



Faculty of Resource Science and Technology

**Chemical Composition of Cave Bats Guano and Its Effects Towards
Plant Growth**

Siti Syamim Nurfatihah Binti Abd Rahman

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Chemical Composition of Cave Bats Guano and Its Effects Towards Plant Growth

Siti Syamim Nurfatihah Binti Abd Rahman

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DECLARATION

I hereby declare that in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



.....
Signature

Name: Siti Syamim Nurfatihah Binti Abd Rahman

Matric No.: 20020354

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date: 5 / 7 / 2025

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To my beloved mother, Putri Zalila Binti Megat Yaacob, gone but never forgotten,

Mama, I made it.

ABSTRACT

Fertilisation is essential for plant development and generally falls into two categories: chemical and organic. While chemical fertilisers are known to significantly enhance plant growth, they are often linked to issues such as soil nutrient depletion and environmental degradation. Organic fertilisers, including chicken manure and cave bat guano, on the other hand, have been recognised for improving soil structure and fertility. Bat guano, the faeces of bats, has been previously reported to be highly beneficial for plant growth. Although multiple studies have discussed the potential of the fertilisers in promoting plant development, comparative studies examining its chemical composition alongside that of commercial fertilisers and chicken manure are limited. Furthermore, the relationship between cave bat guano composition and plant growth performance has not been extensively explored. This study seeks to address these gaps by evaluating the chemical characteristics of cave bat guano and its impact on the growth on chilli plants, while comparing its effects with chemical fertiliser and chicken manure. Guano composition is known to vary depending on bat species, diet, and locality. To gain insight into species contributing to the guano samples, acoustic recordings were collected using a Wildlife Acoustics bat detector and analysed with Kaleidoscope software. Call parameters including frequency range, peak frequency, and pulse duration were assessed, resulting in the identification of 11 bat species across four families: Hipposideridae, Rhinolophidae, Vespertilionidae, and Emballonuridae. For the chemical and plant growth performance analyses, three fertiliser types were used. Guano samples were collected from Wind Cave Nature Reserve and Raya Cave, while chemical fertiliser and chicken manure were sourced from a local agricultural supplier. Soil samples were also chemically analysed to determine the suitability of

the growth medium used in the plant growth study. Chemical analysis followed Malaysian Standard protocols, focusing on organic matter, dry matter, nitrogen (N), phosphorus (P), and potassium (K). Chemical analysis revealed that the chemical fertiliser exhibited the highest Nitrogen, Phosphorus and Potassium content. Raya Cave guano showed higher nutrient levels than Wind Cave samples, suggesting that guano quality is influenced by bat species and habitat. The fertilisers were applied to *Capsicum annuum* L. variety Kulai, with six treatment groups: control (no fertiliser), chemical fertiliser, chicken manure, and bat guano at three application levels (2.5 g, 5.0 g, 7.5 g). Growth parameters—plant height, number of leaves, flower buds, flowers, and fruits—were recorded monthly over a six-month period. The study showed significant differences in plant growth across the various treatments. Chilli plants treated with chemical fertiliser consistently showed the highest growth rates, with significant increases in all the parameters throughout the growth cycle. Bat guano, particularly at the 7.5 g application rate, also promoted strong vegetative and reproductive growth, outperforming chicken manure and the control. Chicken manure produced moderate results, while control plants consistently recorded the lowest growth, reinforcing the importance of fertilisation. Although chemical fertilisers remain the most effective, bat guano presents a viable organic alternative with promising potential for sustainable agriculture. To fully explore its benefits, further research is needed, including trials on a wider range of plant species, variations in guano application rates, assessments of long-term effects on soil health, and evaluations of microbial dynamics. This study highlights the ecological and agricultural value of bat guano towards sustainable farming practices and biodiversity conservation.

Keyword: Bat guano, chemical analysis, chicken dung, N-P-K fertiliser, plant growth

Komposisi Unsur Kimia Guano Kelawar Gua dan Kesannya Terhadap Pertumbuhan Tumbuhan

ABSTRAK

Pembajaan merupakan elemen penting dalam perkembangan tumbuhan. Ia secara amnya terbahagi kepada dua kategori: baja kimia dan baja organik. Baja kimia telah diketahui dapat meningkatkan pertumbuhan tumbuhan secara ketara, namun ia sering dikaitkan dengan masalah seperti penyusutan nutrisi tanah dan kemerosotan alam sekitar. Sebaliknya, baja organik seperti najis ayam dan guano kelawar gua telah diakui dapat meningkatkan struktur dan kesuburan tanah. Guano kelawar, iaitu najis kelawar, telah dilaporkan mempunyai manfaat yang tinggi dalam pertumbuhan tumbuhan. Walaupun terdapat banyak kajian yang membincangkan potensi baja tersebut dalam merangsang pertumbuhan tumbuhan, kajian mengenai perbandingan komposisi kimia guano kelawar gua berbanding baja kimia komersial dan baja ayam masih terhad. Tambahan pula, hubungan antara komposisi guano kelawar gua dan prestasi pertumbuhan tumbuhan masih kurang diterokai. Kajian ini menilai ciri kimia guano kelawar gua serta kesannya terhadap pertumbuhan tanaman pokok cili, di samping membandingkannya dengan baja kimia dan baja ayam. Komposisi guano ditentukan mengikut spesis kelawar, diet, dan lokasi. Untuk memahami spesis kelawar yang menyumbang kepada sampel guano, rakaman gema telah dijalankan menggunakan pengesan kelawar Wildlife Acoustics dan dianalisis menggunakan perisian Kaleidoscope. Parameter panggilan seperti julat frekuensi, frekuensi puncak, dan tempoh denyut telah dinilai dan pengecaman 11 spesies kelawar daripada empat famili: Hipposideridae, Rhinolophidae, Vespertilionidae, dan Emballonuridae telah dikenal pasti. Bagi analisis kimia dan prestasi pertumbuhan tumbuhan, sampel guano dikumpul dari Rizab Alam Semula Jadi Gua Angin dan Gua Raya, manakala baja

kimia dan baja ayam diperoleh daripada pembekal pertanian tempatan. Analisis kimia dijalankan mengikut Piawaian Malaysia, dengan fokus kepada bahan organik, kering, kandungan nitrogen, fosforus, dan kalium. Hasil analisis menunjukkan bahawa baja kimia mengandungi kandungan nitrogen, fosforus dan kalium yang tertinggi. Guano dari Gua Raya menunjukkan paras nutrisi yang lebih tinggi berbanding guano dari Gua Angin, mencadangkan bahawa kualiti guano dipengaruhi oleh spesis kelawar dan habitatnya. Baja-baja ini telah digunakan ke atas Capsicum annuum L. variasi Kulai, dengan enam kumpulan rawatan: kawalan (tanpa baja), baja kimia, baja ayam, dan guano kelawar pada tiga kadar aplikasi (2.5 g, 5.0 g, 7.5 g). Pertumbuhan seperti ketinggian tumbuhan, bilangan daun, putik bunga, bunga, dan buah direkodkan setiap bulan selama enam bulan. Tumbuhan cili yang dirawat dengan baja kimia menunjukkan kadar pertumbuhan tertinggi secara konsisten, dengan peningkatan ketara dalam semua parameter sepanjang pertumbuhan. Guano kelawar, terutamanya pada kadar aplikasi 7.5 g, turut menggalakkan pertumbuhan melebihi prestasi baja ayam dan kumpulan kawalan. Baja ayam menghasilkan pertumbuhan yang sederhana, manakala kumpulan kawalan mencatatkan pertumbuhan paling rendah, menekankan kepentingan pembajaan. Walaupun baja kimia masih paling berkesan, guano kelawar boleh dijadikan sebagai alternatif organik yang berpotensi untuk pertanian mampan. Kajian lanjutan disyorkan untuk menilai kesan jangka panjang terhadap kesihatan tanah, meneroka interaksi mikrob, dan menguji pelbagai jenis tanaman. Kajian ini menyerlahkan nilai ekologi dan pertanian guano kelawar dalam menyokong amalan pertanian mampan dan pemuliharaan biodiversiti.

Kata kunci: Guano kelawar, analisis kimia, baja ayam, baja N-P-K, peningkatan tumbuhan

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LIST OF ABBREVIATIONS

µg	microgram
µm	micrometre
°C	degree celcius
Al	Aluminium
ANOVA	analysis of variance
ASEAN	Association of Southeast Asian Nations
ATP	Adenosine triphosphate
BG	bat guano
BGRC	Bat Guano Raya Cave
BGWCR	Bat Guano Wind Cave Reserve
C	Carbon
Ca	Calcium
CaO	Calcium oxide
CDF	Chicken dung fertiliser
CF	constant frequency
CF	Chemical fertiliser
CFA	Communaute Financiere Africaine
Cl	Chloride

cm	centimetre
cmol	centimole
COI	cytochrome oxidase subunit 1
CRD	Complete randomized design
dB	Decibel
DFA	discriminant function analysis
DNA	Deoxyribonucleic acid
E	East
Fe	Iron
Fend	End Frequency
FM	frequency modulated
Fmax	Maximum Frequency
Fmin	Minimum Frequency
Fppeak	Peak Frequency
Fstart	Start Frequency
g	gram
GA ₃	Gibberellic acid
H ₂ PO ₄ ⁻	Dihydrogen phosphate
ha	hectar

HCl	Hydrochloric acid
HDPE	high-density polyethylene
HoB	Heart of Borneo
HPO_4^{2-}	Hydrogen phosphate
IUCN	International Union for Conservation of Nature
K	potassium
K_2O	Potassium oxide
KCl	Potassium chloride
kg	kilogram
KH_2PO_4	Potassium dihydrogen phosphate
kHz	Kilo Hertz
L	Litre
LSD	Least Significant Differences
m	metre
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
MEA	Millennium Ecosystem Assessment
Mg	Magnesium
mg	milligram

MgO	Magnesium oxide
mL	milliliter
mm	millimeter
Mn	Manganese
MS	Malaysian Standards
N	nitrogen
N	North
Na	Sodium
Na ₂ O	Sodium oxide
NAA	naphthalene acetic acid
NaOH	Sodium hydroxide
NF	no fertiliser
NH ₄ ⁺	Ammonium
nm	nanometer
NO ₃ ⁻	Ammonia
NoB	number of flower buds
NoFl	number of flowers
NoFr	number of fruits
NoL	number of leaves

NPK	Nitrogen Phosphorus Potassium
nr	no records
OM	Organic Matter
P	phosphorus
P ₂ O ₅	Phosphorus pentoxide
PD	Pulse Duration
PH	plant height
PI	Pulse Interval
ppm	parts per million
QCF	quasi-constant frequency
RC	Raya Cave
RM	Ringgit Malaysia
S	Sulphur
s	seconds
SARS-CoV	Severe-Acute-Respiratory-Syndrome-Related Coronavirus
SO ₃	Sulphur trioxide
SORAS	Sarawak Online Research Application System
sp.	species
SPSS	Statistical Package for the Social Sciences

UN	United Nations
USD	United States Dollars
WAV	Waveform Audio File Format
WCNR	Wind Cave Nature Reserve

CHAPTER 1

INTRODUCTION

1.1 Study Overview

Plant growth in agriculture is highly dependent on its physical, chemical, and biological properties. Enhancing these properties can be achieved by adding plant regulators or fertilisers (Bender et al., 1998). The use of fertilisers is crucial in agricultural production to increase plant growth efficiency (Karagoz-Sezer and Hanay, 2020). Fertilisers can be categorised into two types: organic and inorganic. The significance of agriculture in fulfilling the food supply demands of an ever-growing population has led farmers to adopt environmentally friendly plantation techniques (Fernandez et al., 2003). The fertiliser industry has promoted extensive use of chemical fertilisers worldwide, primarily because they are inexpensive and seemingly provide faster results in yield production (Bryant and Kincheloe, 1983).

Chemical fertilisers, including inorganic or synthetic types, are artificially produced and contain essential plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Oo et al., 2019). Another study defined chemical fertilisers as artificial fertilisers containing one or more nutrients (Demirtas et al., 2005). Prolonged use of chemical fertilisers has been found to be ineffective in maintaining soil nutrient levels (Kang et al., 2005). This can significantly impact crop yields as soil fertility deteriorates (Shetty et al., 2013), lead to high salt-toxicity build-up in the soil, and cause imbalances in chemical nutrients that negatively affect plant growth (Poliquit and Calong, 2018). Additionally, environmental concerns such as ecological

imbalance, pollution, biogeochemical cycle disruptions, and global warming have been associated with excessive chemical fertiliser use (Bryant and Kincheloe, 1983; Schroter et al., 2005).

According to Demirtas et al. (2005), organic fertilisers are derived from natural sources such as plant or animal waste, including green manure, livestock manure, and compost. Organic fertilisers release nutrients at a slower rate as they require decomposition by soil microorganisms. While this allows for easier nutrient absorption by plant roots, the absorption process takes longer (Oo et al., 2019). Despite the extended-release period, organic fertilisers provide numerous benefits to soil structure and the environment. Reported advantages include acting as nutrient providers while maintaining soil structure and moisture, as well as enhancing soil fertility and productivity (Kasso and Balakrishnan, 2013; Torres-Jimenez et al., 2020). Furthermore, organic fertilisers have been shown to increase beneficial soil organisms that facilitate nutrient conversion for plant uptake (Hua et al., 2009; Oo et al., 2019).

Organic fertilisers such as compost, green manure, and bioinoculants play a vital role in organic farming management (Thampan, 1993). Several animal-derived fertilisers, including chicken manure and cow dung, are considered among the best organic fertilisers as they provide an abundant source of N, P, K, and other organic matter to the soil (Oo et al., 2019). Organic fertilisers also ensure a steady availability of nutrients with an even uptake by plants, preventing nutrient loss due to rainwater leaching (Linn and Myint, 2018; Oo et al., 2019). The increasing recognition of the benefits of organic fertilisers has prompted efforts to develop superior types of organic fertilisers (Grantina-Ilevina and Ilevinsh, 2015).

Torres-Jimenez et al. (2020) stated that guano provides an excellent alternative to other organic fertilisers. Previous studies have identified bat guano as one of the best fertilisers globally due to its high nitrogen and potassium content (Hutchinson, 1950; Kasso and Balakrishnan, 2013; Sakoui et al., 2020). Bat guano is a preferable alternative to synthetic fertilisers as it does not leave nutrient residues (Diba et al., 2013). It is defined as the excretory matter of bats (Sakoui et al., 2020). The term "guano" originates from the indigenous people of Chile and Peru and was later adopted into the Spanish language. Since the 19th century, over 100,000 tonnes of guano have been exploited in the United States for fertiliser use (Ghanem and Voigt, 2012). In the past, guano was collected for fertiliser production in various countries, including Cuba, Mexico, Jamaica, Brazil, Cambodia, Thailand, and Indonesia (Ghanem and Voigt, 2012; Sothearen et al., 2014; Ferreira, 2019). Tropical countries, in particular, benefit from a continuous supply of guano due to their stable climate (Ferreira, 2019).

Bats, the second largest order of mammals (Order: Chiroptera), comprise more than 1,400 species globally (Nowak, 1999; Ghanem and Voigt, 2012; Sakoui et al., 2020; Torres-Jimenez et al., 2020). They inhabit every continent except some remote oceanic islands and polar regions such as Antarctica (Ghanem and Voigt, 2012; Sakoui et al., 2020). The high diversity of bats in tropical regions (Simmons and Voss, 1998; Bass et al., 2010; Ghanem and Voigt, 2012) is attributed to their varied diets, which have evolved through adaptation and speciation (Voigt and Kingston, 2016; Torres-Jimenez et al., 2020). Bat diets include frugivory, insectivory, nectarivory, sanguivory, and carnivory (Ferreira, 2019; Torres-Jimenez et al., 2020). Their roosting habitats range from caves, tree cavities, and crevices to branches. Heterothermic bat species prefer caves for roosting, breeding, and hibernation (Sakoui et al.,

2020). Large cave colonies, comprising thousands of bats, serve as significant sources of guano, which is an essential biomass for ecosystems (Ghanem and Voigt, 2012; Kasso and Balakrishnan, 2013; Voigt and Kingston, 2016; Torres-Jimenez et al., 2020).

Bats are vital contributors to ecosystems. The Millennium Ecosystem Assessment (MEA) identified bats as providers of all four ecosystem services: (1) provisioning services (food and water supply), (2) regulating services (pollination and pest control), (3) supporting services (seed dispersal), and (4) cultural services (intellectual, academic, or spiritual inspiration) (Millennium Ecosystem Assessment, 2003; Ghanem and Voigt, 2012). Numerous studies support the MEA's recognition of bats' ecological roles, particularly in pollination, pest control, and seed dispersal (Cleveland et al., 2006; Ghanem and Voigt, 2012; Sakoui et al., 2020). However, these contributions are often overlooked due to negative cultural perceptions and a lack of awareness regarding bats' ecological importance (Ghanem and Voigt, 2012).

The use of bat guano as fertiliser is not without its disadvantages. Like bird droppings, bat guano has been reported to contain the infectious fungus *Histoplasma capsulatum*, which can cause histoplasmosis—a lung infection in humans (Tuttle and Moreno, 2005). Declining bat populations due to human and environmental factors may also reduce future guano availability. Public perceptions of bats and their guano are another concern, as studies have found that some farmers lack knowledge about guano usage, while others view it negatively (Torres-Jimenez et al., 2020). Additionally, bat guano emits a strong, pungent odour, which may pose challenges for farmers considering its use as an organic fertiliser (Sridhar et al., 2006).

Research on bat guano properties in Malaysia remains limited, likely due to insufficient education and understanding. However, there has been a recent increase in studies highlighting guano's beneficial effects on plant growth (Grantina-Ievina and Ievinsh, 2015). Historically, guano has been extracted from caves for fertiliser production due to its high nutrient levels (Kasso and Balakrishnan, 2013). The demand for guano-based fertilisers continues to rise due to its effectiveness in enhancing plant growth and yield (Tuttle and Moreno, 2005; Yankova et al., 2016).

1.2 Problem Statements

Fertilisation is vital for plant performance in order to obtain a better yield. Chemical and organic fertilisers are undoubtedly needed in plant management. However, both options are not without their disadvantages. The prolonged use of chemical fertilisers has been found to deteriorate soil health, cause ecological imbalance, and contribute to global warming. The use of organic fertilisers, on the other hand, remains low in the community. This may be due to the limited availability of organic fertilisers, which are restricted in crop production due to geographical constraints, insufficient manure production and management, and low awareness of the value of animal-derived manures.

Previous studies shows that bats' guano positively affects crop growth (Poliquit and Calong, 2018; Jayasvasti and Jayasvasti, 2018; Ferreira, 2019). However, despite extensive research on bat guano utilisation worldwide, studies on the correlation between the chemical composition of cave bat guano and plant growth performance remain insufficient. In Malaysia, specifically, the use of bat guano as manure has not been studied as thoroughly as in

neighbouring countries such as Indonesia (Diba et al., 2013; Misra et al., 2019), Cambodia (Sothearen et al., 2014), and Myanmar (Linn and Myint, 2018). This is reflected in the lack of local knowledge regarding the importance of bat guano in agriculture, as noted by Kingston et al. (2006).

This study aims to bridge the gap in research on the beneficial effects of bat guano on plant growth. Additionally, assessing the availability of bat guano and identifying guano-producing bat species could help establish standards for its commercialisation as a fertiliser

1.3 Research Questions

- I. What are the possible bat species contributing to Wind Cave Nature Reserve (WCNR) and Raya Cave (RC) bats' guano collected through acoustic recordings?
- II. What are the differences in the chemical compositions of WCNR and RC bats' guano with other established commercial fertilisers?
- III. What are the effects of the WCNR and RC bats' guano on plant growth performance compared to other established commercial fertilisers?

1.4 Hypothesis

- H01 There is no significant difference in the bat species contributing to the WCNR and RC bats' guano supplies through acoustic recordings.
- HA1 There is a significant difference in the bat species contributing to the WCNR and RC bats' guano supplies through acoustic recordings.
- H02 There is no significant difference in the chemical compositions of WCNR and RC bats' guano with other established commercial fertilisers.
- HA2 There is a significant difference in the chemical compositions of WCNR and RC bats' guano with other established commercial fertilisers.
- H03 There are no significant effects of the WCNR and RC bats' guano on plant growth performance compared to other established commercial fertilisers.
- HA3 There are significant effects of the WCNR and RC bats' guano on plant growth performance compared to other established commercial fertilisers.

1.5 Objectives

- i. To identify the bat species which are present in the WCNR and RC bats' guano collection sites through acoustic recordings.
- ii. To analyse the chemical compositions of WCNR and RC bats' guano in comparison to other established commercial fertilisers.
- iii. To analyse the effects of the chemical compositions of WCNR and RC bats' guano on plant growth performance in comparison to other established commercial fertilisers.

CHAPTER 2

LITERATURE REVIEW

2.1 Bats in Sarawak

Bats (Order Chiroptera) comprise the second-largest species group, ranked after the order Rodentia (Glover and Altringham, 2008; Morni et al., 2018). They are the most widely distributed mammal group and currently consist of 1,432 recognised species (Simmons and Cirranello, 2023). Bats exhibit the highest diversity in tropical and subtropical areas (Corbet and Hill, 1992; Jones et al., 2002; Mohd Ridwan et al., 2011). Bat species can be distinguished from other mammalian species by their unique ability for true flight, which contributes to their wide distribution. Bats are known to employ various feeding strategies, ranging from consuming plant products (leaves, fruits, flowers, pollen, and nectar) to small animals (insects, small vertebrates, and fish) and even blood (Kunz et al., 1994; Francis, 2008). This diversity in feeding habits underscores the major ecological roles bats play as insect regulators, seed dispersers, and pollinators (Payne et al., 1985).

In Malaysia, specifically, as a hotspot of Old-World bat diversity, the country plays a crucial role in international bat conservation efforts (Findley, 1993). Recent reviews indicate that the IUCN status of the 143 bat species in Malaysia can be categorised as follows: one critically endangered species (*Murina balaensis*), one endangered species (*Hipposideros coxi*), 11 vulnerable species, 14 near-threatened species, 85 least-concern species, 15 data-deficient species, and several species that have not yet been evaluated (Senawi and Ahmad, 2021). In an effort to combat biodiversity loss, the Malaysian government, along with Brunei and Indonesia,

signed the Heart of Borneo (HoB) Initiative in 2007. This initiative aims to conserve 22 million hectares of habitat within the transboundary area of Borneo (World Wildlife Fund for Nature, n.d.).

Borneo is the third-largest island in the world, with dense rainforest coverage spanning approximately 287,000 square miles. It is located southeast of Peninsular Malaysia and southwest of the Philippines (Sada et al., 2019). According to MacKinnon et al. (1996), the hotspots for diversity and endemism in Borneo are found in the northern part of the island, particularly in northern East Kalimantan, Sabah, eastern Sarawak, and Brunei Darussalam (Catullo et al., 2008; Struebig et al., 2012). The tropical rainforest of Borneo is globally renowned for its species diversity and endemism. Bat species constitute one-third of the terrestrial mammals in Borneo, making them a significant component of the forest fauna (Payne et al., 2000; Struebig et al., 2012). According to Senawi and Ahmad (2021) and Phillipps and Phillipps (2018), 99 bat species have been recorded in Borneo. Recent studies on known bat species in Borneo have confirmed a total of 99 bat species on the island. The dominant bat species in Borneo typically belong to the insectivorous families Hipposideridae, Rhinolophidae, and Vespertilionidae (Struebig et al., 2012).

Several research projects in Sarawak have highlighted the high bat biodiversity of the area. In Mount Silabor, 12 trapping nights resulted in the identification of 27 bat species, accounting for 27.27% of the total Bornean bat species (Jinggong et al., 2022). Mohd Ridwan et al. (2011) documented bats in the Wind Cave Nature Reserve in 2008 and 2009, capturing a total of 646 individuals from 17 species across four families over 286 trapping nights. These captures represented 17.7% of the Chiropteran species in Borneo (Mohd Ridwan et al., 2011).

The reserve, which spans 6.16 hectares, is part of the Bau Limestone Area—a narrow limestone belt covering 150 km² in southwest Sarawak. The study recorded that *Penthetor lucasi* was the most abundant bat species in Wind Cave Nature Reserve, followed by *Hipposideros cervinus*. Of the 17 species recorded, 76.5% (13 species) were insectivorous bats, while the remainder were frugivorous. Three of the four frugivorous species recorded—*P. lucasi*, *Cynopterus brachyotis*, *Balionycteris maculata*, and *Eonycteris spelaea*—were captured near the cave entrance, while *P. lucasi* was also found around the cave area. It is estimated that a large roost of *P. lucasi* exists near the cave entrance (Mohd Ridwan et al., 2011).

The records of bats found in the Wind Cave Nature Reserve and the Bau Limestone Area have been extensively studied over the past decade. Records from Hall et al. (2001), Jub et al. (2003), Karim et al. (2004), Pathe et al. (2005), Mohd-Azlan et al. (2005), and Mohd Ridwan et al. (2011) are summarised in Table 2.1. These records indicate that a total of 37 bat species have been captured in the area, with the majority belonging to insectivorous bats (Mohd Ridwan et al., 2011). A broader study on the diversity and distribution of bats across eight localities in Sarawak (Tubau Camp, Batang Ai National Park, Bako National Park, Mount Penrissen, Kubah National Park, Similajau National Park, Lambir Hills National Park, and Mount Murud) documented 45 species from seven families, accounting for 48.9% of the bats known in Borneo. This biodiversity and distribution survey highlighted that Chiropteran diversity in Borneo remains insufficiently studied. Further research on bat diversity in Sarawak is recommended (Jayaraj et al., 2012).

Table 2.1: Previous records of bats (+) in Wind Cave Nature Reserve and Bau Limestone Area (Mohd-Ridwan et al., 2011).

	Mohd- Ridwan et al. (2011)	Mohd- Azlan et al. (2005)	Pathe et al. (2005)	Karim et al. (2004)	Jub et al. (2003)	Hall et al. (2001)
Pteropodidae						
<i>Cynopterus brachyotis</i>	+		+	+	+	+
<i>Cynopterus horsfieldii</i>		+		+	+	
<i>Penthetor lucasi</i>	+	+	+	+	+	+
<i>Rousettus amplexicaudatus</i>		+			+	
<i>Balionycteris maculata</i>	+	+	+	+	+	+
<i>Eonycteris spelaea</i>	+	+		+	+	+
<i>Macroglossus minimus</i>		+	+	+		
Mollosidae						
<i>Cheiromeles torquatus</i>					+	
Emballonuridae						
<i>Emballonura alecto</i>				+		+
Rhinolophidae						
<i>Rhinolophus arcuatus</i>	+	+	+		+	
<i>Rhinolophus borneensis</i>	+	+		+		+
<i>Rhinolophus affinis</i>	+		+			+
<i>Rhinolophus luctus</i>		+	+	+		

Table 2.1: continue

<i>Rhinolophus sedulus</i>		+	+		+	
Nycteridae						
<i>Nycteris tragata</i>				+		
Megadermatidae						
<i>Megaderma spasma</i>				+		+
Hipposideridae						
<i>Hipposideros diadema</i>	+	+	+		+	+
<i>Hipposideros larvatus</i>	+	+	+		+	
<i>Hipposideros dyacorum</i>		+	+		+	
<i>Hipposideros ater</i>		+	+		+	
<i>Hipposideros cineraceus</i>			+		+	
<i>Hipposideros bicolor</i>		+	+		+	
<i>Hipposideros coxi</i>	+	+				
<i>Hipposideros ridleyi</i>	+					
<i>Hipposideros galeritus</i>	+	+	+		+	
<i>Hipposideros cervinus</i>	+	+	+		+	+
Vespertilionidae						
<i>Kerivoula intermedia</i>		+	+		+	
<i>Kerivoula pellucida</i>	+	+			+	
<i>Myotis muricola</i>		+	+		+	

Table 2.1: continue

<i>Myotis ater</i>					+	
<i>Myotis ridleyi</i>		+	+			
<i>Myotis hasselti</i>		+			+	
<i>Myotis horsfieldii</i>	+		+		+	+
<i>Pipistrellus tenuis</i>			+			
<i>Tylonycteris robustula</i>	+					
<i>Tylonycteris pachypus</i>	+					
<i>Glischropus tylopus</i>				+		+
Total number of species	17	23	21	12	23	12
Total number of families	4	4	4	6	5	6

2.2 Caves as one of the habitats of bats

Caves are the main roosting sites for multiple bat species (Arita, 1993). Mickleburgh et al. (2002) mentioned that the survival of many global bat species is dependent on natural caves and underground sites. Cave areas are considered large azonal habitats as they exhibit high environmental similarity across all microclimates (Furey and Racey, 2016). For temperate regions, caves provide hibernation roosting sites and summer breeding sites for some species. For tropical regions, on the other hand, where bats do not hibernate, caves provide stable support for large colonies and have high bat diversity all year round (Monadjem et al., 2020; Furey and Racey, 2016). The species richness of caves studied by Arita (1996) was reportedly low at 3.28% of the average number of species per cave. Twenty-one of the caves studied in the Yucatan region harboured one or two species, while six caves harboured more than seven bat species. The frequency distribution of caves in the Yucatan region is reportedly similar to the whole region studied in Mexico, where only 10% of caves provided refuge for more than six species of bats (Arita, 1993; 1996).

The suitability of caves as roosting sites is greatly affected by the temperatures in the cave system. The pattern of air circulation in caves is one of the factors contributing to temperature inconsistency within a cave system. Temperature selection for bats ranges widely; hence, most bats might not have the same roosting sites even in the same cave (McNab, 1974). Examination of data from Pearson (1962) and Hall (1962) concluded that bats' roosting selection is affected by their weight. *Pipistrellus subflavus*, which is considered the smallest bat in the eastern part of North America, selects the warmest part of the cave, which is the innermost

part. In contrast, *Eptesicus fuscus*, the largest bat in the same region, roosts near the entryway of the cave, which is the coldest part (McNab, 1974). *Craseonycteris thonglongyai*, or Kitti's hog-nosed bat, is known to roost exclusively in caves and exists in only six caves in Thailand (Humphrey and Bain, 1990). Other studies by Arita (1993) reported that 60 out of 134 bat species in Mexico opted for caves as their roosting sites. On top of that, only 27 bat species dwells in caves as their main roost, while others use them occasionally. However, 19 of the cave bat species are considered fragile or vulnerable due to the lack of cave environment protection initiatives to ensure their conservation (Arita, 1993).

McCracken (1989) also mentioned that five endangered species of bats in America use caves as roost sites partly in a year. Arita (1993) recorded the presence of *Mimon bennettii* and *Chrotopterus auritus* in small colonies of less than ten individuals located in Yucatan's caves. One-third of 39 bat species in the temperate region of America are cave dwellers all year round, while only five species of bats opt for caves only for hibernation (McCracken, 1989). Dietz et al. (2009) recorded that out of 40 European bat species, 28 were sighted in caves during hibernation, and only a few were found all year round.

Many bats are known for the ecological services they provide. Prey and predator interactions by *Tadarida brasiliensis* with *Helicoverpa zea* (bollworm moth) and *Chaerephon plicatus* with *Sogatella furcifera* (white-backed planthoppers) are some examples of pest control services provided by cave bats. Fruit pollinators among the bat population are not scarce; a few examples of pollinators among bats are *Eonycteris spelaea*, which is the primary pollinator of durian and tree bean, and other economically important plant species (Leelapaibul et al., 2005; Bumrungsri et al., 2008; 2009; 2013; Wanger et al., 2014; Furey and Racey, 2016). Most

insectivorous bats are commonly associated with caves. Bats from the families of Hipposideridae, Vespertilionidae, Nycteridae, and Rhinolophidae were recorded by Whitaker and Black (1976) in their study on cave bats in Zambia.

Adult lepidopterans, especially moths, were the common food for most of the bat species found in caves. Larvae of the lepidopterans were also included in the diet of *Nycterus thebaica* and *Nycteris woodi* (Whitaker and Black, 1976). These findings were also supported by Vaughan (1977), Bogdanowicz et al. (1999), Syme et al. (2001), and Pavey and Burwell (2005), where some bat species feed largely on moths rather than on beetles. However, clear quantitative data describing the diets of most insectivorous bats are difficult to conclude due to individual, temporal, and geographic variations of bat species (Whitaker and Black, 1976; Whitaker and Tomich, 1983; Brigham, 1990).

Literature associating cave bats with insectivorous bats was done by Lim et al. (2018). Habitat disruption of insectivorous cave bats was discussed in the study. The effect of cave tourism has increased threats to cave bats in East and Southeast Asia (Furey and Racey, 2016). Cave tourism developments impacted their habitat negatively by introducing artificial lighting and altering cave structures, disturbing the flow of cave energy. Additionally, a high number of cave visitors create significant temperature fluctuations, humidity, and carbon dioxide concentration, which usually lead to roost abandonment (Furey and Racey, 2016). In temperate regions, insectivorous bats from Japan migrated around the country to select the optimum temperature for colony establishment. Funakoshi and Uchida (1978) followed the migration of multiple cave-dwelling bats such as *Rhinolophus ferrumequinum nippon*, *Rhinolophus cornutus cornutus*, and *Miniopterus schreibersi fuliginosus* throughout the year. The adaptability of cave

bats in different seasons was deeply discussed in the study. Hibernation activities, habitat selections, and the body temperature of three insectivorous cave bat species were also investigated closely throughout the ecological phenomena (Funakoshi and Uchida, 1978).

For the region of Sarawak particularly, a total of 38 cave bats species over the 80 bats species were recorded (Phillipps and Phillipps, 2016; Senawi and Ahmad, 2021). They were said to be important members of the forest ecosystem which provides multiple ecological services (Jones et al., 2009; Kasso and Balakrishnan, 2013; Rajasegaran et al., 2018). Multiple studies were conducted on cave bats in Sarawak such as Mohd Ridwan et al. (2011) in Wind Cave Nature Reserve, Niah Cave by Medway (1971), Lim et al. (1972), Hall et al. (2002), Nyaun et al. (2004), Mohd Ridwan et al. (2010) and the Bau limestone areas by Hall et al. (2001), Jub et al. (2003), Karim et al. (2004), Mohd-Azlan et al. (2005), Pathe et al. (2005). Mohd Ridwan et al. (2011) mentions that despite numerous studies on bat diversity in limestone areas, it is believed that much remains to be discovered due to the distinct characteristics of the karst habitat. Hence, the preservation of caves as one of bats habitat is of the utmost importance for the conservation of bats (Arita, 1993).

A study on the roost preferences of bats in Wind Cave Nature Reserve found that microclimate temperature preference is correlated with diet (Morni et al., 2018). Bat species that tolerate a wider range of humidity and temperature tend to consume higher-calorie foods, such as *Penthetor lucasi* (frugivorous) and *Megaderma spasma* (insectivorous-carnivorous). In contrast, bats that prefer a narrower range of microclimate conditions are strictly insectivorous. This is because high-calorie foods provide greater energy, enabling bats to withstand temperature variations at their roost sites. Meanwhile, insectivorous bats, which are generally

smaller, are more susceptible to low temperatures, and their diet is influenced by insect availability, especially in temperate regions (Morni et al., 2018). Roost selection within cave areas is also influenced by ambient noise levels. As *Penthetor lucasi* relies on vision rather than acoustic assistance for foraging, this frugivorous species tends to colonize the noisiest roosting areas, with sound levels reaching 78 dB—typically near cave entrances or skylights. In contrast, bats that hunt using echolocation prefer quieter areas with sound levels of 68 dB or below, as excessive noise can interfere with the precise reflection of echolocation waves (Wijayanti, 2011).

Roost selection of bats in Wind Cave Nature Reserve was previously mapped by Shazali (2017) and Shazali et al. (2017) using Light Detection and Ranging (LiDAR) technology, which is an advanced method for investigating complex cave structure. Its ability to generate high-resolution, three-dimensional data makes it especially valuable for ecological research and environmental monitoring (Davies and Asner, 2014). Shazali (2017) recorded the presence of 11 cave-dwelling bat species, including one frugivorous species, *Penthetor lucasi*, and 10 insectivorous species. The mapping of roost sites using the LiDAR system by Shazali (2017) is illustrated in Figure 2.1. Determination of roosting preferences is important to discover social organisations and interactions, distribution of food resources and habitat niche of the bat species (Kunz, 1982).

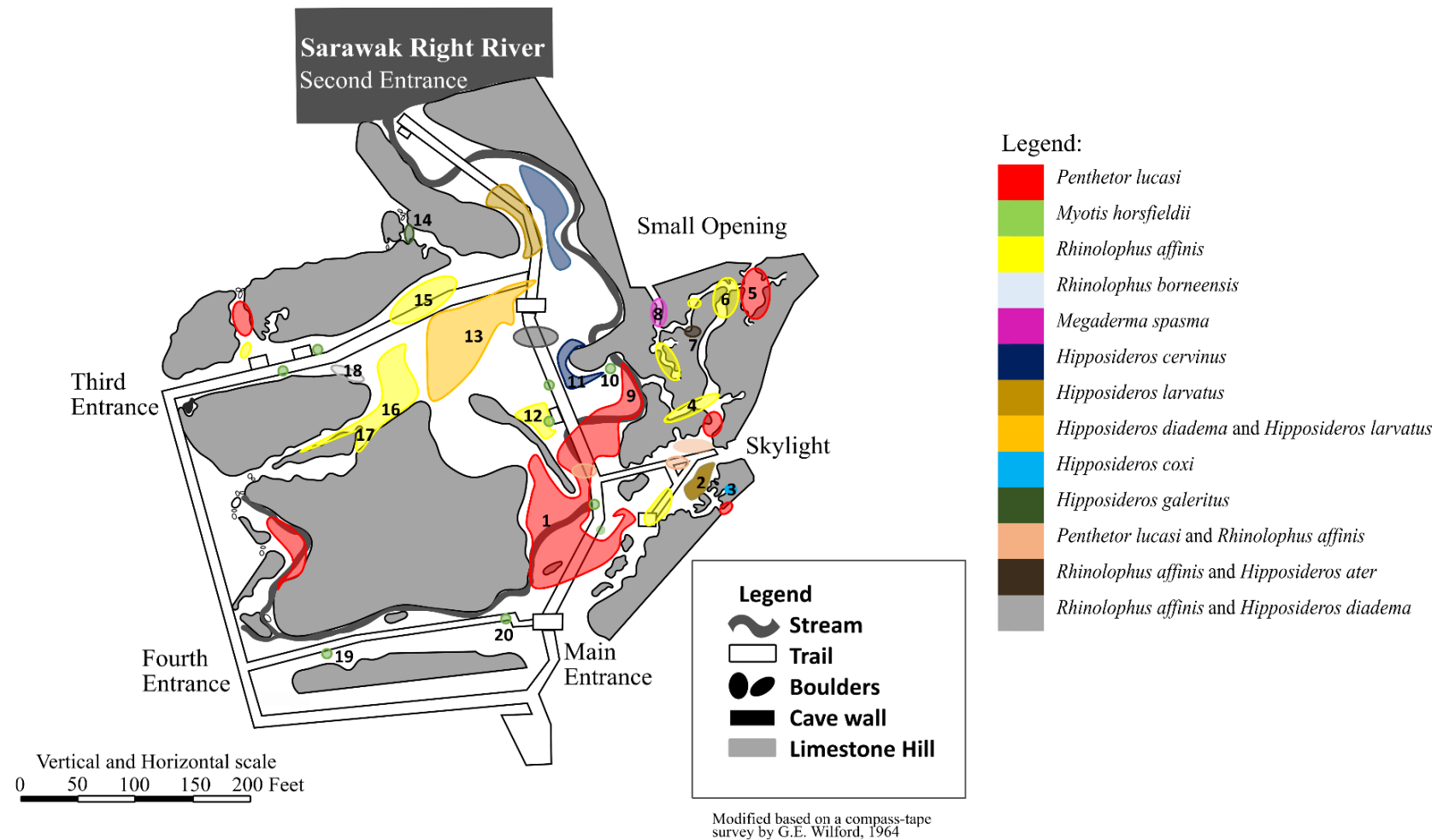


Figure 2.1: Mapping of the roosting sites of 11 bat species recorded using LiDAR. The species present at each roost sites were indicated through the legend as described (Shazali, 2017).

2.3 Frugivorous bats

Bat diets primarily consist of frugivorous (fruit-eating), insectivorous (insect-eating), sanguivorous (blood-feeding), and nectarivorous (nectar-feeding) species (Torres-Jiménez et al., 2020). In the course of evolution, frugivory has developed independently across various vertebrate lineages (Fleming and Kress, 2011). Frugivorous bats play a crucial ecological role as seed dispersers, which is essential for the regeneration of forest habitats (Muscarella and Fleming, 2007). Fruit bats are generally classified within the family Pteropodidae, though some are included in the Phyllostomidae family. These families are geographically separated, with phyllostomid bats found primarily in the Americas, while pteropodid bats are widespread across Asia, Africa, and the Pacific regions (Hodgkison et al., 2003). Although most fruit bats inhabit tropical forests alongside frugivorous birds, studies suggest the existence of a feeding niche separation between them. There is minimal dietary overlap, which is largely influenced by fruit colouration: for instance, bat-preferred figs tend to be yellow to green, whereas bird-preferred figs are typically brightly coloured, particularly in shades of orange and red (Hodgkison et al., 2003).

The diversity and distribution of bats are influenced by factors such as food availability, habitat type, microclimate, and elevation (Maryanto et al., 2011). A study on the habitat preferences of fruit bats in Indonesia revealed that species in Mount Bawakareng utilise a range of habitat types (Yusti et al., 2015). *Thoopterus suhaeniahae* and *T. nigrescens* were observed in mixed garden and pine forest areas, while *Boneia bidens* and *Rousettus celebensis* were found in primary and secondary forests, respectively (Yusti et al., 2015). Previous research has shown that several bat species may inhabit cave environments, including *B. bidens* (Maryanto et al.,

2011), *Penthetor lucasi* (Shazali, 2017), *Cynopterus brachyotis*, *R. amplexicaudatus*, *Eonycteris spelaea*, *Megaerops wetmorei*, and *Ptenochirus jagori* (Tanalgo and Tabora, 2015), as well as *R. leschenaultii* (Nuratiqah et al., 2023). Some of these species are known to roost near cave entrances or large openings, likely to facilitate easier access to foraging areas (Tanalgo and Tabora, 2015).

Unlike insectivorous bats, most fruit bats do not rely on echolocation for navigation or foraging. Pteropodids, in particular, possess strong night vision and are well-adapted to low-light conditions. However, one exception is the cave-dwelling pteropodid genus *Rousettus*, particularly *R. aegyptiacus*, which produces short, broadband clicks for echolocation within the frequency range of 12–70 kHz (Jones and Teeling, 2006). The echolocation system of the Egyptian fruit bat (*R. aegyptiacus*) is notably distinct from that of other echolocating bats, as it does not utilise laryngeal echolocation (producing high-frequency sounds via the larynx). Consequently, the frequencies produced by *R. aegyptiacus* are lower than those typical of echolocating insectivorous bats (Koay et al., 1998).

2.4 Insectivorous bats

Observations on insectivorous bats have concluded that they are generally small (10 to 15 g), especially among aerial feeders (Fenton and Fleming, 1976; Krzanowski, 1977; Barclay and Brigham, 1991). However, the size of the prey and predator correlates with each other, whereby both big and small prey are available for large predators, but only the latter is available for a smaller one. This creates a dietary niche among the cave bats, especially those that coexist in the same cave or habitat (Barclay and Brigham, 1991). Theories regarding the conditions that

affect aerial insectivorous bats' size have been discussed by various researchers. One of the most popular theories suggests that size constraints are due to the demands imposed by flying and foraging. Manoeuvrability and agility for making rapid turns are vital for insectivorous bats to capture flying insects (Norberg, 1986; Norberg and Rayner, 1987). A lower wing loading in insectivorous bats leads to higher manoeuvrability among aerial insectivorous bats (Norberg, 1986; Barclay and Brigham, 1991).

The foraging activities of most aerial insectivorous bats are assisted by their high-frequency sounds (Griffin, 1953; Gould, 1955). Echolocation of these bats, which is used to detect, track, and assess flying insects, is generally higher and more intense at levels exceeding 110 dB (Bogdanowicz et al., 1999). Aldridge and Rautenbach (1987) recorded lower frequency and shallower bandwidth call in bats with higher body mass, aspect ratio, and wing loading. Barclay and Brigham (1991) also support the statement that echolocation call frequencies are negatively correlated with bat body size. That being said, difficulties in establishing the diversity of these small bats are alarming. The traditional capture-release method using mist-netting is inefficient for aerial insectivorous bats. Hence, the utilization of acoustic methods, through echolocation detectors, along with a combination of traditional mist-netting procedures, has been established as one of the best strategies to monitor and estimate aerial insectivorous bats' diversity (Rydell et al., 2002). In previous research, bat identification was the primary step in establishing the experiment's pathway and framework. For that reason, it is imperative to explore multiple methods for bat identification.

2.5 Identification of bats

Bats identification methods have been around even prior to 1989 when the key identification of European bat species was published by Von Helversen (Dietz and Von Helversen, 2004). Morphological characteristics were said to be the basis of the phylogenetic relationship hypothesis (Friedheim, 2016). They have been used for the past 250 years in taxonomy, where individual organisms were defined by their phenotypic characteristics (Hebert and Gregory, 2005). The diversity of organisms can also be primarily identified by morphological characteristics, and they can be further analysed by morphometric methods for description and comparison with other organisms (Rohlf, 1990). This is a fast method for identifying bats, especially in the field. The colouration and morphometric measurements of the forearm and hindfoot of the sample caught are among the proven methods of species identification, particularly for chiropterans (Stadelmann et al., 2007).

Advances in molecular studies, particularly in the field of DNA barcoding, have boosted bat diversity records. Before the breakthrough in the utilization of DNA for taxonomy, only 37 vesper bat species were acknowledged in the Western Palearctic (Europe, North Africa, Arabian Peninsula, and Asia) through morphological means. After the advancement, more than 51 species of bats were identified using mitochondrial DNA sequences. However, the combination of both methods recorded a total of 54 species during the preliminary survey (Mayer et al., 2007). Genetic studies of chiropteran families with revisions of existing scientific names and frequent re-descriptions must be harmonised. Without a proper description of external features, the determination of specimens is not possible (Stadelmann et al., 2007). Hence, it is suggested

that morphological observations of samples paired with molecular analyses significantly improve knowledge of these species, particularly in tropical areas (Ruedi et al., 2015).

Observations of morphology paired with molecular markers have identified at least 11 species of the genus in Madagascar, whereas previous traditional methods only recognised four (Goodman et al., 2011). Aspects considered morphologically in the study were the size of the forearm and colouration. Other than that, the focus on the tragus shape of *Miniopterus* was pertinent. The tragus is an anatomical characteristic proven to establish a second route to the ear canal, allowing the reflection of echoes and assisting in the ability to localise vertical sounds (Lawrence and Simmons, 1982; Chiu and Moss, 2007). Tragus shape has also often been associated with frequency ranges among various species of echolocating bats (Gannon et al., 2001). Hence, the pairing of echolocation or acoustic studies with other identification methods was proven to be beneficial in Ramasindrazana et al. (2015) and other studies with similar goals.

In February 2006, the emergence of a fungal disease caused by the pathogen *Pseudogymnascus destructans* killed over 6 million cave bats. The disease, called white-nose syndrome, was discovered in Howe's Cave, New York, and spread to the bat population across eastern North America (United States Fish and Wildlife Service, 2012; Brown et al., 2017). The extreme decline of bats in the area highlighted the crucial reliance on non-invasive methods for monitoring and research implementation (Brown et al., 2017). Echolocation calls or acoustic identification in bats have been useful in species identification as well as in the study of bat behaviour and ecology (Barclay, 1999; Russo and Voigt, 2016). Some bats emit echolocation calls to navigate in the dark and detect prey or food items. The echolocation frequency reflection allows organisms to perceive their surroundings by creating echo-acoustic images. These calls

are species-specific in relation to their phylogeny, habitat use, and biology (Russo and Voigt, 2016). Voigt-Heucke et al. (2010) suggested that echolocation calls in bats have a dual role, not only for orientation and foraging but also for communication regarding individual identity, gender, and group affiliation.

Taylor et al. (2022) tested the effectiveness of this method by placing a wildlife acoustic detector under bridges in Virginia State and collecting deposited guano to amplify and sequence it using mitochondrial cytochrome oxidase subunit 1 (COI) locus for DNA barcoding. Identification and documentation of foraging activities of bat species were made possible with acoustic methods. Furthermore, their habitat usage and seasonal pattern were apparent and revealed how bat activities vary in space and time (Taylor et al., 2022). The study successfully identified 70 samples of high-quality DNA from 293 guano samples collected. All the species identified were acoustically classified at their respective collection sites (Taylor et al., 2022). Fenton and Simmons (2014) mentioned that over 80% of bats use laryngeal echolocation. The acoustic diversity of bats that produce sound using the larynx was noted to be remarkable. Their call frequencies vary in a range of 8 to more than 200 kHz (Russo et al., 2017). The utilization of echolocation call detection has been widely used to address the difficulties posed by the nocturnal habits of bat groups, especially when direct visualization or capture methods are not reliable.

Identification of bats through echolocation calls can be observed in real time using a call detector and software that displays sonograms (a picture made by collecting information from the echoes bouncing off an object). Sonograms of bat echolocation usually consist of an oscillogram, spectrogram, and power spectrum, where common metrics can be measured for bat

species identification (Fraser et al., 2020). The common metrics used to quantify echolocation parameters are displayed in Figure 2.2 by Fraser et al. (2020). The description of each parameter is as follows: (a) Duration (Dur) – time from the beginning of a call to its end; (b) Inter-pulse interval (IPI) – time between calls; (c) Maximum frequency (Fmax) – highest frequency recorded in the call; (d) Minimum frequency (Fmin) – lowest frequency recorded in the call; (e) Peak frequency (Fppeak) – frequency with the most energy or greatest amplitude (Fraser et al., 2020).

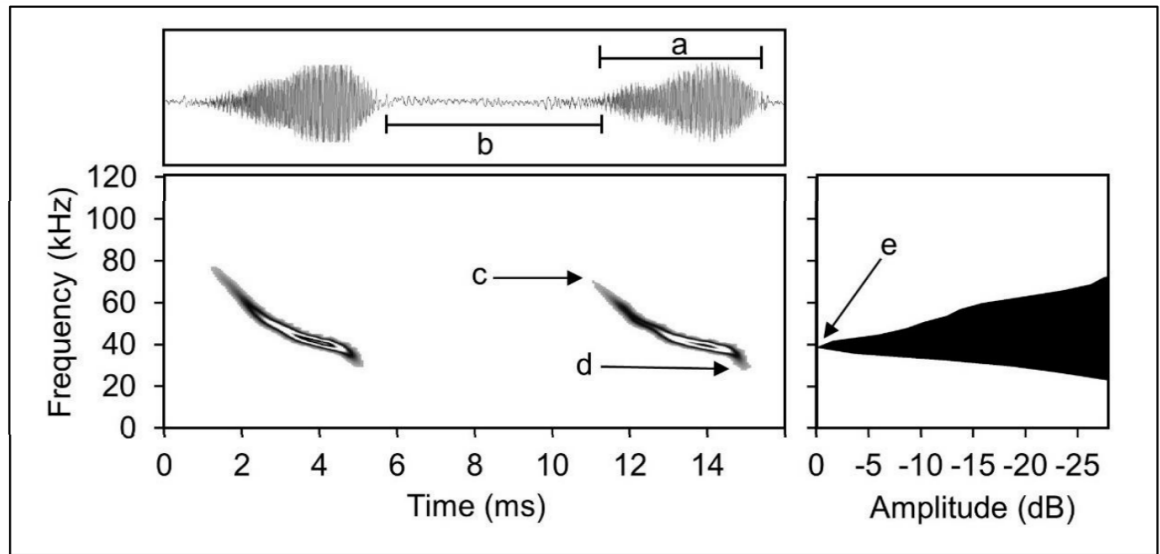


Figure 2.2: Common metrics used in quantifying call structure. *Notes:* (a) Dur; (b) IPI; (c) Fmax; (d) Fmin; (e) Fppeak (Fraser et al., 2020).

Studies on echolocation for identification were largely conducted around the world since the advances of acoustic devices (Russo et al., 2017). Vaughan et al. (1997) recorded the echolocation calls of 15 species of British bats and classify the calls into three different structures which are frequency-modulated calls (FM calls), constant-frequency calls (CF calls)

and an intermediate mixture of both; quasi-constant frequency calls (QCF calls). The structures are described in Figure 2.3 from McArthur (2019).

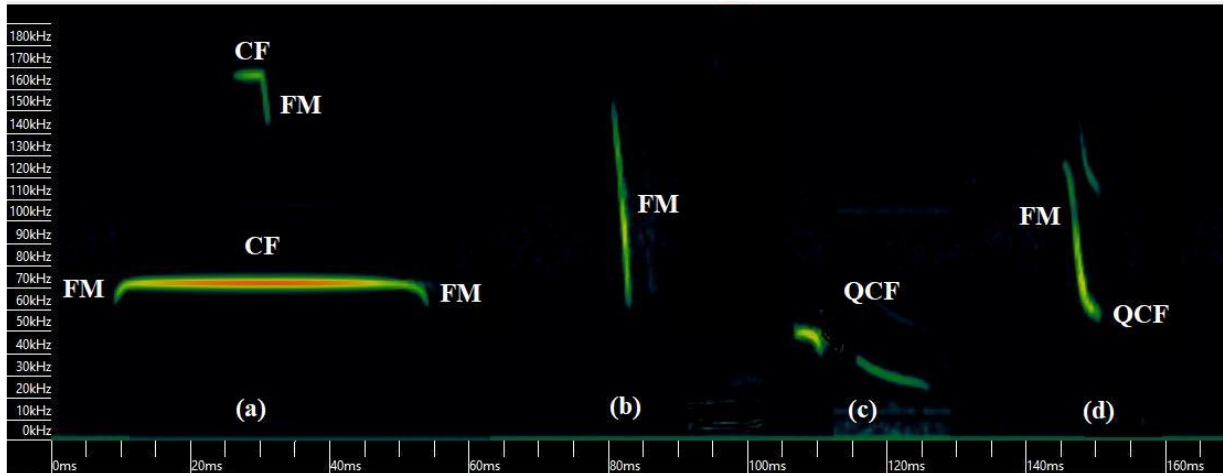


Figure 2.3: Echolocation calls produced by insectivorous bats (a) CF dominated calls with FM components; (b) FM call; QCF calls; (d) FM dominated calls with QCF component (McArthur, 2019)

Intraspecific variation in echolocation calls was found to be present due to habitats, hunting behaviour, and individual identity. Acoustic surveys were found to be challenging due to the uncertainty of species identification in several cases. However, calls of unknown bats can still be analysed and described at the species level accordingly, and they are also a valuable assessment of habitat requirements in the area (Vaughan et al., 1997). Papadatou et al. (2008) also mention that acoustic analysis is a suitable method for species identification when paired with proper databases. Russo and Jones (2002) identified 22 bat species out of 31 recorded species of European bats occurs in Italy by analysing 950 bat call recordings from the area. The analysis of the recordings comprised six parameters: start frequency, end frequency, middle frequency, duration, and inter-pulse interval. The readings were then statistically analysed

through various procedures such as analysis of variance (ANOVA), Mann-Whitney, and Kruskal-Wallis tests, and then applied to multivariate Discriminant Function Analysis (DFA) for cross-validation (Russo and Jones, 2002).

In Sarawak, a comprehensive acoustic survey was conducted in Mulu National Park by McArthur (2019). The survey successfully analysed calls from 31 species belonging to the families Rhinolophidae, Hipposideridae, and Vespertilionidae. The call recordings detected the activities of bats in the national park area, with high activity recorded near rivers, which are known to be vital habitats for wildlife. This proves that acoustic recordings of bats' echolocation can not only provide identification but also offer information on the foraging ecology of the bats studied (McArthur, 2019). However, a few studies also suggested the pairing of visual and acoustic identification with guano collections (Taylor et al., 2022).

2.6 Bats guano

Bat guano can be defined as bats' excrement (Sakoui et al., 2020). The deposition of faeces on cave floors is vital for maintaining invertebrate diversity in caves (Ferreira and Martins, 1999). Bat guano piles are the most common organic matter source, particularly in permanently dry caves in tropical areas where they are produced all year round, acting as the base structure of invertebrate communities (Decu, 1986; Ferreira and Martins, 1998, 1999; Ferreira, 2019). The collection of bat guano for various study implementations is the most accommodating in these circumstances due to easy collection, inexpensive sampling equipment, and minimal stress imposed on the bat colony (Guan et al., 2020). Judy et al. (2010) mentioned that the utilisation of bat guano in identifying bats' diets is the most frequent. However, with

advances in the molecular field of studies, species identification of roosting bats is now possible and has been proven successful (Judy et al., 2010). Multiple research projects, such as Kanuch and Cel'uch (2007) and Puechmaille et al. (2007) had investigated and developed viable methods for protocols on species identification from bat guano using DNA extraction. Guan et al. (2020) mentioned that DNA from individual guano droppings could also be used to identify the sex of the individual, as well as for population genetic analysis, population estimates, and parentage analysis (Vege and McCracken, 2001; Puechmaille and Petit, 2007; Korstian et al., 2013; Viglino et al., 2016; and Swift et al., 2018).

However, the method is not without flaws. The rapid degradation of DNA is a risk for wildlife species as they are exposed to environmental conditions after deposition. DNA extractions from guano were found to be more successful in fresher faecal samples and samples collected in cold or dry climates with low UV exposure. Additionally, DNA compound responses in the samples are target-specific and may vary due to collection and preservation methods (Walker et al., 2019). Moreover, guano samples may produce a small amount of DNA as there is a lower presence of epithelial cells (Vege and McCracken, 2001). Some studies also noted that using universal primers in DNA amplification from bat guano may not be effective for non-invasive samples (Guan et al., 2020). Therefore, the reliability of utilising bat guano should be further explored.

Multiple studies mentioned that bat guano influences trophic dynamics and the community structure of cave habitats and acts as the main energy source in temperate cave ecosystems (Harris, 1970; Gnaspini and Trajano, 2000; Fenolio et al., 2006). Additionally, the condensed and contained structure of bat guano also plays an important role in ecological studies

(Ferreira and Martins, 1999). The accumulation of guano on cave floors, from the roosting sites of bats, creates a reservoir of organic material for microbial growth (Fenolio et al., 2006). Harris (1970) noted the importance of bat guano in the food web of cave invertebrates, which consists of bacteria, fungi, protozoa, nematodes, and arthropods. The physical and chemical properties of guano vary depending on the region, environment, time, and diet of the bats producing the guano (Ferreira and Martins, 1999). The bats' diet also affects guano-associated organism diversity, as assemblages of invertebrates will differ based on guano from different diets (Ferreira and Martins, 1999; Emerson and Roark, 2007).

Morphologically, bat guano consists of elongated and segmented faecal pellets with tiny pits on the surface. The pellets' tips are blunt, and they are easily crushed into powder. Some of the faecal pellets contain shiny bits of insects, and almost all of the pellets collected in previous studies were blackish brown (Sridhar et al., 2006; Shetty et al., 2013). Two previous studies that recorded bat guano morphology found an almost similar measurement of 4.6 ± 0.8 mm in length, with a range of 3.5 to 6 mm, and 1.6 ± 0.24 mm in breadth, with a range of 1.25 to 2 mm, as reported by Sridhar et al. (2006), whereas Shetty et al. (2013) recorded sizes ranging from 9 to 11 mm.

The composition of bat guano shows even greater differences in studies conducted, as it is influenced by multiple factors, such as the bats' diet and the guano's age (Ferreira, 2019; Newman et al., 2018). Bernath and Kunz (1981), Ferreira and Martins (1999), and Chronakova et al. (2009) discovered that fresh guano collected from the surface of guano piles has higher pH, water content, and nitrogen values compared to guano tested deeper in the pile (Newman et al., 2018). However, in general, differences in guano characterisation are highly correlated with

the diet of the bat species. This key feature may assist in identifying prey groups in the local environment, which may lead to strategic conservation approaches (Ware et al., 2020).

The utilisation of bat guano in previous centuries has been recorded in various studies. A few examples include Buchanan (1800), where harvesting guano from *Chaerephon plicatus*, the cave-dwelling Asian wrinkle-lipped bat, had been occurring for decades in Cambodia (Sothearen et al., 2014). Additionally, the extraction of bat guano for fruit production fertiliser in California was recorded from the Carlsbad Caverns in New Mexico between 1903 and 1923. In the 1980s, over 85 tons of bat guano were exploited annually in the now-protected Bracken Caves in Texas (Ghanem and Voigt, 2012). Guano deposits found in natural caves were exploited as commercial manure (Bhat and Sreenivasan, 1990; Sridhar et al., 2006). Countries worldwide, such as Cuba, Jamaica, Thailand, Indonesia, and Brazil, have recorded the usage of bat guano harvests for agricultural purposes (Ferreira, 2019). The market demand for guano products is high, with over 950 bat guano products available as organic fertilisers, with a price range varying from USD 2.50 to 24.00 per kg (approximately RM 11.20 to RM 107.30 per kg) (Kasso and Balakrishnan, 2013). While in Niger, the distribution price of a 70 to 80 kg batch of guano extracted from Magarawa sites may vary from 400 to 500 CFA (approximately RM2.80 to RM3.50), with a rapid rise yearly (Harouna et al., 2021).

Studies in a few nations worldwide have been conducted to assess community perceptions regarding the use of bat guano. Sexton and Stewart (2007) examined community opinions on the presence of bat guano, particularly inside residential areas of Fort Collins, Colorado. Results showed that 76% of respondents had a negative perception of bat guano, while 7% had a positive perception (Sexton and Stewart, 2007). Not just that, many farmers in

Mexico mentioned having chiroptophobia (fear of bats), where they looked at bats with a negative perception due to their physical appearance, which is considered unpleasant and ugly, affecting the percentage of bat guano utilisation in plant growth (Torres-Jimenez et al., 2020). Many of the community members in Fort Collins are doubtful on the usage of bats guano due to the believe that exposure to guano may lead to lung infections (Sexton and Stewart, 2007). Harouna et al. (2021) recorded that 75% of the guano extractors in Magarawa village use protective clothing when handling the guano. Despite this, a high number of extractors noted having respiratory problems, which may be due to the presence of bacteria and fungi in bat habitats (Tuttle and Moreno, 2005; Suwannarong and Schuler, 2016; Harouna et al., 2021).

Torres-Jimenez et al. (2020) reported that understanding of bat guano benefits was left unchecked in the Mexican community, as 59% of the coffee farmers interviewed had little to no knowledge of it that. Some coffee farmers deemed the usage of bat guano as a disadvantage because of the view that organic fertiliser does not facilitate a disease-resistant agroecosystem. However, one farmer noted that guano fertiliser applied to crops could increase the productivity of agroecosystems, including coffee varieties (Torres-Jimenez et al., 2020). On the other hand, guano collection in Magarawa village (a small village, located in the West African region), had complete freedom for guano extraction since they had mastered the techniques of extraction passed down by their elders (Harouna et al., 2021). The market for dry guano and fresh guano extracted from Magarawa is vast in Niger where almost 96.7% of horticulture in Balla and Guidimouni uses guano for the growth of their plants (Harouna et al., 2021).

Meanwhile, in several ASEAN countries, a survey conducted in Pisan, the Philippines, found that 26% of caves in the area were used for guano collection. The study, which focused

on the threats causing the decline of the bat population in the area, noted that the lack of protocols and regulation for guano mining in the area posed a major threat to the population. Sustainability in guano extraction is of the utmost importance to hinder the destruction of geological cave features and prevent damage to bat roosts (Tanalgo et al., 2016). In Thailand, it was reported that guano miners had great appreciation for bats living in their community (Suwannarong and Schuler, 2016).

Suwannarong et al. (2020), in their survey on perceptions of bats in multiple communities around Thailand, reported that several provinces, such as Lopburi and Chiang Rai, conducted guano mining activities managed by Buddhist temples. These activities were considered a source of income for local villagers, who were hired to collect and pack the guano. The price of guano in the area could reach up to 7 Baht per kg (approximately RM0.90). Insectivorous bats of Thailand are more favoured for in guano collection, particularly the species, *Chaerephon plicatus* (Boonkerd et al., 2001; Leelapaibul et al., 2005). All regions apart from the northeastern ones (i.e. Khon Kaen and Ubon Ratchathani) in Thailand utilised bat guano in their agriculture. The enforcement of strict laws on the use of bat-derived substances in Khon Kaen, along with the lack of guano in Ubon Ratchathani caves, was the reason for the absence of bat guano mining in the area (Suwannarong et al., 2020).

Bat guano has been discovered in many caves worldwide and is exploited commercially as manure because of its high nutrient levels (Sridhar et al., 2006; Kasso and Balakrishnan, 2013). Harvesting of bat guano can be done using various methods. Some research on bat guano has been done with samples obtained from the direct collection of guano piles from cave floors (Bird et al., 2007; Wurster et al., 2015; Wasti et al., 2021). Other methods of collection include

the placement of plastic tarps or plates directly underneath bat roosts identified in the cave (McFarlane et al., 2017). In Vietnam, bat guano harvesting is done through the construction of bat houses. The guano collected is sourced economically into the fertiliser market, and the profits are collected by the owner, calculated to be a monthly average of 50 USD per bat house (approximately RM223.50 per bat house) (Dang, 2015).

Due to the stable climate of tropical countries, such as Brazil, the availability of guano remains constant as there is no migration or hibernation of bats, unlike in temperate regions where seasonal changes affect bat populations (Ferreira, 2019). Given this, the tropical country of Malaysia needs to study in depth the qualities of bat guano. In Malaysia, bat guano has been discovered in several localities, particularly in Borneo. McFarlane and Lundberg (2018) suggested the presence of multiple minerals, such as nitrate, phosphate, and sulphate in bat guano collected from several caves in Sarawak, including Racer Cave, Mulu National Park, and Niah Cave.

Wurster et al. (2015) showed that guano from insectivorous bats can be found in limestone caves throughout Malaysia, such as Batu Cave in Peninsular Malaysia, Niah Cave, and Gomantong Cave in Northern Borneo. Another study on bat guano conducted in Southern Borneo used bat guano for radiocarbon dating to record environmental changes (Wurster et al., 2015). Wurster et al. (2015) utilised radiocarbon dating to provide evidence for the presence of a savanna ecosystem in Borneo during the Pleistocene epoch. The most recent findings on bat guano in Malaysia focus on its role as a host for SARS-CoV. In the wake of the Coronavirus pandemic, virology surveillance and studies have been widely conducted throughout Asia. In Borneo specifically, Tan et al. (2021) found a high diversity of alpha and beta coronaviruses at

Wind Cave Nature Reserve which are unique to Borneo Island. In the field of mycology, on the other hand, guano obtained from Madai Cave, Sabah, was found to contain 32 species of fungi, such as *Penicillium* spp., *Aspergillus* spp., and *Purpureocillium lilacinum* (Wasti et al., 2021).

2.7 The chemical composition of bats' guano and its effect on plant growth

Recent studies on the chemical composition of guano provide clear justifications for its use as an organic fertiliser. High levels of organic matter, total nitrogen (N), phosphate (P), potassium (K), and carbon (C) have been reported in numerous studies, including Studier et al. (1994), Sridhar et al. (2006), Emerson and Roark (2007), Suwarno and Idris (2007), Buliga (2010), Shetty and Sreepada (2013), Shetty et al. (2013), Jayasvasti and Jayasvasti (2018), Unal et al. (2018), Oo et al. (2019), Misra et al. (2019), and Ferreira (2019). Most of these studies also mention the presence of secondary nutrients such as calcium (Ca), magnesium (Mg), aluminium (Al), iron (Fe), and sulphur (S), which help regulate soil pH. Additionally, guano is rich in beneficial microbes that enhance nutrient absorption in plants, remove soil toxins, and control fungi and nematodes, thus preventing plant diseases (Shetty et al., 2013; Oo et al., 2019). This was demonstrated in Shetty et al. (2013), where the use of autoclaved soil with guano still resulted in optimal plant growth, reinforcing guano's potential as a biofertiliser.

However, the composition of guano varies depending on the diet, species, age, and locality of bats (Studier et al., 1994; Emerson and Roark, 2007; Vandzurova et al., 2012; Shetty et al., 2013; Jayasvasti and Jayasvasti, 2018). Shetty et al. (2013) highlighted differences in plant growth effects, possibly due to variations in microbial structure in the guano. Differences in guano composition among various species were observed in Misra et al. (2019). For example,

sodium (Na) and potassium (K) levels in the guano of the insectivorous bat *Scotophilus kuhlii* were found to be higher, suggesting that it consumes a significant number of lepidopterans. Similarly, the P content in the guano of the insectivorous bat *Megaderma lyra* was recorded as the highest among all species tested (Misra et al., 2019). Moreover, the higher levels of P, Al, and Fe in fresh guano from the upper layers of guano deposits support the finding that guano composition is influenced by its age (Sakoui et al., 2020).

Comparisons of guano composition based on diet have been explored in several studies. Sridhar et al. (2006) reported that the guano of the insectivorous bat *Hipposideros speoris* contained a higher percentage of N than P. Emerson and Roark (2007) compared the guano of the sanguivorous bat, *Desmodus rotundus* and the frugivorous bat, *Pteropus rodricensis*, revealing that the sanguivorous bat had a higher C level than the frugivorous bat. This may be attributed to the indigestible fruit components consumed by frugivorous bats, which are not included in their faecal matter (Emerson and Roark, 2007). Studier et al. (1994) found that insectivorous or omnivorous bats had higher N levels compared to frugivorous bats, whereas P levels were higher in frugivorous bats than in insectivorous bats (Goveas et al., 2006; Emerson and Roark, 2007). However, Shahack-Gross et al. (2004) did not observe similar results, as their study measured higher P levels in insectivorous bats than in frugivorous bats (Emerson and Roark, 2007).

Findings by McFarlane and Lundberg (2018) on bat guano from caves in Sarawak—specifically Racer Cave, Mulu National Park, and Niah Cave—suggested the presence of diverse chemical compounds. Guano samples from Racer Cave exhibited pink-coloured formations, which were identified via spectral analysis as phosphate minerals. In contrast, bat

guano in Niah Cave, dated at more than 40,000 years, was found to be extensive and stratified. Spectral analysis of samples from Niah Cave detected the presence of fluorine and nitrogen, while further analysis revealed the presence of oxygen (O), P, Ca, and trace amounts of Na, Al, S, chlorine (Cl), and Fe (Dykes, 2007; McFarlane and Lundberg, 2018).

Wurster et al. (2015) demonstrated the elemental composition of guano from insectivorous bats in limestone caves across Malaysia, including Batu Caves in Peninsular Malaysia, Niah Cave, and Gomantong Caves in Northern Borneo, as well as Makangit and Gangub Caves in the Philippines. The examination revealed that C and N levels were highest at the surface of bat guano deposits across all localities. However, both C and N levels decreased with depth, indicating that guano age influences these elements. Furthermore, P levels varied by location. For instance, P levels in bat guano from Batu Caves and Makangit Cave were relatively low, while those in Gomantong and Gangub Caves were moderate. Notably, P levels in samples from Niah National Park were the highest among all sites studied (Wurster et al., 2015). Meanwhile, fresh guano from free-tailed bats collected from Deer Cave, Mulu National Park, contained 7.6% N (McFarlane and Lundberg, 2018).

Understanding the precise nutrient composition of guano could enhance plant growth and improve crop production through its strategic incorporation into agriculture. This ultimately contributes to the sustainable management of fertiliser use (Ferreira, 2019; Misra et al., 2019). The specific nutrient levels recorded in various studies are summarised in Table 2.2. Only the dietary properties from Sridhar et al. (2006) and Sothearen et al. (2014) were included in this dataset. The dietary properties listed in Table 2.2 were supplemented with additional literature, where available, based on species found in regions where bat guano was collected.

Table 2.2: Nutrient levels of bats' guano components from previous studies

Authors	Bat's diet	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Other Nutrients
Sridhar et al. (2006)	Frugivorous bats	11 - 15	65 - 79	11 - 20	nr
	Insectivorous bats	62 - 68	16 - 31	8 - 16	nr
Altintas and Yildiz (2005)	Majorly Insectivorous bats (Paksuz, 2016)	0.97 - 5.60	1.20 - 1.50	0.25 - 0.63	OM: 24 - 79
Ojobor and Egbuchua (2021)	nr	4.6	nr	nr	pH: 5.7 C: 26.4 % C/N: 5.7

Table 2.2: continue

Karagoz-Sezer and Hanay (2020)	nr	8.20	P ₂ O ₅ : 2.00	K ₂ O: 0.5	OM: 67.30 % C/N: 8-15/1 pH: 4.00 Salinity: 1.30 %
Giurgiu and Tamas (2013)	Majorly Insectivorous bats (Csosz et al., 2015; Jerez et al., 2017)	5 - 10	25	nr	nr
Sothearen et al. (2014)	Insectivorous bats (<i>Scotophilus kuhlii</i>)	9.97	3.4	0.4	nr
Yankova et al. (2016)	nr	2	nr	nr	OM: 20 % pH: 4.2 - 6.3

Note. Few information that are not recorded in the studies are marked with (nr)

Determining the specific levels of N, P, and K nutrients in guano can aid plant growth and enhance crop production through suitable guano incorporation, ultimately ensuring sustainable agricultural management in fertiliser usage (Ferreira, 2019; Misra et al., 2019). The primary chemical components vital for regulating plant growth are nitrogen (N), phosphorus (P), and potassium (K). Although nitrogen is abundant in the atmosphere, it is not readily available to plants. It plays a crucial role in plant regulators, being present in proteins and forming essential components of living organisms, such as DNA and chlorophyll. The supply of nitrogen to plants is known to promote leaf growth (Oo et al., 2019). According to Shetty et al. (2013), manure with high nitrogen levels enhances crop growth. When nitrogen is combined with phosphorus, crop yields show increased root growth, dry matter accumulation, and an improved root-to-shoot ratio (Oelhalf, 1978; Poliquit and Calong, 2018).

Phosphorus is an essential component of ATP (an energy carrier required for photosynthesis) and various lipids (Smil, 2000; Jayasvasti and Jayasvasti, 2018; Oo et al., 2019; Isnaeni and Nurhidayah, 2020), highlighting its importance in plant growth. However, phosphorus availability is limited, as it can only be recycled or recovered and cannot be synthetically produced, replaced, or destroyed (Walan, 2013). Phosphorus recovery for fertiliser use can be achieved through extraction from waste, wastewater, or guano (Jayasvasti and Jayasvasti, 2018). The application of phosphorus in plant growth has been documented by Diba et al. (2013) and Oo et al. (2019), who found that phosphorus enhances root development, flowering, seed formation, and fruit production. Sridhar et al. (2006) and Shetty et al. (2013) suggested that phosphorus in manure stimulates shoot budding, root development, and flowering. Walan (2013), as cited in Jayasvasti and Jayasvasti (2018), further supports this by

stating that phosphorus also improves fruit quality and enhances plant disease resistance. Adequate phosphorus availability is crucial for crop maturity and seed formation (Linn and Myint, 2018). Phosphorus is absorbed by plants in its active forms, H_2PO_4^- and HPO_4^{2-} (Diba et al., 2013). Poliquit and Calong (2018) reported that phosphorus-stimulated root development is significantly influenced by organic matter and soil microorganism activity.

The size of grains and seeds depends on the amount of potassium absorbed by plants. Potassium also improves fruit and vegetable quality (Linn and Myint, 2018). According to Oo et al. (2019), potassium promotes stronger stem growth, regulates water movement in plants, and supports fruiting and flowering. Plants absorb potassium in the form of K^+ (Diba et al., 2013). A potassium deficiency can result in shrivelled fruits and seeds (Linn and Myint, 2018). Other essential components in plant regulation include enzymes, calcium (Ca), and magnesium (Mg), which stimulate soil microorganisms, facilitating the release and absorption of nutrients by plants (Yankova et al., 2016).

Numerous plant growth studies have demonstrated positive results when guano is used as an organic fertiliser. Poliquit and Calong (2018) identified several differences on guano application in carrots compared to control treatments. Carrots treated with guano exhibited longer and broader root circumferences, a more balanced accumulation of nitrogen and phosphorus, improved crop growth and development, and more uniform nitrogen release, promoting root growth. Additionally, Linn and Myint (2018) recorded an increase in soil nutrients (pH, moisture, nitrogen levels, available phosphorus and potassium, exchangeable potassium, calcium, and magnesium) when a mixture of guano, wood ash, and a commercial organic fertiliser (Vedagro™) was applied to cassava plants. Higher growth levels and

significant improvements in plant growth were observed in all plant species treated with guano, with statistically significant differences compared to chemical fertilisers (Sakoui et al., 2020).

Sothearen et al. (2014) found that guano application in five economically important plant species in Cambodia—horseradish tree, jackfruit, eggplant, papaya, and longan—yielded impressive results. A significant weekly increase in stem circumference and height was recorded for all plant species treated with guano compared to the control treatment. When compared to chemical fertiliser treatments, three out of five plants fertilised with guano exhibited better growth performance despite guano's lower NPK content (Sothearen et al., 2014). This suggests that the presence of micronutrients and beneficial microflora, absent in chemical fertilisers, played a significant role in plant growth performance (Sridhar et al., 2006; Shetty and Sreepada, 2013). The introduction of guano throughout the study significantly enhanced soil nutrient content, leading to improved plant growth (Sothearen et al., 2014).

Unal et al. (2018) also demonstrated that guano application resulted in increased plant growth compared to the control group. Other supporting studies include Shetty et al. (2013), Almohammed et al. (2014), and Diba et al. (2013). Bat guano is particularly valuable as it provides nutrients that support microfloral growth (Misra et al., 2019). Previous studies indicate that only a small amount of guano is required to influence plant growth (Sridhar et al., 2006; Shetty et al., 2013; Sothearen et al., 2014). Ojobor and Egbuchua (2021) reported that an appropriate mixture of bat guano with other manures can address nutrient deficiencies in crops. Moreover, the suitable proportion of chemical components such as N, P, and K can enhance various plant structures, including shoot length, leaf development, flowering, and disease resistance (Ojobor and Egbuchua, 2021). Additionally, Karagoz-Sezer and Hanay (2020) noted

that studies on guano's influence on plant growth suggest that continuous and sustainable usage can improve soil productivity and nutrient availability.

Shetty et al. (2013) proposed that incorporating guano with high-nitrogen manures could further enhance plant growth performance. However, previous studies have consistently reported that only a small amount of guano is needed to impact plant growth (Sridhar et al., 2006; Shetty et al., 2013; Sothearen et al., 2014). A review of studies on guano application in plant growth shows that it can be used in various forms. Poliquit and Calong (2018), Shetty et al. (2013), and Sothearen et al. (2014) applied guano as a soil-based fertiliser, while Unal et al. (2018) used a water-based mixture through drip irrigation. Other studies incorporated guano with organic waste, such as Diba et al. (2013), who mixed guano with soybean paste, and Linn and Myint (2018), who combined guano with wood ash and sesame meal before application as fertiliser. Variations in guano application methods may influence plant growth, although no definitive studies with consistent variables have been conducted to confirm this. Earlier studies by Sridhar et al. (2006), Shetty et al. (2013), Tasci and Seckin (2013), Almohammed et al. (2014), Sothearen et al. (2014), and Grantina-Ievina and Ievinsh (2015) concluded that research evaluating the effects and mechanisms of bat guano on plant growth performance remains limited and inconclusive.

2.8 The chemical composition of chemical fertilisers and its effect on plant growth

Commercial, chemical, and inorganic fertilisers are an indispensable material treatment in modern agriculture (Ji et al., 2023). Crop productivity and food security in the global market have been maintained largely due to commercial fertilisers (Roberts, 2009). However, concerns

about the effects of fertilisers on the pollution of agricultural areas have been gradually increasing (Vitoria et al., 2004). Numerous studies have discussed the disadvantages of inorganic fertiliser treatments to the ecosystem. Ekinici et al. (2019) discussed the deadly effects on microorganisms and the decline of soil worms and maggots due to the application of high amounts of chemical fertilisers. Moreover, the residual nitrogen in the harvested crop resulting from excessive chemical fertiliser application might lead to water pollution from nitrate leaching and air pollution due to the volatilization of nitrogen into nitrous oxide (Whitehead, 2000; Guler, 2006; Ekinici et al., 2019).

Michalski et al. (2015) also mentioned the negative effects of commercial fertilisers on water supplies. As agriculture is the major source of nitrate in America, eutrophication caused by excess nitrate in lakes, rivers, coastal waters, and estuaries can lead to the destruction of aquatic life as well as hypoxia. In response, the United States Environmental Protection Agency has implemented regulations to address groundwater pollution caused by nitrates in drinking water (Michalski et al., 2015). The European Communities have also established a directive under European Union law to protect waters against nitrate pollution from agriculture, as ingestion of nitrate in high concentrations may cause methemoglobinemia (a blood disorder where an abnormal amount of methaemoglobin is produced) in children and promote stomach cancer (Vitoria et al., 2004).

Nosheen and Bano (2014) noted that excessive use of chemical fertilisers will not only affect the soil's physicochemical properties and pollute the ecosystem, but it will also deplete resources due to their high production cost. In Australia, these fertilisers have also been observed to reduce seedling establishment because of their toxic and osmotic effects. This is more critical

in soils with low moisture, as they have a high concentration of toxic ions and osmotic pressure (Carter, 1987). An increase in the global production of chemical fertilisers has been found to play a vital role in crop yield improvements in China. The use of fertilisers there has contributed to a 40% increase in grain output, which has enabled the nation to feed 22% of the global population. However, the average application of chemical fertilisers in China has far exceeded the international safety limit (225 kg/hm²). Hence, the government of China, through the Ministry of Agriculture, has implemented the 'Zero Growth Action Plan of Fertiliser Use' to increase fertiliser efficiency and promote sustainable development in agriculture (Ji et al., 2023).

The first recorded production of chemical fertilisers dates back to 1840 by Justus Liebig. At the time, superphosphate was introduced to fix ammonia and supply essential elements to plants. Between 1843 and 1853, the commercialization of superphosphate in several Western countries grew rapidly, making it the dominant P fertiliser worldwide for more than 100 years. Along with the development of P fertilisers, K fertilisers also advanced, as records show that Liebig suggested the use of ashes as a K source during this period. Although research on N fixation had started before the 1800s by Joseph Priestly and Henry Cavendish, the N fertiliser industry lagged behind those of the other elements until World War II (Russel and Williams, 1977). Collings (1949) recorded that the first synthetic N fertiliser available on the market was calcium nitrate. A decade later, it was noted that there were 300 liquid mixed fertilisers sold across the United States, a number that further rose to 2,800 by 1975 (Russel and Williams, 1977).

In modern times, the European Communities proposed the classification of commercial fertilisers into two categories: straight fertilisers and compound fertilisers. A straight fertiliser

is characterised by only one primary nutrient, which is N, P, or K, while compound fertilisers contain at least two of the primary nutrients. Compound fertilisers are commonly labelled with three numbers, for instance, 10-10-15 for an NPK fertiliser containing 10% N, 10% P, and 15% K (Vitoria et al., 2004). Wang and Li (2004) noted four types of N fertilisers, namely ammonium chloride, sodium nitrate, ammonium nitrate, and urea. It was highlighted in the study that nitrate is the form of N that is taken up by plants in high percentages. Between 72% and 94% of this nitrate N is then consumed by humans daily through the vegetable portions of their diet. Therefore, several countries, such as Germany, established a standard for accepted levels of N in vegetables to reduce nitrate accumulation. For instance, the limit is 791 mg nitrate N for fresh spinach and 791–1017 mg for fresh lettuce. An imbalance in the N:P ratio in vegetables may also affect nitrate accumulation (Wang and Li, 2004). Hence, establishing the chemical composition of each compound in commercial fertilisers is important to ensure healthy food production.

Despite this, very few studies have examined the chemical analysis of commercial fertilisers, which may be due to the fact that fertiliser grades are often included at the point of sale. Lundstrom and Mehring (1939) found that the average grade of fertilisers consumed in 1934 consisted of 3.52-8.73-5.12 NPK fertilisers and 0-11.36-7.55 PK mixtures. Wheeler (1894) tested 50 different commercial fertilisers and found that the samples contained an average of 3.01% Na₂O, 5.56% P₂O₅, 4.9% CaO, 3.56% K₂O, 3.78% SO₃, 3.30% Cl, and 0.75% MgO. Other research found that the average moisture content was similar across 27 samples of commercial fertilisers, except for concentrated fertilisers, which contained less moisture. In this study, it was found that 62% of the ordinary-grade fertiliser mixtures consisted of ammonium

N, which was considered lower than the higher analysis grade of fertilisers, which contained 80% of total N (Lundstrom and Mehring, 1939).

Recent characterisation of commercial fertilisers analysed P concentrations of 16,388 mg/L, K concentrations of 49,520 mg/L, and N concentrations of 16,500 mg/L from ammonia N (Romero et al., 2013). The pH tested for the fertiliser was 7.01, which is an almost neutral value (Romero et al., 2013). The demand for inorganic fertilisers remains high due to the growing population and the need for food security. Roberts (2009) mentioned that chemical fertilisers cannot be replaced by organic treatments, but the study emphasised the importance of using organic sources when necessary. The study further suggested adopting the fundamental principle of chemical fertiliser management, which is the 4Rs – right source, right rate, right time, and right place (Roberts, 2009).

Several research projects have suggested that chemical fertilisers contribute significantly to the agricultural industry. The increase in crop yield and food production was one of the benefits due to the application of chemical fertilisers (Termine et al., 1987; Fageria et al., 2009). Stewart et al. (2005) represented 362 seasons of crop production analysis and recorded that commercial fertiliser contributed 30–50% of crop yield input. Additionally, Soliman et al. (2016) noted that the application of N fertiliser significantly increased the N content in safflower plants.

The improvement of vegetative growth in plants was largely supported by the increased availability of N for crop uptake through fertilisation using synthetic N fertilisers (Johnson and William, 1997). Ayodele et al. (2015) mentions that high approval should be given to N

fertilisers in particular, as adequate levels of N in plant treatments can stimulate growth and increase fruit yields, particularly for plants with high nutrient demands for development, such as mature pepper plants. The application of synthetic N fertilisers has also been reported to enhance the growth performance of peppers by increasing plant height, branch numbers, and leaf area. N fertilisers are also essential for chlorophyll formation, which gives plants their green colour and enables them to convert sunlight into sugars for growth (Ayodele et al., 2015).

Experiments on the effects of different types of N fertilisers on Peking cabbage and spinach found no significant yield differences between the four types of N fertilisers used, namely sodium nitrate (nitrate N), ammonium nitrate, ammonium chloride (ammonium N), and urea N. However, a substantial increase was observed compared to the control treatment (no N fertilisers), ranging from 101–155% (Wang and Li, 2004). Research on the response of commercial fertilisers to plant growth is often conducted in combination with organic fertilisers. The combined use of animal manures or plant composts with commercial fertilisers has been reported to be beneficial for soils by enhancing nutritional value (Toor, 2009). Ekinici et al. (2019) highlighted that the application of fish manure combined with synthetic N fertilisers on spinach growth produced better results than the application of N fertiliser alone, as shown in Table 2.3.

Table 2.3: Comparison of the growth increment for spinach treated with N fertiliser only and the combination of N fertiliser with fish manure (Ekinici et al., 2019)

Parameter/ Treatment	N fertiliser (%)	N fertiliser+ Fish Manure (%)
Leaf area	12.9	39.7
Stem diameter	5.2	32.3

Table 2.3: continue

Leaf fresh weight	54.9	55.8
Leaf dry weight	88.9	59.5
Root fresh weight	75.2	123.9
Root dry weight	80.0	130.0

Brown et al. (1995) comparing broiler litter and commercial fertiliser for tomato size and marketable yield found that tomatoes treated with broiler litter had a higher marketable fruit yield (65,000 kg/ha) than those treated with commercial fertiliser (52,000 kg/ha). A significant difference in tomato foliar analysis was found when comparing all chemical levels (N, P, Mg, Ca, Fe, and Mn), except for K and Zn levels, between commercial fertilisers and broiler litter in the same study (Brown et al., 1995). Phosphorus fertiliser application in China has resulted in an increased yield of green cabbage and rape by 16.4% and 25.1%, respectively. However, there was no increase in yield for cabbage and spinach crops with the same treatment. Hence, it was deduced that plant growth response to P fertilisers is species-dependent, especially in P-rich soils (Wang and Li, 2004). In India, Toor (2009) suggested that a combination of P application and poultry manure on soybean crops could increase the yield of dry matter and P uptake while simultaneously addressing the problem of P deficiency in soils there.

Although the use of inorganic fertilisers can lead to fertiliser loss and environmental pollution, optimal fertiliser management is vital for improving the quantity and quality of crop yields (Fageria et al., 2009; Jahanzad et al., 2017; Ekinici et al., 2019; Zandvakili et al., 2019).

New alternatives to organic fertilisers are equally important to ensure food production keeps up with the increasing demands of a growing population (Ekinci et al., 2019).

2.9 The chemical composition of chicken manure and its effect on plant growth

Manure can be defined as organic materials or substances that are applied to the soil in order to increase its productivity. It is considered to be a slow-releasing fertiliser, as it provides a constant amount of nutrients over a long period of plant growth. Chicken manure can be described as an organic waste material consisting of faeces and urine from chickens, along with a mixture of bedding materials (wood shavings or sawdust) and feathers (Masarirambi et al., 2012). Recent findings have noted that a single chicken can produce up to 0.5 pounds of fresh manure for each pound of feed consumed. Fresh manure that initially contains 75% moisture can decrease rapidly to 20–40% due to evaporation (Ritz and Merka, 2003). Ritz and Merka (2003) found that a broiler house capable of accommodating 20,000 birds could produce 150 tonnes of chicken litter annually, with a potential value of \$6,150 (approximately RM27,238). Singh et al. (2018) revealed that the chicken manure from Manjung, Perak, in Peninsular Malaysia accounts for 10% of the national chicken production supply. The area's steady population of 14 million chickens can generate approximately 1,400 tonnes of chicken droppings daily. These droppings are sold in their raw state at RM3 per 20 kg (Singh et al., 2018).

The readily available fertiliser in chicken litter helps reduce fertiliser costs in crop production (Ferguson and Ziegler, 2004; Masarirambi et al., 2012). Its use as fertiliser has been steadily increasing in urban areas, possibly due to its high nutrient content, particularly its N, P,

and K levels (Masarirambi et al., 2012; Ravindran et al., 2017). Despite its low price, chicken manure has high potential for biofertiliser production through aerobic composting (Singh et al., 2018). It is one of the most valuable organic fertilisers (Chen and Jiang, 2014). The application of chicken manure in crop production has been proven to increase soil N and P levels and enhance crop yield (Masarirambi et al., 2012). Other studies have also reported that using chicken manure can increase the production of secondary metabolites and boost the activity of antioxidants in selected plants (Thepsilvisut, 2021).

Analysis of the chemical composition of chicken manure has shown significant differences compared to other organic manures (Masarirambi et al., 2012). The chemical analysis of chicken manure in previous studies is recorded in Table 2.4. McCall (1914) stated that several factors could affect the composition of chicken manure, such as the age of the chicken, the manure itself, as well as its storage and handling processes. Studies on the chemical composition of chicken manure over time found that high concentrations of N, P, and organic matter (OM) were detected in fresh manure, while K levels remained nearly constant for up to six months. It was deduced that the nutritional value of chicken manure declines with age (McCall, 1914).

Table 2.4: Chemical Analysis of Chicken Manure Components from Previous Studies

	Nitrogen	Phosphorus	Potassium	Other Nutrients
Masarirambi et al. (2012)	nr	Available P: 4685 µg/kg	Available K: 2149 µg/kg	pH: 8.0
Dikinya and Mufwanzala (2010)	5,954 mg/kg	10,490 mg/kg	Exchangeable K: 1.51 cmol/kg	pH: 7.29 Na: 0.84 cmol/kg Mg: 0.63 cmol/kg Ca: 9.79 cmol/kg
McCall (1914) *				
Fresh	4.5 %	P ₂ O ₅ : 3.0 %	K ₂ O: 1.3 %	O.M.: 53 % Ca: 3.2 % Mg: 0.29 % S: 0.31 %
10 weeks	2.4 %	P ₂ O ₅ : 2.4 %	K ₂ O: 1.8 %	O.M.: 43 %
6 months	1.9 %	P ₂ O ₅ : 2.4 %	K ₂ O: 1.3 %	O.M.: 28.5 %
1 year	1.1 %	P ₂ O ₅ : 1.0 %	K ₂ O: 0.6 %	O.M.: 37 %
Thepsilvisut (2021)	29.3 g/kg	43.2 g/kg	38.8 g/kg	pH: 7.7 O.M.: 559.3 Ca: 47.5 g/kg Mg: 36.2 g/kg
Magid et al. (1993)	3.1 %	Total P: 1.1 Available P: 2700	Total K: 5.4 Available K: 30800	pH: 6.8 C/N: 2:5

Note. Chemical components that are not recorded in the study are marked with (nr)

Studies on the effects of chicken litter on plants are widely recognised by the scientific community. Soil treated with chicken manure was found to decrease pH levels, which may be due to hydrogen ions generated during the nitrification process as NH_4^+ is transformed into NO_3^- . Exchangeable Mg^{2+} and K^+ were recorded to significantly increase from manure application, while exchangeable Ca^{2+} was observed to decrease. The same trend was noted for available P concentration in soil treated with chicken litter. While ammonium nitrogen did not show large variations, the application of chicken manure to tomato plants in the study was found to increase the number of medium and large fruits produced (O'Hallorans and Colberg, 1993). Lettuce grown with chicken manure in Masarirambi et al. (2012) showed a higher number of leaves compared to those treated with chemical fertiliser. It was suggested that these differences were due to the water-holding capacity of the soil. However, weed infestation levels were high in plants receiving the treatment for the same reason.

Other than that, the enhancement of soil biological activity and efficiency was also noteworthy as an advantage of using chicken manure treatment (Masarirambi et al., 2012). Another study on the effects of chicken manure on white mugwort plant growth showed a significant improvement on the properties of the soil samples after treatment, with higher levels of organic matter, total N, available P, and K at all harvesting stages (Thepsilvisut, 2021). Additionally, no distinct negative effects were observed from the application. Mugwort plant growth was significantly enhanced with chicken manure treatments compared to other treatments in the study (Thepsilvisut, 2021).

Another study with similar methods were done on chilli plants where the crops were treated with five different fertilisers, namely vermicompost, chicken dung, peat moss, fermented

fish waste, and cow dung (Khandaker et al., 2017). At the end of the experiment, the highest measurements for plant height, number of leaves, stomatal conductance, chlorophyll content, and number of flower buds were observed in vermicompost-treated plants compared to the other treatments. On the other hand, chilli plants treated with a mixture of vermicompost, and chicken dung showed better fruit production, both in terms of number, size, and quality (Khandaker et al., 2017). This is also supported by other studies such as Khan et al. (2014) and Adejoro et al. (2011) where organic fertilisers showed better plant growth performances. Therefore, due to the high effectiveness of chicken manure as an organic fertiliser, it is essential to assess its suitability in comparison to bat guano in plant growth studies.

2.10 Chilli plants

The chilli plant (*Capsicum annuum* L.) is one of the most widely commercialised and cultivated crops in the world (Hussain and Abid, 2011). It is used in many major industries, such as pharmaceuticals, cosmetics, and food production. The chilli plant, botanically known as *Capsicum annuum* (C. annuum) or *Capsicum frutescens*, belongs to the family Solanaceae, which includes potatoes, tomatoes, and aubergines. These bushy plants can grow to a height of 60 to 80 cm. The plant requires a warm and humid climate for growth, while dry weather is needed for maturity (Hussain and Abid, 2011). The plant originated in Central and South America (Ridzuan et al., 2018).

Approximately 25 varieties of chilli plants are cultivated worldwide, predominantly in tropical regions at altitudes up to 1,800 m, with 1,775 thousand hectares reserved for chilli plantations globally, the yield can amount to over 7,182 thousand tonnes of production. The

world's largest chilli production is centred in India, accounting for 25% of total global production. However, only 4% of the chillies produced in India are exported, either as fresh chillies, chilli powder, stalkless chillies, or green chillies (Hussain and Abid, 2011). While in Nepal, it is cultivated throughout the country in all seasons (Chaudhary et al., 2006). The cultivation of chilli plants in Malaysia is reportedly insufficient to meet local demand. The Department of Agriculture, Malaysia (2018) reported that Malaysia's chilli production in 2014 was 40,520 megatonnes on 3,541.80 hectares of land. Consumption of *Capsicum annuum* in Malaysia was recorded at 1.44 kg per capita (Sahadevan, 1987). Imports of chilli plants from neighbouring countries, such as Thailand, into Malaysia were recorded at 45,900 tonnes in 2005 but decreased to 35,500 tonnes in 2007.

Recently, chilli production in Malaysia has been declining due to high temperatures caused by El Niño, an irregular climatic phenomenon affecting surface temperatures (Usman et al., 2018). Under normal growth conditions, chilli plant yields of 15 to 20 tonnes per hectare are achievable, but, in higher temperatures, 50% yield reduction is likely to happen under stressful conditions (Usman et al., 2018). Salunkhe and Kadam (1998) support this claim, suggesting that soil temperatures above 30°C may inhibit root development.

Almost 40 varieties of *Capsicum annuum* have been cultivated in Malaysia. These varieties are considered either mild or pungent and are used as condiments to enhance the spiciness of food (Juni, 2003). The Malaysian Agricultural Research and Development Institute (MARDI) is responsible for distributing *C. annuum* seeds in Malaysia's agricultural industry. The *C. annuum* Kulai variety was developed by MARDI and is widely cultivated in Malaysia, particularly in Peninsular Malaysia (Usman et al., 2018). Tan et al. (1998) highlighted the

economic importance of cultivating the Kulai variety due to its high market value. Furthermore, in Omar et al. (2008), Kulai seeds were found to have a high germination rate of 90%. The nutritional composition of Malaysian red chillies is recorded in Table 2.5 by Norziah and Ching, 2000. The nutrient profile of red chillies demonstrates that they are a valuable dietary source for human nutrition (Fudholi et al., 2013).

Table 2.5: Nutrient Composition of Malaysian Red Chilli (Norziah and Ching, 2000)

Nutrient	Value
Vitamin C (mg/100g)	175.0
Calcium (mg/100g)	15.0
Iron (mg/100g)	1.8
Ash (mg/100g)	0.9
Lipids (%)	0.7
Protein (%)	2.8
Fibre (%)	4.8

Chilli plants are susceptible to many factors that could reduce production. Physiological and hormonal imbalances, mostly caused by an unsuitable environment, can lead to flower and fruit drop (Rylski, 1973; Erickson and Markhart, 2001; Chaudhary et al., 2006). Plant diseases also pose a major challenge to chilli production. Without proper farming management, chilli plants are vulnerable to various threats, such as pests (e.g., aphids, leafhoppers, crickets), fungal

diseases (e.g., grey mould, Southern blight, Verticillium wilt), bacterial diseases (e.g., bacterial wilt, leaf spot, syringe seedling blight), viruses (e.g., leaf curl, mosaic viruses, Alfalfa mosaic), and nematode diseases (e.g., root-knot, sting nematodes) (Hussain and Abid, 2011).

Studies investigating the use of fertilisers on chilli plants have reported impressive results. Vimala et al. (2007) recorded that the yield per chilli plant in the control group was 0.97 kg/plant, while plants treated with fertilisers produced up to 2.32 kg/plant. Additionally, the number of fruits per plant increased from 1,095 in the control group to 2,805 with fertiliser application. The study mentioned that fertilisers do not affect chilli plant height. In Nepal, commercial chilli cultivators, notably Suryamukhi and Jwala, were studied in two separate growth experiments (Chaudhary et al., 2006; Dahal et al., 2006). In both experiments, Jwala showed higher ascorbic acid content compared to Suryamukhi. Furthermore, the fresh shoot weight of Jwala was significantly higher than that of Suryamukhi. Contrary to this, the fruit yield of Suryamukhi was found to be significantly higher than Jwala (Chaudhary et al., 2006), whereas the opposite was observed in Dahal et al. (2006). This discrepancy may be attributed to the different regions in which the studies were conducted: Chaudhary et al. (2006) carried out their research in Rampur (Chitwan), while Dahal et al. (2006) conducted theirs in Western Terai. Nevertheless, these findings provided valuable insights for Nepal, as *Capsicum annuum* is an important spice crop extensively grown across the country (Chaudhary et al., 2006; Dahal et al., 2006).

Adhikary and Gantayet (2012) found that chilli plants treated with vermicompost exhibited the highest increases over the control group in shoot length, shoot weight, and root length, with percentage increases of 37.05%, 35.75%, and 46.69%, respectively, when

compared to plants treated with cow dung compost and leaf compost. The results from this study are presented in Table 2.6 (Adhikary and Gantayet, 2012). The positive effects of fertiliser application on chilli plants have been well-documented. Hence, the use of bat guano—an organic fertiliser rich in essential nutrients—may enhance chilli yields in Malaysia as well.

Table 2.6: Effect of Leaf Compost, Cow Dung Compost and Vermicompost on 45 Days Old Plants Shoot and Root Length and Shoot and Root Weight of Chilli (*Capsicum annuum*) (Adhikary and Gantayet, 2012)

Control (Garden soil)		Leaf compost	Cow dung compost	Vermicompost	% of or decrease control			
					Increase Control (Garden soil)	over Leaf compost	Cow dung compost	Vermi- compost
Shoot Length (cm)	38.2±0.48	46.26±0.29	48.25±0.16	52.11±0.81	—	21.67	26.90	37.05
Root Length (cm)	16.14±0.16	21.61±0.42	22.82±0.26	24.16±0.36	—	33.89	41.38	49.69
Shoot Weight (mg)	3.86±0.11	4.62±0.28	4.88±0.26	5.24±0.18	—	19.68	26.42	35.75
Root Weight (mg)	0.82±0.02	1.08±0.05	1.16±0.19	1.24±0.26	—	31.70	41.46	51.21

CHAPTER 3

IDENTIFICATION OF BAT SPECIES USING ACOUSTIC RECORDINGS

3.1 Introduction

Bat identification methods have evolved significantly over the years. Prior to 1989, the key identification of European bat species relies on morphological characteristics (Dietz and Von Helversen, 2004). These characteristics have been foundational in understanding the phylogenetic relationships among bats for over 250 years (Friedheim, 2016). The usage of morphometric methods, such as relying on forearm and hindfoot measurements, along with fur colouration has further enhanced species identification, description and comparison (Rohlf, 1990; Hebert and Gregory, 2005; Stadelmann et al., 2007). In the current century, advancements in molecular studies, particularly DNA barcoding, have greatly expanded bat diversity records. DNA barcoding has allowed for the identification of more bat species, surpassing previous morphological methods (Mayer et al., 2007). The combination of morphological observations and molecular analyses has significantly improved species knowledge and differential of cryptic speciation, particularly in tropical regions (Ruedi et al., 2015).

Cryptic species, which are morphologically similar but genetically distinct, highlight the importance of molecular studies in species identification. Molecular techniques have also facilitated revisions of existing scientific names and provided insights into cladogenesis and speciation in bat taxa (Trontelj and Fiser, 2009; Detwiler et al., 2010; Struck et al., 2018; Wei et al., 2022). Genetic data have been particularly useful in delineating species within diverse groups

like the genus *Myotis*, where molecular studies have revealed multiple cryptic species within the genus, challenging traditional morphological subdivisions (Ruedi et al., 2015).

Non-invasive identification methods, such as DNA identification through bat guano collection and acoustic monitoring, have become essential tools in bat research and conservation, especially in light of threats like white-nose syndrome (United States Fish and Wildlife Service, 2012; Brown et al., 2017). The collection of bat guano has been widely used in various studies, particularly in identifying bat diets and genetic analyses (Kanuch and Cel'uch, 2007; Puechmaille et al., 2007; Judy et al., 2010; Guan et al., 2020). DNA extraction from guano samples has facilitated species identification and population genetic studies (Vege and McCracken, 2001; Puechmaille and Petit, 2007; Korstian et al., 2013; Viglino et al., 2016; Swift et al., 2018). Databases like the Species from Faeces website have improved the reliability of bat species identification using guano (Walker et al., 2016). However, the challenges associated with DNA degradation and sample collection had been worrisome in exploring the identification of bat species solely through molecular extraction on guano. Hence, the utilization of echolocation calls recordings, or acoustic identification is the best method for support.

Echolocation calls are species-specific and provide valuable information on bat behaviour and ecology (Barclay, 1999; Russo and Voigt, 2016). Advancements in acoustic technologies have enabled the identification of bats based on their unique echolocation calls. Acoustic surveys have proven effective in identifying bat species and assessing habitat requirements (Vaughan et al., 1997; Russo and Jones, 2002; Papadatou et al., 2008; McArthur, 2019). Borneo rainforest is known for its high level of species diversity and endemism. Struebig et al. (2012) mentioned that species diversity and richness of bats in Borneo are said to represent one-third of the terrestrial mammals. The species of bats that are dominating the island of Borneo typically belonged to the insectivorous

bats, especially from the families of Hipposideridae, Rhinolophidae and Vespertilionidae (Struebig et al., 2012). The objective of the study which focuses on the identification of the bat species present in the cave bats' guano collection sites through acoustic recordings is vital in providing diversity and dietary data of the bats in the analysis.

3.2 Materials and Methods

3.2.1 Acoustic recording collections

To identify bat species, present in the cave, non-invasive methods were done by capturing acoustic recordings of bats. The recordings or echolocation call samples were captured by using an Echo Meter Touch 2 Pro bat detector. The Echo Meter Touch 2 Pro bat detector can capture frequency recordings from 10kHz to 256kHz (Wildlife Acoustics, 2023). The key feature of the detector where it is able to capture quiet and distant echolocation through its low-noise microphone (Wildlife Acoustic, 2024). The acoustic device was connected to a tablet and brought along during the bat guano sample collection and was directed near the plastic tarps placements for an active sampling. Active sampling is the recording of bats echolocation with a handheld detector at a series of points for a fixed period (McArthur, 2019). Two to five minutes recordings in Waveform Audio File Format (WAV) of echolocating bats were captured at each of the tarp placement sites or nearby bats' roosting sites.

3.2.2 Study Sites

Acoustic recordings were taken from two caves around Kuching, Sarawak areas. The first site, Wind Caves Nature Reserve (1°24.915' N and 110°08.109' E), is in Bau. The reserve consists of 6.16 hectares of caves and forest covers and is protected under the Sarawak Forestry Corporation

(Sarawak Forest Department, 1992). The area is included as part of Bau Limestone Area (BLA) which is characterised as a 150 km² narrow belt of limestone covering Southwest Sarawak from the Kalimantan border which is 23 km from Bau area (Mohd Ridwan et al., 2011).

The second site was Raya Cave in Siburan (1° 14' 18.096" N and 110° 25' 46.7184" E). This cave entails a hidden gem of undiscovered diversity in Siburan. The cave is situated between the villages of Chupak and Skuduk off Mile 23, Kuching-Serian Road. Developments of the cave areas were completed in 2013 for the establishment of Skuduk-Chupak Recreational Park (Aubrey, 2013). The sites are as shown in Figure 3.1 with the triangular shape marked for Wind Cave Nature Reserve and the round mark is indicated for Raya Cave.

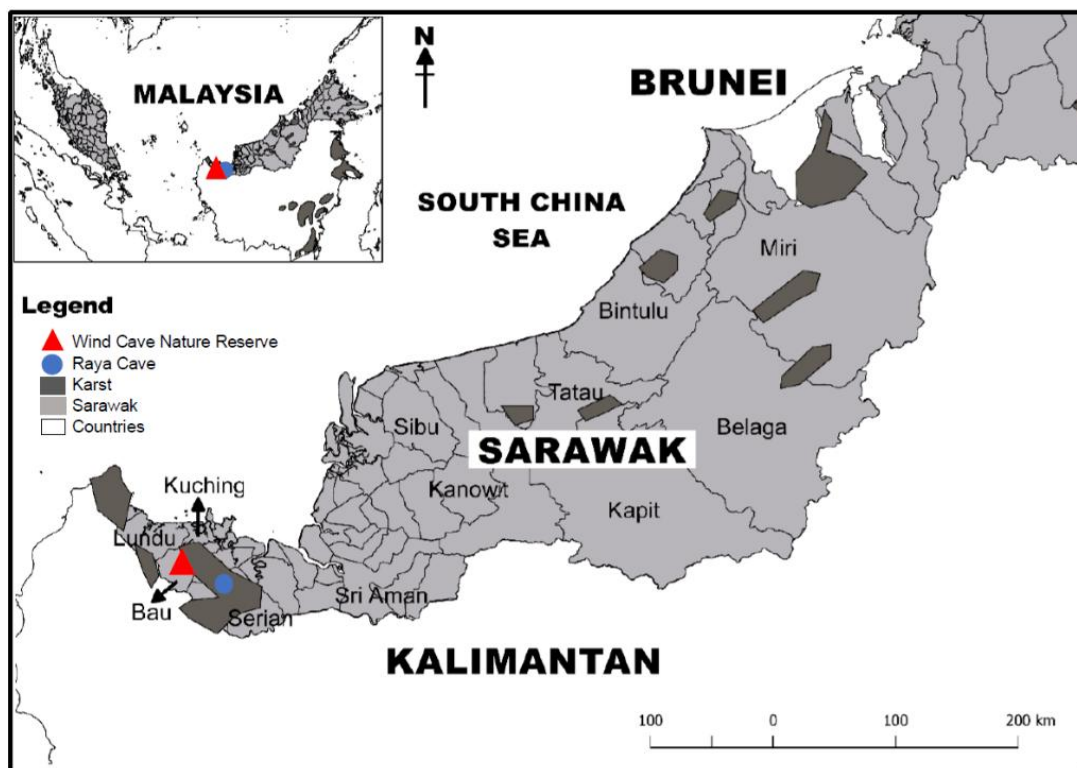


Figure 3.1: Map of Sarawak with the study sites marked according to the legends

3.2.3 Analysis of Acoustic Recordings

The echolocation calls from the wildlife bat detector were analysed using Kaleidoscope software Version 8 on its ultrasonic and acoustic recording files. A sonogram and oscillogram were produced based on the recording of echolocation calls loaded through clicking the ‘Analyse’ button in the software. They were analysed to obtain their parameters (Table 3.1), the common metrics that was used in quantifying the call parameters were also indicated in Figure 2.2 (Chapter 2, subsection 2.4). The call structure of the frequencies was observed to give a primary classification of the bat group.

Table 3.1: List of parameters measured from the call recordings

Key Parameters	Properties	Description
Frequency	Peak frequency (F _{peak})	Frequency with the highest intensity
	Start frequency (F _{start})	Frequency value at the start of the call
	End frequency (F _{end})	Frequency value at the end of the pulse
Duration	Pulse duration (PD)	Duration of a single pulse
	Interpulse interval (IPI)	Time from the start of one pulse to the next pulse

Note: All the frequencies were recorded in kilohertz (kHz) and durations or time were noted in seconds (s).

3.2.4 Comparison with Databases

The structure of the call was observed and was classified into three types as displayed in Figure 2.2 (Chapter 2, subsection 2.4); frequency-modulated calls (FM calls), quasi-constant frequency calls (QCF calls) and constant frequency calls (CF calls); which were used for manual classification. A comparison of the information with a website database of Southeast Asia's bat recordings, ChiroVox were conducted along with references with parameters and call structures from previous research in Sarawak (Gorfol et al., 2022; Jinggong, 2022). Most species were possible to be identified into the species level, but some unidentified species had to be grouped into sonotypes.

3.3 Results

The echolocation survey of free-flying bats from the two sites attained 27 recording files, where 14 recording files were obtained from Wind Cave Nature Reserve and the rest were from Raya Cave. Upon further analysis through the Kaleidoscope software, it was shown that 3 of the overall recordings were noises which indicated that there was no bat echolocation captured in the recordings. Stathopoulos et al. (2018) mentioned that echolocating bats emit call frequencies between 9kHz to 212 kHz. Hence, other calls outside of the range were considered to be noises and not detectable through the software.

3.3.1 Sonogram and Parameters of Bat's Recordings

The spectrograms of the call recordings were analysed to distinguish the sonograms into their different call structure and frequencies. From the 27 recordings, a number of 50 call structures and frequencies were extracted, where at least 2 different variations were found in one recording.

The parameters; Fstart, Fend, Fmax, Fmin, Fppeak, D and PI; of the distinctions were analysed using the ‘analyse selection’ button in Kaleidoscope software. Descriptive analysis for mean, standard deviation and range of the five parameters were calculated through SPSS. The data was tabulated in Table 3.2.

3.3.2 Identification of Bats’ Families and Species

Further groupings were applied to the sonograms to identify the bat species. Past studies noted that the call structure of bats' echolocation can be utilised to identify the bats’ family, while the call properties can be used to identify its species. Species identification of the bats’ calls was done through a comparison of their call frequency recorded in Table 3.2 with previous literature mainly McArthur (2019) and Jinggong (2022) as the data was recorded around Sarawak areas with similar recording and analysis methods. Other than that, further clarification was made through database checking at the ChiroVox website. Groupings of the bats’ family are as follows:

3.3.2.1 Family Hipposideridae: CF-FM Calls

Analysis of the acoustic sampling obtained 18 recordings of the bats in the family Hipposideridae (Old World leaf-nosed bats). The recordings were classified according to their call structure of CF-FM calls. Bats in this family often emit short constant frequency signals at 10ms, then terminate with a frequency-modulated band which enhances the location sweep of the target (Jones and Teeling, 2006) (Figure 3.2).

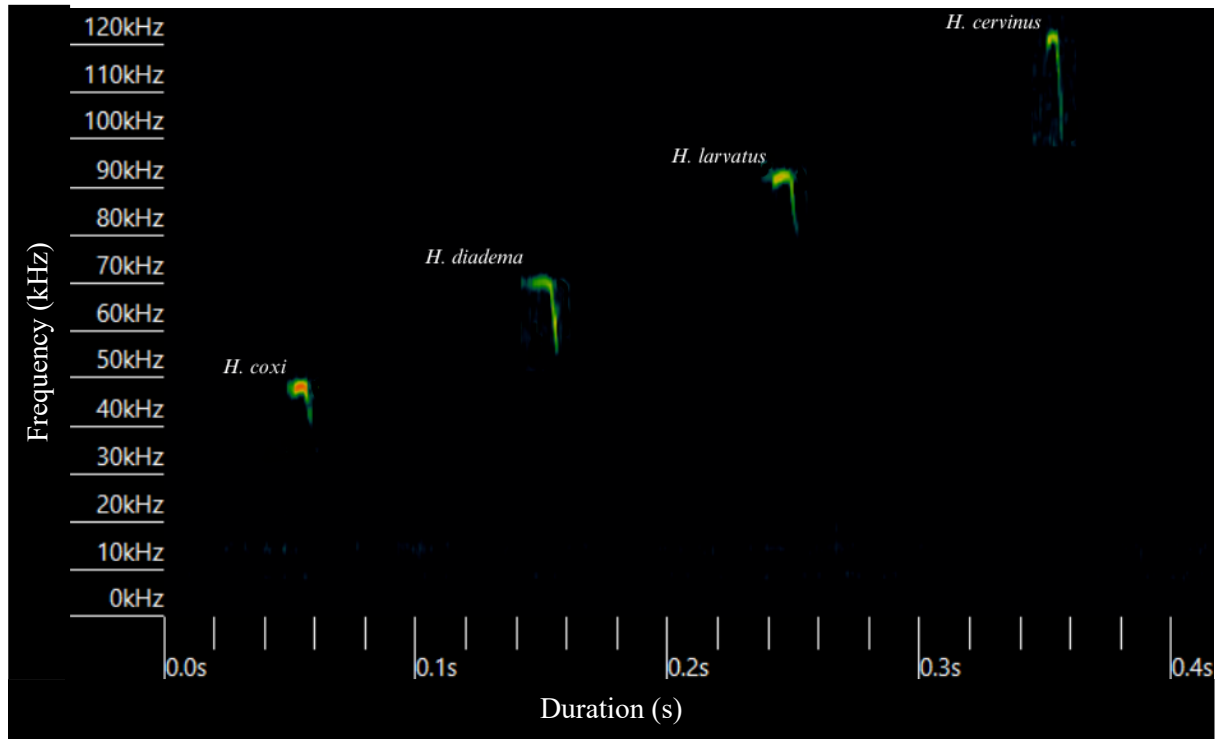


Figure 3.2: Spectrogram of the family Hipposideridae. All the frequencies were recorded in kilohertz (kHz) and durations or time were noted in seconds (s)

From the analysis and comparisons with previous work, four bat species from the family Hipposideridae were identified which are *Hipposiderus cervinus*, *H. coxi*, *H. diadema*, *H. larvatus*. The Fppeak recorded of free-flying *H. cervinus* is at a range of 104.97 kHz to 120.36 kHz according to the data by McArthur (2019), which fits the Fppeak recorded in this study which are $113.72 \text{ kHz} \pm 9.99 \text{ kHz}$. For *H. coxi*, the Fppeak recorded at $47.19 \text{ kHz} \pm 0.33 \text{ kHz}$ fits the range of McArthur (2019) study at 44.65 kHz to 51.45 kHz while *H. diadema* recorded at $69.00 \text{ kHz} \pm 1.04 \text{ kHz}$ in this study fits the range of 65.12 kHz to 69.41 kHz of the stationary *H. diadema* recorded in the McArthur (2019) study. For *H. larvatus* echolocation calls, the identification of calls in the range of $93.35 \text{ kHz} \pm 3.00 \text{ kHz}$ was classified in the species group according to Thabab et al. (2006) where the Fppeak of *H. larvatus* recorded in the study was around 85 kHz or 98 kHz.

3.3.2.2 Family Rhinolophidae: FM-CF-FM Calls

Through analysis of the spectrogram, a number of 25 recordings were captured for the Rhinolophidae family. It was identified through the call structure shape which showed an FM-CF-FM call. Horseshoe bats are known to hunt in low-light conditions or in complete darkness (Jones and Teeling, 2006). They emit their calls using a derived form of echolocation termed ‘constant-frequency echolocation’ with additional narrow frequency modulated band components (Schnitzler and Kalko, 2001). In this call type, the highest frequency is focused consistently on a central frequency throughout the entire call which is around 50 ms for the Rhinolophidae family (Altringham, 2011). CF signals from Horseshoe bats are considered a high-performing signal in locating prey due to their lengths. They often utilise the long CF signals in searching for moving targets in cluttered habitats. Additionally, the long duration of the signal also can distinguish targets from small insects to larger ones (Jones and Teeling, 2006) (Figure 3.3).

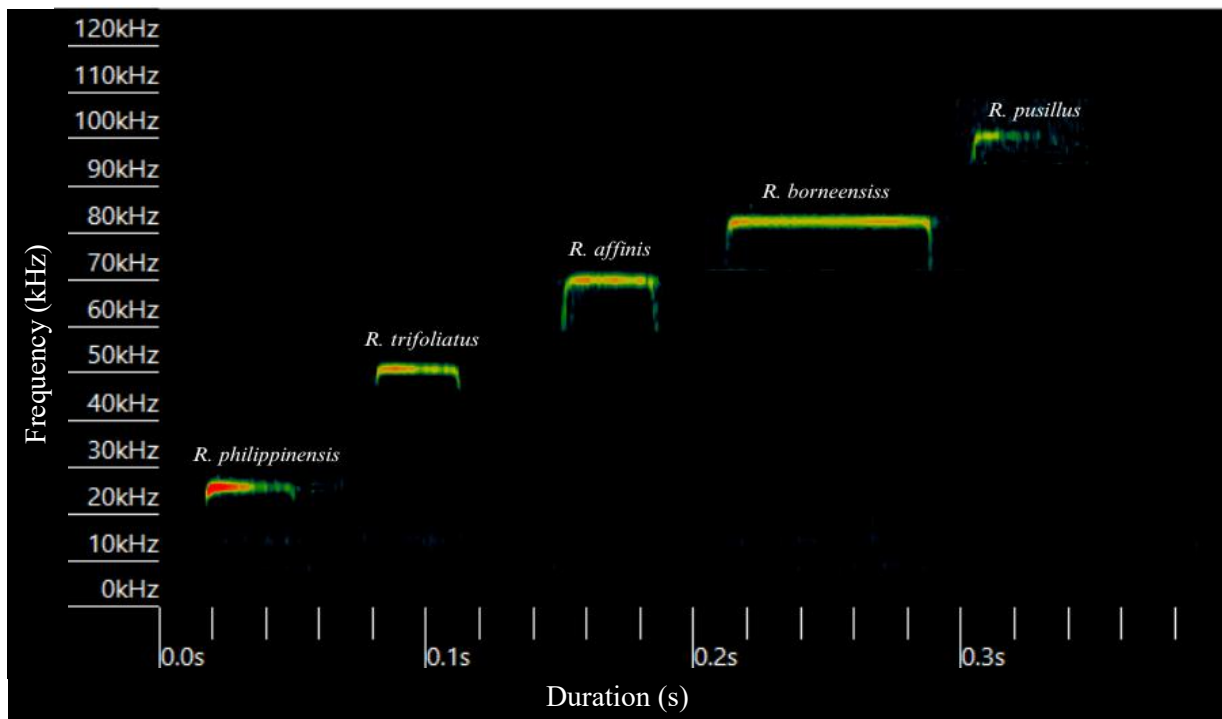


Figure 3.3: Spectrogram of the family Rhinolophidae. All the frequencies were recorded in kilohertz (kHz) and durations or time were noted in seconds (s)

Species identified from this family are *Rhinolophus affinis*, *R. borneensis*, *R. phillippinensis*, *R. pusillus* and *R. trifolius*. Identification of several bat species was referred from McArthur (2019) of the Fppeak range noted in the call measurements tables. The Fppeak of *R. borneensis* noted in the study was found to be at a range of 76.55 kHz to 83.57 kHz for free flight and 78.45 kHz to 83.83 kHz for stationary bats, which was fit to the description of the three recorded calls of *R. borneensis* at a Fppeak at $81.82 \text{ kHz} \pm 0.65 \text{ kHz}$. For *R. phillippinensis*, recorded calls from McArthur (2019) show a range of Fppeak at 32.75 kHz to 34.75 kHz of stationary bats, and 31.67 kHz to 34.09 kHz of free-flying bats, while the single recording of *R. phillippinensis* capture in the study had an Fppeak of 34.73 kHz which might suggest the stationary situation of the individual recorded.

Identification of *Rhinolophus trifolius* were referred to Jingong (2022) where the range of the Fppeak for the species is at 49.33 kHz to 50.03 kHz which was near to the description of *R. trifolius* in the study at $50.98 \text{ kHz} \pm 0.73 \text{ kHz}$. For *R. affinis* on the other hand, multiple studies were reviewed in order to pinpoint the exact range for the acoustic call for Borneo variation of *R. affinis*, Ith et al. (2015) placed the range of *R. affinis* in mainland Southeast Asia at 69 kHz to 84 kHz, with a specification of 77 kHz to 78 kHz for the Thai- Malay border down to peninsular Malaysia. Another study by Ith et al. (2016) recorded a single individual *R. affinis* with the echolocation frequency at 68.9 kHz. With this data, the recordings with the echolocation frequency of $69.23 \text{ kHz} \pm 0.54 \text{ kHz}$ were placed in the *R. affinis* species.

The frequency range of *Rhinolophus pusillus* in this study at $98.69 \text{ kHz} \pm 3.77 \text{ kHz}$ was based on multiple reviews on previous studies where the echolocation range of *R. pusillus* in China recorded in the studies of Jiang et al. (2010) is at a range of 104 kHz to 114 kHz. While in Hughes et al. (2010), the male *R. pusillus* emitted a frequency of 114.8 kHz with 8.78 kHz of standard

deviation while the female *R. pusillus* had a frequency of 113.2 kHz with a standard deviation of 8.57 kHz. Since there was lack of study in the echolocation frequency of *R. pusillus* particularly in Borneo. Therefore, the range of echolocation recordings which falls near to the frequencies in previous studies were noted as *R. pusillus*.

3.3.2.3 Family Vespertillionidae: FM Broadband Calls

Through analysis of the spectrogram, a number of four recordings were captured with short frequency modulated broadband calls that were emitted by bats in the family of Vespertillionidae. The broadband calls detected from this family were specifically utilised for the localization of prey targets. The call types are adapted to localise targets in three-dimensional setting which is vital to refine acoustic performances when flying in clutter. Another possible call structure from this family is a narrowband signal. Many vesper bat species switch to a narrowband signal for detection typically when flying in open spaces. The narrowband signal distinction is its long duration which is affected by the distance of target objects (Schnitzler et al., 2003). The F_{ppeak} range of the recordings were calculated to be $57.50 \text{ kHz} \pm 0.92 \text{ kHz}$. Upon further research and comparisons with previous studies, the recordings were identified to originate from *Myotis muricola*. The F_{ppeak} range of *M. muricola* recorded by McArthur (2019) during an open-space release was at 52.58 kHz to 58.98 kHz which fits the data of this study (Figure 3.4).

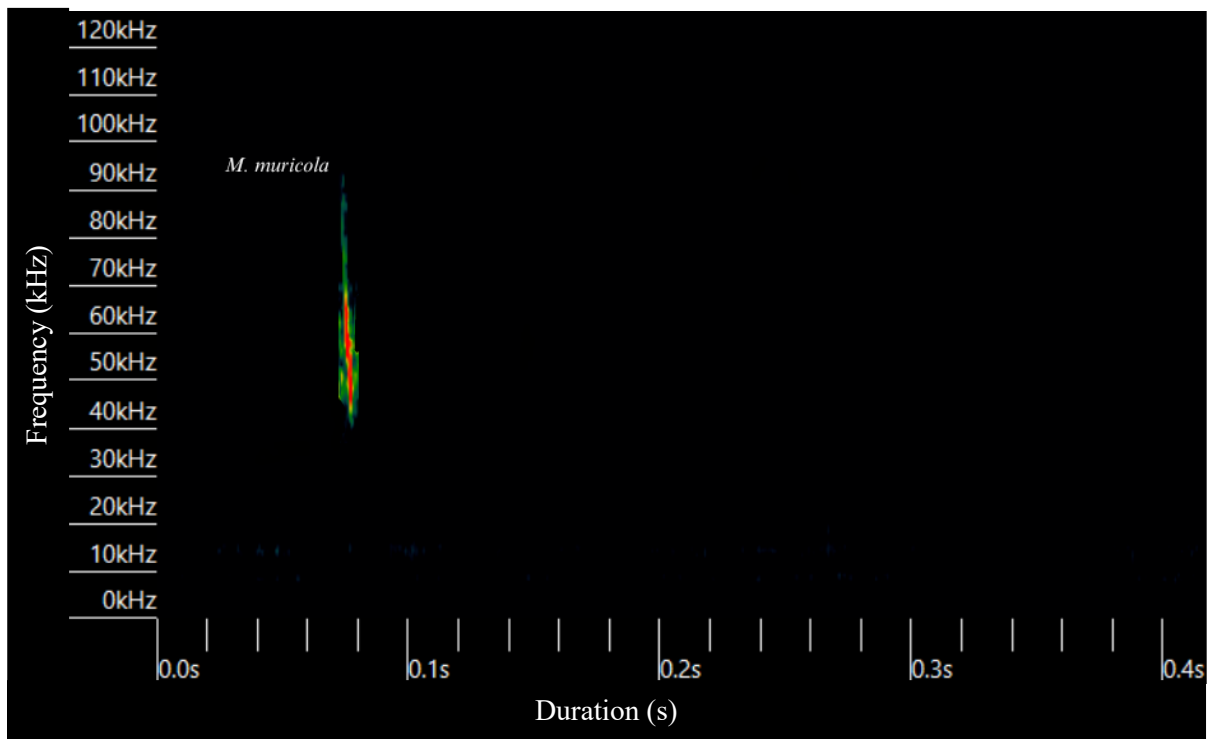


Figure 3.4: Spectrogram of *Myotis muricola*. The frequencies were recorded in kilohertz (kHz) and durations were noted in seconds (s)

3.3.2.4 Family Emballonuridae: QCF Multi Harmonic Calls

The QCF multi-harmonic call was detected in one recording. The spectrogram was analysed and found to be produced by bats from the family Emballonuridae. The call structure is partly constant where its energy is mostly focused on a narrow bandwidth shape (Jones, 1999). This family utilises the call for the detection and classification of targets against background vegetation when foraging in forest edges or gaps. Bats with a shorter QCF pulse and broader FM bandwidth were noted to be more successful in hunting prey closer to vegetation (Schnitzler et al., 2003) (Figure 3.4).

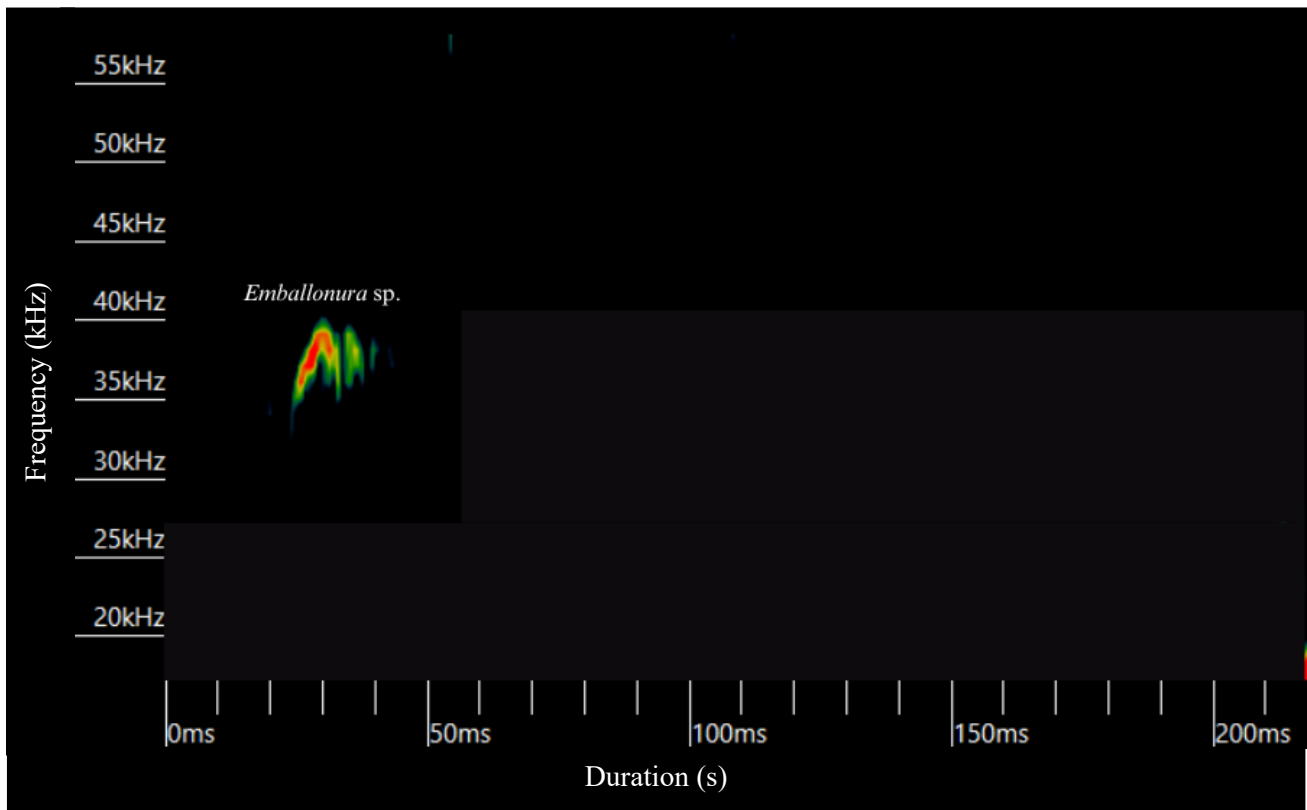


Figure 3.5: Spectrogram of *Emballonura* sp. The frequencies were recorded in kilohertz (kHz) and durations were noted in seconds (s)

Due to a lack of previous data for comparisons, the call was labelled as *Emballonura* sp. Based on the F_{peak} frequency recorded which was 37.88kHz, there is a possibility of the call to be by *E. allecto* or *E. monticola* as the range of F_{peak} of the species was noted to be from 45.05 kHz to 46.57 kHz by McArthur (2019). According to Jingong (2022), the free-flying *Emballonura* sp. recorded in the study had a F_{peak} range of 47.34 kHz to 48.53 kHz. Both of these studies had a higher F_{peak} range than recorded which might indicate other factors of the bat's environment or state during the recording time in this study.

Table 3.2: Measurement of parameters from bats' echolocation calls retrieved from Wind Cave Nature Reserve and Raya Cave

Species Name	N		Fppeak	Fend	Fstart	Fmax	Fmin	PI	D
	WC NR	RC							
Family: Hipposideridae									
<i>Hipposideros cervinus</i>	2	0	113.72 ± 9.99 106.66 – 120.79	125.31 ± 3.02 123.18 – 127.45	101.27 ± 5.19 97.60 – 104.94	69.57 ± 0 70 – 70	68.38 ± 0 68 – 68	19.98 ± 1.13 19.18 – 20.78	8.08
<i>Hipposideros coxi</i>	1	1	47.19 ± 0.33 46.96 – 47.43	50.15 ± 0.35 49.90 – 50.39	40.12 ± 1.38 39.14 – 41.10	47.91 ± 0 48 – 48	43.70 ± 4.37 41 – 47	9.57 ± 0.78 9.02 – 10.13	4.37
<i>Hipposideros diadema</i>	2	1	69.00 ± 1.04 67.81 – 69.64	80.89 ± 15.75 71.43 – 99.07	63.68 ± 17.13 52.59 – 83.42	79.12 ± 16 69 – 98	74.57 ± 17.45 61 – 94	39.53 ± 14.8 22.62 – 50.09	5.77
<i>Hipposideros larvatus</i>	7	4	93.35 ± 3.00 85.87 – 95.98	97.67 ± 3.02 92.96 – 104.21	80.95 ± 2.70 76.07 – 85.13	90.33 ± 10.53 70 – 96	84.30 ± 12.50 56 – 94	34.06 ± 14.56 20.783 – 73.54	5.39
Family: Rhinolophidae									
<i>Rhinolophus affinis</i>	6	8	69.23 ± 0.54 67.72 – 70.00	74.32 ± 1.77 71.67 – 78.28	50.68 ± 2.93 46.18 – 58.46	68.83 ± 5.85 52 – 74	62.76 ± 7.37 45 – 68	60.14 ± 23.85 26.75 – 101.78	15.33

Table 3.2: continue

<i>Rhinolophus borneensis</i>	2	1	81.82 ± 0.65 81.08 – 82.28	85.45 ± 1.43 84.39 – 87.08	73.06 ± 1.02 71.92 – 73.88	83.07 ± 0.51 82 – 83	75.29 ± 5.53 69 – 79	81.23 ± 18.16 61.98 – 98.05	30.36
<i>Rhinolophus phillippinensis</i>	1	0	34.73	38.41	28.38	35.24	34.91	77.27	12.44
<i>Rhinolophus pusillus</i>	0	2	98.69 ± 3.77 96.03 – 101.35	100.91 ± 3.98 98.09 – 103.72	87.94 ± 4.32 84.88 – 91.00	96.40 ± 0 96 – 96	94.13 ± 0 94 – 94	20.66 ± 22.63 4.66 – 36.67	3.63
<i>Rhinolophus triofoliatus</i>	0	5	50.98 ± 0.73 49.90 – 51.67	53.87 ± 0.44 53.57 – 54.55	41.44 ± 3.24 38.41 – 45.50	51.88 ± 0.87 51 – 53	48.27 ± 2.26 46 – 50	52.01 ± 6.64 45.30 – 60.23	8.70
Family: Vespertilionidae									
<i>Myotis muricola</i>	3	1	57.50 ± 0.92 56.94 – 58.87	90.94 ± 5.90 86.35 – 99.32	36.88 ± 2.17 34.247 – 38.89	78.72 ± 14.94 66 – 95	44.31 ± 0.80 44 – 45	53.69 ± 4.65 50.09 – 60.22	2.92
Family: Emballoniridae									
<i>Emballonura</i> sp.	1	0	37.88	40.85	31.56	39.43	36.04	51.69	6.03

Note: The parameters listed are Peak Frequency (F_{peak}), Start frequency (F_{start}), Maximum frequency (F_{max}), Minimum frequency (F_{min}), End frequency (F_{end}), Pulse Interval (PI) and pulse Duration (D). The number of recordings analysed for each species was noted as N. The readings were recorded in kilohertz (kHz), while durations or time were noted in seconds (s). Wind Cave Nature Reserve (WCNR) and Raya Cave (RC). The records are mean readings and standard deviation.

3.4 Discussion

The diversity of bats at the guano collection sites could be established by obtaining acoustic recordings of bats there. The bat identifications noted show the species present in the study and the colonies that contributed to the guano piles in the caves. Acoustic recordings captured at the two sites identified several species of bats. In Wind Cave Nature Reserve, the data was cross-checked with previous diversity survey in that area by Mohd-Ridwan et al. (2011). A new record of *R. phillippinensis* was found through this study, while the other species recorded were present in the previous surveys. For the Raya Cave site, the highest number of recordings was obtained from *R. affinis*, with eight recordings, followed by *R. tridactylus*. Five species were recorded only once, namely *R. luctus*, *R. borneensis*, *M. muricola*, *H. diadema*, and *H. coxi*. However, there was no previous study that can support the findings in this area, as there is an absence of Chiropteran diversity surveys at this cave site. Further diversity studies should be prioritised in Raya Cave to ensure the protection of the bat species there.

The highest number of recordings belonged to the families Hipposideridae and Rhinolophidae, which is consistent with previous studies conducted in Southeast Asia, where cave and forest-dwelling bat species are largely represented by constant frequency (CF) and frequency-modulated (FM-CF-FM) call structures (Jones and Teeling, 2006). The presence of *Hipposideros cervinus*, *H. diadema*, *H. larvatus*, and *H. coxi* aligns with the findings of McArthur (2019), conducted at Mulu National Park, indicating the stable distribution of these bats in Borneo's cave ecosystems. Multiple *Rhinolophus* species such as *R. borneensis*, *R. affinis*, and *R. pusillus* were detected, with their peak frequencies fitting established species-

specific ranges from previous research, supporting the accuracy of species identification through acoustic analysis. The LiDAR survey by Shazali (2017) mapped the roost selection of bats in Wind Cave Nature Reserve (Figure 2.1; Chapter 2, subsection 2.3), initially suggested a high number of *Rhinolophus affinis* would be captured in the study, as the echolocation recording sites were located near the species' roosting areas. However, other species present in the recordings might indicate overlapping flight paths or a mixing of roosting areas for bats inside Wind Cave Nature Reserve.

The FM broadband calls of *Myotis muricola* align with its foraging behaviour in semi-open and cluttered environments (Kingston and Jones, 2009), while the QCF call of *Emballonura* sp. suggests forest-edge activity (Schnitzler et al., 2003). The inconsistency between the peak frequency of *Emballonura* sp. recorded in this study and previous literature could be influenced by environmental factors, behavioural states, or even reflect undocumented regional variation. Similarly, the overlap and slight deviations from literature values highlight the need for region-specific call libraries, particularly in biodiverse areas such as Borneo.

However, the usage of echolocation data to infer bat species affecting the guano is not without limitations. The overlapping foraging activity of bats around the cave during recording sessions may have resulted in the detection of species not directly associated with the guano piles but present within the recording range. Additionally, cave bat detections do not include most frugivorous bats, as they do not exhibit echolocation. The identification of bats is also reliant on comparing frequency ranges with previous research. Species that are rare and lack prior data cannot be identified solely through acoustic recordings. Hence, to overcome these limitations, pairing acoustic recordings with capture–release methods from the field or

referencing previous data on the study sites is essential, particularly for species diversity detection.

For Wind Cave Nature Reserve, where multiple studies have been conducted, secondary data is valuable in evaluating bat species contributing to the collected guano piles. Mohd-Ridwan et al. (2011) and Shazali (2017) were referred to in order to gain some insights on the bat species present. As the acoustic recordings were taken deep within the cave, other bat species that may affect the guano piles collected, according to Shazali (2017), were *Penthetor lucasi*, *Myotis horsfieldii*, *Megaderma spasma* and *Hipposideros ater*. These bat species were not detected in the acoustic recordings, which might be due to several factors, such as their echolocation calls being outside the recording range, or the recordings being overlapped with noise or calls from other bats. The data from Raya Cave, on the other hand, could serve as a foundation for future Chiropteran research in the area. Nevertheless, future research should include a capture-release method for diversity studies in the area to investigate the Chiropteran species present.

From the species list indicated, the guano piles collected were predominantly from insectivorous bats. This would affect the chemical composition of the guano analysis, as it is influenced by the bats' diet (Ferreira, 2019). The acoustic data serves as supporting information on the bat species present in the collected guano. Further chemical analysis could also reveal the relationship between the detected species and the guano's chemical characteristics.

3.5 Conclusion

Through the acoustic recordings, we can determine that the bat guano collected from the cave sites was contributed largely by insectivorous bats from the families Hipposideridae and Rhinolophidae, along with a small number of vesper bats and Emballonura bats. The sonograms observed across the five parameters reflected a higher number of rhinolophids, with *Rhinolophus affinis* recorded as the most frequently detected species overall. *Hipposideros larvatus* accounted for the highest number of recordings at Wind Cave Nature Reserve, while *Emballonura* sp. had the lowest number of recordings overall. The presence of *Myotis* species was also indicated through sonogram analysis at both sites—Wind Cave Nature Reserve and Raya Cave.

The diversity of bat species contributing to guano accumulation at the study sites was effectively investigated through acoustic recordings, revealing valuable insights into the Chiropteran community structure. Wind Cave Nature Reserve showed consistency with previous studies, while the detection of *R. phillippinensis* marked a notable new record. In contrast, the data from Raya Cave provided the first acoustic documentation of bat species in that area, highlighting the need for further diversity assessments. The abundance of species from the families Hipposideridae and Rhinolophidae, along with regionally specific call characteristics, aligns with established echolocation patterns in Southeast Asian cave-dwelling bats. However, limitations such as overlapping call ranges, the absence of frugivorous bat detections, and incomplete species identification due to regional variation highlight the importance of pairing acoustic data with capture-and-release methods. While secondary data

proved useful in confirming bat species at Wind Cave, the lack of historical studies at Raya Cave presents an opportunity for future research.

The study of bat echolocation is important for the non-invasive identification of bats and the protection of their habitats through strategic planning. In the present study, the data served as additional information relating to the chemical composition of bat guano, which is affected by dietary components. With the information at hand from the identification of bat species present at the guano collection sites through acoustic recordings, we could determine the causes of variations in the chemical composition of bat guano collected.

CHAPTER 4

CHEMICAL COMPOSITIONS OF CAVE BATS' GUANO, CHICKEN DUNG, AND CHEMICAL FERTILISER

4.1 Introduction

Fertilisers are substances added to or plants to supply essential nutrients that can promote plant growth. The nutrients typically include nitrogen (N), phosphorus (P) and potassium (K). Fertilisers are the most vital component in obtaining soil fertility, development and optimum moisture (Linn and Myint, 2018). The advantages of fertilisers usage is the replenishment of soil nutrients which may have been depleted from intensive farming (Kang et al., 2005). Fertilisers can also be customised to suit different soil, crops and environmental conditions. However, fertilisers particularly chemical fertilisers can also be the caused of nitrate leaching, which results in nitrate contamination and eutrophication in water bodies (Michalski et al., 2015; Ekinci et al., 2019). Excessive application of fertilisers can reduce soil structure and can also affect the occurrence of soil worms and maggots (Kang et al., 2005; Ekinci et al., 2019). In order to lessen the effects of fertilisation on the environment, the usage of organic-based fertiliser is vital to be utilised (Kasso and Balakrishnan, 2013). Other than that, proper crop management is important with an optimum level of fertiliser application is to be studied (Ekinci et al., 2019; Zandvakili et al., 2019).

Various studies have highlighted the potential of bat guano as an effective organic fertiliser due to its rich chemical composition (Studier et al., 1994; Sridhar et al., 2006; Emerson and Roark, 2007; Suwarno and Idris, 2007; Buliga, 2010; Shetty and Sreepada, 2013; Shetty et

al., 2013; Jayasvasti and Jayasvasti, 2018; Unal et al., 2018; Oo et al., 2019; Misra et al., 2019; and Ferreira, 2019). They have reported high levels of organic matter, total nitrogen (N), phosphate (P), potassium (K), and carbon (C) in bat guano. These studies also mention the presence of other nutrients such as calcium (Ca), magnesium (Mg), aluminium (Al), iron, and sulfur (S) that help regulate soil pH (Shetty et al., 2013; Oo et al., 2019). Additionally, guano also contains beneficial microbes that aid in nutrient absorption, detoxification of soil, and control of fungi and nematodes (Shetty et al., 2013; Oo et al., 2019). Isolation of microbes in bat guano shows that the largest number of bacterial compounds found is from the *Lysinibacillus* and *Paenibacillus* genera (Dimkic et al., 2020). Pseudomonas species were the most abundant species of microbes sampled in deep cave areas. Ascomycetes, the guanophilic fungi, found majorly in the samples tested, where *Penicillium* and *Aspergillus* is the most common. Other microbe species isolated only found *Meyerozyma guilliermondii* to be the only yeast species found (Dimkic et al., 2020).

However, the composition of guano can also vary depending on various factors such as bat diet, species, age, and locality (Studier et al., 1994; Emerson and Roark, 2007; Vandzurova et al., 2012; Shetty et al., 2013; Jayasvasti and Jayasvasti, 2018). Studies show differences in nutrient levels among different bat species, with insectivorous bats having higher sodium (Na) and K levels, and frugivorous bats having higher phosphorus (P) content (Misra et al., 2019). Additionally, the composition of guano can also change with age (Sakoui et al., 2020). Comparisons of guano composition among different diets have been conducted, with studies showing higher N levels in guano from insectivorous bats compared to P (Sridhar et al., 2006). While higher C levels in guano from sanguivorous bats were found when compared to

frugivorous bats (Emerson and Roark, 2007). However, results may vary among studies, with Shahack-Gross et al. (2004) reporting higher P levels in insectivorous bats compared to frugivorous bats.

On the other hand, commercial chemical fertilisers play a significant role in modern agriculture, contributing to crop productivity and food security (Roberts, 2009). However, concerns about their negative effects on the environment, such as water and air pollution (Michalski et al., 2015; Ekinci et al., 2019). Excessive use of chemical fertilisers can lead to soil degradation, water contamination, and health risks (Guler, 2006; Nosheen and Bano, 2014). Recent studies have characterised the chemical composition of commercial fertilisers, highlighting their high concentrations of P, K, and N (Romero et al., 2013). Meanwhile, chicken manure has been recognised as a valuable organic fertiliser due to its high nutrient content, particularly N, P, and K (Masarirambi et al., 2012; Singh et al., 2018). Chicken manure application has been shown to improve soil fertility and crop yield (Masarirambi et al., 2012). However, the composition of chicken manure can vary depending on various factors such as chicken age, diet, and storage conditions (McCall, 1914; Ritz and Merka, 2003).

In summary, bat guano, commercial chemical fertilisers, and chicken manure each have unique chemical compositions and applications in crop agriculture, with guano and manure offering organic alternatives to chemical fertilisers. These organic sources of nutrients can play a vital role in sustainable agricultural practices and environmental conservation. Therefore, the study was conducted to analyse the chemical compositions of cave bats' guano, Bats Guano Wind Cave Nature Reserve (BGWCR) and Bats Guano Raya Cave (BGRC), in comparison to

other commercial fertilisers such as Commercialised Fertiliser (CF) and Chicken Dung Fertiliser (CDF) to establish the chemical composition profile of each material.

4.2 Materials and Methods

4.2.1 Sample Collections

Following the regulations of Wildlife Protection Ordinance 1998, all procurement of animal or plant products from the state of Sarawak requires the issuance of permit from Sarawak government. Hence, the application for a permit to conduct the research was done through the Sarawak Online Research Application System (SORAS). The approval of the application with the issuance permit: SFC.810-4/6/1 (2021) – 51 and WL 27/2021, allows the collection of bats guano samples to be conducted for this study.

4.2.1.1 Bat Guano

Bat guano utilised for the chemical composition analysis were collected from study sites discussed in Chapter 3 under the sub-heading 3.2.1. The collection of cave bats' guano was done by scooping 2 kg of guano from cave floors at a depth of 2 mm using a hand shovel. The cave bats' guano was stored in a zip-lock bag for transportation back to laboratory. Immediately after arrival in the laboratory, 250 g of the samples were sifted in order to separate larger components of the guano. The product was stored in an air-tight container and labelled according to their extraction sites.

4.2.1.2 Chicken Dung Fertiliser

A package of 2 kg chicken dung fertiliser was bought from a nearby agriculture store. The package selected was observed to be intact, airtight and in good condition. Then, the packages were laid horizontally in order to extract the cores from the corners of the packages using a non-corrosive probe. The extracted cores were mixed thoroughly and stored in an air-tight container for analysis. Leftover fertilisers were stored in a zip-lock plastic bag to be used in plant growth experiments.

4.2.1.3 Chemical Fertiliser

Two packages of 1 kg compound fertiliser were purchased from a nearby agriculture store. The packages were labelled with 10-15-15 NPK ratios. The package selected was observed to be intact, airtight and in good condition. Similar to the chicken dung fertiliser samples, representative cores were extracted from the corners of the packages and stored in an air-tight container. Leftover fertilisers were stored in a zip-lock plastic bag to be used in plant growth experiments.

4.2.1.4 Soil Samples for Control Treatment

Soil samples were obtained from bulk clay soil supply purposed for the medium of plant growth experiment. Surface debris such as plants or mulch in the soil were removed. Then, a small spade was inserted at a 3-inch depth in the dry clay soil supply. Slices from the spade were taken as subsamples. Other subsamples from different areas of the soil supply were taken in an even distribution. Any lumps or soil in each of the samples were removed including stones and

debris. The subsamples were mixed together to prepare a single soil sample. The soil sample was placed in an air-tight container for chemical analysis. The clay soil supplied will be used as the plant growth medium.

4.2.2 Chemical Composition of Fertilisers

Analysis of fertilisers was done with reference to the guidelines for analysis of fertiliser by the Department of Standards Malaysia (MS417). The guidelines were retrieved from the National Archive of Malaysia, Kuala Lumpur.

4.2.2.1 Sample Preparation

Preparation of the samples for analysis was done according to MS417: 1976 Clause 5. The fertiliser samples were ground as rapidly as possible with a laboratory mill that would pass through a sieve with 1 mm square apertures. The ground samples were mixed thoroughly before the 250 g portion of the overall sample passed through the sieve again. The samples were stored in an air-tight, non-corrodible container before the next chemical analysis was done. Three replicates of the sample were prepared in order to minimise any errors in analysis. Grinding of the samples was done as rapidly as possible to avoid unnecessary exposure to the environment. This precautionary step is to ensure there is no sudden loss or gain of moisture in the sample.

4.2.2.2 Dry Matter Analysis

The procedure of the analysis was done based on MS417: Part 2: 1994 Clause 3. Determination of the moisture content of fertilisers was done by spreading evenly 5 to 10 g of the sample in a shallow glass dish. The dish was weighed to the nearest mg using a weighing

balance and the original weight (W_1) of the samples was obtained by subtracting the weight of an empty glass dish measured previously. The glass dish with its contents was then heated in the oven at 103 °C for 2 hours. The glass dishes were cooled off in a desiccator before weighing. The processes were then repeated until a constant weight (W_2) was reached. Calculation of the moisture content was done according to the formula below;

$$\text{Moisture content, percent by weight: } \frac{100 \times (W_1 - W_2)}{W_1} \quad \text{Equation 4.1}$$

4.2.2.3 Ash Content or Organic Matter Analysis

The procedure of the analysis was done based on MS417: Part 2: 1994 Clause 5. Around 5 g of dried samples weighed to the nearest mg (W_1) from the dry matter analysis were placed into a pre-weighted silica crucible. The crucibles and their contents were inserted in a muffle furnace. The furnace was set to gradually increase the ignited temperature to 800 °C and maintained it at the temperature for 1 hour. The crucibles were allowed to cool in a desiccator before repeating the procedures again until a constant weight (W_2) was reached. The calculation of ash content analysis is as follows;

$$\text{Ash content: } \frac{100 \times W_1}{W_1} \quad \text{Equation 4.2}$$

4.2.2.4 Nitrogen Analysis

The determination of ammoniacal nitrogen using the distillation method was done based on MS417: Part 3: 1994 Clause 5. Since ammoniacal nitrogen is a strong base, the samples were distilled into acid with a known concentration before determining the percentage of N through acid-base titration. The 1 g of the samples were weighed to the nearest mg (W) and placed into a 250 mg volumetric flask. Distilled water was added to the mark, and the mixture was shaken well to dissolve. Undissolved materials were filtered using filter paper. The 50 mL of the solution was pipetted into a long-necked round-bottomed flask known as a Kjeldahl flask. The 125 mL of water and a few porcelain chips were placed into the flask as well. The flask was labelled as Flask A. Flask A was placed at one end of a delivery tube prepared in the set-up. Another Kjeldahl flask was labelled as Flask B. The 50 mL of standard hydrochloric acid (HCl) solution, 0.1 M were pipetted into the flask with a few drops of indicator solution prepared. The preparation of the mixed indicator solution was done by mixing one part of 0.2 % methyl red with one part of 0.1 % methylene blue in a 95 % alcohol solution. Flask B was placed at the other end of the delivery tube prepared, with its tip dips below the acid surface.

About 23 mL of the 30 % sodium hydroxide (NaOH) solution was transferred into a tap funnel. The solution was then slowly added to the contents of Flask A. Flask A was boiled until at least one-third of the volume was distilled through the delivery and captured in Flask B. Flask B was lowered to remove the tip of the delivery tube out of the content. The heat was turned off. The delivery tube was rinsed with distilled water with the rinsing collected in flask B. Flask F was then titrated with standard NaOH solution, 0.1 M until the colour turned slightly pink. The volume of 0.1 M NaOH solution that was used to neutralise the 0.1 M HCl in the sample titration

was recorded (V_2). The procedure was done for all the fertiliser samples with one blank sample as a control. The volume of 0.1 M NaOH solution used to neutralise the 0.1 M HCl in the titration of control was taken (V_1) for calculation. The calculation of the ammoniacal nitrogen analysis is as follows (atomic weight of N=14.01);

$$\text{Ammoniacal N content (\%)}: \frac{100 \times 14.01 \times (V_1 - V_2)}{W} \quad \text{Equation 4.3}$$

4.2.2.5 Phosphorus Analysis

Analysis of total Phosphate (P_2O_5) of commercial fertilisers was done by referring to the Malaysian Standards MS417: Part 4: 1994 Clause 5. The determination was done through the spectrophotometric molybdovanadophosphate method. This method works by determining the amount of light transmitted by the yellow complex of molybdovanadophosphate which is dependent on P concentration in the solution. A spectrophotometer with light cells was used in the analysis.

Reagents to be used in the analysis were prepared such as the potassium dihydrogen phosphate solution known as the standard P solution. Phosphorus stock solution was first prepared by drying 4.3928 g of potassium dihydrogen phosphate (KH_2PO_4) at 103 °C for 2 hours. The dried compounds were dissolved in distilled water and diluted to 1 L. The P solution was then prepared by diluting 50 mL of the P stock solution with 1 L of distilled water. The standard P solution contained 50 mg/L of P. The molybdovanadate reagent was prepared by

mixing 150 mL of ammonium vanadate solution with 100 mL of ammonium molybdate solution.

Sample preparation for the analysis was classified into two which are the preparation of mixed fertiliser (commercial fertiliser) and organic fertiliser (chicken dung fertiliser and bat guano). The commercial fertiliser sample was prepared by weighing 2.5 g of the sample to the nearest mg (W) and inserting it into a 400 mL beaker. The 30 mL of distilled water was placed in the beaker to wet the solid fertiliser thoroughly; additional 70 mL of distilled water was added with continuous stirring. The mixture was heated to a warm temperature. The 10 mL of concentrated HCl was added, followed by 5 mL of concentrated nitric acid while continuous stirring was applied. The solution was gently boiled for 10 minutes, then it was transferred to 250 mL of volumetric flask after cooling off. The product was further diluted with distilled water up to the mark. Further dilution was carried out to reach 10x of the dilution factor (D). The solution was filtered through a filter paper to ensure the removal of any excess solids in the solution.

Samples of organic fertilisers which are chicken dung fertiliser and bat guano fertiliser were prepared according to the amendment (Amd. 1) attached to MS 417: Part 4: 1994. The total P_2O_5 of the samples were determined from dry-ash samples of the organic fertilisers. The fertilisers were weighed at 5 g to the nearest mg into a pre-weighted crucible. The crucible and its contents were ignited in a muffle furnace with the starting temperature at 350 °C and increasing up to 550 °C. The ignition was maintained at the temperature for 1 hour. The state of the products was observed as a suitable condition for the analysis is a fully ashed product. Organic fertiliser samples that did not reached the state would be reignited for another hour. The

ashed samples were removed and let cool in a desiccator. The 50 mL of 20 % nitric acid was added to the crucible flask before pouring the mixture into a 250 mL volumetric flask. The flask volume was made up to the mark using the nitric acid solution.

The 50 mL of the solutions was pipetted into a 100 mL volumetric flask. Then, 25 mL of molybdovanadate reagent was pipetted into the same flask and both of the solution was diluted together to 100 mL mark using distilled water. The solution was mixed well. A 30-minute buffer time was set before placing the solution in the light cells of the spectrophotometer with the setting of 420 nm wavelength. The standard P solution was tested through the same procedures to obtain the basis of the P ranges set. The concentration of the P (C) was determined from the calibration graph obtained with the standard P solutions. The total P₂O₅ content percentage was calculated by the formula as follows;

$$\text{Total P}_2\text{O}_5 \text{ content (\%)}: \frac{C \times D \times 22.9 \times 100}{W} \quad \text{Equation 4.4}$$

4.2.2.6 Potassium Analysis

Potassium analysis of fertilisers was done by the determination of potash (K₂O) in the samples. The procedures of the analysis were based on MS 417: 1976 Clause 9.4 retrieved from the National Archives Malaysia. In this analysis, the flame photometric method was utilised. The determination of K₂O through this method depends on the measurement of the radiation characteristics emitted from a flame that was sprayed with the samples. The radiation range which suited K₂O determination chosen in the study was between 766 to 770 nm of spectral

range. The procedure started with the preparation of fertiliser samples. The sample was weighed to the nearest centigram, and approximately 10 g of it was gently incinerated at a temperature not exceeding 500 °C to destroy the organic matter. The residue was ground to eliminate any lumps and boiled for 30 minutes with 125 mL of water and 50 mL of ammonium oxalate solution. The solution was then cooled, and a slight excess of ammonium solution was added, then the solution was further cooled to a temperature of 20 °C. After that, the solution was transferred to a 500 mL volumetric flask and diluted to the mark. The solution was mixed, and it was filtered through a dry filter. Successive dilutions were performed, so that the final solution contained approximately 16 ppm of potash. The potash content in the filtrate was determined by the flame photometry method.

The flame photometry instrument was first calibrated by preparing a set of diluted potash (10, 12, 14, 16, 18, and 20 ppm) using the standard potash. The sensitivity of the flame photometry was set to a full-scale deflection which is equivalent to 20 ppm potash solution. The diluted potash was sprayed three times before taking the median reading shown to construct a calibration graph. In order to further ensure that the sensitivity of the flame photometer has not changed, the 20-ppm solution was sprayed again.

For sample analysis, the prepared fertiliser samples diluted to a suitable factor, were sprayed into the flame ignited through the instrument. The graph readings were recorded where the potash content in the sample solution could be approximated. Next, two further dilutions of the standard potash solution which contained 1 ppm more and 1 ppm less than the potash estimated in the sample were prepared. The low-standard solution was first sprayed into the flame, following the sample solution prepared and lastly, the higher-standard solution was

sprayed into the flame. This procedure was repeated three times and the median result for each of the three readings was taken. The potash content of the fertiliser solution was calculated from the proportionality of the radiation readings of the three different fertiliser samples gained from the predicted potash content.

4.2.3 Chemical Composition of Soil

Analysis of soil was done with reference to the guidelines for analysis of fertiliser by the Department of Standards Malaysia (MS678) for N, P, and K analysis. The guidelines were retrieved from the National Archive of Malaysia, Kuala Lumpur. Procedures of dry matter analysis and organic matter analysis that were not included in the standards were conducted based on previous research in the field.

4.2.3.1 Sample Preparation

The soil samples were air-dried at room temperature. The dried samples were mixed well and crushed to a smaller size. The samples were mixed until a homogenous sample was obtained. The 200 g of the samples were stored in a clean labelled container for testing.

4.2.3.2 Dry Matter Analysis

The 50 g of soil was weighed and placed into pre-weighed dishes. The soil was then placed in an oven with a temperature of 105 °C and left overnight. The dishes were removed from the oven and were allowed to cool. The weight of the dishes after drying was taken and the final weight was calculated by the differences between the final weight of the soil and the

weight of the soil prior to drying (Journal of Visualized Experiments Science Education Database, 2023).

4.2.3.3 Soil Organic Carbon Analysis

The soil organic matter analysis was conducted based on Estefan et al. (2013). The 1 g of air-dried soil through the soil preparation was weighed (W) into a 500 mL Erlenmeyer flask. The 10 mL potassium dichromate solution (V_1) was added followed by 20 mL of concentrated H_2SO_4 . The flask was shaken gently before being left at room temperature for 30 minutes to allow the reaction of the chemicals to take place. Then, 200 mL of distilled water with 5 mL of phosphoric acid was added. The mixture was allowed to cool. Next, 10 to 15 drops of diphenylamine indicator prepared beforehand were added. The content was titrated with 0.5 M ferrous ammonium sulphate solution until the colour of the content changed from purple to green. The volume of the ferrous ammonium sulphate sample titrated was recorded (V_2) for calculation. A blank determination was carried out with the same procedure. The calculation of the total organic carbon in percentage is as follows (N_1 = normality of potassium dichromate, N_2 = normality of ferrous ammonium sulphate);

$$\text{Total Organic Carbon content (\%)}: \frac{3 \times (N_1 V_1 - N_2 V_2) \times 100}{W \times 1000} \quad \text{Equation 4.5}$$

4.2.3.4 Total Nitrogen Analysis

Nitrogen analysis of the soil samples was determined by distillation method. The method was completed by first conducting the Kjeldahl digestion method (MS 678: Part II: 1980). Then, the solution was distilled using a Markham apparatus. An indicator was first prepared by dissolving 0.25 g of methylene blue and 0.75 g of methyl-red components in 1 L of 95 % ethyl alcohol.

The soil samples were ground to pass through a 212 μm sieve. The 0.25 g of the ground soil samples (W) were placed into a 50 mL Kjeldahl flask along with 2 mL of sulphuric acid. The mixture was allowed to stand for 30 minutes with frequent shaking. About 0.3 g of sodium thiosulphate was added to the flask before heating on a digestion rack in the fume cupboard. When the fuming commences at the neck of the flask, a buffer time of 5 minutes was given for the digestion to concur before cooling the digestion rack for a while. During the short break, 1 g of sodium sulphate was added to the mixture and heated until the mixture turned white. The flask was further heated for 90 minutes, then cooled off and the sides of the flask were washed down with a small amount of distilled water.

The digested mixture was transferred into a Markham distillation apparatus. Excess traces of solution from the flasks were removed using a minimal amount of water. The 12 mL of 30 % sodium hydroxide and distilled water was added to the flask. A 50 mL conical flask was prepared with the content of 5 mL of 3 % boric acid and a few drops of methyl red indicator prepared previously. The Kjeldahl flask was heated with its distillate collected in the conical flask. The distillate mixed with boric acid was titrated with a known concentration of 0.01 M

HCl until the mixture turned slightly pink. The volume of 0.1 M HCl used in the titration was recorded for calculation (V). To narrow the possibility of errors, a blank sample was carried out according to the procedures of N analysis. Calculation of the soil N content percentage is as follows (1 mL of 0.01 M HCl = 0.00014 g N);

$$\text{N content in soil (\%)}: \frac{100 \times 0.00014 \times V}{W} \quad \text{Equation 4.6}$$

4.2.3.5 Total Phosphorus Analysis

The determination of total P in soil samples was measured through the colourimetric method (MS 678: Part VIII: 1980). A digestion mixture was prepared by adding 20 mL of concentrated sulphuric acid into 20 mL of perchloric acid. The mixture was cooled to room temperature before being used. Other reagents prepared, are molybdate-sulphuric acid solution, ascorbic acid solution and standard solution of P. To prepare a molybdate-sulphuric acid solution, an ammonium molybdate solution was readied by dissolving 25 g of ammonium molybdate in 200 mL of water heated to 60 °C. The mixture was then filtered using a filter paper. Next, a dilution of 1 L of sulphuric acid (H₂SO₄) was prepared from 280 mL of concentrated H₂SO₄. When both of the solutions cooled, the ammonium molybdate solution was added to the H₂SO₄ solution with a continuous gentle shaking. The mixture of the two solutions which was known as 2.5 % ammonium molybdate solution in 5 M H₂SO₄ (molybdate-sulphuric acid solution) was diluted to 1 L in a volumetric flask. The solution was further diluted by taking 20 mL of molybdate-sulphuric acid solution into 400 mL of distilled water. Next, the preparation of ascorbic acid solution was done by dissolving 1 g of ascorbic acid in 100 mL of water. Lastly,

a standard solution of P was prepared by taking stock standard solution 100 ppm, (0.4394 g of KH_2PO_2 dissolved in 1 L distilled water) and diluting it in a suitable amount to obtain 0, 2, 4, 5, 6, 8, and 10 ppm of P.

The procedure of P analysis of soils was initiated with the digestion step, where 2.0 g of air-dried soil collected was placed in a Kjeldahl flask with 6 mL of the digestion mixture prepared prior along with 2 glass beads. The Kjeldahl flask was heated moderately for 2 hours. A small amount of digestion mixture was used if necessary to wash down undigested materials and the neck of the flask. The product of digestion was cooled before transferring it to a 100 mL volumetric flask. Distilled water was added to make up the solution to the mark. The solution was filtered using a filter paper and 10 mL of the filtrated solution was collected for analysis.

The collected solution was pipetted into a 50 mL volumetric flask. The 10 mL of ammonium molybdate solution followed by 5 mL of ascorbic acid was added to the flask. The 50 mL mark of the flask was made up with distilled water. The solution was mixed thoroughly. The standard P solutions in the different concentrations were prepared similarly to the collected solution. The blue colour of the phosphorus was allowed to develop for 5 minutes before placing it in the 4 cm cell absorptiometer. The absorptiometer measured the blue colour intensity of the solution using a red filter (660 m μ). The P calibration curve was obtained from the P concentrations of the standard solutions prepared. Calculations of the total P in soil is as follows;

$$\text{Total P in soil (ppm): } 50 \times \text{Concentration of the extract} \quad \text{Equation 4.7}$$

4.2.3.6 Potassium Analysis

The determination of exchangeable K in soil was done through the flame photometry method (MS 678: Part IV: 1980). First, the soil samples were prepared through a leaching process. A leaching column of 2 cm diameter and 20 cm length was packed with filter paper that was previously washed with acid. 10 g of soil was weighed (W) and placed in the leaching column. A 100 mL of 1 M ammonium acetate reagent was placed in a 350 mL Erlenmeyer flask fitted with an outlet tube. The flask was inverted over the soil column. Collection of the leachate was dropped in a 100 mL volumetric flask. Air entry into the flask was permitted by ensuring an irregular shape of the outlet tube end was present in order for a smooth and continuous leaching process. The process was left for 5 hours and ended when the flask was empty. The leachates were set aside for the determination of potassium. Next, the reagents used in the procedure of K determination were prepared. A standard stock solution was prepared by dissolving 1.90 g of oven-dried KCl in 1 L distilled water. The stock solution that contained 1000 ppm of K was diluted with a 10x dilution factor to 100 ppm of K. Then, a working standard of 0 to 10 ppm was prepared by mixing the stock solution in a suitable dilution factor into 1 M ammonium acetate solution.

The flame photometry instrument was first calibrated by preparing a set of diluted K (10, 12, 14, 16, 18, and 20 ppm) using the standard stock solution. The sensitivity of the flame photometry was set to a full-scale deflection which is equivalent to 20 ppm K solution. The diluted K was sprayed three times before taking the median reading shown to develop a calibration graph. In order to further ensure that the sensitivity of the flame photometer has not changed, the 20-ppm solution was sprayed again. For sample analysis, the leachate soil samples

diluted to a suitable factor, were sprayed into the flame ignited through the instrument. The graph readings were recorded where the K content in the sample solution could be approximated (C). The calculation of K milliequivalents in 100 g soil is as follows (Atomic mass of K= 39.0983):

$$\text{K content in soil: } \frac{100 \times C}{W \times K} \quad \text{Equation 4.8}$$

4.2.4 Statistical Analysis

Statistical analysis of the chemical compositions was done by inputting the results in SPSS Version 36 software and Microsoft Excel. Coefficient of determination (r^2) values between the chemical analyses were obtained from linear regression. Analysis of variance (ANOVA) through one-way ANOVA calculation was performed for analysing the characterization of the fertilisers and soil nutrient data.

4.3 Results

4.3.1 Dry Matter Analysis

The CF has the highest dry matter content ($94.833 \% \pm 0.167 \%$) compared to another samples (Figure 4.1). The BGRC has recorded lowest at $65.933 \% \pm 0.219 \%$. Other samples recorded $86.167 \% \pm 0.133 \%$ for CDF and $69.467 \% \pm 0.463 \%$ for BGWCR. Analysing dry matter in fertiliser is vital as it enables accurate measurement of the nutrient content of the fertiliser and allows the user to measure the application rates of the treatment accordingly. This

approach also helps in ensuring the correct amount of nutrients received by plants for optimal growth and avoiding over-fertilization or under-fertilization (Rotz et al., 2005). Alternatively, the moisture content in bat guano samples specifically BGRC are the highest at 34.067 %, when compared to the other samples (CF: 5.167 %, CDF: 13.833 %, BGWCR: 30.533 %). Determination of moisture content can be done by subtracting dry matter content from 100 % of the overall content. Determining the moisture content in fertiliser is important for several reasons. Firstly, it helps in determining the quality and stability of the fertiliser. Excessive moisture content can lead to caking, clumping, and reduced shelf life of the fertiliser. On the other hand, insufficient moisture content can affect the solubility and effectiveness of the fertiliser. Additionally, knowing the moisture content is important for proper storage and transportation of the fertiliser to prevent spoilage and degradation (Wang et al., 2019).

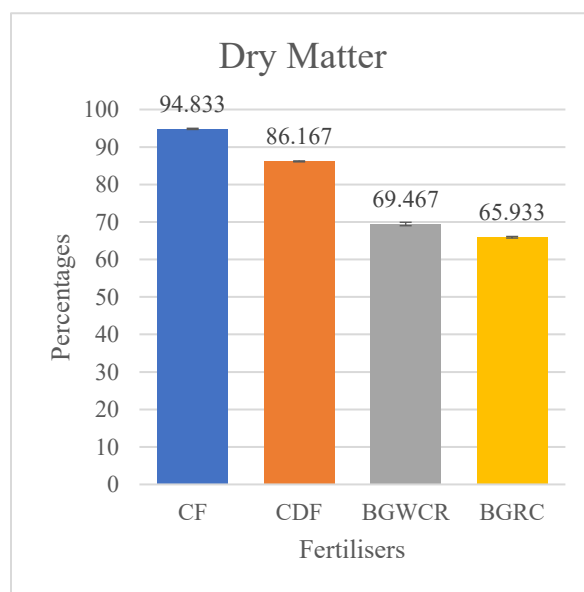


Figure 4.1: The mean of dry matter content for fertilisers in percentages (%). Bars represented standard errors of differences between the means. *Notes:* Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano Wind Cave Reserve (BGWCR), and Bat Guano Raya Cave (BGRC).

4.3.2 Organic Matter Analysis

The CDF has the highest organic matter content ($33.63 \% \pm 0.578 \%$) compared to another samples (Figure 4.2). Meanwhile, CF has recorded lowest at $3.9 \% \pm 0.321 \%$. Other samples recorded $10.7 \% \pm 0.208 \%$ and $18.93 \% \pm 0.491 \%$ for BGWCR and BGRC, respectively. The reading shows low C concentration in commercial fertiliser as it is manufactured to contain synthetic macronutrients such as N, P, and K. Compounds containing C are originated mostly from living organisms and their by-products. The analysis of organic matter provides important input on the composition and nutritional content of the fertilisers. Studies on the mineral levels of bat guano found an organic matter percentage in bat guano is at a range of 24 to 79 % (Altintas and Yildiz, 2005). Another study reflected the findings of the organic matter level where it was measured to be at 20 % (Yankova et al., 2016). Analysis on organic matter percentages of chicken dung fertilisers particularly tested after one year was found to be almost similar range with the analysis at 37 % (McCall, 1914).

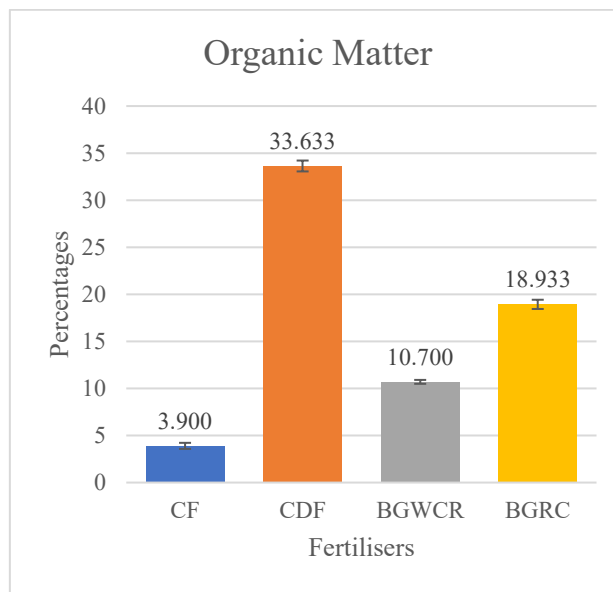


Figure 4.2: The mean of organic matter content for fertilisers in percentages (%). Bars represented standard errors of differences between the means. *Notes:* Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano Wind Cave Reserve (BGWCR), and Bat Guano Raya Cave (BGRC).

4.3.3 Nitrogen Content Analysis

The CF has the highest N content ($10 \% \pm 0.1 \%$) compared to another samples (Figure 4.4). Meanwhile, CDF has recorded lowest at $2.55 \% \pm 0.021 \%$. Other samples recorded $2.957 \% \pm 0.029 \%$ and $3.47 \% \pm 0.052 \%$ for BGWCR and BGRC, respectively. The readings reflected high level of N content in CF making it a superior N fertiliser source. Previous studies of N content discovered a range which fitted the calculation in this study at 0.97 to 5.60 % (Altintas and Yildiz, 2005). Other studies also found the N content to be at 2 %, which correlate with these findings (Yankova et al., 2016). Figure 4.3 shows the N content among the sample's groups. The N content analysed found that chicken dung fertiliser at 10 weeks had 2.4 % of N percentage which supports the suitability of N content findings in this study (McCall, 1914).

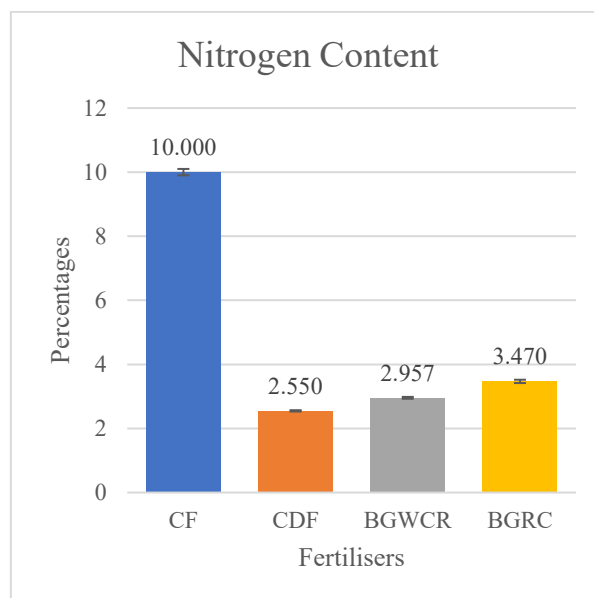


Figure 4.3: The mean of nitrogen content for fertilisers in percentages (%). Bars represented standard errors of differences between the means. *Notes:* Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano Wind Cave Reserve (BGWCR), and Bat Guano Raya Cave (BGRC).

4.3.4 Phosphorus Content Analysis

The CF has the highest P content ($14.733 \% \pm 0.219 \%$) compared to another samples (Figure 4.4). The CDF has recorded lowest at $1.523 \% \pm 0.007 \%$. Other samples recorded $2.247 \% \pm 0.123 \%$ for BGRC and $2.073 \% \pm 0.043 \%$ for BGWCR. The importance of P in plant treatments was mentioned in previous studies where high levels of N pairs with P can cause an increment of root growth and improvement on roots to shoot ratio of plants (Poliquit and Calong, 2018). The P content in several studies shows similarity to this study, where analysis of P percentage from bats guano obtained a level of 1.20 to 1.50 % (Altintas and Yildiz, 2005). Other than that, Karagoz-Sezer and Hanay (2020) analysed the content of 2.00 % of P_2O_5 in their study of the chemical composition of bats guano. For chicken dung fertilisers, the percentage of the

fertilisers found in the study approximately fits the 1.0 % of P_2O_5 found in the chicken dung fertiliser at one year (McCall, 1914).

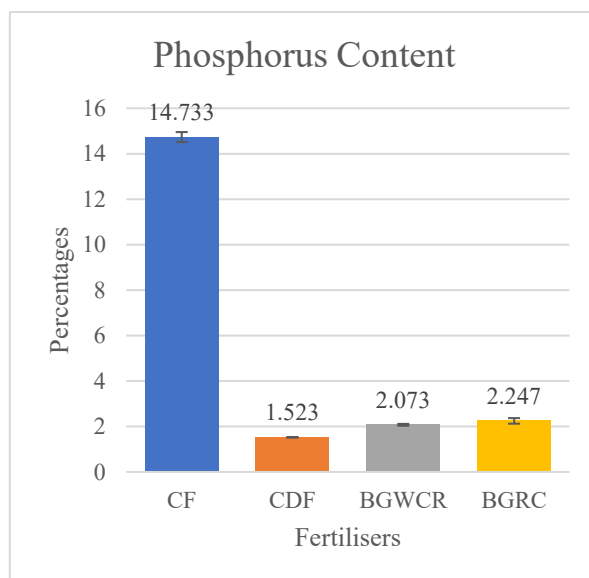


Figure 4.4: The mean of phosphorus content for fertilisers in percentages (%). Bars represented standard errors of differences between the means. *Notes:* Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano Wind Cave Reserve (BGWCR), and Bat Guano Raya Cave (BGRC).

4.3.5 Potassium Content Analysis

The CF has the highest K content ($15.767 \% \pm 0.033 \%$) compared to another samples (Figure 4.5). The BGWCR has recorded lowest at $0.397 \% \pm 0.017 \%$. Other samples recorded $0.857 \% \pm 0.047 \%$ for BGRC and $2.243 \% \pm 0.003 \%$ for CDF. Low K content of bats' guano found in this study correlates with previous studies 0.25% to 0.63% (Altintas and Yildiz, 2005); 0.5% (Karagoz-Sezer and Hanay, 2020); and 0.4% (Sothearen et al., 2014). Despite the reviews in previous studies that bat guano contains high amount of K, this study does not reflect the statement (Hutchinson, 1950; Kasso and Balakrishnan, 2013; Sakoui et al., 2020). This might

be due to the diet of bats from the guano collected. High K levels in bats guano might suggest the consumption of a considerable number of lepidopteran species (Misra et al., 2019). A different diet consumption of bats in the study might explain the low levels of K content in bats' guano. In previous research on the correlation of the macronutrient content in chicken dung fertiliser with time, a range of 0.6 to 1.3 % of K_2O was found in the fertiliser, which does not correlate with the analysis shown (McCall, 1914). A newer study on the percentages of macronutrients for chicken dung fertilisers which could support the percentage measure in this study was not found.

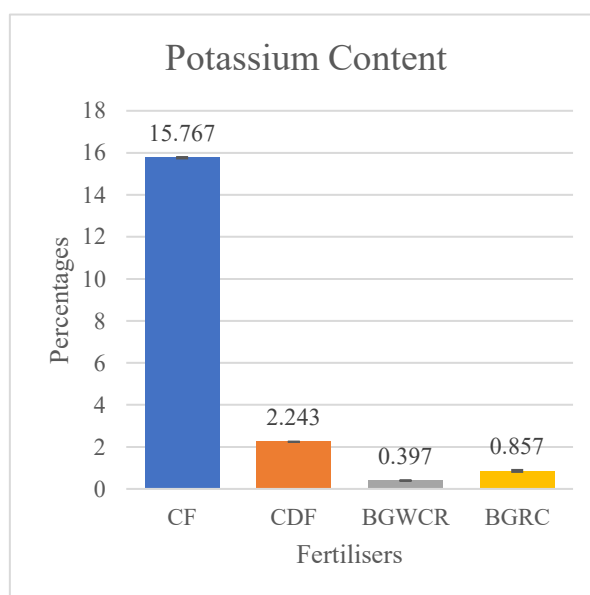


Figure 4.5: The mean of potassium content for fertilisers in percentages (%). Bars represented standard errors of differences between the means. *Notes:* Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano Wind Cave Reserve (BGWCR), and Bat Guano Raya Cave (BGRC).

4.3.6 Soil Samples Properties as Control

The analysis of soil samples properties served as a basis for plant growth studies requirement. The soil used in the study was analysed for its dry matter, organic matter, total nitrogen, available phosphorus, and exchangeable potassium. pH levels of the soil were found to be acidic at 4.8 to 5.7 pH levels. A suitable pH of 6.5 to 7 was considered to be desirable in chilli plant growth. The N level was recorded at $0.073 \% \pm 0.153 \%$, available phosphorus at $0.933 \% \pm 0.808 \%$, and exchangeable potassium at 0.08% .

Table 4.1: Chemical characteristics of soil sample

Parameter	Measurements (%)
pH	4.8 – 5.7
Dry Matter	70.3 ± 0.953
Organic Matter	0.72 ± 0.02
Total Nitrogen	0.073 ± 0.153
Available Phosphorus	0.933 ± 0.808
Exchangeable Potassium	0.08 ± 0.000

4.4 Discussions

Chemical analysis of the samples shows the highest readings for CF, with dry matter content recorded at $94.833\% \pm 0.167\%$, organic matter content at $33.63\% \pm 0.578\%$, N content at $10\% \pm 0.1\%$, P content at $14.733\% \pm 0.219\%$, and K content at $15.767\% \pm 0.033\%$. This is expected, as CF is commercially manufactured to contain high levels of N, P, and K. The CF used in the study had a labelled ratio of 15-15-15 N-P-K; however, the analysis revealed an actual NPK ratio of 10-15-15. The 5 % reduction in N may be attributed to oxidation of the fertiliser samples due to improper storage. Ensuring airtight storage of fertilisers is crucial to prevent microbial growth, which can initiate the mineralisation process. Nitrogen losses are associated with ammonia absorbed for microbial growth, which is then released after the death of microbial cells (Stella et al., 2019).

McCall (1914) previously examined the degradation of macronutrients in CDF over time. A fresh supply of CDF typically contains higher macronutrient levels, primarily due to mineralisation, particularly from microbial activity (Stella et al., 2019). However, a fixed degradation timeline could not be determined through comparison with this study alone, as further research with that specific objective is required. Furthermore, no recent study using the same methodology was available for direct comparison. Hence, McCall's (1914) study can only serve as a reference when considering other literature discussing the chemical composition of chicken dung fertiliser.

Regarding the organic fertiliser derived from bat guano, several studies have reported the influence of diet on its chemical composition. Frugivorous bats are typically identified by

higher P levels in their guano, whereas insectivorous bats tend to produce guano with higher N content relative to P (Sridhar et al., 2006). In comparison to this study, slightly higher N levels were observed in both BGWCR (N: $2.55 \% \pm 0.029 \%$, P: $2.073 \% \pm 0.043 \%$) and BGRC (N: $3.47 \% \pm 0.052 \%$, P: $2.247 \% \pm 0.123 \%$), which might indicate a higher proportion of insectivorous bat faecal matter. Nevertheless, confirming this would require complementary identification studies, whether invasive or non-invasive.

In terms of soil chemical composition, soil analysis revealed acidic pH levels (4.8–5.7) and low fertility, with N at 0.073 %, P at 0.933 %, and K at 0.08 %. The critical level of N required for optimal plant growth is 3 %, while the critical range for K content lies between 0.75 and 1.25 % (Bush et al., 2016). Therefore, the low nutrient and pH levels indicate that the soil has poor fertility and would benefit from the application of fertiliser treatments to enhance plant growth. Fertilisation is a crucial process involving the addition of substances to soil or plants to provide essential nutrients for growth. However, the use of chemical fertilisers has long been debated, as it has been linked to environmental issues such as nitrate contamination, pollution, and nutrient loss (Ekinici et al., 2019).

Technological advancements in organic materials have increasingly influenced the agricultural market. The implementation of organic fertilisers should be promoted more widely within the farming community. Several initiatives ought to be undertaken to encourage their use. Proper crop management courses should be promoted among aspiring agriculturists to support sustainable plantation practices. Standards for the management of both organic and chemical fertilisers should be outlined by relevant authorities, and legislation governing fertilisation practices should be enacted to control the overuse of chemical fertilisers.

Future research on the chemical composition of bat guano should explore the influence of diet on nutrient content. Although a few studies have examined the relationship between diet and guano composition (Sridhar et al., 2006), targeted research on Malaysian cave bat guano communities is still lacking. Additionally, guano processing methods could be refined through further study to identify optimal storage practices that stabilise nutrient content. Lastly, examining the microbial and enzymatic benefits of guano in enhancing soil nutrients offers potential for future research. This would help to understand the broader influence of substances beyond NPK values and their role in soil fertility.

4.5 Conclusion

The analysis of chemical fertilisers revealed high nitrogen, phosphorus, and potassium levels, with low organic matter. This is expected for synthetic fertilisers aimed at promoting plant growth. In contrast, organic fertilisers, including bat guano, showed lower N-P-K values but slightly higher nitrogen levels. Phosphorus levels were higher for BGRC BGWCR, compared CDF. Not just that, Potassium levels were higher in CDF than in BGWCR and BGRC. The variation in N-P-K content among bat guano samples from different sites likely stems from differences in bat diets, guano age, species, and locality. Finally, Raya Cave guano had higher nitrogen, phosphorus and potassium levels than Wind Cave Reserve guano.

Soil sample analyses revealed low levels of total N, available P, exchangeable K, and organic matter, suggesting limited fertility in untreated soil. The similarity in dry matter between soil samples and the BGWCR sample may indicate a high moisture content in both. These

findings underscore the importance of assessing fertiliser and soil quality to optimise their application and to evaluate their overall impact on plant growth.

Promoting the use of organic fertilisers through education, proper management standards, and supportive legislation is vital for achieving sustainable agricultural practices. Additionally, further research into the chemical properties and processing methods of bat guano—particularly within the Malaysian context—could showcase its potential as a valuable organic fertiliser and support healthier, more resilient soil systems beyond standard nutrient metrics.

CHAPTER 5

EFFECTS OF CAVE BATS' GUANO, CHICKEN DUNG, AND CHEMICAL FERTILISER ON CHILLI PLANT

5.1 Introduction

Chilli plants are known to be vulnerable to various physical and chemical factors (including physiological conditions, environmental influences, and pests) that can affect production (yield) (Rylski, 1973; Rylski and Halevy, 1975; Erickson and Markhart, 2001; Chaudhary et al., 2006; Hussain and Abid, 2011). The application of fertilisers has been shown to positively influence chilli plant performance by increasing growth and yield. For example, Vimala et al. (2007) found that fertilised chilli plants produced significantly higher fruit yields, with an average of 2.32 kg per fertilised plant, compared to 0.97 kg per plant in the control group (without fertilisation). Similarly, studies involving the application of triacontanol, naphthalene acetic acid (NAA), and gibberellic acid (GA_3) on two different chilli cultivars, 'Pusa Jwala' (a Cayenne-type chilli) and Suryamukhi, have shown variations in plant performance, including ascorbic acid content and fresh shoot weight (Dahal et al., 2006; Chaudhary et al., 2006).

Studies on the application of organic fertilisers, such as vermicompost and guano, have also demonstrated positive effects on plant growth. Adhikary and Gantayet (2012) reported significant increases in shoot length, shoot weight, and root length in chilli plants treated with vermicompost compared to control plants. Guano, which is rich in nitrogen (N), phosphorus (P), and potassium (K), has been shown to enhance plant growth and improve soil productivity in various studies (Sothearen et al., 2014; Unal et al., 2018; Ferreira, 2019; Misra et al., 2019).

Furthermore, the application of guano has been associated with increased growth, nutrient uptake, and disease resistance in various plant species, including carrots, cassava, and other economically important crops in Cambodia (Sothearen et al., 2014; Linn and Myint, 2018; Poliquit and Calong, 2018). Studies have also suggested that combining guano with other organic fertilisers can further enhance its effectiveness in promoting plant growth (Karagoz-Sezer and Hanay, 2020; Ojobor and Egbuchua, 2021).

The effects of chicken manure on plant growth have also been studied extensively. Chicken manure has been shown to improve soil properties, increase nutrient availability, and enhance plant growth in various crops, including tomatoes, lettuces, and chillies (O'Hallorans and Colberg, 1993; Masarirambi et al., 2012; Khandaker et al., 2017; Thepsilvisut, 2021). Although chemical fertilisers have been widely used in agriculture and have shown benefits in increasing crop yield and food production, their excessive use can lead to environmental pollution and nutrient depletion (Fageria et al., 2009; Jahanzad et al., 2017; Ekinici et al., 2019). Therefore, exploring alternative organic fertilisers such as guano and chicken manure offers sustainable solutions for improving soil fertility and plant growth while minimising negative environmental impacts. Further research into their effectiveness and optimal application methods is essential for advancing sustainable agricultural practices. Consequently, this study was conducted to examine the effects of cave bat guano, in comparison with other established commercial fertilisers, on plant growth performance—particularly in chilli plants.

5.2 Materials and Methods

5.2.1 Location and Duration of Experiment

The study was conducted in growth house (600 cm x 236 cm) at Centre for Pre-University Studies, Universiti Malaysia Sarawak (1.4607°N, 110.4517°E). The study was conducted for 2 times life cycle, 3 months for each cycle (6-month from 20th September 2022 to 10th March 2023) (Table 5.1).

Table 5.1: The timeline and growth cycle of chili plant

Date	Growth Cycle	Notes
September 2022	• Germination (2 weeks)	300 seeds were germinated in a germination tray using peat moss as a growth medium.
October 2022	• Transferring plantlets • Start of acclimatization (one month) • First plant measurements	The plantlets were transferred to 16x16 polybags with sifted clay soil as the growth medium.
November 2022	• Second plant measurements • First plant treatment	Fertilisers were treated on the plants accordingly (Table 5.2).
December 2022	• Third plant measurements • Second plant treatment	Measurements of plants were taken for analysis.

Table 5.1: continue

January 2023	• Fourth plant measurements • Third plant treatment	
February 2023	• Fifth plant measurements • Fourth plant treatment	
March 2023	• Final plant measurements • Plant harvests	Plant measurements were recorded. All the plant parts were harvested and placed in a paper bag.

5.2.2 Experimental Design and Treatments

A Complete Randomized Design (CRD) were applied in the experiment as it is the best fit design in this study with treatments of similar experimental units. The performance of chilli plants growth was monitored for six months (2 times life cycle, 3 months for each cycle). Treatments were applied on the total of 162 chilli plants where three replicates of three plant per treatments for three harvest periods as shown in Table 5.2. Different rates of bat guano were applied on the plants in order to identify its suitability as a fertiliser with an optimum effect. The fertilisers used for the treatment were collected following the method in Chapter 4 under the sub-heading 4.2.1. The treatments were applied monthly for 5 months commencing from the end of acclimatization period.

Table 5.2: List of treatment on chili plant

No.	Treatment	Weight (g)	Code	Plant count
1	No Fertiliser (Control)	0.0	NF	27
2	Commercial Fertiliser	5.0	CF	27
3	Chicken Dung Fertiliser	5.0	CDF	27
4	Bats Guano	2.5	BG1	27
5	Bats Guano	5.0	BG2	27
6	Bats Guano	7.5	BG3	27
Total				162

Notes: No Fertiliser (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3).

5.2.3 Plant Preparation

The effects of cave bats' guano, chicken dung, and chemical fertiliser on plant growth performance was conducted using chilli plant (*Capsicum annuum* L. var. Kulai). Red Pepper (code: 108) seeds for chilli plants from Green World brand were purchased from Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor. The plant preparation was done by preparing the growth medium. The growth medium was prepared by sifting clay soil supplies which is known for its fine particles and ability to hold moisture and nutrients well. The soil sifted was ensured to be uniformed and free from debris. The soils were then finely crushed to break down any remaining clumps. A refined soil texture was vital in

order to provide better aeration for root penetration. The clay soil was mixed thoroughly for a consistent growth medium content. The soil was filled in 16'' x 16'' polybags made of high-density polyethylene (HDPE) which are durable and resistant to moisture and chemicals.

The seeds for seedling were initially placed between damped tissue papers for one week for pre-germination. An approximate number of 300 pre-germinated seeds were sown in three germination trays using peat moss as a growth medium. The germination trays were placed under the shed for four weeks for its development. Once the seedlings developed, plantlets were transferred into the polybags filled with the growth medium that was prepared earlier for acclimatization.

5.2.4 Maintenance and Sampling

Plants were maintained through daily observation, watering, weeding, and biweekly ploughing of soils. Other than that, the plant was observed closely to prevent any diseases or pathological symptoms.

Harvesting was done after each 3-months period of the cycle completed. The fruits were harvested from each plant and placed in labelled paper bags. The plants were removed from each polybag carefully ensuring the roots were still intact. Immediately after the harvest, the plants were cleaned and separated into leaves, stems, and roots in labelled paper bags. The paper bags containing the plants' parts were brought to the laboratory for further processing and storage. The weight of the materials was obtained using an electronic weighing machine. The total plant dry weight was obtained by placing it in a Forced Draft Oven at 40°C until constant weight was achieved. Dried samples were ground to fine powder 1.0 mm using the Cyclotec

Grinder, (Sample Mill, FOSS). The samples were then kept in a 6 cm x 9 cm zip lock bag in a cool and dry temperature until further analysis.

5.2.5 Measurements and Analysis

The measurements of plant height (PH) (cm), number of leaves (NoL), number of flower buds (NoB), number of flowers (NoFl), and number of fruits (NoFr) were taken each month. The data were inputted into Microsoft Excel spreadsheet to establish a clear statistical format before being imported into SPSS Version 36 Software to run several statistical tests including three-factor analysis of variance (ANOVA) using a general linear model to analyse the effects of three independent categorical variables on dependent variable. The post hoc test which is Fisher's Least Significant Difference (LSD) was done to identify the specific group which differs from each other where the mean difference is considered significant at $P < 0.05$. Additionally, correlation analysis was tested using the same software to assess the relationships between the variables and their significance in the experiment.

5.2.5.1 Plant Height (cm)

The plant heights (PH) were taken monthly using a standard 3.5 m pocket measuring tape. The measurement was done from the plant base to the tip of the longest tip using a ruler during its juvenile stage. Height of matured plants was measured from the ground level to the tip of the highest leaf with the usage of industrial measuring tape. Three measurements were taken to ensure minimal errors were in effect.

5.2.5.2 Number of Leaves

The number of leaves (NoL) were counted manually from the first month of plantation which was after the germination of chilli plants.

5.2.5.3 Number of Flower Buds and Number of Flowers

Number of flower buds (NoB) and flowers (NoFl) were counted manually weekly as it emerged.

5.2.5.4 Number of Fruits

Number of fruits (NoFr) were counted manually as it emerged starting from the third month to the sixth month during harvest.

5.3 Results

5.3.1 Plant Height

All germinated seeds were recorded at similar heights after acclimatisation, measuring 5 cm in M1. In M2, plants treated with CF recorded the highest plant height (PH) at 18.333 ± 5.31 cm and continued to increase in M3 and M4 to 28.185 ± 8.473 cm and 36.519 ± 11.706 cm, respectively. The plants treated with CF further increased in height in M6, reaching 42 ± 7.149 cm (Table 5.3). Conversely, the control plants (NF) recorded the lowest PH throughout the study. In M2, the PH for NF was recorded at 9.4 ± 1.9828 cm, increasing slightly in M3 and M4 to 10.844 ± 2.1846 cm and 11.407 ± 2.8387 cm, respectively (Table 5.3). Meanwhile, plants treated with the organic fertiliser CDF showed lower PH than CF, but higher than bat guano and

the control. The PH was recorded at 15.57 ± 3.8883 cm in M2, 19.741 ± 6.8786 cm in M3, 25.759 ± 12.3579 cm in M4, 27.63 ± 12.9047 cm in M5, and 39.7 ± 10.2567 cm in M6. Among the plants treated with bat guano, BG3 showed growth trends similar to CDF. Plants treated with BG3 recorded PH values of 12.741 ± 4.1286 cm in M2, 15.519 ± 5.2431 cm in M3, 19.278 ± 6.5696 cm in M4, 24.685 ± 9.6538 cm in M5, and 32.654 ± 6.5044 cm in M6 (Table 5.3).

The ANOVA table comparing PH between the treatment groups (Table 5.4) shows significant differences ($P < 0.001$), with an F-value of 52.507. In Fisher's LSD analysis for PH (Appendix D1), the study observed that CF recorded the highest PH, significantly different from other treatments at $P < 0.05$. Meanwhile, the control plants (NF) recorded the lowest PH. A non-significant difference was observed between the control plants (NF) and BG1, with $P = 0.073$. In conclusion, for the PH parameter, CF recorded the highest, the control plants (NF) the lowest, and no significant difference was found between the control plants (NF) and BG1.

Table 5.3: Changes in the PH as affected by the treatment types observed throughout the cycle of 6 months.

	M1	M2	M3	M4	M5	M6
NF	5	9.4 ± 1.983	10.844 ± 2.185	11.407 ± 2.839	10.981 ± 3.39	0
CF	5	18.333 ± 5.31	28.185 ± 8.473	36.519 ± 11.706	35.13 ± 11.156	42 ± 7.149
CDF	5	15.57 ± 3.888	19.741 ± 6.879	25.759 ± 12.358	27.63 ± 12.905	39.7 ± 10.257
BG1	5	10.852 ± 3.502	12.259 ± 3.977	14.296 ± 5.027	14.889 ± 5.956	38

Table 5.3: continue

BG2	5	11.248 ± 2.74	13 ± 2.728	15.981 ± 3.321	18.685 ± 7.345	34.333 ± 7.638
BG3	5	12.741 ± 4.129	15.519 ± 5.243	19.278 ± 6.57	24.685 ± 9.654	32.654 ± 6.504

Notes: Month 1 (M1), Month 2 (M2), Month 3 (M3), Month 4 (M4), Month 5 (M5), Month 6 (M6), No Fertiliser or Control (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3). Full readings of PH are shown in Appendix B.

Table 5.4: ANOVA results showing the effect of different fertiliser treatments on plant height (PH)

Parameters		Sum of Squares	df	Mean Square	F	Sig.
PH	Between Groups	25068.082	5	5013.616	52.507	<0.001
	Within Groups	79824.643	836	95.484		
	Total	104892.724	841			

5.3.2 Number of Leaves

All germinated seeds were recorded to have the same leaf count after acclimatisation in M1 and M2, with 2 leaves. In M2, plants treated with CF recorded a higher number of leaves (NoL) at 6.67 ± 1.217 and continued to increase in M3 and M4 to 12.59 ± 6.429 and 29.33 ± 16.291 , respectively. The plants treated with CF further increased in M6 to 49.6 ± 25.851 (Table 5.5). Conversely, the control plants (NF) recorded the lowest NoL throughout the study. In M2, the NoL for NF was recorded at 6.37 ± 1.074 , but decreased in M3 and M4 to 5 ± 2.353 and 4.07 ± 2.526 , respectively (Table 5.5). Meanwhile, plants treated with the organic fertiliser BG3

showed a lower NoL than CF, but higher than CDF and the control. The plants treated with BG3 recorded NoL values of 6.52 ± 1.051 in M2, 8.59 ± 0.2707 in M3, 10.63 ± 5.884 in M4, 16.04 ± 12.945 in M5, and 25.85 ± 16.587 in M6. Among the organic fertiliser treatments, CDF showed similar consistency to BG3. Plants treated with CDF were recorded at 6.48 ± 1.445 in M2, 8.44 ± 4.108 in M3, 12.33 ± 8.334 in M4, 9.26 ± 9.816 in M5, and 15.0 ± 6.819 in M6 (Table 5.5).

The ANOVA table for the comparison of NoL between the treatment groups (Table 5.6) shows significant differences ($P < 0.001$) between the groups, with the F-test at 32.977. In Fisher's LSD analysis for NoL (Appendix D2), the study observed that CF recorded the highest NoL compared to other treatments at $P < 0.001$. Meanwhile, the control plants (NF) recorded the lowest. A non-significant difference was found between the control plants (NF) and BG1 at $P = 0.142$. Additionally, the mean difference in NoL for CDF compared to BG1, BG2, and BG3 was also found to be not significant, at $P = 0.111$, $P = 0.716$, and $P = 0.062$, respectively. In conclusion, for the NoL parameter, CF recorded the highest, NF the lowest, and no significant difference was found between the control plants (NF) and BG1, as well as between the organic fertiliser CDF and bat guano treatments.

Table 5.5: Changes in the NoL as affected by the treatment types observed throughout the cycle of 6 months.

	M1	M2	M3	M4	M5	M6
NF	2	6.41 ± 1.074	5 ± 2.353	4.07 ± 2.526	2.52 ± 3.332	0
CF	2	6.67 ± 1.217	12.59 ± 6.429	29.33 ± 16.291	30.22 ± 26.243	49.6 ± 25.851

Table 5.5: continue

CDF	2	6.37 ± 1.445	8.44 ± 4.108	12.33 ± 8.334	9.26 ± 9.816	15 ± 6.819
BG1	2	6.48 ± 1.578	6.52 ± 3.155	6.78 ± 4.594	6.81 ± 6.355	34
BG2	2	6.37 ± 1.214	7.67 ± 2.542	9.11 ± 6.29	10.15 ± 7.784	26.67 ± 7.506
BG3	2	6.52 ± 1.051	8.59 ± 0.271	10.63 ± 5.884	16.04 ± 12.945	25.85 ± 16.587

Notes: Month 1 (M1), Month 2 (M2), Month 3 (M3), Month 4 (M4), Month 5 (M5), Month 6 (M6), No Fertiliser or Control (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3). Full readings of NoL are shown in Appendix B.

Table 5.6: ANOVA results showing the effect of different fertiliser treatments on number of leaves (NoL)

Parameters		Sum of Squares	df	Mean Square	F	Sig.
NoL	Between Groups	18151.393	5	3630.279	32.977	<0.001
	Within Groups	92031.497	836	110.086		
	Total	110182.890	841			

5.3.3 Number of Flower Buds

In M3, plants treated with CF recorded a higher number of buds (NoB) at 26, which continued to increase in M4 to 35 buds. The NoB in plants treated with CF then decreased in M5 and M6 to 20 and 10 buds, respectively (Table 5.7). In contrast, the control plants (NF)

recorded the lowest NoB throughout the study, with no flower buds produced at any stage. Meanwhile, plants treated with the organic fertiliser BG3 showed a lower NoB than CF, but higher than CDF and the control. The plants treated with BG3 recorded a NoB of 21 in M5 and 7 in M6. Among the organic fertiliser treatments, CDF showed similar consistency to CF. Plants treated with CDF recorded an increase in NoB starting in M3 with 1, rising to 6 in M4, 12 in M5, and 1 in M6 (Table 5.7).

The ANOVA table for the comparison of NoB between the treatment groups (Table 5.8) shows significant differences ($P < 0.001$), with the F-test value at 12.893. In Fisher's LSD analysis of NoB (Appendix D3), the study observed that CF recorded the highest NoB compared to other treatments at $P < 0.001$, while the control plants (NF) recorded the lowest. Another significant difference was observed between the NoB of NF and BG3, at $P = 0.041$. In conclusion, for the NoB parameter, CF recorded the highest, NF the lowest, and significant differences were found only between the control plants (NF) and BG3, and CF with other treatments.

Table 5.7: Changes in the NoB as affected by the treatment types observed throughout the cycle of 6 months.

	M1	M2	M3	M4	M5	M6
NF	0	0	0	0	0	0
CF	0	0	26	35	20	10
CDF	0	0	1	6	12	1

Table 5.7: continue

BG1	0	0	0	0	2	0
BG2	0	0	0	0	6	4
BG3	0	0	0	0	21	7

Notes: Month 1 (M1), Month 2 (M2), Month 3 (M3), Month 4 (M4), Month 5 (M5), Month 6 (M6), No Fertiliser or Control (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3). Full readings of NoB are shown in Appendix B.

Table 5.8: ANOVA results showing the effect of different fertiliser treatments on number of flower buds (NoB)

Parameters		Sum of Squares	df	Mean Square	F	Sig.
NoB	Between Groups	38.939	5	7.788	12.893	<0.001
	Within Groups	504.981	836	0.604		
	Total	543.920	841			

5.3.4 Number of Flowers

In M3, plants treated with CF recorded a higher number of flowers (NoFl) at 5, which continued to increase in M4 to 19 flowers. The NoFl in plants treated with CF then continued to decrease in M5 and M6 to 11 and 2 flowers, respectively (Table 5.9). In contrast, the control plants (NF) recorded the lowest NoFl throughout the study, with no flowers produced at any stage. Meanwhile, plants treated with the organic fertiliser BG3 showed a lower NoFl than CF, but higher than CDF, other bat guano treatments, and the control. The plants treated with BG3

initially did not record any NoFl in M3 and M4. However, in M5 and M6, plants treated with BG3 produced 13 flowers and 2 flowers, respectively. Plants treated with CDF recorded a gradual increase in NoFl starting in M3, with 1 flower recorded in each of M3, M4, and M5 (Table 5.9).

The ANOVA table for the comparison of NoFl between the treatment groups (Table 5.10) shows significant differences ($P < 0.001$), with the F-test value at 9.853. In Fisher's LSD analysis of NoFl (Appendix D4), the study observed that plants treated with CF recorded the highest NoFl compared to other treatments at $P < 0.05$. Another significant difference was observed in the mean NoFl for BG3 compared to other treatment types at $P < 0.05$. Meanwhile, mean differences between the control plants (NF) and other treatment types were not significant. In conclusion, for the NoFl parameter, CF recorded the highest, NF the lowest, and significant differences were observed only between BG3 and CF with other treatments.

Table 5.9: Changes in the NoFl as affected by the treatment types observed throughout the cycle of 6 months.

	M1	M2	M3	M4	M5	M6
NF	0	0	0	0	0	0
CF	0	0	5	19	11	2
CDF	0	0	1	1	1	0
BG1	0	0	0	0	1	0
BG2	0	0	0	0	1	1

Table 5.9: continue

BG3	0	0	0	0	13	4
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Notes: Month 1 (M1), Month 2 (M2), Month 3 (M3), Month 4 (M4), Month 5 (M5), Month 6 (M6), No Fertiliser or Control (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3). Full readings of NoFl are shown in Appendix B.

Table 5.10: ANOVA results showing the effect of different fertiliser treatments on number of flowers (NoFl)

Parameters		Sum of Squares	df	Mean Square	F	Sig.
NoFl	Between Groups	7.219	5	1.444	9.853	<0.001
	Within Groups	122.505	836	0.147		
	Total	129.724	841			

5.3.5 Number of Fruits

In M3, plants treated with CF recorded a higher number of fruits (NoFr) at 13 fruits, which continued to increase in M4 to 19 fruits. The NoFr in plants treated with CF then continued with 4 fruits and 6 fruits recorded in M5 and M6, respectively (Table 5.11). Conversely, the control plants (NF) and BG1 recorded the lowest NoFr throughout the study, with no fruits produced at any stage. Meanwhile, plants treated with the organic fertiliser BG3 showed a lower NoFr than CF, but higher than CDF, other bat guano treatments, and the control. The plants treated with BG3 did not record any NoFr in M3, but this increased to 4 fruits in M4,

5 fruits in M5, and 8 fruits in M6. Plants treated with CDF recorded a gradual increase in NoFr, starting from 1 fruit each in M4 and M5, and 2 fruits in M6 (Table 5.11).

The ANOVA table for the comparison of NoFr between the treatment groups (Table 5.12) shows significant differences ($P < 0.001$), with the F-test value at 8.822. In Fisher's LSD analysis of NoFr (Appendix D5), the study observed that plants treated with CF recorded the highest NoFr compared to other treatments at $P < 0.05$. Another significant difference was found in the mean NoFr between NF and BG3 at $P = 0.05$. Meanwhile, the mean difference between the control plants (NF) and BG1 was recorded as non-significant, with a value of $P = 1.000$. In conclusion, for the NoFr parameter, CF recorded the highest, NF the lowest, and significant differences were observed between NF and BG3, and between CF and the other treatments.

Table 5.11: Changes in the NoFr as affected by the treatment types observed throughout the cycle of 6 months.

	M1	M2	M3	M4	M5	M6
NF	0	0	0	0	0	0
CF	0	0	16	19	4	6
CDF	0	0	0	1	1	2
BG1	0	0	0	0	0	0
BG2	0	0	0	0	0	0
BG3	0	0	0	5	4	8

Notes: Month 1 (M1), Month 2 (M2), Month 3 (M3), Month 4 (M4), Month 5 (M5), Month 6 (M6), No Fertiliser or Control (NF), Chemical Fertiliser (CF), Chicken Dung Fertiliser (CDF), Bat Guano (2.5 g) (BG1), Bat Guano (5.0 g) (BG2), and Bat Guano (7.5 g) (BG3). Full readings of NoFr are shown in Appendix B.

Table 5.12: ANOVA results showing the effect of different fertiliser treatments on number of fruits (NoFr).

Parameters		Sum of Squares	df	Mean Square	F	Sig.
NoFr	Between Groups	10.708	5	2.142	8.822	<0.001
	Within Groups	202.960	836	0.243		
	Total	213.669	841			

5.3.6 Control Treatment

The correlation coefficient of plants in the control treatment (no fertiliser) was analysed and is presented in Table 5.3. The results showed that plant height (PH) and number of leaves (NoL) were not significantly correlated, with $r = 0.762$; $P > 0.05$. This indicates that an increase in PH does not necessarily correlate with an increase in NoL. Additionally, the N-P-K levels remained unchanged throughout the experiment, as no fertiliser treatment was applied to the plants up to harvest. Furthermore, the reduction in N-P-K levels was not calculated, as the study considered environmental influences as non-manipulated variables. In this experiment, the non-significant correlation between PH and NoL may be attributed to the naturally low levels of N-P-K in the growth media. This result is consistent with previous reports highlighting poor performance of plants in control treatments (no fertiliser), such as carrots (*Daucus carota* L.) in Poliquit and Calong (2018), and mung beans (*Vigna radiata*) in Shetty et al. (2013).

Table 5.13: Correlation of plant growth and N-P-K composition variables in control treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	a							
P	a	a						
K	a	a	a					
PH	a	a	a	1.000				
NoL	a	a	a	0.762	1.000			
NoB	a	a	a	a	a	a		
NoFl	a	a	a	a	a	a	a	
NoFr	a	a	a	a	a	a	a	a

Note: All analyses are correlation coefficients using the Pearson Correlation Test. ^aCannot be computed because at least one of the variables is constant, Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.3.7 Chemical Fertiliser

The correlation coefficient of plants treated with chemical fertiliser was analysed and is presented in Table 5.4. The results showed a significant correlation between plant height (PH) and the N, P, and K contents, with $r = 0.920$, $r = 0.917$, and $r = 0.920$; $P \leq 0.01$, respectively. This positive correlation indicates that the higher the N-P-K levels, the taller the chilli plants will grow. This finding is supported by Javid et al. (2005), which stated that treatments on *Zinnia* (*Zinnia elegans*) containing high nitrogen levels combined with optimum amounts of

phosphorus and potassium can substantially improve crop growth. Additionally, Yenesew and Tilahun (2009) also noted the positive influence of phosphorus fertiliser on the height of maize crops. The results also showed that the number of leaves (NoL) and the N, P, and K contents were significantly correlated ($P \leq 0.01$), with $r = 0.984$, $r = 0.985$, and $r = 0.984$, respectively. This finding is supported by Olowoboko et al. (2017), which stated that an increase in nitrogen application rate leads to an increase in the number of leaves per plant. The report also highlighted that increasing phosphorus levels can enhance plant growth by stimulating microbial activity involved in the mineralisation and availability of nutrients. High potassium content in the treatments also affected plant height and leaf growth due to its role in maintaining water potential, turgor pressure, and cell elongation, all of which are supported by the availability of potassium in the treatments (Olowoboko et al., 2017).

The analysis also revealed a significant correlation between NoL and PH ($P \leq 0.01$), with $r = 0.906$. This correlation is supported in Falster and Westoby (2003), who reported that increased leaf production enhances light interception and photosynthetic activity, ultimately contributing to increased plant height. Another significant correlation was recorded between the number of buds (NoB) and the number of flowers (NoFl), with $r = 0.882$; $P \leq 0.05$. This suggests that a higher bud count leads to a higher flower count, which is consistent with the active growth phase when flower development begins with bud formation (Reid, 2003).

In this experiment, a non-significant correlation ($P > 0.05$) was observed between NoB, NoFl, and the number of fruits (NoFr), and the N-P-K levels. The correlation coefficients between NoB and N, P, and K were $r = 0.352$, $r = 0.348$, and $r = 0.352$, respectively. For NoFl, the correlations with N, P, and K were $r = 0.299$, $r = 0.297$, and $r = 0.299$, respectively. Finally,

for NoFr, the correlations with N, P, and K were $r = 0.238$, $r = 0.235$, and $r = 0.238$, respectively. However, these results contrast with Jiang et al. (2010), who reported that nitrogen, phosphorus, and potassium treatments were effective in the development of quality chrysanthemum flowers. The difference may be attributed to the onset of the plant's senescence stage after the fourth month of fertiliser application, leading to a reduction in the production of buds, flowers, and fruits. This explanation is supported by Das et al. (2023), who found that the application of N-P-K fertilisers on Indian spinach resulted in a decline in plant growth after the third month.

Table 5.14: Correlation of plant growth and N-P-K composition variables in chemical fertiliser treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	1.000							
P	1.000**	1.000						
K	1.000**	1.000**	1.000					
PH	0.920**	0.917**	0.920**	1.000				
NoL	0.984**	0.985**	0.984**	0.906*	1.000			
NoB	0.352	0.348	0.352	0.641	0.372	1.000		
NoFl	0.299	0.297	0.299	0.556	0.367	0.882*	1.000	
NoFr	0.238	0.235	0.238	0.535	0.273	0.926	0.717	1.000

Note: All analyses are correlation coefficients using the Pearson Correlation Test. *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.3.8 Chicken Dung Fertiliser

The correlation coefficient of plants treated with chicken dung fertiliser was analysed and is presented in Table 5.5. The results showed a significant correlation between plant height (PH) and N, P, and K content, at $r = 0.984$, $r = 0.965$, and $r = 0.984$; $P \leq 0.01$, respectively. The application of chicken dung fertiliser has therefore been proven effective in promoting plant growth, in accordance with Thepsilvisut (2021), where the application of chicken manure on white mugwort led to improved plant height and overall biomass compared to control groups without fertiliser or those treated only with chemical fertilisers. Dubey et al. (2023) also found that the application of chicken dung fertiliser resulted in greater plant height compared to control and cow manure treatments in *Amaranthus dubius* L., with goat manure yielding the highest plant height measurements.

The results also showed that the number of leaves (NoL) and N, P, and K content were significantly correlated ($P \leq 0.05$), at $r = 0.913$, $r = 0.886$, and $r = 0.913$, respectively. According to Dubey et al. (2023), chicken manure treatment resulted in a higher number of leaves compared to no fertiliser and cow dung treatments. While goat manure led to the maximum number of leaves, chicken manure still demonstrated a notable increase in leaf production, confirming its beneficial role in promoting leaf growth. The analysis further showed that the number of fruits (NoFr) and N, P, and K content were significantly correlated ($P \leq 0.01$), at $r = 0.946$, $r = 0.962$, and $r = 0.946$, respectively. This indicates that chicken manure fertiliser positively affects fruit production in plants. According to Thepsilvisut (2021), chicken manure applied to white mugwort resulted in the highest fresh weight throughout the growing season. Additionally, Ibrahim et al. (2013) reported that the use of chicken dung fertiliser enhanced the

antioxidant activity of plants and improved secondary metabolite production compared to chemical fertiliser treatments.

In this experiment, a significant correlation was also recorded between NoL and PH at $r = 0.961$; $P \leq 0.01$. This positive correlation was similarly reported by Emam et al. (2020) and Thepsilvisut (2021), involving the application of chicken manure to plant growth. A significant correlation was also observed between NoFr and PH at $r = 0.912$; $P \leq 0.05$. The nutrient richness of chicken manure, which promotes vegetative growth, flowering, fruit production, and overall plant health, can enhance soil conditions and, in turn, support taller plant growth and higher fruit yields (Moridani et al., 2023). Meanwhile, NoL and NoFr also showed a significant correlation at $r = 0.862$; $P \leq 0.05$. Several studies have shown that high applications of chicken manure led to increased chlorophyll production and higher leaf counts, resulting in a larger photosynthetic surface area. This improves the plant's ability to capture sunlight, convert it into energy, and ultimately results in higher yields (Taokaenchan et al., 2020; Thepsilvisut, 2021).

However, a non-significant correlation ($P > 0.05$) was recorded between the number of buds (NoB), the number of flowers (NoFl), and N-P-K levels. The correlation coefficients between NoB and N, P, and K were $r = 0.398$, $r = 0.384$, and $r = 0.398$, respectively. Meanwhile, the correlation coefficients between NoFl and N, P, and K were $r = 0.167$, $r = 0.126$, and $r = 0.167$, respectively. The non-significant correlations for NoB and NoFl with N-P-K may be due to the low counts of flower buds and flowers in plants treated with chicken dung fertiliser (CDF). The incorporation of CDF into the growth media might be affecting individual plants differently, as the development of flower buds into flowers is regulated by plant hormones such as auxins, gibberellins, and cytokinins. This suggests that, while chicken manure provides nutrients to

support vegetative growth and bud formation, the conversion of buds into flowers can vary depending on how these hormones interact within the plant's physiological context (Srivastava, 2002).

Table 5.15: Correlation of plant growth and N-P-K composition variables in chicken dung fertiliser treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	1.000							
P	0.996**	1.000						
K	1.000**	0.996**	1.000					
PH	0.984**	0.965**	0.984**	1.000				
NoL	0.913*	0.886*	0.913*	0.961**	1.000			
NoB	0.398	0.384	0.398	0.357	0.280	1.000		
NoFl	0.167	0.126	0.167	0.199	0.267	0.684	1.000	
NoFr	0.946**	0.962**	0.946**	0.912*	0.862*	0.340	0.000	1.000

Note: All analyses are correlation coefficients using the Pearson Correlation Test. * Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed),^a Cannot be computed because at least one of the variables is constant, Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.3.9 Bat Guano 1 (2.5 g)

The correlation coefficient of plants treated with 2.5 g of bat guano was analysed and is presented in Table 5.6. The results showed a significant correlation between plant height (PH)

and nitrogen (N), phosphorus (P), and potassium (K) content, at $r = 0.908$, $r = 0.913$, and $r = 0.910$; $P < 0.05$, respectively. This correlation indicates that the increase in plant height is attributed to the high nutrient content of bat guano fertiliser applied to the plants. Palita et al. (2021) found that okra treated with a low rate of cave bat guano showed lower plant growth and fruit production compared to treatments with a higher rate. The study attributed these differences to the nutrient content of bat guano, which is rich in N, P, and K.

The results also showed that the number of leaves (NoL) and N, P, and K content were significantly correlated ($P \leq 0.05$) at $r = 0.826$, $r = 0.842$, and $r = 0.830$, respectively. The application of bat guano in the growth medium has been proven to positively influence leaf production during plant growth. According to Afa (2016), the cultivation of spring onion using bat guano treatments demonstrated that a lower amount of guano led to a reduced number of leaves, although the difference was not significant compared to treatments with higher guano levels.

Simultaneously, a significant correlation was also observed between NoL and PH at $r = 0.985$; $P \leq 0.01$. In addition to plant phenology, which naturally influences the relationship between leaf number and stem height, the increased availability of nutrients in the growth media supports leaf development. This, in turn, improves the plant's energy capacity to enhance stem height (Dimande et al., 2023). Another significant correlation was recorded between the number of flower buds (NoB) and the number of flowers (NoFl) at $r = 1.000$; $P < 0.01$. This strong correlation is justifiable as the growth and development of flower buds are crucial in determining the eventual number of *Geranium koreanum* flowers (Lu et al., 2023).

However, non-significant correlations ($P > 0.05$) were recorded between NoB and NoFl and the N-P-K levels. The correlation coefficients for NoB and N, P, and K were $r = 0.302$, $r = 0.297$, and $r = 0.302$, respectively. Similarly, the correlations between NoFl and N, P, and K were $r = 0.302$, $r = 0.297$, and $r = 0.302$, respectively. This may be due to the low level of N-P-K in the 2.5 g bat guano treatment, which may not have significantly affected flower bud development or flower production. Korn et al. (2023) also reported that lower application rates of bat guano in tomato cultivation resulted in lower fruit productivity.

Non-significant correlations ($P > 0.05$) were also recorded between NoB and the number of fruits (NoFr), and PH at $r = -0.043$. Meanwhile, non-significant correlations were observed between NoL and NoB, and between NoL and NoFl, both at $r = -0.152$; $P > 0.05$. These results may be attributed to limited nutrient allocation (particularly N-P-K) during the budding, flowering, and fruiting stages. In some cases, plants may prioritise vegetative growth over reproductive development, resulting in discrepancies between flower bud and flower numbers, even when nutrients are applied (Zhang and Wei, 2022). This phenomenon may also affect fruit production in plants treated with 2.5 g of bat guano. Additionally, the failure of some flowers to develop into chilli fruits may be linked to nutrient deficiencies in the media. Adequate levels of essential nutrients, particularly N-P-K, are crucial for flower development and fruit set. Therefore, deficiencies or imbalances in these nutrients can inhibit the successful transition from flowers to fruits (Marschner, 2011).

Table 5.16: Correlation of plant growth and N-P-K composition variables in bat guano (2.5 g) treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	1.000							
P	0.992**	1.000						
K	1.000**	0.994**	1.000					
PH	0.908*	0.913*	0.910*	1.000				
NoL	0.826*	0.842*	0.830*	0.985**	1.000			
NoB	0.302	0.297	0.302	-0.043	-0.152	1.000		
NoFl	0.302	0.297	0.302	-0.043	-0.152	1.000**	1.000	
NoFr	a	a	a	a	a	a	a	a

Note: All analyses are correlation coefficients using the Pearson Correlation Test. * Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ^aCannot be computed because at least one of the variables is constant, Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.3.10 Bat Guano 2 (5.0 g)

The correlation coefficient of plants treated with 5.0 g of bat guano was analysed and is presented in Table 5.7. The results showed a significant correlation between plant height (PH) and nitrogen (N), phosphorus (P), and potassium (K) content at $r = 0.967$; $P \leq 0.01$, respectively. Bat guano was shown to have a positive effect on plant height, as reported by Sothearen et al. (2014), where four out of five plant species studied in the experiment—including *Artocarpus*

heterophyllus, *Solanum melongena*, *Carica papaya*, and *Dimocarpus longan*—experienced greater weekly height increments compared to control plants.

The results also showed that the number of leaves (NoL) and N, P, and K content were significantly correlated ($P \leq 0.01$) at $r = 0.920$, $r = 0.924$, and $r = 0.921$, respectively. Poliquit and Calong (2018) similarly reported that an optimal ratio of bat guano and soil in the growth medium can enhance foliage production. Therefore, the findings of this study align with their report, indicating that the higher the bat guano content incorporated into the growth medium, the greater the number of leaves produced by the plants. At the same time, the results showed that the number of flowers (NoFl) and N, P, and K content were significantly correlated at $r = 0.837$, $r = 0.850$, and $r = 0.840$; $P \leq 0.05$, respectively. The number of flowers increased in response to rising N-P-K levels. Shetty et al. (2013) also reported the positive effect of guano treatment on flowering in a study on *Vigna radiata*. In that study, *Vigna radiata* treated with guano and autoclaved soil exhibited enhanced growth, particularly in flowering, compared to control plants grown only in autoclaved soil.

In this experiment, NoL and PH were also significantly correlated at $r = 0.988$; $P \leq 0.01$. This correlation indicates that during plant development, an increase in plant height contributes to greater leaf production and vice versa (Taiz et al., 2015). Furthermore, the number of flower buds (NoB) and number of flowers (NoFl) were significantly correlated at $r = 0.971$; $P \leq 0.01$. These correlations reflect the reproductive potential of the plant, in which a high number of buds increases the potential for flower formation (Taiz et al., 2015).

However, a non-significant correlation ($P > 0.05$) was recorded between NoB and N-P-K levels. The correlation coefficients between NoB and N, P, and K were $r = 0.744$, $r = 0.753$, and $r = 0.745$, respectively. This may be related to the low number of flower buds recorded during the study. Theoretically, under optimal conditions of temperature, light, and nutrient availability, the development time from flower buds to flowers may be shortened (Dorji et al., 2005). Therefore, the lack of fruit yields observed in this study might be attributed to insufficient nutrient availability in the growth media, which may have been inadequate to support the development of chillies from flowers (Poorter and Nagel, 2000).

Table 5.17: Correlation of plant growth and N-P-K composition variables in bat guano (5.0 g) treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	1.000							
P	0.998	1.000						
K	1.000**	0.999**	1.000					
PH	0.967**	0.967**	0.967**	1.000				
NoL	0.920**	0.924**	0.921**	0.988**	1.000			
NoB	0.744	0.753	0.745	0.648	0.570	1.000		
NoFl	0.837*	0.850*	0.840*	0.789	0.737	0.971**	1.000	
NoFr	a	a	a	a	a	a	a	a

Note: All analyses are correlation coefficients using the Pearson Correlation Test. * Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ^a Cannot be computed because at least one of the variables is constant, Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.3.11 Bat guano 3 (7.5 g)

The correlation coefficient of plants treated with 7.5 g of bat guano was analysed and is presented in Table 5.8. The results show a significant correlation between plant height (PH) and nitrogen (N), phosphorus (P), and potassium (K) content at $r = 0.993$, $r = 0.988$, and $r = 0.992$; $P \leq 0.01$, respectively. Plant height was significantly increased by the N-P-K concentration in the growth media. Other studies have also shown that nutrient-rich fertilisers, such as bat guano, can effectively enhance plant growth, including plant height. Specifically, guano treatments at optimal concentrations have been observed to increase plant height. For instance, research on the application of guano has demonstrated improvements in the height of various plants due to its rich nutrient profile, which includes essential macro- and micronutrients that promote healthy and robust plant development (Adam et al., 2020).

The results also showed significant correlations ($P \leq 0.01$) between the number of leaves (NoL) and N-P-K levels at $r = 0.986$, $r = 0.987$, and $r = 0.986$, respectively. Bat guano has been shown to significantly affect the number of leaves in this plant. Studies have demonstrated that the application of bat guano can lead to an increase in leaf count due to its rich nutrient content, which enhances plant growth and development. For example, a study on hybrid sunflower cultivars found that bat guano treatments resulted in higher leaf area indices compared to controls, indicating more robust leaf development (Sothearen et al., 2014).

At the same time, the results also showed that the number of fruits (NoFr) and N, P, and K content were significantly correlated at $r = 0.925$, $r = 0.932$, and $r = 0.926$; $P \leq 0.01$, respectively. A study on okra (*Abelmoschus esculentus*) demonstrated that applying bat guano

as a fertiliser significantly increases fruit yield. In that study, bat guano applied at 5 tonnes/ha and 10 tonnes/ha resulted in higher fruit yields compared to the control treatment, which did not receive any guano. Dimande et al. (2023) reported that the highest okra yields, averaging over 10 tonnes/ha, were observed in plots with higher rates of guano application.

In this study, NoL and PH were also significantly correlated at $r = 0.983$; $P \leq 0.01$. The increase in plant height could be due to the synergistic effect of energy production derived from the plant's photosynthetic capacity, which is a result of enhanced leaf development (Taokaenchan et al., 2020). The number of flower buds (NoB) and number of flowers (NoFl) were also significantly correlated at $r = 1.000$; $P \leq 0.01$. A study on apricots highlighted the significant effects of flower bud development on the final number of flowers. The study indicated that the quality and weight of flower buds, influenced by nutrient storage and carbohydrate metabolism, play a crucial role in flower formation and the overall reproductive success of the plant (Zhang and Wei, 2022).

Additionally, significant correlations were recorded between NoFr and PH at $r = 0.894$; $P \leq 0.05$, and between NoFr and NoL at $r = 0.898$; $P \leq 0.05$. These correlations suggest the influence of plant height and number of leaves on fruit production, specifically in plants treated with 7.5 g of bat guano. The significant correlation might be attributed to the photosynthetic capabilities of plants treated with guano, which aided in energy generation and consequently improved yield production (Palita et al., 2021).

However, non-significant correlations ($P > 0.05$) were recorded between NoB and NoFl and N-P-K levels. The correlation coefficients between the number of flower buds and N, P,

and K were $r = 0.561$, $r = 0.564$, and $r = 0.561$, respectively. Meanwhile, for the number of flowers and N, P, and K, the correlations were $r = 0.543$, $r = 0.546$, and $r = 0.543$, respectively. The non-significant correlation could be due to nutrient imbalances in individual plants, which in turn affect flower production. Excessive or insufficient amounts of these nutrients can disrupt the balance needed for optimal flowering. For example, research in potato farming indicates that high nitrogen levels encourage vegetative development rather than reproductive growth. This imbalance can hinder the plant's ability to produce flowers and fruits, thus affecting overall yield (Hopkins et al., 2020).

Table 5.18: Correlation of plant growth and N-P-K composition variables in bat guano (7.5 g) treatment

	N	P	K	PH	NoL	NoB	NoFl	NoFr
N	1.000							
P	0.999**	1.000						
K	1.000**	0.999**	1.000					
PH	0.993**	0.988**	0.992**	1.000				
NoL	0.986**	0.987**	0.986**	0.983**	1.000			
NoB	0.561	0.564	0.561	0.574	0.543	1.000		
NoFl	0.543	0.546	0.543	0.557	0.523	1.000**	1.000	
NoFr	0.925**	0.932**	0.926**	0.894*	0.898*	0.425	0.406	1.000

Note: All analyses are correlation coefficients using the Pearson Correlation Test. * Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ^a Cannot be computed because at least one of the variables is constant, Nitrogen (N), Phosphorus (P), Potassium (K), Plant Height (PH), Number of Leaves (NoL), Number of Flower Buds (NoB), Number of Flowers (NoFl), and Number of Fruits (NoFr).

5.4 Discussion

The results of plant growth performance demonstrate the efficacy of chemical fertiliser (CF). When observing the growth trends of chilli plants across all parameters, those treated with CF consistently outperformed other treatments in the study. Vegetative growth, reproductive development, and yield production were significantly increased with CF treatments. This finding aligns with previous studies that highlighted the positive impact of chemical fertilisers on crop growth (Javid et al., 2005; Yenesew and Tilahun, 2009).

BG3, or bat guano at 7.5 g, was found to perform better than other organic treatments (CDF, BG1, and BG2) in most parameters, such as plant height, number of leaves, and number of fruits. An increase in flower buds and flower counts was also observed under BG3 treatment during the later stages of the growth cycle. Although BG3 was not as effective as chemical fertiliser in promoting plant responses, a gradual but steady improvement was evident. This suggests its potential as a sustainable and eco-friendly fertiliser. These findings support the assertion that higher application rates of bat guano can enhance crop production (Dimande et al., 2023).

Treatments involving chicken dung fertiliser (CDF) showed moderate effects on chilli plant growth. Although it performed better than the control and BG1 treatments, it did not significantly outperform BG3 or CF. Notable improvements in vegetative growth were observed during the early stages of the cycle. However, this trend did not extend to flower and fruit production. This could indicate a slower nutrient release or a nutrient imbalance in the CDF treatment when compared with CF and BG3. Organic fertilisers are considered slow releasing,

which benefits crop growth and soil nutrition by extending nitrogen availability (Masarirambi et al., 2012). Nevertheless, identifying a suitable organic fertiliser that provides an optimal release rate while being environmentally safe remains essential for meeting crop production demands.

Control treatments (NF) showed consistent underperformance throughout the cycle. No flowering or fruiting was recorded in plants that received no treatment. Without fertiliser, the nutrient levels in the soil were insufficient to support plant growth. Previous studies have established the critical levels of nutrients required for optimal plant growth—N: 3%, K: 0.75 to 1.25% (Bush et al., 2016). The absence of supplementation severely affected crop growth, with untreated plants deteriorating over time (Poliquit and Calong, 2018; Shetty et al., 2013).

Statistical comparisons of growth parameters revealed significant differences in all treatments involving CF. In contrast, some comparisons among lower-performing treatments (NF, BG1, and BG2) were not statistically significant, suggesting similar performance likely due to low nutrient availability. In terms of correlations between N-P-K levels and plant growth parameters, a strong and significant relationship was found between plant height and the levels of nitrogen, phosphorus, and potassium for all treatments except the control. This implies that fertiliser treatments significantly promote stem elongation, likely due to their N-P-K content. Potassium, in particular, is known to support vegetative growth and cell elongation (Olowoboko et al., 2017). Similarly, strong correlations between the number of leaves and N-P-K levels across fertilised treatments suggest that leaf production is highly influenced by nutrient availability. This supports the roles of nitrogen in leaf development, phosphorus in root and

energy transfer, and potassium in overall plant health (Sothearen et al., 2014; Ojobor and Egbuchua, 2021).

A significant correlation was also found between flower buds and flowers, indicating that nearly all flower buds developed into flowers. This is consistent with previous research, which showed that the growth and development of flower buds determine the final number of flowers (Lu et al., 2023). However, the non-significant correlations between N-P-K levels and both flower buds and flower counts suggest that other factors may influence flower initiation in this study. Flowering may be more dependent on hormonal balances, photoperiod, or internal carbohydrate levels than on N-P-K levels at the tested fertiliser rates (Zhang and Wei, 2022).

Significant positive correlations between fruit production and N-P-K levels were also revealed through the correlation coefficient analysis. This indicates that higher nutrient levels contribute to increased fruit yields. High nutrient availability not only affects the number of fruits but also supports sustained fruit development and maturation (Ayodele et al., 2015). The significant correlations between fruit yield and both plant height and number of leaves suggest that vegetative vigour influences reproductive success. Well-developed plants with a greater number of photosynthetic tissues (more leaves) and stronger structural support (taller stems) are more likely to produce higher fruit yields due to greater energy synthesis (Taokaenchan et al., 2020; Thepsilvisut, 2021; Palita et al., 2021).

Therefore, applying fertilisers with higher N-P-K values at optimal rates has been shown to positively influence stem growth, foliage development, and yield production. While chemical fertilisers remain the most effective in this study, organic alternatives such as bat guano warrant

further exploration for their environmental benefits. Concerns over water and air pollution caused by chemical fertilisers underline the importance of sustainable practices (Ekinici et al., 2019). Additionally, the low cost and wide availability of bat guano make it an attractive alternative for sustainable farming, especially among smallholder farmers. In some regions, guano can be obtained for less than RM 5.00 per kilogram, highlighting its potential as a cost-effective and environmentally friendly fertiliser (Suwannarong et al., 2020; Harouna et al., 2021).

5.5 Conclusion

In conclusion, this study highlights the significant impact of fertiliser treatments on the growth performance and productivity of chilli plants. The analysis of plant height (PH) revealed that commercial fertiliser (CF) consistently produced the tallest plants throughout the growth cycle, with a noticeable increase in height observed month by month. In contrast, plants in the control treatment (no fertiliser, NF) consistently recorded the lowest height measurements, demonstrating the importance of fertilisers in promoting plant growth. Organic fertilisers, such as chicken dung fertiliser (CDF) and bat guano (BG), also positively influenced plant height, with a steady increase observed in stem height.

Similarly, the analysis of the number of leaves demonstrated the influence of fertilisers on foliage production, with CF producing the highest number of leaves compared to other treatments. The control treatment (NF) again resulted in the lowest leaf counts, reinforcing the role of fertilisers in stimulating vegetative growth. While CDF and BG3 also enhanced leaf production, CF remained the most effective overall. Moreover, the analysis of flower buds and

flower counts revealed significant differences among the treatments, with CF again leading in both parameters, followed by BG3 in promoting flower development. Fruit yield analysis further underscored the importance of fertiliser, with CF producing the highest number of fruits, followed by BG3, which also showed promising results in enhancing fruit production.

The significant correlations observed between N-P-K levels and various growth traits such as plant height, leaf number, and fruit yield highlight the crucial role of these nutrients in chilli plant development. However, the lack of significant correlations between N-P-K levels and flower bud or flower counts suggests that other physiological or environmental factors may influence floral induction. Overall, while chemical fertilisers remain the most effective, bat guano emerges as a viable, cost-effective, and environmentally friendly alternative for sustainable agriculture. Its ability to promote plant growth with a reduced ecological footprint supports the need for further research and optimisation for broader agricultural use.

Future studies should investigate the application of a wider range of bat guano treatments, extending up to 10 g per plant, to identify the optimal dosage for maximising growth and yield. Furthermore, prolonged comparisons between chemical fertiliser and bat guano treatments are recommended to evaluate their long-term effects on soil nutrient composition. These studies should also incorporate post-harvest soil analyses, focusing particularly on microbial activity and micronutrient dynamics, to provide deeper insights into the sustainability and ecological implications of each fertiliser type.

CHAPTER 6

GENERAL DISCUSSION

6.1 General discussion

The application of different fertiliser treatments had a notable effect on the growth trends of chilli plants, with chemical fertiliser (CF), which contained the highest levels of N-P-K (N: 10 %, P: 14.733 %, K: 15.767 %), demonstrating the most significant positive impact across all measured growth parameters. Plants treated with CF showed a consistent and progressive increase in plant height (PH) following the start of the monthly treatments, with PH values steadily rising from month to month. Similarly, the number of leaves (NoL) in CF-treated plants was the highest among all treatments, with a continuous increase observed throughout the growth cycle. The findings align with previous research highlighting that high nitrogen content, combined with optimal levels of phosphorus and potassium, substantially enhances crop growth and productivity (Javid et al., 2005).

In terms of reproductive development, CF treatments led to the highest number of flower buds (NoB) and flowers (NoFl), with the number of flower buds peaking before declining in later months, following the natural flowering cycle. The number of flowers also rose initially before decreasing in the subsequent months. Fruit production (NoFr) followed a similar trend, with CF-treated plants producing the highest number of fruits during the peak months, followed by smaller yields later in the cycle. Yield production was recorded to have a significant impact from the usage of chemical fertiliser (Ji et al., 2023). The correlation coefficient analysis confirmed a strong and significant relationship between plant height and the levels of nitrogen,

phosphorus, and potassium, highlighting the critical role of nutrient availability in promoting robust vegetative growth and overall plant development. Hence some studies mentioned the irreplaceability of chemical fertiliser due to the demand of food security, however, the usage of organic fertilisers should be utilised where it is necessary (Roberts, 2009; Ji et al., 2023).

The best organic fertiliser option identified in this study was bat guano applied at 7.5 g per plant. Two types of bat guano were analysed for their chemical composition. Bat guano collected from Wind Cave Nature Reserve had N-P-K values of $2.957 \% \pm 0.029 \%$, $2.073 \% \pm 0.043 \%$, and $0.397 \% \pm 0.017 \%$, respectively, while bat guano from Raya Cave had a chemical composition of N: $3.47 \% \pm 0.052 \%$, P: $2.247 \% \pm 0.123 \%$, and K: $0.857 \% \pm 0.047 \%$. This indicates that the bat guano from Raya Cave possessed higher chemical values than that from Wind Cave Nature Reserve. These differences could be influenced by environmental factors at the collection sites or by the diet and species of the bats producing the guano (Ferreira and Martins, 1999; Shetty et al., 2013; Jayasvasti and Jayasvasti, 2018).

When comparing the acoustic data recorded from the two caves, similar bat colonies were found inhabiting both areas, with only a few species recorded exclusively in one cave. Species recorded at Wind Cave Nature Reserve (WCNR) but not at Raya Cave (RC) included *Hipposideros cervinus*, *Rhinolophus philippinensis*, and *Emballonura* sp. Conversely, *Rhinolophus pusillus* and *Rhinolophus trifolius* were recorded at RC but not at WCNR. Previous surveys of WCNR by Mohd-Ridwan et al. (2011) also did not report the presence of *R. pusillus* and *R. trifolius*. Analysis of the bat guano revealed higher nitrogen (N) levels compared to phosphorus (P) and potassium (K) levels at both sites, supporting the likelihood of a predominantly insectivorous bat colony presence, as insectivorous or omnivorous bats

typically produce guano with higher nitrogen content compared to frugivorous bats (Studier et al., 1994).

However, several bat species recorded in earlier studies were not detected in the acoustic recordings of this study (Mohd-Ridwan et al., 2011). Previous records indicated the presence of frugivorous bats near WCNR, which could partly explain the differences in the N-P-K composition of bat guano between the two sites. Meanwhile, no prior records of frugivorous bats were available for RC, as this study represents the first bat survey conducted there. Environmental influences, such as the presence of skylights mapped by LiDAR in WCNR (Shazali, 2017), may also affect the chemical composition of bat guano, as sunlight and aeration can alter nutrient levels. Furthermore, human activities, particularly cave tourism, may disrupt the cave's ecological balance, potentially impacting the N-P-K values of guano (Furey and Racey, 2016).

N-P-K values are crucial for the plant growth particularly in the chilli plant treatments examined in this study. The application of 7.5 g of bat guano demonstrated a consistent improvement in chilli plant growth compared to other organic fertiliser treatments. Plants treated with BG3 showed a steady increase in plant height and leaf production across the growth cycle. Robust increases in plant height and the number of leaves were observed throughout the treatment months, supporting previous findings that optimum levels of bat guano significantly enhance plant height and leaf development (Sothearen et al., 2014; Adam et al., 2020).

In reproductive development, plants treated with BG3 recorded higher flower bud counts in month 5 and a reduced number in month 6. These plants also produced more flowers in month

5 before declining in month 6. Fruit production was initially absent in month 3 but began to increase in month 4, continuing to rise in the following months. Other studies have similarly reported that the application of higher levels of bat guano can enhance fruit yield (Dimande et al., 2023). Correlation coefficient analysis revealed significant positive relationships between plant height, number of leaves, number of fruits, and N-P-K content, highlighting the critical role of nutrient availability in plant development.

Among the organic fertiliser treatments, chicken dung fertiliser (CDF) exhibited consistent growth trends similar to those observed with the BG3 treatment. Dubey et al. (2023) also reported that the application of chicken dung fertiliser resulted in greater plant height compared to the control treatment and cow manure. Plants treated with CDF showed steady development in plant height over the months. Similarly, the number of leaves increased progressively, reflecting improved vegetative growth. Reproductive development also followed an upward trend, with flower buds (NoB) beginning to emerge in month 3, increasing through month 5, before declining slightly in month 6. The number of flowers (NoFl) also began to appear from month 3, with a consistent presence across the following months. Fruit production (NoFr) started to increase from month 4, continuing into months 5 and 6. The nutrient richness of chicken manure, which promotes vegetative growth, flowering, fruit production, and overall plant health, can enhance soil conditions and, in turn, support taller plant growth and increased fruit yield (Moridani et al., 2023).

When comparing the three treatments, chemical fertiliser was found to be more effective than the two organic fertilisers—bat guano and chicken dung fertiliser—in terms of fast-growing effects of plants. However, the environmental drawbacks associated with chemical

fertilisers outweigh their rapid crop growth benefits. Prolonged use of chemical fertilisers has been shown to be ineffective in sustaining soil nutrient levels (Kang et al., 2005). This can lead to significant negative impacts on crop yield as soil fertility declines (Shetty et al., 2013) and may also result in a build-up of salt toxicity in the soil, causing nutrient imbalances that hinder plant growth (Poliquit and Calong, 2018). Additionally, the use of chemical fertilisers contributes to broader environmental concerns, including ecological imbalance in agricultural lands, pollution, disruptions to biogeochemical cycles, and global warming (Bryant and Kincheloe, 1983; Schroter et al., 2005).

The use of organic fertilisers such as bat guano (7.5g) and chicken dung is considerably more beneficial to the environment. Based on the findings of this study, bat guano at the 7.5g application rate demonstrated more significant effects on plant growth compared to chicken dung fertiliser. In Kuching area, bat guano collected from Raya Cave was found to have a higher nutrient content than that from Wind Cave Nature Reserve. The predominance of insectivorous bats, contribute to higher nitrogen levels in the guano, as indicated by the chemical analysis. Future studies could explore comparisons between guano from insectivorous and frugivorous bats to better understand differences in chemical composition and their effects on plant growth. Additionally, research into the application of various types and concentrations of organic fertilisers could provide further insight into identifying the most effective options available on the market.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 General Conclusion

Through acoustic monitoring and secondary data from previous records, it was found that there are differences in bat species contributing to the guano deposits at Wind Cave Nature Reserve (WCNR) and Raya Cave (RC). These differences in bat species are crucial, as they influence the chemical composition of the guano produced. Chemical analysis revealed significant differences between the guano from WCNR and RC, as well as when compared to other established commercial fertilisers. The guano from RC, in particular, exhibited higher levels of essential nutrients when compared to guano collected from WCNR. Which supports the idea that the locality and species composition plays a role in determining the nutrient quality of guano. Furthermore, plant growth trials demonstrated that bat guano at an optimised rate significantly enhanced plant performance compared to other organic fertilisers. While it may not be more superior than chemical fertilisers, it provides a better alternative for a long-term treatment. Plants treated with bat guano at the rate of 7.5g, showed notable improvements in plant height, leaf development, and fruit production, thereby confirming its potential as a high-value organic fertiliser.

Based on this study, bat guano has similar implication on plant growth performance when compared with chicken dung fertilisers. The objectives of this study were achieved where the analysis of chemical compositions (dry matter, organic matter, nitrogen content, phosphorus content, and potassium content) of cave bats guano was done compared to other established

commercial fertilisers. Other than that, the effects of chemical compositions of bat guano on *Capsicum annuum* L. var. Kulai plant growth performance (plant height, number of leaves, number of flower buds, number of flowers, and number of fruits) and comparisons with other established commercial fertilisers were measured accordingly throughout 6 months of the growth cycle.

7.2 Identification of bat species using acoustic recordings

The study of bats' echolocation calls is crucial for non-invasive identification of bat species and habitat protection. The sonograms analysed revealed key parameters such as peak frequency, start frequency, end frequency, pulse duration, and interpulse interval, which helped in identifying and classifying the bat species present. The identification process indicated a higher number of Rhinolophids, with *Rhinolophus affinis* having the highest combined counts. *Hipposiderus larvatus* was recorded the most at Wind Cave Nature Reserve, whereas *Emballonura* sp. had the lowest counts across both sites. The presence of *Myotis* species was also confirmed through sonogram analysis.

Acoustic recordings proved to be an effective method for evaluating the diversity of bat species contributing to guano piles at the study sites, providing important insights into the composition of bat communities. Observations from Wind Cave Nature Reserve were largely in line with previous research, though the identification of *Rhinolophus phillippinensis* marked a new finding. Conversely, the data from Raya Cave represented the first acoustic record of bat species in that location, highlighting the necessity for further surveys to explore bat diversity there. The dominance of *Hipposideridae* and *Rhinolophidae* species, together with site-specific

echolocation features, is consistent with known acoustic behaviour of Southeast Asian cave-dwelling bats. However, factors such as overlapping echolocation frequencies, the absence of frugivorous species in the recordings, and limitations in species identification due to regional call variation demonstrate the importance of combining acoustic methods with physical capture-and-release techniques. While historical data supported species verification at Wind Cave, the lack of the information for Raya Cave presents a valuable area for future investigation.

Understanding the variations in bat guano's chemical composition due to dietary differences is essential. The identification of bat species through acoustic recordings provided additional information related to the chemical composition of the bat guano collected. This information is vital for strategic planning in bat habitat conservation. By identifying the bat species present at the guano collection sites, researchers can determine the causes of differences in the chemical composition of the bat guano. This study highlights the importance of non-invasive techniques in bat conservation and provides crucial data for protecting bat habitats through informed decision-making.

7.3 Chemical compositions of cave bats' guano, chicken dung and chemical fertiliser

The chemical analysis confirmed that synthetic fertilisers contained high concentrations of nitrogen (N), phosphorus (P), and potassium (K), as expected from commercially manufactured products designed to maximise plant growth. However, they also showed notably low organic matter content. In contrast, all organic fertilisers—including chicken dung fertiliser (CDF) and bat guano—shows lower dry matter and nutrient levels overall. Between the organic options, bat guano samples demonstrated slightly higher nitrogen and phosphorus contents

compared to CDF, while CDF contained a higher potassium concentration. Among the two bat guano sources, the sample from Raya Cave showed higher N, P, and K levels than that from Wind Cave Nature Reserve, likely influenced by factors such as bat diet, guano age, species composition, and local environmental conditions.

Lastly, the soil samples analysis shows low levels of total N, available P, and exchangeable K. The organic matter percentage of soil samples was also analysed to be the lowest among other fertiliser treatments. However, the dry matter of soil samples is approximately similar to the dry matter of BGWCR which could suggest a high level of moisture content of soil samples. Assessing the quality of fertilisers and soil samples is vital to ascertain the level of fertiliser usage, quality and impact of the treatments on plant growth performance generally. Promoting the use of organic fertilisers through education, standardised management, and supportive legislation is essential for sustainable agricultural practices. Concurrently, further research into the chemical composition and processing of bat guano, particularly in Malaysian contexts, could enhance its potential as a valuable organic fertiliser and contribute to improved soil health beyond conventional nutrient parameters.

7.4 Effects cave bats' guano, chicken dung, and chemical fertiliser on chilli plant

This study highlights the significant influence of fertiliser treatments on the growth performance and productivity of chilli plants. Analysis of plant height revealed that chemical fertiliser (CF) consistently produced the tallest plants throughout the growth cycle, with a steady monthly increase. In contrast, the control group (no fertiliser, NF) recorded the lowest plant heights, highlighting the critical role of fertilisers in supporting vegetative development.

Organic fertilisers such as chicken dung fertiliser (CDF) and bat guano (BG) also contributed positively to stem growth, with a progressive increase in height observed over time.

Similarly, leaf count analysis shows that fertiliser application significantly boosted foliage production. CF resulted in the highest number of leaves, while NF again showed the lowest values, confirming the necessity of nutrient supplementation for optimal vegetative output. Both CDF and BG3 enhanced leaf production, though CF remained the most effective treatment. Flower bud and flower count analyses also revealed substantial differences among treatments, with CF leading in both parameters, followed by BG3, which showed promising results in stimulating floral development. Fruit yield analysis further reinforced the importance of fertilisation, with CF producing the highest number of fruits, closely followed by BG3.

Significant correlations between N-P-K concentrations and key growth traits—such as plant height, leaf number, and fruit yield—signifies the essential role these macronutrients play in plant development. However, the absence of strong correlations between N-P-K levels and floral parameters suggests that additional physiological or environmental factors may influence flowering. While chemical fertilisers demonstrated the highest effectiveness overall, bat guano presents itself as a viable, cost-effective, and environmentally sustainable alternative. Its ability to support plant growth while minimising ecological harm opens up area for continued research and potential integration into sustainable agricultural practices.

7.5 Recommendations for future research.

Several additional treatments and parameters could be implemented to further explore the overall effects of cave bats' guano on plant growth performance. Studies on a variety of

organic fertilisers could serve as a foundation for determining the most effective and suitable organic fertiliser options available on the market. The study could incorporate a variety of plant species—such as spinach, brinjal, banana, and papaya—to evaluate the broader applicability of bat guano as a fertiliser in agriculture. Different application rates of bat guano, extending up to 10 g, should be tested to determine the optimal dosage for promoting plant growth.

Long-term comparative studies between chemical fertilisers and bat guano are also recommended to assess their respective impacts on soil nutrient profiles over time. These studies should include post-harvest soil analyses to gain a more comprehensive understanding of each fertiliser's contribution to soil health and sustainability. Other abiotic factors, such as light and water, could be incorporated to assess their combined effects with fertiliser treatments on plant performance under realistic environmental conditions. Furthermore, the presence of microelements and beneficial microbes also plays a role in plant health; thus, analyses of these components in the growth media would provide valuable insights.

To effectively evaluate the impact of bat guano on soil health—and to detect any potential deterioration in soil quality caused by chemical fertilisers—monthly chemical analyses of the plant growth media throughout the growth cycle are recommended. From a biodiversity perspective, the use of molecular identification techniques, following appropriate protocols, would complement acoustic recordings and enhance the accuracy of bat species identification, contributing to habitat studies and diversity records. Finally, conducting a public survey to assess the knowledge and acceptance of bat guano use among the Malaysian community would support the development of more effective strategies for education and advocacy, particularly in promoting bat conservation initiatives in Malaysia.

REFERENCES

- Adam, A. H., Adam, A., & Magid, F. M. (2020). Efficacy of bat guano on growth and yield of some hybrid sunflower (*Helianthus annuus*, L.) cultivars in Sudan. *Journal of Agronomy and Research*, 3(2), 28-38.
- Adejoro, S. A., Okunlola, A. I., & Fakanlu, G. (2011). Evaluation of some manure types for the growth and yield of watermelon in Southwestern Nigeria. *Researcher*, 3, 393-395.
- Adhikary, S. P., & Gantayet, P. K. (2012). Studies on the influence of organic fertilisers on the growth and some biochemical parameters of chilli (*Capsicum annum* L. Var). *The Bioscan*, 7(2), 255-258.
- Afa, M. (2016). The effect of natural guano organic fertiliser on growth and yield of spring onion (*Allium fistulosum* L.). *Agrotech Journal*, 1(1), 26-32.
- Aldridge, H. D. J. N., & Rautenbach, I. L. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *The Journal of Animal Ecology*, 56(3), 763-778.
- Almohammed, A. N., Almehemdi, A. F., & AlAjeelee, R. K. (2014). Impact of bat guano *Otonycteris hemprichii* Camd and seaweed extract on some growth and yield traits of barakaseed *Nigella sativa* L. *Journal of Biology, Agriculture and Healthcare*, 4(1), 57-65.
- Altintas, A., & Yildiz, G. (2005). Mineral levels of bat guano. *Veterinary Journal of Ankara University (Turkey)*, 52(1), 1-5.
- Altringham, J. D. (2011). *Bats: from evolution to conservation*. New York: Oxford University Press.

- Arita, H. T. (1993). Conservation biology of the cave bats of Mexico. *Journal of Mammalogy*, 74(3), 693-702.
- Arita, H. T. (1996). The conservation of cave-roosting bats in Yucatan, Mexico. *Biological Conservation*, 76(2), 177-185.
- Ayodele, O. J., Alabi, E. O., & Aluko, M. (2015). Nitrogen fertiliser effects on growth, yield and chemical composition of hot pepper (Rodo). *International Journal of Agriculture and Crop Sciences*, 8(5), 666.
- Barclay, R. M. (1999). Bats are not birds—a cautionary note on using echolocation calls to identify bats: a comment. *Journal of Mammalogy*, 80(1), 290-296.
- Barclay, R. M., & Brigham, R. M. (1991). Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small?. *The American Naturalist*, 137(5), 693-703.
- Bass, M. S., Finer, M., Jenkins, C. N., Kreft, H., Cisneros-Heredia, D. F., McCracken, S. F., Pitman, N. C. A., English, P. H., Swing, K., Villa, G., Fiore, A. D., Voigt, C. C., & Kunz, T. H. (2010). Global conservation significance of Ecuador's Yasuní National Park. *Public Library of Science One*, 5(1), e8767.
- Bender, D. J., Contreras, T. A., & Fahrig, L. (1998). Habitat loss and population decline: a meta-analysis of the patch size effect. *Ecology*, 79(2), 517-533.
- Bernath, R. F., & Kunz, T. H. (1981). Structure and dynamics of arthropod communities in bat guano deposits in buildings. *Canadian Journal of Zoology*, 59(2), 260-270.
- Bhat, H. R., & Sreenivasan, M. A. (1990). Records of bats in Kyasanur Forest disease area and environs in Karnataka state, India, with ecological notes. *Mammalia*, 54 (1), 69-106.

- Bird, M. I., Boobyer, E. M., Bryant, C., Lewis, H. A., Paz, V., & Stephens, W. E. (2007). A long record of environmental change from bat guano deposits in Makangit Cave, Palawan, Philippines. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 98(1), 59-69.
- Bogdanowicz, W., Fenton, M. B., & Daleszczyk, K. (1999). The relationships between echolocation calls, morphology and diet in insectivorous bats. *Journal of Zoology*, 247(3), 381-393.
- Boonkerd, K., Wanghongsa, S., & Pimmanrojukul, W. (2001). Management of bat caves. *National Parks and Wildlife Research Division. Annual Report. Royal Forest Department, Bangkok. (In Thai)*, 33-45.
- Brigham, R. M. (1990). Prey selection by big brown bats (*Eptesicus fuscus*) and common nighthawks (*Chordeiles minor*). *The American Midland Naturalist*, 154(1), 73-80.
- Brown, J. E., Gilliam, C. H., Shumack, R. L., Porch, D. W., & Donald, J. O. (1995). Comparison of broiler litter and commercial fertiliser on production of tomato, *Lycopersicon esculentum*. *Journal of Vegetable Crop Production*, 1(1), 53-62.
- Brown, V. A., Willcox, E. V., Fagan, K. E., & Bernard, R. F. (2017). Identification of southeastern bat species using non-invasive genetic sampling of individual guano pellets. *Journal of Fish and Wildlife Management*, 8(2), 632-639.
- Bryant, J. P., & Kincheloe, S. (1983). Fertiliser minerals. *Industrial Minerals and Rock*, 5(1), 233-241
- Buliga, C. (2010). *Guano exploitation in Madagascar. Independent Study Project (ISP) Collection. 904.* [online] Available at: https://digitalcollections.sit.edu/isp_collection/904 [Accessed on 5 September 2021].

- Bumrungsri, S., Harbit, A., Benzie, C., Carmouche, K., Sridith, K., & Racey, P. (2008). The pollination ecology of two species of *Parkia* (Mimosaceae) in southern Thailand. *Journal of Tropical Ecology*, 24(5), 467-475.
- Bumrungsri, S., Lang, D., Harrower, C., Sripaoraya, E., Kitpipit, K., & Racey, P. A. (2013). The dawn bat, *Eonycteris spelaea* Dobson (Chiroptera: Pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. *Acta Chiropterologica*, 15(1), 95-104.
- Bumrungsri, S., Sripaoraya, E., Chongsiri, T., Sridith, K., & Racey, P. A. (2009). The pollination ecology of durian (*Durio zibethinus*, Bombacaceae) in southern Thailand. *Journal of Tropical Ecology*, 25(1), 85-92.
- Carter, D. L. (1987). Water relations and irrigation. *Wheat and Wheat Improvement*, 13(2), 390-395.
- Catullo, G., Masi, M., Falcucci, A., Maiorano, L., Rondinini, C., & Boitani, L. (2008). A gap analysis of Southeast Asian mammals based on habitat suitability models. *Biological Conservation*, 141(11), 2730-2744.
- Chaudhary, B. R., Sharma, M. D., Shakya, S. M., & Gautam, D. M. (2006). Effect of plant growth regulators on growth, yield and quality of chilli (*Capsicum annuum* L.) at Rampur, Chitwan. *Journal of the Institute of Agriculture and Animal Science*, 27, 65-68.
- Chen, Z., & Jiang, X. (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilisers: a review. *Agriculture*, 4(1), 1-29.
- Chiu, C., & Moss, C. F. (2007). The role of the external ear in vertical sound localization in the free flying bat, *Eptesicus fuscus*. *The Journal of the Acoustical Society of America*, 121(4), 2227-2235.

- Chronakova, A., Horak, A., Elhottova, D., & Kristufek, V. (2009). Diverse archaeal community of a bat guano pile in Domica Cave (Slovak Karst, Slovakia). *Folia Microbiologica*, 54, 436-446.
- Cleveland, C. J., Betke, M., Federico, P., Frank, J. D., Hallam, T. G., Horn, J., Lopez, J. D. Jr., McCracken G. F., Medellin, R. A., Moreno-Valdez, A., Sansone, C. G., Westbrook, J. K., & Kunz, T. H. (2006). Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. *Frontiers in Ecology and the Environment*, 4(5), 238-243.
- Corbet, G. B., & Hill, J. E. (1992). *The mammals of the Indomalayan region: A systematic review*. Oxford University Press.
- Dahal, K. C., Sharma, M. D., Dhakal, D. D., & Shakya, S. M. (2006). Evaluation of heat tolerant chilli (*Capsicum annuum* L.) genotypes in Western Terai of Nepal. *Journal of the Institute of Agriculture and Animal Science*, 27, 59-64.
- Das, U., Hansdah, B., Behera, S. S., & Ray, R. C. (2023). Life cycle assessment of biofuels derived from Mahua (*Madhuca* species) flowers and seeds: key issues and perspectives. In *Green Approach to Alternative Fuel for a Sustainable Future* (pp. 13-23). Elsevier.
- Davies, A. B., & Asner, G. P. (2014). Advances in animal ecology from 3D-LiDAR ecosystem mapping. *Trends in Ecology & Evolution*, 29(12), 681-691.
- Decu, V. (1986). Some considerations on the bat guano synusia. *Travail du Institut de Spéologie "Emile Racovitza"*, 25(1), 41-51.
- Demirtas, E. I., Nuri, A. R. I., Arpacioğlu, A., Harun, K. A. Y. A., & Ozkan, C. F. (2005). *Degisik Organik Kokenli Gübrelerin Kimyasal Özellikleri* [Chemical properties of different organic manures]. *Derim*. 22(2): 47-52.

- Department of Standards Malaysia. (n.d.). MS417: Analysis of fertiliser. Retrieved from National Archives of Malaysia.
- Department of Standards Malaysia. (n.d.). MS678: Recommended methods for soil analysis. Retrieved from National Archives of Malaysia.
- Detwiler, J. T., Bos, D. H., & Minchella, D. J. (2010). Revealing the secret lives of cryptic species: examining the phylogenetic relationships of echinostome parasites in North America. *Molecular Phylogenetics and Evolution*, 55(2), 611-620.
- Diba, P. F., Susatyo, E. B., & Pratjojo, W. (2013). *Peningkatan Kadar N, P Dan K pada Pupuk Organik Cair dengan Pemanfaatan Bat Guano* [Enhancement of N, P and K levels in liquid organic fertiliser using bat guano]. *Indonesian Journal of Chemical Science*, 2(1), 56-60.
- Dietz, C., Dietz, I., Ivanova, T., & Siemers, B. M. (2009). Seasonal and regional scale movements of horseshoe bats (*Rhinolophus*, Chiroptera: Rhinolophidae) in northern Bulgaria. *Nyctalus (NF)*, 14(1), 52-64.
- Dietz, C., & Von Helversen, O. (2004). Illustrated identification key to the bats of Europe. [online] Available at: https://www.researchgate.net/publication/274838308_Illustrated_Identification_key_to_the_Bats_of_Europe_-_complete_pdf [Accessed on 3 August 2022].
- Dikinya, O., & Mufwanzala, N. (2010). Chicken manure-enhanced soil fertility and productivity: Effects of application rates. *Journal of Soil Science and Environmental Management*, 1(3), 46-54.
- Dimande, P., Arrobas, M., & Rodrigues, M. A. (2023). Effect of Bat Guano and Biochar on Okra Yield and Some Soil Properties. *Horticulturae*, 9, 728.

- Dimkic, I., Stankovic, S., Kabic, J., Stupar, M., Nenadic, M., Ljaljevic-Grbic, M., Zikic, V., Vujisic, L., Tesevic, V., Vesovic, N., Pantelic, D., Savic-Sevic, S., Vukojevic, J., & Curcic, S. (2020). Bat guano-dwelling microbes and antimicrobial properties of the pygidial gland secretion of a troglophilic ground beetle against them. *Applied Microbiology and Biotechnology*, 104(9), 4109-4126.
- Dorji, K., Behboudian, M. H., & Zegbe-Dominguez, J. A. (2005). Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial rootzone drying. *Scientia horticulturae*, 104(2), 137-149.
- Dubey, R. P., Chethan, C. R., Choudhary, V. K., & Mishra, J. S. (2023). A review on weed management in millets. *Indian Journal of Weed Science*, 55(2), 141-148
- Dykes, A. P. (2007). Mass movements in cave sediments: investigation of a ~ 40,000-year-old guano mudflow inside the entrance of the Great Cave of Niah, Sarawak, Borneo. *Landslides*, 4(3), 279-290.
- Ekinci, M., Atamanalp, M., Turan, M., Alak, G., Kul, R., Kitir, N., & Yildirim, E. (2019). Integrated use of nitrogen fertiliser and fish manure: Effects on the growth and chemical composition of spinach. *Communications in Soil Science and Plant Analysis*, 50(13), 1580-1590.
- Emam, M. S. A., Elsayed, T., & Mamdoh Mohamed Hamed, L. (2020). Sweet corn growth performance and Rhizosphere microbial densities in response to mineral and organic amendments. *Egyptian Journal of Soil Science*, 60(1), 43-52.
- Emerson, J. K., & Roark, A. M. (2007). Composition of guano produced by frugivorous, sanguivorous, and insectivorous bats. *Acta Chiropterologica*, 9(1), 261-267.

- Erickson, A. N., & Markhart, A. H. (2001). Flower production, fruit set, and physiology of bell pepper during elevated temperature and vapor pressure deficit. *Journal of the American Society for Horticultural Science*, 126(6), 697-702.
- Estefan, G., Sommer, R., & Ryan, J. (2013). *Methods of soil, plant, and water analysis. A Manual for The West Asia and North Africa region*. [e-book] Beirut, Lebanon: International Centre for Agricultural Research in the Dry Areas (ICARDA). Available through: Monitoring Evaluation and Learning <<https://repo.mel.cgiar.org/items/14bb2a3-fdc9e-455b-99e3-97f27ceb9e42>> [Accessed on 15 January 2024].
- Fageria, N. K., Filho, M. B., Moreira, A., & Guimarães, C. M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, 32(6), 1044-1064.
- Falster, D. S., & Westoby, M. (2003). Plant height and evolutionary games. *Trends in Ecology & Evolution*, 18(7), 337-343.
- Fenolio, D. B., Graening, G. O., Collier, B. A., & Stout, J. F. (2006). Coprophagy in a cave-adapted salamander; the importance of bat guano examined through nutritional and stable isotope analyses. In *Proceedings of the Royal Society B: Biological Sciences*, 273(1585), 439-443.
- Fenton, M. B., & Fleming, T. H. (1976). Ecological interactions between bats and nocturnal birds. *Biotropica*, 8(2), 104-110.
- Fenton, M. B., and Simmons N. B. (2014). *Bats: a world of science and mystery*. Chicago: University of Chicago Press.
- Ferguson, J. J., & Ziegler, M. R. (2004). Guidelines for Purchase and Application of Poultry Manure for Organic Crop Production: HS973/HS217, 4/2004. *EDIS*, 2004(7).

- Fernandez, N., Viciana, V., & Drovandi, A. (2003). Assessment of the total environmental impact of agrochemicals in the Mendoza River basin. *Project OEI. Mendoza, Argentina*.
- Ferreira, R. L. (2019). Guano communities. In *Encyclopedia of caves* (William, B. W., David C. Culver and Tanja Pipan (pp. 474-484). Academic Press.
- Ferreira, R. L., & Martins, R. P. (1998). Diversity and distribution of spiders associated with bat guano piles in Morrinho cave (Bahia State, Brazil). *Diversity and Distribution*, 4(5), 235-241.
- Ferreira, R. L., & Martins, R. P. (1999). Trophic structure and natural history of bat guano invertebrate communities, with special reference to Brazilian caves. *Tropical Zoology*, 12(2), 231-252.
- Findley, J. S. (1993). *Bats: A Community Perspective*. Cambridge, Great Britain: Cambridge University Press.
- Fleming, T. H., & Kress, W. J. (2011). A brief history of fruits and frugivores. *Acta Oecologica*, 37(6), 521–530.
- Francis, C. M. (2008). A field guide to the mammals of SouthEast Asia. London, United Kingdom: New Holland Publishers.
- Friedheim, S. (2016). Comparison of species identification methods: DNA barcoding versus morphological taxonomy. *Horizons*, 1(1), 74-86.
- Fudholi, A., Othman, M. Y., Ruslan, M. H., & Sopian, K. (2013). Drying of Malaysian *Capsicum annum* L. (red chili) dried by open and solar drying. *International Journal of Photoenergy*, 2013(1), 167895.
- Funakoshi, K., & Uchida, T. (1978). Studies on the physiological and ecological adaptation of temperate insectivorous bats: III. Annual activity of the Japanese house-dwelling bat,

- Pipistrellus abramus*. *Journal of the Faculty of Agriculture, Kyushu University*, 23(1), 95-115.
- Furey, N. M., & Racey, P. A. (2016). Conservation ecology of cave bats. In C. C. Voigt & T. Kingston (Eds.), *Bats in the Anthropocene: Conservation of bats in a changing world* pp. 463-500. New York: Springer Nature.
- Gannon, W. L., Sherwin, R. E., DeCarvalho, T. N., & Ofarrell, M. J. (2001). Pinnae and echolocation call differences between *Myotis californicus* and *M. ciliolabrum* (Chiroptera: Vespertilionidae). *Acta Chiropterologica*, 3(1), 77-91.
- Ghanem, S. J., & Voigt, C. C. (2012). Increasing awareness of ecosystem services provided by bats. *Advances in the Study of Behavior*, 44, 279-302.
- Giurgiu, A., & Tamas, T. (2013). Mineralogical data on bat guano deposits from three Romanian caves. *Studia Universitatis Babes-Bolyai Geologia*, 58(2), 13-18.
- Glover, A. M., & Altringham, J. D. (2008). Cave selection and use by swarming bat species. *Biological Conservation*, 141(6), 1493-1504.
- Gnasplini, P., & Trajano, E. (2000). Guano communities in tropical caves. In *Subterranean ecosystems* (Wilkins, H., Culver, D. C., & Humphreys, W. F.), pp. 251-268. Amsterdam: Elsevier Press.
- Goodman, S. M., Ramasindrazana, B., Maminirina, C. P., Schoeman, M. C., & Appleton, B. (2011). Morphological, bioacoustical, and genetic variation in *Miniopterus* bats from eastern Madagascar, with the description of a new species. *Zootaxa*, 2880(1), 1-19.
- Gould, E. (1955). The feeding efficiency of insectivorous bats. *Journal of Mammalogy*, 36(3), 399-407.

- Goveas, S. W., Miranda, E. C., Seena, S., & Sridhar, K. R. (2006). Observations on guano and bolus of Indian flying fox, *Pteropus giganteus*. *Current Science*, 90(2), 160-162.
- Grantina-Ievina, L., & Ievinsh, G. (2015). Microbiological characteristics and effect on plants of the organic fertiliser from vermicompost and bat guano. *Research for Rural Development*, 1, 95-101.
- Griffin, D. R. (1953). Bat sounds under natural conditions, with evidence for echolocation of insect prey. *Journal of Experimental Zoology*, 123(3), 435-465.
- Guan, X., Britzke, E. R., Piaggio, A. J., Bergman, D. L., Van Pelt, L., & Lance, R. F. (2020). Genetic assays for guano-based identification of species and sex in bats of the United States and Canada. *Journal of Mammalogy*, 101(4), 970-978.
- Guler, S. (2006). Developments on fertiliser consumption of the world and Turkey. *Anadolu Tarım Bilimleri Dergisi*, 21(2), 243-248.
- Hall, J. S. (1962). *A life history and taxonomic study of the Indiana bat, Myotis sodalis*. (Publication No. 6100137) [Doctoral dissertation, University of Illinois]. Urbana-Champaign ProQuest Dissertations & Theses.
- Hall, L. S., Richards, G., & Abdullah, M. T. (2002). The bats of Niah national park, Sarawak. *Sarawak Museum Journal*, 52(78), 255-282.
- Hall, L. S., Tuen, A. A., Ketol, B., Sait, I., & Abdullah, M. T. (2001). Senarai spesies kelawar di Gua Angin, Bau. *Unpublished report. Universiti Malaysia Sarawak, Kota Samarahan*.
- Harouna, K. A., Tidjani, A. D., Fanna, A. G., & Yadji, G. (2021). Bats guano from Magarawa in Niger: exploitation, marketing and peasant perception. *International Journal of Innovation and Applied Studies*, 32(3), 426-436.

- Harris, J. A. (1970). Bat-guano cave environment. *Science*, 169(3952), 1342-1343.
- Hebert, P. D., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54(5), 852-859.
- Hodgkison, R., Balding, S. T., Zubaid, A., & Kunz, T. H. (2003). Fruit bats (Chiroptera: Pteropodidae) as seed dispersers in a lowland Malaysian rain forest. *Biotropica*, 35(4), 491–502.
- Hopkins, B. G., Stark, J. C., & Kelling, K. A. (2020). Nutrient management. In J. C. Stark, M. Thornton & P. Nolte (Eds.), *Potato Production Systems* (pp. 155-202). Springer Cham.
- Hua, L., Wu, W., Liu, Y., McBride, M. B., & Chen, Y. (2009). Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. *Environmental Science and Pollution Research*, 16(1), 1-9.
- Hughes, A. C., Satasook, C., Bates, P. J., Soisook, P., Sritongchuay, T., Jones, G., & Bumrungsri, S. (2010). Echolocation call analysis and presence-only modelling as conservation monitoring tools for Rhinolophoid bats in Thailand. *Acta Chiropterologica*, 12(2), 311-327.
- Humphrey, S. R., & Bain, J. R. (1990). *Endangered Animals of Thailand*. Sandhill Crane Press: Gainesville, Florida
- Hussain, F., & Abid, M. (2011). Pest and diseases of chilli crop in Pakistan: a review. *International Journal of Biology and Biotechnology*, 8(2), 325-332.
- Hutchinson, G. E. (1950). Survey of contemporary knowledge of biogeochemistry. *The Biogeochemistry of Vertebrate Excretion*, 70(4), 329.

- Ibrahim, M. H., Jaafar, H. Z., Karimi, E., & Ghasemzadeh, A. (2013). Impact of organic and inorganic fertilisers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth). *Molecules*, 18(9), 10973-10988.
- Isnaeni, S., & Nurhidayah, S. (2020). Respon tanaman jagung manis (*Zea mays* L.) terhadap pemberian pupuk guano kelelawar dan pupuk guano walet. *Jurnal Agroteknologi*, 11(1), 33-38.
- Ith, S., Bumrungsri, S., Furey, N. M., Bates, P. J., Wonglapsuwan, M., Khan, F. A. A., Thong, V. D., Soisook, P., Satasook, C., & Thomas, N. M. (2015). Taxonomic implications of geographical variation in *Rhinolophus affinis* (Chiroptera: Rhinolophidae) in mainland Southeast Asia. *Zoological Studies*, 54, 1-29.
- Ith, S., Bumrungsri, S., Thomas, N. M., Bates, P. J., Willette, D. A., Khan, F. A. A., Wonglapsuwan, M., Soisook, P., Maryanto, I., Huang, J. C., & Furey, N. M. (2016). Geographical variation of *Rhinolophus affinis* (Chiroptera: Rhinolophidae) in the Sundaic subregion of Southeast Asia, including the Malay peninsula, Borneo and Sumatra. *Acta Chiropterologica*, 18(1), 141-161.
- Jahanzad, E., Barker, A. V., Hashemi, M., Sadeghpour, A., Eaton, T., & Park, Y. (2017). Improving yield and mineral nutrient concentration of potato tubers through cover cropping. *Field Crops Research*, 212, 45-51.
- Javid, Q. A., Abbasi, N. A., Saleem, N., Hafiz, I. A., & Mughal, A. L. (2005). Effect of NPK fertiliser on performance of Zinnia (*Zinnia elegans*) wirlyging shade. *International Journal of Agriculture and Biology*, 7(3), 471-473.

- Jayaraj, V. K., Laman, C. J., & Abdullah, M. T. (2012). A predictive model to differentiate the fruit bats *Cynopterus brachyotis* and *C. cf. brachyotis* forest (Chiroptera: Pteropodidae) from Malaysia using multivariate analysis. *Zoological Studies*, 51(2), 259-271.
- Jayasvasti, I., & Jayasvasti, M. (2018). Bat guano as the component of fertiliser or the health hazard? *Southeast Asian Journal of Tropical Medicine and Public Health*, 49(2), 331-339.
- Ji, X., Xu, J., & Zhang, H. (2023). Environmental effects of rural e-commerce: A case study of chemical fertiliser reduction in China. *Journal of Environmental Management*, 326, 116713.
- Jiang, T., Metzner, W., You, Y., Liu, S., Lu, G., Li, S., Wang, L., & Feng, J. (2010). Variation in the resting frequency of *Rhinolophus pusillus* in Mainland China: effect of climate and implications for conservation. *The Journal of the Acoustical Society of America*, 128(4), 2204-2211.
- Jinggong, E. R. (2022). *Acoustic survey on insectivorous bats activity pattern at contrasting elevation in Kubah National Park* (Publication No. 40483) [Masters' Thesis, Universiti Malaysia Sarawak]. UNIMAS Institutional Repository.
- Jinggong, E. R., Rajasegaran, P., Morni, M. A., William-Dee, J., Denel, A., & Khan, F. A. A. (2022). Bats of Mount Silabur, Sarawak, and its potential for conservation. *Borneo Journal of Resource Science and Technology*, 12(1), 141-156.
- Jones, G., Jacobs, D. S., Kunz, T. H., Willig, M. R., & Racey, P. A. (2009). Carpe noctem: the importance of bats as bioindicators. *Endangered Species Research*, 8(1), 93-115.
- Jones, G., & Teeling, E. C. (2006). The evolution of echolocation in bats. *Trends in Ecology & Evolution*, 21(3), 149-156.

- Jones, K. E., Purvis, A., MacLarnon, A. N. N., Bininda-Emonds, O. R., & Simmons, N. B. (2002). A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biological Reviews*, 77(2), 223-259.
- Journal of Visualized Experiments Science Education Database. (2023). *Determination of moisture content in soil*. [online]. Available at: <https://www.jove.com/v/10011/determination-of-moisture-content-in-soil> [Accessed on 2 November 2023].
- Jub, N., Barapoi, I. P., Abdul Aziz, N. A., Jalaweh, N., Hamdan, F. Z., Tuen, A. A., Mohd-Azlan, J., Laman, C. J., & Abdullah, M. T. (2003). Diversity of small mammals in Bau limestone habitat, Sarawak. In *Natural Resources, Environment and Development Conference. Kuching*.
- Judy, D. J., Sparks, D. W., Whitaker, J. O. Jr., & Oyler-McCance, S. J. (2010). Bat guano is useful for more than diet studies. In *Proceedings of the Indiana Academy of Science* (Vol. 119, No. 1, pp. 95-98).
- Juni, J. (2003). *Germination of Capsicum annuum L. seeds after accelerated ageing and storage*. (Publication No. 18069) [Final Year Project Report, Universiti Malaysia Sarawak]. UNIMAS Institutional Repository.
- Kang, G. S., Beri, V., Sidhu, B. S., & Rupela, O. P. (2005). A new index to assess soil quality and sustainability of wheat-based cropping systems. *Biology and Fertility of Soils*, 41(6), 389-398.
- Kanuch, P., & Cel'uch, M. (2007). Bat assemblage of an old pastured oak woodland (Gavurky Protected Area, central Slovakia). *Vespertilio*, 11, 57-64.

- Karagoz-Sezer, K., & Hanay, A. (2020). Effect of bat guano on cation exchange capacity, some macro nutrient content and reaction of soil (pH). *Ataturk Universitesi Ziraat Fakultesi Dergisi*, 51(2), 140-144.
- Karim, C., Tuen, A. A., & Abdullah, M. T. (2004). Mammals. *Sarawak Museum Journal*, 6, 221 - 234.
- Kasso, M., & Balakrishnan, M. (2013). Ecological and economic importance of bats (Order Chiroptera). *International Scholarly Research Notices*, 2013(1), 187415.
- Khan, A., Shah, S. N. M., Rab, A., Sajid, M., Ali, K., Ahmed, A., & Faisal, S. (2014). Influence of nitrogen and potassium levels on growth and yield of chillies (*Capsicum annuum* L.). *International Journal of Farming and Allied Sciences*, 3(3), 260-264.
- Khandaker, M. M., Rohani, F., Dalorima, T., & Mat, N. (2017). Effects of different organic fertilisers on growth, yield and quality of *Capsicum annuum* L. Var. Kulai (Red Chilli Kulai). *Biosciences Biotechnology Research Asia*, 14(1), 185-192.
- Kingston, T., Akbar, Z., Lim, G., & Hatta, F. (2006). From research to outreach: environmental education materials for the bats of Malaysia. In *Proceedings of the Best of Both Worlds Conference for Environmental Education for Sustainable Development*.
- Koay, G., Heffner, R. S., & Heffner, H. E. (1998). Hearing in a Megachiropteran Fruit Bat (*Rousettus aegyptiacus*). *Journal of Comparative Psychology*, 112(4), 371-382.
- Korn, L., Ngang, C., Ader, D. R., & Srean, P. (2023). Bat guano application rate in horticulture in Cambodia: an experiment with tomato. *Editorial Board*, 15(11), 24.
- Korstian, J. M., Hale, A. M., Bennett, V. J., & Williams, D. A. (2013). Advances in sex determination in bats and its utility in wind-wildlife studies. *Molecular Ecology Resources*, 13(5), 776-780.

- Krzanowski, A. (1977). Weight classes of palearctic bats. *Acta Theriologica*, 22(27), 365-370.
- Kunz, T., Pierson, E., & Nowak, R. M. (1994). *Walker's bats of the world*. RM Nowak. *Bats of the world: An introduction*. Baltimore: The Johns Hopkins University Press.
- Kunz, T. H. (1982). Roosting ecology of bats. In T.H. Kunz (Eds.), *Ecology of bats*, (pp. 1-55). Springer Cham.
- Lawrence, B. D., & Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *The Journal of the Acoustical Society of America*, 71(3), 585-590.
- Leelapaibul, W., Bumrungsri, S., & Pattanawiboon, A. (2005). Diet of wrinkle-lipped free-tailed bat (*Tadarida plicata* Buchannan, 1800) in central Thailand: insectivorous bats potentially act as biological pest control agents. *Acta Chiropterologica*, 7(1), 111-119.
- Lim, B. L., Chai, K. S., & Muul, I. (1972). Notes on food habits of bats from the fourth division, Sarawak. *Sarawak Museum Journal*, 20, 351-357.
- Lim, T., Cappelle, J., Hoem, T., & Furey, N. (2018). Insectivorous bat reproduction and human cave visitation in Cambodia: A perfect conservation storm?. *Public Library of Science One*, 13(4), e0196554.
- Linn, K. Z., & Myint, P. P. (2018). Study on the effectiveness of natural organic fertilisers on cassava (*Manihot esculenta* Crantz.) cultivation. *Asian Journal of Soil Science and Plant Nutrition*, 3(3), 1-10.
- Lu, W., Han, Z., Liu, Q., Wang, K., Liu, Q., & Song, X. (2023). Development of Flowers Buds and Mixed Buds in the Dichasial Inflorescence of *Geranium koreanum* Kom. (Geraniaceae). *Plants*, 12(18), 3178.

- Lundstrom, F. O., & Mehring, A. L. (1939). Complete composition of commercial mixed fertilisers. *Industrial & Engineering Chemistry*, 31(3), 354-361.
- MacKinnon, K., Hatta, G., Halim, H., & Mangalik, A. (1996). *The ecology of Kalimantan* (Vol. 3). Oxford: Oxford University Press.
- Magid, H. A., Sabrah, R. E. A., Rabi, R. K., El Nadi, A. R. H., & Aal, S. A. (1993). Biodegradation of municipal refuse and chicken manure in a winter-wheat ecosystem in Saudi Arabia. *Journal of Arid Environments*, 25(4), 411-419.
- Marschner, H. (Ed.). (2011). *Marschner's mineral nutrition of higher plants*. United States: Academic Press.
- Maryanto, I., Yani, M., Priyono, S. N., & Wiantoro, S. (2011). Altitudinal distribution of fruit bats in Lore Lindu National Park, Central Sulawesi, Indonesia. *Mammal*, 22(1), 167-177.
- Masarirambi, M. T., Dlamini, P., Wahome, P. K., & Oseni, T. O. (2012). Effects of chicken manure on growth, yield and quality of lettuce (*Lactuca sativa* L.) 'Taina' under a lath house in a semi-arid sub-tropical environment. *Agriculture & Environmental Science*, 12(3), 399-406.
- Mayer, F., Dietz, C., & Kiefer, A. (2007). Molecular species identification boosts bat diversity. *Frontiers in Zoology*, 4, 1-5.
- McArthur, E. (2019). *Acoustic survey on habitat use and activity patterns of insectivorous bats in a riverine forest at Gunung Mulu National Park, Sarawak, Malaysia*. (Publication No. 27512) [Masters' Thesis, Universiti Malaysia Sarawak]. UNIMAS Institutional Repository.

- McCall, W. W. (1914). Chicken manure. *General Home Garden Series No. 2*. College of Tropical Agriculture and Human Resources, University of Hawaii.
- McCracken, G. F. (1989). Cave conservation: special problems of bats. *American National Speleological Society Bulletin*, 51, 47–51
- McFarlane, D. A., & Lundberg, J. (2018). New records of guano-associated minerals from caves in northwestern Borneo. *International Journal of Speleology*, 47(2), 1.
- McFarlane, D. A., Lundberg, J., & Rentergem, G. V. (2017). Preliminary observations on tropical bat caves as biogeochemical nitrogen sinks. In *Proceedings of the 17th International Congress of Speleology*, 157- 160.
- McNab, B. K. (1974). The behavior of temperate cave bats in a subtropical environment. *Ecology*, 55(5), 943-958.
- Medway, L. (1971). Observations of social and reproductive biology of the bent-winged bat *Miniopterus australis* in northern Borneo. *Journal of Zoology*, 165(2), 261-273.
- Michalski, G., Kolanowski, M., & Riha, K. M. (2015). Oxygen and nitrogen isotopic composition of nitrate in commercial fertilisers, nitric acid, and reagent salts. *Isotopes in Environmental and Health Studies*, 51(3), 382-391.
- Mickleburgh, S. P., Hutson, A. M., & Racey, P. A. (2002). A review of the global conservation status of bats. *Oryx*, 36(1), 18-34.
- Millenium Ecosystem Assessment [MEA]. (2003). *Ecosystems and Human Well-being: A Framework for Assessment*. Washington, DC: Island Press.
- Misra, P. K., Gautam, N. K., & Elangovan, V. (2019). Bat guano: a rich source of macro and microelements essential for plant growth. *Annals of Plant and Soil Research*, 21(1), 82-86.

- Mohd-Azlan, J., Neuchlos, J., & Abdullah, M. T. (2005). Diversity of Chiropterans in limestone forest area, Bau, Sarawak. *Malaysian Applied Biology*, 34(1), 59.
- Mohd Ridwan, A. R., Tingga, R. C. T., Azhar, M. I., Hasan, N. H., & Abdullah, M. T. (2011). Bats of the Wind Cave Nature Reserve, Sarawak, Malaysian Borneo. *Tropical Natural History*, 11(2), 159-175.
- Mohd Ridwan, A. R., Tingga, R. C. T., Noor Haliza, H., Sigit, W., Anang, S. A., Eileen, L., Besar, K., Huzal, I. H., & Abdullah, M. T. (2010). Diversity of bats in two protected limestone areas in Sarawak, Malaysia. *Sarawak Museum Journal*, 88, 209-246.
- Monadjem, A., Taylor, P. J., & Schoeman, M. C. (2020). *Bats of Southern and Central Africa: A Biogeographic and Taxonomic Synthesis*. South Africa: Wits University Press.
- Moridani, M. J., Sadeghi, S. M., Roshan, N. M., Ashouri, M., & Doroudian, H. R. (2023). Effects of organic and chemical fertilisers on yield and yield components of rice in different moisture stress conditions. *Gesunde Pflanzen*, 75(5), 1799-1811.
- Morni, M. A., Khan, F. A. A., Rosli, Q. S., Dee, J. W., Tingga, R. C. T., & Rahman, M. R. A. (2018). Bats roost site preferences in Wind Cave Nature Reserve, Bau, Sarawak. *Malaysian Applied Biology*, 47, 57-64.
- Muscarella, R., & Fleming, T. H. (2007). The role of frugivorous bats in tropical forest succession. *Biological Reviews*, 82(4), 573–590.
- Newman, M. M., Kloepper, L. N., Duncan, M., McInroy, J. A., & Kloepper, J. W. (2018). Variation in bat guano bacterial community composition with depth. *Frontiers in Microbiology*, 9, 914.
- Norberg, U. M. (1986). Evolutionary convergence in foraging niche and flight morphology in insectivorous aerial-hawking birds and bats. *Ornis Scandinavica*, 17(3), 253-260.

- Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 316(1179), 335-427.
- Norziah, M. H., & Ching, C. Y. (2000). Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry*, 68(1), 69-76.
- Nosheen, A., & Bano, A. (2014). Growth enrichment of *Carthamus tinctorius* (L) and reduction in dosage of chemical fertilisers with application of plant growth promoting rhizobacteria. *International Journal of Agronomy and Agricultural Research*, 4(6), 75-84.
- Nowak, R. M. (1999). *Walker's Mammals of the World* (Vol. 1). Baltimore, Maryland: John Hopkins University Press.
- Nuratiqah, A. R., Senawi, J., Syedaziz, S. S., Kamal, S. N. A. S. A, & Zubaid, A. (2023). Updated checklist of bats from the Batu Caves karst massif, Selangor, Peninsular Malaysia. *Malayan Nature Journal*, 75(1), 99-107.
- Nyaun, I., Junior, J. L., Ku, S. S., & Majupin, R. (2004). *A brief study on small mammals of Niah National Park. Unpublished report. Universiti Malaysia Sarawak, Kota Samarahan*
- Oelhalf, C. R. (1978). *Organic matter*. United States: Allanheld, Osmun and Co. Publisher.
- O'Hallorans, J. M., & Colberg, O. (1993). Effect of chicken manure on chemical properties of a Mollisol and tomato production. *Journal of Agriculture-University of Puerto Rico*, 77, 181-181.

- Ojobor, S. A., & Egbuchua, C. N. (2021). Influence of formulated palm mill effluent and bat guano mixture on maize performance and soil chemical properties in Delta State, Nigeria. *Indian Journal of Agricultural Research*, 56(1), 28-32.
- Olowoboko, T. B., Onasanya, O. O., Salami, O. T., & Azeez, J. O. (2017). Growth and uptake in maize as influenced by NPK fertiliser in green house experiment. *International Journal of Plant & Soil Science*, 17(3), 1-10.
- Omar, S. R., Ahmed, O. H., Saamin, S., & Majid, N. M. A. (2008). Gamma radiosensitivity study on chili (*Capsicum annuum*). *American Journal of Applied Sciences*, 5(2), 67-70.
- Oo, A. L., Khine, T. M., & Aye, T. T. (2019). Preparation and characterization of organic fertilisers made from bat guano, rice husk ash and groundnut leaves. *Journal Myanmar Academy of Arts and Sciences*, 17(1B), 393-405.
- Palita, S. K., Panigrahi, R., & Panda, D. (2021). Potentiality of bat guano as organic manure for improvement of growth and photosynthetic response in crop plants. In *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 91(1), 185-193.
- Papadatou, E., Butlin, R. K., & Altringham, J. D. (2008). Identification of bat species in Greece from their echolocation calls. *Acta Chiropterologica*, 10(1), 127-143.
- Pathe, I. N., Julaihi, A. M., Ketol, B., Imat, A., Ahmad Mazlan, A. F., Omar, K. A., Ab Manaf, M. A., Muhammad Razaki, M. S., Wan Rahim, W. R., & Abdullah, M. T. (2005). Brief survey of small mammals of Jambusan Cave. Bau, Sarawak. In *Proceedings of the Eighth Hornbill Workshop on Protected Areas and Biodiversity Conservation. Kuching*.
- Pavey, C. R., & Burwell, C. J. (2005). Cohabitation and predation by insectivorous bats on eared moths in subterranean roosts. *Journal of Zoology*, 265(2), 141-146.

- Payne, J., Francis, C. M., & Phillipps, K. (1985). *A field guide to the mammals of Borneo*. Sabah: The Sabah Society.
- Payne, J., Francis, C. M., Phillipps, K., & Kartikasari, S. N. (2000). Mamalia di Kalimantan, Sabah. Sarawak dan Brunei Darussalam, Wildlife Conservation Society Indonesia Program, Jakarta
- Pearson, E. W. (1962). Bats hibernating in silica mines in southern Illinois. *Journal of Mammalogy*, 43(1), 27-33.
- Phillipps, Q., & Phillipps, K. (2016). *Phillipps' field guide to the mammals of Borneo and their ecology: Sabah, Sarawak, Brunei, and Kalimantan* (Vol. 105). New Jersey: Princeton University Press.
- Phillipps, Q., & Phillipps, K. (2018). *Phillipps' field guide to the mammals of Borneo and their ecology: Sabah, Sarawak, Brunei, and Kalimantan*. John Beaufoy Publishing Ltd.
- Poliquit, D., & Calong, E. (2018). Bat guano levels of application influencing carrot (*Daucus carota* L.) growth and yield performance. *Countryside Development Research Journal*, 6(1), 23-29.
- Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Functional Plant Biology*, 27(12), 1191-1191.
- Puechmaille, S. J., Mathy, G., & Petit, E. J. (2007). Good DNA from bat droppings. *Acta Chiropterologica*, 9(1), 269-276.
- Puechmaille, S. J., & Petit, E. J. (2007). Empirical evaluation of non-invasive capture-mark-recapture estimation of population size based on a single sampling session. *Journal of Applied Ecology*, 44, 843-852.

- Rajasegaran, P., Shazali, N., & Khan, F. A. A. (2018). Microclimate and physiological effects in the roosts of cave dwelling bats: implications in roost selection and conservation in Sarawak, Malaysian Borneo. *Zoological Science*, 35(6), 521-527.
- Ramasindrazana, B., Rakotondramanana, C. F., Schoeman, M. C., & Goodman, S. M. (2015). Evidence of echolocation call divergence in *Hipposideros commersoni* sensu stricto (E. Geoffroy, 1803) from Madagascar and correlation with body size. *Acta Chiropterologica*, 17(1), 85-94.
- Ravindran, B., Mupambwa, H. A., Silwana, S., & Mnkeni, P. N. (2017). Assessment of nutrient quality, heavy metals and phytotoxic properties of chicken manure on selected commercial vegetable crops. *Heliyon*, 3(12), e00493.
- Reid, M. S. (2003, August). Flower development: from bud to bloom. In *VIII International Symposium on Postharvest Physiology of Ornamental Plants* 669 (pp. 105-110).
- Ridzuan, R., Rafii, M. Y., Ismail, S. I., Mohammad Yusoff, M., Miah, G., & Usman, M. (2018). Breeding for anthracnose disease resistance in chili: Progress and prospects. *International Journal of Molecular Sciences*, 19(10), 3122.
- Ritz, C. W., & Merka, W. C. (2003). *Maximizing poultry manure utilization through nutrient management planning*. The University of Georgia College of Agricultural & Environmental Sciences. Retrieved May 29, 2024, from <https://esploro.libs.uga.edu/>
- Roberts, T. L. (2009). The role of fertiliser in growing the world's food. *Better Crops*, 93(2), 12-15.
- Rohlf, F. J. (1990). Morphometrics. *Annual Review of ecology and Systematics*, 21, 299-316.

- Romero, C., Ramos, P., Costa, C., & Márquez, M. C. (2013). Raw and digested municipal waste compost leachate as potential fertiliser: comparison with a commercial fertiliser. *Journal of Cleaner Production*, 59, 73-78.
- Rotz, C. A., Buckmaster, D. R., & Comerford, J. W. (2005). A beef herd model for simulating feed intake, animal performance, and manure excretion in farm systems. *Journal of Animal Science*, 83(1), 231-242.
- Ruedi, M., Csorba, G., Lin, L. K., & Chou, C. H. (2015). Molecular phylogeny and morphological revision of *Myotis* bats (Chiroptera: Vespertilionidae) from Taiwan and adjacent China. *Zootaxa*, 3920(1), 301-342.
- Russel, D. A., & Williams, G. G. (1977). History of chemical fertiliser development. *Soil Science Society of America Journal*, 41(2), 260-265.
- Russo, D., Ancillotto, L., & Jones, G. (2017). Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. *Canadian Journal of Zoology*, 96(2), 63-78.
- Russo, D., & Jones, G. (2002). Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. *Journal of Zoology*, 258(1), 91-103.
- Russo, D., & Voigt, C. C. (2016). The use of automated identification of bat echolocation calls in acoustic monitoring: a cautionary note for a sound analysis. *Ecological Indicators*, 66, 598-602.
- Rydell, J., Arita, H. T., Santos, M., & Granados, J. (2002). Acoustic identification of insectivorous bats (order Chiroptera) of Yucatan, Mexico. *Journal of Zoology*, 257(1), 27-36.

- Rylski, I. (1973). Effect of night temperature on shape and size of sweet pepper (*Capsicum annuum* L.). *Journal of the American Society for Horticultural Science*, 98(2), 149-152.
- Rylski, I., & Halevy, A. H. (1975). Optimal environment for set and development of sweet pepper fruit. *Acta Horticulture*, 42, 55-62.
- Sada, C., Alas, Y., & Anshari, M. (2019). Indigenous people of Borneo (Dayak): Development, social cultural perspective and its challenges. *Cogent Arts & Humanities*, 6(1), 1665936.
- Sahadevan, N. (1987). *Green fingers: A total commitment to the development of farming*. Malaysia: Sahadevan Publications Sdn. Bhd.
- Sakoui, S., Derdak, R., Addoum, B., Serrano-Delgado, A., Soukri, A., & El Khalfi, B. (2020). The life hidden inside caves: Ecological and economic importance of bat guano. *International Journal of Ecology*, 2020(5), 1-7.
- Salunkhe, D. K., & Kadam, S. S. (1998). Celery and other salad vegetables. In D. K. Salunkhe & S. S. Kadam (Eds.), *Handbook of Vegetable Science and Technology* (pp. 541-615). Boca Raton: CRC Press.
- Sarawak Forest Department. (1992). *Proposal to constitute Wind cave and Fairy Cave as Nature Reserve*. National Park Wildlife Office Forest Department, Sarawak.
- Schnitzler, H. U., Moss, C. F., & Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution*, 18(8), 386-394.
- Schroter, D., Cramer, W., Leemans, R., Prentice, I. C., Araujo, M. B., Arnell, N. W., Bondeau, A., Bugmann, H., Carter, T. R., Gracia, C. A., Vega-Leinert, A. C. D. L., Erhard, M., Ewert, F., Glendining, M., House, J. I., Kankaanpää, S., Klein, R. J. T., Lavorel, S.,

- Lindner, M., ... Zierl, B. (2005). Ecosystem service supply and vulnerability to global change in Europe. *Science*, 310(5752), 1333-1337.
- Senawi, J., & Ahmad, N. (2021). *Kelawar Malaysia / Bats of Malaysia*. Kementerian Tenaga dan Sumber Asli, Malaysia.
- Sexton, N. R., & Stewart, S. C. (2007). *Understanding knowledge and perceptions of bats among residents of Fort Collins, Colorado*. US Geological Survey.
- Shahack-Gross, R., Berna, F., Karkanas, P., & Weiner, S. (2004). Bat guano and preservation of archaeological remains in cave sites. *Journal of Archaeological Science*, 31(9), 1259-1272.
- Shazali, N. (2017). *Bats' Roost Selection and Population Count using Light Detection and Ranging (LiDAR) System in Wind Cave Nature Reserve*. (Publication No. 23843) [Masters' thesis, Universiti Malaysia Sarawak]. UNIMAS Institutional Repository.
- Shazali, N., Chew, T. H., Shamsir, M. S., Tingga, R. C. T., Mohd-Ridwan, A. R., & Khan, F. A. A. (2017). Assessing bat roosts using the LiDAR system at wind cave nature reserve in Sarawak, Malaysian Borneo. *Acta Chiropterologica*, 19(1), 199-210.
- Shetty, S., & Sreepada, K. S. (2013). Prey and Nutritional Analysis of *Megaderma lyra* Guano from The West Coast of Karnataka, India. *Advances in BioResearch*, 4(3).
- Shetty, S., Sreepada, K. S., & Bhat, R. (2013). Effect of bat guano on the growth of *Vigna radiata* L. *International Journal of Scientific and Research Publications*, 3(3), 1-8.
- Simmons, N. B., & Cirranello, A. L. (2023). Bat species of the world: a taxonomic and geographic database. Version 1.4. Accessed on August 21, 2023.
- Simmons, N. B., & Voss, R. S. (1998). The mammals of Paracou, French Guiana, a Neotropical lowland rainforest fauna. Part 1, Bats. Bulletin of the AMNH; no. 237.

- Singh, G., Shamsuddin, M. R., & Lim, S. W. (2018, December). Characterization of chicken manure from manjung region. In *IOP conference series: materials science and engineering* (Vol. 458, No. 1, p. 012084). IOP Publishing.
- Smil, V. (2000). Phosphorus in the environment: Natural flows and human interferences. *Annual Review of Energy and the Environment*, 25(1), 53-88.
- Soliman, E., Dawood, R. A., El-Far, I. A., & Bashir, M. I. (2016). Effect of NPK fertilization in the newly reclaimed soil on productivity of grain sorghum. *Assiut Journal of Agricultural Sciences*, 47, 363-376.
- Sothearen, T., Furey, N. M., & Jurgens, J. A. (2014). Effect of bat guano on the growth of five economically important plant species. *Journal of Tropical Agriculture*, 52(2), 169-173.
- Sridhar, K. R., Ashwini, K. M., Seena, S., & Sreepada, K. S. (2006). Manure qualities of guano of insectivorous cave bat *Hipposideros speoris*. *Tropical and Subtropical Agroecosystems*, 6(2), 103-110.
- Srivastava, L. M. (2002). *Plant Growth and Development: Hormones and Environment*. United States: Academic Press.
- Stadelmann, B., Lin, L. K., Kunz, T. H., & Ruedi, M. (2007). Molecular phylogeny of New World Myotis (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Molecular phylogenetics and evolution*, 43(1), 32-48.
- Stathopoulos, V., Zamora-Gutierrez, V., Jones, K. E., & Girolami, M. (2018). Bat echolocation call identification for biodiversity monitoring: a probabilistic approach. *Journal of the Royal Statistical Society Series C: Applied Statistics*, 67(1), 165-183.

- Stella, T., Mouratiadou, I., Gaiser, T., Berg-Mohnicke, M., Wallor, E., Ewert, F., & Nendel, C. (2019). Estimating the contribution of crop residues to soil organic carbon conservation. *Environmental Research Letters*, 14(9), 094008.
- Stewart, W. M., Dibb, D. W., Johnston, A. E., & Smyth, T. J. (2005). The contribution of commercial fertiliser nutrients to food production. *Agronomy Journal*, 97(1), 1-6.
- Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., Kistenich, S., Larsson, K. H., Liow, L. H., Nowak, M. D., Stedje, B., Bachmann, L., & Dimitrov, D. (2018). Finding evolutionary processes hidden in cryptic species. *Trends in Ecology & Evolution*, 33(3), 153-163.
- Struebig, M. J., Božek, M., Hildebrand, J., Rossiter, S. J., & Lane, D. J. (2012). Bat diversity in the lowland forests of the Heart of Borneo. *Biodiversity and Conservation*, 21, 3711-3727.
- Studier, E. H., Seveck, S. H., Keeler, J. O., & Schenck, R. A. (1994). Nutrient levels in guano from maternity colonies of big brown bats. *Journal of Mammalogy*, 75(1), 71-83.
- Suwannarong, K., Chanabun, S., Kanthawee, P., Khiewkhern, S., Boonyakawee, P., Suwannarong, K., Saengkul, C., Bubpa, N., & Amonsin, A. (2020). Risk factors for bat contact and consumption behaviors in Thailand: A quantitative study. *BMC Public Health*, 20, 1-13.
- Suwannarong, K., & Schuler, S. (2016). Bat consumption in Thailand. *Infection Ecology & Epidemiology*, 6(1), 29941.
- Suwarno & Idris, K. (2007). Potensi dan kemungkinan penggunaan guano secara langsung sebagai pupuk di Indonesia [Potential and possibility of direct use of guano as fertiliser in Indonesia]. *Jurnal Ilmu Tanah dan Lingkungan*, 9(1), 37-43.

- Swift, J. F., Lance, R. F., Guan, X., Britzke, E. R., Lindsay, D. L., & Edwards, C. E. (2018). Multifaceted DNA metabarcoding: Validation of a noninvasive, next-generation approach to studying bat populations. *Evolutionary Applications*, 11(7), 1120-1138.
- Syme, D. M., Fenton, B. M., & Zigouris, J. (2001). Roosts and food supplies ameliorate the impact of a bad summer on reproduction by the bat, *Myotis lucifugus* LeConte (Chiroptera: Vespertilionidae). *Ecoscience*, 8(1), 18-25.
- Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant Physiology and Development* (6th ed.). Sunderland: Sinauer Assoc Inc.
- Tan, C. S., Noni, V., Sathiya Seelan, J. S., Denel, A., & Anwarali Khan, F. A. (2021). Ecological surveillance of bat coronaviruses in Sarawak, Malaysian Borneo. *BMC Research Notes*, 14, 1-6.
- Tanalgo, K. C., & Tabora, J. A. G. (2015). Roosting ecology of cave-dwelling bats in southern Mindanao, Philippines. *Journal of Threatened Taxa*, 7(2), 6946–6953.
- Tanalgo, K. C., Teves, R. D., Salvana, F. R. P., Baleva, R. E., & Tabora, J. A. G. (2016). Human-bat interactions in caves of South-Central Mindanao, Philippines. *Wildlife Biology in Practice*, 12(1), 1-14.
- Taokaenchan, N., Areesrisom, P., & Satienperakul, S. (2020). Vitamin C, chlorophyll content and total phenolic compounds in *Artemisia lactiflora* as affected by different organic fertilisers. *Chiang Mai University Journal Natural Science*, 19(4), 1029-1041.
- Tasci, E., & Seckin, D. B. (2013). Guano-induced germination and responses of wheat seedlings to guano under water stress treatments. *Journal of the Faculty of Agriculture/Ziraat Fakültesi Dergisi*, 8(2), 44.

- Taylor, H., Powers, K. E., & Hallerman, E. M. (2022). *Development of a bat guano and acoustic sampling testing protocol to identify species occupying Virginia Department of Transportation bridges* (No. FHWA/VTRC 22-R20). Virginia Transportation Research Council (VTRC).
- Termine, E., Lairon, D., Taupier-Letage, B., Gautier, S., Lafont, R., & Lafont, H. (1987). Yield and content in nitrates, minerals and ascorbic acid of leeks and turnips grown under mineral or organic nitrogen fertilizations. *Plant Foods for Human Nutrition*, 37, 321-332.
- Thabab, A., Rossiter, S. J., Kingston, T., Zhang, S., Parsons, S., Mya, K. M., Akbar, Z., & Jones, G. (2006). Genetic divergence and echolocation call frequency in cryptic species of *Hipposideros larvatus* sl. (Chiroptera: Hipposideridae) from the Indo-Malayan region. *Biological Journal of the Linnean Society*, 88(1), 119-130.
- Thampan, P. K. (Ed.). (1993). *Organics in soil health and crop production*. Peekay Tree Crops Development Foundation.
- Thepsilvisut, O. (2021). Effect of chicken manure and biochar on growth, pigment concentration and mineral composition in green oak lettuce grown in acid soil. *Soil Use and Management*, 30(2), 182-188.
- Toor, G. S. (2009). Enhancing phosphorus availability in low-phosphorus soils by using poultry manure and commercial fertiliser. *Soil Science*, 174(6), 358-364.
- Torres-Jimenez, M. G., Murrieta-Galindo, R., Bolívar-Cimé, B., Wojtarowski-Leal, A., & Piñar-Álvarez, M. Á. (2020). Coffee farmers' perception of bat guano as fertiliser in agroecosystems of Mexico. *Regions and Cohesion*, 10(1), 22-35.

- Trontelj, P., & Fiser, C. (2009). Perspectives: cryptic species diversity should not be trivialised. *Systematics and Biodiversity*, 7(1), 1-3.
- Tuttle, M. D., & Moreno, A. (2005). *Cave-dwelling bats of Northern Mexico: Their value and conservation needs*. Bat Conservation International.
- Unal, M., Can, O., Can, B. A., & Poyraz, K. (2018). The effect of bat guano applied to the soil in different forms and doses on some plant nutrient contents. *Communications in Soil Science and Plant Analysis*, 49(6), 708-716.
- United States Fish and Wildlife Service. (2012, January 17). *North America bat death toll exceeds 5.5 million from white-nose syndrome*. U.S. Fish and Wildlife Service.
- Usman, M. G., Rafii, M. Y., Martini, M. Y., Yusuff, O. A., Ismail, M. R., & Miah, G. (2018). Introgression of heat shock protein (Hsp70 and sHsp) genes into the Malaysian elite chilli variety Kulai (*Capsicum annuum* L.) through the application of marker-assisted backcrossing (MAB). *Cell Stress and Chaperones*, 23(2), 223-234.
- Vandzurova, A., Pilis, V., Backor, P., Judova, J., Javorsky, P., Faix, S. P. P., & Pristas, P. (2012). Microflora of the bat guano. *Folia Vetrinaria*, 56(2), 68-69.
- Vaughan, N., Jones, G., & Harris, S. (1997). Identification of British bat species by multivariate analysis of echolocation call parameters. *Bioacoustics*, 7(3), 189-207.
- Vaughan, T. A. (1977). Foraging behaviour of the giant leaf-nosed bat (*Hipposideros commersoni*). *African Journal of Ecology*, 15(4), 237-249.
- Vege, S., & McCracken, G. F. (2001). Microsatellite genotypes of big brown bats (*Eptesicus fuscus*: Vespertilionidae, Chiroptera) obtained from their feces. *Acta Chiropterologica*, 3(2), 237-244.

- Viglino, A., Caniglia, R., Ruiz-Gonzalez, A., Russo, D., Galaverni, M., Toffoli, R., Culasso, P., Bernardi, P. D., Patriarca, E., Agnelli, P., Farina, F., Mastrobuoni, G., Martinoli, A., & Randi, E. (2016). What can we learn from faeces? Assessing genotyping success and genetic variability in three mouse-eared bat species from non-invasive genetic sampling. *Hystrix*, 27(2), 150.
- Vimala, P., Melor, R., Shokri, O. A., & Balasubramaniam, P. (2007). Effect of organic and inorganic fertilisers on growth, yield and nutrient content of bird chilli (*Capsicum frutescence*). *Journal of Tropical Agriculture and Food Science*, 35(1), 29.
- Vitoria, L., Otero, N., Soler, A., & Canals, A. (2004). Fertiliser characterization: isotopic data (N, S, O, C, and Sr). *Environmental Science & Technology*, 38(12), 3254-3262.
- Voigt, C. C., & Kingston, T. (2016). *Bats in the Anthropocene: Conservation of bats in a changing World*. [e-book] New York: Springer Nature. Available through: OAPEN Library <<https://library.oapen.org/>> [Accessed on 3 October 2022].
- Voigt-Heucke, S. L., Taborsky, M., & Dechmann, D. K. (2010). A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Animal Behaviour*, 80(1), 59-67.
- Walan, P. (2013). Modelling of peak phosphorus: A study of bottlenecks and implications for future production. *Unpublished report. Uppsala University*.
- Walker, F. M., Tobin, A., Simmons, N. B., Sobek, C. J., Sanchez, D. E., Chambers, C. L., & Fofanov, V. Y. (2019). A fecal sequel: Testing the limits of a genetic assay for bat species identification. *Public Library of Science One*, 14(11), e0224969.

- Walker, F. M., Williamson, C. H., Sanchez, D. E., Sobek, C. J., & Chambers, C. L. (2016). Species from feces: order-wide identification of Chiroptera from guano and other non-invasive genetic samples. *Public Library of Science One*, *11*(9), e0162342.
- Wang, L. S., Wang, R. J., Lu, C. P., Wang, J., & Huang, W. (2019). Rapid determination of moisture content in compound fertiliser using visible and near infrared spectroscopy combined with chemometrics. *Infrared Physics & Technology*, *102*, 103045.
- Wang, Z., & Li, S. (2004). Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. *Journal of Plant Nutrition*, *27*(3), 539-556.
- Wanger, T. C., Darras, K., Bumrungsri, S., Tschardtke, T., & Klein, A. M. (2014). Bat pest control contributes to food security in Thailand. *Biological Conservation*, *171*, 220-223.
- Ware, R. L., Garrod, B., Macdonald, H., & Allaby, R. G. (2020). Guano morphology has the potential to inform conservation strategies in British bats. *Public Library of Science One*, *15*(4), e0230865.
- Wasti, I. G., Khan, F. A. A., Bernard, H., Hassan, N. H., Fayle, T., & Sathiya Seelan, J. S. (2021). Fungal communities in bat guano, speleothem surfaces, and cavern water in Madai cave, Northern Borneo (Malaysia). *Mycology*, *12*(3), 188-202.
- Wei, C., Sangster, G., Olsson, U., Rasmussen, P. C., Svensson, L., Carey, G. J., Leader, P. J., Zhang, R., Chen, G., Song, G., Lei, F., Wilcove, D. S., Alstrom, P., & Liu, Y. (2022). Cryptic species in a colorful genus: Integrative taxonomy of the bush robins (Aves, Muscicapidae, Tarsiger) suggests two overlooked species. *Molecular Phylogenetics and Evolution*, *175*, 107580.
- Whitaker, J. O., & Black, H. (1976). Food habits of cave bats from Zambia, Africa. *Journal of Mammalogy*, *57*(1), 199-204.

- Whitaker, J. O. Jr., & Tomich, P. Q. (1983). Food habits of the hoary bat, *Lasiurus cinereus*, from Hawaii. *Journal of Mammalogy*, 64(1), 151-152.
- Whitehead, D. C. (2000). *Nutrient elements in grassland: Soil-plant-animal relationships*. CABI Publishing.
- Wijayanti, F. (2011). Biodiversitas dan Pola Pemilihan Sarang Kelelawar: Studi Kasus di Kawasan Karst Gombang Kabupaten Kebumen Jawa Tengah. *Unpublished report*. Institut Pertanian Bogor.
- Wildlife Acoustic (2023). *Echo Meter Touch 2 and 2 Pro for ANDROID User Guide*. [online] Available at: <https://www.wildlifeacoustics.com/uploads/user-guides/EMT-ANDROID-GUIDE-EN-12112023.pdf> [Accessed on 23 February 2025].
- Wildlife Acoustic (2024). *Echo Meter Touch 2 Pro*. [online] Available at: <https://www.wildlifeacoustics.com/products/echo-meter-touch-2-pro> [Accessed on 23 February 2025].
- World Wildlife Fund [WWF]. (n.d.). *Heart of Borneo*. [online] Available at: https://wwf.panda.org/discover/knowledge_hub/where_we_work/borneo_forests/ [Accessed on 3 September 2022].
- Wurster, C. M., Munksgaard, N., Zwart, C., & Bird, M. (2015). The biogeochemistry of insectivorous cave guano: a case study from insular Southeast Asia. *Biogeochemistry*, 124(1), 163-175.
- Yankova, P., Naskova, P., Malcheva, B., & Plamenov, D. (2016). Impact of the biological fertilisers on the microorganisms and the nutrient elements in the soil. *International Journal of Current Research*, 8, 39681-39686.

- Yenesew, M., & Tilahun, K. (2009). Yield and water use efficiency of deficit-irrigated maize in a semi-arid region of Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 9(8), 1635-1651.
- Yusti, E., Maryanto, I., & Suryobroto, B. (2015). The fruit bats (Megachiroptera, Pteropodidae) from Mount Bawakaraeng, South Sulawesi. *Borneo Science*, 36(1), 33–43.
- Zandvakili, O. R., Barker, A. V., Hashemi, M., Etemadi, F., Autio, W. R., & Weis, S. (2019). Growth and nutrient and nitrate accumulation of lettuce under different regimes of nitrogen fertilization. *Journal of Plant Nutrition*, 42(14), 1575-1593.
- Zhang, Q. P., & Wei, X. (2022). Transcriptome analysis of flower bud identified genes associated with pistil abortions between long branches and spur twigs in apricots (*Prunus armeniaca* L.). *Public Library of Science One*, 17(8), e0273109.

Appendix A- Growth House Layout with the polybags marked accordingly

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
i																											
ii																											
iii																											
iv																											
v																											
vi																											

Legend:

	A	No Fertilizer	-
	B	Commercialized Fertilizer	5 g
	C	Chicken Dung (Unfertilized)	5 g
	Da	Bat's Guano A1 (WCR)	2.5 g
	Ea	Bat's Guano A2 (WCR)	5 g
	Fa	Bat's Guano A3 (WCR)	7.5 g
	Db	Bat's Guano B1 (GR)	2.5 g
	Eb	Bat's Guano B2 (GR)	5 g
	Fb	Bat's Guano B3 (GR)	7.5 g

Appendix B1 – Plant growth results for Month 1 to Month 3

Date		3 Oct 2022		9 Nov 2022		1 Dec 2022				
Px	Tx	PH	NoL	PH	NoL	PH	NoL	NoFB	NoFl	NoFr
i	1	NF	5	2	15.5	4	15.3	5		
	2	CF	5	2	14	4	31	15		
	3	CDF	5	2	13.2	7	17	8		
	4	BG1	5	2	14.5	4	16	8		
	5	BG2	5	2	14	8	15.5	9		
	6	BG3	5	2	14.5	6	17	10		
	7	NF	5	2	8.8	7	10	2		
	8	CF	5	2	20.5	6	27	9		
	9	CDF	5	2	14.2	7	15	6		
	10	BG1	5	2	9	8	8.5	5		
	11	BG2	5	2	11.2	6	13	6		
	12	BG3	5	2	14.5	6	15.5	9		
	13	NF	5	2	10.5	7	13	6		
	14	CF	5	2	14	7	33	23		

	15	CDF	5	2	19	7	22	12			
	16	BG1	5	2	10.5	5	11	4			
	17	BG2	5	2	13.5	5	16	8			
	18	BG3	5	2	13	5	16	10			
	19	NF	5	2	11.5	8	11	8			
	20	CF	5	2	20.5	7	33	17			
	21	CDF	5	2	13.5	8	16	8			
	22	BG1	5	2	10.5	6	14	9			
	23	BG2	5	2	14.5	8	16.5	12			
	24	BG3	5	2	14	6	17.5	11			
	25	NF	5	2	8	6	9.5	5			
	26	CF	5	2	16.5	5	20	7			
	27	CDF	5	2	20	7	33.5	10			
ii	1	CF	5	2	16	5	20.5	5			
	2	CDF	5	2	15	8	15	6			
	3	BG1	5	2	12	6	14	7			
	4	BG2	5	2	14	7	15.5	7			

	5	BG3	5	2	14	6	17	9			
	6	NF	5	2	9.5	8	14.5	10			
	7	CF	5	2	20.5	8	36	23	3	1	
	8	CDF	5	2	19.5	6	26	10			
	9	BG1	5	2	11	9	14	4			
	10	BG2	5	2	13	8	14	7			
	11	BG3	5	2	10	8	12	6			
	12	NF	5	2	9.5	6	11	4			
	13	CF	5	2	25	6	27	3			
	14	CDF	5	2	17	7	20	9			
	15	BG1	5	2	13	7	13	3			
	16	BG2	5	2	14	6	16	7			
	17	BG3	5	2	11.5	6	15	12			
	18	NF	5	2	9.5	7	11	5			
	19	CF	5	2	31	6	31	17			
	20	CDF	5	2	11	4	13	5			
	21	BG1	5	2	8	7	8	4			

	22	BG2	5	2	12	5	14	6			
	23	BG3	5	2	16	8	21	8			
	24	NF	5	2	13	7	14	8			
	25	CF	5	2	20	6	36	22	3		2
	26	CDF	5	2	18.5	5	27.5	14			
	27	BG1	5	2	7	8	9.5	7			
iii	1	CDF	5	2	12	3	15	6			
	2	BG1	5	2	9.5	7	10.5	3			
	3	BG2	5	2	9	6	10	8			
	4	BG3	5	2	7	7	8	6			
	5	NF	5	2	7	8	8	2			
	6	CF	5	2	20	8	35	16			
	7	CDF	5	2	18	7	26.5	13			
	8	BG1	5	2	16	6	18	9			
	9	BG2	5	2	18	5	19	10			
	10	BG3	5	2	7	6	8	5			
	11	NF	5	2	9	8	10	3			

	12	CF	5	2	15	4	30	9			
	13	CDF	5	2	7	5	8.5	4			
	14	BG1	5	2	13	6	13	5			
	15	BG2	5	2	7	4	10	5			
	16	BG3	5	2	20	8	24.5	11			
	17	NF	5	2	8	6	9	2			
	18	CF	5	2	22	6	29	16	2		1
	19	CDF	5	2	15	9	18	13			
	20	BG1	5	2	7	6	9.5	2			
	21	BG2	5	2	12	5	13	8			
	22	BG3	5	2	11	7	12	9			
	23	NF	5	2	9	5	10	5			
	24	CF	5	2	26	6	29	14			
	25	CDF	5	2	11	5	13	2			
	26	BG1	5	2	23	8	25	16			
	27	BG2	5	2	12	6	13	4			
iv	1	BG1	5	2	9	6	9	9			

	2	BG2	5	2	12	5	14	7			
	3	BG3	5	2	25	5	30	12			
	4	NF	5	2	12	7	13.5	5			
	5	CF	5	2	18	6	22.5	9			
	6	CDF	5	2	19	8	25	13			
	7	BG1	5	2	9	8	9.5	5			
	8	BG2	5	2	11	7	12	9			
	9	BG3	5	2	14	8	17	4			
	10	NF	5	2	10	5	13	6			
	11	CF	5	2	14	8	18.5	7			
	12	CDF	5	2	19	6	21.5	9			
	13	BG1	5	2	10	4	14	9			
	14	BG2	5	2	11	8	13	10			
	15	BG3	5	2	14.5	6	15.5	6			
	16	NF	5	2	11	6	11	10			
	17	CF	5	2	25	8	46	20	4		1
	18	CDF	5	2	26	8	29	14			

	19	BG1	5	2	11	9	15	7			
	20	BG2	5	2	11.5	9	13	8			
	21	BG3	5	2	16	7	19.5	11			
	22	NF	5	2	8	7	10.5	4			
	23	CF	5	2	20	8	24	9			
	24	CDF	5	2	13	7	14	3			
	25	BG1	5	2	13	4	15.5	5			
	26	BG2	5	2	11	6	13	4			
	27	BG3	5	2	16	7	21	12			
v	1	BG2	5	2	10	6	14	7			
	2	BG3	5	2	9	7	10.5	8			
	3	NF	5	2	9	8	10	4			
	4	CF	5	2	21	7	32.5	10			
	5	CDF	5	2	18	6	30	14	1	1	
	6	BG1	5	2	6	8	7	5			
	7	BG2	5	2	14	7	15	7			
	8	BG3	5	2	6	8	7.5	4			

	9	NF	5	2	7	8	8	6			
	10	CF	5	2	7	6	11.5	9			
	11	CDF	5	2	15	6	15	3			
	12	BG1	5	2	10	6	10	4			
	13	BG2	5	2	9	7	13	14			
	14	BG3	5	2	13	6	18	10			
	15	NF	5	2	10	6	12	7			
	16	CF	5	2	25	7	47	20	10	4	8
	17	CDF	5	2	19	8	34	17			
	18	BG1	5	2	10	8	12.5	9			
	19	BG2	5	2	7	7	9.5	10			
	20	BG3	5	2	12	7	13.5	13			
	21	NF	5	2	8	7	9	5			
	22	CF	5	2	11	8	21	6			
	23	CDF	5	2	11	4	12.5	7			
	24	BG1	5	2	15	7	17	12			
	25	BG2	5	2	10	7	12.5	8			

	26	BG3	5	2	11	6	14.5	9			
	27	NF	5	2	8	6	8	4			
vi	1	BG3	5	2	8	6	10.5	4			
	2	NF	5	2	8	6	9.5	1			
	3	CF	5	2	16	6	18	7			
	4	CDF	5	2	15	6	17.5	6			
	5	BG1	5	2	10.5	4	11	5			
	6	BG2	5	2	10	6	11	5			
	7	BG3	5	2	11	4	13	7			
	8	NF	5	2	7	6	10	2			
	9	CF	5	2	13	6	16	5			
	10	CDF	5	2	15	7	18	5			
	11	BG1	5	2	10.5	6	11	8			
	12	BG2	5	2	6	6	6.5	4			
	13	BG3	5	2	13	8	14.5	11			
	14	NF	5	2	7	8	7	3			
	15	CF	5	2	16	6	35	24	4		4

	16	CDF	5	2	13.5	5	15	4			
	17	BG1	5	2	8	4	8.5	9			
	18	BG2	5	2	7	7	8	12			
	19	BG3	5	2	15	6	21	9			
	20	NF	5	2	10	6	11	6			
	21	CF	5	2	15	8	29.5	12			
	22	CDF	5	2	13	6	15.5	7			
	23	BG1	5	2	7	8	7	3			
	24	BG2	5	2	10	5	11	5			
	25	BG3	5	2	8	6	9.5	6			
	26	NF	5	2	10	7	14	7			
	27	CF	5	2	13	5	22	6			

Notes: CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g), PH= Plant Height, NoL= Number of Leaves, NoFB= Number of Flower Buds, NoFl= Number of Flowers, NoFr= Number of Fruits.

Appendix B2- Plant growth results for Month 4 to Month 6

Date			8 Jan 2023					8 Feb 2023					12 Mac 23				
Px	Tx		PH	No L	NoFB	NoF l	NoF r	PH	No L	NoFB	NoF l	NoF r	PH	No L	NoFB	NoF l	No Fr
i	1	NF	17	8				20	10								
	2	CF	47	30	8		1	46	27								
	3	CDF	18	12				25.5	10								
	4	BG1	17.5	11				14.5	4								
	5	BG2	14.5	9				19	6								
	6	BG3	14.5	0				15	0								
	7	NF	9.5	2				8.5	0								
	8	CF	28	8				23	6								
	9	CDF	18	4				13.5	4								
	10	BG1	10	2				10	2								
	11	BG2	13	3				13	6								
	12	BG3	17	8				17	12								
	13	NF	11	5				10	0								

	14	CF	46	63	3			46	24								
	15	CDF	24	11				21	0								
	16	BG1	19	3				8.5	0								
	17	BG2	15	2				11	0								
	18	BG3	16	6				13.5	0								
	19	NF	12	1				12.5	0								
	20	CF	39	21				41	22								
	21	CDF	26	10				37	17								
	22	BG1	15	10				17	12								
	23	BG2	16	20				32	22				41	34	4	1	
	24	BG3	20	2				23.5	7								
	25	NF	8	4				9	1								
	26	CF	20	5				22.5	6								
	27	CDF	45	24	3			49	37	3			49.5	24			1
ii	1	CF	18	19				39	50	3		3	42	56			3
	2	CDF	17	4				15	0								
	3	BG1	17.5	6				17	4								

	4	BG2	20	9				21	12								
	5	BG3	22	14				31	25	2	2	1	31	9			
	6	NF	17	9				15.5	14								
	7	CF	37	32	3		1	17	1								
	8	CDF	27	14				29	19								
	9	BG1	13	3				13	0								
	10	BG2	17	3				17	5								
	11	BG3	18	11				22	12								
	12	NF	11	5				11	2								
	13	CF	28	8				30	22								
	14	CDF	20	8				19	0								
	15	BG1	9	3				8	0								
	16	BG2	15	2				15	0								
	17	BG3	19	13				25	25								
	18	NF	14	7				9	3								
	19	CF	39	36		4		46	80				47	43			
	20	CDF	13	4				12	0								

	21	BG1	13	3				14	6								
	22	BG2	13	11				17	12								
	23	BG3	19	13				42	33	4			41	53			
	24	NF	17	9				19	5								
	25	CF	50	34		1	2	46	0								
	26	CDF	47	27				43	0								
	27	BG1	15	10				18	8								
iii	1	CDF	15	3				16	2								
	2	BG1	10	3				12	2								
	3	BG2	12	4				10	0								
	4	BG3	11	7				13	8								
	5	NF	8	3				6	0								
	6	CF	41	45	1			39	57				36	65			
	7	CDF	38	20				47	13								
	8	BG1	17	7				18	5								
	9	BG2	21	13				12	17								
	10	BG3	7	5				10	5								

	11	NF	10	3				9	0								
	12	CF	39	34	2			44	70	1	3		46	55	1		1
	13	CDF	9.5	8				12	2								
	14	BG1	15	7				11	1								
	15	BG2	9.5	3				7	0								
	16	BG3	29	12			2	29	11	1			27.5	11			
	17	NF	14	4				12	2								
	18	CF	44	27	2			35	21	1	2		36	32	1		
	19	CDF	42	26				44	18	2			25	6			
	20	BG1	10	4				16	7								
	21	BG2	17	10				19	10								
	22	BG3	19	12				20	18								
	23	NF	10	0				9	1								
	24	CF	36	37	2	1		35	42	1	1		30	0			
	25	CDF	19	7				16	0								
	26	BG1	33	17				40	24	2	1		38	34			
	27	BG2	15	11				28	15								

iv	1	BG1	9	0				11	4								
	2	BG2	13	5				15	3								
	3	BG3	35	29			3	42	15		2		44	33	3		
	4	NF	14	3				10	0								
	5	CF	35	44				37	72				45	40			
	6	CDF	31	16				36	16	2		1	35	13			
	7	BG1	10	2				10	5								
	8	BG2	14	2				13	3								
	9	BG3	13	6				12.5	0								
	10	NF	13.5	7				13	4								
	11	CF	19	8				18	0								
	12	CDF	27	4				28	3								
	13	BG1	16	9				17	8								
	14	BG2	15	6				22	9								
	15	BG3	20	10				32	15	2	1		27	14			1
	16	NF	11	4				12	2								
	17	CF	61	44	1	3	2	56	39								

	18	CDF	45	19				43	18								
	19	BG1	18	6				20	10								
	20	BG2	22	15				38	24	2	1		36	19			1
	21	BG3	26	9				34	20		1		38	37	1	1	
	22	NF	10	2				10	1								
	23	CF	29	24				17	37	1							
	24	CDF	16	4				22	6								
	25	BG1	15	2				13	2								
	26	BG2	13	8				26	9								
	27	BG3	30	12				27	15				23	0			
v	1	BG2	17.5	18				16	25								
	2	BG3	24	18				33	47	1	3	2	33	50			4
	3	NF	10	3				11	2								
	4	CF	44	35		3	2	39	13								
	5	CDF	38	23			1	37	15	3			40	13	1		1
	6	BG1	8	7				15	7								
	7	BG2	20	14				26	27	4			26	27			

	8	BG3	26	18				33	31	2	3	1	36	16			1
	9	NF	9	6				7	7								
	10	CF	17	11				16	9								
	11	CDF	16	6				18	4								
	12	BG1	11	7				14	10								
	13	BG2	16	7				28	8								
	14	BG3	21	9				21	6								
	15	NF	15	5				15	1								
	16	CF	57	61	4	2	5	50	96	4	1		55	75	2		1
	17	CDF	52	28	3	1		50	25	2	1		49	19			
	18	BG1	14	12				12	3								
	19	BG2	19	12				20	15								
	20	BG3	13	13				24	24	3			35	26	3	2	
	21	NF	9	1				10	0								
	22	CF	35	23	1	1	1	37	8			1					
	23	CDF	13	6				20	8								
	24	BG1	19	19				17	25								

	25	BG2	17	10				18	10								
	26	BG3	21	14				39	43	4			37	42			2
	27	NF	8	4				8	4								
vi	1	BG3	10	2				9	0								
	2	NF	9.5	2				9	0								
	3	CF	36	48	2	2		39	44	1	2		38	36	3		
	4	CDF	31	23				41	23								
	5	BG1	12	6				10	4								
	6	BG2	11	7				16	5								
	7	BG3	13	12				14	0								
	8	NF	9.5	2				11	2								
	9	CF	18	4				19	7								
	10	CDF	16	5				14	0								
	11	BG1	13	4				14	6								
	12	BG2	17	5				7.5	6								
	13	BG3	16	9				24	14	2			25	30		1	
	14	NF	8	0				6	0								

15	CF	46	44	2	2	5	36	17									
16	CDF	18	10				25	10									
17	BG1	17	10				18	10									
18	BG2	23	29				19	12									
19	BG3	26	7				27	17		1		27	15				
20	NF	11	5				11	3									
21	CF	44	17	3			36	19									
22	CDF	14	7				13	0									
23	BG1	10	10				14	15									
24	BG2	16	8				19	13									
25	BG3	15	16				34	30									
26	NF	12	6				13	4									
27	CF	33	30	1			39	27	8	2		45	94	3	2	1	

Notes: CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g), PH= Plant Height, NoL= Number of Leaves, NoFB= Number of Flower Buds, NoFl= Number of Flowers, NoFr= Number of Fruits.

Appendix C- Effect of Treatments and Months on Growth Performance of Chilli Plants

	PH	NoL	NoFB	NoFl	NoFr
Treatment					
NF	9.527	4.05	0.00	0.00	0.00
CF	25.831	18.42	0.63	0.26	0.31
CDF	19.489	7.94	0.14	0.02	0.03
BG1	11.654	5.93	0.01	0.01	0.00
BG2	13.251	7.49	0.07	0.01	0.01
BG3	16.956	10.26	0.19	0.11	0.11
Months					
M1	5.000	2.00	0.00	0.00	0.00
M2	13.024	6.47	0.00	0.00	0.00
M3	16.591	8.14	0.17	0.04	0.10
M4	20.540	12.04	0.25	0.12	0.15
M5	22.000	12.50	0.38	0.17	0.06
M6	37.000	31.91	0.69	0.22	0.53
Interaction					
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001
Months	<0.001	<0.001	<0.001	<0.001	<0.001
Tx X M	<0.001	<0.001	<0.001	<0.001	<0.001

Notes: CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g), M1= Month 1, M2= Month 2, M3= Month 3, M4= Month 4, M5= Month 5, M6= Month 6, PH= Plant Height, NoL= Number of Leaves, NoFB= Number of Flower Buds, NoFl= Number of Flowers, NoFr= Number of Fruits.

Appendix D1 – Post Hoc test of Least Significant Differences on Plant Height measurements

		Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
Tx (I)	Tx (J)				Lower Bound	Upper Bound
NF	CF	-16.3044 [*]	1.1687	<0.001	-18.598	-14.010
	CDF	-9.9619 [*]	1.1787	<0.001	-12.275	-7.648
	BG1	-2.1277	1.1872	0.073	-4.458	0.202
	BG2	-3.7248 [*]	1.1829	0.002	-6.047	-1.403
	BG3	-7.4294 [*]	1.1629	<0.001	-9.712	-5.147
CF	NF	16.3044 [*]	1.1687	<0.001	14.010	18.598
	CDF	6.3425 [*]	1.1578	<0.001	4.070	8.615
	BG1	14.1766 [*]	1.1664	<0.001	11.887	16.466
	BG2	12.5796 [*]	1.1621	<0.001	10.299	14.861
	BG3	8.8750 [*]	1.1418	<0.001	6.634	11.116
CDF	NF	9.9619 [*]	1.1787	<0.001	7.648	12.275
	CF	-6.3425 [*]	1.1578	<0.001	-8.615	-4.070
	BG1	7.8342 [*]	1.1765	<0.001	5.525	10.143
	BG2	6.2371 [*]	1.1722	<0.001	3.936	8.538
	BG3	2.5325 [*]	1.1520	0.028	0.271	4.794
BG1	NF	2.1277	1.1872	0.073	-0.202	4.458
	CF	-14.1766 [*]	1.1664	<0.001	-16.466	-11.887
	CDF	-7.8342 [*]	1.1765	<0.001	-10.143	-5.525
	BG2	-1.5970	1.1807	0.177	-3.914	0.720
	BG3	-5.3017 [*]	1.1607	<0.001	-7.580	-3.023

BG2	NF	3.7248*	1.1829	0.002	1.403	6.047
	CF	-12.5796*	1.1621	<0.001	-14.861	-10.299
	CDF	-6.2371*	1.1722	<0.001	-8.538	-3.936
	BG1	1.5970	1.1807	0.177	-0.720	3.914
	BG3	-3.7046*	1.1563	0.001	-5.974	-1.435
BG3	NF	7.4294*	1.1629	<0.001	5.147	9.712
	CF	-8.8750*	1.1418	<0.001	-11.116	-6.634
	CDF	-2.5325*	1.1520	0.028	-4.794	-0.271
	BG1	5.3017*	1.1607	<0.001	3.023	7.580
	BG2	3.7046*	1.1563	0.001	1.435	5.974

All analyses are least significant difference results using Post Hoc Fischer's Least Significant Difference Test, *The mean difference is significant at the 0.05 level, *Notes:* Tx= Treatment type, NF= No Fertiliser, CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g).

Appendix D2 - Post Hoc test of Least Significant Differences on Number of Leaves

		Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
Tx (I)	Tx (J)				Lower Bound	Upper Bound
NF	CF	-14.369 [*]	1.255	<0.001	-16.83	-11.91
	CDF	-3.891 [*]	1.266	0.002	-6.38	-1.41
	BG1	-1.875	1.275	0.142	-4.38	0.63
	BG2	-3.434 [*]	1.270	0.007	-5.93	-0.94
	BG3	-6.205 [*]	1.249	<0.001	-8.66	-3.75
CF	NF	14.369 [*]	1.255	<0.001	11.91	16.83
	CDF	10.478 [*]	1.243	<0.001	8.04	12.92
	BG1	12.494 [*]	1.252	<0.001	10.04	14.95
	BG2	10.935 [*]	1.248	<0.001	8.49	13.38
	BG3	8.164 [*]	1.226	<0.001	5.76	10.57
CDF	NF	3.891 [*]	1.266	0.002	1.41	6.38
	CF	-10.478 [*]	1.243	<0.001	-12.92	-8.04
	BG1	2.016	1.263	0.111	-0.46	4.50
	BG2	0.457	1.259	0.716	-2.01	2.93
	BG3	-2.314	1.237	0.062	-4.74	0.11
BG1	NF	1.875	1.275	0.142	-0.63	4.38
	CF	-12.494 [*]	1.252	<0.001	-14.95	-10.04
	CDF	-2.016	1.263	0.111	-4.50	0.46
	BG2	-1.559	1.268	0.219	-4.05	0.93
	BG3	-4.330 [*]	1.246	0.001	-6.78	-1.88

BG2	NF	3.434*	1.270	0.007	0.94	5.93
	CF	-10.935*	1.248	<0.001	-13.38	-8.49
	CDF	-0.457	1.259	0.716	-2.93	2.01
	BG1	1.559	1.268	0.219	-0.93	4.05
	BG3	-2.771*	1.242	0.026	-5.21	-0.33
BG3	NF	6.205*	1.249	<0.001	3.75	8.66
	CF	-8.164*	1.226	<0.001	-10.57	-5.76
	CDF	2.314	1.237	0.062	-0.11	4.74
	BG1	4.330*	1.246	0.001	1.88	6.78
	BG2	2.771*	1.242	0.026	0.33	5.21

All analyses are least significant difference results using Post Hoc Fischer's Least Significant Difference Test, *The mean difference is significant at the 0.05 level, *Notes:* Tx= Treatment type, NF= No Fertiliser, CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g).

Appendix D3 - Post Hoc test of Least Significant Differences on Number of Flower Buds

		Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
Tx (I)	Tx (J)				Lower Bound	Upper Bound
NF	CF	-0.628*	0.093	<0.001	-0.81	-0.45
	CDF	-0.143	0.094	0.128	-0.33	0.04
	BG1	-0.015	0.094	0.876	-0.20	0.17
	BG2	-0.072	0.094	0.441	-0.26	0.11
	BG3	-0.189*	0.092	0.041	-0.37	-0.01
CF	NF	.628*	0.093	<0.001	0.45	0.81
	CDF	.485*	0.092	<0.001	0.30	0.67
	BG1	.613*	0.093	<0.001	0.43	0.79
	BG2	.555*	0.092	<0.001	0.37	0.74
	BG3	.438*	0.091	<0.001	0.26	0.62
CDF	NF	0.143	0.094	0.128	-0.04	0.33
	CF	-0.485*	0.092	<0.001	-0.67	-0.30
	BG1	0.128	0.094	0.171	-0.06	0.31
	BG2	0.070	0.093	0.450	-0.11	0.25
	BG3	-0.046	0.092	0.613	-0.23	0.13
BG1	NF	0.015	0.094	0.876	-0.17	0.20
	CF	-0.613*	0.093	<0.001	-0.79	-0.43

	CDF	-0.128	0.094	0.171	-0.31	0.06
	BG2	-0.058	0.094	0.539	-0.24	0.13
	BG3	-0.174	0.092	0.059	-0.36	0.01
BG2	NF	0.072	0.094	0.441	-0.11	0.26
	CF	-0.555*	0.092	<0.001	-0.74	-0.37
	CDF	-0.070	0.093	0.450	-0.25	0.11
	BG1	0.058	0.094	0.539	-0.13	0.24
	BG3	-0.117	0.092	0.205	-0.30	0.06
BG3	NF	.189*	0.092	0.041	0.01	0.37
	CF	-0.438*	0.091	<0.001	-0.62	-0.26
	CDF	0.046	0.092	0.613	-0.13	0.23
	BG1	0.174	0.092	0.059	-0.01	0.36
	BG2	0.117	0.092	0.205	-0.06	0.30

All analyses are least significant difference results using Post Hoc Fischer's Least Significant Difference Test, *The mean difference is significant at the 0.05 level, *Notes:* Tx= Treatment type, NF= No Fertiliser, CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g).

Appendix D4 - Post Hoc test of Least Significant Differences on measurements Number of Flowers

Tx (I)	Tx (J)	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
					Lower Bound	Upper Bound
NF	CF	-0.255*	0.046	<0.001	-0.35	-0.17
	CDF	-0.021	0.046	0.643	-0.11	0.07
	BG1	-0.007	0.047	0.874	-0.10	0.08
	BG2	-0.014	0.046	0.755	-0.11	0.08
	BG3	-0.115*	0.046	0.012	-0.20	-0.03
CF	NF	.255*	0.046	<0.001	0.17	0.35
	CDF	.234*	0.045	<0.001	0.14	0.32
	BG1	.248*	0.046	<0.001	0.16	0.34
	BG2	.241*	0.046	<0.001	0.15	0.33
	BG3	.140*	0.045	0.002	0.05	0.23
CDF	NF	0.021	0.046	0.643	-0.07	0.11
	CF	-0.234*	0.045	<0.001	-0.32	-0.14
	BG1	0.014	0.046	0.760	-0.08	0.10
	BG2	0.007	0.046	0.880	-0.08	0.10
	BG3	-0.093*	0.045	0.039	-0.18	0.00
BG1	NF	0.007	0.047	0.874	-0.08	0.10
	CF	-0.248*	0.046	<0.001	-0.34	-0.16
	CDF	-0.014	0.046	0.760	-0.10	0.08
	BG2	-0.007	0.046	0.877	-0.10	0.08
	BG3	-0.108*	0.045	0.018	-0.20	-0.02

BG2	NF	0.014	0.046	0.755	-0.08	0.11
	CF	-0.241*	0.046	<0.001	-0.33	-0.15
	CDF	-0.007	0.046	0.880	-0.10	0.08
	BG1	0.007	0.046	0.877	-0.08	0.10
	BG3	-0.100*	0.045	0.027	-0.19	-0.01
BG3	NF	.115*	0.046	0.012	0.03	0.20
	CF	-0.140*	0.045	0.002	-0.23	-0.05
	CDF	.093*	0.045	0.039	0.00	0.18
	BG1	.108*	0.045	0.018	0.02	0.20
	BG2	.100*	0.045	0.027	0.01	0.19

All analyses are least significant difference results using Post Hoc Fischer's Least Significant Difference Test, *The mean difference is significant at the 0.05 level, *Notes:* Tx= Treatment type, NF= No Fertiliser, CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g).

Appendix D5 - Post Hoc test of Least Significant Differences on Number of Fruits

Tx (I)	Tx (J)	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
					Lower Bound	Upper Bound
NF	CF	-0.310*	0.059	<0.001	-0.43	-0.19
	CDF	-0.029	0.059	0.631	-0.15	0.09
	BG1	<0.001	0.060	1.000	-0.12	0.12
	BG2	-0.007	0.060	0.903	-0.12	0.11
	BG3	-0.115	0.059	0.050	-0.23	0.00
CF	NF	.310*	0.059	<0.001	0.19	0.43
	CDF	.282*	0.058	<0.001	0.17	0.40
	BG1	.310*	0.059	<0.001	0.19	0.43
	BG2	.303*	0.059	<0.001	0.19	0.42
	BG3	.195*	0.058	0.001	0.08	0.31
CDF	NF	0.029	0.059	0.631	-0.09	0.15
	CF	-0.282*	0.058	<0.001	-0.40	-0.17
	BG1	0.029	0.059	0.630	-0.09	0.15
	BG2	0.021	0.059	0.718	-0.09	0.14
	BG3	-0.086	0.058	0.138	-0.20	0.03
BG1	NF	<0.001	0.060	1.000	-0.12	0.12
	CF	-0.310*	0.059	<0.001	-0.43	-0.19
	CDF	-0.029	0.059	0.630	-0.15	0.09
	BG2	-0.007	0.060	0.903	-0.12	0.11
	BG3	-0.115	0.059	0.050	-0.23	0.00

BG2	NF	0.007	0.060	0.903	-0.11	0.12
	CF	-0.303*	0.059	<0.001	-0.42	-0.19
	CDF	-0.021	0.059	0.718	-0.14	0.09
	BG1	0.007	0.060	0.903	-0.11	0.12
	BG3	-0.108	0.058	0.065	-0.22	0.01
BG3	NF	0.115	0.059	0.050	0.00	0.23
	CF	-0.195*	0.058	0.001	-0.31	-0.08
	CDF	0.086	0.058	0.138	-0.03	0.20
	BG1	0.115	0.059	0.050	0.00	0.23
	BG2	0.108	0.058	0.065	-0.01	0.22

All analyses are least significant difference results using Post Hoc Fischer's Least Significant Difference Test, *The mean difference is significant at the 0.05 level, *Notes:* Tx= Treatment type, NF= No Fertiliser, CF= Chemical Fertiliser, CDF= Chicken Dung Fertiliser, BG1= Bat Guano (2.5 g), BG2= Bat Guano (5.0 g), BG3= Bat Guano (7.5 g).