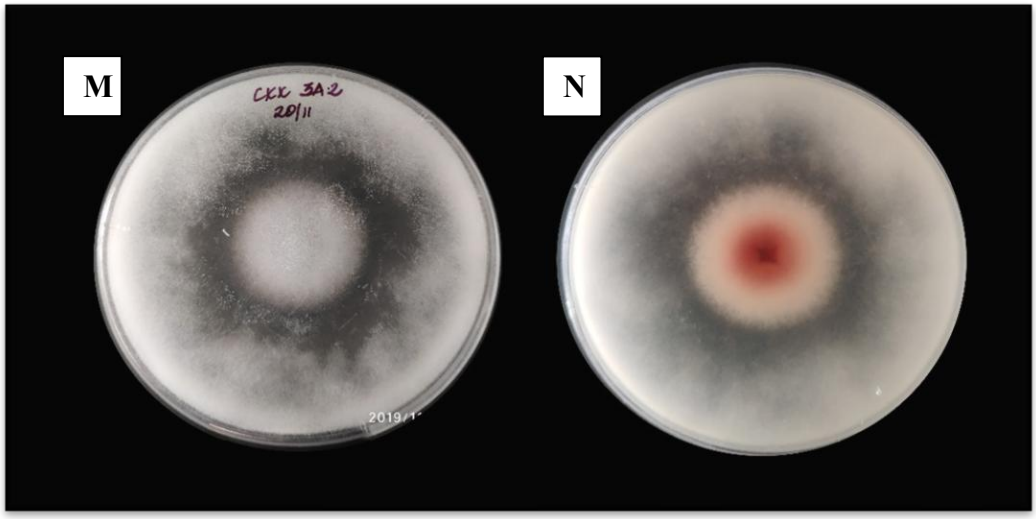


Fig 4.13: Colony picture-front (M) and back (N) of *Clonostachys rosea*

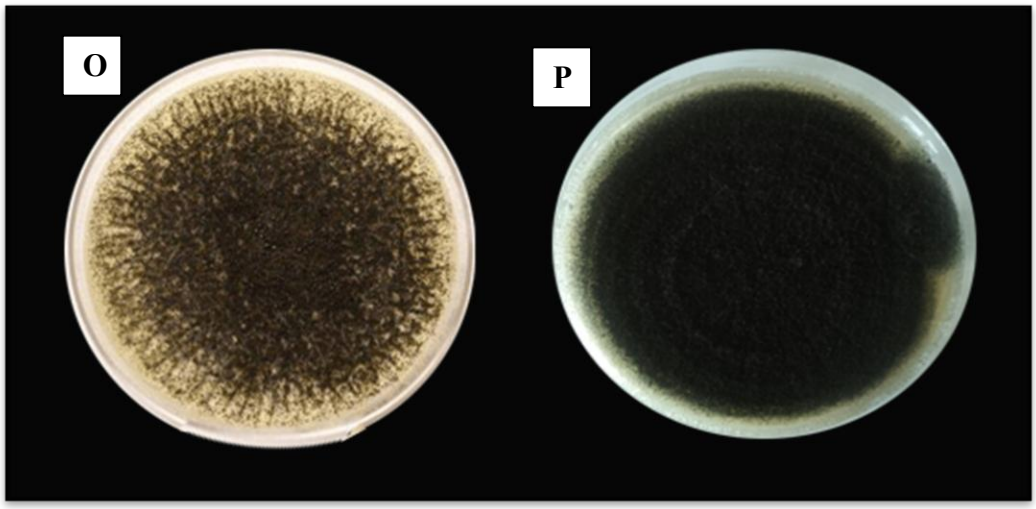


Fig 4.14: Colony picture-front (O) and back (P) of *Aspergillus niger*

Table 4.3 Prevalance of fungal isloates form collected ginger specimens from different districts of Mizoram.										
Districts	**Village**	**Isolate Code**	***F. solani***	***F. oxysporum***	***P. myriotylum***	***P. aphanidermatum***	***C. rosea***	***N. Intermedia***	***S. rofslii***	***A.niger***
Aizawl	**Saikhamakawn**	**AZLSKK**	+	-	-	-	-	-	-	-
	Hlimen	**AZLHL**	+	-	-	-	-	-	-	-
	Samtlang	**AZLST**	+	+	-	-	-	-	-	-
	Lungleng-I	**ALLL1**	-	-	-	-	-	-	-	-
	Lungleng 2	**AZLLL2**	+	-	-	-	-	-	-	-
	Hualngohmun	**ALZHH**	-	+	-	-	-	-	-	-
	Melthum	**AZLMT**	+	-	-	-	-	-	-	-
	Melriat	**AZLMR**	+	-	-	-	-	-	-	-
	Kelsih	**AZLKS**	-	-	-	-	-	-	-	-
	Muallungthu	**AZLMLT**	+	-	-	-	-	-	-	-
	Falkawn	**AZLFK**	-	+	-	-	-	-	-	-
	Tachhip	**AZLTC**	+	-	-	-	-	-	-	-
	Aibawk	**AZLAB**	-	-	-	-	-	-	-	-
Serchhip	**Pangzawl**	**SCPZ**	+	+	+	+	-	-	-	-
	Serchhip	**SCSC**	+	+	+	+	-	-	-	-
	Serchhip AOC	**SCAOC**	+	+	+	+	-	-	-	-
	Serchhip P&E	**SCPE**	+	+	-	-	-	-	-	-
	Keitum 1	**SCKT1**	+	+	-	+	+	-	-	-
	Keitum 2	**SCKT2**	+	-	+	+	-	-	-	-
	E. Lungdar	**SCEL**	+	-	+	-	-	-	-	-
	Khawlailung	**SCKLL**	+	+	+	+	-	-	-	+
	Chhiahtlang	**SCCTL**	+	+	-	+	-	-	-	-
	Chhingchhip	**SCCTL**	+	-	+	+	-	-	-	-
	Hualtu	**SCHT**	+	+	+	+	-	-	-	-
	Thenzawl 1	**SCTZ1**	+	-	-	+	-	-	-	-
	Neihloh	**SCNL**	+	+	+	-	-	-	-	-
	Thenzawl 2	**SCTZ2**	+	+	+	+	-	-	+	-
	E. Lungdar 1	**SCEL1**	+	+	-	+	-	-	-	-
	E. Lungdar 2	**SCEL2**	+	+	+	+	-	-	-	-

Districts	Village	Isolate Code	*F. solani*	*F. oxysporum*	*P. myriotylum*	*P. aphanidermatum*	*C. rosea*	*N. Intermedia*	*S. rofslii*	*A.niger*
Champhai	Kahrawt	CKH	+	+	+	+	-	-	-	-
	Zote	CZT	+	+	-	-	-	-	-	-
	Aiduzawl	CTADZ	+	-	+	-	-	-	-	-
	S. Khawbung	CSK	+	-	+	-	-	-	-	-
	Dungtlang	CDT	+	-	-	+	-	+	-	-
	Tualcheng	CTC	+	-	-	+	-	-	-	-
	Thekte	CTT	+	+	+	+	-	-	-	-
	Kelkang	CKK	+	+	+	-	-	-	-	-
	Dinthar	CDT	+	+	-	+	+	-	-	-
	Thekte	CTK	+	+	+	-	-	-	-	-
	Vanzau	CVZ	+	+	+	+	-	-	-	-
	Farkawn	CFK	+	+	+	+	-	-	-	-
	Vangchhia	CVC	+	+	+	-	-	-	-	-
	Sesih	CSS	+	+	+	+	-	-	-	-
	Tuipui	CTP	+	+	+	+	-	-	-	-
	Khuangleng	CKL	+	+	+	+	-	-	-	-
	Hruaikawn	CHK	+	+	+	-	-	-	-	-
	IB Veng	CIB	+	+	+	+	-	-	-	-
	Lungphunlian	CLPL	+	+	+	+	-	-	-	-
	Vanzau	CVZ	+	+	+	+	-	-	-	-
	Lungtan	CLT	+	+	+	+	-	-	-	-
	Chiahpui	CCP	+	+	+	-	-	-	-	-
	Dilkawn	CDK	+	+	+	-	-	-	-	-
	Khuangphah	CKP	+	+	+	-	-	-	-	-
Khawzawl	Kawlkulh 1	CKK1	+	+	+	+	-	-	-	-
	Kawlkulh 2	CKK2	+	+	+	+	-	-	-	-
	Kawlkulh 3	CKK3	+	+	+	+	-	+	-	-
	Kawlkulh 4	CKK4	+	+	+	-	-	-	-	-
	Dulte	CCD	+	+	+	+	-	-	+	-
	Puilo	CPL	+	+	+	+	+	-	-	-
	Vanchengpui	CVC	+	+	+	+	-	-	-	-

Districts	Village	Isolate Code	*F. solani*	*F. oxysporum*	*P. myriotylum*	*P. aphanidermatum*	*C. rosea*	*N. Intermedia*	*S. rofslii*	*A.niger*
Khawzawl	Chhawrtui	CCT	+	+	+	-	-	-	-	-
	Chawngtlai	CCTL	+	+	+	+	-	-	-	-
	Biate	CBT	+	+	+	+	-	-	-	-
	Sialhawk	CSH	+	+	+	+	-	-	-	-
	Khawzawl 1	CCK1	+	+	+	+	-	-	-	-
Saitual	Saitual 1	CST1	+	+	+	-	-	-	-	-
	Saitual 2	CST2	+	+	+	+	-	-	-	-
	Saitual 3	CST3	+	+	+	+	-	+	-	-
	Rulchawm	CRC	+	+	-	+	-	-	-	+
	Lenchim	CLC	+	+	+	-	-	-	-	-
	Keifang	CKF	+	+	-	+	-	-	-	-
	Ruallung	CRLL	+	+	-	+	+	-	-	-
	Mualpheng	CMP	+	+	+	+	-	-	-	-
	Tawizo	CTZ	+	+	+	+	-	-	-	-
	Maite	CMT	+	+	+	-	-	-	-	-
Kolasib	N. Hlimen	CNH	+	-	-	+	-	-	-	-
	Thingthleh	CTT	-	-	+	-	-	-	-	-
	Lungdai	CLD	-	-	-	-	-	-	-	-
	Serkhan	CSK	+	-	-	-	-	-	-	-
	Nisapui	CNSP	+	-	+	-	-	-	-	-
	Zanlawn	CZL	-	+	+	-	-	-	-	-
	Bilkhawthlir	CBKT	-	+	+	-	-	-	-	-
	Kawnpui	CKP	+	+	+	-	-	-	-	-
	Bairabi	CBB	-	-	+	-	-	-	-	-
	Lungmuat	CLM	-	-	+	-	-	-	-	-
Mamit	Chuhvel	CHV	+	-	+	-	-	-	-	-
	Suarhliap	CSH	+	+	+	-	-	-	-	-
	Saikhawthlir	CSKT	+	+	-	-	-	-	-	-
	Zamuang	CZM	+	-	-	-	-	-	-	-
	Rengdil	CRD	-	-	-	-	-	-	-	-
	Kawrthah	CKT	+	-	-	+	-	-	-	-

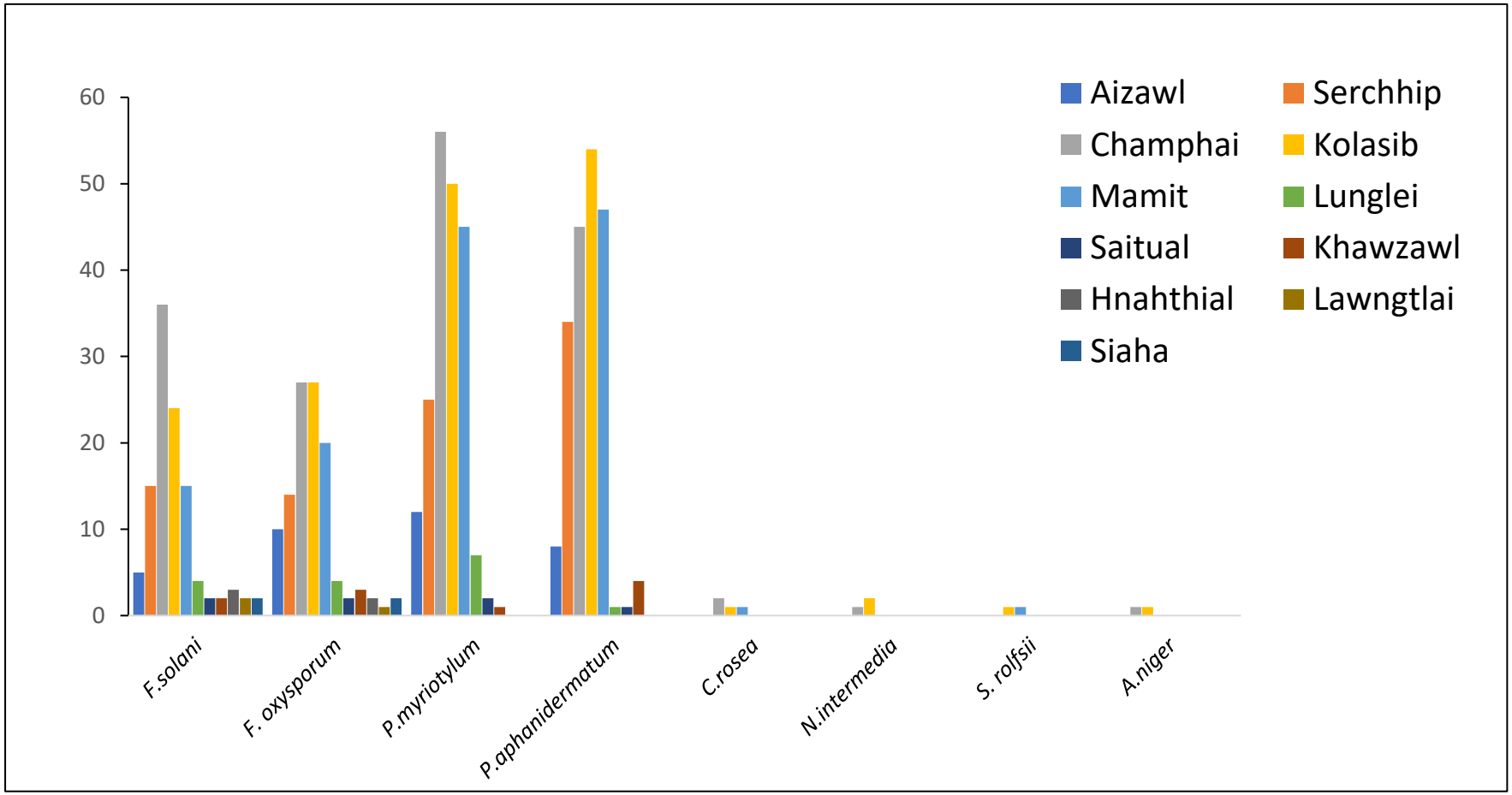
Districts	Village	Isolate Code	*F. solani*	*F. oxysporum*	*P. myriotylum*	*P. aphanidermatum*	*C. rosea*	*N. Intermedia*	*S. rofslii*	*A.niger*
Mamit	Vawngawnzo	CVAZ	+	-	+	-	-	-	-	-
	Damdiai	CDD	+	-	-	-	-	-	-	-
	Kananthar	CKTT	+	-	-	-	-	-	-	-
Lunglei	Haulawng	CHL	-	-	-	+	-	-	-	-
	Ramlaitui	CRLT	+	-	+	+	-	-	-	-
	Thangpui	CTP	+	-	-	-	-	-	-	-
	Mualthuam	CMT	-	+	-	+	-	-	-	-
	Chhipphir	CCPP	-	+	-	+	-	-	-	-
	Sekhum	CSK	+	+	-	-	-	-	-	-
Hnahthial	Leite 1	CLT1	-	+	-	+	-	-	-	-
	Leite 2	CLT2	+	+	-	-	-	-	-	-
	New Ngharchhip 1	CNC1	-	+	-	+	-	-	-	-
	New Ngharchhip 2	CNC2	-	+	+	+	-	-	-	-
	Hnanhthial 1	CHT1	-	+	+	-	-	-	-	-
	Hnanthial 2	CHT2	+	+	+	-	-	-	-	-
Lawngtlai	Lawngtlai 1	CLT1	-	+	+	+	-	-	-	-
	Lawngtlai 2	CLT2	-	+	-	-	-	-	-	-
	Lawngtlai 3	CLT3	-	-	-	-	-	-	-	-
	Lawngtlai 4	CLT4	-	-	-	-	-	-	-	-
	Lawngtlai 5	CLT5	+	-	-	-	-	-	-	-
Siaha	Siaha 1	CSH1	-	+	+	-	-	-	-	-
	Siaha 2	CSH2	-	-	-	-	-	-	-	-
	Siaha 3	CSH3	+	-	+	-	-	-	-	-
	Siaha 4	CSH4	-	-	-	-	-	-	-	-
	Siaha 5	CSH5	+	-	-	-	-	-	-	-
Total			**110**	**112**	**205**	**198**	**4**	**3**	**2**	**2**

Table 4.4 : Percentage incidence of fungal pathogens from different districts of Mizoram.

Percentage incidence of fungal isolates per district								
Districts	**No of species isolated per disrict**							
	F.oxysporum	***F.solani***	***P. myriotylum***	***P. aphanidermatum***	***C. rosea***	***N.intermedia***	***S.rolfsii***	***A.niger***
Aizawl	5	10	12	8	-	-	-	-
Serchhip	15	14	25	34	-	-	-	-
Champhai	**36**	**27**	**56**	45	**2**	1	-	1
Khawzawl	24	**27**	50	**54**	1	**2**	1	1
Saitual	15	20	45	47	1	-	1	-
Kolasib	4	4	7	1	-	-	-	-
Mamit	2	2	2	1	-	-	-	-
Lunglei	2	3	1	4	-	-	-	-
Hnahthial	3	2	-	-	-	-	-	-
Lawngtlai	2	1	-	-	-	-	-	-
Siaha	2	2	-	-	-	-	-	-
Total isolates	**110**	**112**	**198**	**198**	**4**	**3**	**2**	**2**
Percentage incidence	**F.oxysporum**	**F.solani**	**P. myriotylum**	**P. aphanidermatum**	**C. rosea**	**N.intermedia**	**S.rolfsii**	**A.niger**
Aizawl	1.92	3.85	4.62	3.08	-	-	-	-
Serchhip	4.69	4.38	7.81	10.63	-	-	-	-
Champhai	7.50	5.63	11.67	9.38	0.42	-	-	0.21
Khawzawl	**10.00**	**11.25**	20.83	22.50	0.42	**0.83**	0.42	**0.42**
Saitual	7.50	10.00	**22.50**	**23.50**	**0.50**	0.50	**0.50**	-
Kolasib	1.82	1.82	3.18	0.45	-	-	-	-
Mamit	1.11	1.11	1.11	0.56	-	-	-	-
Lunglei	1.67	2.50	0.83	3.33	-	-	-	-
Hnahthial	2.50	1.67	2.50	2.50	-	-	-	-
Lawngtlai	2.00	1.00	1.00	1.00	-	-	-	-
Siaha	2.00	2.00	2.00	-	-	-	-	-

Percentage Incidence (PI)=Total no. of isolates/Total no of plant

Fig 4.15 : District wise occurrence and distribution of fungal isolates from different districts of Mizoram



A total of six distinct species of fungal pathogens were meticulously isolated from the ginger cultivation fields located in the region of Mizoram, which include the following: *Fusarium solani, Fusarium oxysporum, Pythium myriotylum, Pythium aphanidermatum, Clonastachys rosea, Nuerospora intermedia, Sclerotium roflsii*, and *Aspergillus niger*. Among the eleven districts that were rigorously examined during the study, it was observed that Champhai, Serchhip, Saitual, and Khawzawl were predominantly afflicted with significant symptoms that were indicative of infections by *F. solani*, *F. oxysporum*, *P. myriotylum*, and *P. aphanidermatum*, which are known to severely impact the health of ginger crops. These particular districts are recognized as the primary ginger-growing regions in Mizoram, where the agricultural practices are heavily focused on the cultivation of this economically important crop.

In Khawzawl District, the most pronounced levels of infection were recorded for *F. solani*, which exhibited an alarming infection rate of 11.25%, while *N. intermedia* and *A. niger* showed lower but still notable infection rates of 0.83% and 0.42%, respectively. Conversely, in Saitual District, the fungal pathogen *P. myriotylum* was observed to have a strikingly high infection prevalence of 22.50%, accompanied by *P. aphanidermatum*, which demonstrated an even higher infection rate of 23.50%, while *C. rosea* and *S. roflsii* were found to have lower infection rates of 0.50% each. Additionally, the presence of rhizome rot, attributed to Pythium species, was notably found to be associated with infestations caused by rhizome flies of the *Mimegralla* species, which were present in sporadic patches across nearly all of the surveyed districts.

It is, however, important to highlight that no significant association between the infestation of fusarium species and the presence of rhizome flies was detected during the course of this study, indicating a potential divergence in the ecological interactions between these pathogens and their respective insect vectors.

Invitro-Pathogenecity test

All of the various fungal genera that were examined in this study were determined to exhibit pathogenic characteristics when introduced to fresh ginger rhizomes, with the pathogenicity indices exhibiting a notable range that spanned from 10.56% for the specific strain of *Neurospora intermedia*, all the way up to a much higher pathogenicity index of 33.24% for another strain of *Pythium aphanidermatum*, which was observed at Champhai district, as illustrated in Table 4.6. Among the fungal genera analyzed, both *Pythium myriotylum* and *Pythium aphanidermatum* were found to possess relatively elevated pathogenicity indices of 33.24% and 30.73%, respectively, while *Fusarium* was noted to have a slightly lower pathogenicity index of 25.78% for *Fusarium solani* and 23.65% for *Fusarium oxysporum*, indicating a significant variance in the level of pathogenicity exhibited by these fungal species.

The pathogenicity assessments that were conducted unequivocally demonstrated that every fungal isolate derived from the ginger rhizomes that had undergone spoilage was capable of inducing rot in otherwise healthy fresh ginger rhizomes, thereby implicating these fungal pathogens in the deterioration process of ginger. Notably, *Pythium* spp. was identified as the fungal genus responsible for a comparatively greater degree of ginger deterioration when the rhizomes were subjected to conditions related to drying and storage, while *Fusarium* spp. was found to be particularly detrimental, indicating a differential impact based on environmental factors. In addition to the aforementioned fungi, *Sclerotium rolfsii* and *Clonostachys rosea* also contributed significantly to the deterioration of ginger, although to a lesser extent compared to *Fusarium* and *Pythium*, while *Neurospora intermedia* was observed to be the least aggressive in terms of spoilage and rot, suggesting a hierarchy of pathogenicity among these fungal genera.

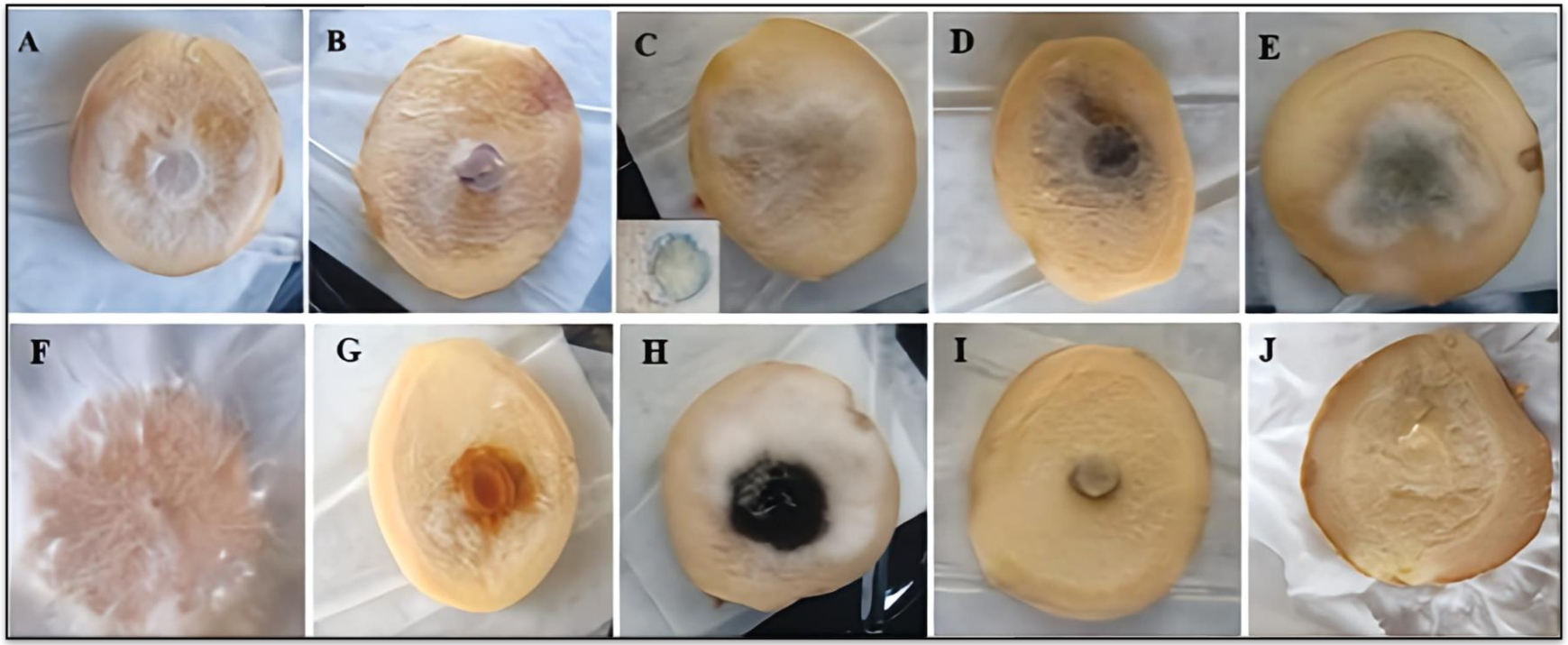


Fig 4.16 (A-J): Results of the pathogenicity tests for colonization of cut ginger.

A. *Fusarium oxysporum*; **B**. *Fusarium oxysporum* **C**. *Fusarium solani* (in sporodochia formation); **D**. *Pythium myriotylum*; **E**. *Pythium aphanidermatum*; **F.** *Clonostachys rosea*; **G** *Sclerotium rolfsii*; **H**. *Aspergillus niger*; **I**. *Neurospora intermedia*; **J**. *Control*

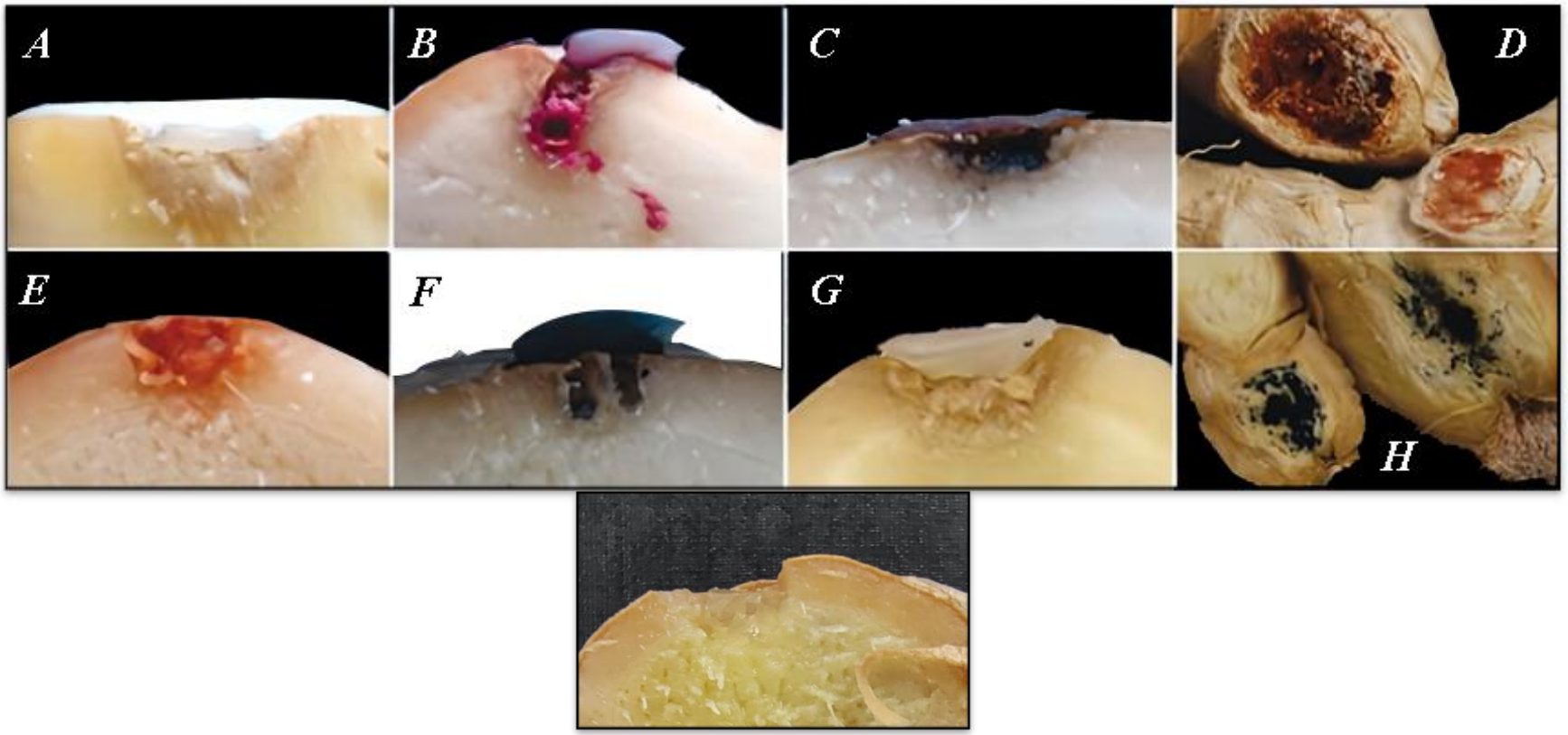


Fig 4.17 (A-I): Results of the pathogenicity tests for colonization of the punctured surface and rot of ginger rhizomes inoculated with mycelial discs with a crosssectional view of the rhizomes inoculated with **A.** *F.oxysporum;* **B.** *F.* solani **C.** *P. myriotylum;* **D.** *P. aphanidermatum;* **E.** *C,rosea* **F.** *S. rolfsii ;* **G**. *N.intermedia;* **H.** *A. niger;* **I.** *Control*

Table 4.5 : Pathogenicity test of fungi isolated from ginger rhizomes		
Fungal Species	**Pathogenicity Index (%)**	**Characteristics of Rhizome rot**
F.oxysporum	20.83	Dry rot, yellow margin
	23.65	Wet rot, brown color at the margin
	22.33	Dry rot , pale brown
	1.63 (Control)	No rot induced
F. solani	22.17	Dry rot, dark brown
	21.02	Dry rot, brown at the margin
	25.78	Dry rot, pale to dark brown
	1.33 (Control)	No rot induced
P.myriotylum	**30.73**	Wet rot, brown color at the margin
	25.36	Wet rot, brown to dark brown
	25.78	
	1.1 (Control)	No rot induced
P. aphanidermatum	29.63	Wet rot, brown color at the margin
	33.24	Wet rot, brown to dark brown
	27.14	
	1.55 (Control)	No rot induced
C.rosea	**17.75**	Dry rot, tellow margin
	16.33	
	12.4	
	1.87 (Control)	No rot induced
N.intermedia	**11.23**	Dry rot, yellow margin
	10.56	
	10.89	
	1.24 (Control)	No rot induced
S.roflsii	**19.33**	Dry rot , black margin
	12.32	
	16.52	
	1.39 (Control)	No rot induced
A.niger	20.7	Dry rot, black color
	16.36	
	18.33	
	1.27 (Control)	No rot induced

4.4 Summary and Conclusion

A significant multitude of fungal species have been documented across various regions of the globe as responsible agents in the manifestation of diseases affecting ginger, a widely cultivated and economically important plant. These pathogenic fungi are categorized into several distinct genera, which notably include *Aspergillus*, *Sclerotium, Penicillium, Pythium, Fusarium*, and *Phyllosticta*, as referenced in the scholarly works of **Dake (1995)**, **Pawar *et al.* (2008)**, **Moreira *et al.* (2013)**, and **Meenu and Kaushal (2017)**. In the present investigation, a variety of species belonging to the genera *Fusarium*, *Pythium*, *Neurospora*, *Clonostachys*, *Aspergillus*, and *Sclerotium* were systematically isolated and rigorously identified from ginger samples that exhibited signs of disease, which included not only the leaves and stems but also the rhizomes, in addition to the surrounding soil samples that were collected in proximity to the afflicted plants.

The characteristics pertaining to the colony and spores have proven to be invaluable taxonomic features that aid in the differentiation of fungal species, as articulated by **Johnston and Jones (1997)** and **Photita *et al.* (2005)**. Nevertheless, it is critical to note that certain features, such as the coloration of the colony and the structural attributes of the conidiophores associated with various species, tend to exhibit a degree of overlap, thereby complicating the identification process. Furthermore, it is worth mentioning that individuals within the same species can display a range of morphological variations, including discrepancies in conidial shapes and differences in colony coloration. Consequently, it becomes increasingly evident that relying solely on colony and spore characteristics may not provide a definitive means of distinguishing between different species within this complex fungal kingdom. In general, while it has been feasible to differentiate between individual isolates of fungal specimens based on the morphology of colonies and spores, this method has proven less effective when attempting to distinguish between species that belong to the same genus.

The pathogenicity assays conducted during this research demonstrated unequivocally that all fungal isolates obtained from the leaves, shoots and rhizomes exhibiting symptoms of damage and rotting located at the designated study sites

exhibited the capability to infect and subsequently induce rot in otherwise healthy, fresh rhizomes. In this context, it was observed that *Pythium myriotylum* and *Pythium aphanidermatum* were responsible for a comparatively higher degree of spoilage, which was subsequently followed by the actions of *Fusarium* and *Penicillium*, indicating a spectrum of pathogenic potential among these fungal genera. The relatively low pathogenicity indices recorded for the fungi involved in this study may be attributed to several factors, including the natural microbial activity inherent to ginger, the microaerobic conditions established as a result of sealing with agar blocks, and the fibrous structural characteristics of ginger rhizomes that may hinder fungal invasion.

The pathogenicity exhibited by the genus *Fusarium* aligns with the findings presented by **Stirling (2004)**, who undertook similar pathogenicity assessments and documented that both *Fusarium oxysporum* and other species within the *Fusarium* genus were indeed pathogenic to ginger rhizomes. Furthermore, the pathogenicity investigations conducted by **Yusuf and Okusanya (2008)** on *Sclerotium rolfsii*, *Aspergillus niger*, and *Clonostachys rosea*, which were isolated from decaying ginger, indicated that these particular fungal species were confirmed to possess pathogenic capabilities towards ginger plants as well. It is noteworthy that despite the fact that *Pythium* spp. and *Fusarium* spp. were identified as being relatively the most pathogenic among the fungi studied, they were also found to be isolated more frequently at the study sites, suggesting a complex interplay between pathogenic potential and prevalence.

Most of the fungi examined in this research exhibited nearly indistinguishable types of rot, predominantly characterized as dry rot, although they did differ in coloration. Specifically, the dry rot induced by *Fusarium solani* and *Fusarium solani* was observed to range in color from pale brown to dark brown, while *Pythium aphanidermatum* and *Pythium aphanidermatum* was noted to develop dry rot that was distinctly covered with a white mould, adding further complexity to the identification process. Aspergillus was responsible for producing dry rot that manifested as a brownish-yellow to dark brown hue, contributing to the diversity of fungal effects on ginger. Similarly, *Neurospora intermedia* was found to induce dry rot that presented a

brown coloration, reinforcing the notion of color as a distinguishing feature among the rot types. In a parallel observation, both *Aspergillus niger* and *Sclerotium rolfsii* produced similar types of dry rot, characterized by a black coloration, demonstrating that even within different genera, there can be overlaps in the types of rot caused. The rots induced by the fungi associated with ginger spoilage in this current study were found to be comparable to the results documented in the work of **Cherian (2002)**, indicating a consistency in findings across different studies in the realm of fungal pathogenicity.

Morphological identification has historically served as the foundational pillar upon which the intricate fields of fungal taxonomy and pathogen diagnosis have been built, providing a framework for understanding various fungal species. This time-honored approach encompasses the examination of observable characteristics, which include, but are not limited to, attributes such as the coloration of fungal colonies, the rate at which they grow, the dimensions of their spores, their distinctive shapes, and the identification of specific reproductive structures that may be present. In particular, when considering the fungal pathogens that pose a threat to ginger (*Zingiber officinale*), such as *Fusarium solani* and *Fusarium oxysporum*; *Pythium aphanidermatum* and *Pythium myriotylum* it is evident that these morphological methods have been widely and extensively employed for both the diagnosis and the classification of these detrimental organisms.

Nevertheless, in spite of its historical relevance and the successes it has achieved, morphological identification is not without its shortcomings; it is plagued by a myriad of limitations that significantly undermine its reliability and the accuracy of its results when it comes to identifying fungi that adversely affect ginger crops. The inherent complexity associated with fungal pathogens, coupled with their considerable genetic diversity, as well as the significant influence exerted by various environmental factors, underscores the pressing need to integrate advanced molecular identification techniques into our diagnostic processes in order to effectively address and surmount these challenges.

CHAPTER 5

Molecular identification and phylogeny of insect pests and isolated fungi from ginger

5.1 General Introduction

5.1.1 Molecular identification of insect pest using Cytochrome c Oxidase Subunit I (*COI*) primers

Molecular identification methodologies are fundamentally dependent upon the use of specific genetic markers that enable the precise differentiation of various species with a high degree of accuracy, which is crucial in numerous biological contexts. Among the myriad of genetic markers utilized in this capacity, the cytochrome c oxidase subunit I (*COI*) gene has notably emerged as the preeminent standard, particularly within the specialized fields of insect taxonomy and the identification of pest species that pose significant challenges to agriculture and biodiversity. The *COI* gene itself is classified as a mitochondrial gene, which encodes for a vital subunit of the enzyme cytochrome c oxidase, a protein that plays an indispensable role in the intricate process of cellular respiration by facilitating the transfer of electrons in the mitochondrial electron transport chain. The efficacy of the *COI* gene in the accurate identification of species has been robustly validated through a multitude of extensive research studies and has subsequently led to its endorsement as the standard genetic marker for DNA barcoding by the Barcode of Life Data Systems (BOLD) initiative, as articulated in the foundational work by **Hebert *et al*. (2003).**

The cytochrome c oxidase subunit I (*COI*) gene is an integral component of the mitochondrial genome, distinguished by its elevated copy number and maternal inheritance patterns. This gene encodes a subunit of cytochrome c oxidase, which serves as a critical enzyme in the process of cellular respiration. *COI* primers are conventionally designed to amplify a region of approximately 650 base pairs (bp) within this gene, commonly referred to as the "barcode region," which encompasses the following characteristics.

The *COI* gene encompasses flanking regions that exhibit a high degree of conservation across a wide array of taxa, facilitating the development of universal primers **(Folmer *et al*., 1994).** These primers, such as those formulated by Folmer et al., demonstrate efficacy across numerous insect orders.

Embedded within the conserved structure, the *COI* gene contains ample variability that enables the differentiation of species, including those that share close phylogenetic relationships. This interplay of conservation and variability is crucial for precise species identification and the conduct of phylogenetic analyses **(Ratnasingham & Hebert, 2007).**

The specific primers designed for the *COI* gene target a highly conserved region of this gene, which significantly aids in the amplification of DNA sequences that exhibit a high degree of variability among different species while simultaneously maintaining a relatively stable sequence within individual species. This unique equilibrium between genetic variability and conservation is precisely what renders *COI* primers exceptionally well-suited for the identification of insect pests, particularly those species that are closely related or are cryptic in nature, thereby complicating traditional identification methods.

Consequently, the implementation of molecular identification techniques utilizing the *COI* gene not only enhances the accuracy of species differentiation but also contributes to a deeper understanding of biodiversity, ecological interactions, and the management of pest populations in various ecosystems. The growing reliance on such molecular tools underscores the importance of integrating advanced genetic methodologies into traditional taxonomic practices to address the challenges posed by rapidly changing environmental conditions and the continuous emergence of new pest species. In summary, the incorporation of the *COI* gene as a benchmark for species identification represents a significant advancement in the field of molecular biology, with far-reaching implications for conservation efforts and pest management strategies worldwide.

Consequently, it is imperative that a comprehensive and rigorous study be conducted to elucidate and clarify this particular issue regarding the diversity of pests.

The primary objective of this study is to determine the species of insect pest that are infesting *Zingiberaceae* in the ginger growing districts of Mizoramm, employing both morphological and molecular characteristics for accurate identification. Moreover, it is important to note that the molecular identification utilizing mitochondrial cytochrome c oxidase subunit I (*mtCOI*) characters on the insect pest of ginger in Mizoram has not been previously undertaken, and the relevant information regarding the base nucleotide sequences has not been located in the National Center for Biotechnology Information (NCBI) GenBank database. Therefore, conducting identification through *mtCOI* analysis is essential to gain a deeper understanding of the phylogenetic relationships among these species within the broader context of *Zingiberaceae* plant interactions.

5.1.2 Molecular identification of fungal pathogens using Internal Transcribed Spacer (*ITS*) primers

The accurate identification of fungi is an essential component in the broader context of comprehending their various ecological functions, the potential pathogenic effects they may exert, and their diverse applications within the field of biotechnology. Conventional methods of morphological identification frequently prove inadequate, primarily due to the significant phenotypic plasticity exhibited by fungi, the existence of cryptic species that can complicate classification, and the inherent limitations associated with visual taxonomic approaches that rely on observable characteristics. Consequently, molecular techniques have become absolutely crucial in this field of study, with the Internal Transcribed Spacer (*ITS*) region of ribosomal DNA (rDNA) standing out as the most prevalently utilized genetic marker for the purpose of accurately identifying fungal species **(Schoch *et al*., 2012).** The primers designed to amplify the *ITS* region are characterized by their high specificity, versatility, and effectiveness, enabling researchers to differentiate between various fungal taxa, which ranges from precise identification at the species level to more complex analyses involving higher-order phylogenetic relationships among diverse fungal groups.

The Internal Transcribed Spacer (*ITS*) region occupies a significant position within the ribosomal DNA (*rDNA*) repeat unit, being situated between the highly

conserved ribosomal RNA genes that correspond to the small subunit, specifically the 18S gene, and the large subunit, which is represented by the *28S* gene. The *ITS* region itself is composed of two distinct variable regions, designated as *ITS1* and *ITS2*, which are strategically separated by the conserved 5.8S *rRNA* gene, thus creating a unique arrangement that combines both conserved and variable genetic segments. This particular configuration of genetic material not only facilitates the binding of primers but also offers an invaluable degree of resolution at the species level, a fact that has been extensively documented in the literature **(White *et al*., 1990).**

The flanking ribosomal genes, namely the 18S and 28S, exhibit a high degree of conservation across various fungal taxa, providing a solid foundation for the design of universal primers, particularly those identified as *ITS1* and *ITS4*. These universally applicable primers are specifically engineered to amplify the entirety of the ITS region, which includes not only *ITS1* and *ITS2* but also the intervening *5.8S* gene, thereby making them exceptionally suitable for a wide array of fungal species **(White *et al*., 1990)**.

The regions designated as *ITS1* and *ITS2* are characterized by significant variability between different fungal species, while simultaneously maintaining a degree of conservation within individual species. This inherent variability is of paramount importance, as it plays a crucial role in the differentiation of closely related taxa and the identification of cryptic species that may otherwise go unrecognized in taxonomic studies **(Schoch *et al*., 2012)**.

The specificity of *ITS* primers is remarkably high, ensuring that they are predominantly applicable to fungal DNA while minimizing the potential for cross-amplification with non-fungal DNA sequences. Such specificity is particularly critical when analyzing mixed samples, which are frequently encountered in environments like soil or plant tissues, where fungal DNA may be present alongside DNA from a myriad of other organisms **(Gardes & Bruns, 1993)**.

The *ITS* region is universally present in all fungal organisms, encompassing a wide range of groups, including *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Chytridiomycota*. This universal characteristic of the *ITS* region enables the utilization

of *ITS* primers across the entire fungal kingdom, thereby facilitating comparative studies and large-scale assessments of biodiversity that are crucial for ecological research **(*Schoch et al*., 2012).**

In terms of taxonomic resolution, the *ITS* region offers the highest level of specificity for species-level identification when compared to other genetic markers, such as the large subunit (LSU) or the small subunit (SSU) ribosomal DNA. This elevated resolution is especially valuable for the identification of various types of fungi, including pathogenic species, endophytes, and mycorrhizal fungi that play essential roles in ecological interactions **(Nilsson *et al*., 2008).**

The abundance of reference sequences associated with the *ITS* region is unparalleled, making it the most extensively sequenced genetic marker for fungi. With millions of *ITS* sequences readily accessible in databases such as GenBank and UNITE, this wealth of reference data allows for the confident matching of newly obtained *ITS* sequences, which is critical for ensuring accurate species identification in mycological research **(Abarenkov *et al*., 2010).**

Beyond merely facilitating species identification, the *ITS* region also serves as a valuable tool for elucidating evolutionary relationships among various fungal taxa. The significant variability present within the *ITS1* and *ITS2* regions enables researchers to construct phylogenetic trees that effectively resolve evolutionary relationships at multiple taxonomic levels, thus contributing to our understanding of fungal evolution and diversity **(Schoch *et al*., 2012)**.

Another noteworthy aspect of *ITS* primers is their robustness, particularly when it comes to amplifying fungal DNA from complex environmental samples, which may include substrates such as soil, plant roots, or even airborne particles. Their effectiveness in detecting fungal DNA, even when present in low concentrations or within mixed samples, renders them invaluable for conducting ecological studies that aim to assess the role of fungi in various ecosystems **(Smith & Peay, 2014).**

The molecular characterization of fungal pathogens in ginger is imperative for comprehending and addressing the maladies that jeopardize its cultivation. Conventional methodologies frequently prove inadequate in identifying cryptic

species and mixed infections; however, molecular techniques, especially those focused on the *ITS* region, facilitate accurate, swift, and dependable identification. This advancement allows for the timely identification of pathogens, guides the formulation of targeted disease management approaches, and bolsters sustainable practices in ginger cultivation. Through the integration of molecular identification with phylogenetic analysis, researchers are able to elucidate the evolutionary interrelations among pathogens, thereby enhancing global initiatives aimed at plant disease management and agricultural sustainability.

5.1.3 Phylogenetic analysis

Phylogenetic trees serve as a fundamental element within the fields of molecular biology and evolutionary studies, providing an illustrative framework that encapsulates the intricate evolutionary connections that exist among various organisms. These trees, which are meticulously constructed from sequences of genes, facilitate researchers in their endeavors to trace the intricate divergences in lineage, comprehend the complex genetic interrelationships, and unravel the extensive evolutionary narrative that characterizes different species throughout history. With the emergence and proliferation of sophisticated molecular methodologies, alongside advancements in bioinformatics, the significance of phylogenetic trees has escalated to an indispensable status across a multitude of scientific disciplines, which encompass systematics, ecology, epidemiology, and conservation biology **(Felsenstein, 1985).**

Through the meticulous examination of both conserved and variable regions present within genetic sequences, phylogenetic trees offer profound insights into the intricate aspects of species taxonomy, the rich tapestry of genetic diversity, and the underlying patterns of evolution that govern life on Earth. Furthermore, these trees are of paramount importance in a variety of practical applications, which include the identification of pathogenic organisms, the investigation of pathways through which diseases are transmitted, and the formulation of informed strategies aimed at conservation efforts. This introduction serves to underscore the critical importance of constructing phylogenetic trees utilizing gene sequences, while accentuating their

diverse applications and overarching significance, all supported by relevant references drawn from scholarly literature that enrich the discourse surrounding this topic.

The construction of phylogenetic trees is fundamentally reliant on the utilization of gene sequences that function as molecular markers, which are essential for discerning evolutionary relationships. These molecular markers encompass a range of genetic components, including ribosomal DNA segments such as the Internal Transcribed Spacer (ITS), mitochondrial genes like Cytochrome Oxidase I (*COI*), and various protein-coding genes, including *rbcL* and *matK*. The sequences derived from these markers are subjected to rigorous analyses aimed at identifying both similarities and differences, which are subsequently employed to infer the evolutionary relationships that exist among taxa. In contemporary phylogenetic studies, molecular data have largely replaced traditional morphological traits as the primary basis for analysis, owing to their superior objectivity, enhanced resolution, and their remarkable capacity to capture genetic alterations that may not be readily apparent in phenotypic characteristics **(Nei and Kumar, 2000).**

Phylogenetic trees, which represent intricate branching diagrams, serve as fundamental constructs in the field of taxonomy, as they facilitate the classification and categorization of diverse organisms based on the genetic similarities and differences that exist among them. The discipline of molecular phylogenetics has significantly advanced our understanding by revealing cryptic species that were previously obscured from view, as well as clarifying the ambiguities that often arise in the delineation of species boundaries, a task that traditional morphological methods have frequently struggled to accomplish **(Hebert *et al*., 2003)**. Through the meticulous mapping of genetic divergences, these phylogenetic trees elucidate the complex evolutionary pathways that genes, populations, and species have traversed over time. This understanding has proven to be critically important in the realms of adaptive radiations, speciation events, and the maintenance of genetic continuity across successive generations **(Felsenstein, 1985).**

Moreover, phylogenetic trees assume a pivotal role in the identification of pathogens and in the meticulous tracking of the evolutionary trajectories of agents

responsible for diseases. The ability of these trees to distinguish between closely related strains provides invaluable insights into the origins and propagation mechanisms of infectious diseases, thereby enhancing our comprehension of their dynamics.

In addition, the field of phylogenetics serves to identify species and lineages that are evolutionarily distinct, which is instrumental in prioritizing conservation efforts aimed at preserving biodiversity. The genetic diversity that is inferred from phylogenetic trees plays a significant role in guiding decisions related to the protection of populations that are at an elevated risk of extinction **(Faith, 1992)**.

Furthermore, the comparative analysis of gene sequences across different species reveals a plethora of conserved genes and functional motifs, which illuminate our understanding of gene function, evolutionary constraints, and the underlying molecular mechanisms that govern these processes. Phylogenetic trees are also utilized to investigate the geographic distribution of genetic lineages, thereby tracing the intricate patterns of migration and diversification that species have undergone over extended periods of time.

The precision and accuracy of phylogenetic trees are profoundly influenced by the selection of genes utilized in their construction. The ideal genes selected for phylogenetic analysis should exhibit a judicious balance between conserved regions, which provide stability, and variable regions that allow for the resolution of evolutionary relationships, thereby facilitating primer binding and ensuring robust evolutionary inference. Among the genes that have gained prominence in phylogenetic studies, a few notable examples include:

The Internal Transcribed Spacer (ITS) Region, which is extensively employed within the field of mycology and is renowned for providing high-resolution insights at the species level **(Schoch *et al.*, 2012)**. The Cytochrome c Oxidase subunit I (*COI*) Gene, recognized as the standard for animal barcoding, which offers reliable amplification processes and effective species discrimination **(Hebert *et al.*, 2003)**. The 16S ribosomal RNA (*16S rRNA*) gene, which is commonly utilized in prokaryotic

studies and serves as a vital tool for the exploration of microbial diversity and the elucidation of phylogenetic relationships **(Janda & Abbott, 2007)**.

5.2 Materials and methods

5.2.1 DNA isolation of Insect pest

The insect pest collected from the ginger farms were taken for molecular identification at Research and Instrumentation Center, Department of Zoology, Pachhunga University College, Aizawl Mizoram. DNA isolation was carried out using the protocols as described by **Rivero *et al*., 2004.**

The process of extracting deoxyribonucleic acid (DNA) was meticulously conducted utilizing samples of insects that had been systematically collected from natural field environments. In order to prevent any potential contamination from blood that might compromise the integrity of the DNA samples, legs and tissues of the collected insect specimens were considered for initiating the DNA isolation procedure.

The initial conditions required for the extraction of DNA were established as follows: individual specimens were strategically placed in 1.5 ml microcentrifuge tubes that were kept on ice to maintain their cellular integrity, and subsequently, 20 µl of lysis buffer, specifically formulated to have a pH of 8.0 (50 mM Tris, 5 mM EDTA, 100 mM NaCl, and 1% SDS), was added to each tube to facilitate cell lysis. Each sample was then manually homogenized using Micropestle (HiMedia) and sterile pointed scissors, to ensure thorough breakdown of the tissue. An additional 100 µl of lysis buffer was added to each tube to enhance the extraction process. Subsequently, 5µl RNaseA (HiMedia) at the concentration was of 10mg/ml was added to remove RNA contamination from the samples. The tubes were incubated at 37^{o}C for 2 hours in a hot water bath (NUVE ST-30) after which 20µl of proteinase K (HiMedia) at the concentration of 2 mg/ml were introduced into the mixture and incubated at 55^{o}C for 3 hours in a water bath to complete the enzymatic digestion. Equal amount of Phenol:Chloroform:Isoamyl alcohol (PCI) to efficiently separate DNA from proteins, lipids, and other contaminants, ensuring that the extracted DNA is suitable for downstream applications such as PCR, sequencing, and cloning. The tubes were then centrifuges at 12000 rpm for 5 minutes (Eppendorf Centrifuge 5430R). The upper

layer of the supernatant was transferred onto fresh centrifuge tube and equal volume of PCI was added and centrifuged again at 12000rpm for 15 minutes. The supernatant was collected onto fresh centrifuge tubes and double the volume of ice-cold ethanol (HiMedia) was added onto it and incubated overnight at -20ºC (Haier HCF-230HTQ) in order to precipate the DNA. Once the precipitation process was complete After overnight incubation, the tubes were then taken out and centrifuged at 12000 rpm for 10 minutes. Once the precipitation process was complete, the samples were centrifuged at 12,000rpm for 10 minutes at 4 degrees Celsius to pellet the DNA. The resulting DNA pellet was meticulously washed with 70% ethanol, allowed to air dry, and then finally resuspended in 20 µl of sterilized water to prepare it for downstream applications.

Following this enzymatic treatment, the purified DNA was separated via electrophoresis on 1% agarose gels, a standard analytical technique, which allowed for visualization by staining with Sybr Safe solution in accordance with established protocols as referenced in Coen et al. (1982). After completion of the electrophoresis, the samples were preserved at -80 degrees Celsius to maintain their stability and viability for future analysis.

5.2.2 PCR amplification

The Cytochrome c oxidase subunit I (COI) regions were amplified by PCR using Primers HCO2198 and LCO1490 **(Folmer *et al.*,1994)**. The PCR reaction was carried out using a ProFlex PCR System (Applied Biosystems, Life Technologies). A reaction volume of 25 µl containing 9.5 ml of nuclease free water, 12.5 ml of PCR MasterMix (Takara), 1 ml each of primer (10 pmole) [LCO1490 (5'- **GGT CAA CAA ATC ATA AAG ATA TTG G**-3') and HCO2198 (3'- **TAA ACT TCA GGG TGA CCA AAA AAT CA**-5')] and 1 ml of DNA template was used. Amplification was performed in a thermal cycler programmed for initial denaturation at 95°C for 2 min., followed by 35 cycles of denaturation at 95°C for 30 sec., annealing at 55°C for 30 sec., extension at 72°C for 1 minute. and a final extension at 72°C for 5 min. PCR products were separated by electrophoresis in 1.4% agarose gels, stained with SYBR Safe DNA Gel Stain (Invitrogen). Electrophoretic migration was carried out for 30

min. at 150 V. The amplified products were visualized and photographed UV Transilluminator (Vilber E-Box CX5.TS Edge). A 100 bp DNA Ladder (GBiosciences) was used to estimate the size of PCR products. Each amplification reaction was replicated three times.

5.2.3 Sequencing and phylogenetic analysis

Sequencing and phylogenetic analysis are critical components in the field of molecular biology, particularly for understanding evolutionary relationships among various organisms. In this study, the polymerase chain reaction (PCR) products that correspond to the Cytochrome oxidase c Sub-Unit I (COI) region were meticulously dispatched to Agrigenome Labs Pvt. Ltd. located in Kerala, as well as to Eurofins Genomics India Pvt. Ltd., Bangalore, specifically for the purpose of sequencing those PCR fragments. To ensure the accuracy and reliability of the data, the software program known as BioEdit Sequence Alignment Editor, version 7.0.5.3, was employed to meticulously edit and align the sequence files obtained from the sequencing process.

Following the alignment of the sequences, a comprehensive analysis was conducted using the BLAST (Basic Local Alignment Search Tool) search functionality available at the National Center for Biotechnology Information's (NCBI) GenBank nucleotide database, which is instrumental in identifying sequence similarities among the submitted sequences. Subsequently, sequences exhibiting a high degree of similarity were systematically downloaded from the GenBank database for the subsequent construction of a phylogenetic tree, which serves as a visual representation of the evolutionary relationships among the analyzed sequences. The generation of the phylogenetic tree was accomplished utilizing the neighbor-joining method, a widely recognized algorithm in the field, and this analytical process was facilitated through the use of the Molecular Evolutionary Genetics Analysis (MEGA) software, specifically version 11.

This comprehensive approach not only enhances our understanding of the genetic relationships but also contributes significantly to the broader field of evolutionary biology. The results obtained from this phylogenetic analysis can potentially have far-reaching implications for the study of biodiversity and the

evolutionary processes that shape the genetic makeup of various species. Therefore, the combination of advanced sequencing techniques and sophisticated computational tools is essential for elucidating the complexities inherent in phylogenetic relationships among organisms.

5.2.4 DNA isolation of Fungal samples

The process of DNA extraction was conducted utilizing a modified version of the methodology originally established by **Cenis in 1992**, wherein a total of 100mg of wet weight, specifically from the mycelial mat, was systematically collected from the liquid culture medium by employing centrifugation (Eppendorf Centrifuge 5430R) at a high speed of 13,000 revolutions per minute for a duration of 5 minutes. Following this initial step, the resulting mycelia pellet underwent a thorough washing procedure with 500 ml of 1xTE buffer, after which it was subjected to another round of centrifugation (Eppendorf Centrifuge 5430R) at the same speed of 13,000 rpm for an additional 5 minutes, thereby ensuring the removal of any residual contaminants. Upon discarding the supernatant, a carefully measured volume of 300ml of extraction buffer (200 mM Tris-HCl adjusted to a pH of 8.5, 25 mM EDTA at pH 8.0, 250 mM sodium chloride, and 0.5% sodium dodecyl sulfate) was introduced to the pellet, and the mycelium was subsequently ground by hand using a conical grinder for an extensive period of 10 minutes to facilitate the disruption of cellular structures. In the following phase of the procedure, 150 µl of 3M sodium acetate, meticulously adjusted to a pH of 5.2, was incorporated into the mixture, which was then incubated at a sub-zero temperature of -20ºC (Haier HCF- 230HTQ) for a period of 10 minutes, ensuring optimal conditions for subsequent reactions. Once this incubation was complete, the sample was again subjected to centrifugation at 13,000 rpm for 5 minutes, leading to the separation of the supernatant from the solid components. The supernatant, which contained desired genetic material, was then carefully transferred into a new 1.5 ml tube, to which an equal volume of isopropanol was added, and the mixture was allowed to remain undisturbed at room temperature for a duration of 15 minutes to facilitate the precipitation of nucleic acids. After this period, the precipitated DNA was collected by subjecting the mixture to centrifugation at 13,000 rpm for a prolonged duration of 10 minutes, which efficiently pelleted the DNA for further purification. Subsequently,

the DNA pellet was washed twice with 70% ethanol in order to remove any remaining contaminants and impurities, thereby enhancing the purity of the extracted genetic material. Once the washing steps were completed, the final DNA pellet was allowed to air-dry to remove excess ethanol, after which it was re-suspended in 40 milliliters of 1xTE buffer to ensure the stability and usability of the extracted DNA. Finally, the reconstituted DNA solution was stored at a temperature of -20 degrees Celsius until it was required for future experimental applications or analyses, thereby preserving its integrity for subsequent molecular biology techniques.

5.2.5 PCR amplification

The Internal Transcribed Spacer (*ITS*) regions were amplified by PCR using Primers *ITS1* and *ITS4* **(Zarzoso, 1999)**. The PCR reaction was carried out using a ProFlex PCR System (Applied Biosystems, Life Technologies). A reaction volume of 25 µl containing 9.5 ml of nuclease free water, 12.5 ml of PCR MasterMix (Takara), 1 ml each of primer (10 pmole) [ITS1 (5'- **TCCGTAGGTGAACCTGCGG**-3') and ITS4 (3'- **TCCTCCGCTTATTGATATGC**-5')] and 1 ml of DNA template was used. Amplification was performed in a thermal cycler programmed for initial denaturation at 95°C for 5 min., followed by 35 cycles of denaturation at 95°C for 30 sec., annealing at 56°C for 30 sec., extension at 72°C for 45 seconds and a final extension at 72°C for 7 min. PCR products were separated by electrophoresis in 1.4% agarose gels, stained with SYBR Safe DNA Gel Stain (Invitrogen). Electrophoretic migration was carried out for 30 min. at 150 V. The amplified products were visualized and photographed UV Transilluminator (Vilber E-Box CX5.TS Edge). A 100 bp DNA Ladder (GBiosciences) was used to estimate the size of PCR products. Each amplification reaction was replicated three times.

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5.3 Results and Discussions

5.3.1 Molecular analysis and phylogeny of collected samples

The sequences that were generated as part of this study underwent a comprehensive comparison with the closest known species available in the GenBank database, utilizing the highly effective BLASTN algorithm as a means of identifying and matching genetic sequences.

In order to achieve a comprehensive and nuanced understanding of the evolutionary relationships and classifications of the insect and fungal sequences that were generated as a result of this study, phylogenetic methodologies that are fundamentally rooted in the neighbor-joining algorithm were meticulously employed, thereby facilitating an insightful comparison with the previously published sequences of the *COI* gene sequences for insects and *ITS* gene sequences for fungi. To perform the necessary multiple sequence alignment and subsequently construct the phylogenetic tree, the FASTA format was utilized, and this process was executed using

the advanced features available in the MEGA10 software, specifically the version 10.2, which is renowned for its robust analytical capabilities. This methodological approach not only enhances the accuracy of the phylogenetic inference but also underscores the significance of integrating computational tools in the realm of molecular biology, as they allow researchers to draw meaningful conclusions from complex biological data.

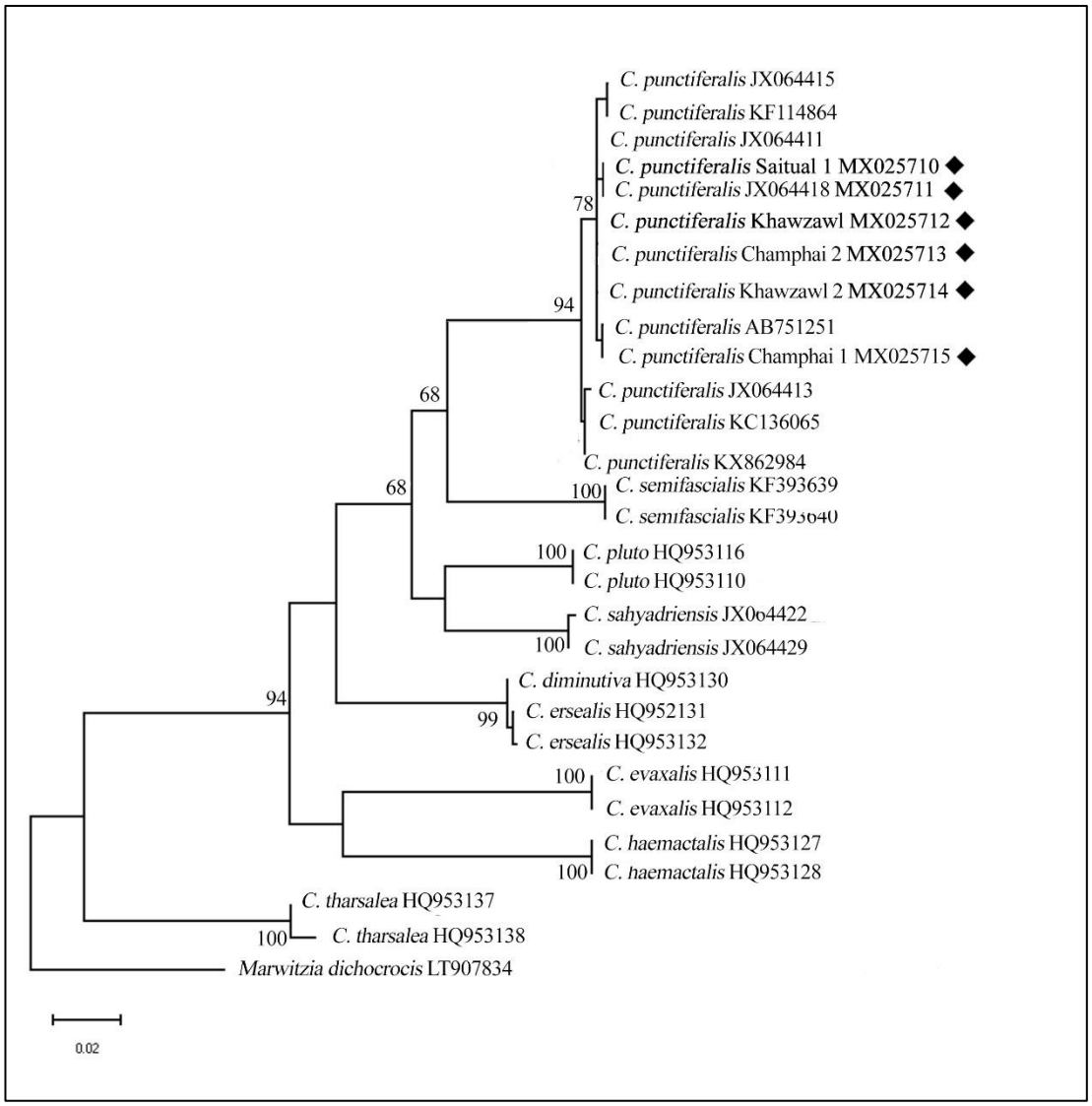

Fig 5.1: Maximum likelihood tree of *Conogethes punctiferalis* isolates inferred from *COI* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The phylogenetic tree constructed based on the mitochondrial cytochrome c oxidase subunit I (commonly referred to as CO1 or MT-CO1) gene offers significant

and profound insights into the intricate genetic relationships that exist within the diverse genus known as *Conogethes*. This meticulously crafted tree encompasses not only our primary samples—specifically, the specimens of *C. punctife*ralis collected from the Khawzawl region (which includes two distinct samples Accession No. MX025712 and MX025714), Champhai area (which also contains two individual samples with Accession No. MX025713, MX025715) and Saitual area (Accession No. MX025710)—but it also incorporates a multitude of reference sequences derived from various geographical locales and different species within the *Conogethes* genus. The MT-CO1 gene, characterized by its elevated mutation rate and the unique nature of mitochondrial inheritance, serves as a highly effective DNA barcode, allowing for the precise differentiation of closely related insect species, which is crucial for taxonomic studies.

In this comprehensive phylogenetic reconstruction endeavor, our key samples have been distinctly marked with black diamonds for the sake of clarity and ease of interpretation: these include *C. punctiferalis* Khawzawl (MX025712), *C. punctiferalis* Khawzawl 2 (MX025714), *C. punctiferalis* Champhai 1 (MX025715), *C. punctiferalis* Champhai 2 (MX025713) and C. punctiferalis Saitual (MX025710). These samples are strategically positioned within a prominent clade that also encompasses additional *C. punctiferalis* reference sequences sourced from various regions, including India, with a notable example being from Saitual (designated as MX025710), as well as globally accessible sequences available in the GenBank database. It is particularly noteworthy that the phylogenetic tree exhibits a bootstrap value of 78, which lends moderate support to the cluster that includes our samples, thereby indicating a degree of confidence in the reliability of this grouping.

The arrangement of the Khawzawl and Champhai samples in close proximity to *C. punctiferalis* specimens from Saitual and other distant locations within this specific clade strongly implies that they are indeed conspecific, which means that they belong to the same species, notwithstanding the potential for minor genetic divergences that may arise due to geographic isolation or localized adaptations. This interpretation is further substantiated by the observation of close genetic distances

among these sequences, which is elegantly illustrated by the relatively short branch lengths observed in the phylogenetic tree.

Furthermore, this cluster of *C. punctiferalis* is distinctly separate from other species within the *Conogethes* genus, such as *C. semifascialis, C. pluto, C. sahyadrensis, C. diminutiva, C. eresalis, C. evaealis, C. haemactalis*, and *C. tharsalea*, each of which forms well-supported and monophyletic groups characterized by high bootstrap values, predominantly exceeding the thresholds of 94 to 100. These elevated support values significantly enhance the confidence in the validity of species boundaries and underscore the reliability of the CO1 gene as a powerful molecular marker for elucidating both intra- and interspecific relationships within the *Conogethes* genus.

One of the particularly noteworthy observations derived from this phylogenetic tree is that while *C. punctiferalis* displays some degree of internal structuring, as evidenced by the presence of sub-clades exhibiting varying bootstrap values such as 94 and 68, the species does not demonstrate sufficient divergence among the analyzed samples that would suggest the presence of cryptic speciation within the individuals studied. Our samples from Saitual, Khawzawl and Champhai do not establish a separate sub-clade; rather, they integrate seamlessly with varying lineages of *C. punctiferalis*, reflecting genetic variation that, while notable, does not justify the assignment of a distinct taxonomic status.

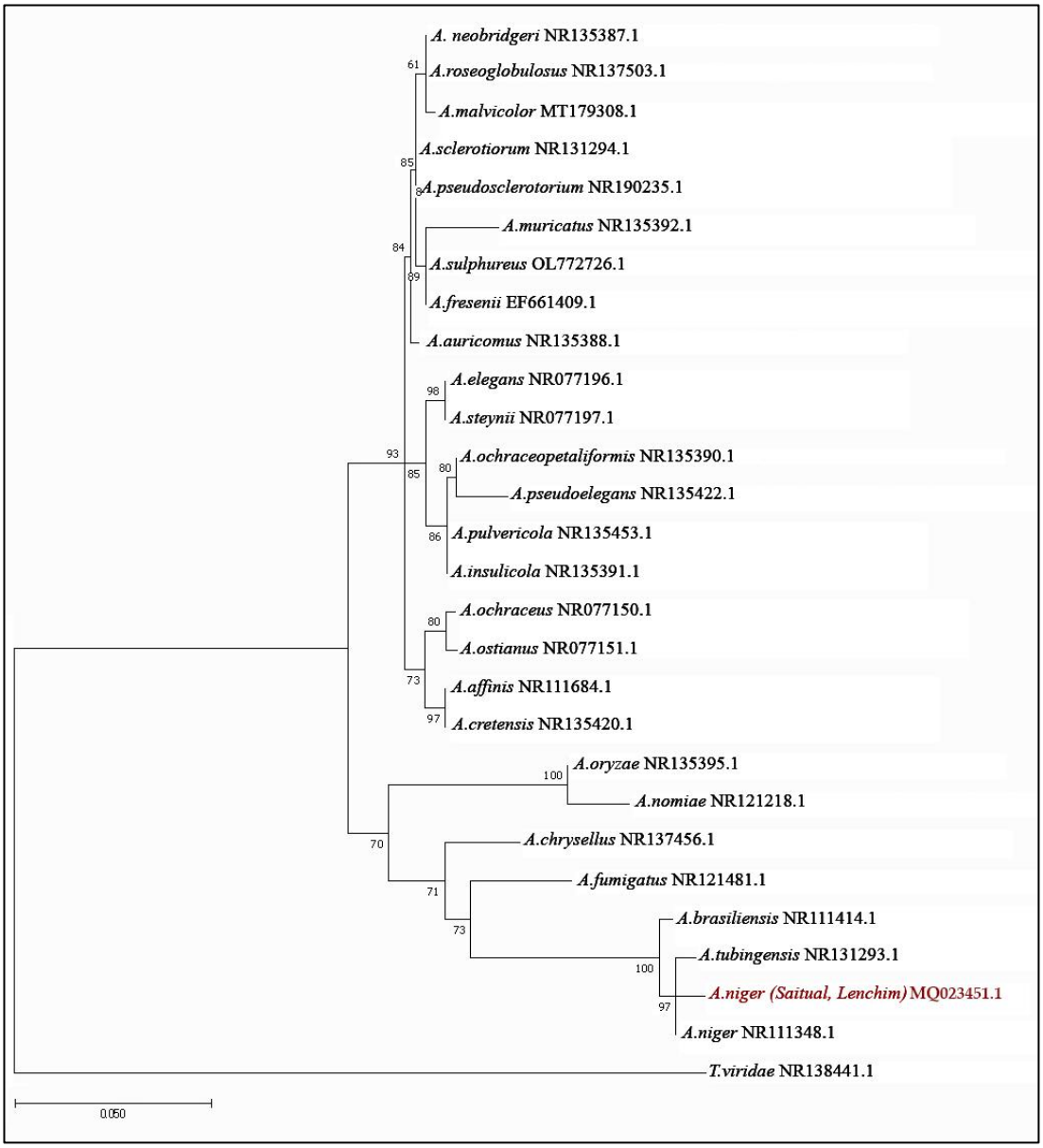

Fig 5.2: Maximum likelihood tree of *Aspergillus niger* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The phylogenetic tree that has been meticulously constructed in this study is primarily founded upon the analysis of the Internal Transcribed Spacer 1 (ITS1) region of ribosomal DNA, which is recognized as a highly valuable and widely utilized molecular marker within the field of mycology, particularly for the identification of fungal species and the execution of phylogenetic analyses due to its notable variability observed among species that are closely related to one another. Within the confines of this phylogenetic tree, the principal subject of investigation is the isolate *Aspergillus*

niger (Saitual, Lenchim) Accession No. MQ023451.1, which has been rigorously compared to reference sequences obtained from a variety of Aspergillus species that have been retrieved from the National Center for Biotechnology Information (NCBI) database. Furthermore, the tree strategically incorporates *Trichoderma viridae* (NR138441.1) as an outgroup, serving the critical purpose of establishing a root for the tree, thereby ensuring that the evolutionary inferences drawn from this analysis possess clear directionality and are grounded in a robust framework.

The ITS1 sequence associated with *A. niger* (Saitual, Lenchim) displays a notable degree of genetic proximity to two additional *A. niger* reference sequences, specifically *A. niger* NR111348.1 and *A. niger* NR121481.1, collectively forming a well-supported clade that boasts an impressive bootstrap value of 97%, which serves to underscore the high level of confidence that can be placed in this particular phylogenetic grouping. This pronounced clustering serves as a compelling confirmation that the *A. niger* isolate originating from Saitual, Lenchim is indeed accurately classified as *Aspergillus niger*, and it further indicates that there exists a significant degree of sequence homology between this isolate and the reference strains mentioned previously. Such findings lend substantial support to the classification of this organism within the Nigri section of the *Aspergillus* genus, which is well-known for encompassing black-spored species that are not only of industrial importance but also occasionally exhibit pathogenic characteristics.

A more in-depth examination of the phylogenetic tree reveals that the aforementioned *A. niger* clade is situated within a broader assemblage that includes other closely related species, such as *A. brasiliensis* (NR111414.1) and *A. tubingensis* (NR131293.1). These species frequently exhibit morphological similarities to *A. niger*, and the phylogenetic tree serves as a valuable tool for delineating their genetic distinctions. The positioning of *A. fumigatus* (NR121481.1) in a nearby branch, characterized by a lower bootstrap value of 71%, signifies a more distant phylogenetic relationship, yet it still remains situated within the broader context of the *Aspergillus* genus, highlighting the intricate web of relationships among these fungal species.

The phylogenetic tree additionally provides a comprehensive illustration of the genetic diversity that exists within the *Aspergillus* genus. A multitude of species, including *A. ochraceus, A. flavus, A. oryzae, A. elegans,* and *A. auricomus*, form distinct clades that are bolstered by strong bootstrap support, which reflects the evolutionary divergence that has occurred across this expansive genus. For instance, the clade comprised of *A. oryzae*, *A. nomiae*, and *A. chrysellus* is supported by a remarkable bootstrap value of 100%, which unequivocally indicates a very close genetic relationship among these species. Another noteworthy cluster that has been identified includes *A. elegans*, *A. steynii*, and *A. ochraceopetaliformis*, which again exhibits high internal support, a critical aspect for comprehending the intricate species-level relationships and overarching evolutionary patterns that characterize this group.

The outgroup, *Trichoderma viridae*, is distinctly separated from all the Aspergillus species represented in the tree, thereby validating its role as a phylogenetic root and reinforcing the monophyly of the Aspergillus genus in the context of this analysis. Its remote placement within the phylogenetic framework confirms that all other species depicted in the tree are more closely related to one another than they are to Trichoderma, thus providing further clarity regarding the evolutionary relationships among these fungal entities.

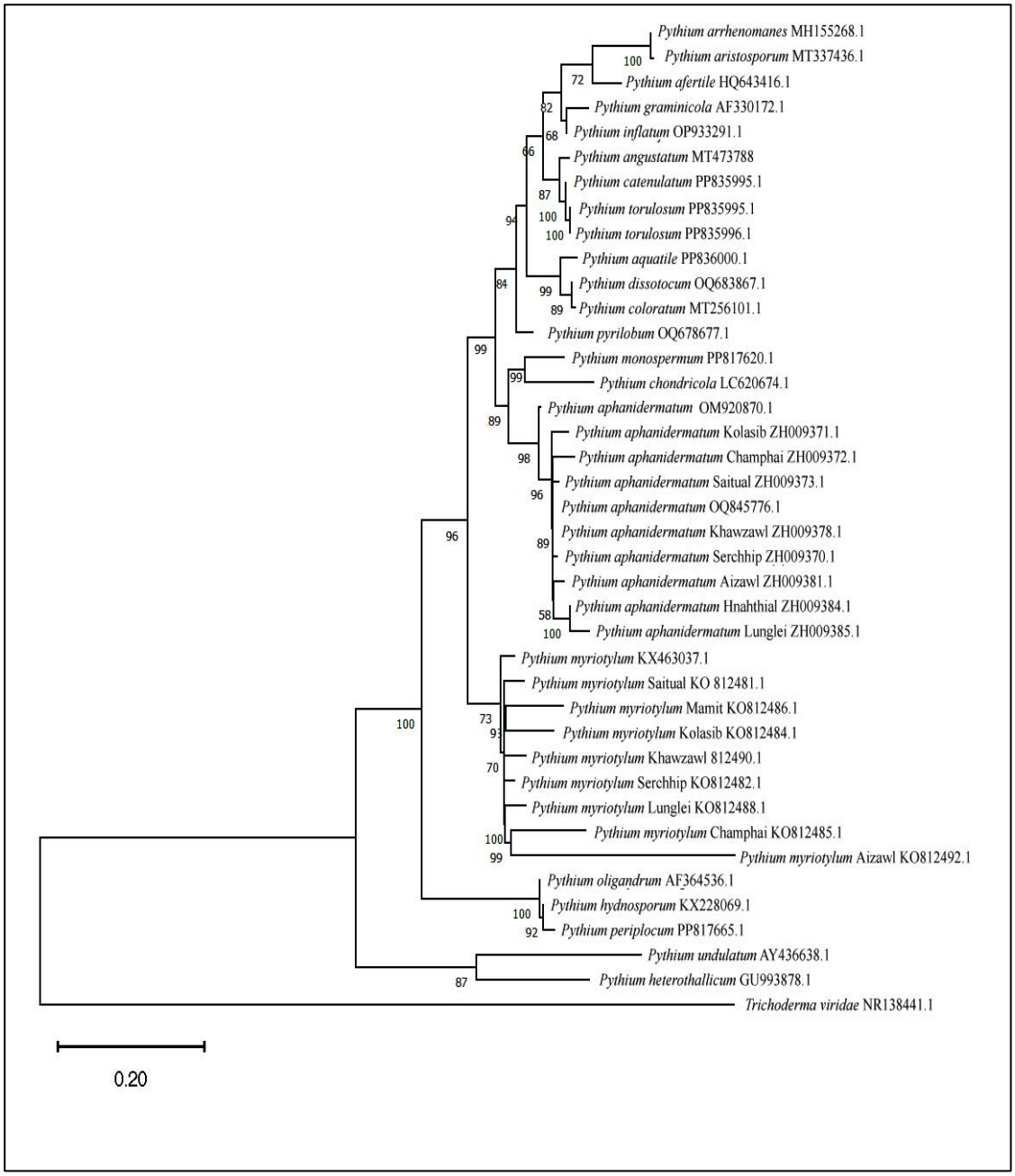

Fig 5.3: Maximum likelihood tree of *Pythium myriotylum* and *Pythium aphanidermatum* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The phylogenetic tree that has been meticulously presented here is constructed utilizing the Internal Transcribed Spacer 1 (ITS1) gene region, which is recognized as a highly reliable and widely accepted molecular marker specifically employed for the identification and classification of fungi and oomycetes. The ITS1 region is strategically situated between the 18S and 5.8S ribosomal RNA (rRNA) genes within

the nuclear ribosomal DNA (rDNA) framework, and it exhibits a remarkable degree of variability among different species, thereby facilitating comprehensive and robust phylogenetic analyses. Within this particular phylogenetic tree, the principal emphasis is directed towards the isolates of *Pythium myriotylum* and *Pythium aphanidermatum*, which have been meticulously collected from a variety of districts in Mizoram, India, including but not limited to Saitual, Mamit, Kolasib, Khawzawl, Serchhip, Lunglei, Champhai, and Aizawl. These isolates are methodically compared against reference sequences derived from the *Pythium* genus with the objective of elucidating and determining the genetic relationships that exist among them. Furthermore, *Trichoderma viridae* (NR138441.1) is employed as an outgroup in this study, which serves the essential role of a taxonomic anchor that roots the tree and helps to establish the evolutionary direction of the relationships depicted within the tree.

The isolates of *Pythium aphanidermatum* that have been sourced from Kolasib (ZH009371.1), Champhai (ZH009372.1), Saitual (ZH009373.1), Khawzawl (OM920870.1 and ZH009378.1), Serchhip (ZH009370.1), Aizawl (ZH009381.1), Hnahthial (ZH009384.1), and Lunglei (ZH009385.1) exhibit a tendency to cluster together within a singular, strongly supported clade. The close genetic proximity observed among these isolates not only confirms their identification as *P. aphanidermatum* but also suggests that there is a remarkably low level of intraspecific divergence present among the samples that have been collected from geographically disparate yet climatically analogous regions throughout Mizoram. Additionally, this grouping aligns closely with a reference strain (OM920870.1), which further fortifies the assertion regarding the accurate identification of these isolates. The close clustering of samples originating from diverse districts such as Kolasib, Champhai, and Lunglei indicates that *P. aphanidermatum* possesses a broad and consistent distribution across the entirety of the state of Mizoram.

Conversely, the isolates of *Pythium myriotylum* that have been sourced from Saitual (KO812481.1), Mamit (KO812486.1), Kolasib (KO812484.1), Khawzawl (KO812490.1), Serchhip (KO812482.1), Lunglei (KO812488.1), Champhai (KO812485.1), and Aizawl (KO812492.1) also form a distinct and separate clade. This specific group is robustly supported with bootstrap values achieving a remarkable

100%, particularly highlighted by the close clustering of the isolates from Lunglei and Champhai. The exceptionally high bootstrap values recorded among the samples of P. myriotylum imply a very high degree of genetic similarity, which strongly suggests that this particular species is also widely and homogeneously distributed throughout the region of Mizoram. The close phylogenetic relationship of all these isolates with the reference sequence of *P. myriotylum* (KX446307.1) serves to confirm their taxonomic identity and indicates that there is a low level of genetic variation among the field samples that were analyzed.

Upon conducting a comparative analysis of *P. aphanidermatum* and *P. myriotylum,* it becomes abundantly evident that while both species exhibit a coherent genetic structure throughout the region of Mizoram, they are distinctly separated within the phylogenetic tree, thereby forming two clearly delineated clades. The separation between these two clades is marked by a branch that is strongly supported by a high bootstrap value (100%), which underscores the evolutionary divergence that exists between these two species. This divergence is reflective of their unique biological characteristics and ecological roles. Specifically, *P. aphanidermatum* is typically associated with environments characterized by elevated temperatures and is well-known for its role in causing root rot in a variety of crops, whereas *P. myriotylum* tends to prefer moist and warm conditions and is frequently linked to damping-off diseases that affect seedlings. Despite the overlapping geographic distributions of these two species, their genetic divergence implies a significant degree of ecological niche differentiation and potentially varying host preferences that warrant further investigation.

Furthermore, within the taxonomic group classified as *Pythium myriotylum*, there exists a discernible presence of minor subclades, which highlights the complexity of genetic relationships within this group; notably, the isolates derived from the regions of Saitual and Mamit have been observed to cluster together, exhibiting a bootstrap support value of 73%, while the isolates originating from Kolasib and Serchhip form a separate cluster with a bootstrap support of 70%. These findings suggest the existence of slight intraspecific genetic variation that may be attributed to geographical distribution and the specific microecological factors that influence these isolates in

their respective environments. A similar pattern of minor branching is also evident within the clade of *Pythium aphanidermatum*, where the isolates obtained from Champhai and Kolasib have been identified as forming a well-supported subgroup, which strongly indicates the presence of regional lineage differentiation within the context of the state of Mizoram.

In addition to the aforementioned species, the presence of other *Pythium species, specifically Pythium graminicola, Pythium inflatum, Pythium dissotocum, and Pythium torulosum,* contributes significantly to a more comprehensive taxonomic framework for the evaluation of the collected isolates, thereby enhancing the understanding of the ecological dynamics at play. These additional species are positioned on distinct branches and clades within the phylogenetic tree, which serves to validate the separation and integrity of the *P. aphanidermatum* and *P. myriotylum* groups, further corroborating their status as distinct taxa within the broader Pythium genus.

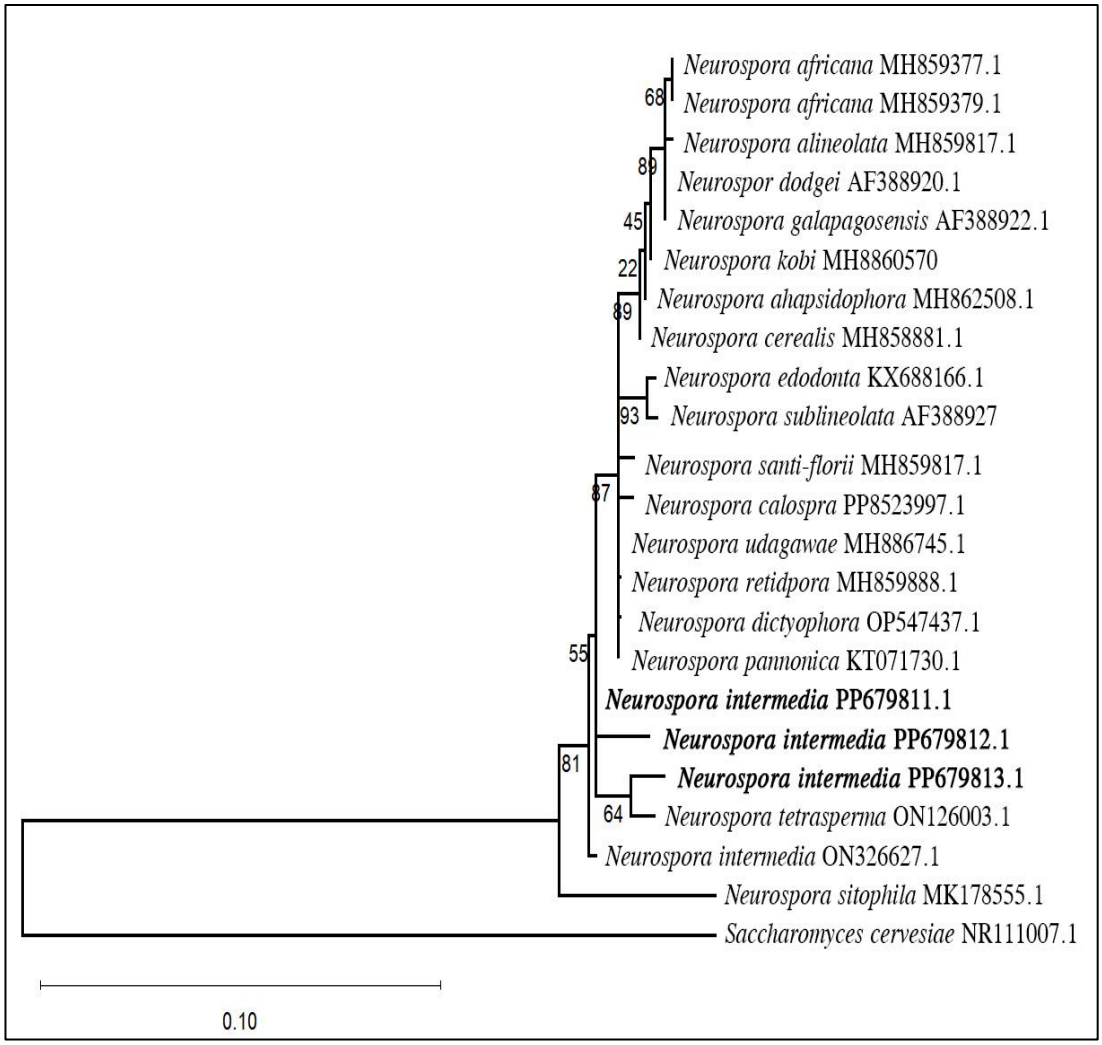

Fig 5.4: Maximum likelihood tree of *Neurospora intermedia* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The three bolded entries — *Neurospora intermedia* PP679811.1, PP679812.1, and PP679813.1 — represent the main isolates analyzed in this study, while the remaining sequences are reference strains retrieved from the NCBI database. This tree helps in understanding the genetic affiliation and taxonomic placement of the isolated strains within the broader *Neurospora* genus.

The three main isolates group tightly together in a well-supported clade (bootstrap value of 81), indicating a high degree of genetic similarity among them. Their placement within the *Neurospora intermedia* cluster confirms that these isolates indeed belong to the same species. This cluster is further closely related to *Neurospora*

tetrasperma (ON126003.1) and another *N. intermedia* reference sequence (ON326627.1), forming a broader lineage of closely allied species within the *Neurospora* genus. The bootstrap value of 64, though moderate, supports this grouping and suggests that *N. tetrasperma* and *N. intermedia* share a recent common ancestor, which aligns with previous studies indicating overlapping evolutionary histories and partial reproductive compatibility between these two taxa.

The broader phylogenetic tree reveals several distinct clades representing different *Neurospora* species. For instance, *Neurospora africana* (MH859377.1 and MH859379.1) forms a well-supported cluster with *Neurospora lineolata* (MH859817.1) and *N. dodgei* (AF388920.1), reflecting their close evolutionary relationship. Another well-supported clade includes *Neurospora galapagosensis*, *N. kobi*, and *N. ahaspidophora*, all forming a sister group with high bootstrap values, suggesting a shared lineage. *Neurospora sublineolata* and *N. edodonta* also form a strong cluster (bootstrap 93), further confirming the robustness of species-level identification in this group.

The placement of *Neurospora pannonica* (KT071730.1) just outside the *N. intermedia* cluster, along with *N. dictyophora* and *N. retipodra*, suggests a more distant yet still notable relationship to the main isolates. These species may share some morphological or ecological traits with *N. intermedia* but are genetically distinct enough to form their own subclade. Additionally, *N. calospora*, *N. udagawae*, and *N. santi-florii* cluster together, with *N. calospora* (bootstrap value of 87) forming a tight relationship with *N. santi-florii*, further indicating evolutionary divergence within this complex genus.

Neurospora sitophila (MK178555.1), although part of the genus, lies on a separate branch closer to the base of the tree. This separation implies a more distant relationship from the *N. intermedia* group and suggests greater genetic divergence. The tree is rooted using *Saccharomyces cerevisiae* (NR111007.1) as an outgroup, which helps establish the direction of evolutionary branching and provides context for interpreting ancestral versus derived lineages within *Neurospora*.

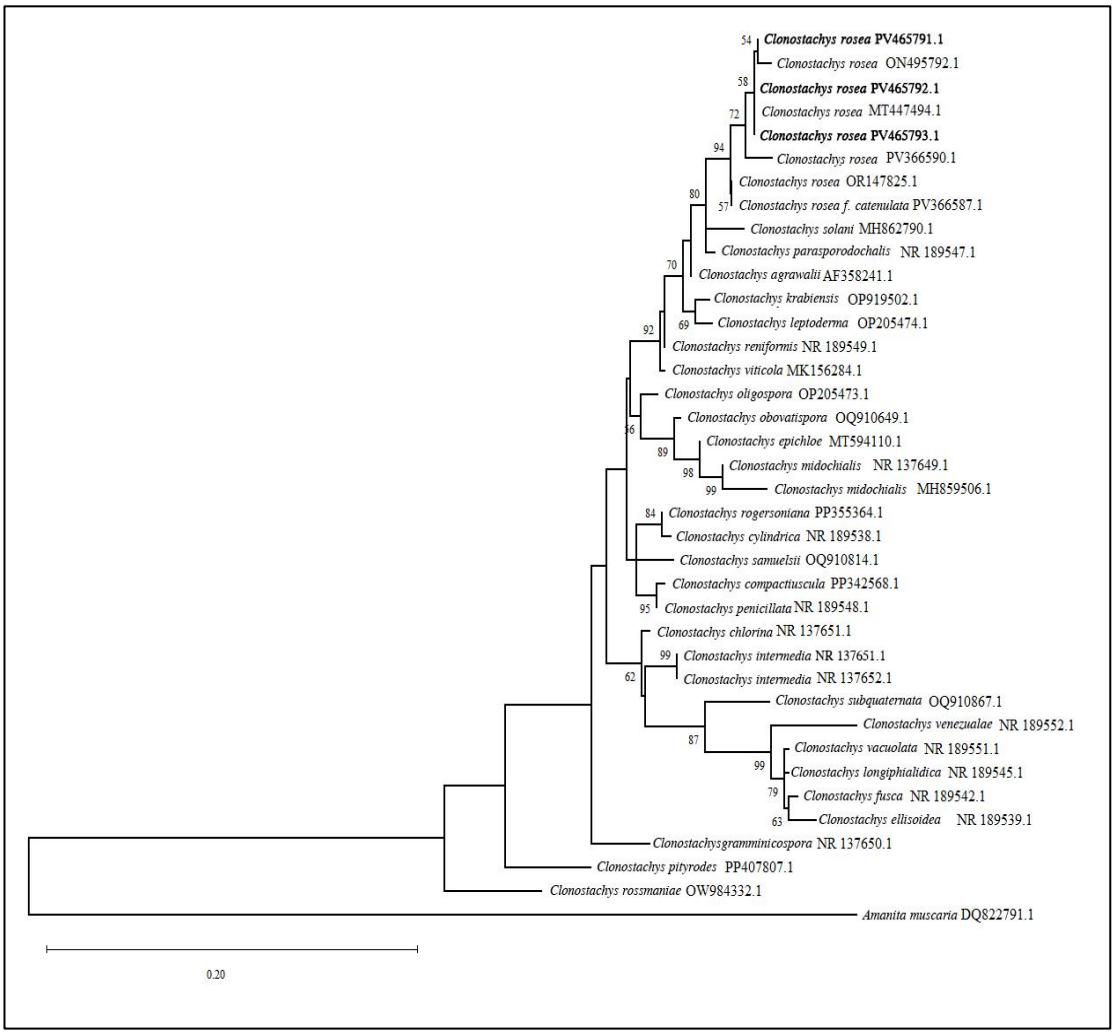

Fig 5.5: Maximum likelihood tree of *Clonostachys rosea* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The phylogenetic tree presented herein illustrates the intricate evolutionary relationships that exist among a variety of species encompassed within the genus *Clonostachys*. This particular depiction places a significant emphasis on the identification and precise placement of three isolates that have been bolded for prominence*: Clonostachys rosea* PV465791.1, PV465792.1, and PV465793.1. These isolates represent the primary samples that are currently under rigorous investigation, while the remaining sequences included in the tree are reference strains that have been systematically retrieved from the National Center for Biotechnology Information (NCBI) database. To enhance the analytical depth of this phylogenetic exploration, the tree has been thoughtfully rooted using *Amanita muscaria* (DQ827791.1) as an

outgroup, which serves as a distant point of reference that aids in determining the trajectory of evolutionary divergence throughout the tree's structure.

The three isolates that have been highlighted in bold exhibit a notable tendency to cluster tightly together alongside other reference sequences of *Clonostachys rosea*, thereby forming a distinct clade that is well-supported by the available data. This clustering strongly indicates that these isolates are genetically consistent with the established lineage of *C. rosea*, thereby confirming their identification as bona fide members of this particular species. Furthermore, the bootstrap values that have been calculated for the *C. rosea* clade, which range from 54 to 88, provide a spectrum of moderate to strong support for this grouping, thereby reinforcing the reliability and robustness of the phylogenetic structure that has been delineated.

In close proximity to this clade, one can observe *Clonostachys rosea* f. catenulata, as well as other closely related species such as *C. solani* and *C. parasporosphoralis*, which collectively contribute to the formation of a broader lineage that includes *C. rosea* and its various phenotypic variants. This phylogenetic arrangement suggests that these species may share a relatively recent common ancestor and could potentially exhibit overlapping morphological or ecological traits, which further enriches the understanding of their evolutionary pathways.

Additionally, other species within this genus, such as *C. chlorina, C. penicillata*, and *C. compactiuscula*, have been found to form separate and well-supported clades, which serve to highlight the genetic divergence that exists between them and *C. rosea*.

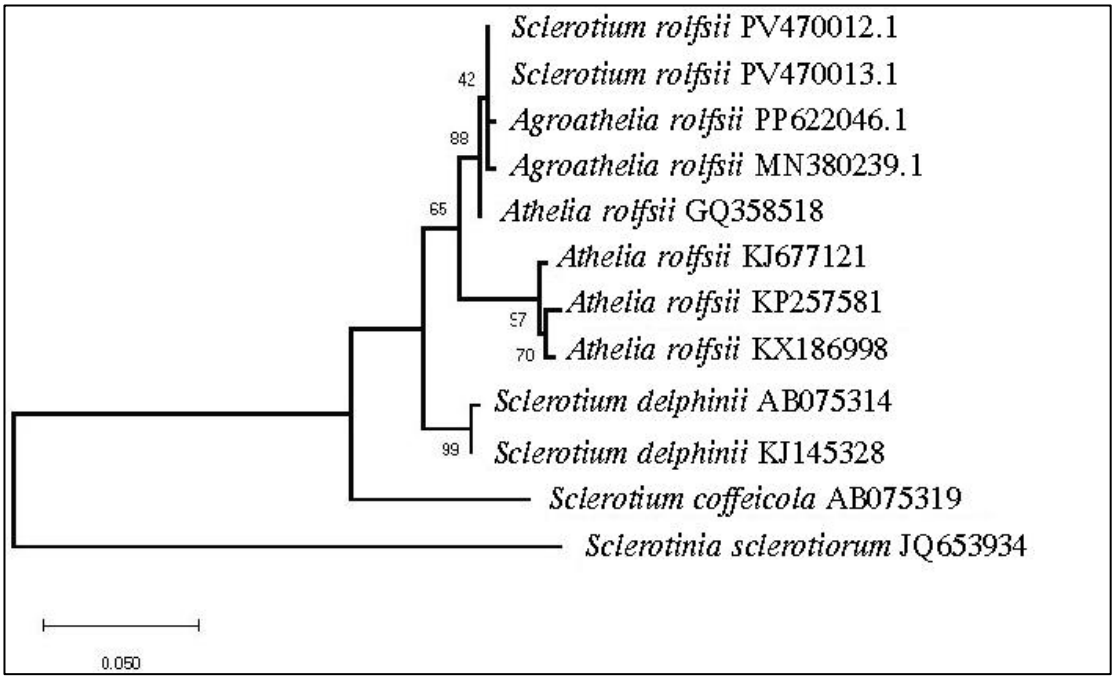

Fig 5.6: Maximum likelihood tree of *Sclerotium rolfsii* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The phylogenetic tree that has been constructed serves as a comprehensive illustration of the intricate evolutionary relationships that exist among the various species that are categorized within the *Sclerotium–Athelia–Agroathelia* complex, with a particular emphasis placed on two primary isolates that are being examined in this study: *Sclerotium rolfsii* PV470012.1 and PV470013.1. These specific isolates are strategically positioned within the uppermost clade of the tree, alongside a number of other reference sequences that have been previously identified as belonging to *Agroathelia rolfsii* and *Athelia rolfsii*, which strongly suggests a close genetic relatedness among these entities and provides substantial support for their classification within the broader *Sclerotium rolfsii* complex.

The observed clustering of the *S. rolfsii* isolates in conjunction with the reference sequences for *Agroathelia rolfsii* (specifically, PP622046.1 and MN380239.1) as well as *Athelia rolfsii* (which include GQ358518, KJ677121, KP257581, and KX186998) is indicative of the well-documented taxonomic revisions in which *Sclerotium rolfsii* is recognized as the anamorphic, or asexual, stage of the species known as *Athelia rolfsii*. The robustness of these groupings is further

emphasized by the high bootstrap values that have been recorded (for instance, values of 88, 85, and 70), which provide compelling evidence supporting the assertion that these isolates are genetically consistent with their corresponding taxonomic identities.

In the lower portion of the phylogenetic tree, one can observe the presence of more distantly related species, including *Sclerotium delphinii* and *Sclerotium coffeicola*, both of which are organized into separate clades that are bolstered by strong support evidenced by a bootstrap value of 99, thereby confirming their genetic divergence from the other studied isolates. Additionally, *Sclerotinia sclerotiorum* is utilized as an outgroup, effectively rooting the tree and facilitating the distinction of the evolutionary lineage pertaining to the isolates under investigation.

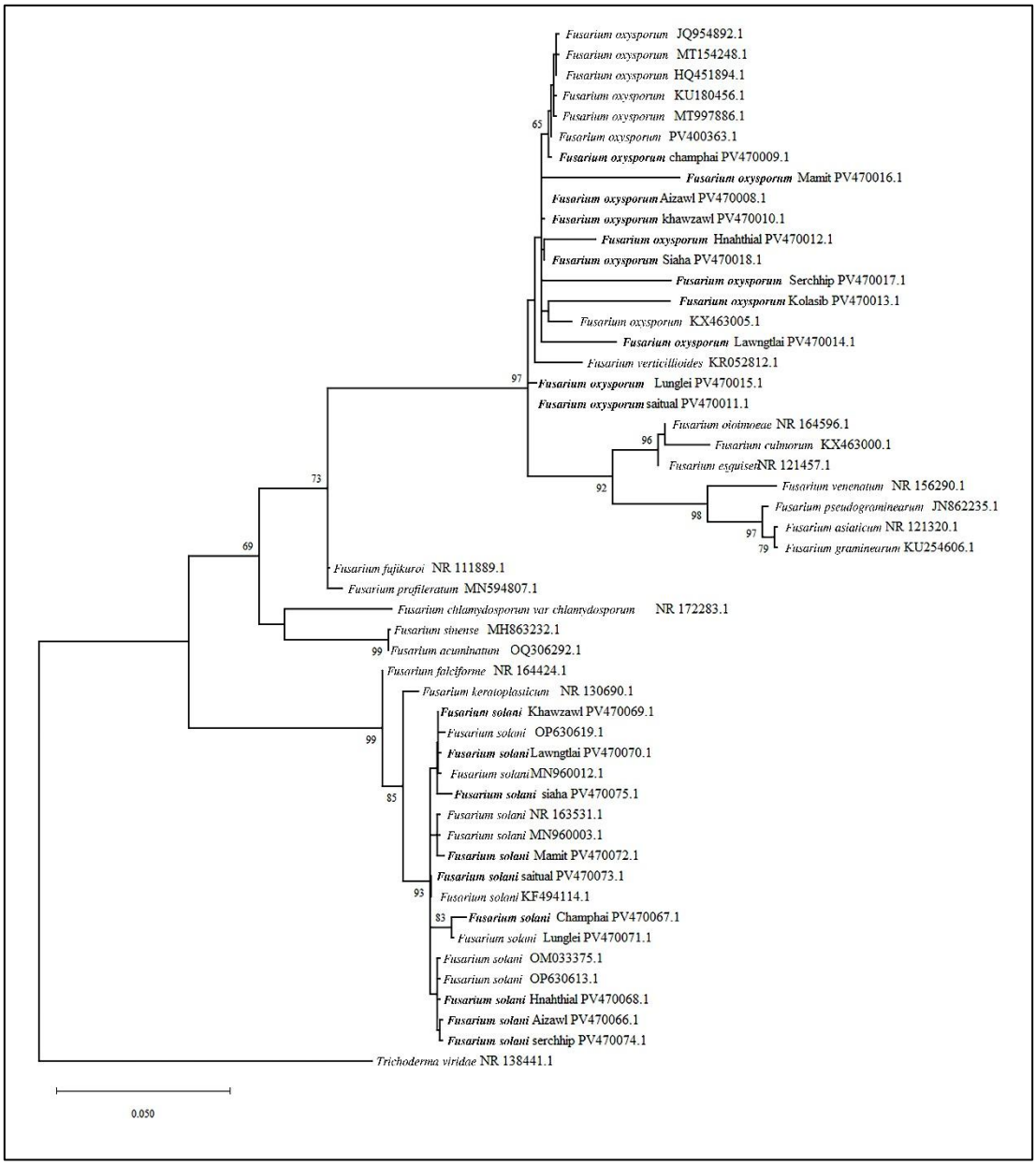

Fig 5.7: Maximum likelihood tree of *Fusarium oxysporum* and *Fusarium solani* isolates inferred from *ITS1* gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The provided phylogenetic tree delineates the evolutionary connections among various *Fusarium* species, particularly emphasizing the isolates of *Fusarium solani* and *Fusarium oxysporum* (highlighted in bold italics), which constitute the principal samples for this investigation. The other sequences were sourced from the NCBI database and are utilized as reference sequences. The tree is anchored with *Trichoderma viride* (NR138441.1) employed as an outgroup, establishing a framework

for evolutionary analysis and affirming the monophyletic configuration of the *Fusarium* clades.

The *Fusarium oxysporum* isolates—Aizawl PV470008.1, Khawzawl PV470010.1, Hnahthial PV470012.1, Siaha PV470018.1, Serchhip PV470017.1, Kolasib PV470013.1, Lawngtlai PV470014.1, Lunglei PV470015.1, and Saitual PV470011.1—converge within a highly supported clade (bootstrap values reaching 97). These isolates exhibit close genetic affinities with reference strains such as *F. oxysporum* JQ954892.1, MT511424.1, HQ415894.1, and KU186564.1, signifying notable genetic congruence. Their close-knit clustering and adjacency to established *F. oxysporum* sequences corroborate precise identification and indicate minimal intraspecific variation, notwithstanding their collection from various geographical locales in Mizoram.

This restricted divergence may suggest that these isolates possess a recent common ancestor or exhibit significant gene flow between populations across different locations. These observations bolster the premise that *F. oxysporum* represents a broadly adapted species with a relatively stable genetic framework, particularly in the examined regions.

The *Fusarium solani* isolates—Khawzawl PV470069.1, Lawngtlai PV470070.1, Siaha PV470075.1, Mamit PV470072.1, Saitual PV470073.1, Champhai PV470067.1, Lunglei PV470071.1, Hnahthial PV470065.1, Aizawl PV470066.1, and Serchhip PV470074.1—likewise constitute a robustly supported clade (bootstrap values up to 99), closely aligned with GenBank references such as MN906012.1, OP603611.1, OM033375.1, and NR165331.1. These findings affirm the taxonomic classification of the isolates within *F. solani* and substantiate their identification.

Although originating from diverse geographic backgrounds, these isolates exhibit minimal divergence, akin to the *F. oxysporum* samples. Nevertheless, the F. solani group manifests slightly more branching diversity than *F. oxysporum*, potentially indicative of greater adaptability or varied evolutionary pressures across the environmental contexts of Mizoram.

Both *F. solani* and *F. oxysporum* isolates are characterized as monophyletic, denoting that each group constitutes a singular, distinct evolutionary lineage. The elevated bootstrap values within each clade reinforce strong genetic cohesion and dependable phylogenetic classification. Nevertheless, *F. solani* seems to exhibit marginally more subclade diversification, implying either elevated mutation rates or adaptations to micro-environmental variations.

While both species illustrate close affiliations with their respective reference sequences from NCBI, *F. solani* isolates appear to be more widely dispersed within their clade, suggesting a higher level of intraspecies diversity compared to *F. oxysporum*, which remains more compactly clustered.

The isolates of *Fusarium oxysporum* exhibit a pronounced affinity towards specific sequences such as HQ415894.1 and KU186564.1, which not only validates their identity but also implies the presence of conserved genetic traits that may play a significant role in their evolutionary biology. In contrast, the isolates of *Fusarium solani* demonstrate a close relationship with reference sequences MN906012.1 and NR165331.1, which serves to confirm their accurate identification while also indicating a slightly increased level of genetic branching, a phenomenon that could potentially be attributed to variations that are specific to certain regional environments or host interactions.

5.4 Summary and Conclusion

In conclusion, the phylogenetic analysis conducted on the MT-CO1 gene provides robust support for the identification of the Saitual, Khawzawl and Champhai samples as belonging to the species *C. punctiferalis*. Their strategic placement within a broader, moderately supported clade of *C. punctiferalis* sequences serves to underscore their genetic similarity to global populations of this particular species. Additionally, this phylogenetic tree effectively distinguishes *C. punctiferalis* from other closely related congeneric species, thereby affirming the utility of CO1 gene analysis in both species identification and the exploration of genetic diversity within the genus *Conogethes*.

The phylogenetic analysis grounded in the ITS1 region effectively corroborates the molecular identity of the *Aspergillus niger* (Saitual, Lenchim) isolate, as it confidently clusters this isolate with established sequences of A. niger. This phylogenetic framework serves not only to affirm the species identity but also to shed light on the broader evolutionary relationships that exist within the diverse genus Aspergillus, thereby underscoring the utility of the ITS1 region as a powerful and essential tool in the realms of fungal taxonomy and molecular systematics, which are critical for advancing our understanding of fungal biodiversity and evolution.

Analysis based on the ITS1 region has successfully validated the precise identification of *Pythium aphanidermatum* and *Pythium myriotylum* isolates that have been collected from various districts within the state of Mizoram, thereby contributing to the existing body of knowledge regarding these important pathogens. Both species have been observed to form well-supported and genetically coherent clades, which exhibit minimal intraspecific variation across the different regions from which the samples were obtained. The phylogenetic tree generated through this analysis reveals a distinct evolutionary divergence between these two species, despite their overlapping geographical ranges, highlighting the intricate patterns of biodiversity present in this region. This study serves to underscore the utility of the ITS1 region as a powerful tool for resolving taxonomic identities and elucidating the evolutionary relationships that exist within the genus *Pythium*, thereby providing invaluable insights into their biodiversity, geographical distribution, and potential pathogenic behaviors within the ecological context of the Mizoram region.

The phylogenetic tree confirms that the three main isolates belong to *Neurospora intermedia*, showing high genetic similarity and clustering with known reference sequences. The tree also reveals well-supported relationships among various *Neurospora* species, highlighting evolutionary divergence and speciation within the genus. These findings provide strong molecular evidence supporting the identification and classification of the isolated strains and contribute to a broader understanding of *Neurospora* diversity and evolution.

The phylogenetic tree vividly demonstrates that the three primary isolates are not only closely related to one another, but also fall firmly within the complex of *Clonostachys rosea*. This particular phylogenetic placement is entirely consistent with their molecular identity and significantly contributes to the overarching understanding of species diversity and taxonomy within the intricate genus *Clonostachys*.

The phylogenetic tree unequivocally confirms that the isolates PV470012.1 and PV470013.1 are closely associated with the known sequences of *S. rolfsii* and *Athelia rolfsii*, thereby supporting not only accurate species identification but also validating their appropriate placement within the *Athelia rolfsii* complex, which is crucial for understanding their evolutionary context.

The findings obtained from this comprehensive phylogenetic analysis provide compelling evidence that the predominant isolates of *Fusarium solani* and *Fusarium oxysporum* sourced from the Mizoram region are genetically aligned with reference strains that are recognized globallyAlthough both fungal species exhibit a robust level of internal genetic consistency, it is noteworthy that *F. solani* reveals a somewhat greater degree of genetic variation compared to *F. oxysporum*, which may be a reflection of ecological influences or factors related to specific host organisms. This comparative analysis highlights the critical role that molecular methodologies play in the precise identification of fungi, as well as in the assessment of biodiversity within agricultural landscapes, thereby underscoring the necessity for continued research in this vital area of study.

Summary

1. The research endeavor presented herein concentrates on the prevalence, systematic collection, and both morphological and molecular identification of the various insect pests and fungal pathogens afflicting ginger crops in the region of Mizoram, situated in North-East India, which is recognized as a significant part of the Indo-Burma biodiversity hotspot, renowned for its rich ecological diversity and unique species assemblages.

2. The process of identifying insect pests that are associated with ginger fields suffering from various diseases in Mizoram is of paramount importance for developing a comprehensive understanding of pest dynamics and subsequently formulating effective and sustainable pest control strategies. In particular, this study meticulously focused on three principal insect pests that are detrimental to ginger cultivation: *Conogethes punctiferalis*, *Mimegralla coeruleifrons*, and specific species belonging to the genus *Holotrichia*.

3. The accurate identification of these pests was achieved through a rigorous combination of direct field collection, detailed morphological examination, and advanced molecular characterization methodologies, all conducted in accordance with established entomological protocols and standards.

4. Field surveys were systematically conducted during the active cropping season in the prominent ginger-growing regions of Mizoram, where careful attention was paid to environmental conditions and pest prevalence. The insects were collected utilizing a multifaceted approach that included techniques such as sweep netting, handpicking, and the deployment of light traps, as articulated in the seminal work of **Southwood and Henderson (2000).**

5. Notably, light traps proved to be especially effective in capturing nocturnal insect species, including various members of *Holotrichia*, while sweep nets were employed during the early morning and late afternoon hours to

successfully capture active flying species such as *Conogethes punctiferalis* and *Mimegralla coeruleifrons*. Upon collection, the insects were subsequently placed in specially designed kill jars containing ethyl acetate and later preserved in a solution of 70–95% ethanol, thereby facilitating both morphological and molecular analyses in subsequent phases of the study.

6. The preserved insect specimens underwent detailed examination under a high-quality stereomicroscope, allowing for precise taxonomic identification through the application of standard taxonomic keys and guides.

7. *Conogethes punctiferalis* was distinctly identified based on its characteristic morphological features, which include striking yellow forewings adorned with black dots and an average wingspan that ranges from 20 to 24 mm, in alignment with the detailed descriptions provided in the comprehensive review by **Mally *et al*. (2019).**

8. *Mimegralla coeruleifrons*, which is classified within the family Sciomyzidae, was identified through a careful assessment of its morphological characteristics, including its distinctive metallic blue coloration and specific wing venation patterns, as thoroughly described in the authoritative work of **Steyskal (1981).**

9. For the identification of *Holotrichia* species, the focus was primarily directed towards analyzing the shape and structure of the clypeus, the characteristics of the antennae club, and the patterns of elytral punctation, with references made to the taxonomic keys established by **Roonwal and Chatterjee (1958).** Additionally, photographic documentation and extensive morphometric data were diligently recorded to facilitate comparisons with existing published descriptions and classifications.

10. In conjunction with morphological analysis, molecular techniques were employed to substantiate species identity and resolve any ambiguities

associated with morphotypes that were difficult to classify. Genomic DNA was meticulously extracted from the legs of the collected insects. The mitochondrial cytochrome oxidase subunit I (COI) gene, which is widely utilized in the realm of DNA barcoding, was amplified using universal primers LCO1490 and HCO2198.

11. The polymerase chain reaction (PCR) conditions were meticulously standardized, and the resultant amplified products were visualized on 1.5% agarose gels that were stained with ethidium bromide to facilitate easy observation of the bands. Following this, the purified PCR products underwent sequencing and were subsequently analyzed using the BLAST algorithm against the NCBI GenBank database to confirm species-level identity with a high degree of accuracy and reliability.

12. Moreover, phylogenetic analysis was conducted to evaluate the genetic relationships among the collected specimens, providing deeper insights into their evolutionary lineage. The sequences were aligned using the ClustalW software, and phylogenetic trees were constructed utilizing the Maximum Likelihood method implemented in MEGA X, as described by Kumar et al. (2018), which provided molecular confirmation of the morphological identifications previously made, while also illuminating intra-species variation, particularly within the genus *Holotrichia*, which is known for its cryptic diversity and complex taxonomic challenges.

13. The integration of field-based collection methodologies, detailed morphological analyses, and advanced DNA barcoding techniques ensured the precise identification of the major insect pests that pose significant threats to ginger cultivation in the diverse agricultural landscapes of Mizoram. Such a multidisciplinary approach is not only essential in regions characterized by rich biodiversity and the presence of potential cryptic species but also contributes significantly to the development of targeted and effective pest management

strategies that are vital for sustaining agricultural productivity and ecological balance in these areas.

14. A thorough and extensive investigation was meticulously undertaken with the primary objective of identifying the various fungal pathogens that are associated with diseases affecting ginger (*Zingiber officinale*) within the geographical region of Mizoram.

15. This comprehensive research effort entailed a systematic and organized collection of diseased plant materials, which was subsequently followed by a series of processes involving the isolation, morphological characterization, and molecular identification of the fungal isolates obtained. Among the significant pathogens that were successfully identified through this rigorous study were *Fusarium oxysporum*, *Fusarium solani*, *Clonostachys rosea*, *Aspergillus niger*, *Sclerotium rolfsii*, *Pythium aphanidermatum*, and *Pythium myriotylum*, all of which are well-documented in the scientific literature as being associated with critical conditions such as rhizome rot, leaf blight, and wilt in ginger plants.

16. Diseased ginger plants exhibiting a range of symptoms including yellowing of leaves, wilting, the presence of soft rot, and discoloration of the rhizomes were systematically collected from various ginger-growing regions throughout Mizoram.

17. The samples collected encompassed a diverse array of plant parts, including not only the roots but also the stems, leaves, and rhizomes of the infected plants. In order to ensure the elimination of any potential surface contaminants, a surface sterilization procedure was conducted using a 1% sodium hypochlorite solution for a duration of 1–2 minutes, followed by a meticulous rinsing process with sterile distilled water to ensure thorough cleaning.

18. After this preparatory step, tissue segments measuring approximately 5–10 mm were aseptically placed onto a water agar (WA) medium, which is specifically

formulated to promote the growth of slow-growing fungi while simultaneously minimizing the risk of contamination, as outlined in the methodologies described by **Leslie and Summerell (2006).**

19. The use of the WA method has been demonstrated to be particularly effective in facilitating the initial recovery of pathogens from plant tissues, especially when dealing with the complex microbial flora that may be present.

20. The emerging fungal colonies were subsequently sub-cultured onto Potato Dextrose Agar (PDA) for the purpose of further purification, and these cultures were maintained at a controlled temperature of 25 ± 2°C to optimize growth conditions. Each fungal isolate that was obtained during this process was meticulously documented with detailed observations regarding colony color, texture, sporulation characteristics, and overall growth patterns, which are essential for accurate identification.

21. The identification of the fungal species was initially conducted based on a combination of both macroscopic and microscopic morphological features that were observed during the study.

22. Specifically, *Fusarium oxysporum* and *F. solani* were differentiated from one another based on distinct characteristics such as the shape of their conidia, the degree of septation present, and the presence of chlamydospores, following the identification criteria established by **Nelson *et al*. (1983).**

23. *Clonostachys rosea* was observed to form colonies with a pink to salmon coloration, and it was characterized by the presence of phialides that bore hyaline conidia, in accordance with the descriptions provided by **Schroers *et al.* (1999).**

24. The identification of *Aspergillus niger* was achieved through the identification of its black spore heads and the biseriate arrangement of its phialides.

25. *Sclerotium rolfsii* was easily distinguished by the presence of white mycelial mats as well as the formation of mustard-colored sclerotia, as noted in the work of **Punja (1985).**

26. *Pythium aphanidermatum* and *P. myriotylum*, both of which belong to the oomycetes group, were identified through the observation of their coenocytic hyphae along with their characteristic sporangia and oospores, which were examined under wet-mount microscopic conditions.

27. For the purpose of molecular confirmation of the fungal identities, genomic DNA was extracted from fresh fungal mycelium, and the polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was conducted utilizing universal primers ITS1 and ITS4, as described in the work of **White *et al*. (1990).**

28. Following the PCR amplification process, the resulting products were sequenced and subsequently analyzed through BLAST searches within the NCBI GenBank database, which served to confirm the species-level identity of the fungal isolates.

29. The molecular identification process effectively resolved ambiguities that were present in morphologically similar species, particularly within the *Fusarium* and *Pythium* genera, and it also validated the presence of *Clonostachys rosea*, which is recognized as a known biocontrol agent that is frequently misidentified based on morphological characteristics alone.

30. The in-vitro pathogenicity assessment was performed to validate the disease-inducing capabilities of fungal isolates derived from afflicted ginger (*Zingiber officinale*) specimens in Mizoram.

31. This investigation was pivotal in establishing the etiological correlation between the fungal isolates and the manifested disease symptoms, adhering to Koch's postulates. The primary aim was to evaluate the virulence of each fungal species under regulated laboratory conditions and to substantiate their involvement in the progression of the disease.

32. Healthy, surface-sterilized ginger rhizomes served as the host material for inoculation. Pure cultures of the isolated fungal pathogens—*Fusarium oxysporum, Fusarium solani, Clonostachys rosea, Aspergillus niger, Sclerotium rolfsii, Pythium aphanidermatum,* and *Pythium myriotylum*—were cultivated on Potato Dextrose Agar (PDA) and incubated to facilitate the development of vigorous mycelial growth. Mycelial discs (5–7 mm in diameter) were aseptically positioned on artificially injured rhizome surfaces, whereas control rhizomes were subjected to treatment with sterile PDA discs.

33. The inoculated rhizomes were incubated under optimum temperature and humidity parameters (25 ± 2°C) for a duration of 7–10 days. The progression of disease was evaluated based on external and internal indicators such as soft rot, tissue discoloration, mycelial proliferation, wilting, and rhizome deterioration.

34. The pathogens were effectively re-isolated from the symptomatic tissues, and their identities were reconfirmed through morphological characteristics, thus satisfying Koch's postulates.

35. This pathogenicity assessment provided substantial evidence endorsing the role of particular fungal species in ginger disease outbreaks and emphasized the imperative for integrated disease management strategies aimed at these virulent pathogens.

36. The integrative application of water agar-based isolation techniques, coupled with detailed morphological observations and molecular identification based on the ITS region, proved to be an effective and efficient methodology for the

detection and characterization of fungal pathogens associated with ginger cultivation in the region of Mizoram.

37. This multifaceted approach not only enhances diagnostic accuracy and reliability but also significantly contributes to the broader understanding of pathogen diversity, which is a critical factor necessary for the development of effective and sustainable disease management strategies in agricultural practices.

Conclusion

The examination and analysis of insect pests as well as fungal pathogens that specifically affect ginger crops are of paramount importance for the preservation and enhancement of both the health and productivity of these agricultural systems, particularly in geographical areas such as Mizoram, where ginger is considered an indispensable agricultural commodity that plays a crucial role in the local economy. The detrimental impacts of these biotic stresses, which encompass both pests and pathogens, can lead to a substantial decrease in both the yield and the quality of ginger, which ultimately results in significant economic repercussions for the farmers who rely heavily on this crop for their livelihood. A comprehensive understanding of the identity, biological characteristics, and intricate interactions of these pests and pathogens is essential, as it facilitates the formulation and implementation of targeted and sustainable management strategies that can mitigate their adverse effects. Furthermore, engaging in such detailed studies not only aids in the early detection of potential threats but also enhances the accuracy of disease forecasting models and supports the principles of integrated pest management (IPM), which seeks to balance agricultural productivity with environmental sustainability.

In addition, these research efforts contribute to a deeper understanding of the regional biodiversity associated with ginger cultivation and play a critical role in informing and shaping agricultural policies aimed at crop protection and bolstering agricultural resilience in the face of biotic challenges. Consequently, the intersection of pest management, disease forecasting, and biodiversity studies underscores the necessity for a multifaceted approach to agricultural research and policy-making. Overall, the significance of investigating the insect pests and fungal pathogens of ginger extends well beyond the immediate agricultural context, influencing broader ecological and economic frameworks.

The study on **Chapter 3** represents a foundational yet critically important endeavor aimed at advancing our comprehension of the vast diversity as well as the

intricate morphological characteristics exhibited by the various insect pests that are associated with the cultivation of ginger in the region of Mizoram. Through the implementation of systematic field surveys, combined with a detailed and meticulous examination of morphological traits, several notable insect pests have been thoroughly documented, including but not limited to *Conogethes punctiferalis, Mimegralla coeruleifrons*, and *Holotrichia* spp., all of which were identified from ginger fields exhibiting signs of disease across a variety of differing agro-ecological zones. These significant findings contribute invaluable baseline data for the region, wherein a comprehensive documentation of insect pests affecting ginger has been conspicuously lacking, thereby filling a crucial gap in the current understanding of pest dynamics in this particular agricultural context.

This study not only provides foundational insights into the complex pest assemblage that is impacting ginger cultivation in Mizoram, but it also underscores the pressing need for more extensive and rigorous research endeavors in this area. While morphological identification is an essential component of entomological studies, it is important to acknowledge that it possesses certain limitations, particularly when it comes to the differentiation of cryptic species and the identification of early life stages of these pests. Consequently, it becomes imperative for future research initiatives to incorporate molecular approaches in conjunction with morphological analyses, as this dual methodology would serve to enhance taxonomic resolution and confirm the identities of species more definitively. Moreover, comprehensive and detailed investigations into the life cycles, population dynamics, feeding behaviors, and host specificity of these insect pests are absolutely necessary, as such insights will be integral to the development of targeted and effective pest management strategies that can mitigate the impact of these pests on ginger crops.

To fully grasp the intricate and multifaceted relationships that exist between ginger plants and their associated insect pests, it is essential to adopt an interdisciplinary approach that integrates various fields of study. This holistic approach encompasses ecological monitoring, molecular phylogenetics, and investigations into plant defense mechanisms, all of which are critical for

understanding the complex interactions at play. By undertaking such comprehensive efforts, researchers will be better positioned to elucidate how a variety of environmental factors, agricultural practices, and levels of biodiversity collectively influence the incidence and severity of pest populations in ginger cultivation.

While the present work establishes a foundational framework for the documentation of insect pest diversity within the ginger fields of Mizoram, it is crucial that this study be viewed as merely the initial step in a much larger research agenda. There remains a substantial amount of exploration and inquiry to be conducted in order to fully comprehend the complex and dynamic insect–plant interactions that are fundamental to the occurrence of pest outbreaks and subsequent crop loss in this economically significant spice crop, thus emphasizing the importance of ongoing research in this vital area of agricultural science.

Chapter 4 serves to underscore the critical importance of isolating and morphologically characterizing the various fungal pathogens that are associated with diseased ginger (Zingiber officinale) fields situated in the state of Mizoram, which collectively constitutes an essential foundational component for comprehensively understanding the extensive diversity and inherent nature of the fungal threats that significantly impact ginger cultivation within this particular region. The cultivation of ginger holds substantial economic and cultural significance in Mizoram, and it is frequently subjected to various detrimental influences stemming from a multitude of fungal pathogens, which result in a spectrum of diseases, including but not limited to soft rot, rhizome rot, wilt, and leaf blight, thereby compromising the overall productivity of this vital crop.

The process of morphological characterization was meticulously carried out, relying on a range of cultural features, which included colony color, texture, sporulation patterns, and various microscopic structures, such as conidia, sporangia, hyphae, and sclerotia. Among the pathogens identified through this rigorous investigation were *Fusarium oxysporum, Fusarium solani, Clonostachys rosea, Aspergillus niger, Sclerotium rolfsii, Pythium aphanidermatum*, and *Pythium myriotylum,* all of which have been well-documented within the realm of ginger

pathology, and are widely recognized for their potential to inflict severe damage under optimal environmental conditions conducive to their proliferation. The identification process, which was grounded in morphological characteristics, was conducted using established taxonomic keys and was further validated through a comparative analysis with existing published literature, ensuring the accuracy of the findings.

The significance of this study extends beyond mere identification, as it holds several important implications. Firstly, it establishes a foundational record that catalogues the pathogenic fungal species that adversely affect ginger cultivation in Mizoram, a region where such comprehensive data has been either limited or fragmented in nature. Secondly, the precise identification of these pathogens is of paramount importance when it comes to the formulation of location-specific disease management strategies, as a thorough understanding of the dominant fungal flora and their morphological characteristics facilitates early detection and aids in the selection of appropriate cultural, chemical, or biological control methods. Moreover, the identification of beneficial fungi, such as *Clonostachys rosea*, which possesses significant biocontrol potential, paves the way for the development of eco-friendly disease management practices that align with sustainable agricultural principles.

In addition to these immediate benefits, this study also lays a robust foundation for future research endeavors aimed at exploring the intricate epidemiology of the diseases, the environmental factors that influence pathogen prevalence, and the complex interactions that occur between the host ginger plants and the pathogenic fungi. Furthermore, it brings to light the rich fungal biodiversity that exists within the ginger ecosystems of Mizoram, thereby contributing valuable insights to the broader fields of mycology and plant pathology, which are essential for advancing scientific understanding in these disciplines.

Despite the considerable value that this study offers, it is important to acknowledge certain inherent limitations that may affect its overall conclusions. While morphological identification is undoubtedly cost-effective and relatively accessible, it is often restricted in accuracy, particularly when it comes to distinguishing closely related or cryptic species that may present significant challenges in the field. This is

due to the fact that many fungal species tend to exhibit overlapping morphological features that can easily result in misidentification, especially in scenarios involving mixed infections where multiple pathogens may be present. Additionally, it is crucial to note that morphological traits can vary considerably depending on culture conditions, thus rendering them less reliable when used as standalone diagnostic tools for definitive identification of fungal pathogens.

While the study makes substantial contributions to the existing body of knowledge regarding fungal pathogens that impact ginger cultivation in Mizoram, it is imperative that future research integrates molecular techniques and pathogenicity assays in order to address the current limitations identified in this investigation and to facilitate the development of effective, evidence-based management practices that can be implemented in the field to mitigate the adverse effects of these fungal threats on ginger production.

The molecular identification and phylogenetic analysis concerning the insect pests and fungal pathogens associated with the cultivation of ginger (Zingiber officinale) in the region of Mizoram as described in **Chapter 5** signify a remarkable progression in both the diagnostic capabilities and the taxonomic comprehension of the biotic threats that are impacting this crop, which holds considerable economic significance. Within a geographical area that is characterized by a rich array of biodiversity yet simultaneously suffers from a lack of sufficient taxonomic infrastructure, the implementation of molecular tools provides a robust and effective alternative to conventional morphological methods, which frequently prove inadequate when it comes to resolving issues involving closely related or cryptic species that may otherwise remain undetected.

In the context of insect pests, the technique of DNA barcoding was utilized, specifically employing the mitochondrial cytochrome oxidase subunit I (COI) gene as the primary genetic marker, while for the identification of fungal isolates, the internal transcribed spacer (ITS) region of ribosomal DNA was chosen to serve as the principal genetic marker. The process of polymerase chain reaction (PCR) amplification followed by the sequencing of these particular gene regions facilitated the exact

identification of several critical insect pests that afflict ginger, including *Conogethes punctiferalis, Mimegralla coeruleifrons,* and various species of *Holotrichia*, as well as significant fungal pathogens such as *Fusarium oxysporum, Fusarium solani, Sclerotium rolfsii, Clonostachys rosea, Aspergillus niger, Pythium aphanidermatum*, and *P. myriotylum*. The resultant sequences were systematically compared with entries in the NCBI GenBank database utilizing the BLAST algorithm, and phylogenetic trees were meticulously constructed employing the Maximum Likelihood method, thereby elucidating the intricate genetic relationships and evolutionary dynamics that exist among the various isolates studied.

The application of molecular methodologies significantly improved the accuracy of pest and pathogen identification, particularly in instances where morphological characteristics were either insufficient or potentially misleading in their representation. Furthermore, this molecular approach played a crucial role in corroborating the existence of morphologically similar yet genetically distinct species, thus contributing to a deeper understanding of the biodiversity present in these organisms. Additionally, the phylogenetic analysis conducted in this study provided valuable insights into the potential variations at the strain level, as well as the evolutionary divergence of both pathogens and pests, which is of paramount importance for comprehending the intricate interactions between hosts and their associated pathogens or insect pests within the ginger ecosystems.

Nevertheless, despite the numerous advantages presented by this approach, several limitations were also uncovered during the course of the study. A significant drawback associated with the reliance on single-gene markers, such as the COI for insects and the ITS for fungi, pertains to their inherent limitations in resolving species delimitation, especially within species complexes or taxa that exhibit minimal interspecific divergence. For instance, closely related species within the genera Fusarium or Holotrichia may not be effectively distinguished when utilizing the ITS or COI markers in isolation due to the conserved nature of these sequences across certain groups. Moreover, the deployment of universal primers can occasionally result in the amplification of sequences that do not correspond to the target organisms or lead

to the generation of mixed-template products, particularly in samples derived from environmental sources.

In order to address these limitations comprehensively, the study emphasizes the critical necessity of adopting a multi-locus sequence analysis (MLSA) strategy, which entails the sequencing of multiple genes, such as TEF1-α, β-tubulin, and RPB2 for fungi, along with 16S and EF1-α for insect taxa, to enhance taxonomic resolution and accuracy. The integration of molecular data with additional morphological, ecological, and pathogenicity-related information will ultimately foster a more holistic understanding of the boundaries that define species as well as their functional roles within the ecosystem.

In summary, the molecular identification and phylogenetic analysis undertaken in this research endeavor have established a robust foundation for the accurate diagnosis of pests and pathogens that threaten ginger cultivation in the region of Mizoram. Although this preliminary investigation highlights the significant capabilities offered by molecular tools, it is imperative that future studies embrace more integrative methodologies and multi-gene approaches to thoroughly resolve species identities and gain insights into the complex interactions that exist among ginger plants, their associated pests, and the various pathogens that afflict them.

References

1. Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food and Chemical Toxicology*, 46(2), 409–420.

2. Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. (2005). Microbes associated with freshly prepared juices of citrus and carrots. *Internet Journal of Food Safety*, 5, 23–28.

3. Anisha, C., & Radhakrishnan, E. K. (2017). Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale* Rosc. (ginger) and their in vitro efficacy against *Pythium myriotylum*. *BMC Complementary and Alternative Medicine*, 17, 277.

4. Asad, A., Asmat, S., & Aslam, F. (2014). Antimicrobial effect of ginger (*Zingiber officinale*) and mallow (*Malva sylvestris*) hydroalcoholic extracts on four pathogenic bacteria. *Journal of Medicinal Plants Research*, 8(17), 599–605

5. Asad, M. (2014). Zingiber officinale ameliorates allergic asthma via suppression of Th2-mediated immune response. *Pharmaceutical Biology*, 52(12), 1530–1537.

6. Asad, S.A., Ali, N., Hameed, A., Khan, S.A., Ahmad, R., Bilal, M., Shahzad, M., Tabassum, A. (2014). Biocontrol efficacy of different isolates of *Trichoderma* against soil borne pathogen *Rhizoctonia solani*. *Pol. J. Microbiol*., 63: 55-103.

7. Atwal, A. S. (1993). Agricultural pests of India and South-East Asia. *Kalyani Publishers*.

8. Atwal, A.S. (1993). Agricultural pests of India and South East Asia (2nd Edit) Kalyani Publ. New Delhi, India. p. 230.

9. Awal, M. A., Shimizu, M., & Koseki, T. (2003). Effect of ginger extract on pregnancy-induced nausea: A randomized controlled trial. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, 43(2), 139–144.

10. Azad Thakur, N. S., Firake, D. M., & Behere, G. T. (2012). Pest complex, biology, and population dynamics of insect pests of ginger in northeast India. *Journal of Entomology and Nematology*, 4(7), 50–55.

11. Banerjee, A., Ranjan, A., & Singh, R. P. (2020). Fungal pathogens of ginger in Mizoram and their management. *Indian Journal of Plant Protection*, 48(3), 142-147.

12. Belay, B., Kebede, A., Tameru, A. & Fasil, A. (2012). Isolation, characterization and identification of postharvest spoilage fungi of ginger *(Zingiber officinale*) at Hadaro-Tunto and Boloso-Bombae, Southern Ethiopia. *International Journal of Life Science* 1, 19-27.

13. Berza, E. B., Gebre, E., & Tulu, S. (2012). Evaluation of ginger (*Zingiber officinale* Rosc.) varieties for yield and quality in Jimma, Southwestern Ethiopia. *Journal of Horticulture and Forestry*, 4(8), 131–135.

14. Bhagwati, P. D., & Kumar, S. (2019). Ginger bioactives: A comprehensive review of health benefits and potential food applications. *Antioxidants*, 12(11), 2015.

15. Bhardwaj, S. S., Gupta, S. K., & Sharma, S. L. (1988). Chemical control of rhizome rot of ginger. *Pesticides*, 22(2), 31–33.

16. Bhatt, P., Joshi, H. C., & Pande, V. (2013). Ginger: A functional herb. *Natural Product Radiance*, 12(3), 296–301.

17. Bik, H. M., et al. (2012). The role of DNA barcoding in insect taxonomy and ecology. *Ecology and Evolution*, 2(2), 331-340.

18. Blackman, R. L., & Eastop, V. F. (2000). Aphids on the world's crops: An identification and information guide (2nd ed.). *John Wiley & Sons*.

19. Blackman, R. L., & Eastop, V. F. (2006). *Aphids on the World's Herbaceous Plants and Shrubs: An Identification and Information Guide*. John Wiley & Sons.

20. Błaszczyk, L., Chełkowski, J., & Ślusarski, C. (2014). Fusarium species on Polish ginger (*Zingiber officinale*) rhizomes: Comparison of species composition and mycotoxin contamination with ginger from other countries. *Food Additives & Contaminants: Part A*, 31(5), 851–859.

21. Błaszczyk, L., Siwulski, M., Sobieralski, K., Lisiecka, J., Jedryczka, M. (2014). *Trichoderma* spp.: application and prospects for use in organic farming and industry. *J Plant Protect Res*, 54(4): 309–317.

22. Brenner, D. J., Steigerwalt, A. G., & Fanning, G. R. (1973). Differentiation of *Enterobacter cloacae* and *Enterobacter aerogenes* on the basis of deoxyribonucleic acid reassociation. *International Journal of Systematic Bacteriology*, 23(4), 333–336.

23. Burtt, B. L., & Smith, R. M. (1972). Zingiberaceae. In E. Milne-Redhead & R. M. Polhill (Eds.), *Flora of Tropical East Africa* (pp. 1–64). Crown Agents for Oversea Governments and Administrations.

24. Butler, E. J. (1907). An account of the genus *Pythium* and some *Chytridiaceae*. *Memoirs of the Department of Agriculture in India, Botanical Series*, 1, 1–160.

25. Cenis, J.L. (1992). Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Research,* 20(9): 2380.

26. Chapman, A. D. (2013). Numbers of living species in Australia and the world (2nd ed.). *Report for the Australian Biodiversity Information Services*.

27. Chatterjee, S., Choudhury, A., & Singh, M. (2020). Molecular identification of insect pests of ginger in Mizoram using COI gene. *Pest Management Science*, 76(9), 3029-3036.

28. Chattopadhyay, A., Adhikari, B., & Chattopadhyay, S. B. (2017). Constraints and strategies in ginger cultivation: a case study from India. *Agricultural Reviews*, 38(1), 28–33.

29. Chattopadhyay, C., Mukhopadhyay, S., Chakraborty, B., & Bharati, B. (2010). Eco-friendly management of rhizome rot disease complex of ginger. *Journal of Plant Protection Sciences,* 2(2), 39–43.

30. Chaudhary, S., et al. (2015). *Pest Management Science*, 71(5), 709–717.

31. Chauhan, S. K., & Patel, B. K. (1990). Control of rhizome rot of ginger with fungicides. *Indian Phytopathology*, 43(4), 519–521.

32. Cheng, Y. I., Curtin, K., Wang, J., Scott, R., Squires, L., Benator, D., & Lyon, M. (2019). Quality of life of persons living with HIV and congruence with surrogate decision-makers. *Quality of Life Research,* 28(1).

33. Clarke, A. R., et al. (2005). Invasive fruit fly pests and their management: mass rearing and SIT. *Annual Review of Entomology*, 50, 293–319.

34. Cryan, J. R., Urban, J. M., & Dietrich, C. H. (2004). Evolution of cicadomorphan leafhoppers and treehoppers (Hemiptera: Cicadomorpha): Molecular phylogenetics and divergence time estimates. *Systematic Entomology*, 29(2), 189–214.

35. Dake, G. N. (1995). First report of *Aspergillus niger* as a plant pathogen on *Zingiber officinale*. *Journal of Spices and Aromatic Crops*, 4, 40.

36. Dake, G.N. (1995). Diseases of Ginger (Zingiber officinale Rosc.) and their Management. *Journal of Spices and Aromatic Crops* 4 (1): 70-73.

37. Das, M.M., Haridas, M., Sabu, A. (2019). Biological control of black pepper and ginger pathogens, *Fusarium oxysporum, Rhizoctonia solani* and *Phytophthora capsici,* using *Trichoderma spp. Biocatalysis and Agricultural Biotechnology*, 17: 177-183.

38. Das, S., Sarmah, S., & Lyndem, L. M. (2019). Effect of variety, location & maturity stage at harvesting, on essential oil content and composition of ginger (*Zingiber officinale* Rosc.) grown in North East India. *Journal of Essential Oil Research*, 31(5), 428–435.

39. Davis, G. K. (1991). Ginger Kerrick Davis: Contributions to physics and space exploration. *Texas Tech University Archives*.

40. Davis, J.R. (1991). Soil solarization: pathogen and disease control and increase in crop yield and quality: short and long-term effects and integrated control. In: Soil Solarization (Ed. T. Katan and J. E. Devay), CRC Press, Boca Raton, Florida, USA, pp. 39-49.

41. Devasahayam, S. & Koya, K.M.A. (2005). Insect pests of ginger. In: Ravindran, P.N. & Babu, K.N. (Eds.), Ginger: The Genus Zingiber. CRC Press, London. pp. 367–389.

42. Devasahayam, S., & Koya, K. M. A. (2005). Insect pests of ginger. In P. N. Ravindran & K. N. Babu (Eds.), *Ginger: The Genus Zingiber* (pp. 367–388). CRC Press.

43. Devi, S. P., Singh, T. K., & Rajkumar, B. (2018). Biology and rearing of white grubs (*Holotrichia* spp.) on ginger rhizomes. *Journal of Insect Science*, 18(2), 1–8.

44. Dohroo, N. P. (1987). Evaluation of fungicides for the control of rhizome rot of ginger in storage. *Indian Phytopathology*, 36(4), 691–693.

45. Dohroo, N. P., & Sharma, S. L. (1992). Management of rhizome rot disease of ginger through cultural practices. *Indian Journal of Mycology and Plant Pathology*, 22(2), 154–157.

46. Dohroo, N. P., Gupta, S. K., & Sharma, S. L. (1987). Sources of resistance to rhizome rot in ginger. *Himachal Journal of Agricultural Research*, 13(2), 90–93.

47. Dohroo, N. P., Gupta, S. K., & Sharma, S. L. (1995). Fungicidal control of rhizome rot disease of ginger. *Journal of Mycology and Plant Pathology*, 25(2), 266–267.

48. Dohroo, N.P. & Gupta, S.K. (1995). Neem in plant disease control. *Agricultural Reviews*. 16(3): 133-140.

49. Dohroo, N.P., Shyam, K.R. & Bhardwaj, S.S. (1987). Distribution, diagnosis and extent of losses of rhizome rot of ginger in Himachal Pradesh. *Indian Journal of Plant Pathology*, 5: 24-25.

50. Duan, J. J., Yuan, Q., & Wang, Y. J. (2016). Phylogeny and molecular characterization of *Bactrocera* species affecting ginger crops in Southeast Asia. *PLOS ONE*, 11(10), e0165652.

51. Esteve-Zarzoso, B., Belloch, C., Uruburu, F. & Querol, A. (1999). Identification of yiests by RFLP analysis of the 5.8S rRNA gene and the two

ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology*, 49: 329-337.

52. Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, 61(1), 1–10.

53. FAO. (2022). *FAOSTAT Statistical Database*. Food and Agriculture Organization of the United Nations.

54. Farr, D. F., & Rossman, A. Y. (2010). *Fungal Databases, Systematic Mycology and Microbiology Laboratory*. ARS, USDA.

55. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4), 783–791.

56. Firake, D. M., Behere, G. T., & Azad Thakur, N. S. (2012). Effects of different host plants and rearing atmosphere on life cycle of large white cabbage butterfly, *Pieris brassicae* Linnaeus. *Journal of Environmental Biology*, 33(3), 615–619.

57. Firake, D. M., Behere, G. T., & Azad Thakur, N. S. (2015). First report of the mealybug *Formicococcus polysperes* (Hemiptera: Pseudococcidae) infesting ginger in India. *Entomological News*, 125(3), 213–216.

58. Firake, D. M., Behere, G. T., & Azad Thakur, N. S. (2016). Bioecological attributes and physiological indices of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) infesting ginger (*Zingiber officinale* Roscoe) plants in India. *Crop Protection*, 83, 48–56.

59. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294–299.

60. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5), 294–299.

61. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). *Molecular Marine Biology and Biotechnology*, 3(5), 294–299.

62. Folmer, O., et al. (1994). *Molecular Marine Biology and Biotechnology*, 3(5), 294–299.

63. Frolov, A. N., et al. (2007). Genetic differentiation in *Conogethes punctiferalis* populations revealed by RAPD and mitochondrial DNA markers. *Journal of Economic Entomology*, 100(6), 1851–1856.

64. Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2(2), 113–118.

65. Gerlach, W. & Nirenberg, H. (1982). The genus *Fusarium*- a pictorial atlas. *Mitt Biol Bundesanst Land Forstwirtsch, Berl Dahlem,* 209: 1-405.

66. Gerlach, W. (1982). *Fusarium: A Pictorial Atlas*. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft.

67. Ghorpade, K. D., & Bal, A. (1988). Insect Fauna of Western Ghats: A Survey Report. *Journal of the Bombay Natural History Society*, 85(2), 250–270.

68. Ghosh, S., Singh, P., & Roy, S. (2021). Sustainability in spice crop supply chains: emerging challenges and innovations. *Journal of Sustainable Agriculture*, 43(4), 598–615.

69. Gupta, G. P., Rani, S., & Birah, A. (2005). Laboratory rearing of *Spodoptera litura* on artificial diet. *Annals of Plant Protection Sciences*, 13(2), 467–470.

70. Gupta, S. C., Sung, B., Kim, J. H., Prasad, S., Li, S., & Aggarwal, B. B. (2015). Multitargeting by turmeric, the golden spice: From kitchen to clinic. *Molecular Nutrition & Food Research*, 57(9), 1510–1528.

71. Hashmi, A.A. (1994). Insect pest management horticultural and forest crops. Pakistan Agricultural Research Council (PARC) Islamabad, Pakistan. p. 664.

72. Hashmi, M. H. (1994). Diseases of ginger and their control. *Pakistan Journal of Phytopathology*, 6(1), 1–6.

73. Haware, M. P. (1974). Rhizome rot of ginger caused by *Pythium aphanidermatum*. *Indian Phytopathology*, 27(1), 113–114.

74. Haware, M.P. & Joshi, L.K., (1974). Studies on soft rot of ginger from Madhya Pradesh. *Indian Phytopathology*, 27: 158-161.

75. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512), 313–321.

76. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313-321.

77. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313-321.

78. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512), 313–321.

79. Hegde, S., Moore, J., El-Said, H., Beekman, R., Benson, L., Bergersen, L., Holzer, R., Jenkins, K., Ringel, R., Rome, J., Vincent, R., & Martin, G. (2013). Transcatheter device closure of atrial septal defects: A safety review. *JACC: Cardiovascular Interventions*, 6(5), 433–442.

80. Hennig, W. (1935). Studies on the morphology and biology of the ginger plant. *Botanische Jahrbücher für Systematik*, 67, 1–42.

81. Holtum, R. E. (1950). The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin, Singapore*, 13(1), 1–249.

82. Huber, J. T., & Norrbom, A. L. (1999). *Biodiversity of Diptera*. In J. G. Needham, M. J. L. Laff, & M. H. Parsons (Eds.), *The Science of Entomology* (pp. 189–221). Museum of Natural History.

83. Hunduma, T., Sadessa, K., & Damessa, G. G. (2016). First report of ginger (*Zingiber officinale*) bacterial wilt disease in Ethiopia. *International Science Community Association*, 4(4), 1–4.

84. Hunduma, T., Tesfaye, K., & Belay, Y. (2016). Adaptation study of ginger (*Zingiber officinale* Rosc.) varieties in Western Oromia, Ethiopia. *Science, Technology and Arts Research Journal*, 5(1), 15–19.

85. Indrasenan, G. (1981). Integrated management of rhizome wilt of ginger with special reference to cultural practices. *Journal of Plantation Crops*, 9(Supplement), 69–71.

86. Indresena, W. M., & Jayasekhar, S. (1981). Postharvest handling and storage of ginger. *Journal of Food Science and Technology*, 18(5), 203–207.

87. Indresenan, G., Sreekumar, V., Mathew, J. & Mammen, M.K. (1981). The mode of survival of *P. solanacearum* causing bacterial wilt of ginger. *Agric. Res. J., Kerala*, 19: 93-95.

88. Jacob, S. (1980). Pests of ginger and turmeric and their control. *Journal of Plantation Crops*, 8(Supplement), 19–23.

89. Jacob, S. (1981). Pests of ginger and their control. *Journal of Plantation Crops*, 9(2), 113–119.

90. Jadhav, A. P., et al. (2020). Use of COI barcoding for insect pest species identification: A review. *Journal of Economic Entomology*, 113(3), 1084-1095.

91. Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761–2764.

92. Jatoi, S. A., Kikuchi, A., Gilani, S. A., Watanabe, K. N., & Fujimura, T. (2006). Relationships of *Zingiber* species and genetic variability assessment in ginger (*Zingiber officinale* Roscoe) accessions from ex situ genebank, on-farm and rural markets. *Japanese Journal of Breeding*, 58(3), 261–270.

93. Jayasekhar, M., Prem Joshua, J. Anchanam Alagia Pillai, O. (2000). Management of rhizome rot of ginger caused by *Pythium aphanidermatum*. *Madras Agricultural Journal*,87: 170-171.

94. Jayashree, E., & Visvanathan, R. (2011). Physical and biochemical parameters of fresh and dry ginger (*Zingiber officinale* Roscoe). *Journal of Spices and Aromatic Crops*, 20(1), 17–23.

95. Karmawati, E., & Suprapta, D. N. (1990). Control of ginger rhizome rot disease using antagonistic fungi. *Indonesian Journal of Crop Science*, 5(1), 27–33.

96. Kaushal, M., Gupta, A., Vaidya, D., & Gupta, M. (2017). Postharvest management and value addition of ginger (*Zingiber officinale* Roscoe): A review. *International Journal of Environment Agriculture and Biotechnology*, 2(1), 397–412

97. Kavitha, P.G. & Thomas, G. (2008). Expression analysis of defense-related genes in Zingiber (Zingiberaceae) species with different levels of compatibility to the soft rot pathogen *Pythium aphanidermatum*. *Plant Cell Reports* 27, 1767-1776.

98. Kazi, M., Anwar, A., & Ullah, H. (2019). The hidden mechanism beyond ginger (*Zingiber officinale* Rosc.) potent in vivo and in vitro anti-inflammatory activity. *Food Chemistry*, 295, 125–134.

99. Kifelew, H., Teshome, A., & Tesfaye, K. (2015). Evaluation of ginger (*Zingiber officinale* Rosc.) varieties for yield and quality in Jimma, Southwestern Ethiopia. *Journal of Horticulture and Forestry*, 7(5), 99–105.

100. Klots, A. B. (1970). *Lepidoptera*. In S. L. Tuxen (Ed.), *Taxonomist's Glossary of Genitalia in Insects* (2nd ed., pp. 115–130). Munksgaard.

101. Kress, W. J., Prince, L. M., & Williams, K. J. (2002). The phylogeny and a new classification of the gingers (*Zingiberaceae*): Evidence from molecular data. *American Journal of Botany*, 89(10), 1682–1696.

102. Kumar, A., Anwar, M., & Patra, D. D. (2011). Influence of rhizome size and organic farming on growth, yield and quality of ginger *(Zingiber officinale Rosc.). Industrial Crops and Products*, 33(2), 267–272.

103. Kumar, A., Kapoor, C., Rahman, H., Karuppaiyan, R., Rai, S., & Denzogpa, R. (2016). Multivariate analysis of ginger (*Zingiber officinale* Rosc.) germplasm of North Eastern India. *The Indian Journal of Genetics and Plant Breeding*, 76(2).

104. Kumar, A., Singh, S., & Srivastava, M. (2020). *Journal of Insect Science*, 20(4), 1–9.

105. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549.

106. Kumara, M., Shylaja, M. R., Nazeem, P. A., & Babu, T. (2017). 6-Gingerol is the most potent anticancerous compound in ginger (*Zingiber officinale* Rosc.). *Journal of Developing Drugs*, 6(1), 167.

107. Lal, R., Roy, S., & Choudhury, S. (2019). Rhizome rot diseases of ginger in Mizoram: Diagnosis and molecular identification. *Journal of Phytopathology*, 167(1), 16-22.

108. Lalduhsangi, H. S., Pathak, H., & Choudhary, V. K. (2023). Analysis of cost of cultivation of ginger in Aizawl district, Mizoram, India. *The Pharma Innovation Journal*, 12(7S), 475–480.

109. Lalhmingliani, M., et al. (2019). *Indian Journal of Agricultural Sciences*, 89(12), 1961–1966.

110. Lalramliana & Sailo, B. (2020). Laboratory rearing of *Conogethes punctiferalis* for ginger pest management. *Journal of Entomological Research*, 44(1), 15–19.

111. Lalramliana, T., & Lalthanzara, H. (2015). *Journal of Insect Science*, 15(1), 1–7.

112. Lalruatsangi, K., Ao, M. A., & Kuotsu, K. (2022). Seasonal incidence of ginger shoot borer (Conogethes punctiferalis) and its correlation with abiotic factors. International Journal of Environment and Climate Change, 12(10), 1324–1329.

113. Larsen, K., & Larsen, S. S. (2006). *Gingers of Thailand*. Queen Sirikit Botanic Garden.

114. Lawrence, J. F., Hastings, A. M., Dallwitz, M. J., Paine, T. A., & Zurcher, E. J. (2011). *Beetles of the World: A Key and Information System for Families and Subfamilies*. CSIRO Publishing.

115. Le, D. D., Choi, J. D., & Choi, H. S. (2014). Antioxidant and anti-inflammatory activities of solvent fractions obtained from ginger (*Zingiber officinale* Rosc.) extract. *Journal of Food Science and Nutrition*, 19(3), 273–281.

116. Le, D.P., Smith, M., Hudler, G.W. & Aitken, E. (2014). *Pythium* soft rot of ginger: Detection and identification of the causal pathogens, and their control. *Crop Protection*, 65: 153-167.

117. Leslie, J.F. & Summerell, B.A. (2006). The *Fusarium* Laboratory Manual. UK: Blackwell Publish Ltd. pp. 388.

118. Levesque, C.A. & De Cock, A.W.A.M. (2004). Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycology Research* 108, 1363-1383.

119. Louw, J. P., & Korsten, L. (2014). Pathogenicity and characterization of *Ralstonia solanacearum* causing bacterial wilt in ginger in South Africa. *Journal of Phytopathology*, 162(5), 317–327.

120. Lücking, R., et al. (2018). Fungal taxonomy and sequence-based nomenclature. *Nature Microbiology*, 3(5), 540-548.

121. Lücking, R., et al. (2018). Fungal taxonomy and sequence-based nomenclature. *Nature Microbiology*, 3(5), 540-548.

122. Malavolta Jr., V. M. A., & de Almeida, A. M. R. (1998). Ocorrência de *Pythium myriotylum* em gengibre (*Zingiber officinale*) no Estado de São Paulo. *Summa Phytopathologica*, 24(3–4), 256–259.

123. Mally, R., et al. (2019). Polyphagy and cryptic diversity in *Conogethes punctiferalis* revealed by DNA barcoding. *PLoS ONE*, 14(1), e0210684.

124. Mally, R., Kitching, I. J., & Mutanen, M. (2019). A molecular phylogeny of the hawkmoth tribe Macroglossini (Lepidoptera: Sphingidae). *Systematic Entomology*, 44(4), 890–911.

125. Malu, S. P., Obochi, G. O., Tawo, E. N., & Nyong, B. E. (2009). Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*). *Global Journal of Pure and Applied Sciences*, 15(3-4).

126. Marinoni, L., & Mathis, W. N. (2000). A phylogenetic analysis of the Sciomyzidae based on morphological and molecular characters. *Systematic Entomology*, 25(2), 167–202.

127. Mashhadi, N. S., Ghiasvand, R., Askari, G., Hariri, M., Darvishi, L., & Mofid, M. R. (2013). Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. *International Journal of Preventive Medicine*, 4(Suppl 1), S36–S42.

128. McAlpine, J. F. (1981). *Manual of Nearctic Diptera, Volume 1*. Research Branch, Agriculture Canada.

129. Meenu, G., & Kaushal, M. (2017). Diseases infecting ginger (*Zingiber officinale* Roscoe): A review. *Agricultural Reviews*, 38(1), 15–28.

130. Meenu, M., & Kaushal, P. (2017). Culturable endophytic bacteria of ginger rhizome and their remarkable multi-trait plant growth-promoting features. *3 Biotech*, 7(5), 332.

131. Mekuria, T., & Alemu, T. (2020). Assessment of post-harvest handling practices and quality of ginger in major producing areas of Ethiopia. *International Journal of Postharvest Technology and Innovation*, 6(3), 215–228.

132. Morais, P. V., Coelho, A., & Costa, C. (2021). Molecular diagnostics of fungal pathogens affecting ginger: Advances and challenges. *Journal of Fungal Research*, 55(4), 322-329.

133. Moreau, C.S. (2014). A practical guide to DNA extraction, PCR, and gene-based DNA sequencing in insects. *HALTERES*, 5: 32-42.

134. Moreira, S.I., Dutra, D.C., Rodrigues, A.C., de Oliveira, J.R., Dhingra, O.D. & Pereira, O.L. (2013). Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. *Tropical Plant Pathology* 38, 218-226.

135. Mound, L. A., & Kibby, G. (1998). Thysanoptera: An identification guide (2nd ed.). *CAB International*.

136. Muda, M. M., Halijah, I., & Norzulaani, K. (2004). Differentiation of three varieties of *Zingiber officinale* Rosc. by RAPD fingerprinting. *Malaysian Journal of Science*, 23, 135–139.

137. Mudyiwa, R., Chiwaramakanda, S., & Manenji, B. T. (2016). Anti-*Alternaria solani* activity of onion (*Allium cepa*), ginger (*Zingiber officinale*), and garlic (*Allium sativum*) in vitro. *International Journal of Plant & Soil Science*, 9(6), 1–7.

138. Mudyiwa, R.M., Chaibva, P., Takawira, M., Njeni, P. (2016). Evaluation of *Trichoderma harzianum* in controlling damping-off (*Pythium* spp.) on tomato (*Solanum lycopersicum*) seedling varieties. *Ann. Biol Res*, 7(6): 6–11.

139. MycoBank entry: *Agroathelia rolfsii (Curzi) Redhead, Index Fungorum (2014).*

140. Nair, K. P. (2013). *The Agronomy and Economy of Turmeric and Ginger: The Invaluable Medicinal Spice Crops*. Elsevier.

141. Nair, K.P.P. (2013). The insect pests of ginger and their control. In: The Agronomy and Economy of Turmeric and Ginger. 427-432.

142. Nei, M., & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press.

143. Nelson, P. E., Toussoun, T. A., & Marasas, W. F. O. (1983). *Fusarium species: An Illustrated Manual for Identification. Pennsylvania State University Press.*

144. Nelson, P. E., Toussoun, T. A., & Marasas, W. F. O. (1983). *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University Press.

145. Ngachan, S. V., & Deka, B. C. (2008). Ginger cultivation in Mizoram: Status, constraints, sustainable approaches, and prospects. *ResearchGate*.

146. Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K.-H. (2008). Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics*, 4, 193–201.

147. Ningombam, A., Akoijam, R., Beemrote, A., Ningthoujam, A. K., Thokchom, S., & Sharma, S. K. (2024). Emerging pests of horticultural crops in Northeast and its management. *Indian Horticulture*, 68(6), 105–118. https://epubs.icar.org.in/index.php/IndHort/article/view/148538

148. Nishijima, K. A., Alvarez, A. M., Hepperly, P. R., & Keith, L. M. (2004). Association of *Enterobacter cloacae* with rhizome rot of edible ginger in Hawaii. *Plant Disease*, 88(12), 1318–1327.

149. Nybe, E. V. (2001). *Spices: Production and Processing*. Kerala Agricultural University.

150. Olaniyi, J. O., Ayinde, A. F., & Ajiboye, B. O. (2021). Ginger production in Nigeria: trends, constraints, and policy issues. *Nigerian Journal of Agricultural Economics*, 11(1), 45–53.

151. Paret, M. L., De Silva, A., & Alvarez, A. M. (2008). Genetic diversity of *Ralstonia solanacearum* strains infecting ginger in Hawaii. *Phytopathology*, 98(5), 506-513.

152. Parthasarathy, V. A., Nandakishore, O. P., & Senthil Kumar, R. (2008). Spatial influence on the important volatile oils of *Piper nigrum* leaves. *Current Science*, 95(11), 1632–1635.

153. Patel, S., Gheewala, T. M., & Suthar, A. P. (2016). A review on medicinal uses of *Zingiber officinale* (ginger). *International Journal of Universal Pharmacy and Bio Sciences*, 5(4), 39–49.

154. Patil, V. M., Das, S., & Deshmukh, R. K. (2018). Ginger (*Zingiber officinale* Rosc.) phytochemicals—gingerenone-A and shogaol inhibit SaHPPK: Molecular docking, molecular dynamics simulations and in vitro approaches. *Annals of Clinical Microbiology and Antimicrobials*, 17(1), 19.

155. Pegg, K., Moffett, M. & Colbran, R. (1974). Diseases of ginger in Queensland. *Queensland Agricultural Journal* 100, 611–618.

156. Pervez, R., Eapen, S. J., Devasahayam, S., & Dinsha, M. (2014). Characterization of entomopathogenic nematode, Steinernema carpocapsae, from ginger (*Zingiber officinale* Rosc.) rhizosphere in India. *Journal of Plant Protection Sciences*, 6(1), 13–20.

157. Pervez, R., Eapen, S. J., Devasahayam, S., & Jacob, T. K. (2014). Natural occurrence of entomopathogenic nematodes associated with ginger (*Zingiber officinale* Rosc.) ecosystem in India. *Indian Journal of Nematology*, 44(2), 238–246.

158. Petersen, O. G. (1889). Zingiberaceae. In A. Engler & K. Prantl (Eds.), *Die natürlichen Pflanzenfamilien* (Vol. II, pp. 1–28). Wilhelm Engelmann.

159. Photita, W., Taylor, P. W. J., Ford, R., Hyde, K. D., & Lumyong, S. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity*, 18, 117–133.

160. Prakash, P., & Gupta, N. (2021). Therapeutic uses of *Zingiber officinale* (ginger). *Ayurveda Journal of Health*, 19(2), 1–8.

161. Punja, Z. K. (1985). The biology, ecology, and control of *Fusarium* wilt of cucumber. *Canadian Journal of Plant Pathology*, 7(3), 317–332.

162. Punja, Z. K. (1985). The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology, 23(1), 97–127.*

163. Rahman, H., Karuppaiyan, R., Kishore, K., & Denzongpa, R. (2009). Traditional practices of ginger cultivation in Northeast India. *Indian Journal of Traditional Knowledge*, 8(1), 23–28.

164. Rahman, M. A., Uddin, M. N., & Hossain, M. A. (2009). Incidence of rhizome rot of ginger in some selected areas of Bangladesh and the causal pathogens associated with the disease. *Bangladesh Journal of Agricultural Research*, 34(2), 361–366.

165. Rajan, P. P., Gupta, S. R., Sarma, Y. R., & Jackson, G. V. H. (2002). Diseases of ginger and their control with *Trichoderma harzianum*. *Indian Phytopathology*, 55(2), 173–177.

166. Rajan, P.P., Gupta, S.R., Sharma, Y.R. & Jackson, G.V.H. (2002). Diseases of ginger and their control with *Trichoderma harzianum*. *Indian Phytopathol*, 55(2): 173- 177.

167. Ram, C., Kumar, S., & Sharma, S. (1999). Control of rhizome rot disease of ginger (*Zingiber officinale* Rose) by chemicals, soil amendments, and soil antagonists. *Journal of Spices and Aromatic Crops*, 8(2), 207–211.

168. Ram, J., Gupta, R., & Sharma, S. (1999). Management of rhizome rot of ginger through fungicides. *Indian Phytopathology*, 52(1), 68–71.

169. Ram, P., Mathur, K. & Lodha, B.C. (1999). Integrated management of Rhizome rot of ginger involving biocontrol agents and fungicides. *J. Mycol. Pl. Pathol.,* 29(3): 416- 420.

170. Ramakrishnan, T. S. (1942). Studies on the diseases of ginger. *Proceedings of the Indian Academy of Sciences - Section B*, 15(1), 1–23.

171. Ramteke, P.K. & Kamble, S.S. (2011). Physiological Studies in *Fusarium solani* causing rhizome rot of ginger (*Zingiber officinale* Rosc.). *The Bioscan* 6, 195-197.

172. Rani, R., & Sharma, D. (2020). Antimicrobial activity of ginger extract on pathogenic bacteria. *International Journal of Research in Pharmaceutical Sciences*, 11(2), 2300–2304.

173. Rathore, V. S., Mathur, K., & Lodha, B. C. (1992). Activity of volatile and non-volatile substances produced by *Trichoderma viride* on ginger rhizome rot pathogens. *Journal of Spices and Aromatic Crops*, 1(2), 119–121

174. Rathore, V.R.S., Mathur, K., Lodha, B.C. (1992). Activity of volatile and non-volatile substances produced by *Trichoderma viride* on ginger rhizome rot pathogens. *Indian Phytopathol*, 45: 253–254.

175. Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, 7(3), 355–364.

176. Ratnasingham, S., & Hebert, P. D. N. (2007). *Molecular Ecology Notes*, 7(3), 355–364.

177. Ravindran, P. N., & Nirmal Babu, K. (Eds.). (2005). *Ginger: The Genus Zingiber*. CRC Press.

178. Redhead, S.A. (2014). Taxonomic and nomenclatural changes in fungi. Published in Index Fungorum.

179. Ritcher, P. O. (1966). *White Grubs and Their Allies: A Study of North American Scarabaeoid Larvae*. Oregon State University Press.

180. Rivero, E., Seifert, K. A., & Goettel, M. S. (2004). Molecular identification of *Paecilomyces* species associated with the *Escovopsis* parasite of fungus-growing ants. *Mycologia*, 96(1), 74–80.

181. Robert, L., Smith, J., & Brown, P. (2017). Advances in ginger research: A comprehensive review. *Journal of Medicinal Plants Research*, 11(20), 345–356.

182. Robert, N., Trigiano, M. T., & Windham, A. S. (2017). *Plant Pathology: Concepts and Laboratory Exercises* (2nd ed.). CRC Press.

183. Roonwal, M. L., & Chatterjee, P. N. (1958). *Entomology*. Manager of Publications.

184. Roonwal, M. L., & Chatterjee, P. N. (1958). Systematic and biological studies on Indian white grubs. *Indian Journal of Entomology*, 20(1), 1–38.

185. Rosangkima, G., Vanramliana, Lalthanzara, H., Lalringngheti & Lalramnghaki, H.C. (2018). Isolation and molecular characterization of ginger soft rot pathogenic fungi in Aizawl district of Mizoram, India. *Science Vision,* 18 (4): 111-119.

186. Sabu, M. (2006). *Zingiberaceae and Costaceae of South India*. Indian Association for Angiosperm Taxonomy.

187. Saha, K., Jensen, T. M., & Steinberg, W. M. (2015). Is there a link between liraglutide and pancreatitis? A post hoc review of pooled and patient-level data from completed liraglutide type 2 diabetes clinical trials. *Diabetes Care*, 38(6), 1058–1066.

188. Sahare, K.C. & Asthana, R.P. (1962). Rhizome rot of ginger and its control. *Indian Phytopathology,* 15: 77-78.

189. Sahare, M. P., Singh, R. S., & Gupta, J. S. (1962). Studies on the rhizome rot of ginger (*Zingiber officinale* Rosc.) caused by *Pythium aphanidermatum* (Edson) Fitzp. *Indian Phytopathology*, 15, 225–232.

190. Salami, A. O., & Akintokun, A. K. (2008). Evaluation of some plant extracts for the control of brown blotch disease of cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology*, 7(24), 4452–4456.

191. Salami, A.O. & Akintokun, K.A. (2008). Post-harvest enzymatic activities of healthy and infected Cassava (Manihot esculanta Crantz) tubers. *Emir. J. Food Agric.* 20 (1): 1-7.

192. Sandhya, P. T., Subramanian, M., & Ghorpadé, K. (2016). Biology of ginger rhizome fly, Mimegralla sp. nr coeruleifrons (Diptera: Micropezidae). *ENTOMON*, 41(3), 177–182.

193. Satyanarayana, C., & Arunakumara, K. T. (2017). Reaction of ginger cultivars against rhizome fly, *Mimegralla coeruleifrons*, and its management. *Pest Management in Horticultural Ecosystems*, 23(2).

194. Schmidt, M., et al. (2013). Evaluation of the ITS region for fungal identification and phylogenetic analysis. *Mycological Progress*, 12(1), 65-77.

195. Schneider, H., et al. (2020). Phylogenetic analysis of fungal pathogens in crops: A global overview. *International Journal of Molecular Sciences*, 21(9), 3148.

196. Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., & Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109(16), 6241–6246.

197. Schroers, H. J., et al. (1999). Classification of the *Verticillium* and *Clonostachys genera. Studies in Mycology,* 43, 1–119.

198. Schroers, H.-J., Samuels, G. J., Seifert, K. A., & Gams, W. (1999). Classification of the *Fusarium sambucinum* species complex. *Studies in Mycology*, 45, 1–172.

199. Seifert, K. A. (1996). *Fusarium: Paul E. Nelson Memorial Symposium*. APS Press.

200. Selvakumar, K., Baskar, V., Madhan, R., & Srinivasan, G. (2016). Study on improving bioavailability ratio of anti-inflammatory compound from ginger through nano transdermal delivery. *International Journal of Research in Ayurveda and Pharmacy*, 7(5), 84–88.

201. Selvakumar, R., Singh, A.K., Raja, P. (2013). Identification of potential *Trichoderma* sp. for management of rhizome rot of ginger in Arunachal Pradesh. *Environ Ecol*, 31(1): 67–70.

202. Senapati, A. K., & Ghose, S. (2005). Screening of ginger varieties against rhizome rot disease complex in Eastern Ghat high land zone of Orissa. *Indian Phytopathology*, 58(4), 437–439.

203. Senapati, A.K. & Ghose, S. (2005). Screening of ginger varieties against rhizome rot disease complex in eastern ghat highland zone of Orissa. *Indian Phytopathology* 58, 437-439.

204. Sharma, B.K. (1998). Antifungal properties of bio-control agents and plant extracts against casual fungi of yellows and rhizome rot of ginger. *J. Bio-control*. 12(1): 77-78.

205. Sharma, B.K. (1998). Antifungal properties of biocontrol agents from plant extracts against casual fungi of yellows and rhizome rot of ginger. *Journal of Biological Control*. 12: 77-80.

206. Sharma, P., Singh, R., & Singh, D. (2015). Ginger and its constituents: Role in prevention and treatment of gastrointestinal cancer. *Gastroenterology Research and Practice,* 2015, Article 142979.

207. Sharma, S. K., Dohroo, N. P., & Gupta, S. K. (1998). Management of rhizome rot complex of ginger in nursery. *Journal of Mycology and Plant Pathology*, 28(3), 315–316.

208. Sharma, S. L., & Dohroo, N. P. (1980). Fungicidal control of rhizome rot of ginger. *Pesticides*, 14(12), 24–25.

209. Sharma, Y. R., Pandey, K. K., & Dev, B. P. (1995). Rhizome rot disease of ginger and its management. *Journal of Spices and Aromatic Crops*, 4(1), 56–59.

210. Shashank, P. R., et al. (2014). *Zootaxa*, 3866(1), 101–117.

211. Shimelis, T. (2021). Spices production and marketing in Ethiopia: A review. *Cogent Food & Agriculture*, 7(1), 1915558.

212. Simmonds, N. W. (1955). Botany of ginger. *The Ginger Industry*, 3–14.

213. Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). *Annals of the Entomological Society of America*, 87(6), 651–701.

214. Singh, B., Bansal, M., & Meena, K. (2015). Phylogenetic analysis of *Fusarium* species infecting ginger in India and Southeast Asia. *Journal of Applied Microbiology*, 118(1), 231-240.

215. Singh, P., & Rembold, H. (1992). Artificial diet for mass rearing of *Helicoverpa armigera*. *Journal of Applied Entomology*, 113(1–5), 429–433.

216. Singh, P., Jain, K., & Kaur, G. (2018). Ginger (Zingiber officinale): A noble herbal remedy. *International Journal of Current Microbiology and Applied Sciences*, Special Issue-7, 4065–4077.

217. Singh, R. K., & Singh, A. K. (2017). Ginger cultivation and its uses. *Agricultural Reviews*, 38(1), 1–5.

218. Singh, R., et al. (2020). *Current Science*, 119(7), 1155–1160.

219. Skidmore, A.M. & Dickinson, C.H. (1976). Colony interaction and hyphal interference between *Septoria nodorum* and phyloplane fungi. *Trans. Brit. Mycol. Soc.* 66: 57-64.

220. Smith, D. P., & Peay, K. G. (2014). Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLOS ONE*, 9(2), e90234.

221. Sontakke, B. K. (2000). *Insect Pests of Tropical Crops*. Agrotech Publishing Academy.

222. Southwood, T. R. E., & Henderson, P. A. (2000). *Ecological Methods* (3rd ed.). Blackwell Science.

223. Southwood, T. R. E., & Henderson, P. A. (2000). *Ecological Methods* (3rd ed.). Blackwell Science.

224. Spices Board. (2007). *Spices Board of India*. Ministry of Commerce and Industry, Government of India.

225. Sreedharan, A., & Krishnamurthy, K. (2016). In vitro rearing of rhizome maggot (*Formosina flavipes*) infesting ginger. *Indian Journal of Entomology*, 78(2), 189–193.

226. Steyskal, G. C. (1981). *A bibliography of the biosystematic literature on the family Tephritidae (Diptera) 1960–1979*. U.S. Department of Agriculture.

227. Steyskal, G. C. (1981). *Manual of Nearctic Diptera, Volume 1*. Agriculture Canada.

228. Stirling, A. M. (2004). The causes of poor establishment of ginger (*Zingiber officinale*) in Queensland, Australia. *Australasian Plant Pathology*, 33(2), 203–210.

229. Stirling, G. R. (2004). *Biological Control of Plant-Parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture*. CABI Publishing.

230. Stirling, G. R., Turaganivalu, U., Stirling, A. M., Lomavatu, M. F., & Smith, M. K. (2009). Rhizome rot of ginger (*Zingiber officinale*) caused by *Pythium myriotylum* in Fiji and Australia. *Australasian Plant Pathology*, 38(5), 453–460.

231. Stirling, G.R., Turaganivalu, U., Stirling, A.M., Lomavatu, M.F. & Smith, M.K. (2009). Rhizome rot of ginger (*Zingiber officinale*) caused by *Pythium myriotylum* in Fiji and Australia. *Australias Plant Pathology* 38, 453-460.

232. Taberlet, P., et al. (2012). *Trends in Ecology & Evolution*, 27(4), 223–232.

233. Tawnenga, L., & Vanlaltluanga, C. (2016). *Indian Journal of Entomology*, 78(4), 372–375.

234. Thakur, N. S. A., Firake, D. M., & Behere, G. T. (2019). Pest complex, biology, and population dynamics of insect pests of ginger in northeast India. *Journal of Entomology and Nematology*, 4(7), 50–55.

235. Thomas, K. M. (1938). A disease of ginger (Zingiber officinale Rosc.) caused by Pythium sp.. *Proceedings of the Indian Academy of Sciences*, Section B, 7(3), 139–147.

236. Thomas, K.M., (1938). Administration Report of the Government Mycologist, Madras, 1937- 38.

237. Trigiano, M. T., & Ownley, B. H. (2017). *Plant Pathology Concepts and Laboratory Exercises*

238. Trujillo, E. E. (1964). *Diseases of ginger (Zingiber officinale) in Hawaii* (Circular 62). Hawaii Agricultural Experiment Station, University of Hawaii.

239. Utpala, P., Johny, A. K., Parthasarathy, V. A., Jayarajan, K., & Madan, M. S. (2006). Diversity of ginger cultivation in India—a GIS study. *Journal of Spices and Aromatic Crops*, 15(2), 93–99.

240. Varadarasan, S., Kumar, N., & Prakash, S. (2000). Postharvest management of ginger. *Indian Horticulture*, 45(3), 17–18.

241. Wang, C. C., Chen, L. G., Lee, L. T., & Yang, L. L. (2003). Effects of 6-gingerol, an antioxidant from ginger, on inducing apoptosis in human leukemic HL-60 cells. *In Vivo*, 17(6), 641–645.

242. Wang, C. C., Chu, C. Y., & Lin, S. Y. (2003). Effect of ginger on gastric emptying and motility in healthy humans. *European Journal of Gastroenterology & Hepatology*, 15(5), 455–458.

243. Wang, Y., Li, W., & Zhang, L. (2019). China's ginger export competitiveness in the global market. *Journal of International Agricultural Trade and Development*, 15(2), 107–118.

244. Webster, J., & Weber, R. (2007). *Introduction to Fungi* (3rd ed.). Cambridge University Press.

245. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications, Academic Press, 315–322.

246. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press.

247. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press.

248. Yang, Y. S., Kim, Y. H., & Kim, J. C. (1988). Studies on the storage diseases of ginger rhizomes. *Korean Journal of Plant Pathology*, 4(1), 1–7.

249. Yella, L., Meenu, M., & Jebasingh, T. (2006). Effect of biofertilizers on growth and yield of ginger (*Zingiber officinale* Rosc.). *Journal of Spices and Aromatic Crops*, 15(2), 128–131.

250. Yusuf, C. & Okusanya, B.A.D. (2008). Fungi associated with the storage rot of Yam (*Discorea rotundata poir*) in Yola, Adamaula state. *Journal of sustainable Development in Agriculture and Environment.* 3 (2): 99-103.

251. Yusuf, N. A., & Okusanya, B. O. (2008). Fungi associated with the storage rot of yam (*Dioscorea rotundata* Poir) in Yola, Adamawa State. *Journal of Applied Biosciences*, 7(2), 159–165.

252. Zakir, M., Hailemichael, G., Seyoum, M., & Addisu, M. (2018). Status of ginger (*Zingiber officinale*) research and production challenges and future prospects in Ethiopia: A review. *Academic Research Journal of Agricultural Science and Research*, 6(5), 276–290.

253. Zarzoso, M. M., & Ramirez, C. (1999). Yeast identification using molecular tools: A simple approach for identifying species. *Antonie van Leeuwenhoek*, 75(2), 141–149.

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PAPER PRESENTED IN SYMPOSIUM/CONFERENCE

- Oral Presentation at 2nd Annual Convention of North East (India) Academy of Science and Technology (NEAST) & International Seminar on Recent Advances in Science and Technology (ISRAST) organized by NEAST, Mizoram University from 16th -18th November 2020 on the topic **"Isolation and Molecular Characterisation of Ginger (*Zingiber officinale.* Rosc.) Soft Rot Pathogenic Fungi from Champhai District of Mizoram, India."**

- Poster presentation at the 4th Mizoram Science Congress 2022 held between 24-25 November 2022 on the topic **"In-vitro pathogenicity test of fungal samples causing soft rot disease in ginger (Zingiber officinale.rosc) isolated from Champhai and Serchhip Districts of Mizoram, North East India."** organised by Mizoram Science, Technology and Innovation Council (MISTIC) in collaboration with Mizoram Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Bodiversity and Nature ConservationNetwork (BIOCONE) and Mizoram Information & Technology Society (MITS)

- Oral presentation at PUC Research Conclave 2023 (An International Workshop on Research and Development) organized by Research and Development Cell, Pachhunga University College during 24th-25th January 2023 on the topic **" Molecular characterisation and Molecular Phylogeny of Insect pest and Fungal apthogens of ginger of Mizoram."**

- Oral Presentation at the International Conference on Conservation of Biodiversity in Genomics Era, held at Pachhunga University College during 22nd – 23rd March 2024 on the topic **"Molecular phylogeny of Insect pest and Fungal Pathogens of Ginger (*Zingiber officinale* Rosc.) of Champhai and Serchhip Districts of Mizoram, India."**

TRAINING/WORKSHOP ATTENDED

1. State Level Workshop cum Awareness Programme on '*Tinospora cordifolia'* held ao November 19, 2019 organised by Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University and State Medicinal Plants Board (SMPB), Mizoram, Directorate of AYUSH.
2. 'Applications of Cold Plasma in Agriculture, Food and Medicine' organized by Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University, Aizawl, on June 17, 2021.
3. Hands on workshop in Molecular Phylogenetics sponsored by DBT-Advanced State Level Biotech Hub and Mizoram University, from 9^{th} – 14^{th} March 2023.

LIST OF PUBLICATIONS

1. Isolation and Molecular Characterisation of Ginger (*Zingiber officinale.* Rosc.) Soft Rot Pathogenic Fungi from Champhai District of Mizoram, India. *Science and Technology Journal* Vol. 9 Issue: 2 July 2021 ISSN: 2321-3388 .

2. Antifungal potential of entomopathogenic bacteria, *Photorhabdus* and Xenorhabdus (Morganellaceae) against pathogenic fungi. Journal of Applied Biology and Biotechnology Vol. x(x),pp. 1-13, 2025

3. Physico-chemical variability in the fruits of Theichhungsen (*Haematocarpus validus* (Miers.) Bakh. F. ex. Forman)—A potential source of natural food colour. *Journal of Applied Horticulture*, 24(1), 73–76. https://doi.org/10.37855/jah.2022.v24i01.13

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DEGREE : DOCTOR OF PHILOSOPHY

DEPARTMENT : HORTICULTURE, AROMATIC AND MEDICINAL PLANTS

TITLE OF THE THESIS : MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INSECT PESTS AND FUNGAL PATHOGENS OF GINGER (*ZINGIBER OFFICINALE* ROSCOE) OF MIZORAM.

DATE OF ADMISSION : 29.7.2019

APPROVAL OF RESEARCH PROPOSAL:

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2. **BOS** : 15.04.2020
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ABSTRACT

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INSECT PESTS AND FUNGAL PATHOGENS OF GINGER (*Zingiber officinale* Roscoe) OF MIZORAM

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF HORTICULTURE, AROMATIC AND MEDICINAL PLANTS

SCHOOL OF EARTH SCIENCE & NATURAL RESOURCES MANAGEMENT

APRIL, 2025

ABSTRACT

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INSECT PESTS AND FUNGAL PATHOGENS OF GINGER (*Zingiber officinale* Roscoe) OF MIZORAM

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In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants (HAMP) of Mizoram University, Aizawl

Ginger, scientifically known as *Zingiber officinale* Rosc., is recognized as one of the most commercially significant crops that are cultivated in the state of Mizoram, where the unique and favorable agro-climatic conditions significantly facilitate its large-scale agricultural production. Nevertheless, the overall productivity and quality of ginger cultivated in this particular region are profoundly influenced by a variety of insect pests that tend to attack the crop during its various growth stages throughout the cultivation cycle. Among the most notable of these insect pests are the shoot borer, identified as *Conogethes punctiferalis*, the rhizome maggot, referred to scientifically as *Formosina flavipes*, and an assortment of aphid species, thrips, and white grubs, all of which pose substantial threats to the ginger crop.

These pests inflict direct damage to the plants by boring into them, feeding on the tender shoots and rhizomes, and engaging in sap-sucking behaviors, all of which can lead to stunted growth, curling of leaves, and a significant reduction in the overall yield of rhizomes produced. Furthermore, the injuries inflicted by these insect pests often serve as entry points for secondary infections, particularly those caused by fungal and bacterial pathogens, which can further exacerbate the already considerable crop losses suffered by farmers. The challenging topography of the hilly terrain, when combined with traditional farming practices and a lack of comprehensive pest monitoring systems, creates significant obstacles for effective pest management strategies in Mizoram. In addition to these challenges, the absence of scientific documentation regarding the diversity, life cycles, and ecological behavior of insect pests affecting ginger in the region serves to hinder the development of effective control strategies that could mitigate the impact of these pests. Consequently, this study is designed to identify and characterize the major insect pests that affect ginger cultivation in Mizoram, utilizing both morphological and ecological approaches, thereby aiming to support the formulation of integrated pest management practices that are specifically tailored to the unique conditions of the region.

The cultivation of ginger in Mizoram is also markedly threatened by an array of fungal pathogens that can lead to substantial yield losses and the production of poor-quality rhizomes, which are detrimental to the economic viability of this crop. Among the most concerning fungal diseases impacting ginger production are soft rot, which is caused by various species of *Pythium*, rhizome rot attributed to the *Fusarium* species,

and leaf spot diseases caused by *Phyllosticta zingiberi*, all of which have been identified as significant threats. These fungal pathogens thrive exceptionally well in the warm and humid climatic conditions typical of Mizoram, often resulting in rapid and widespread disease outbreaks, particularly under poorly drained or perpetually moist soil conditions that exacerbate their proliferation. The symptoms associated with these diseases typically manifest as yellowing and wilting of the leaves, the softening and decay of the rhizomes, and overall stunted growth of the ginger plants, which can severely impact the health of the crop.

Traditional farming methods, coupled with a conspicuous lack of effective disease management strategies, frequently aggravate the severity of the problem, leading to diminished yields and compromised quality. Despite the considerable economic importance of ginger as a crop, it is noteworthy that there exists a significant gap in the research focused on the identification and control of fungal pathogens within this region. A more profound understanding of the morphological and molecular characteristics of these pathogenic fungi is vital for the early detection of outbreaks and the development of targeted, eco-friendly management practices that can effectively mitigate the impacts of these diseases. Therefore, this study is dedicated to the isolation, identification, and characterization of the fungal pathogens that affect ginger cultivation in Mizoram, with the overarching goal of contributing to sustainable disease control measures that can enhance the resilience of ginger production in the region.

Objective 1: Collection and morphological characterization of insect pest of ginger.

This comprehensive research endeavor was meticulously executed with the primary objective of systematically collecting and thoroughly morphologically characterizing the predominant insect pests that are known to infest ginger crops in various selected districts within the state of Mizoram. Ssystematic field surveys were conducted during the peak active growing season for ginger, specifically within the major cultivation areas, which notably include the districts of Aizawl, Kolasib, Serchhip, Lunglei, Champhai, Hnahthial, Khawzawl, Saitual, Siaha, Lawngtlai. In the process of gathering insect specimens, various collection methods were employed,

which included hand-picking, sweep netting, and pitfall trapping techniques, all of which were utilized during the different growth stages of the ginger crop to ensure a comprehensive sampling of the pest population. The specimens that were collected throughout the survey were preserved in a solution of 70% ethanol to maintain their integrity, and they were subsequently examined meticulously under a stereo zoom binocular microscope to facilitate detailed morphological analysis. The identification and classification of the collected specimens were conducted employing standard entomological keys along with relevant taxonomic literature, which enabled researchers to accurately categorize the specimens up to the family or species level, thereby providing a robust taxonomic framework for the study.

The findings from this extensive study revealed the existence of several major and minor insect pests that significantly impact ginger cultivation within the region. Among the most ubiquitous of these pests was the shoot borer, scientifically known as *Conogethes punctiferalis*, which inflicts damage on young ginger shoots and causes noticeable wilting, leading to compromised plant health. Moreover, white grubs from the family Scarabaeidae, which are known to feed on the roots and rhizomes of the ginger plants, were frequently observed in the surveyed ginger fields, particularly in regions characterized by poorly drained soil conditions that exacerbate pest prevalence. Each specimen collected during the research was meticulously documented with great attention to detail, encompassing crucial aspects such as body morphology, antenna type, wing structure, color pattern, and other distinguishing characteristics that aid in accurate identification. Additionally, photographic records were systematically maintained throughout the study to support visual identification and provide a comprehensive pictorial reference for future entomological research.

The morphological characterization of these insect pests is of paramount importance, as it plays a critical role in facilitating early diagnosis and the implementation of effective management strategies aimed at mitigating pest-related damage. This study represents the first comprehensive account of the insect pest complex associated with ginger cultivation in Mizoram, thus offering invaluable baseline data that can serve as a foundation for subsequent research endeavors in this field. Gaining a deeper understanding of the identity and biological characteristics of these pests is essential for the development of integrated pest management (IPM)

strategies that are not only sustainable but also environmentally friendly, thereby promoting the long-term health of ginger crops. The findings emanating from this study will not only assist farmers in recognizing pests early and implementing timely interventions but will also provide crucial support to agricultural extension agencies in devising control measures that are specifically tailored to the unique conditions of the region.

In conclusion, the extensive collection and thorough morphological characterization of insect pests associated with ginger crops in Mizoram have unveiled a rich and diverse pest fauna that necessitates ongoing monitoring and scientific management interventions. This research highlights the critical need for continuous surveillance, increasing farmer awareness, and the integration of traditional morphological data all aimed at achieving precise identification and effective control of the various pest species that adversely affect ginger cultivation in the region.

Objective 2: Isolation and morphological characterization of fungal pathogen of ginger.

This comprehensive research endeavor was meticulously undertaken with the primary objective of isolating and morphologically characterizing the various fungal pathogens that are intricately associated with diseased ginger plants specifically located in the region of Mizoram. To achieve this, extensive field surveys were systematically conducted across the key ginger-growing districts, which notably include Aizawl, Serchhip, Kolasib, Lunglei, Lawngtlai, Siaha, Saitual, Champhai, Khawzawl, Hnahthial and Mamit districts ensuring a thorough examination of the most significant areas for ginger cultivation. During these surveys, samples of infected rhizomes, shoots and leaves that exhibited a range of symptoms, such as pronounced yellowing, wilting, soft rot, and distinct leaf spots, were meticulously collected and subsequently transported to the laboratory for in-depth analysis and investigation. The process of isolating the fungal pathogens was executed employing standard tissue segment and plating techniques on the widely recognized Potato Dextrose Agar (PDA) medium, which is known for its effectiveness in fostering fungal growth. Following this, the plates containing the isolated fungi were incubated under strictly controlled

environmental conditions, and as a result, the emerging fungal colonies were subsequently sub-cultured to yield pure cultures that could be further examined.

The isolated fungi underwent a detailed characterization process based on a variety of macroscopic features, which included but were not limited to colony color, texture, growth rate, pigmentation, and spore production, all of which are critical for understanding the biological attributes of these pathogens. Additionally, a microscopic examination was conducted using the lactophenol cotton blue staining technique, which facilitated the observation of key structural components, including hyphae, conidia, sporangia, chlamydospores, and the patterns of septation that are essential for taxonomic classification. The identification of the fungi was carried out utilizing established mycological keys and subsequently compared with existing literature to ensure accurate confirmation of the species. Among the major fungal pathogens identified during the course of this study were *Fusarium oxysporum*, *Fusarium solani*, *Sclerotium rolfsii*, *Neurospora intermedia*, *Aspergillus niger*, *Clonostachys rosea, Pythium aphanidermatum* and *Pythium myriotylum*, all of which were found to be significantly associated with distinctive symptoms, such as the soft, water-soaked rot of rhizomes attributed to *Pythium*, vascular wilt and dry rot caused by *Fusarium*.

The morphological characterization of these fungi serves as a fundamental basis for gaining a deeper understanding of the etiology of ginger diseases prevalent in Mizoram, thereby highlighting the importance of such studies in the field of plant pathology. Accurate identification of these pathogens is essential not only for facilitating early diagnosis but also for enabling effective disease forecasting and the strategic development of targeted control methodologies that can mitigate the impact of these pathogens. This study represents the first systematic documentation of the major fungal pathogens affecting ginger in Mizoram, thus providing invaluable baseline data that will be instrumental for future molecular studies and integrated disease management approaches aimed at combating these challenges. The findings generated from this research will significantly assist farmers, researchers, and extension workers alike in designing sustainable, region-specific strategies that are geared towards disease prevention and control, ultimately contributing to the enhancement of ginger productivity and the improvement of farmers' livelihoods within the region.

Objective 3: Molecular identification and phylogeny of insect pests and isolated fungi from ginger.

Traditional methods of species identification, which primarily rely on the morphological characteristics of organisms, often exhibit a significant lack of precision and accuracy, particularly when it comes to cryptic species that are difficult to differentiate or during the early developmental stages of these organisms where distinguishing features may not be fully developed. Consequently, the incorporation of molecular tools, which enable a more detailed and nuanced approach to species identification, has become not only beneficial but essential for achieving accurate results in species identification as well as for elucidating the complex phylogenetic relationships that exist among various species. The primary objective of this study was to systematically identify the predominant insect pests and the fungal pathogens that are associated with ginger cultivation in the region of Mizoram, employing advanced molecular markers—specifically, the Cytochrome c oxidase subunit I (COI) gene for the identification of insects and the Internal Transcribed Spacer (ITS) region for the identification of fungi—and to construct phylogenetic trees that would facilitate a deeper understanding of their evolutionary relationships and lineage.

In order to collect the necessary data, comprehensive field surveys were meticulously conducted across several key ginger-growing regions within Mizoram, which are renowned for their ginger production. During these surveys, various insect pests, including shoot borers, rhizome maggots, and aphids, were systematically collected at different growth stages of the ginger crop to ensure a representative sampling of the pest population. Concurrently, diseased ginger samples exhibiting various symptoms such as soft rot, rhizome decay, and leaf spots were also collected, and fungal pathogens were isolated through the application of standard tissue isolation techniques on Potato Dextrose Agar (PDA) media to facilitate accurate identification. The extraction of DNA from both insect and fungal specimens was performed using the CTAB method for insect samples and commercial DNA extraction kits for fungal samples, ensuring the integrity and quality of the genetic material for subsequent analysis. The Polymerase Chain Reaction (PCR) was executed using specific COI primers (LCO1490/HCO2198) for insects and ITS1/ITS4 primers for fungi to amplify the target regions of interest. The amplified products were subsequently sequenced and

subjected to analysis using the BLAST algorithm to confirm the identity of the species represented in the samples.

The results of the molecular analysis unveiled the presence of several notable insect pests, including *Conogethes punctiferalis*, *Mimegralla coeruleifrons*, and various species of aphids, all of which were successfully confirmed through COI sequencing techniques that underscored their identity. In a similar vein, the fungal isolates that were identified included significant pathogens such as *Fusarium oxysporum*, *Fusarium solani*, *Sclerotium rolfsii*, *Neurospora intermedia*, *Aspergillus niger*, *Clonostachys rosea, Pythium aphanidermatum* and *Pythium myriotylum* all of which were accurately identified based on their respective ITS sequences.

Phylogenetic trees were constructed using MEGA software (version 10.2) employing the Neighbor-Joining method, complete with bootstrap values that assessed the reliability and robustness of the clades identified in the analysis. The resulting phylogenetic trees clearly delineated the boundaries between species and confirmed the taxonomic placement of both the insect and fungal organisms within their respective clades, enhancing our understanding of their evolutionary relationships.

This study vividly illustrates the significant utility of molecular markers in improving the accuracy and reliability of pest and pathogen identification, which is critically important for the development of effective pest management and disease control strategies in agricultural contexts. The integration of COI and ITS gene-based identification not only resolved the ambiguities that often arise from morphological similarities but also provided valuable insights into the genetic diversity and evolutionary lineage of the organisms studied. The findings of this research constitute a foundational database that can serve as a springboard for future studies focused on the biodiversity, ecological interactions, and control strategies pertaining to ginger-associated pests and pathogens within the geographical context of Mizoram.

In conclusion, the molecular characterization conducted using COI and ITS markers has proven to be a reliable and efficient methodology for the identification and comprehension of the phylogenetic relationships of both insect pests and fungal pathogens that affect ginger cultivation in the region of Mizoram. The insights gleaned from this research will be instrumental in guiding targeted interventions aimed at promoting sustainable ginger cultivation practices within the region, ultimately

contributing to the enhancement of agricultural productivity and the economic welfare of local farmers.