

ENTOMOPHAGY PRACTICES AND NUTRITIONAL PROFILING OF CERTAIN EDIBLE INSECTS AS ALTERNATIVE SOURCES OF FOOD SECURITY IN NAGALAND

by

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This is to certify that the thesis entitled “**Entomophagy practices and nutritional profiling of certain edible insects as alternative sources of food security in Nagaland**” is an original research work carried out by **Ms. Patricia Kiewhuo** (Regd. No. Ph.D./ZOO/00291 Dated 28/08/2018) under my supervision in the Department of Zoology, Nagaland University, Lumami. She has fulfilled all the requirements of Ph.D. regulations for submission of the thesis. The content of this thesis is original and has not been submitted elsewhere for the award of any degree or distinction. The thesis is therefore forwarded for adjudication and consideration for the award of the degree of Doctor of Philosophy (Ph.D) in Zoology under Nagaland University.

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Chapter 1 Introduction 1.1 1.2 Contents 1.3 1.4 1.5 1.6 1.7 1.8 General Introduction Mass rearing of insects and associated safety measures Insect farming and its prospect Nutrient content in insects Conservation and sustainable usage of insects Review of Literature Scope of the study in Nagaland Objectives 1.1 General introduction The most challenging task of the present-day world is to fulfill

the United Nations' Sustainable Development Goals (SDGs) of 'no poverty,' 'zero hunger', and 'good health' by 2030; being 85

highly concerned about

under-nutrition (micronutrient deficiency), over- nutrition (obesity) and protein – energy malnutrition 98

that directly affects human health (Imathiu, 2020). About half of child deaths globally are caused by undernutrition, which disturbs

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**Dedicated to my Father, Mr. Kereiselie Kiewhuo, and my
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Contents

	List of Content	Page No
	List of Tables	i-vi
	List of Figures	vii-xii
	List of Abbreviations	xiii
Chapter 1	Introduction	1-22
1.1	General Introduction	1-4
1.2	Mass rearing of insects and associated safety measures	4-6
1.3	Insect farming and its prospect	6-8
1.4	Nutrient content in insects	9-11
1.5	Conservation and sustainable usage of insects	12-13
1.6	Review of Literature	13-20
1.7	Scope of the study in Nagaland	20-22
1.8	Objectives	22
Chapter 2	Diversity and distribution of edible insects in Nagaland	23-67
2.1	Introduction	23-26
2.2	Material and Method	26-30
2.2.1	Study area	26-27
2.2.2	Survey	28
2.2.3	Informants	29
2.2.3.1	General informants	29
2.2.3.2	Key informants	30
2.3	Results	30-62
2.3.1	Diversity of edible insects	30-57
2.3.3	Preparation methods for consumption	57-59
2.3.3.1	Boiling	58
2.3.3.2	Fried	58
2.3.4	Preservation methods of edible insects	59
2.3.4.1	Smoking	59
2.3.4.2	Sun drying	
2.3.4.3	Frying	59

2.3.4.4	Pickled	59
2.3.5	Socioeconomic value of edible insects	59-62
2.4	Discussion	63-67
Chapter 3	Traditional rearing techniques of (a) <i>Vespa mandarinia</i> Smith (1852) and (b) <i>Thysia wallichii</i> Hope (1831)	68-107
	(a) <i>Vespa mandarinia</i> Smith (1852)	68
3.1 (a)	Introduction	68-69
3.2(a)	Materials and methods	69-70
3.2.1(a)	Study area	69
3.2.2(a)	Methodology	69-70
3.3(a)	Results and discussion	70-86
3.3.1(a)	Locating the hornet nest	73-76
3.3.2(a)	Trapping of hornet workers and queen	76-77
3.3.3(a)	Construction of nesting site and rearing details	77-79
3.3.4(a)	Harvesting	79-80
3.3.5(a)	Socio-economy	80-85
3.3.6(a)	Future prospects	85-86
	<i>Thysia wallichii</i> (Hope, 1831)	87
3.1(b)	Introduction	87-88
3.2(b)	Materials and Methods	89-91
3.2.1 (b)	Study area	89
3.2.2(b)	Methodology	89-91
3.2.3(b)	Use value	91
3.2.4(b)	Proximate analysis	91-92
3.3 (b)	Result and discussion	92-107
3.3.1(b)	Inducing or facilitating the infestation of the host plant	93-95
3.3.2(b)	Mating and oviposition	95-98
3.3.3(b)	Harvesting	98-100
3.3.4(b)	Traditional laws protecting the domesticators	100-101
3.3.5(b)	Socio-Economic potential	101-102

	3.3.6(b)	Consumption process	103-107
Chapter 4		Ecology and Life history of <i>Tarbinskiellus portentosus</i> (Lichtenstein, 1796): a relished edible insect in Nagaland, Northeast India	108-123
	4.1	Introduction	108-110
	4.2	Materials and Method	110-113
	4.2.1	Study area	110
	4.2.2	Sample collections	110-111
	4.2.3	Rearing	111-113
	4.2.4	Observations of burrows in the wild	113
	4.3	Result	113-120
	4.3.1	Life stages	113-115
	4.3.2	Nymphs	115-117
	4.3.3	Adults	118
	4.3.4	Ecology of <i>T. portentosus</i>	119-121
	4.4	Discussion	121-123
Chapter 5		Quantitative characterization of microbial load in wild-harvested edible insects of Nagaland, India	124-134
	5.1	Introduction	124-125
	5.2	Materials and Method	126-127
	5.2.1	Insect samples	126
	5.2.2	Plate counts	126
	5.2.3	Statistical analyses	127
	5.3	Results and Discussion	127-134
Chapter 6		Nutritional and antinutritional properties of certain edible insects in Nagaland, India	135-168
	6.1	Introduction	135-136
	6.2	Materials and methods	135-142
	6.2.1	Insect samples collection and extraction	136-138
	6.2.2	Crude protein	138
	6.2.3	Crude lipid	138-139
	6.2.4	Total carbohydrate	139

6.2.5	Total phenol	139
6.2.6	Total antioxidant	139-140
6.2.7	Screening of Amino acids	140-141
6.2.8	Screening of Fatty acids	141
6.2.9	Statistical analysis	142
6.3	Results	142-161
6.3.1	Total protein content (mg/g)	142-143
6.3.2	Total lipid content (mg/g)	144-145
6.3.3	Carbohydrate content (mg/g)	145-146
6.3.5	Total phenol content (mg/g)	147-148
6.3.6	Total antioxidants (mg/g L-ascorbic acid equivalent)	149-150
6.3.7	Screening of amino acids in edible insects	151-157
6.3.7.1	Essential amino acids (ug/100g) in fresh form	151-152
6.3.7.2	Essential amino acids (ug/100g) in roasted form	152-153
6.3.7.3	Non-essential amino acids (ug/100g) in fresh form	153-155
6.3.7.4	Non-essential amino acids (ug/100g) in roasted form	155-157
6.3.8	Screening of fatty acids (ug/100g)	157-161
6.3.8.1	Fatty acid (ug/100g) profile in fresh form	157-159
6.3.8.2	Fatty acid (ug/100g) profile in roasted form	159-161
6.4	Discussion	161-167
Chapter 7	Summary and Conclusion	168-175
	References	177-220
	List of Publications	221
	Conferences/Seminars/Workshops presented/attended	222-225
	Academic Awards/achievements	226

List of tables

Table No	Name	Page No
Table 2.1	Indigenous tribal inhabitants of Nagaland (as per census of India 2011)	27
Table 2.2	Sampled Villages across twelve districts of Nagaland.	28
Table 2.3	Insects consumed District wise	31-36
Table 2.4	List of insects belonging to order Orthoptera	38
Table 2.5	Insects list of the order Hemiptera	41
Table 2.6	Insects list of order Hymenoptera	44
Table 2.7	Insects lists of the order Coleoptera	47
Table 2.8	Insect list of order Odonata	49
Table 2.9	Insects lists of the order Lepidoptera	51
Table 2.10	Insects list of the order Mandotea, Isoptera, and Diptera	52
Table 2.11	Market value of commonly available edible insects in Nagaland	61
Table 3.1(a)	Demographic patterns and status of the informants	72
Table 3.2(a)	Comparative presentation of protein content (mean \pm standard deviation) of <i>Vespa mandarinia</i> (larva and pupa) with those of <i>V. mandarinia</i> , <i>Vespa velutina nigrithorax</i> , <i>Apis mellifera ligustica</i> and <i>Tenebrio molitor</i> .	86
Table 3.1(a)	Details of the information's collected from the sampled villages	92
Table 3.2(b)	Demographic patterns of informants in the study	92
Table 4.1	The instars of <i>T. portentosus</i> with details of body weight, body length, antennal length, and cerci length	116
Table 5.1	Insect samples tested for the presence or absence of microbial load (+ shows the presence of a single colony, ++ shows the presence of double colonies).	127
Table 5. 2	Morphological characters of microflora extracted from different insect samples.	130
Table 5. 3	Microbial counts (log cfu/g) from insect samples	132
Table 6.1	Essential amino acid (ug/100g) profile of different edible insects in fresh form	151

Table 6.2	Essential amino acid (ug/100g) profile of different edible insects in roasted form	152-153
Table 6.3	Non-essential amino acid (ug/100g) profile of different edible insects in fresh form	154
Table 6.4	Non-essential amino acids (ug/100g) profile of different edible insects in roasted form.	156
Table 6.5	Fatty acid (ug/100g) profile in fresh form of edible insects	158
Table 6.6	Fatty acids (ug/100g) profile in roasted form of edible insects	160

List of Figures

Figure No	Name	Page No
Figure 1.1	Most commonly edible insects order-wise (Source: The Seattle Globalist)	2
Figure 1.2	Environmental impact of different animals. Source: Bloomberg (2018)	4
Figure 1.3	Nutrients in edible insects; Source: Zhou et al., 2022.	9
Figure 1.4	Edible insects as a source of biologically active substances and properties. Source: Castro-López et al., 2020.	10
Figure 2.1	Study area covering twelve (12) districts of Nagaland.	27
Figure 2.2	A- <i>A. domesticus</i> ; B- <i>Gryllus</i> species; C- <i>M. cincticornis</i> ; D- <i>Teleogryllus</i> sp. E. <i>T-occipitalis</i> F- <i>T. orientalis</i> G- <i>T. portentosus</i> H-G. <i>orientalis</i> . I- <i>O. hyla</i> J- <i>M. bivattus</i> K- <i>H. banian</i> L- <i>Attractomorpha</i> Sp.M- <i>Chondracris rosea</i> N-C. <i>robustus</i> O- <i>G. africanus africanus</i> P- <i>O. fuscovittata</i> Q-A. <i>exaltata</i> R- <i>P. infumata</i> S- <i>Melanoplus</i> sp. T- <i>M. nipponensis</i> U- <i>M. elongata</i> V- <i>P. titan</i> W- <i>E. securigera</i> X- <i>Tettigonia</i> species Y. <i>S.monstrosus</i> .	39
Figure 2.3	A-D. <i>hardwickii</i> B- <i>T. pruinosa</i> C- <i>D. intemerata</i> D- <i>D. oopaga</i> E- <i>Pomponia</i> sp. F-P. <i>repanda</i> . G-C. <i>ianus</i> H-C. <i>chinensis</i> I-C. <i>singhalanus</i> J-A. <i>nepalensis</i> K-N. <i>meleagris</i> L-A. <i>phasiana</i> M- <i>D. planiventris</i> N-C. <i>hilaris</i> O-C. <i>siccifolia</i> P-E. <i>fullo</i> Q-E. <i>grossipes</i> R- <i>Dolycoris</i> sp. S-U. <i>montana</i> T-T. <i>javanica</i> . U-L. <i>indicus</i> V-L. <i>ruber</i>	42
Figure 2.4	A- <i>Apis cerana indica</i> B- <i>A. dorsata dorsata</i> C- <i>A. dorsata laboriasa</i> D- <i>A. florum</i> E- <i>L. arcifera</i> F- <i>L. camifrons</i> G- <i>X. violacea</i> H- <i>O. smaragdina</i> I- <i>P. varia</i> J- <i>P. olivaceus</i> K-P. <i>stigma</i> L- <i>Polistes</i> Sp. M-R. <i>rufoplagiata</i> N- <i>Ropalida</i> sp. O- <i>Vespula</i> sp P-P. <i>barthelemyi</i> Q- <i>V. affinis</i> indosinensis R- <i>V. auraria</i> S-V. <i>basalis</i> T- <i>V. ducalis</i> U- <i>V. mandarinia</i> V-V. <i>tropica tropica</i> W-V. <i>soror</i> X- <i>V. orbata</i> Y- <i>V. bicolor</i>	45

Figure 2.5	A- <i>Anoplophora</i> sp B- <i>B. rubus</i> C- <i>B. parryi</i> D- <i>B. rufumaculata</i> E- <i>O. brunneum</i> F-A. <i>chalybaeus</i> G- <i>T. wallichii</i> H- <i>B. lineolata</i> I-C. <i>limbatus</i> J-C. <i>tripunctatus lateralis</i> K-S. <i>manipurensis</i> L-R. <i>ferrugineus</i> M- <i>H.cashmirensis</i> N-X. <i>gideon</i> O-L. <i>stigma</i> P- <i>Holotricha</i> sp.	48
Figure 2.6	Photo plate of odonates A- <i>D. trivialis</i> B- <i>O. pruinsum</i> C- <i>N. fulvia</i> D- <i>C. servilia servilia</i> E- <i>P. congenera</i> F- <i>O. sabina sabina</i> G- <i>O. triangulare triangular</i> H- <i>P. flavescens</i> .	50
Figure 2.7	Photo plate for Lepidoptera. A- <i>Cossus</i> sp. B- <i>Malacosoma</i> sp. C-S. <i>cynthia ricini</i> D. <i>A. assamensis</i> E- <i>O. fuscidentalis</i> F- <i>P. cruegeri</i> G- <i>E.torus</i> H- <i>A. myllida</i> .	51
Figure 2.8	A- <i>H. coarctata</i> SaussureB- <i>T. sinensis</i> SaussureC- <i>Macrotermes</i> sp., D- <i>Tipula</i> sp.	53
Figure 2.9	Use value of edible insects in Dimapur, Kohima, Peren, and Phek district of Nagaland.	55
Figure 2.10	Use value of edible insects in Wokha, Zunheboto,Mokokchung, and Kiphire district of Nagaland.	56
Figure 2.11	Use value of edible insects in Mon, Noklak, Tuensang, and Longleng district of Nagaland	56
Figure 2.12	Average use value of edible insects across Nagaland.	57
Figure 2.13	Average use value of insects in different district of Nagaland.	57
Figure 2.14	Boiled form of edible insects (A) <i>S. cynthia ricini</i> (B) <i>Malacosoma</i> sp.(C) <i>D. trivialis</i> nymph (D). <i>V. mandarinia</i> (E) <i>Cossus</i> sp. Larvae	58
Figure 2.15	Fried form of edible insects (A) <i>O. fuscidentalis</i> larvae (B) <i>O. hyla</i> (C) <i>L.indicus</i> (D) <i>Cossus</i> sp. larvae	58
Figure 2.16	A-B Fried and packed <i>O.hyla</i> for sale C. Honey products of <i>Apis cerana indica</i> and <i>L. canifrons</i> sold atHornbill festival. D. Fragments of raw honey in the comb of <i>A. cerana indica</i> sold at Hornbill festival E. Empty combs of <i>V.mandarinia</i> hanging at the entrance of a home as decoration F. Exoskeleton of green click beetle used in earning by Chang	62

	tribe	
Figure 2. 16	Insects sold in the market (A) <i>S. cynthia ricini</i> (B) <i>A.nepalensis</i> (C) <i>O.smaragdina</i> (D) <i>T. wallichii</i> (E) <i>Cossus</i> sp. larvae (F) <i>O.hyla</i> (G) <i>C.limbatus</i> (H) <i>D. trivialis</i> (I) <i>L. indicus</i>	62
Supplementary Figure 2.1	A-B. <i>A.cerana indica</i> honey collection; C. Handpicking of <i>O.fuscidentalis</i> larva; D. Smoking of <i>V.mandarinia</i> nests for brood collection; E. Collection of aquatic insects(<i>L. ruber</i> , <i>C.limbatus</i> , <i>H.caschmerensis</i>) using bamboo weaved baskets; F. Collection of <i>Macrotermes</i> sp. using nets,G-H. Rearing of <i>A. cerana indica</i> within bamboo weaved rooms with an opening for exit and entry small enough only for bees to pass and not hornets. I-J. Traditional methods of rearing <i>L. camifrons</i> in wooden and bamboo boxes. K-L Rearing of <i>S. cynthia ricini</i> at home.	67
Figure 3.1(a)	Map of the study area showing the location of surveyed villages under Kohima and Dimapur, Nagaland.	71
Figure 3.2(a)	A-D. <i>Vespa mandarinia</i> larvae, pupae and adult sold at local market, Kohima, Nagaland.	72
Figure 3.3(a)	Preparation of new rearing site for hornets: (A) a hollow pit of 60-75 cm for new rearing site; (B), (C) comb collected from the wild is tied onto a strong wood and hanged downward; (D) bamboo slices placed horizontally to maintain a strong foundation for the hornets to move in and out of the hive.	76
Figure 3.4(a)	Traditional trapping method of <i>Vespa mandarinia</i> : (A) hornet biting a bait made of cotton balls soaked in sugar syrup; (B) white ductile material tied on the hornet which acts as visual markers until nests is located; (C) hideouts near the nesting site of hornets; (D) collection of milky sap from <i>Ficus</i> sp.; (E) hornets captured by glue trap method; (F) folded bamboo sticks with trapped hornets; (G) sugar	78

syrup is fed to the hornets to keep them alive; (H) newly constructed structure to rear the hornet; (I) large hornet comb harvested after successful rearing.

Figure 3.5(a)	Comparative analysis of the market value of hornet grubs with other	82
Figure 3.6(a)	Informant's preference of consumption	82
Figure 3.7(a)	(A, B). Exotic ' <i>khwidi</i> ' giant hornet (cooked with bamboo shoot and local spices) dish, a delicacy among the Naga communities.	83
Figure 3. 1(b)	Map of the study area showing the location of surveyed villages under Kohima and Dimapur, Nagaland	90
Figure 3.2(b)	Flow chart showing semi-domestication steps of <i>T. wallichii</i> by indigenous people of Nagaland.	93
Figure 3.3(b)	A- <i>A. pennata</i> tree and leaves; B- <i>T. wallichii</i> adult and larva; C- Cut wedged shape end of <i>A. pennata</i> ; D- <i>T. wallichii</i> adults on host tree; E- Hole created in the host tree by adults for egg-laying; F- sawdust expelled from the host tree by feeding larva.	96
Figure 3.4(b)	A-B. Larva of <i>T. wallichii</i> were found infesting the host plant, C-D. Stem of <i>A. pennata</i> kept horizontally and vertically in open space at home for domestication	98
Figure 3.5(b)	A- <i>A. pennata</i> stem stacked for harvesting; B- A domesticator collecting the larva using woolen gloves; C- Collected on the clean ground; D-E, Larva kept along with sawdust in a plastic container and sackcloth; F- Dead larva turned black.	100
Figure 3.6(b)	Market value (in rupees) of <i>T. wallichii</i> compared to other conventional meat sources in Nagaland.	102
Figure 3.7(b)	Different modes of consumption of <i>T. wallichii</i> larvae.	103
Figure 3.8(b)	Proximate composition of <i>T. wallichii</i> larvae	105
Figure 4. 1	Map showing the sample collection areas at Zaphumi and Lumami, village, Zunheboto, Nagaland.	110

Figure 4. 2	(A) Collection of the egg using high-intensity light; (B) Water sprayed to keep the soil moist for eggs. (C) The round container; (D) Rectangular box for rearing adult's crickets.	112
Figure 4. 3	A) Day 1- Oblong compact yellowish in color; (B) Day 7- Yellowish-white in color and bent at the center; (C) Day 13 – more slender and thinner; (D) Day 20 – Eyes become visible, egg white;(E) Day 28 – Egg white and more compact, body appendages visible; (F-H) Day 29 to 31- Brown dirty look on the inside with stretch marks, cracks appear.	115
Figure 4.4	(A-G) The seven instars and (H) an adult <i>T. portentosus</i>	116
Figure. 4. 5	Increase body weight (mg) of <i>T. portentosus</i> during different stages of development.	117
Figure 4. 6	(A) Adult female and (B) Adult male and (C) lateral view of female <i>T. portentosus</i> .	118
Figure 4. 7	(A) Heap of loose soil mounted around the burrow's entrance; (B) <i>T. portentosus</i> embedded at the end of the burrow; (C) Food stored in burrows.	120
Figure 4. 8	(A) A male <i>T. portentosus</i> ; (B) Adult <i>T. portentosus</i> digging its burrow.	121
Figure 5.1	Microbial count (log cfu/g) in fresh and roasted insect samples	132
Figure 6.1	A-N Samples of (A) <i>Tarbinskelius portentosus</i> , (B) <i>Mecopoda nipponensis</i> , (C) <i>Schizodactylus monstrosus</i> , (D) <i>Aspongus nepalensis</i> , (E) <i>Lethoceros indicus</i> (F) <i>Laccotrephes ruber</i> , (G) <i>Oecophylla samaragdina</i> , (H) <i>Provespa barthelemyi</i> (I) Wood larva (J) <i>Cybister limbatus</i> (K) <i>Hydrophylus caschmirensis</i> , (L) Dragon fly, (M) <i>Cossus</i> sp. and (N) <i>Samia cynthia ricini</i> .	138
Figure 6.2	Variations of Protein content in fresh, fried, and roasted forms of insect	143
Figure 6.3	Various edible insects with their protein content in fresh	143

	friend and roasted forms.	
Figure 6.4	Variations of lipid content in fresh, fried, and roasted forms of insects	144
Figure 6.5	Various edible insects with their lipid content in fresh friend and roasted forms.	145
Figure 6.6	Variations of carbohydrate content in fresh, fried, and roasted forms of insects	146
Figure 6.7	Various edible insects with their carbohydrate content in fresh, fried and roasted forms..	146
Figure 6.8	Average amount of total phenol in fresh, fried, and roasted forms of insects.	147
Figure 6.9	Various edible insects with their total phenol content in fresh fried and roasted forms	148
Figure 6.10	Total phenol content in various stages of development of <i>S. Cynthia ricini</i> and <i>P. barthelemyi</i>	148
Figure 6.11	Average amount of total antioxidant in fresh, fried, and roasted forms.	149
Figure 6.12	Various edible insects with total antioxidant content in fresh fried and roasted forms	150
Figure 6.13	Total antioxidant content in various stages of development of <i>S. Cynthia ricini</i> and <i>P. barthelemyi</i>	150
Figure 6.14	Non-essential amino acid chromatogram in the fresh (F) form of <i>O. fuscidentalis</i> , Wood larva, <i>A. nepalensis</i> , and <i>P. barthelemyi</i>	155
Figure. 6.15	Amino acid chromatogram in the roasted (R) form of <i>O. fuscidentalis</i> , Wood larva, <i>O. smaragdina</i> , and <i>P. barthelemyi</i>	157
Figure 6.16	Fatty acid chromatogram in fresh (F) form of <i>A. nepalensis</i> , <i>S. cynthia ricini</i> , <i>P. barthelemyi</i> , and <i>O. smaragdina</i>	159
Figure 6.17	Fatty acid chromatogram in the roasted (R) form of <i>O. fuscidentalis</i> , <i>A. nepalensis</i> , <i>M. elongata</i> , and <i>L. indicus</i> .	161

List of Abbreviations

ANOVA	:	Analysis of the Variance
AOAC	:	Association of Official Analytical Chemists
FAO	:	Food and Agricultural Organization
CFU	:	Colony forming Units
UV	:	Use Value
SPSS	:	Statistical Package for the Social Sciences
FCR	:	Folin-Ciocalteu reagent
GC MS	:	Gas Chromatography Mass Spectrometer
MUFA	:	Monounsaturated fatty acids
PUFA	:	Polyunsaturated fatty acids
TMUFA	:	Monounsaturated fatty acids
WHO	:	World Health Organization

Chapter 1

Introduction

Contents

-
- 1.1 *General Introduction*
 - 1.2 *Mass rearing of insects and associated safety measures*
 - 1.3 *Insect farming and its prospect*
 - 1.4 *Nutrient content in insects*
 - 1.5 *Conservation and sustainable usage of insects*
 - 1.6 *Review of Literature*
 - 1.7 *Scope of the study in Nagaland*
 - 1.8 *Objectives*
-

1.1 General introduction

The most challenging task of the present-day world is to fulfill the United Nations' Sustainable Development Goals (SDGs) of 'no poverty,' 'zero hunger,' and 'good health' by 2030; being highly concerned about under-nutrition (micronutrient deficiency), over-nutrition (obesity) and protein – energy malnutrition that directly affects human health (Imathiu, 2020). About half of child deaths globally are caused by undernutrition, which disturbs the long-term health of populations and the physical and cognitive development of children (Black et al., 2008, 2013). One of the primary causes of food insecurity is the lack of access to healthy, affordable, and improved food sources that are diverse enough to form a balanced diet, including protein. With the estimated increase of the human population to reach 9 billion by the year 2050 (Van Huis et al., 2013), it is necessary to search for alternative protein food sources to solve the problem of human undernutrition worldwide. Thus the world is now looking for mitigating strategies that must be executed to provide quality and safe food regularly. However, the continuous pressure to increase food production is challenged by land degradation, urban expansions, climate change, and water scarcity (Nellemann et al., 2009). Also, expansions of food production are the primary source of greenhouse gas emissions, with livestock products being one of the most significant contributors that trouble the ideas of modern sustainable means of livelihood (FAO, 2017). Further, due to the decline in the availability of agricultural land, as well as the growing demand for meat and other environmental challenges, finding an alternative source of protein has become a priority for countries all over the world. In this context, edible insects have gained great relevance as a food source as they align with sustainability goals for food production, such as reducing natural resource use (Gravel and Doyen, 2020).

Entomophagy, the practice of eating insects, is known in many parts of the world. In countries such as Africa, Asia, South America, and Australia, insects are a regular part of traditional food (Mitsuhashi, 1997; Van Huis, 2003; Nonaka, 2009; Mozhui et al., 2022; Kiewhuo et al., 2022). Approximately 2,000 species of insects are edible and considered food and feed worldwide (Jongema, 2012). Edible insects occupy a wide range of insect orders. The most consumed groups of edible insects include beetles (Coleoptera, 31%); caterpillars (Lepidoptera, 18%); bees, wasps, and ants (Hymenoptera, 14%); and followed by grasshoppers, locusts and crickets (Orthoptera, 13%); cicadas, leafhoppers, planthoppers, scale insects and true bugs (Hemiptera, 10%); termites (Isoptera, 3%); dragonflies (Odonata, 3%); flies (Diptera, 2%) and 5% of other orders (Figure 1.1) (Van Huis, 2013). The availability of these insects depends substantially on the geographical distributions of host plants, seasons, and harvesting period (Chakravorty, 2014).

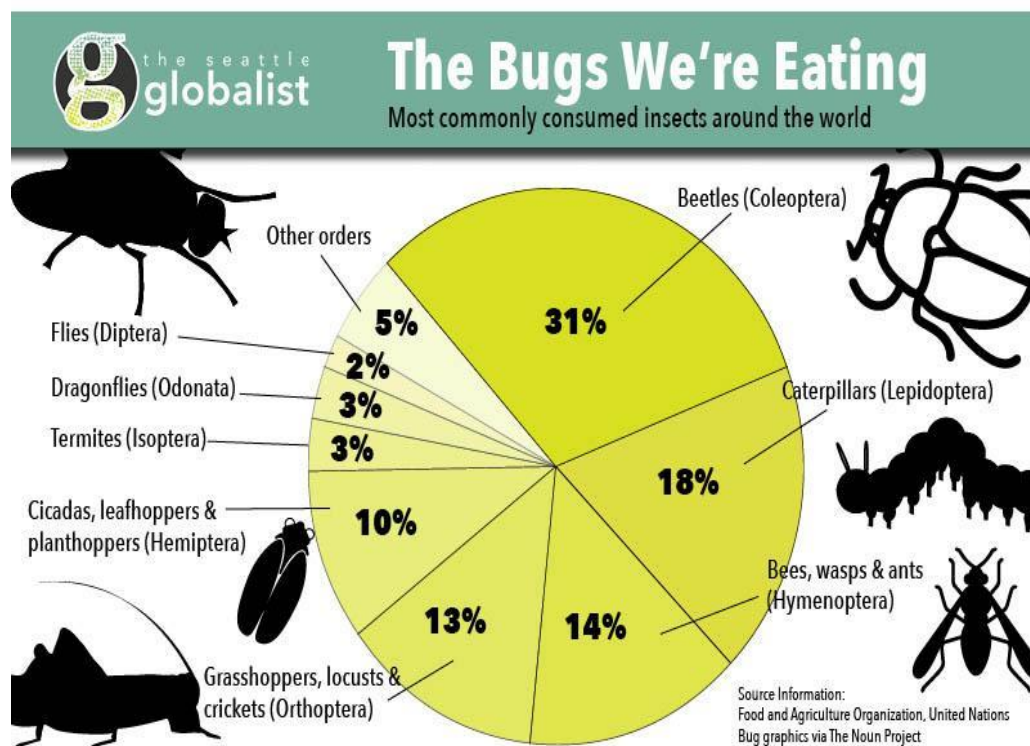


Figure 1.1. Most commonly edible insects order-wise (Source: The Seattle Globalist).

Archaeological evidence and analysis of fossils show that entomophagy practices have evolved throughout human evolution (Sutton, 1995; Ramos-Elorduy, 2009; Fontaneto et al., 2011) as has been resurrected by the pioneering work of Holt (1885), Bergier (1941), and DeFoliart (1989). The prehistoric people used higher animal bones to make tools to acquire insects, as evidenced by Plio-Pleistocene tools to dig termites in Sterkfontein Valley (dErrico, 2001). With the religious and cultural development through advancement of time, the use of insects as food has been discouraged to prevent animal sacrifice in rituals or just for disgust of eating insects (Meyer-Rochow, 2009). Colonization also played a significant role in eroding entomophagy (Yen, 2009). However, in recent years, Entomophagy practices have gained significant momentum with increasing interest in their high nutritional value that provides alternatives to conventional animal protein sources and the potential to address environmental sustainability concern. Edible insects are being recognized as a powerful dietary practice that can replace other animal-based proteins. Across various cultures and religions, edible insects contribute to food security and offer a rich source of protein, vitamins, and minerals. Compared to other conventional meat sources such as chicken, pig, and cow, the environmental benefits of rearing edible insects like crickets and mealworms, include less greenhouse gas emission, less feed requirement, less space required, and fewer water requirements (Figure 1.2).

Farming's environmental impact by species





	 Insect	 Chicken	 Pig	 Cow
Greenhouse gases released per kg of live weight, g	2	NA	1,130	2,850
Feed required per kg of live weight, kg	1.7	2.5	5	10
Land required per g of protein, m ²	18	51	63	254
Water required per g of protein, li	23	34	57	112

Figure 1.2. Environmental impact of different animals. Image: Bloomberg

They require less feed and produce less methane, a potent greenhouse gas (Oonincx and de Boer, 2012). Insect farming requires less land compared to traditional livestock farming. It is particularly advantageous in regions with limited agricultural land (Van Huis, 2013). Insects generally require less water for their growth and maintenance than traditional livestock like cattle and pigs (Makkar and Heuze, 2014). Insect farming has a smaller ecological footprint and can help reduce the pressure on forests and natural habitats, often cleared for livestock grazing and feed production (Van Huis et al., 2013). Insects can be reared on organic waste materials, such as food scraps and agricultural by-products, contributing to waste reduction and recycling (Diener et al., 2011).

1.2 Mass rearing of insects and associated safety measures

Mass rearing of edible insects is the most effective way of producing insects, making them readily available for human consumption and as pet feeds. However, precautionary measures should be considered during rearing as insects are kept in

artificially modified habitats, making them very prone to infections, which can pass on the disease to the farmers and consumers (Vega and Kaya, 2012). Studies on disease control in insects' population systems in history focused on two main species that are domesticated, i.e., silkworm (*Bombyx mori*, Linnaeus 1758) and honeybee (*Apis mellifera*, Linnaeus 1758), making them models for studies (James and Li, 2012).

Safety concerns are associated with the consumption of insects, such as contaminating chemical and biological agents (Garofalo et al., 2019). The increasing introduction of insects into the human diet increases attention to their safety concerns. The safety of edible insects needs proper evaluation by monitoring the presence of harmful microorganisms (Baiano, 2020). Banjo et al. (2006) reported that insects could harbour many kinds of pathogenic bacteria and fungi, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Aspergillus niger*. Food safety is influenced by the way how it is produced, stored, processed, and eaten. The rearing environment, rearing procedures, hygiene measures, and insect feed are known to affect the microbiota of insect species (Wynants et al., 2018). The study concluded that possible contamination with microbiological hazards is influenced by the nature and the hygienic conditions of the substrate and the farming environment (Van der Fels-Klerx et al., 2018).

Pesticides used against insects such as grasshoppers and locusts become toxic residues when consumed by humans (Van Huis, 2003; Yen, 2009). It is reported that proper heat treatment for detoxifying African silkworms that contain high protein sources makes the worm safe for consumption (Nishimune et al., 2000). The boiling, freezing, and heating are considered necessary preparatory processing methods for insects to kill microbes before consumption (Grabowski and Klein, 2017; Ad'amek et al., 2019). While investigating the microbial community composition of two common

edible insect species mealworms and superworms, Osimani et al. (2018) highlighted the presence of various bacteria, some of which could be of concern regarding food safety. Stoops et al. (2016) also examined the microbial populations in mealworm larvae and crickets, recorded the presence of potentially harmful bacteria, and emphasized the importance of proper processing and hygiene practices. The European Food Safety Authority (EFSA) 2016 published a risk profile addressing the safety of using insects in food and feed. It covers potential hazards, including microbial contamination, providing valuable insights into the topic. Microbial contamination can vary depending on insect species, production methods, and processing practices. While edible insects are consumed safely by many communities, it is essential to follow proper hygiene, processing, and cooking practices to minimize the risk of microbial contamination and ensure food safety.

1.3 Insect farming and its prospect

In earlier times, the collection methods did not prioritize the sustainability aspects of the insect population, as the majority of the gathered insects were intended for personal use only. The question of whether edible insects would be accessible was seldom a concern. Nevertheless, the escalating commercialization of edible insects and the recent surging demand for specific species have resulted in more intensive harvesting approaches being adopted in certain regions. These circumstances have prompted researchers to question ecological soundness and long-term sustainability (Shrestha and Bawa, 2013; Yen and Ro, 2013). Edible insects are sourced by three main strategies: wild harvesting, semi-domestication of insects in the wild, and farming. Semi-domestication and farming have the potential to provide a more sustainable food supply; however, globally, 92% of species are wild-harvested (Yen, 2015).

Since ancient times, insects have been domesticated, and the commonly domesticated insects for food or their products are *Apis mellifera*, Fabricius, 1793, silkworm, palm weevil, and caterpillars. Crickets are another commonly reared insect around the world for food and feed. Thailand is the largest producer of crickets and has 20,000 farmers producing 7,500 tons of crickets annually (Hanboonsong et al., 2013). Farming insects is the most effective way to produce insects to meet the market demands by constant supply. Globally, 92% of edible insect species are wild-harvested, but semi-domestication and farming can provide a food supply in a more sustainable way (Yen, 2015). The most commonly used commercial insects in mass production are mulberry silkworm, waxworm, yellow mealworm, house cricket, black soldier fly, housefly (indoor farming), palm weevil, bamboo caterpillar, weaver ant, grasshopper (outdoor farming), eri silkworm, muga silkworm, giant hornet, and termite (wild farming) (Gahukar, 2016; Cortes et al., 2016).

An assessment of insect market value at different levels in various places shows the potential of edible insects to uplift the domesticator at local and industrial levels (Van Huis et al., 2013). For example, one kg of termites is sold at € 10 in Kenya; Yellow mealworms and lesser mealworms are sold in the Netherlands at € 4.85 per 50 gm. Similarly, in the Lao People's Democratic Republic, grasshoppers are sold at € 8–10 per kg. In Cambodia, a can of fried crickets containing 150–200 g is sold at € 0.40–0.70. In Cameroon, by selling honeybees and termites, local vendors generate 2-3, 5-10, and 16-20 USD (Tamesse et al., 2018). Different edible insects and their market value are reported from different parts of the world. Odongo et al. (2018) reported that in the Lake Victoria basin, one kilogram of *Ruspolia differens*, is sold at \$ 3.00, which is comparable to or higher than one kg of beef (\$ 3.50) and one kg of fish (\$ 1.95). Analysis of market players and opportunities of *R. differens* traders in the Lake

Victoria basin in Uganda and Burundi reveals a supply chain of collectors, wholesalers, retailers, and consumers for these insects. Each commercial collector earns up to \$ 2696.00 per swarming season, while wholesalers earn \$ 2633.00.

The Global Market Insights (2018) has assessed the significant growth of edible insect market with a projected turn over of \$710 million in 2024. North America's market is expected to surge by over 43.5%, while the Asia Pacific region, particularly Thailand and China, could exceed \$270 million. This growth is fueled by a rising aversion to processed foods and a growing demand for high-protein diets. The availability of insects in the market hinges on mass rearing, semi-domestication, or wild procurement during scarcity. However, collecting insects from the wild remains challenging due to unpredictable locations and population densities (Gahukar, 2011). In India, particularly in Northeast region, where entomophagy practices are prevalent, substantial market potential exists for socioeconomic development through the promotion of edible insects. Despite the popularity of insect consumption, seasonal availability limits widespread access, and large-scale production remains elusive. Concerted efforts are needed to bridge the gap between indigenous entomophagy practices and technological intervention. Successful mass rearing emphasizes on thorough understanding biology, growth, and reproduction of edible insects. Species selection should prioritize the factors like short life cycles, palatability, and nutritional content. Life cycle assessments are essential for evaluating the environmental, economic, and social impacts of insect farming compared to traditional livestock production. Advancing the mass rearing of edible insects requires a multifaceted approach of integrating traditional knowledge with scientific innovation. By studying preferred species and optimizing production methods, we can enhance productivity, sustainability, and ultimately the livelihoods of insect farmers.

1.4 Nutrient content in insects

The scientific merit of entomophagy has by now been well established by documenting the undisputed nutritional value of many edible insects, which contain high amounts of protein, fats, carbohydrates, and significant percentages of amino acids, vitamins, minerals, and unsaturated fatty acids and serve as a good nutritional source in many developing countries around the world (Ashiru, 1988). Insects can source essential vitamins and minerals, including B vitamins (e.g., B12, riboflavin), iron, zinc, and magnesium (Finke, 2002).

The protein content in edible insects may vary in different species, but it is often comparable to or even higher than that of traditional meat sources like beef, chicken, or fish (Ooninx et al., 2010). Many edible insects also contain all essential amino acids, making them a complete protein source (House, 2016). Edible insects can provide healthy fats, including unsaturated fats such as omega-3 and omega-6 fatty acids (Rumpold and Schlüter, 2013). They are also good sources of vitamins, minerals, and many other essential nutrients (Figure 1.3).

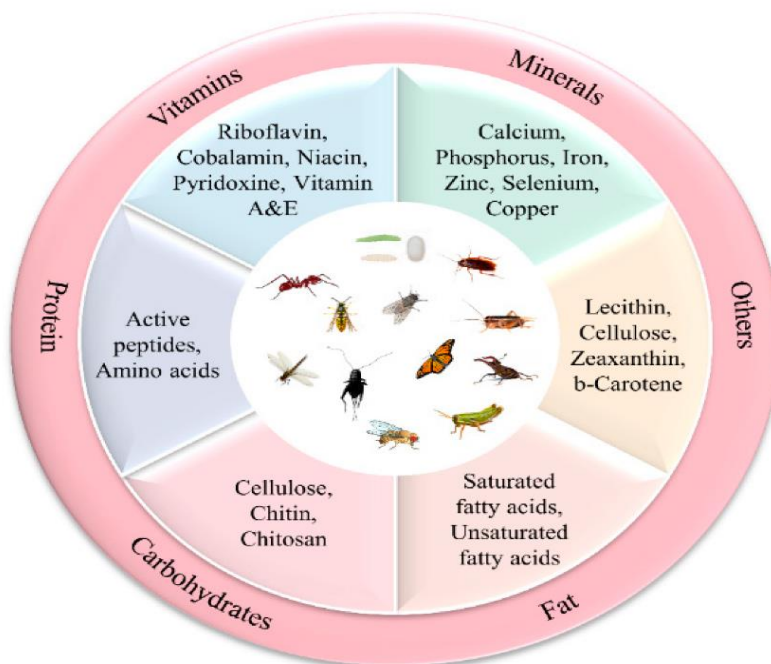


Figure 1.3. Nutrients in edible insects; Source: Zhou et al., 2022.

Insect consumers are mostly unaware of the nutritional aspects of eating insects, however prefer to eat them because of their taste. Generally, the taste of insects varies depending on the species, the stage of development (larva, pupa, adult, or egg), and the food eaten by the insects (Ramos-Elorduy, 1996). Various nutritional requirements like Sugar (honey ants, sugar bags, and lerps) and fats (grubs) are provided by insects to humans (Yen, 2009). For vegetarians, insects can serve as an alternative to protein since it is more acceptable unlike conventional meat sources. While insects serve as a rich source of nutrients, biologically active compounds such as antifungal, anti-inflammatory, antioxidant, antibacterial, and antidiabetic are also reported to be present in insects and their derived products (Figure 1.4) (Prakash and Bhargava, 2014; Tang et al., 2015; Józefik and Engberg, 2017; Wu et al., 2018).

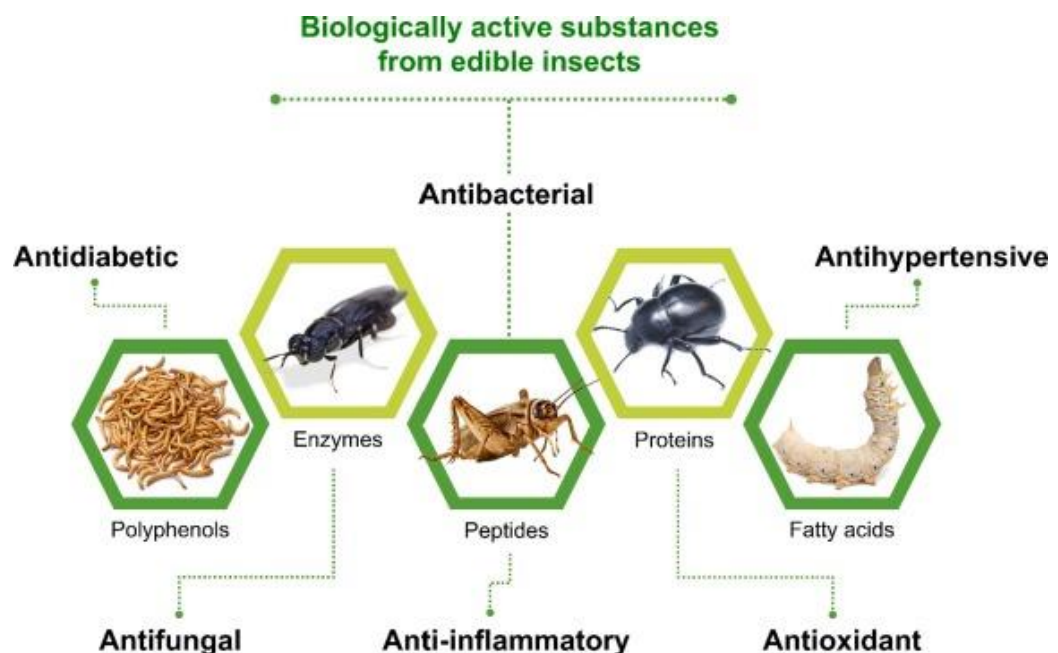


Figure 1.4 Edible insects as a source of biologically active substances and properties.

Source: Castro-López et al., 2020.

Various factors influence the nutritional composition of insects, including their diet (Pearincott, 1960), life stage (Downer and Matthews, 1976; Ali and Ewiess, 1977), rearing conditions (Finke and Oonincx, 2017), and processing methods prior to consumption (Dreyer and Wehmeyer, 1982; Batterham et al., 1986; Jensen and Lieberoth, 2019). In terms of nutritional content, insects are known to be highly nutrient-dense (DeFoliart, 1992). Typically, the highest fat storage occurs in the final larval or nymphal stage (Fast, 1970), while carbohydrates are present in smaller quantities (Finke, 2002; Finke, 2013). On a dry matter basis, protein and fat content in insects ranges from 25% to 75% (Oonincx and Van der Poel, 2011; Oonincx and Dierenfeld, 2012) and from 10% to 70% (Bukkens, 1997; Yang et al., 2006a; Finke, 2013) respectively.

Analysis of the nutrient content of edible insects has been conducted worldwide, including North-East India (Ramos-Elorduy et al., 1997; Raksakantong et al., 2010; Chakravorty et al., 2011; Shantibala et al., 2014; Elhassan et al., 2019). Studies reveal that insects contain a variety of fatty acids, including monounsaturated and polyunsaturated fats (Payne et al., 2016). However, the nutrient composition varies depending on the species of insect, its developmental stages, habitat, and dietary habits, highlighting the need for further research into the potential of edible insects as a nutrient source.

Limited evidence exists on the effects of insect processing methods, although it is likely similar to that of other human foods and animal feeds (Ssepuuya et al., 2017). The nutrient content of insects can also change with different preparation methods. For instance, a study on four edible insects using traditional processing techniques indicated a decrease in fat content (0.8-51%) and an increase in crude protein (1.2-22%) (Nyangena et al., 2020). Fat content was highest in raw products and decreased in the

following order: toasting, boiling, oven-drying, and sun-drying. Additionally, the fatty acid profiles of insects are influenced by types of insects as well as the cooking methods, as fats can be absorbed from cooking oils (Oliveira et al., 1976).

1.5 Conservation and sustainable usage of insects

Due to the increase in human population and commercialization of insects, traditional regulations have been weakened, and more plants are cut for harvesting caterpillars (Mbata and Chidumayo, 2003). For instance, the urban population's high demand for mopane worms has caused overexploitation, forcing harvesters to shift from subsistence harvesting to commercializing them in markets in Namibia (Thomas, 2013). However, in some parts of the world, traditional practices of sustainable usage of insects are followed. For example, palm weevil larvae are acquired by cutting the tree trunks for infestation and collecting them after 6 weeks (Johnson, 2010). In Northern Zambia, an edible caterpillar is managed traditionally by monitoring the forest's development and abundance, protecting host plants and eggs from forest fires, and allowing only temporal harvest (Mbata et al., 2002).

Insects generally provide services to the ecosystem through pollination, composting, and pest control (Losey and Vaughan, 2006). However, several human activities pose a threat to the insect population. Collecting insects for food may also directly undermine the viability of the insect population (Choo, 2008). Overexploitation of insects is another major challenge for the present and future of entomophagy, specifically if the harvesting of individuals of a species population exceeds their regeneration capacity (Cerritos, 2009). Additionally, if the practice of insect collection becomes less selective, the stability and regeneration of the population are threatened (Illgner and Nel, 2000; Latham, 2003; Ramos Elorduy, 2006), as evident when a matured insect is collected before its first mating or before eggs are laid (Cerritos,

2009). Other activities such as deforestation, pollution, and forest destruction have stressed the insect population (Ramos Elorduy, 2006; Schabel, 2006). The cutting down of host trees to increase or allow infestation has further reduced the insect population.

As the ecological services provided by insects are vital for human life, the need to conserve insects and their habitats has recently gained attention (DeFoliart, 2005; Samways, 2007). “Flagship species” are promoted to arouse public interest in conservation efforts (Simberloff, 1998). Identifying “Umbrella species” by conservation biologists serves as the representative species that, when protected, can indirectly help several co-occurring species along with their habitats (Roberge and Angelstam, 2004). Along with the large animals like pandas and tigers, edible insects can also be used as flagship species or Umbrella species protecting other resources and should be given important attention (DeFoliart, 2005; Yen, 2009).

1.6 Review of Literature

1.6.1 International

The diversity of insects is reported to be more than 1.1 million, comprising 70-95% of the total animal kingdom (Chapman, 2009), and there could be more than 6 million species globally (Hamilton et al., 2010). The international status of entomophagy is rapidly evolving, driven by growing interest in sustainable food sources. While challenges remain, including consumer acceptance and regulatory aspects, the potential benefits of edible insects for nutrition, food security, and the environment are becoming increasingly recognized. Entomophagy has been practiced for centuries in various cultures around the world. Insects are a traditional food source in Thailand, Mexico, Ghana, and Australia, among others. They hold cultural significance and are often considered delicacies (Van Huis, 2013). The culinary

exploration of edible insects has gained momentum in various parts of the world, showcasing different insect consumption practices.

Records of edible insects' diversity exploration are reported from different parts of the world. A comprehensive study on the inventory of edible insects has been made through a "List of edible insects of the world" that include beetles (659), Caterpillars (362), Ants, bees and wasps (321), Grasshoppers and Locusts (278), True bugs (239), Dragonflies (61), Termites (59), Flies (37), Cockroaches (37), Spiders (15) and others (45) (Jongema, 2017). Edible insects are mostly consumed in Asia, Central and Southern Africa, Australia, and Latin America (MacEvilly, 2000; Gullan and Cranston, 2014). In America alone, 679 edible insects were recorded, followed by Africa (524), Asia (349), Australia (152), and 41 species in Europe (Ramos- Elorduy, 2005). In a comprehensive study, Sirimungkararat et al. (2010) documented 194 edible insect species in Thailand. Likewise, China boasts 178 recorded species (Chen et al., 2009), while Brazil reveals a culinary diversity with 95 edible insect varieties (Ramos-Elorduy, 2006). The Amazonian region stands out for its utilization of approximately 209 edible insect species, with the predominantly consumption of ants, butterflies, wasps, termites, and bees (Johonson, 2010). Meanwhile, Borneo showcases an edible insect repertoire with 80 recorded species (Chung, 2010). In the Democratic Republic of Congo, Lepidopterans are the most popular edible insects, comprising 60% of the 148 recorded species. Maneechan and Prommi (2023) reported the presence of 64 genera of edible insects in the rice fields in Thailand. While exploring the edible insect biodiversity and anthropo-entomophagy practices in Kalehe and Idjwi territories, DR Congo, Ishara et al. (2023) reported that *R.differens* and *Macrotermes subhyalinus* Rambur (1842), is the most preferred insects in the area.

In Democratic Republic of Congo, Bomolo et al. (2017) reported a list of eleven edible insect species belonging to four families, based on seasonal availability, community preference, and willingness to consume them. In a study on the consumption and rearing of insects in Asia, Africa, and Europe, Raheem et al. (2019) reported that over one thousand edible insects are consumed in some stages of their life cycle and play an important role in ensuring food security. Edible insects have been part of Mexican cuisine since ancient times. The consumption of ants, grasshoppers (known as "chapulines"), and agave worms is common. These insects are used in traditional dishes like tacos, tamales, and salsas (Ramos-Elorduy, 2009). Termites, caterpillars, and crickets are often collected in the wild during specific seasons and are prepared in various ways, including roasting and boiling (Van Huis et al., 2013). Aboriginal communities in Australia have a history of consuming witchetty grubs, honey ants, and other insects as part of their traditional diet which are nutrient-rich and important food sources in arid regions (Yen et al., 2018). Rice bugs, *Leptocorisa oratorius* Fabricius (Coreidae), and *Nezara viridula* Linnaeus, 1758 (Pentatomidae), are delicacies in Borneo. The bugs are mashed with salt and chilies, steamed in bamboo, and served as a condiment (Chung, 2010).

The global perspective on insect consumption is evolving, with regulatory frameworks adapting to include insects as a viable food source. Organizations such as the FAO and EU have actively developed guidelines and regulations for insect consumption (Halloran et al., 2018). Payne et al. (2016) reported that the nutritional composition of insects is highly diverse compared to conventional meat sources and could help combat undernutrition. Nsevolo et al. (2022), while studying the protein content of selected edible insects in DPR Congo, reported that edible insects are a nutrient-rich source of food containing 46.1% to 52.9% (dry matter) protein and call for

enhancing cross-border trade of species linked to potential economic, social, and ecological benefits. While studying the nutritive value in cricket (*Gryllobates sigillatus*), larvae of mealworm (*Tenebrio molitor*), and adult locust (*Schistocerca gregaria*), Zielińska et al. (2015) reported that protein and fat content ranges from 52.35 to 76% and from 12.97–24.7% respectively; also these insects are very rich in minerals such as magnesium, copper, iron, and zinc. While assessing the bioavailability of nutrients in edible insects, Ojha et al. (2021) reported that nutritional composition varies depending on the species, diet, developmental stage, sex, and the growth environment of insects.

Kolobe et al. (2023) reported that insects such as mealworms, grasshoppers, and termites are rich in polyunsaturated fatty acids, and lipids and fatty acids profiles are altered by applying different processing methods. While studying the nutritional composition and antioxidant properties of insects, Park et al. (2023) reported that protein content and antioxidant activity were highest in rice locust and white-spotted beetle larvae and further opined that edible insects can be used in the development of nutritionally fortified foods for the public or in functional foods for elderly and medical patients.

While compiling nutrient compositions for 236 edible insects, Rumpold and Schlüter (2013) suggested that many edible insects provided satisfactory amounts of energy and protein and meet amino acid requirements for humans, high in monosaturated and/or poly-saturated fatty acids, and were rich in micronutrients such as copper, iron, manganese, magnesium, phosphorus, selenium, and zinc as well as riboflavin, pantothenic acid, biotin and in some cases, folic acid. High activity of heat-resistant thiaminase was reported in African silkworm pupa by Nishimune et al. (2000).

In many parts of the world, harvesting and consumption of edible insects happens throughout the year. Meutchieye et al. (2016) reported that the insect harvesters

use four methods to collect the insects: (1) semi-domestication, (2) hand picking, (3) light trapping, and (4) net trapping. Early mornings and evenings are considered the ideal times for the collection of insects such as *A.ruficornis*, and *Acoryphanigrovariegatatibialis* Kirby (1902), *Locusta migratoria migratoria* Linnaeus, 1758 when they are inactive. Van Huis (2003) reported that in East Africa, the indigenous people collect *Chaoborus* sp. by sweeping them off from the rocks and bushes by whirling baskets. In Thailand, Crickets are collected by poured water into burrows and when they come out, they are handpicked (Hanboonsong, 2010). Insects are collected mainly depending on their habitat. In China, Dragonflies, including the larger green emperor dragonfly *Anaxguttatus* Burmeister (Aeshnidae) and the red-veined drop wing *Trithemis arteriosa* Burmeister (1839), are collected in paddy fields (Feng et al., 2000).

In Japan, insects like hornet larvae, silkworm pupae, and aquatic bugs have been consumed for centuries. They are often served in regional dishes, preserved in soy sauce, or incorporated into other foods (Durst et al., 2010). Entrepreneurs and startups are delving into innovative approaches to incorporate insects into mainstream food products, exemplified by creating protein bars and snacks (House, 2018). In Thailand, various types of edible insects, such as crickets, grasshoppers, and silkworms, are often deep-fried and seasoned with spices and are consumed as snacks or incorporated into dishes, and are also sold in markets in the street stalls (Yhoung-aree, 2010).

Nonaka (1996) reported that in the Kalahari region of South Africa, caterpillar intestines are extracted and then roasted amidst hot ashes and sand. Sun-dried caterpillars are preserved in bags and consumed as needed. Occasionally, dried caterpillars are crushed into powder and simmered with watermelon for consumption. While studying the different drying methods on nutritional compositions of edible

insects, Yisa et al. (2023) reported that drying methods did not significantly affect proximate composition, available lysine, and protein digestibility. They further emphasized that sun-drying with prior boiling could be a cost-effective and affordable alternative to freeze-drying and oven-drying for preserving edible insects.

1.6.2 National

India is a tropical country and diverse forms of insects are found throughout the year due to the harmonious coexistence between humans and natural resources. Meyer-Rochow and Chakravorty (2013) reported that Indian tribals practice entomophagy with various insects containing valuable proteins, digestible fats, vitamins, and minerals, however, with modernization, these old-aged practices are beginning to be discarded. Viewing the entomophagy practices, Chakravorty et al. (2014) enlisted 255 edible insect species including Coleoptera (34%), orthoptera (24%), Hemiptera (17%), Hymenoptera (10%), Odonata (8%), Lepidoptera (4%), isoptera (2%), and Ephemeroptera (1%) taken as food by different tribes of India. Among the list, the most popular groups are termites, locusts, weaver ants, bees, cinnamon bugs, silkworm pupae, mealworms, grasshoppers, crickets, mole crickets, petaloid bugs, etc. Sharma and Banu (2019) also documented the use of edible insects in Kerala, Karnataka, Tamil Nadu, and Telangana. According to Roy and Rao (1957), the Muria Tribe in Madhya Pradesh is known to consume ants as food. In Orissa, Srivastava et al. (2009) reported using red ants and termites. Sathe (2015) reported a total of 18 edible insects from Western Maharashtra. In West Bengal, Seni (2017) documented the tribal people consuming red ants' eggs and larvae. Entomophagy practices have been documented in central India (Madhya Pradesh, Chhattisgarh, Jharkhand) by Bhowate and Kumar (2020), as well as in Odisha and West Bengal by Jena et al. (2020). Despite documentation of entomophagy practices in certain parts of the country, especially

among tribal communities, there is very limited information on mass rearing and nutritional profiling of edible insects.

1.6.3 North-East India

In the predominantly tribal-populated regions of the Northeastern states of India, entomophagy practices are widespread. The indigenous people's selection of insects as food is influenced by factors such as the insects' taste, availability, nutritional value, habits, and cultural traditions (Pradhan et al., 2022). Chakravorty et al. (2014) reported the insect diversity in various regions of Northeast India, such as Arunachal Pradesh (158 species), Nagaland (41 species), Manipur, Assam (38 species), Meghalaya (16 species). Das et al (2023), in their study on the edible insect diversity and entomophagy attitudes of ethnic people in Manas National Park, Assam, reported a total of 22 species. Among these, Orthopterans were the most abundant with eight (8) species, followed by Hymenoptera (4), Hemiptera (3), Lepidoptera (2), Blattodea (2), and one species each from Coleoptera, Odonata, and Mantodea. In Assam, 80 edible insect species have been reported by Das and Hazarika (2019) and Borah et al. (2020).

In Manipur, 119 species of edible insects were reported by Kapesa et al. (2020) and Kom and Arunkumar (2020). In Arunachal Pradesh, entomophagy practices by the Nyishi tribe have been reported by Chakravorty et al. (2013). Recently, Baruah and Bhattacharyya (2019) and Chakravorty et al. (2019) reported 109 species of edible insects from Arunachal Pradesh.

While studying the nutritional content of five edible insects (*Diplonychus rusticus* Fabricius 1781, *Lethocerus indicus*, *Laccotrephes* sp. *Ranatra* sp. *Cybister* sp.) in Assam Sarmah et al. (2022), reported high protein content (50.03 to 57.67%), fats, carbohydrate, and crude fiber, etc. and also high energy content (331.98 to 506.38 kJ/100 g). Chakravorty et al. (2014) assessed the nutritional composition of the short-

horned grasshopper, *Chondacris rosea*, and mole cricket *Brachytrupes orientalis* in Arunachal Pradesh and recorded 68.88 and 65.74% crude protein, 7.88 and 6.33% fat, 12.38 and 8.75% crude fiber, 4.16 and 4.33% ash, and 6.69% and 15.18% carbohydrate, respectively. These findings collectively underscore the nutritional richness of aquatic insects, highlighting their potential as sustainable and nutritious food resources in the context of food security and alternative protein sources. Sarmah et al. (2022) reported that phenol and flavonoid content in edible insects reflects their high antioxidant activity (80.82 to 91.47% DPPH inhibition). Further other antinutritional compounds such as Tannin (18.50 to 60.76 mg tannic acid equivalent/100 g), phytic acid (11.72 to 97.30 mg/100 g), and oxalic acid (2.93 to 5.34 mg/100 g) were registered below the toxic level (0.52% or 520 mg/100 g).

1.7 Scope of the study in Nagaland

Nagaland is a hilly state located in the extreme North-Eastern end of India, having an area of 16,579 km² and a home of 16 major tribes with a population of 1,980,602 (As per the 2011 census). The entomophagy practices are relatively common among different tribes of Nagaland, particularly those living in rural areas and traditionally consume insects as supplementary animal protein. In a preliminary report, Meyer-Rochow and Chakarvorty (2013) identified 42 species of insects used as food by Ao-Nagas. Mozhui et al. (2020) recorded 106 edible insects belonging to 9 orders viz: Odonata, Orthoptera, Mantodea, Isoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera among seven tribal communities from certain selected districts of Nagaland. A few preliminary reports are also available on the nutritional benefit of certain edible insect species of Nagaland (Singson et al., 2016; Pongener et al., 2019; Aochen et al., 2020).

Edible insects are natural renewable resources that are not only considered as good

sources of high content of proteins, minerals and vitamins but also have medicinal and socio-religious value. Although they are highly relished by the different ethnic groups of Nagaland, proper scientific explorations and a comprehensive understanding on distribution and diversity of edible insects encompassing all tribes throughout the state of Nagaland are not yet completed. Also, the heterogeneity in the collection and consumption of insects and the role of sharing cultural and ecological knowledge among the tribes must be properly documented as knowledge transfer between generations passes orally. With its rich cultural diversity and unique ecological conditions, Nagaland presents an opportune environment for exploring traditional rearing methods of edible insects and for the feasibility of mass rearing of certain edible insects along with their life cycle studies. Further, investigating and integrating traditional knowledge into modern practices could enhance the region's efficiency and sustainability of edible insect production. Along with this, it is also imperative to improve the current methods of collection, storage, and preservation of edible insects and evaluate the biochemical and nutritional value of edible insects and their products. The insects could be a base for new food products of considerable nutritive value and have the potential for exploitation to combat nutritional deficiencies that are of public health concern. Since insects contribute significantly to the food security and livelihood of the poor as sources of protein, carbohydrates, and vitamins, and also traditional medicines, the scientific investigation on the biochemical contents of edible insects would provide a reliable source of nutrition to people for better health. Thus, being in large numbers, insects present a potential sustainable food source for humans. Rearing and cultivation of insects in a scientific manner will be beneficial for both the natural conservation of insect fauna and forest. It is therefore important to establish a uniform database of edible insects and document different features of entomophagy that have

significant nutritional, economic, and ecological benefits as well as play important roles in socio-cultural integration among different tribes in Nagaland. Hence, the present study was conducted with the following objectives.

1.8 Objectives

- Diversity and distribution of edible insects in different districts of Nagaland along with consumption, processing, and use pattern of edible insects among different tribes.
- Traditional rearing techniques of *Vespa mandarinia* Smith (1852) and *Thysia wallichii* Hope (1831).
- Biology and Ecology of *Tarbinskiellus portentosus* Lichtenstein (1796)
- Quantitative characterization of microbial load in wild-harvested edible insects of Nagaland.
- Assessment of nutritional and antinutritional properties of certain edible insects in different levels of processing

Chapter 2

Diversity and distribution of edible insects in Nagaland

Contents

2.1 *Introduction*

2.2 *Materials and Methods*

2.3 *Results*

2.4 *Discussion*

2.1 Introduction

Entomophagy refers to the inclusion of insects in the human diet (Evans et al., 2015). Various ethnic groups across the globe incorporate insects into their culinary traditions (Feng et al., 2018) which include more than 2,000 species of insects consuming worldwide (Jongema, 2015). Edible insects are among the most important bioresources being promoted to address global food and nutritional security (Premalatha et al., 2011; Halloran et al., 2015; Kinyuru et al., 2015). Worldwide edible insects are regularly consumed by 2 billion people (Rumpold and Schlüter, 2013; Van Huis and Oonincx, 2017) for their nutritional value and taste (Kourimsa and Adámková, 2016; Mishyna and Chen, 2020). Typically, insects are not consumed out of necessity to prevent starvation; instead, some research indicates that they are primarily gathered and eaten when other animal food sources are extremely scarce or completely unavailable (Dufour, 1987).

Entomophagy practices are more prevalent in the tropical countries owing to the fact that (a) insects tend to be larger in size, which facilitates their harvesting; (b) insects often congregate in large numbers so large quantities can be collected in a single harvest; and (c) a variety of insects are available all year round (van Huis et al., 2013). Understanding the diversity and distribution of edible insects emerge with great interest due to their many advantages: (i) they are an important source of protein, fat, carbohydrate and other nutrients; (ii) their use as food has ecological advantages over conventional livestock and, in the long run, economic benefits; (iii) their rich species diversity and large populations bring diversity in diet menu (Seni, 2017). Diversity of insects is a combination of species richness (the number of species present), evenness or equitability (and the distribution of individuals among the species). Insect diversity is an important factor in the balance of environmental conditions (Yi et al., 2012).

Worldwide, the most consumed groups of edible insects include beetles (Coleoptera, 31%), caterpillars (Lepidoptera, 18%), and bees, wasps, and ants (Hymenoptera, 14%), followed by grasshoppers, locusts and crickets (Orthoptera, 13%), cicadas, leafhoppers, planthoppers, scale insects and true bugs (Hemiptera, 10%), termites (Isoptera, 3%), dragonflies (Odonata, 3%), flies (Diptera, 2%) and 5% other orders (Van Huis, 2013). The availability of some edible insects depends on the geographical distribution of their host plants and seasonality (Chakravorty, 2013) and correlates with their harvesting period (Takeda and Sato, 1993). Various ethnic communities across India have long incorporated edible insect species into their traditional diets. Roy and Rao (1957) documented the consumption of certain ant species as food by the Muria tribes in Madhya Pradesh. Srivastava et al. (2009) observed the consumption of red ants and termites by ethnic groups in Odisha and the consumption of red ant eggs (*Demta* sp.) by tribal communities in Jharkhand. Sathe (2015) identified 18 edible insect species in the Kolhapur region of Western Maharashtra. Samuel et al. (2016) noted the utilization of *Macrotermes* sp., *Oecophylla smargadina*, and *Apis* sp. (honey, bee larvae, and eggs) as food by ethnic groups in the Western Ghats. Similarly, Seni (2017) reported the consumption of red ant eggs and larvae by tribal communities in West Bengal.

While the practice of entomophagy is less widespread in other regions of India *i.e.* Madhya Pradesh, Tamil Nadu, Kerala, and Odisha, it enjoys greater popularity in North-East India, particularly among ethnic communities in Arunachal Pradesh, Manipur, Assam, and Nagaland. Additionally, it is embraced to a lesser extent by ethnic communities in Mizoram and Meghalaya (Chakravorty, 2014). The diverse ethnic groups of the North–Eastern part of India have a unique culture of food intake, and insect-eating is most prevalent, particularly among rural tribal people having a long-

cultured history (Hazarika et al., 2020). Gope and Prasad (1983) documented 20 edible insect species as significant food sources among various ethnic groups in Manipur. Meyer-Rochow (2005) reported over 60 edible insect species used as food by the Meitei people of Manipur and the Khasi people of Meghalaya. Subsequently, a total of 41 insect species, spanning 8 orders under 36 genera and 24 families, were reported from the ethnic communities of Manipur (Shantibala et al., 2012). In Arunachal Pradesh, a total of 109 edible insect species are enlisted with Odonata in major consumption list and followed by Orthoptera, Hemiptera, Hymenoptera, and Coleoptera (Baruah and Bhattacharyya, 2019; Chakravorty et al., 2019). Further, wide variations in the consumption and utilization of edible insect species are observed among ethnic tribes of Arunachal Pradesh. As per record, utilization of edible insects among different tribes are 66 species in the Nyshi tribe, 60 species in the Galo tribe, 53 insect species in the Adi tribe, 49 species in the Apatani tribe, 20 species in the Wangcho tribe, 16 species in the Nocte tribe, 15 species in the Chakma tribe, 14 species in the Singpho tribe, 12 species in the Deori tribe, and 9 species in the Tangsa tribe (Chakravorty et al., 2011; 2013; 2018). In a study from Baksa District of Assam, Das (2019) recorded 30 number of edible insects of which Orthopterans order shared with maximum number of 10 species followed by Hymenoptera of 6 species, Coleopteran of 5 species then Hemipterans by 3 species and Lepidoptera by 2 species and then Odonota, Mantodea, Blattellidae and Isopteran by 1 species respectively. Hazarika et al. (2020) assessed the diversity and seasonal abundance of edible insects from a UNESCO Natural World Heritage Site located in Manash, Assam and recorded 22 species of edible insects belonging to fifteen families and eight orders. Singh and Babu (2021) recorded thirty-one species belonging to nineteen families and seven orders from the multi-use ecosystem in South Manipur.

In Nagaland, insects are part of the daily diet and have been used traditionally along with the main dish eaten with local beverages or as a side dish along with other staple food. However, with the fast-shifting culture to compromise with new generation, there is a risk of losing the traditional knowledge of practicing entomophagy that are mostly passed on orally since time immemorial. It is therefore urgent need to have comprehensive assessment of diversity and distribution of edible insects across the different geographical areas of Nagaland to understand the varying nature of entomophagy among the different tribes of Naga. This chapter deals with documentation on collecting methods, seasonal availability, insect abundance, distribution and diversity in different habitats and geographical areas of Nagaland.

2.2. Material and method

2.2.1 Study area

Nagaland is the 16th state in India, covering an area of 16,579 square kilometers, and it shares its borders with Assam, Manipur, Arunachal, Manipur, and Myanmar (Figure 2.1). It is situated between 96-98degrees east longitude and 26.6-27.4 degrees north latitude. The population of Nagaland stands at approximately 1.9 million people, at a 1:0.9 male: female ratio.

The state's economy relies heavily on agriculture, accounting for 70% of its economic activity. Additionally, forestry, insurance, real estate, tourism, and small cottage industries play significant roles in its economic landscape. Approximately one-sixth part geographical areas of Nagaland is covered by tropical and sub-tropical evergreen forests, featuring diverse flora including palms, bamboo, rattan, timber, and mahogany forests. While some forested areas have been cleared for jhum cultivation, many regions are characterized by scrub forests, tall grasses, and reeds. Nagaland is divided in to 12 districts and is home to 16 major tribes, including the Angami, Ao,

Chakhesang, Chang, Kachari, Khiamniungan, Konyak, Kuki, Lotha, Phom, Pochury, Rengma, Sangtam, Sumi, Yimkhiung, and Zeliang (Table 2.1).

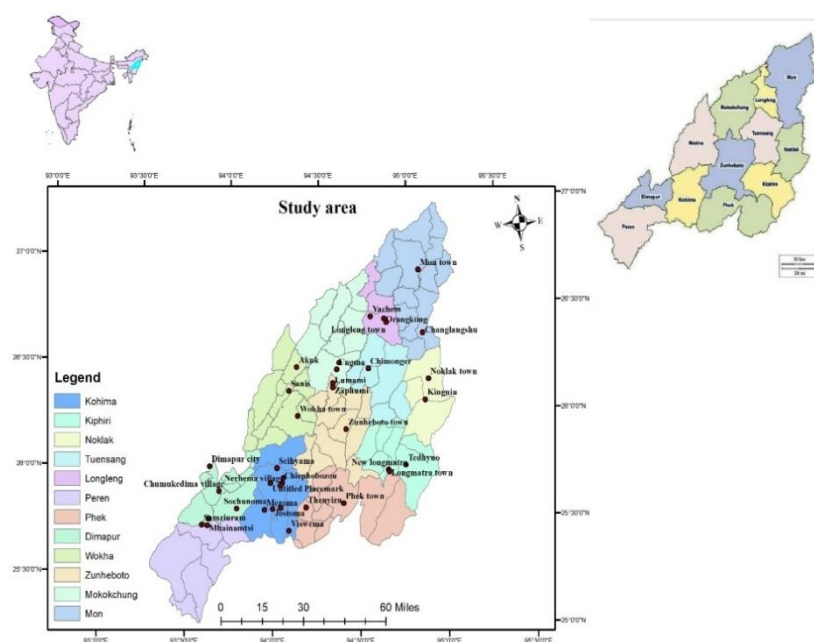


Figure 2.1. Study area covering twelve (12) districts of Nagaland.

Table 2.1. Indigenous tribal inhabitants of Nagaland (as per census of India 2011).

District	Major Tribes	Total area (Sq.Km)	Population	General climate	Temperature (°C)
Dimapur	Angami, Kachari, Kuki, Lotha, Sumi	927	378,811	Warm,temperate	20-35
Kiphire	Sangtam, Yimkhiung	1130	74,004	Warm,temperate	20-30
Kohima	Angami, Rengma	1463	267,988	Warm,temperate	20-30
Longleng	Phom	562	50,484	Warm,temperate	20-30
Mokokchung	Ao	1615	194,622	Warm,temperate	20-30
Mon	Konyak	1786	250,260	Warm,temperate	20-30
Peren	Zeliang	23000	95,219	Warm,temperate	20-30
Phek	Chakhesang, Pochury	2026	163,418	Warm,temperate	20-30
Tuensang	Chang,Sangtam, Yimkhiung	2536	196,596	Warm,temperate	20-30
Wokha	Lotha	1628	166,343	Warm,temperate	20-30
Zunheboto	Sumi	1255	140,757	Warm,temperate	20-30
Noklak	Khiamniungam	1152	59,300	Warm,temperate	20-30

2.2.2 Survey

Based on the earlier record on availability and utilization of edible insects restricted to only seven ethnic groups in the state (Mozhui et al., 2020), the present study on entomophagy practices was further expanded to all districts and conducted among different tribal communities using a structured questionnaire following Tongco (2007). The sampling was carried out in the twelve districts of Nagaland taking randomly selected villages from each district and information were collected from different ethnic tribal inhabitants (Table 2.2). Before, field visitation, villages and town heads were pre-informed through telephonic conversations to extract maximum knowledge from the informants. Further research materials were referred by using secondary data from books, the internet, journals, and articles (McDonald et al., 2003).

Table 2.2. Sampled Villages across twelve districts of Nagaland

District	Villages/Town
Kohima	Nerhema Village, Seihyama Village, Chiechama Village, Viswema Village, Mezoma Village, Jotsoma Village, Zhadima Village, Chiephobozou town, and Kohima town,
Dimapur	Sochunoma village, Dimapur town, and Chumukedima town,
Phek	Thenyizu village and Phek town
Mon	Changlangshu village, Mon town
Mokokchung	Ungma village and Mokokchung town
Peren	Samziuram village, Mhainamtsi village and New Jalukie town
Zunheboto	Zaphumi village, Lumami village and Zunheboto town
Tuensang	Chimonger village and Tuensang town
Noklak	Kingniu village and Noklak town
Kiphiri	Longmatra village, Dedhyuo village and Longmatra town
Longleng	Orangkong village, Yachem Village and Longleng town
Wokha	Akuk village, Sanis Village and Wokha town

2.2.3 Informants

2.2.3.1 General informants

Nagaland is inhabited by different major tribes and sub-tribes, (Table 2.1) inhabiting 12 districts *i.e* Dimapur, Kohima, Wokha, Mokokchung, Kiphire, Longleng, Tuensang, Noklak, Mon, Phek, Peren, and Zunheboto. Primary data was collected based on a predesigned questionnaire. Data collections involve in-depth personal interviews, group discussions, and observations. The present study employed a combination of multi-stage random sampling and purposive sampling techniques. Multi-stage sampling involves multiple phases to progressively reduce the sample size at each stage, as described by Kothari (2014). On the other hand, Purposive sampling is a method used to select informants in ethno-studies based on their possession of traditional knowledge, as outlined by Tongco (2007). In this approach, we selected and identified individuals who are willing to share information based on their expertise and experience, and accordingly knowledge on entomophagy practices were collected from them. A total of 1200 general informants irrespective of village and town dwellers were random selected for interview purpose. Insects were generally classified into 20 broader groups, such as grasshoppers, crickets, mole crickets, weaver ants, water beetles, giant water bugs, wasps and hornet, silkworms, bamboo borers, wood borer, carpenter worms, cicadas, water scorpions, stink bug, dragon flies, termites, mantis, katydids, caterpillars and rhino beetles for use value estimation. Secondary data were obtained through various books, journals, news articles, government office sources, the internet, etc. The use value of different groups of insects was calculated using the formula given by Phillips et al. (1994).

Use value = $\Sigma U/n$, where ‘U’ is the number of informants per citation and ‘n’ is the total number of informants.

2.2.3.2 Key informants – Using a purposive sampling method, a total of 40 key informants were interviewed, which included elderly people, insect collectors, insect domesticators, and local vendors.

2.3. Results

2.3.1 Diversity of edible insects

A total of 108 edible insects are recorded from different districts of Nagaland belonging to 9 insect orders. Orthopterans and Hymenoptera accounted for the highest number of insects (25 each), followed by Hemiptera (22), Coleoptera (16), Odonata (8), Lepidoptera (8), Mantodea (2), Isoptera (1), and Diptera (1). A maximum number of edible insects were recorded in Kohima, Dimapur, and Wokha districts, with 104 species each, followed by Phek (103) and Zunheboto (102) districts. However, comparatively less number of edible insects were recorded from Longleng (50), Noklak (47), Tuensang (47) and Kiphire (44) districts. Thirty three (33) insects species were found in all the districts of Nagaland. Insects such as *P. titan*, *S. monstrous*, *Dolycoris* sp. and *P. cruegeri* are considered eaten only in Phek, Dimapur, Zunheboto, Wokha, Kohima, and Peren districts (Table 2.3).

Table 2.3. List of insects consumed insampled districts of Nagaland

Sl no	Order	Insects name	Phk	Kma	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
1		<i>Acheta domesticus</i> Linn.	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X	X
2		<i>Gryllus</i> sp. Linn.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
3		<i>Meloimorpha cincticornis</i> Walker	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
4		<i>Teleogryllus</i> sp.	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	X	X
5		<i>Teleogryllus occipitalis</i> Serville	✓	✓	✓	✓	✓	✓	✓	X	X	✓	X	✓
6		<i>Tarbinskiellus orientalis</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓
7		<i>Tarbinskiellus portentosus</i> Lichtenstein	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓
8		<i>Gryllotalpa orientalis</i> Burmeister	✓	✓	✓	X	X	✓	✓	✓	X	✓	X	X
9		<i>Oxya hyla</i> Serville	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
10	Orthoptera	<i>Melanoplus bivittatus</i> Say	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
11		<i>Hieroglyphus banian</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
12		<i>Attractomorpha</i> Sp. Saussure	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
13		<i>Chondracris rosea</i> Serville	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X
14		<i>Choroedocus robustus</i> De Geer	✓	✓	✓	✓	✓	✓	✓	X	X	✓	X	X
15		<i>Gastrimargus africanus africanus</i> Sassure	✓	✓	✓	✓	✓	✓	✓	X	X	✓	X	X
16		<i>Oxya fuscovittata</i> Marschall	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X
17		<i>Acrida exaltata</i> Walker	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
18		<i>Phlaeoba infumata</i> Brunner	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
19		<i>Melanoplus</i> sp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X

Table 2.3 conti..

Order	Insects name	Phk	Km	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
Orthoptera	<i>Mecopoda nipponensis</i> Haan	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	<i>Mecopoda elongata</i> Linn.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	<i>Pseudophyllus titan</i> White	✓	✓	X	✓	X	✓	X	X	X	X	X	X
	<i>Elimaea securigera</i> Brunner	X	✓	✓	✓	✓	✓	✓	X	X	X	X	X
	<i>Tettigonia</i> species	✓	✓	X	✓	✓	✓	✓	X	X	✓	X	X
	<i>Schizodactylus monstrosus</i> Drury	X	✓	✓	✓	X	X	✓	X	X	X	X	X
26	<i>Darthula hardwickii</i> Gray	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
27	<i>Tibicen pruinosa</i> Say	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
28	<i>Dundubia intemerata</i> Walker	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
29	<i>Dundubia oopaga</i> Distant	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓
30	<i>Pomponia</i> sp. Walker	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
31	<i>Pycna repanda</i> Amyot and Serville	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
32	<i>Coridius janus</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓
33	<i>Coridius chinensis</i> Dallas	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓
34	<i>Coridius singhalanus</i> Dist.	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓
35	<i>Aspongopus nepalensis</i> Westwood	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
36	<i>Notobitus meleagris</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
37	<i>Anoplocnemis phasiana</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X
38	<i>Dalader planiventris</i> Westwood	✓	✓	✓	✓	✓	✓	X	✓	✓	X	X	X

Table 2.3 conti..

Sl no	Order	Scientific name	Phk	Kma	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
39		<i>Chinavia hilaris</i> Say	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
40		<i>Cyclopelta siccifolia</i> Westwood	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
41		<i>Erthesina fullo</i> Thunberg	✓	✓	✓	✓	✓	X	X	X	X	✓	X	X
42		<i>Eurostus grossipes</i> Dallas	✓	X	✓	✓	✓	✓	X	X	X	X	X	X
43	Hemiptera	<i>Dolycoris</i> sp.	✓	✓	✓	✓	X	✓	X	X	X	X	X	X
44		<i>Udongo montana</i> Distant	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓
45		<i>Tessaratomia javanica</i> Thunberg	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓
46		<i>Lethocerus indicus</i> Lepeltier andServille	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
47		<i>Laccotrephes ruber</i> Linn.	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
48		<i>Apis cerana indica</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
49		<i>Apis dorsata dorsata</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
50		<i>Apis dorsata laboriosa</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
51		<i>Apis florae</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
52		<i>Lepidotrigona arcifera</i> Cockerell	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
53	Hymenoptera	<i>Lophotrigona canifrons</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
54		<i>Xylocopa violacea</i> Linn.	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X
55		<i>Oecophylla smaragdina</i> Fabr.	X	✓	✓	✓	✓	✓	✓	X	X	X	X	X
56		<i>Parapolybia varia</i> Fabr.	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X
57		<i>Polistes olivaceus</i> De Geer	✓	✓	✓	✓	✓	✓	X	X	✓	X	X	X

Table 2.3 conti..

Sl no	Order	Scientific Name	Phk	Kma	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
58		<i>Polistes stigma</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	X
59		<i>Polistes</i> sp.	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X
60		<i>Ropalida rufoplagiata</i> Cameron	✓	✓	✓	✓	✓	✓	X	X	X	✓	X	X
61		<i>Ropalida</i> sp.	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X
62		<i>Vespula</i> sp.	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
63		<i>Provespa barthelemyi</i> du Buysson	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
64		<i>Vespa affinis indosinensis</i> Perez	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
65	Hymenoptera	<i>Vespa auraria</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
66		<i>Vespa basalis</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
67		<i>Vespa ducalis</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
68		<i>Vespa mandarinia</i> Smith	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
69		<i>Vespa tropica tropica</i> Linn.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
70		<i>Vespa soror</i> du Buysson	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
71		<i>Vespa orbata</i> du Buysson	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
72		<i>Vespa bicolor</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
73		<i>Anoplophora</i> sp. Hope	✓	✓	✓	✓	✓	✓	✓	X	✓	X	✓	✓
74	Coleoptera	<i>Batocera rubus</i> Linn.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
75		<i>Batocera parryi</i> Hope	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓

Table 2.3 conti..

Sl no	Order	Sicentific name	Phk	Kma	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
76		<i>Batocera rufomaculata</i> De Geer	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
77		<i>Orthosoma brunneum</i> Forster	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
78		<i>Aplosonyx chalybaeus</i> Hope	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	X
79		<i>Thysia wallichi</i> Hope	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
80		<i>Batocera lineolata</i> Chevrolat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
81		<i>Cybister limbatus</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
82	Coleoptera	<i>Cybister tripunctatus</i> lateralis Fabr.	✓	✓	✓	X	✓	✓	✓	X	X	X	X	X
83		<i>Sandracottus manipurensis</i> Vazirani	✓	✓	✓	X	✓	✓	✓	X	X	X	X	X
84		<i>Rynchophorus ferrugineus</i> Olivier	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
85		<i>Hydrophilus cashmirensis</i> Redtenbacher	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X
86		<i>Xylotropes gideon</i> Linn.	✓	✓	✓	✓	✓	X	✓	X	X	✓	X	X
87		<i>Lepidiota stigma</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	X	X	✓	X	X
88		<i>Holotricha</i> sp.	✓	X	✓	X	✓	✓	✓	X	X	X	X	X
89		<i>Diplacodes trivialis</i> Rambur	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
90		<i>Orthetrum pruinatum</i> neglectum Rambur	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X
91	Odonata	<i>Neurothemis fulvia</i> Drury	✓	✓	✓	✓	X	✓	✓	X	X	X	X	X
92		<i>Crocothemis servilia</i> Drury	✓	✓	✓	✓	X	✓	✓	X	X	X	X	X
93		<i>Potamarcha congener</i> Rambur	✓	✓	✓	✓	X	✓	✓	X	X	X	X	X

Table 3 conti..

Sl no	Order	Scientific name	Phk	Kma	Dmp	Zbn	Mkg	Wkh	Pern	Lon	Tsg	Mon	Kph	Nlk
94	Odonata	<i>Orthetrum sabina sabina</i> Drury	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
95		<i>Orthetrum triangulare triangulare</i> Sely	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
96		<i>Pantala flavescens</i> Fabr.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
97	Lepidoptera	<i>Cossus</i> sp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
98		<i>Malacosoma</i> sp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
99		<i>Samia cynthia ricini</i> Biosduval	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
100		<i>Antheraea assamensis</i> Helfer	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
101		<i>Omphisa fuscidentalis</i> Hampson	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
102		<i>Pericyma cruegeri</i> Butler	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
103		<i>Erionota torus</i> Evans	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
104		<i>Antheraea mylitta</i> Drury	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
105		<i>Hierodula coarctata</i> Saussure	X	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓
106		<i>Tenodera sinensis</i> Saussure	X	X	X	✓	✓	✓	✓	✓	✓	✓	✓	✓
107	Isoptera	<i>Macrotermes</i> sp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
108	Diptera	<i>Tipula</i> sp. Linn.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Nlk=Noklak, Kph=Kiphire, Mon, Tsg=Tuensang, Lon=Lonleng, Pern=Peren, Wkh=Wokha, Mkg=Mokokchung, Zbn=Zunheboto, Dmp=Dimapur, Kma=Kohima, Phk=Phek; ✓=Present, X= Absent

Orthoptera: A total of twenty-five (25) Orthopteran species belonging to the families Gryllidae, Gryllotalpidae, Acrididae, Tettigoniidae, and Schizodactylidae are eaten as food in Nagaland (Table 2.4, Figure 2.2). Among the orthopterans, *O. hyla*, *M. niponensis*, and *M. elongatae* are consumed across all districts. *P titan* is eaten in Phek, Kohima, Zunheboto, and Wokha districts. The least number of Orthopterans are eaten in the Noklak and Kiphire districts, whereas Phek, Dimapur, and Kohima boast the highest number of edible orthopterans (Table 2.3). Orthopterans are available from the beginning of early summer till the early winter *i.e.* June to November. Crickets such as *A. domesticus*, *Gryllus sp.*, *M. cincticornis*, and *Teleogryllus sp.* are found in abundance in different habitats, including homes, kitchen gardens, and fields, and can be easily located and handpicked for consumption because of the sharp distinct chirps they produce. *T. orientalis*, *T.portentosus*, and *S. monstrosus* are dug out from burrows using spades.

The mole cricket *G. orientalis* is found in water and close to water sources in paddy fields. Grasshoppers and crickets are found in a wide range of habitats such as fields, and grasses near human habitations having light sources at night. *O. hyla*, *M. bivattus*, *H. banian*, *E. securigera* and *O. fuscovittata* are also considered as pests for the damage they cause to paddy fields when present in large amount. These grasshoppers are collected early in the morning or late at night while found seated on the branches of rice stems. Katydidids such as *M. nipponensis*, *P.titan*, *E. securigera* are mainly collected at night using a high-intensity light source by locating the chirping sound it makes. During the daytime, katydidids are found only in few numbers as they camouflage with the host plant on which they perch.

Table 2.4. List of insects belonging to order Orthoptera

Scientific Name	Common name	Family	Edible stage	Habitat	Available Months
<i>A. domesticus</i>	House cricket	Gryllidae	Adult	Fields	June - Sep
<i>Gryllus sp.</i>	Field cricket	Gryllidae	Adult	Field	June - Sep
<i>M. cincticornis</i>	Cricket	Gryllidae	Adult	Grass, Field	June - Sep
<i>Teleogryllus sp.</i>	Cricket	Gryllidae	Adult	Grass, field	June - Sep
<i>T. occipitalis</i>	Cricket	Gryllidae	Adult	Grass, field	June - Sep
<i>T. orientalis</i>	Cricket	Gryllidae	Adult	Soil	June - Sep
<i>T. portentosus</i>	Soil cricket	Gryllidae	Nymph Adult	Soil	June - Sep
<i>G. orientalis</i>	Mole cricket	Gryllotalpidae	Adult	Water	Aug - Sep
<i>O. hyla</i>	Short-horned Grasshopper	Acrididae	Adult	Field	June - Sep
<i>M. bivattus</i>	Two stripped Grasshoppers	Acrididae	Adult	Field	June - Sep
<i>H. banian</i>	Rice grasshopper	Acrididae	Adult	Field	June - Sep
<i>Attractomorpha Sp.</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>C. rosea</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>C. robustus</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>G. africanus</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>Africanus</i>					
<i>O. fuscovittata</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>A. exaltata</i>	Chinese Grasshopper	Acrididae	Adult	Field	June - Sep
<i>P. infumata</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>Melanoplus sp.</i>	Grasshopper	Acrididae	Adult	Field	June - Sep
<i>M. nipponensis</i>	Katydid	Tettigoniidae	Adult	Grass, field	June - Sep
<i>M. elongate</i>	Katydid	Tettigoniidae	Adult	Grass, field	June - Sep
<i>P. titan</i>	Katydid	Tettigoniidae	Adult	Grass, field	June - Sep
<i>E. securigera</i>	Katydid	Tettigoniidae	Adult	Grass, field	June - Sep
<i>Tettigonia sp.</i>	Katydid	Tettigoniidae	Adult	Grass, fields	June - Sep
<i>S. monstrosus</i>	Sand cricket	Schizodactylidae	Adult	Underground	June - Aug



Figure 2.2. A- *A. domesticus*; B- *Gryllus* species; C-*M. cincticornis*; D-*Teleogryllus* sp. E. *T-occipitalis*; F-*T. orientalis*; G- *T. portentosus*; H-*G. orientalis*; I- *O. hyla* J- *M. bivattus*; K- *H. banian*; L- *Attractomorpha* Sp.; M- *Chondracris rosea*; N-*C. robustus*; O-*G. africanus africanus*; P- *O. fuscovittata*; Q-*A. exaltata*; R- *P. infumata*; S- *Melanoplus* sp.; T- *M. nipponensis*; U- *M. elongate*; V- *P. titan*; W- *E. securigera*; X- *Tettigonia* sp.; Y. *S.monstrosus*.

Hemiptera: The edible hemipterans comprise twenty-two (22) species belonging to Aetalionidae, Cicadidae, Dinidoridae, Pentatomidae, Belostomatidae, and Nepidae families (Table 2.5 Figure 2.3). Species such as *D. hardwickii*, *T. pruinose*, *D. intemerata*, *Pomponia sp.*, and *P. repanda* are eaten as food in all the districts of Nagaland. While they are eaten in all other districts, in Lonleng district *C. ianus*, *C. chinensi*, *U. montana*, and *T. javanica* are not eaten as food. Among the hemipterans, *Dolycoris sp.* is considered edible in only four districts viz. Phek, Kohima, Dimapur, Zunheboto and Wokha.

Species like *C. Tibicen pruinosa*, *D. Intemerata*, *D. oopaga*, *Pomponia sp.*, and *P. repanda* are available from August to November. Nymphs are collected from the soil, while adults (Cicada), identified by the noise they produce and often found on trees, are captured using long stick poles coated with glue on the top. In contrast, *L. ruber* is prevalent during the dry months of October to January. An effective method for collecting hemipterans (adult cicadas) is to locate them in abundance near light sources at night. Stink bugs (Pentatomidae) are known for their pungent smell which becomes a reason for acceptability by different people. They are available throughout the year and are usually handpicked from host plants. The treehopper *D. hardwickii* is preferred in their nymphal stage because it is less pungent. *L. indicus* is most abundantly found from December to March. The fruity aroma of these edible insects makes them suitable for creating chutneys and relished by different tribes across Nagaland.

Table 2.5. Insects list of the order Hemiptera

Scientific name	Common name	Family	Consumed stage	Habitat	Available months
<i>D. hardwickii</i>	Treehopper	Aetalionidae	Nymph, Adult	Tree	Sept-Oct
<i>T. pruinosa</i>	Cicada	Cicadidae	Nymph, Adult	underground, tree	Aug-Nov
<i>D.intemerata</i>	Cicada	Cicadidae	Nymph, Adult	underground, tree	Aug-Nov
<i>D. oopaga</i>	Cicada	Cicadidae	Nymph, Adult	Underground, tree	Aug-Nov
<i>Pomponia sp.</i>	Cicada	Cicadidae	Nymph, Adult	Underground, tree	Aug-Nov
<i>P. repanda</i>	Cicada	Cicadidae	Nymph, Adult	Underground, tree	Aug-Nov
<i>C. ianus</i>	Stink bug	Dinidoridae	Adult	Tree	April-July
<i>C. chinensis</i>	Red pumpkin stink bug	Dinidoridae	Adult	Tree	April - July
<i>C. singhalanus</i>	Stink bug	Dinidoridae	Adult	Tree	Feb-Mach
<i>A. nepalensis</i>	Black stinkbug	Dinidoridae	Adult	Tree	Nov-Jan
<i>N. meleagris</i>	Leaf footed bug	Coreidae	Adult	Tree	Aug-Sept
<i>A. phasiana</i>	Coreid bug	Coreidae	Adult	Tree	May-Aug
<i>D. planiventris</i>	Dead leaf footed bug	Coreidae	Adult	Tree	May-July
<i>C. hilaris</i>	Green stink bug	Pentatomidae	Adult	Tree	May-July
<i>C. siccifolia</i>	Stink bug	Pentatomidae	Adult	Tree	May-July
<i>E. fullo</i>	Yellow-spotted stink bug	Pentatomidae	Adult	Tree	Aug-Sept
<i>E. grossipes</i>	Pentatomide Bug	Pentatomidae	Adult	Tree	May-July
<i>Dolycoris sp.</i>	Shield Bug	Pentatomidae	Adult	Tree	April-Nov
<i>U. Montana</i>	Stink bug	Pentatomidae	Adult	Tree	April-Nov
<i>T. javanica</i>	Litchi stink bug	Tessartomida	Adult	Tree	May-July
<i>L. indicus</i>	Giant water bug	Belostomatidae	Adult	Ponds, Paddy fields	Dec-March
<i>L. ruber</i>	Water scorpion	Nepidae	Adult	Paddy fields	Oct-Jan

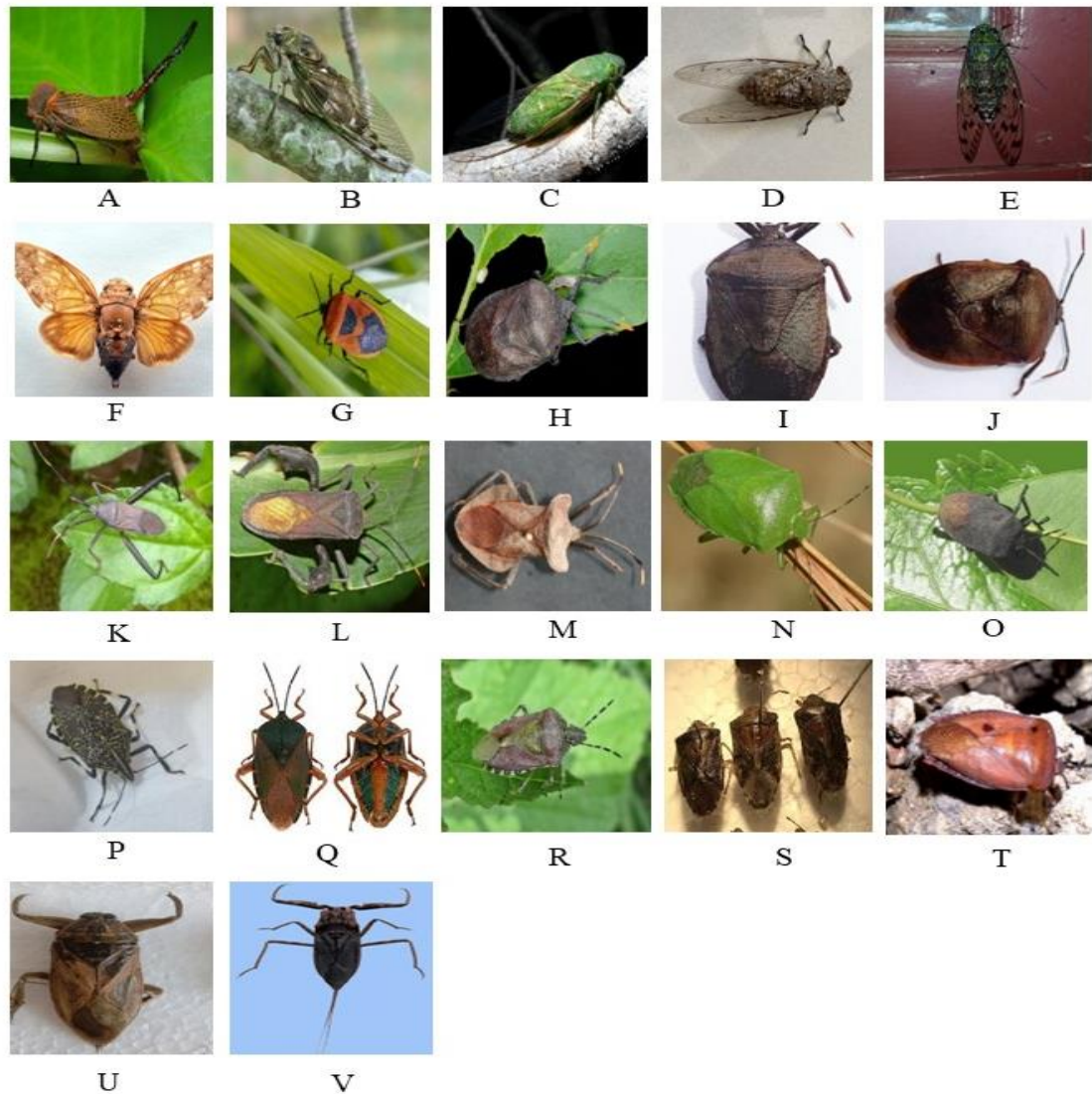


Figure 2.3. A-D. *hardwickii*; B- *T. pruinose*; C- *D. intemerata*; D- *D. oopaga*; E- *Pomponia* sp.; F- *P. repanda*; G- *C. ianus*; H- *C. chinensis*; I- *C. singhalanus*; J- *A. nepalensis*; K-N. *meleagris*; L- *A. phasiana*; M- *D. planiventris*; N- *C. hilaris*; O- *C. siccifolia*; P- *E. fullo*; Q- *E. grossipes*; R- *Dolycoris* sp.; S- *U. montana*; T- *T. javanica*; U- *L. indicus*; V- *L. ruber*.

Hymenoptera: Twenty-five (25) hymenopterans belonging to the families Apidae, Formicidae, and Vespidae are consumed as part of their diet by the indigenous people in Nagaland (Table 2.6, Figure 2.4). Hymenopterans are the most commonly available edible insects across Nagaland. Sixteen (16) species of Hymenopterans are recorded as edible in all the districts of Nagaland. The highest numbers of Hymenoptera are

considered a delicacy in Phek, Kohima, Dimapur, Mokokchung, Zunheboto, and Wokha Peren (Table 2.3). The least edible hymenopterans were recorded in the Noklak, Kiphire, Mon, and Tuensang districts. Insects such as *A. cerana indica*, *A. dorsata dorsata*, *A. dorsata laboriosa*, *A. florum*, *L. arcifera*, and *L. canifrons* are available for consumption all throughout the year. Also, the products of these insects (honey) are considered an important medicine for treating different ailments (fever, cold coughs, stomach ache, ulcers, etc.). Hymenopterans insects are eaten by boiling or frying and due to their sweet taste in nature, the larva and pupa are also eaten raw. In Nagaland, *A. cerana indica* is the most commonly reared bee followed by the stingless bee, *L. arcifera*, which are kept in wooden rectangular boxes or log hives. Honey is extracted by putting the comb in a thin linen cloth, squeezing them, and the filtered honey is stored in plastic and glass bottles for usage.

The weaver ant *O. smaragdina* (Formicidae) tastes sourly and is consumed at all stages. It is usually collected from May to July by cutting the branch containing the nests and dipping it into a bucket filled with water to avoid being bitten by the adults. Wasp and hornets are available all throughout the year depending on the species. Nocturnal wasps, *P. barthelemyi* are usually collected during the day by smoking the nests, where adults are sedated. While the broods of other hornets and wasps, such as *V. auraria*, *V. affinis indosinensis*, etc. are collected at night. Bees, wasps, and hornets including species like *V. mandarinia*, *V. soror*, and *V. orbata*, that build underground nests, are collected by smoking into the nest entrance until the adults are sedated. Thereafter, nests are dug open with a spade, and the brood and the adults are handpicked. While *L. canifrons*, the stingless bee is collected from underground nest by simply digging open the nests.

Table 2.6. Insects list of order Hymenoptera

Scientific name	Common name	Family	Consumed stages	Habitat	Available months
<i>A. cerana indica</i>	Indian honey Bee	Apidae	All	Tree	Jan-Dec
<i>A. dorsata dorsata</i>	Giant honey Bee	Apidae	All	Rocks	Sept-May
<i>A. dorsata laboriasa</i>	Himalayan honey bee	Apidae	All	Rocks	Sept-May
<i>A. florum</i>	Dwarf honey Bee	Apidae	All	Tree	Sept-Feb
<i>L. arcifera</i>	Stingless bee	Apidae	Honey	Tree	Sept-Feb
<i>L. canifrons</i>	Underground stingless bee	Apidae	Honey	Underground	Sept-Feb
<i>X. violacea</i>	Violet carpenter bee	Apidae	All	Tree	May-Oct
<i>O. smaragdina</i>	Weaver ant	Formicidae	Queen, pupa, larva	Tree	May-July
<i>P. varia</i>	Lesser paper Wasp	Vespidae	All	Tree	May-Sept
<i>P. olivaceus</i>	Common paper Wasp	Vespidae	All	Tree	May-Sept
<i>P. stigma</i>	Paper wasp	Vespidae	All	Tree	May-Sept
<i>Polistes</i> sp.	Paper wasp	Vespidae	All	Tree	May-Sept
<i>R. rufoplagiata</i>	Paper wasp	Vespidae	All	Tree	May-Sept
<i>Ropalida</i> sp.	Paper wasp	Vespidae	All	Tree	Sept-Feb
<i>Vespa</i> sp.	Paper wasp	Vespidae	All	Tree	Sept-Feb
<i>P. barthelemyi</i>	Nocturnal wasp	Vespidae	All	Tree	Sept-Feb
<i>V. affinis</i>	Lesser branded wasp	Vespidae	All	Tree	Sept-Feb
<i>V. indosinensis</i>					
<i>V. auraria</i>	Hornet	Vespidae	All	Tree	Sept-Feb
<i>V. basalis</i>	Hornet	Vespidae	All	Tree	May-Nov
<i>V. ducalis</i>	Hornet	Vespidae	All	Tree	May-Nov
<i>V. mandarinia</i>	Giant hornet	Vespidae	All	Underground	Sept-Feb
<i>V. tropica tropica</i>	Greater branded hornet	Vespidae	All	Tree	May-Nov
<i>V. soror</i>	Hornet	Vespidae	All	Tree, Underground	Sept-Feb
<i>V. orbata</i>	Hornet	Vespidae	All	Underground	May-Nov
<i>V. bicolor</i>	Hornet	Vespidae	All	Tree	May-Nov

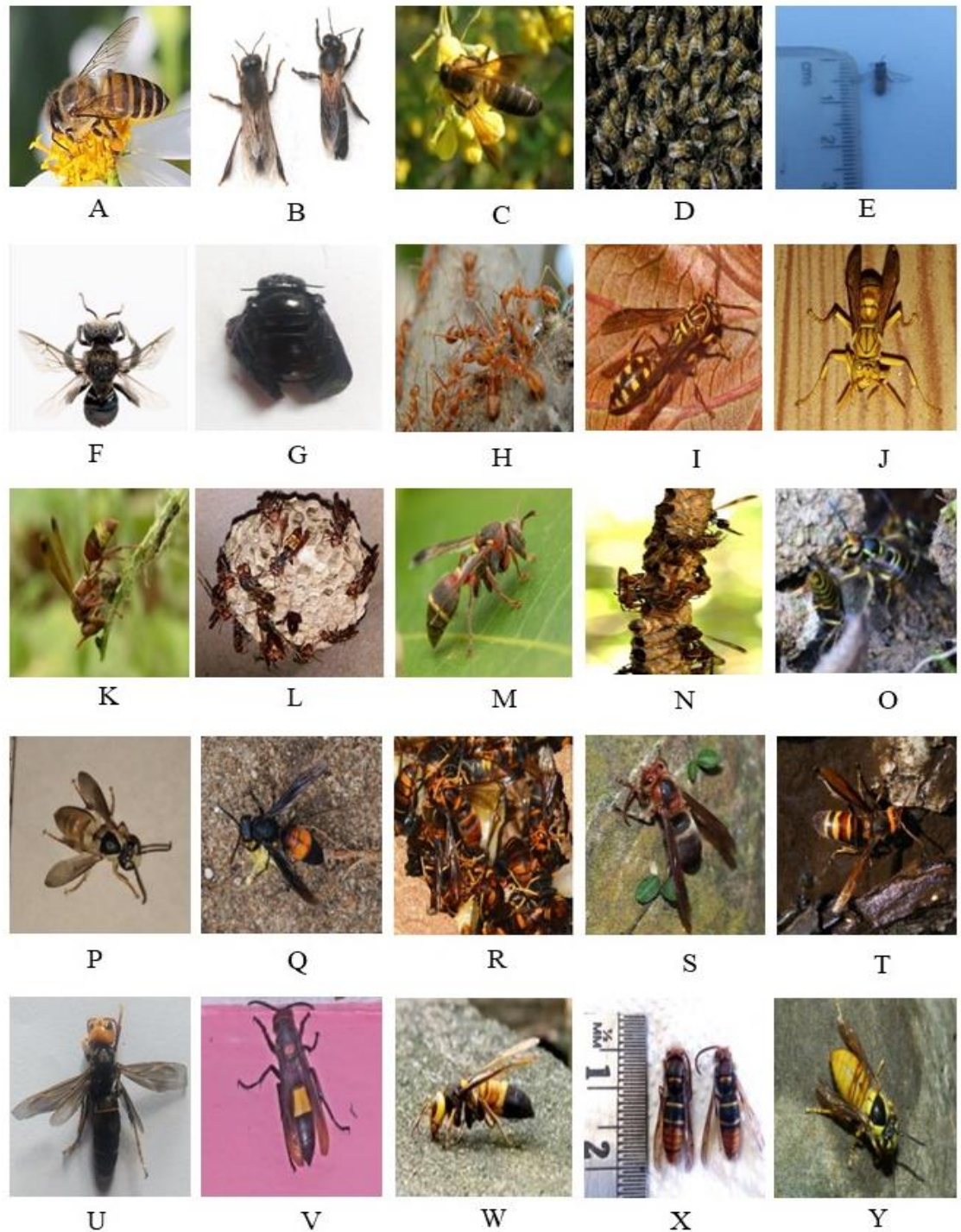


Figure 2.4. A-*Apis cerana indica*; B- *A. dorsata dorsata*; C- *A. dorsata laboriosa*; D-*A. florea*; E-*L. arcifera*; F- *L. camifrons*; G- *X. violacea*; H- *O. smaragdina*; I- *P. varia*; J- *P. olivaceus*; K- *P. stigma*; L-*Polistes* Sp.; M- *R. rufoplagiata*; N-*Ropalida* sp.; O-*Vespa* sp; P- *P. barthelemyi*; Q-*V. affinis indosinensis*; R- *V. Auraria*; S- *V. basalis* T-*V. ducalis*; U- *V. mandarinia* V-*V. tropica tropica* W-*V. soror* X- *V. orbata* Y- *V. bicolor*

Coleoptera: 16 species of coleopterans belonging to the families Cerambycidae, Dytiscidae, Curculionidae, Hydrophilidae, and Scarabaeidae were recorded in the present study (Table 2.7 Figure 2.5). Species such as *B. rubus*, *O. brunneum*, *T. wallichii*, and *B. lineolata* are eaten in all the 12 districts. While species such as *C. tripunctatus*, *S. manipurensis*, and *H. cashmirensis* are considered debile in only six districts (Table 2.3). Coleopterans (Mostly wood borers) larvae are typically harvested from infested trees in their natural habitat. Species such as *Anoplophora* sp., *O. brunneum* and *B. lineolata* are consumed in all stages of their development, whereas others like *B. rubus* Linn, *B. parryi*, *B. rufomaculata*, and *T. wallichii* are primarily consumed during their pupal and larval stages. These larvae are available for consumption from June to October and are gathered by handpicking them from leaves or by splitting open the wood using tools like machetes or axes.

The aquatic insects such as *C. limbatus*, *C. tripunctatus lateralis*, *S. manipurensis* and *H. cashmirensis* are collected during the months of June to December using bamboo weave baskets or simply handpicked by reducing the volume of water. The wings of the insects are removed and either fried or cooked with bamboo shoot or axone by adding water.

X. gideon, *L. stigma*, and *Holotricha* sp. can be seen inhabiting host trees like *Acacia penata*, *Quercus* sp., and *Albizia chinensis*. They are typically gathered by shaking the tree branches and carefully picked between June and August, after which they are either roasted or fried for consumption.

Table 2.7. Insects lists of the order Coleoptera

Scientific name	Common name	Family	Consumed stages	Habitat	Available months
<i>Anoplophora sp.</i>	Long horn Beetle	Cerambycidae	All	Tree	June - Oct
<i>B. rubus</i>	Long horn Beetle	Cerambycidae	Larva	Tree	June - Oct
<i>B. parryi</i>	Long horn Beetle	Cerambycidae	Larva	Tree	June - Oct
<i>B. rufomaculata</i>	Long horn Beetle	Cerambycidae	Larva	Tree	June - Oct
<i>O. brenneum</i>	Long horn Beetle	Cerambycidae	All	Tree	June - Oct
<i>A. chalybaeus</i>	Corn borer	Cerambycidae	Larva	Tree	June - Oct
<i>T. wallichii</i>	Longhorn beetle	Cerambycidae	Larva	Tree	June - Oct
<i>B. lineolata</i>	Longhorn beetle	Cerambycidae	All	Tree	June - Oct
<i>C. limbatus</i>	Water Beetle	Dytiscidae	Adult	Water	June - Dec
<i>C. tripunctatus lateralis</i>	Beetle	Dytiscidae	Adult	Water	June - Dec
<i>S. manipurensis</i>	Aquatic Beetles	Dytiscidae	Adult	Water	June - Dec
<i>R. ferrugineus</i>	Palm Weevil	Curculionidae	All	Tree	Oct - Nov
<i>H. cashmirensis</i>	Scavenging water beetle	Hydrophilidae	Adult	Water	June - Dec
<i>X. gideon</i>	Rhinoceros Beetle	Scarabaeidae	Adult, Larva	Grass	June - Aug
<i>L. stigma</i>	White grub	Scarabaeidae	Adult	Grass	June - Aug
<i>Holotricha sp.</i>	White grub	Scarabaeidae	Adult	Grass	June - Aug

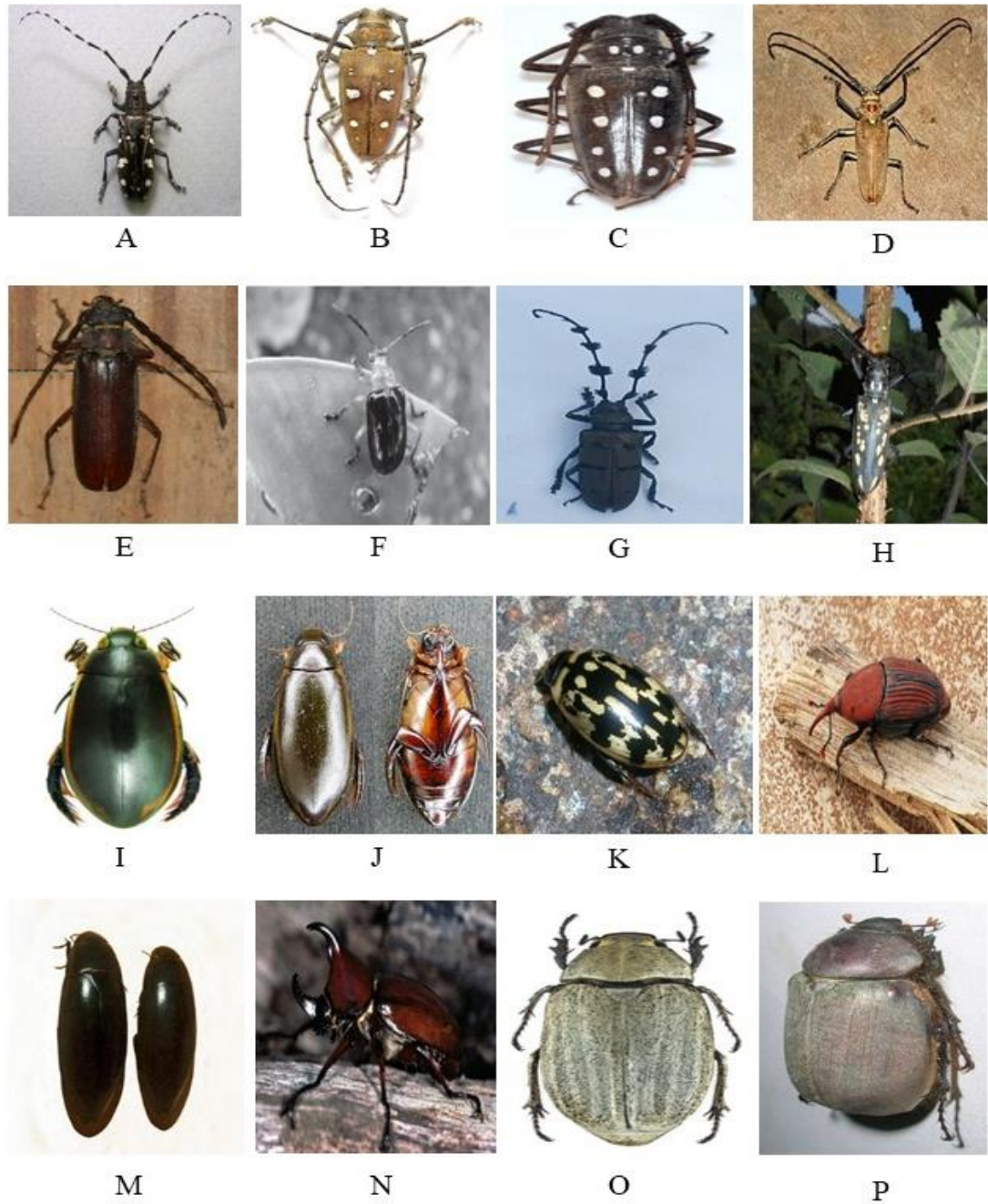


Figure 2.5. A-*Anoplophora* sp.; B-*B. rubus*; C-*B. parryi*; D- *B. rufumaculata*; E- *O. brunneum*; F-*A. chalybaeus*; G- *T. wallichii*; H- *B. lineolate*; I-*C. limbatus*; J-*C. tripunctatus lateralis*; K-*S. manipurensis*; L-*R. ferrugineus*; M- *H. cashmirensis*; N- *X.gideon*; O- *L. stigma*; P- *Holotricha* sp.

Odonata: Eight (8) species namely *D. trivialis*, *O. pruinosum*, *N. fulvia*, *C. servilia*, *P. congenera*, *O. sabina*, *O. triangulare triangulare*, *P. flavescens*, belonging to the family

Libellulidae under order Odonata were recorded in the present investigation (Table 2.8, Figure 2.6). All these species are commonly called dragon fly, they are mostly found during summer season. The adult dragonflies are found hovering around near water bodies like ponds, ditches, and paddy fields while the nymphal stages are aquatic in nature. Nymphs are collected using bamboo-weaved baskets (Supplementary figure 2.1) or simply handpicked by decreasing the volume of the water. Entomophagy practices of Odonata are mostly seen around the regions where indigenous people practice paddy field. In districts such as Phek, Kohima, Dimapur, Peren, and Wokha most of the Odonata species are edible. While in Tuensang, Mon, Kiphire, and Noklak, none of the Odonata insects are consumed, this could be attributed due to lack of terrace farming practices in the regions because nymphal stages of Odonates are completely aquatic in nature.

Table 2.8. Insect list of order Odonata

Scientific name	Common name	Family	Consumed stage	Habitat	Available season
<i>D. trivialis</i>	Blue Percher	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>O. pruinosa neglectum</i>	Common red skimmer	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>N. fulvia</i>	Fulvous forest skimmer	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>C. servilia</i>	Dragon fly	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>P. congenera</i>	Dragon fly	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>O. sabina sabina</i>	Slender skimmer	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>O. triangularet triangulare</i>	Yellow-tailed ashy skimmer	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov
<i>P. flavescens</i>	Globe skimmer	Libellulidae	Nymph, Adult	Ponds, Ditches	July- Nov

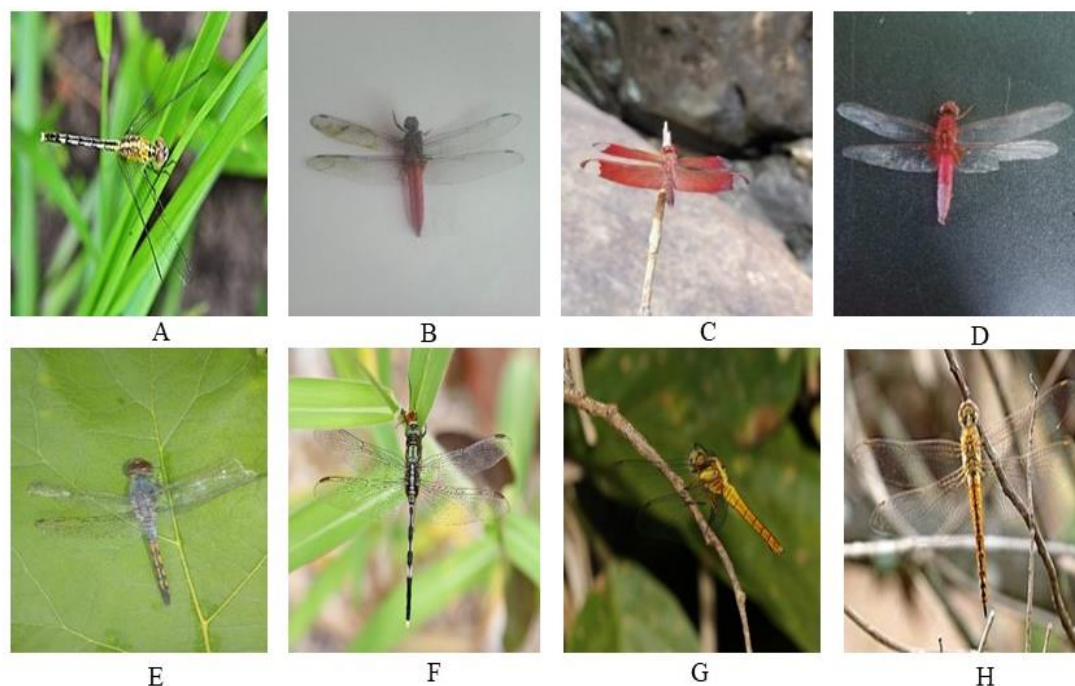


Figure 2.6. Photo plate of odonates A- *D. trivialis*; B- *O. pruinosum*; C- *N. fulvia*; D- *C. servilia servilia*; E- *P. congenera*; F- *O. sabina sabina*; G- *O. triangulare triangulare*; H- *P. flavescens*.

Lepidoptera: Eight (8) species of Lepidopterans belonging to the families Cossidae, Lasiocampidae, Saturniidae, Crambidae, Saturniidae, Hesperidae were considered as edible in Nagaland. *Cossus* sp. and *O. fuscidentalis* larvae are the most common edible Lepidopterans recorded, highly preferred by most people due to their good smell and taste. They are handpicked from oak trees and bamboo trees by splitting open the trees. *E. torus*, and *P. cruegeri* are the least known, considered edible in only four and five districts respectively (Table 2.3). Most lepidopterans are available from May to November (Table 2.9). Rearing of *S. cynthia ricini* is most popular among most of the tribal people for silk production and the green pupae as bi-product are readily available as the most relish item for consumption. The pupae are also abundantly found from May to July in local markets and commands higher prices. The pupae of this silkworms are typically boiled, fried, or roasted before being consumed. Pupae and larvae of

Malacosoma sp., *P. cruegeri*, and *E. torus* are usually available from May to November and are usually handpicked from trees.

Table 2.9. Insects lists of the order Lepidoptera

Sl. No	Scientific names	Common names	Family	Consumed stage	Habitat	Available months
1	<i>Cossus</i> sp.	Moth	Cossidae	Larva, pupa	Tree	Aug-Nov
2	<i>Malacosoma</i> sp.	Tent caterpillar	Lasiocampidae	Larva, pupa	Tree	May-Sept
3	<i>S. cynthia ricini</i>	Eri silkworm	Saturniidae	pupa	Tree	Jan-Dec
4	<i>A. assamensis</i>	Muga Silkworm	Saturniidae	pupa	Tree	Jan-Dec
5	<i>O. fuscidentalis</i>	Bamboo borer	Crambidae	larva	Bamboo	Aug-Oct
6	<i>P. cruegeri</i>	Moth	Saturniidae	larva	Tree	April-May
7	<i>E. torus</i>	Rounded palm redeye	Hesperiidae	larva	Tree	Sept-Nov
8	<i>A. mylitta</i>	Silkworm	Saturniidae	Pupa	Tree	April-July



Figure 2.7. Photo plate for Lepidoptera. A- *Cossus* sp.; B-*Malacosoma* sp.; C-*S. cynthia ricini*; D. *A. assamensis*; E- *O. fuscidentalis*; F- *P. cruegeri*; G- *E. torus*; H- *A. myllida*.

Mantodea: Under order Mantodea, *H. coarctata* and *T. sinensis* are considered edible in Nagaland (Table 2.10, Figure 2.8-A,B), however, in Phek, Kohima, Dimapur, Peren, and Tuensang districts, these insects are not consumed (Table 2.3). These insects inhabit mostly grassland areas and are eaten during their nymphal, larval and adult stages (Figure 2.8-C). They are mostly available during May-September. The sole isopteran species consumed by the Naga tribal community is *Macrotermes* sp. (commonly called termites), a member of the Termitidae family, and it is available from November to December (Table 2.10). *Macrotermes* sp. is considered edible and relished as nutritious by the people from all the districts of Nagaland. During the swarming season, the tribal inhabitants locate the entrances of the burrows and handpick the winged termites as they emerge out of the burrow. Alternatively, people also place nets around the burrow's entrances using long sticks to capture all flying termites. Also, the only dipteran species identified as edible is the larva of *Tipula* sp.(Figure 2.8-D) These larvae are found in small streams and paddy fields, and the Naga tribe harvests them by either lifting rocks or using bamboo-woven baskets. The *Tipula* sp. is considered edible in only six districts, namely Phek, Kohima, Dimapur, Zunheboto, Wokha, and Peren. The larvae are prepared for consumption by removing their entire digestive tract and are available from September to January.

Table 2.10. Insects list of the order Mandotea, Isoptera, and Diptera

Scientific name	Order	Common name	family	Consume stages	Habitat	Available months
<i>H. coarctata</i>	Mantodea	Giant Asian Mantis	Mantidae	Nymph, adult	Grass	May-Sept
<i>T. sinensis</i>	Mantodea	Chinese mantis	Mantidae	Nymph, Adult	Grass	May-Sep
<i>Macrotermes</i> sp.	Isoptera or Blattodea	Termite	Termitidae	Larva, adult	Underg -round	Nov-Dec
<i>Tipula</i> sp. Linn.	Diptera	Cranefly	Tipulidae	Larva	Water	Sept-Jan

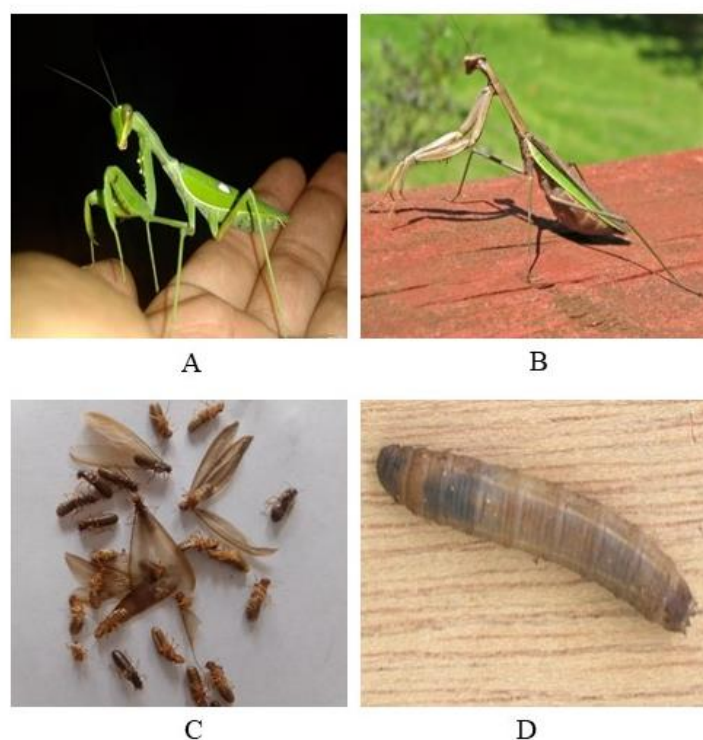


Figure 2.8. A- *H. coarctata*; B- *T. sinensis*; C- *Macrotermes* sp.; D- *Tipula* sp.

2.3.2 Insects use value

Based on the grouping of the insects by the locals, twenty (20) groups of edible insects were considered to determine their used value. Depending on the tribes, their preferences and insect availability, use value differs widely across different districts of Nagaland. In Dimapur, highest use value was recorded for insects such as Grasshoppers (1.00), Weaver ants (0.98), Water beetles (0.94), Wasp and hornets (0.92) and Silkworm (0.9). In Phek and Kohima districts, a maximum number of insects (15 and 12, respectively) with the highest use value was observed (Figure 2.9-B and D). In these two districts least number of use values was recorded for Weaver ants and Mantis. In the Peren district, only one group of insects (Grasshopper) has a use value of 1, while a minimum use value was noted for Mantis, Katydid, Caterpillar, and Rhino beetle (Figure 2.9-C). In Wokha district, insects with highest use value were recorded for Grasshopper (0.9), Cicada (0.9) and Silkworm (0.87) however, minimum use value was recorded for insects such as Mantis (0.1), Mole cricket (0.4), Caterpillar (0.4), and

Rhino beetle (0.4). In Zunheboto use value ranges from 0.3-0.9, where the minimum was recorded for Termites, the maximum was recorded for Weaver ants, Wasps and Hornets, and Katydid. In Mokokchung, the use value ranges from 0.0-0.9, with the minimum value recorded for Rhino beetle (0.00) and the maximum for Grasshoppers (0.9), Silkworms (0.9), and Termites (0.9). In Kiphire district, insects such as Mole cricket (0.2), Mantis (0.2), Water scorpion (0.24), and Wood borers (0.28) has the minimum use value while Bamboo borer, Caterpillar, and Grasshoppers have the maximum use value of 0.72, 0.68, and 0.68 respectively (Figure 2.10-D). Maximum use value in Grasshoppers (0.9), Wasp (0.9), hornets (0.9) and Silkworm (0.9) and minimum in Mole cricket, Water beetles, Giant water bug, Water scorpion and Dragon flies were recorded in Mon, Noklak, Tuensang, and Longleng districts (Figure 2.11-A, B, C, D). Grasshoppers, Crickets, Cicada, Dragon flies, Termites, Katydid, and Caterpillar are most common and highly relished insects among all Naga tribes as indicated by the highest use value, while least use value was observed for Weaver ants, Dragon flies, and Rhino beetle, Mantis etc. Further, wide variations of insect's preferences were also observed among different districts, for instance, Mole crickets are consumed widely in Phek (0.6), Kohima (0.8), Zunheboto (0.8), Dimapur (0.7), showing high use value while in Kiphiri (0.2) and Tuensang(0.2) least use value was observed for this species. Similarly, Weaver ants are consumed or used widely in Dimapur (0.9), Peren (0.9), Zunheboto (0.9) districts and least in Noklak (0.2), Kohima (0.5), Longleng (0.5), and Wokha (0.6) districts. The average use value of insects across Nagaland ranges from 0.28 ± 0.11 to 0.88 ± 0.3 (Figure 2.12), having shown the highest use value in wasps and hornet (0.88 ± 0.3), followed by grasshopper (0.83 ± 0.26), Silkworm (0.83 ± 0.32), Termites (0.72 ± 0.33), Crickets (0.7 ± 0.24), Bamboo borer (0.67 ± 0.28), Wood borers (0.65 ± 0.28), Cicada (0.64 ± 0.31). The minimum use value

was in Rhino beetle (0.28 ± 0.11), Mantis (0.34 ± 0.24), Dragon flies (0.44 ± 0.31), Mole cricket (0.44 ± 0.26), water scorpion (0.48 ± 0.34), carpenter worm (0.48 ± 0.26), water beetle (0.53 ± 0.14), Giant water bug (0.53 ± 0.13), etc. Average use value of edible insects across the districts was highest in Phek (0.93 ± 0.18), followed by Kohima (0.88 ± 0.22), Dimapur (0.73 ± 0.25), Zunheboto (0.7 ± 0.11), and showed comparative low value in Noklak (0.39 ± 0.19), Kiphire (0.4 ± 0.19), Mon (0.41 ± 0.19), Tuensang (0.44 ± 0.17), and Longleng (0.46 ± 0.36) districts (Figure 2.13).

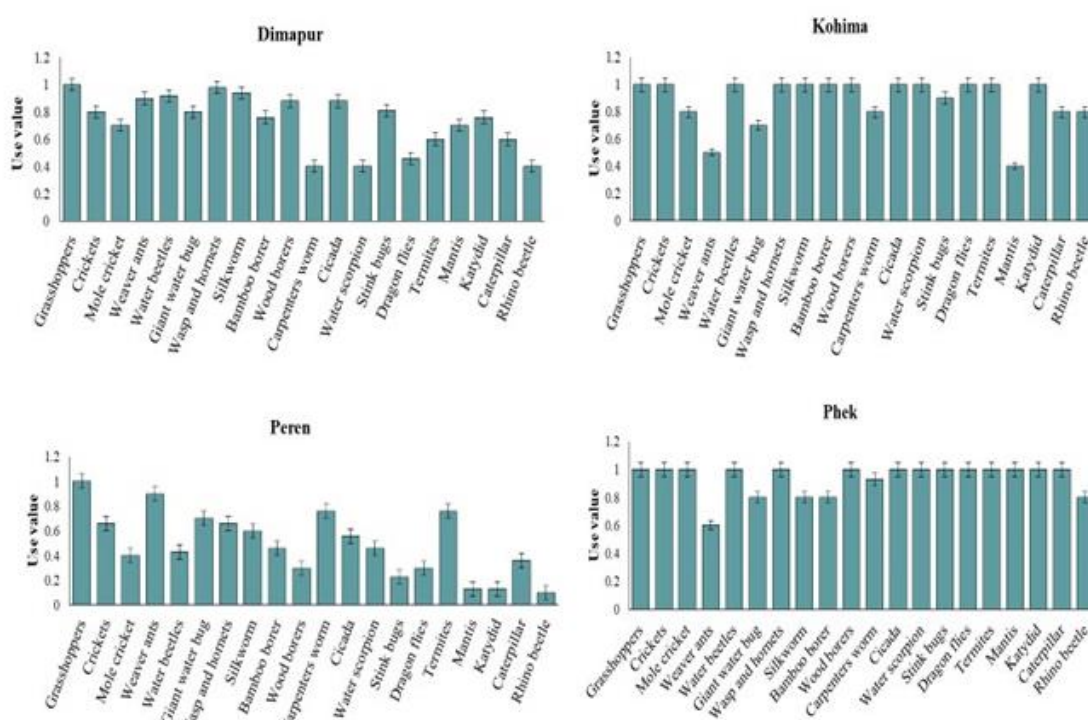


Figure 2.9. Use value of edible insects in Dimapur, Kohima, Peren, and Phek district of Nagaland.

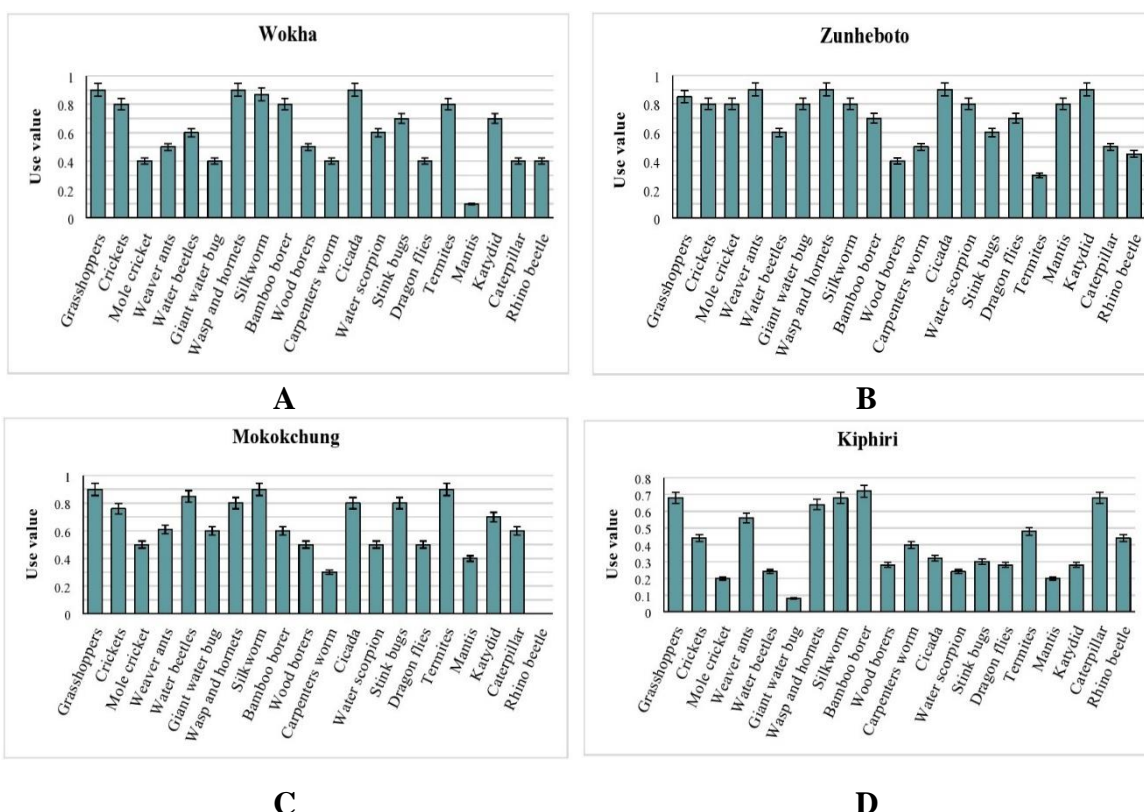


Figure 2.10. Use value of edible insects in Wokha, Zunheboto, Mokokchung, and Kiphire district of Nagaland.

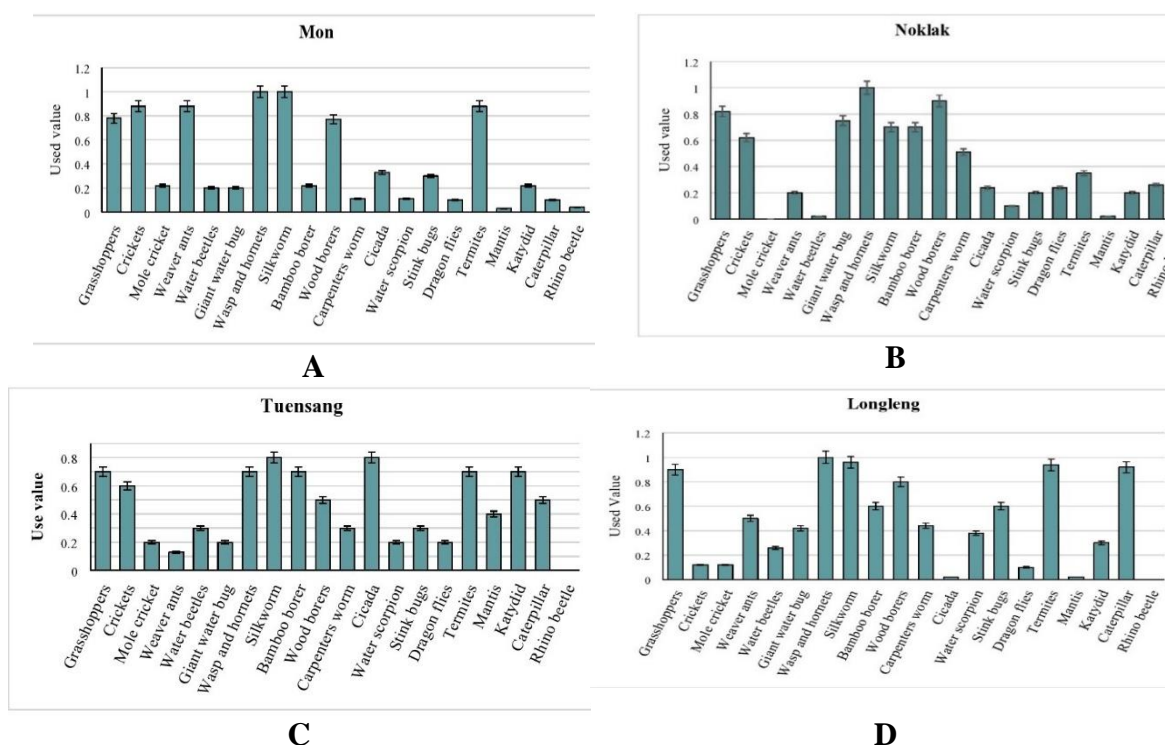


Figure 2.11. Use value of edible insects in Mon, Noklak, Tuensang, and Longleng district of Nagaland

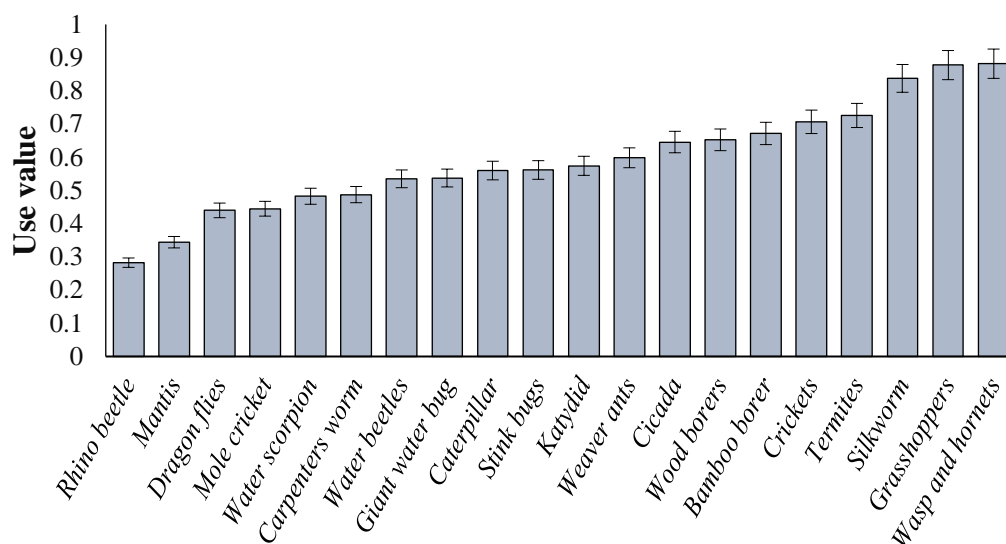


Figure 2.12. Average use value of edible insects across Nagaland.

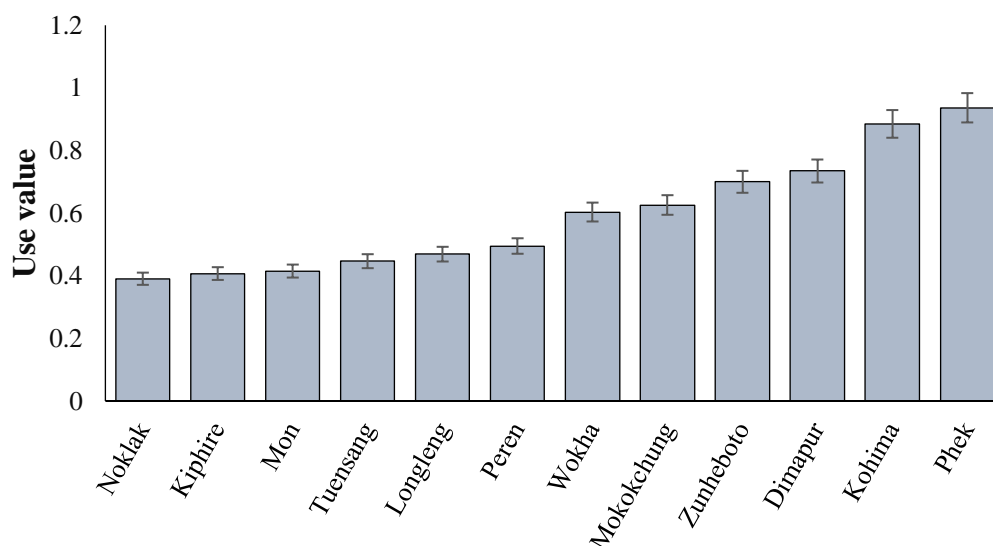


Figure 2.13. Average use value of insects in different district of Nagaland.

2.3.3 Preparation methods for consumption

Depending on the preferential consumption of insects, processing and preparation methods varies among the insects and tribes. Usually, edible insects are boiled, fried, roast, made into chutney or steamed. Due to its distinctive sweet and fruity smell, certain insects such as *A. cerana indica* and larva of *Cossus* sp. larva are eaten raw.

2.3.3.1 Boiling- Edible insects are usually boiled with local spices such as fermented bamboo shoots, axone, ginger, and garlic. Most common insects that are boiled for consumption include hornets (*V. ducalis*, *V. basalis*, *V. mandarinia*, etc) wasps (*P. barthlemyi*) Dragon fly (*D. trivialis*, *O. pruinosum*, *N. fulvia*), Crickets (*A. domesticus*, *Gryllus sp.*, *M. cincticornis*, *T. occipitalis*, *G. orientalis*, *T. orientalis*, *T. portentosus*), and Wood borers (*Anoplophora sp.*, *B. rubus*, *B. parryi*, *B. rufomaculata*, *Orthosoma brunneum*, *A. chalybaeus*, *T. wallichii*, *B. lineolata*) (Figure 2.14).

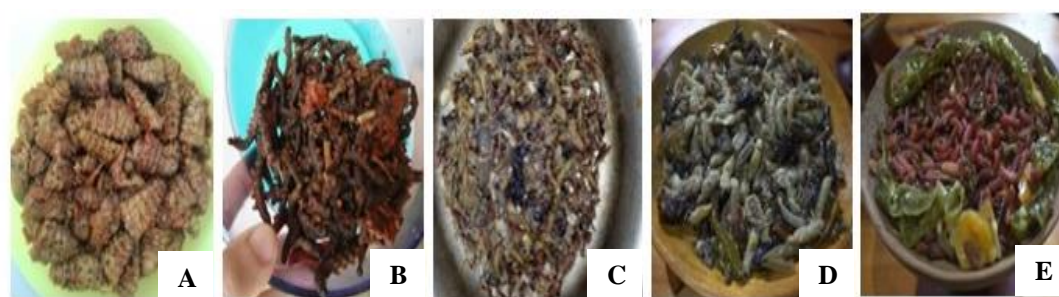


Figure 2.14. Boiled form of edible insects (A) *S. cynthia ricini* (B) *Malacosoma* sp. (C) *D. trivialis* nymph (D) *V. mandarinia* (E) *Cossus* sp. Larvae

2.3.3.2 Fried- Insects such as Bamboo borer (*O. fuscidentalis*), grasshoppers (*O. hyla*, *A. exaltata*, *H. banian*), Crickets (*A. domesticus*, *T. occipitalis*, *T. portentosus*) are fried in the cooking oil and are ready for consumption (Figure 2.15). The ingredients used in the frying differ, depending on the consumer's preferences, however, the most used spices include onion, ginger, garlic, salt, pepper, etc.



Figure 2.15. Fried form of edible insects. A- *O. fuscidentalis* larvae; B- *O. hyla*; C- *L. indicus*; D- *Cossus* sp. larvae.

Depending on the preferences same group of insects can be cooked in different methods. Along with other forms of preparations insects such as katydids (*M. nipponensis*, *M. elongata*, *P. titan*) Mantis (*H. coarctata*, *T. sinensis*), and wood borer larva (*T. wallichii*, *A. chalybaeus*) can also be eaten in roasted form. Due to their distinct aroma stink bugs (*C. ianus*, *C. chinensis*, *C. singhalanus*, and *U. montana*), *Lethocerus indicus*, and *Cossus* larvae are used for chutney in fresh form or after toasting in fire. Also, the larva of *O. fuscidentalis*, *V. mandarinia*, and *P. barthelemyi* can also be consumed after being steamed under hot ashes for 10 to 15 minutes.

2.3.4 Preservation methods of edible insects

3.4.1 Smoking- Adult insects are smoked over fireplaces by placing them on bamboo trays and once dried the insects are used for self-consumption or sold in the market. This method is commonly used for insects such as *Macrotermes* sp.

2.3.4.2 Sun drying- This method is usually applied for insects such as stink bugs (*Udongo montana*). Insects are either dried freshly or are dried after boiling. Insects such as sting bugs are usually sundried.

2.3.4.3 Frying- This method is applied to insects such as *O. fuscidentalis* larva which are fried and stored for self-consumption or sold in the market.

2.3.4.4 Pickled- The larvae and pupae of *V. mandarinia*, as well as the pupae of *S. cynthia ricini*, are fried and then placed in bottles with ingredients like chili, bamboo shoots, ginger, and garlic. The mixture is filled with mustard oil until fully immersed, and the container is sealed tightly. This concoction can be stored for approximately two months or more depending on the hygiene of the storage facility.

2.3.5 Socio economic value of edible insects

Edible insects and their products fetch a very high price in the rural as well as urban markets (Table 2.11, Figure 2.16 and 2.17). Majority of insects are harvested from the wild according to the seasonal availability. However, only a few insects (*S. cynthia ricini*, *A. cerana indica*, *L. canifrons*) are reared at home and products are used for self consumption or for sale at the markets. For example, silkworm pupae, which are the byproduct of silk production, are the most delicious food among people, also they generate a good amount of income for the domesticator (INR-700-800/kg). Honey, a product of Bee culture, is a highly sought-after nutritious food for human consumption. They are available more often compared to other wild harvested insects as they are domesticated or semi-domesticated, also fetching a high price (Rs. 900–2000 ml/750ml) (Figure 2.16). Insects such as *V. mandarinia*, *T. wallichii* are semi-domesticated and harvested for self-consumption or sold at high prices per comb (INR 5000 to 25,000) or in Kilograms (kg) (INR 800 to 1000) respectively. Variation in market price of insects depends on how they are collected, availability, taste, size or consideration as healthy food. In case of *P. barthelemyi*, *V. mandarinia*, and *A. cerana indica*, collection involves risks of being stung; *L. canifrons*, and *T. wallichii* require manual efforts in collection so market price is very high. Grasshoppers (INR 100-300/250gm) and *Macrotermes* sp. (INR 150- 200/250gm) are easily available during its peak season so it is low priced. Cost of honey extracted from *A. cerana indica* (INR 900-1000/750ml) is lower than that of *L. canifrons* (INR 2000/750ml) as the former is domesticated and the later is collected from wild species having very infrequent chance of being found. *S. cynthia ricini* (500-600 /kg), *O. fuscidentalis* (INR 400-600/250gm) are highly preferred because of its good taste. *L.indicus* (INR 15-20/piece) is most preferred among other aquatic insects and *T. portentosus* (INR 150-200/300gm) is preferred above other

crickets because of its large size. *Cossus* sp. (INR 4,000/500gm) larva is a good medicinal food while *S. cynthia ricini* is consumed and sought after as a better protein diet over other meat such as beef, pork, and chicken.

Table 2.11. Market value of commonly available edible insects in Nagaland

Scientific name	Insect Stage/ state	Source	Quantity	Price in INR	
				Rural	Urban
<i>S. cynthia ricini</i>	Pupa Fresh	Domesticated at home	1 kg	400 – 500	700 - 800
<i>O. fuscidentalis</i>	Larva / Fresh	Wild	250 gm	250 – 300	400 - 600
<i>Cossus</i> sp.	Larva/ Fresh	Wild	500 gm	3500	4000
<i>P. barthelemyi</i>	All/ Fresh	Wild	Per comb range	300 – 1500	500 – 2000
<i>V. mandarinia</i>	All/ Fresh	Semi-domesticated	Per comb range	3000 to 20,000 Or 15 to 20 per larva or pupa	5000-25,000
Dragonfly	Nymph/ Fresh	Wild	200gm	-	100 - 150
Grasshoppers	Adult / Dried	Wild	250gm	-	200 – 300
Grasshoppers	Adult/ fresh	Wild	250gm		100 – 200
<i>T. portentosus</i>	Adult/ Fresh	Wild	300gm	-	150 – 200
<i>Macrotermes</i> sp.	All/ Dried	Wild	250gm	-	150 – 200
<i>L. indicus</i>	Adult/ Fresh	Wild	Per piece	15	20
<i>L. ruber</i>	Adult/ Fresh	Wild	250gm		150 - 200
Stink bugs	Adults/ Dried	Wild	250gm		200 – 300
<i>T. wallichii</i>	Larva/ Fresh	Semi-domesticated	1kg	500 – 600	800 – 1000
Water beetles	Adult/ Fresh	Wild	250gm		200 – 250
<i>A. cerana indica</i>	Honey	Domesticated	750ml	600 – 800	900 – 1000
<i>L. camifrons</i>	Honey	Domesticated or wild	750ml	1000	2000
<i>O. smaragdina</i>	All/ Fresh	Wild	250gm		150 – 200
Tent caterpillar	Larva/ Dried	Wild	300gm		300 – 400



Figure 2.16. A-B Fried and packed *O. hyla* for sale; C- Honey products of *Apis cerana indica* and *L. canifrons* sold at Hornbill festival; D- Fragments of raw honey in the comb of *A. cerana indica* sold at Hornbill festival; E- Empty combs of *V. mandarinia* hanging at the entrance of a home as decoration; F- Exoskeleton of green click beetle used in earning by Chang tribe



Figure 2.17. Insects sold in the market; A- *S. cynthia ricini*; B- *A. nepalensis*; C- *O. smaragdina*; D- *T. wallichii*; E- *Cossus* sp. larvae; F- *O. hyla*; G- *C. limbatus*; H- *D. trivialis*; I- *L. indicus*

2.4 Discussion

The varietal use of edible insect species by the tribal of Nagaland shows their importance throughout the year's daily diet. Differences in geographical locations, market channels, and familiarity with a particular insect play an important role in the consumption preferences among the people across Nagaland. In the present study, 108 edible insects have been recorded, including two additional species of an earlier report of 106 species in Nagaland (Mozhui et al., 2020). The wide diversity of entomophagy practices in Nagaland is comparable with and shows similar patterns in the use of edible insects among the different ethnic communities in the northeast part of India. Nyishi and Galo tribes of Arunachal Pradesh consume about 102 species of insects of which, 40 belong to the order Coleoptera; 26 Orthoptera; 12 Hymenoptera; 8 Hemiptera; 5 Homoptera; 3 Ephemeroptera; 4 Odonata; 2 Plecoptera; 3 Dictyoptera; 2 Isoptera; and 3 Diptera (Chakravorty et al., 2013). Different tribes of Dhemaji, Morigaon, Udalguri, Baksa, and Karbi Anglong districts of Assam consume around 67 species of edible insects that come under 8 orders and 27 families (Doley and Kalita, 2012; Rahman et al., 2018; Thangjam and Kadam, 2020). In Manipur, 41 insect species belonging to 8 orders, 24 families and 36 genera have been identified as edible of which hemiptera contribute maximum number of edible species (10) and the orders Dictyoptera and Isoptera enlist the lowest number. Meitei, Tarao, Tangkhul, Chothe, and Thadou groups in Manipur consume around 28-30 insect species, surpassing other ethnic groups (Shantibala et al., 2012; Singh et al., 2013). Similarly, ethnic tribes in Arunachal Pradesh, such as Nyishi, Galo, Adi, Wangcho, Nocte, Shingpo, Tangsa, Deori, and Chakma, consume approximately 65 species of edible insects from 9 orders and 24 families (Chakravorty et al., 2013; Singh et al., 2013). A comparative assessment among the north eastern states shows that consumption of edible insect is the highest in

Nagaland with a record of 108 numbers belonging to 9 orders viz: Orthopterans and Hymenoptera (25 each) followed by Hemiptera (22), Coleoptera (16), Odonata (8), Lepidoptera (8), Mantodea (2), Isoptera (1), and Diptera (1). In Nagaland, entomophagy practiced by different tribes varied according to the lifestyles and its association with nature.

Entomophagy in Nagaland mostly depend on cultural practices, farming pattern or seasonal availability of edible insects. For instance, aquatic insects such as *L. indicus*, *L. ruber*, *C. limbatus*, *H. cashmirensis*, and dragonfly nymphs are rarely available and less eaten in the districts of Mon, Tuensang, Noklak, Kiphiri, Longleng as the people from these districts sparsely practice terrace farming that are primary habitat for these aquatic insects. However, people living in districts that are well connected by roads practice terrace cultivation and have a diverse entomophagy practice. Also, exposure, availability, and shared knowledge of edible insects, particularly in urban markets such as Kohima, Dimapur, Mokokchung, and Wokha districts, are attributed to more diverse entomophagy practices and higher use value found in these districts. Culture also plays a major role in determining the use of insects as food, people in the districts of Phek, Kohima, Wokha, Mokokchung, and Zunheboto are familiar with the use of edible insects as food, having better knowledge of its seasons for collection. People from districts such as Kiphiri, Peren, Noklak, Mon, and Tuensang, being rich in bio-resources, utilize more of bigger mammals for food and undermine the use of smaller arthropods such as insects for food. Edible insects such as *S.cynthia ricini*, *L. camifrons*, and *A. cerana indica* are domesticated at home for their pupa or for honey as food and domesticators receive support from the government through sericulture departments and Nagaland Honey Bee Keeping Mission (NHBM).

In India, there's been a study looking into how factors like plant availability and seasonal changes influence the diversity of insects (Kumari and Priya, 2022). During the rainy season, which spans from June to September, coleopterans are quite common, whereas hymenopterans, like bees and wasps, thrive more in the winter months, from November to February. In September and October, acridids such as grasshoppers and locusts become abundant. And interestingly, crickets, known as Gryllids, hit their peak during the summer months, from May to August. These findings shed light on the intricate relationship between insects and their environment (Chakravorty et al., 2013; Van Huis and Oonincx, 2017). Another contributing factor for increased consumption of insects is the livelihood of certain tribal communities as certain tribes engage in harvesting and gathering insects, which they then sell in local markets (Chakravorty et al., 2013, 2019). Some other tribes supplement their income by collecting insects from their agricultural lands as part time occupation, and still, others opt to buy insects from local markets rather than collecting them themselves (Shantibala et al., 2012; Chakravorty et al., 2013; Mozhui et al., 2017). The present study found that most edible insect vendors in urban areas do not collect the insects by themselves but rather buy from the insect collectors or from the rural market. As the insects passed on from rural areas to urban markets, the price of the insets also increased substantially (Table 2.11).

For tribal communities across Indian states, insect consumption depends on their culture and socio-economic practices (Meyer-Rochow and Chakravorty, 2013; Chakravorty, 2014; Kom and Arunkumar, 2020) and in Nagaland too, insects are associated with festivals as they are cooked as special delicacies during weddings or offered as priced gifts to well-wishers during festivals. Indigenous people Assam prefer taking red ants during Bohag-Bihu festival (Loganathan and Haldhar, 2020) and in Arunachal Pradesh *A. nepalensis* is associated with the Unying-Aran festival of the Adi

tribes (Megu et al., 2018). For some people, insects serve as the major nutrient source in their daily diets (Mozhui et al., 2017; Megu et al., 2018), some consume insects as an optional food (Mitra et al., 2020), while other people eat insects as a delicacy (Paul and Dey, 2011; Mitra et al., 2020; Mozhui et al., 2017, 2020). Further, preferential eating of insects by different groups of people also depends on availability and familiarity with the insects. For instance, the Meitei people of Manipur living in the valley prefer aquatic insects, while the Kom tribe inhabiting in the hills does not prefer aquatic insects. However, the Thangkhul tribe living on the banks of hilly rivers prefers both aquatic and terrestrial insects (Kom and Arunkumar, 2020). The districts of Nagaland like Kohima and Dimapur which have a diverse population of tribes show maximum diversity of insect consumption compared to other districts inhabited by a single or fewer tribes. In various Indian states, the consumption of insects by tribal communities is influenced by their cultural and socio-economic practices (Meyer-Rochow and Chakravorty, 2013; Chakravorty, 2014; Kom and Arunkumar, 2020).



Supplementary Figure 2.1. A-B- *A. cerana indica* honey collection; C- Handpicking of *O. fuscidentalis* larva; D- Smoking of *V. mandarinian* nests for brood collection; E- Collection of aquatic insects (*L. ruber*, *C. limbatus*, *H. caschmerensis*) using bamboo weaved baskets; F- Collection of *Macrotermes* sp. using nets; G-H- Rearing of *A. cerana indica* within bamboo weaved rooms with an opening for exit and entry small enough only for bees to pass and not hornets; I-J- Traditional methods of rearing *L. camifrons* in wooden and bamboo boxes; K-L- Rearing of *S. cynthia ricini* at home.

Chapter 3

Traditional rearing techniques of (a) *Vespa mandarinia* Smith (1852) and (b) *Thysia wallichii* Hope (1831)

Contents		3.1(a)	<i>Introduction</i>
	(a) <i>Vespa</i>	3.2(a)	<i>Materials and Methods</i>
	<i>mandarinia</i>	3.3(a)	<i>Results</i>
	Smith (1852)	3.4 (a)	<i>Discussion</i>
	<hr/>		
	(b) <i>Thysia</i>	3.1(b)	<i>Introduction</i>
	<i>wallichii</i>	3.2(b)	<i>Materials and Methods</i>
	Hope (1831)	3.3(b)	<i>Results</i>
	3.4 (b)	<i>Discussion</i>	

***Vespa mandarinia* Smith (1852)**

3.1 (a) Introduction

Hornets of the genus *Vespa* (Vespidae: Vespinae) are large predatory, eusocial wasps native to Asia and Europe. They are known to prey on other insects especially honey bees, e.g. *Apis mellifera* L. (Smith-Pardo et al., 2020) and pose a danger to humans on account of their painful sting and extreme reactions (like anaphylactic shock) to it (George et al., 2008). Among the hornets, the Asian giant hornet *Vespa mandarinia* Smith, is the world's largest species measuring up to 5 cm in length (Ono et al., 2003). Originally described from eastern China (Zhejiang) in terms of its colour but without any detailed reference to its morphological characteristics (Archer, 1995), *V. mandarinia* is now known to be widespread in China and to occur in India, Nepal, Bhutan, Japan, Korea, Taiwan, Thailand, Myanmar, Malaysia, Laos, Indonesia, Philippines and parts of Russia (Srinivasan and Kumar, 2010). Depending on the species, wasp nests can be attached to tree branches and shrubs, found in crevices, under eaves or underground (Smith- Pardo et al., 2020). While *Vespa fervida* (another hornet species) inhabit forests as well as urban areas, *V. mandarina* is found mainly in hilly environments of an altitude of 850-1,900 m with nests frequently located below ground in fields and uncultivated areas making it rather difficult to locate them. *V. mandarinia* nests are large, sometimes containing over 1000 workers and being constructed 6-60 cm below the ground in spaces between decaying roots and in abandoned small mammal burrows (Archer, 2008).

Despite their formidable defensive behaviour, the larval, pupal and even adult *V. mandarinia* are considered by members of all ethnic groups in Nagaland as a highly tasty and nutritious seasonal food (Mozhui et al., 2017, 2020). Documenting the local knowledge on trapping and rearing methods, market values of this hornet species can

serve as the baseline or way forward for the creation of innovative new and superior collection methods, tools and improved rearing techniques, which could help uplift the livelihood of the rural poor and, in general, boost the economy of the state. The tribal people of Nagaland possess unique traditional knowledge on rearing practices of various hornet species to sustainably use them as food and part of traditional remedies in treating diseases. Semi-domestication of *V. mandarinia* in their natural habitat has also been traditionally practiced by various ethnic groups of Nagaland and therefore, documenting this knowledge and expertise is important before such valuable information gets lost or is forgotten.

3.2(a) Materials and methods

3.2.1(a) Study area

Nagaland is a state in the North-East region of India, situated between longitudes 93°20' and 95°15' E and latitudes 25°6' and 27°4' N. It covers an area of 16,579 sq. km with valleys, meandering streams, high mountains, and deep gorges and has a population of approximately 2.3 million. The characteristically hilly topography of the state exhibits rugged terrain, broken by deep gorges and steep slopes with varied altitude from 199 to 3,841 m.a.s.l. Due to the humid sub-tropical and warm-temperate climatic condition, the state can be considered to be part of the North-East India biodiversity hotspot (Myers et al., 2000).

3.2.2(a) Methodology

To document the traditional rearing and harvesting of *V. mandarinia*, extensive field surveys were conducted between April 2018 and August 2019 in fourteen villages viz., Chiephobozou, Chiechama, Jakhama, Jotsoma, Khonoma, Kigwema, Mima, Nerhema, Phesama, Seiyhama, Sirhima, Socūnoma, ToupHEMA and Viswema of Kohima and Dimapur districts (Figure 3.1(a)).

The data presented in this study are based on onsite field visits, and personal interviews with sixty informants (45 male and 15 female) belonging to the age group of the 20-80-year-olds. Of the informants, 17% were illiterate, 60% had below high school education and 23% had higher education. All the informants belong to the ‘Angami’ community, a major tribe in Nagaland mostly inhabiting in Kohima district and parts of Dimapur district known the *Chakroma Angamis* (Figure 3.1(a)). The informants included hornet farmers/collectors, entrepreneurs and Naga traditional cuisine experts. Prior to field visitations, the informants were informed through telephonic communications and through the village head man for interviews. Questions were asked on how hornets could be located, captured, semi-domesticated and harvested. The informants were also questioned on their opinion of hornets as a source of food as well as their role in the socio-economic realm. Further, the market value of hornets was also assessed along with other conventional meat items available in different places in Nagaland. In order to establish hornets as an important nutritional food (in addition to more conventional items), hornet brood was collected from a Super market in Kohima during the month of November. Thereafter the hornet samples were kept in a freezing box, brought to the laboratory and stored at -80 °C until further analysis. The crude protein content of *V. mandarinia* (larvae and pupae) was determined (in triplicate) based on dry matter (1 g) and assessed on the total nitrogen percentage multiplied by the specific nitrogen to a protein conversion factor of 6.25 (AOAC, 1990) using Kel Plus automatic nitrogen/protein estimation system (ISO 9001:2015 certified & CE certified; Pelican Equipments, Chennai, India) and calculated as mean \pm SD.

3.3(a) Results and discussion

In Nagaland, preferences in edible insect consumption are mainly due to six reasons: (1) availability of the insect species; (2) size of the insects, as generally larger

insects are preferred for consumption; (3) taboos associated with the insect species; (4) one's own palatability/taste preference; (5) market value of the insect species; and (6) traditional ethno-medicinal knowledge associated with the insect species (Mozhui et al., 2020). While every Naga tribe has a preference of its own with regard to insect consumption (Mozhui et al., 2017), the giant hornet (*V. mandarinia*) is a delicacy for all ethnicities and their larvae, pupae, and the adult are prepared by frying or cooking with local spices thereby replacing conventional meat sources such as beef, chicken, pork, etc. (Mozhui et al., 2020). In the present study, differences based on gender and age group among the informants with respect to hornet harvesting and consumption were observed (Table 3.1(a)). From the informant analysis, it is obvious that hornet harvesting is only done by male of all age groups. Regarding consumption of hornet grubs as food, both male and female informants under the age group 20-30 years showed maximum interest with 50 and 42%, respectively, while informants (male and female) under the age group 61-70 years and 71-80 year-olds showed lesser interest.

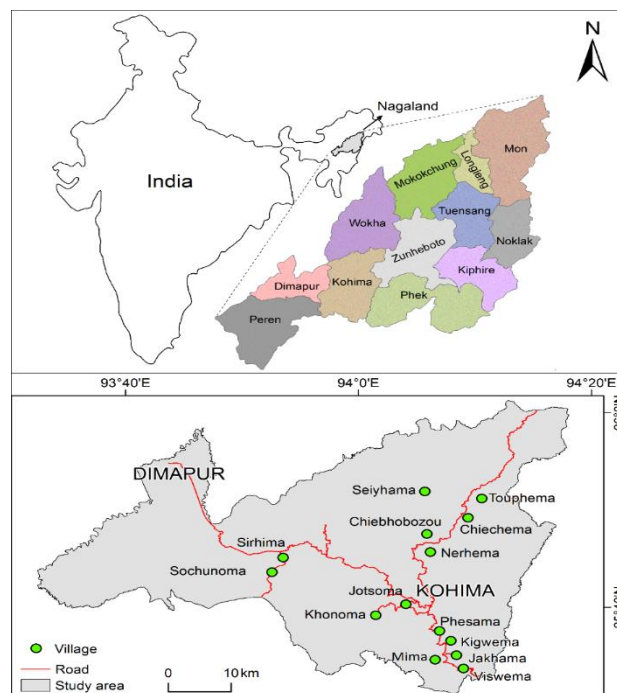


Figure 3.1(a). Map of the study area showing the location of surveyed villages under Kohima and Dimapur, Nagaland.

As the gathering of hornet colony is laborious and involves risks of being stung, only men are involved in the process while women are usually involved in marketing/selling the harvested hornet combs/nests. Although *V. mandarinia* is available throughout the year, sequential growth events of colony or comb cannot be observed except during the introduction of new colony and at the time of harvesting, because most of the artificially constructed rearing sites are underground. Also, frequent disturbance to the colony will disturb the normal environment needed for the colony to grow well, at the same time making the adult more aggressive towards the rearers. *V. mandarinia* is most abundant from September to February (time when harvested hornets are made available at local markets) and consequently sought after predominantly during that time of the year (Figure 3.2(a)A-D). Collection sites are usually easily accessible to indigenous people who know the habits of this species well and are aware of their hiding places and when, where and how to best collect them.

Table 3.1(a) Demographic patterns and status of the informants.

Age group (years)	20-30		31-40		41-50		51-60		61-70		71-80	
Gender	M	F	M	F	M	F	M	F	M	F	M	F
Harvesting (%)	27	0	6	0	33	0	20	0	7	0	7	0
Consumption (%)	50	42	14	22	11	17	11	13	7	3	7	3

M=Male, F=Female



Figure 3.2(a) (A-D). *Vespa mandarinia* larvae, pupae and adult sold at local market, Kohima, Nagaland.

For the Nagas, practices of rearing *V. mandarinia* in manmade structures begins during the end of April to June. The rearing site is excavated down to 60-75 cm (Figure 3.3(a)-A), sometimes a round cylindrical sheet of tin open from top and bottom is inserted into the underground cavity. The tin sheet is introduced in order to enhance/improve the shape of the comb. Some loose soil is then filled into the structure after which a single comb (collected from the wild along with workers) is tied to a strong wood, placed horizontally on top of the underground structure and allowed to hang downward (Figure 3.3(a)-B and 3.3(a)-C). After all of these arrangements are done, slices of bamboos are placed horizontally next to each other covering the whole of the opening from the top with just a little opening on one side as entrance for the hornet colony (Figure 3.3(a)-D). On top of this a polythene/plastic sheet is spread and loose soil is used for leveling the toppings.

After the structure is ready, the queen and workers are introduced into their new site from the entrance. Once the hornets are introduced and rearing begins, a frequent check of the hive is essential and an increase of the hornet population is indicated by an increasing amount of soil pellets dumped outside the nest by workers. More details on the traditional rearing techniques practiced in Nagaland will be given in the following sections.

3.3.1(a) Locating the hornet nest

The age-old tactics and ways of locating and capturing hornets are passed on from generation to generations. Adult workers of *V. mandarinia* are usually found feeding on the sap of Oak trees (*Quercus* sp.). Crushed crickets, usually *Tarbiskiellus portentosus* (Lichtenstein), and grasshoppers such as *Oxya hyla* (Serville) or cotton balls soaked in sugar syrup are used as baits to attract the hornets. In the initial stage, the hornet queens appear in the month of April and are seen collecting sap for herself on

Oak trees. On sighting the queen, the hornet hunters wait nearby to observe its movements. The queen returns only after an interval of approximately 1 hour, as it is engaged in making a new colony and visits the Oak tree for about 5-6 times in a day. Once the first batch of eggs hatches and gradually develops into adult, the queen rests in the nest and the adult workers begins to emerge out of the nest to collect feed for the new colony. The hunters reported that since hornet colonies are small during the time of collection (for semi-domestication) i.e. 1st and 2nd week of May, hornet workers do not travel far away from their nests to collect sap. During this period, the workers are baited in order to locate the nests in the wild. When the number of workers is still small, the hornets travel only within a range of 500 m away from its nest to collect feeds and usually visits the Oak trees for about 15-20 times in a day. When the hornet colony increases, the workers can travel up to 8 km to 10 km in search of feed (for a colony with 300 to 400 adult workers, distance travelled is approximately 2 km, for a colony with 1000 workers, they travel up to 8 to 10 km).

Before the bait is set for nest location, the nearby forest area is cleared to make it easier for the hunters to chase the hornet. The hunters wait for the workers to come down at a reachable distance where they can introduce the baits attached to a long bamboo stick (3-4.5 m in length) while it feeds on the sap of an Oak tree. The hunters reported that with experience, they know exactly when to bait the workers i.e. when the hornet has collected the sap, the tip of the abdomen becomes pointed and shiny; the hornet then uses its leg to clean itself and even loses balance as it tries to fly away. Immediately, on perceiving the baits, the hornet diverts its attention, climbs onto the baits for a closer investigation and grips the bait to taste it (Figure 3.4(a)-A). While the hornet is fully engrossed in assessing the bait, a fine loop of human hair together with a feather or light polythene strip is gently pushed over the gaster (i.e. posterior end of the

hornet) without touching it until it reaches the constricted area of the petiole (Figure 3.4(a)-B). The hair strand loop is then tightened and acts as a visual marker for the nest hunters. After visual markers are laid on the workers, the hunters follow the hornet to the nests which is usually less than 500 m.

A different way of locating the nest does not involve marking the insect, but rendering its provision more visible, e.g. a small piece of meat to which a tiny ribbon or a light feather are attached will be grabbed by a hornet and when it flies away with the morsel, the wasp hunter can easily follow the feather or the ribbon attached to the bait and in this way locate the hornet's nest (Mozhui et al., 2017). In Japan, a similar capture method of attaching a tiny white ribbon to the bait and following the wasp where it takes the bait has been reported by Nonaka (2010). In Japan as well as Nagaland locating the hornet nest requires teamwork involving 3-4 persons; someone is needed to set the bait, someone to trail the adult hornet as it flies to its nests after foraging and someone to give signals to others regarding the whereabouts of the hornets till the nests is located. The coordination among the team is very important especially in the steep forested slopes, firstly the most experienced person sets the bait, once the baited hornet takes off, the baiter would shout giving direction of its flight to the next person stationed at an assumed flight path at the same time trying to follow the hornet till the second person has spotted it. At further location the third person would do the same thing till the next person gets a visual of the hornet and continues till the nests is located. In most of the cases the hunters do not see each other due to thick forest but they communicate by calling out each other in the process. The certainty of locating the nest depends on the experience and skills of each team member. Once the nest is located, the hunters make hideouts for themselves near the nests with nets of mesh sizes small enough to protect them against the hornet attacks and wearing special protective

clothing to capture the hornets for rearing (Figure 3.4(a)-C). The time taken for setting the bait, clearing the forests till locating the nests can take up to 1 to 5 hours.

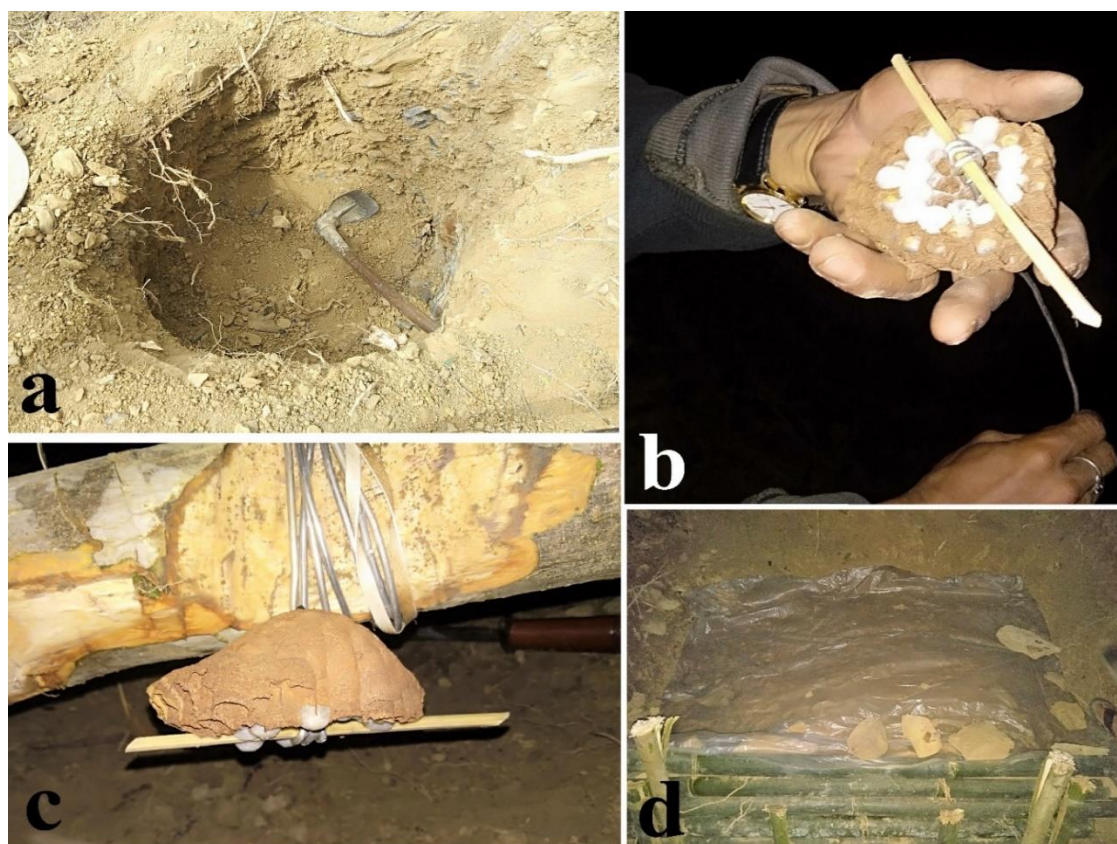


Figure 3.3(a) Preparation of new rearing site for hornets: (A) a hollow pit of 60-75 cm for new rearing site; (B), (C) comb collected from the wild is tied onto a strong wood and hanged downward; (D) bamboo slices placed horizontally to maintain a strong foundation for the hornets to move in and out of the hive.

3.3.2(a) Trapping of hornet workers and queen

Since *V. mandarina* constructs its nest underground, once the nest is located, hunters dig out small portion of the soil around its entrance. Two trapping method viz., the bottle trap and the glue trap are employed to catch the gravid queen and workers for rearing. The bottle trap method is mostly carried out at night as it involves the use of light to trap the hornets. In the bottle trap method, a transparent plastic bottle whose mouth is placed over the hornet's nest entrance is used, after which, a light is projected

through the other end of the bottle that attracts and provokes the queen and the workers to emerge from their nests, ultimately falling into the bottle. The process is repeated until the desired queen and up to nine to ten workers are caught.

In the glue trap method, glue (locally made from the sap of *Ficus* sp.) is applied to one end of a twig/bamboo that is then placed at the nest entrance (Figure 3.4(a)-D and 3.4(a)-E). Once a hornet gets stuck, others become attracted presumably by pheromones emitted by the trapped individual. The captured hornets are gently placed in between folded bamboo sticks (that have been inserted into a banana stem) and tied down loosely to keep them intact and alive (Figure 3.4(a)-F). After successful trapping, the hornets are fed with sugar syrup to keep them alive (Figure 3.4(a)-G) until the adults are introduced to their new nesting site by slowly untying the folded bamboo sticks and placing it at the entrance one at a time (Figure 3.4(a)-H).

3.3.3(a) Construction of nesting site and rearing details

Getting large number of larvae and pupae of hornets in the wild is often uncertain due to their unpredictable locations and population density. Semi-domestication of hornets using wooden boxes, would allow a continuous supply rather than relying on natural harvesting. Hence many farmers in Nagaland prefer rearing of *V. mandarinia*, which, however, requires a certain combination of tender loving care, originality and ingenuity. In Japan too, semi domestication of hornets (*Vespula* spp.) in wooden hive boxes has been reported (Nonaka, 2010; Van Itterbeeck et al., 2021). Certain locations are best sited for hornet hives to thrive well. Therefore, selection of a site is very important for successful hornet rearing and a site is selected based on three important points. Firstly, hornet colonies should be separated by 2-3 km from each other to avoid conflicts between nests and allow each colony an adequate area to forage in. Otherwise, there is the possibility that a dominant colony destroys the weaker one

(Nonaka, 2009). Secondly, the rearing site should be far from human habitation because of the hornets' aggressive temperament and potent sting. Tribal people in Nagaland construct artificial structures in areas with little intensity of air, as harsh winds slow down the hornets' daily activities such as bringing in food, removing soil, etc. Also, to prevent the inflow of water into the hives during the rainy season, rearing structures need to be well covered because combs soak up the water easily and then breaks off, compelling the hornets to flee in search of a new nesting site. Looking into its potential economic value, some farmers resort to many practices of wild harvesting of the hornets which can lead to ultimate habitat destruction.



Figure 3.4(a) Traditional trapping method of *Vespa mandarinia*; (A) hornet biting a bait made of cotton balls soaked in sugar syrup; (B) white ductile material tied on the hornet which acts as visual markers until nests is located; (C) hideouts near the nesting site of hornets; (D) collection of milky sap from *Ficus* sp.; (E) hornets captured by glue trap

method; (F) folded bamboo sticks with trapped hornets; (G) sugar syrup is fed to the hornets to keep them alive; (H) newly constructed structure to rear the hornet; (I) large hornet comb harvested after successful rearing.

3.3.4(a) Harvesting

In about four to six months, the fully developed nests are harvested for personal consumption as well as for commercial purpose (Figure 3.4(a)-I). Harvesting of the hornet brood combs involves several techniques with planning and coordination among the harvesters. A different method of smoking out is applied to drive away/kill the workers which is a method resembling that of the Japanese practice where in the entrance of the nest, smoke is generated to sedate the wasps inside the nest. Collection is preferred at night when the workers are resting (Nonaka, 2010) which make it safer for the harvester to collect the brood without being stung. In Nagaland, no special protective suits are worn while harvesting hornets. The hunters simply wear thick clothing and doesn't leave any skin exposed in order to avoid being stung. Sometimes, they wear woollen gloves to be more cautious, however the hunters prefer not to wear gloves because it may damage the hornet's body while harvesting/collection. However, in Japan, as *V. mandarinia* are dangerous, hunters wear specially ordered protective suits when collecting hornet nests during the day time (Nonaka, 2010), even when collecting wasps (*Vespula* sp.) especially during day time, the collector wears purpose-made protective clothing (Payne and Evans, 2017). Further, as there are reports where sight-threatening injury is caused due to venom of *V. mandarinia* (Hirano and Tanikawa, 2020), it is advised that special protective eye gear as well as suits are to be worn while collecting hornets. We also found through the survey that if any hunters are being stung while collecting hornets, they are being compensated with an amount of Rs. 5,000 by the rearer/owner. Presently, there are no reports of hornet stings causing

serious health issues among the informants. However, at any instance when being stung by the hornets, firstly they just press the stung body part and oozes out the venom quickly and then apply ointment or take painkillers and take complete rest for 5 days. As hornet stings are painful, the hunters are very careful not to get stung when collecting.

It is important to note that while harvesting, the queen along with some workers is left behind which may construct a new nest if the harvest took place prior to the end of the hornet season, because hornet nest are annual constructions. Only mated queens survive the winter and form a new colony in the spring. The traditional practices of rearing or semi domesticating *V. mandarinia* helps conserving the species and at the same time keeping the population of *V. mandarinia* hornets under control.

3.3.5(a) Socio-economy

V. mandarinia is a promising alternative to conventional food items in relation to protein supplementation and to improve the socio-economic condition of the rural poor in Nagaland. In fact, farming communities are willing to rear hornets for commercial purpose with a hope of removing the negative stigma associated with eating insects and show urban consumers how healthy the habit of eating insects is. Many people are directly collecting and selling larvae, pupae and adult of hornets as a source of income for their livelihood and thus the marketing of hornets form an integral part in the socio-economic lives of the Naga people. Comb size ranges from 30-90 cm in diameter. Annual comb harvesting depends on size i.e. the bigger the comb size, the fewer the total number of combs. Generally, eight to nine combs measuring 30-45 cm in diameter are harvested, each of which fetching an amount of Rs. 10,000-50,000. However, when comb diameters of 90 cm are encountered, only three to four combs will be present. Hornet combs are rated by counting the number of larvae and pupae

present in them (a single larva/pupa cost Rs. 15-20). Therefore, it is estimated that 1 kg of hornet (one single hornet larva weighs average 2.42 ± 0.39 g and pupa weighs 1.72 ± 0.36 g), fetches an average amount of Rs. 7,290 which in comparison to other conventional meat sources has high market value (Figure 3.5(a)). Due to its high market value, these insects are often used as valuable gifts to well-wishers. Hornet larvae, pupae and adults are taken as fried, roasted or raw food items. Some are cooked directly at the market stalls and it is therefore possible to taste them fresh.

The demand for these insects at the local markets is increasing due to their high nutritive value and their excellent taste, which consumers relish. In the present study, we observed that there are differences in the preference for consumption. Hornet grubs are cooked with salt, chili, ginger, garlic and bamboo shoots; they may be also consumed as fried (fried in a little amount of oil with added local spices like ginger and garlic). In some cases, hornets are simply taken raw or roasted over fire and consumed. Most of the informants responded that they prefer cooked hornets (32%), followed by fried (29%), roasted (27%) and raw (12%) (Figure 3.6(a)). Our observations describe the special characteristics of the Naga way of capturing and rearing *V. mandarinia*, but Naga wasp and hornet dishes are also quite unique. Recipes for hornet dishes vary greatly from tribe to tribe and household to household, bringing a rich and significant existence of culinary culture into the social sphere.

Giant hornet larva becomes a prized Naga delicacy that catches a great deal of attention when the larvae are cooked with pork and traditional flavourings such as bamboo shoots or when they are steamed by wrapping them in banana leaves over hot ashes for 15-20 minutes. While fresh hornet larvae, harvested between October and November are considered to be the tastiest, the demand for their dried version has become increasingly popular as a food item and dried larvae are often sold out in a

matter of days. A Bengaluru-based entrepreneur from Nagaland sells packages of 85 grams of dried giant hornet in Karnataka (South India) for Rs. 500 (Kagti, 2016). Another popular dish is the exotic ‘*khwidi*’ (giant hornet larvae served among the Angami community), i.e. a boiled and cooked preparation of giant hornet larvae with tomato slices and bamboo shoots, served only to very distinguished guest (Figure 3.7(a)-A and 3.7(a)-B). Although there is no particular festival especially involving insect dishes and products, the tribal people do get an opportunity to show case and sell their different kinds of edible insects and insect products of which giant hornet dishes are some of them.

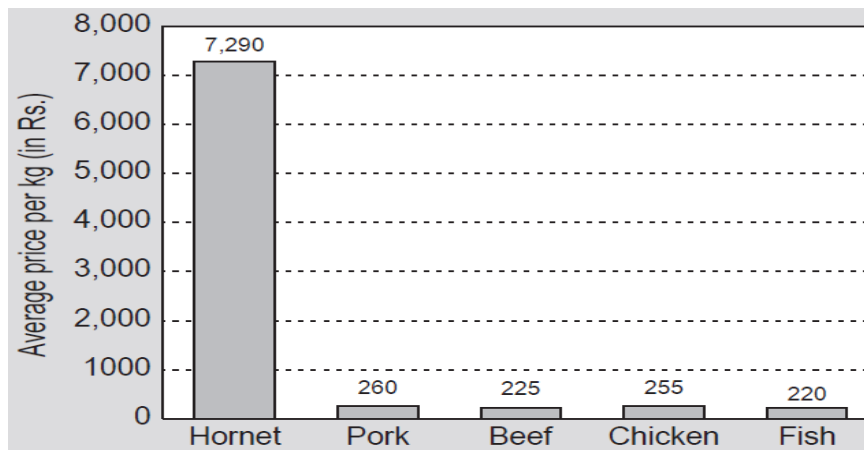


Figure 3.5(a) Comparative analysis of the market value of hornet grubs with other conventional meat sources.

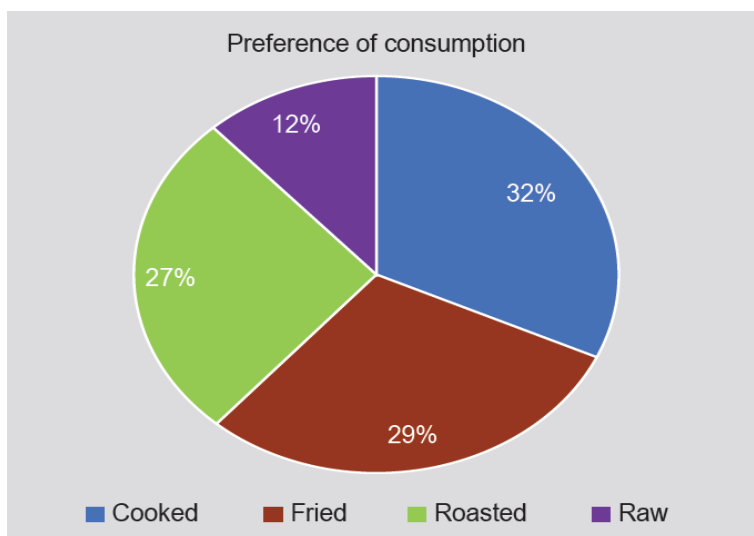


Figure 3.6(a) Informant’s preference of consumption

In mountainous areas of Japan, pupae of the common wasp *Vespula flavipes* as well as other *Vespula* species (Van Itterbeeck et al., 2021) and *V. mandarinia* larvae are usually boiled with soy sauce, fried with salt or are even boiled to a hard consistency with soybean sauce, sugar and sake (Nonaka, 2010). Other wasp species like *Provespa anomala*, *Vespa* sp. and *Ropalida* spp. (Vespidae) broods are often eaten raw or boiled with rice in Borneo (Chung, 2010). While *V. mandarinia* and other edible insects are either consumed alive right after being caught (raw) or consumed after further processing such as cooking, baking, frying, pickling, fermenting or drying in different regions of the world (Kourimska and Adamkova, 2016), the acceptability depends not just on the taste and price (Ghosh et al., 2018), but also largely on the insects' market availability. The categorisation as an acceptable food product, moreover, is shaped by marketing strategies, mode of preparation and the educational level of the consumer clientele (Sogari et al., 2017).



Figure 3.7(a) **a** and **b**-Exotic ‘*khwidi*’ giant hornet (cooked with bamboo shoot and local spices) dish, a delicacy among the Naga communities.

Hornets as edible forest insects play an important role in economically poor rural communities where household-level foods and nutritional insecurity are associated not just with an energy and nutrient intake gap, but also orthodox traditional food

consumption patterns and socio-economic status (Parappurathu et al., 2019). In many parts of the world, wasps are also a part of the traditional armamentarium to fight various diseases and a wide range of disorders (Meyer-Rochow, 2017) and in Japan some people at home prepare fermented giant hornet *shouchuu* brew (Japanese liquor similar to vodka) for health-related benefits as the venom-infused liquor is said to make the skin more beautiful, boost recovery from fatigue and prevent ‘lifestyle disease’ (Spooky, 2013). Different wasp products also have a history of use in traditional medicine e.g. hornet juice which contain amino acids found in the trophallaxis fluid produced by *V. mandarinia* is marketed for boosting endurance (Brock et al., 2021). The empty combs are also used as home decorations due to the intricate structured cells of the comb giving the latter a beautiful and attractive appearance.

V. mandarinia contains good amount of protein both in larvae (52.82%) and pupae (60.99%) and can be regarded as a valuable protein source. Comparison of the protein content of hornet species of Nagaland with those from other geographical regions highlights the variation which may be due to places, habitat and species. For instance, the protein content of *V. mandarinia* larva (52.82%) in the present study is less than of those that from Korea (59.7%) whereas it was found to be higher than that of larval *Vespa velutina nigrithorax* (48.64%) (Jeong et al., 2020). Edible *Tenebrio molitor* (52.23%) and *A. mellifera ligustica* (35.2%) from Korea also contains less protein (Ghosh et al., 2016; Wu et al., 2020) than Nagaland *V. mandarinia* larvae, highlighting the nutritional value of the latter (Table 3.2(a)).

Further socio-economic development especially in the tribal communities away from urban centres is possible through village level activities such as cooperative societies or small-scale enterprises to control the sustainability of collecting wild insects, the marketing and export of such insects and insect-derived foods and to

support outdoor rearing and processing of certain insects. Activities as described can offer employment opportunities to men and women alike, but initiatives are still largely lacking in developing countries where government support and encouragement through financial subsidies would be needed. Obviously, in order to bolster progress in family livelihood (Gahukar, 2020), economic gains alone are not enough and need to be integrated with a sustainable ecosystem and a clean and unpolluted environment.

3.3.6(a) Future prospects

Even though, the scope and nature of the ecosystem services provided by different kinds of wasp species are not well understood and overlooked, they are known to contribute in regulating, provisioning, supporting and cultural services of the ecosystem (Brock *et al.*, 2021). Mass rearing of different insect species have many advantages over livestock production and therefore needs to be addressed for future food security. Commercialisation of the hornet culture may be possible by shifting the controlled rearing from small units to an industrial phase with adoption of economical mass production on a large scale (Hardouin, 1995) for which a detailed study of the ecology and biology of an insect is required (Yen, 2010). Recently, low-cost techniques for mass rearing of edible insects, including crickets, grasshoppers, ants and the giant water bug, have successfully been developed in China, Korea and Thailand (Boulidam, 2010; Feng *et al.*, 2009; Kim *et al.*, 2008) and are in the process of being developed in particular for *V. velutina* but other species as well (Van Itterbeeck *et al.*, 2021). Along with traditional harvesting and management practices, promotional and educational extension services should include assurance of quality and safety of insect products. It is time we recognise the benefits provided by hornets and link the potential of these bio-resources in a sustainable manner with economic prosperity rather than pursuing them as pests to be eradicated.

Table 3.2(a) Comparative presentation of protein content (mean \pm standard deviation) of *Vespa mandarinia* (larva and pupa) with those of *V. mandarinia*, *Vespa velutina nigrithorax*, *Apis mellifera ligustica* and *Tenebrio molitor*.

Insect name	Nagaland	Korea			
<i>V. mandarinia</i>	<i>V. mandarinia</i>	<i>V. velutina nigrithorax</i>	<i>A. mellifera ligustica</i>	<i>T. molitor</i>	
Jeong <i>et al.</i> , 2020	Jeong <i>et al.</i> , 2020		Ghosh <i>et al.</i> , 2016	Wu <i>et al.</i> , 2020	
Larva	52.82 \pm 2.20	59.7	48.64	35.3	52.23
Pupa	60.99 \pm 1.01	–	–	45.9	–

Thysia wallichii Hope (1831)

3.1(b) Introduction

The collecting techniques of the past, when most of what had been assembled was for home consumption and the human population density of the region was low, did not take sustainability of the insect resource into consideration. The availability of an edible insect was rarely in doubt. However, the growing commercialization of edible insects and the increasing demand for preferred species in recent years have led to more aggressive harvesting practices in some areas. This has caused some researchers to raise questions in relation to ecological integrity and sustainability (Shrestha and Bawa, 2013; Yen and Ro, 2013). There is particular concern in areas where modern harvesting techniques (e.g., large-scale light traps, odoriferous attractants, etc.) are used to collect substantially larger numbers of insects than in the past.

Environmental pollution, population increase, water availability, and misuse of land are inexorably driving humans to take on important challenges related to sustainability. In the near future, one can expect to see a significant increase in food and feed demands and, in this situation, the efficient and sustainable use of insects as a source of protein and other nutrients has been invoked as a possible strategic solution as early as 1975 (Meyer-Rochow, 1975). As a candidate for noteworthy growth, insect farming promises significant benefits to the agro-food industry, offering interesting opportunities for implementing a circular economy (Cadinu et al., 2020). Unfortunately, since little is known of the population dynamics of many insect species, including those in connection with *Thysia wallichii*, it is difficult to know with certainty whether human collecting beetles and their larvae will have serious impacts on long-term sustainability.

Many species of long-horned beetles are invasive pests causing significant economic damage in agro-forestry systems. As they spend the majority of their life-

cycle concealed inside natural wood or wooden packaging materials, they are largely protected from adverse environmental conditions and pesticide sprays (Jucker et al., 2020). However, they are also the most commonly eaten insect group in Nagaland besides caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leaf and plant hoppers, scale insects and true bugs, termites, dragonflies and flies (Mozhui et al., 2020).

The use of the larvae of cerambycid species such as *Anoplophora* sp., *Batocera rubus*, *Batocera parryi*, *Batocera rufomaculata*, *Cerosterna* sp., *Xylorhiza* sp., and *Orthosoma brunneum* as food is reported from different parts of India particularly Arunachal Pradesh, Assam, Bihar, Meghalaya, Nagaland, Sikkim, Uttar Pradesh, West Bengal, and Mizoram (Annandale, 1912; Bhavan, 2016; Chakravorty et al., 2011; Meyer-Rochow and Changkija, 1997; Mitra et al., 2016, 2017; Mozhui et al., 2020). However, globally, all studies related to *T. wallichii* is specifically directed towards faunistic surveys, taxonomic approach, morphological characterization and geographical distribution. The present study will be the first report to document its utilization as a nutritious food. Apart from generally documenting the use of edible insects (Mozhui et al., 2020) and describing traditional rearing practices of *Vespa mandarinia*, (Kiewhuo et al., 2022), studies on rearing techniques for other edible insects is rarely available in Nagaland, let alone elsewhere. The large forest cover with abundant host plants of *Acacia pennata* in Nagaland makes it a suitable hub for the egg-laying activity of *T. wallichii* and to increase the population of the species, but their host plants also need to be managed to enhance and improve their attractiveness for cerambycids like *T. wallichii*. Here, in the present study, the traditional semidomestication methods of *T. wallichii* is documented and an appropriate technology for mass production is described along with assessing the market value and nutrient content of *T. wallichii*.

3.2(b) Materials and Methods

3.2.1 (b) Study area

Lying between a longitude of 93°20'N and a latitude of 95°15'E the state of Nagaland has an area of 16, 579 sq. km and is gifted with mountains and valleys having humid subtropical and warm-temperate climatic conditions. The study was undertaken within the geographical location of the districts of Kohima and Dimapur. Kohima is situated the north of Japfu Barail intersection and has an average elevation of 1261 m (4137 feet) and lies between latitudes 25°6' and 27°4'N, and longitudes 93°20'E and 95°15'E. It features a very moderate version of a humid subtropical climate with hot rainy summers and very cool winters. December to February is considered the coldest period while June to August are the hottest months when the temperature rises to as high as 34 degrees Celsius. The Dimapur district is located in the southwest of Nagaland between 25°48' and 26°00'N and 93°30' and 93°54'E. The vast majority of this area is flat with the Dhansiri River, a tributary of the Brahmaputra River flowing east of the city. Dimapur is hot and humid in summer and moderately cold in winter.

3.2.2(b) Methodology

A pre-designed questionnaire incorporating the questions related to the host plant, methods of collection of *T. Wallichii* larvae, semi domestication and rearing techniques, seasonal availability, and the larvae as nutritional source as well as their role in the socio-economic realm was prepared for data collection. With prior permission through telephonic communications and also through the village headmen, field surveys were conducted from March 2019 to October 2021 in fourteen villages viz., Chiephobozou, Chiechama, Jakhama, Jotsoma, Khonoma, Kigwema, Mima, Nerhema, Phesama, Seiyhama, Sirhima, Socūnoma, ToupHEMA and Viswema of Kohima and Dimapur districts (Figure 3.1(b)) to document the process of semi-domestication and

harvesting of *T. Wallichii*. The initial survey among the ‘Angami’ community, (a major tribe inhabiting the Kohima district and parts of Dimapur district in Nagaland) who reside mainly in these 14 villages had revealed that, semi-domestication of *T. wallichii* was practiced only in 5 villages namely Chiephobozou, Chiechama, Nerhema, Seiyhama and Touphema. It was then decided to restrict the detailed survey to these

villages with 10 persons representing each village. Other villages were not included in the survey. Thus, we had responses from ten persons per village.

In total, fifty villagers were interviewed, comprising 15 rearers/collectors, 5 sellers and 30 general informants. The 15 rearers and collectors were from the two villages Chiephobozou and Chiechama and considered key informants on the basis of their profound knowledge and experience

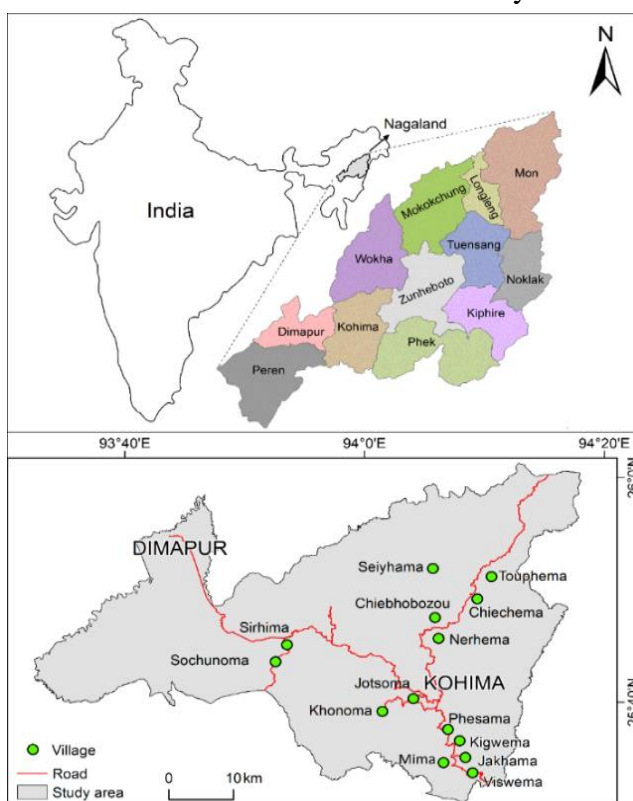


Figure 3.1(b) Map of the study area showing the location of surveyed villages under Kohima and Dimapur, Nagaland

dealing with the semi-domestication of *T. wallichii*. The information from the 30 general informants was gathered to evaluate the “Use Value” of *T. wallichii* and the responses from the 5 sellers were used to obtain some information on aspects of marketing (Table 3.1(b)). The demographic pattern of the informants in the present study is presented in Table 3.2(b).

The data were collected by following the purposive sampling method given by Tuckett (2004). The fifty informants (28 male and 22 female) ranged in age from 15 to 100 years. Of the informants, 10 were illiterate and 17 below high school education, 18 were graduates and 5 post graduates (Table 3.2(b)). Market surveys were carried out to assess the economic value of wood borer larvae along with other conventional meat items available at the supermarket and Mao market of Kohima, the capital city of Nagaland. Fresh and larvae of *T. wallichii* were collected from a semi-domestication site (Chiephobozou, Kohima) to determine nutritional parameters.

3.2.3(b) Use value: The use value of *T. wallichii* larva was calculated using the formula given by Phillips et al. (1994) [$Use\ value = \Sigma U/n$, where ‘*U*’ is the number of informants per citation and ‘*n*’ is the total number of informants].

3.2.4(b) Proximate analysis: *T. wallichii* eggs hatch in the month of February and 7 larvae for analysis were collected during the month of September, 2021, when they were approximately 7 months old. The seven larvae, being of the same age and from the same environment, were collectively analysed. Moisture and ash content of the larva was determined using the AOAC (2000) method. The gut of *T. wallichii* larva was removed and cleaned properly for further analysis. The crude protein content of the larvae was determined based on dry matter (1g) and assessed on the total nitrogen percentage multiplied by the specific nitrogen to a protein conversion factor of 6.25 (AOAC, 1990) using Kel Plus automatic nitrogen/protein estimation system (ISO9001:2015 certified & CE certified; Pelican Equipments, Chennai, India) and calculated as mean \pm S.D. Total lipid was estimated by the Sulpho-Phospho-Vanillin (SPV) method given by Bligh and Dyer (1959). All nutritional analysis was done in triplicate.

Table 3.1(b) Details of the information's collected from the sampled villages

Type of informants	Number	Purpose	Villages
General informants	30	For estimation of use value only	Chiephobozou, Chiechama, Nerhema, Seiyhama, ToupHEMA
Vendors	5		Chiephobozou, Chiechama
Key informants	15	Documentation of semi-domestication of <i>T. wallichii</i>	Chiephobozou, Chiechama

Table 3.2(b) Demographic patterns of informants in the study

Educational status	Illiterate	10
	High school	17
	Graduate	18
	Post-graduate	5
Age	15 to 30	23
	31 to 60	16
	61 to 100	11
Gender Informants	Male	28
	Female	22
	General informants	35
	<i>T. wallichii</i> rearers	15

3.3 (b) Result and discussion

Beetles and other insects are not commonly reared in their countries of origin, but rather by private and professional insect growers and enthusiasts around the world. Japan, Taiwan, and the Republic of Korea have strong beetle-farming communities (mainly for Lucanidae, Cetoniidae and Dynastidae); numerous insect shops in larger cities and a number of magazines devoted to the topic of beetle-growing and beetle-selling testify to this. There is, however, good potential for rearing these insects in their countries of origin, for example by providing suitable sites for growing them.

Perhaps the earliest attempts to provide edible beetle species with substrates for their larvae to grow on were those reported by Reim (1962) from West Australian

Aborigines. A study by Sauvard (2007) on bark beetles categorized their life cycle into four phases: reproduction, development, maturation, and dispersal. The reproductive phase covers the period when the adults arrive on the host tree for mating and egg laying. The developmental phase is purely endophytic, hidden from view, and the larvae, and pupae are unable to survive outside the host tree. After the final larval moult, the maturation phase occurs followed by the dispersal phase. In the maturation phase sclerotization, development of functional wings, and genitals takes place. Dispersal sees the mature adults seek suitable places to breed. The traditional knowledge involved in the semi-domestication of *T. wallichii* is discussed below with the flow chart (Figure 3.2(b)).

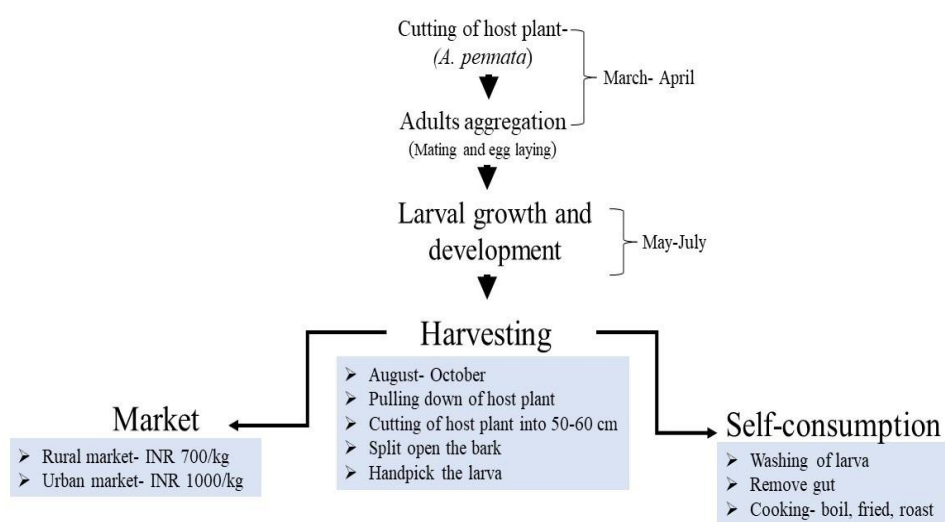


Figure 3.2(b) Flow chart showing semi-domestication steps of *T. wallichii* by indigenous people of Nagaland.

3.3.1(b) Inducing or facilitating the infestation of the host plant

The host plant on which *T. wallichii* (Figure 3.3(b)-B) adults lay their eggs is *Acacia pennata* (Linn.), locally named “Siikhru-o” by the Angami (Figure 3.3(b)-A). The plant belongs to the family Fabaceae, and is a native to south and south-east Asia. It is a shrublike, small tropical climber used as a source of food in Thailand, and Laos and as a

supplier of medicine in India (Chun-Lin, 1995; Johnson 2002; Khare 2007). The large prickly climbing shrub is found at an altitude of 800-1500 meters and is widely distributed in India, Bhutan, Myanmar *etc.* (Aye et al., 2019). The plant suffocates and disturbs other valuable plants and trees due to its climbing nature.

Semi-domestication of insects involves the manipulation of the environment to procure insects and examples include (a) palm trees being cut down to attract weevils to lay their eggs in and to collect the larvae from (Van Itterbeeck and van Huis, 2012), (b) to collect and remove *Vespa mandarinia* hornets from the wild and to rear their brood, highly appreciated as food, under the protection and control of a rearer (Kiewhuo et al., 2022) and (c) to place desired species of beetles on selected host trees that their edible larvae develop in as once practiced by West Australian Aborigines (Reim, 1962).

The flow chart for the domestication of *T. wallichii* is provided in Figure 3.2. During the months of April-May the farmers locate or select *Acacia pennata* in the forest to initiate the semi-domestication process of *T. wallichii*. The process starts by cutting the trunk at a height of 100 cm above the ground using a sharp machete, disconnecting the lower parts to avoid termite infestation. The upper end of the *A. pennata* which still remains entangled or twisted, hangs from the supporting tree and is prepared for *T. wallichii* (wood worm) infestation by inflicting vertical cuts with a machete forming V-shapes tapering towards the ground (Figure 3.3(b)-C). Faleiro et al. (2002) in coconut plantations from Goa (India) and Choo et al. (2009) in the Amazon region of Venezuela reported similar cuts on palm tree trunks to attract palm weevils. Preparation of the host plant for raising *T. wallichii* wood borers is laborious and accomplished by a group of two to three people during the dry and humid days of April and May. The sliced ends of the host plants release exudates from the plant tissues

which on fermentation emit strong odours and release pheromones (Borden, 1984; Wood et al., 1986) which attract adult beetles (Figure 3.3(b)-D).

3.3.2(b) Mating and oviposition

Mature stages of *T. wallichii* are first noticed during the early weeks of January and the beetles are then seen visiting the host plant that has been prepared or altered for them by humans. However, the laying of eggs does not occur immediately. On detecting the cut portion of the host plant, adult beetles visit the area to check the suitability for egg laying. Once the tree sap is in a semi-dry state, which takes about two weeks, *T. wallichii* adults aggregate due to the release of pheromones (Borden, 1984; Wood et al., 1986), and begin to mate and lay eggs as they prefer freshly dead bark (Bense, 1995). A study on the Pine engraver *Ips pini* (Say) (Coleoptera; Scolytidea) has shown that breeding takes place on recently dead or weak trees which exhibit fewer defence reactions (Anderson, 1948; Wood, 1982). It has also been reported that *R. palmarum* weevils arrive 2 to 24 hours after cutting of a host tree while *R. barbirostris* weevils start colonization 1 to 2 days after the palm tree has been cut down (Choo et al., 2009).

Oviposition of eggs on the host trees is preceded by chewing the bark or by simply laying the eggs on already existing cavities. Once the adult has laid its eggs, the larvae emerge from the eggs after about 4 days to one week mainly depending on the temperature as observed by the domesticators. The eggs and first instar larvae are rarely noticed because they are well hidden in crevices of the tree bark (Gardiner, 1966) until they gradually move inward into the bark of the host plant as they chew through it. Larvae are then found between the bark and the heartwood of the tree during July-August (Figure 3.4(b)-A and 3.4(b)-B) and they do not come out until September-October. The presence and sizes of the larvae are indicated by dark brown pieces of chewed tree bark near the infested tree (Figure 3.3(b)-E and 3.3(b)-F). Domesticators

carefully look for such traces to confirm an infestation or colonization has taken place. They visit the rearing site once in a week to check the progress of larval development and mark the occupied area to claim the possession of infested host plants.

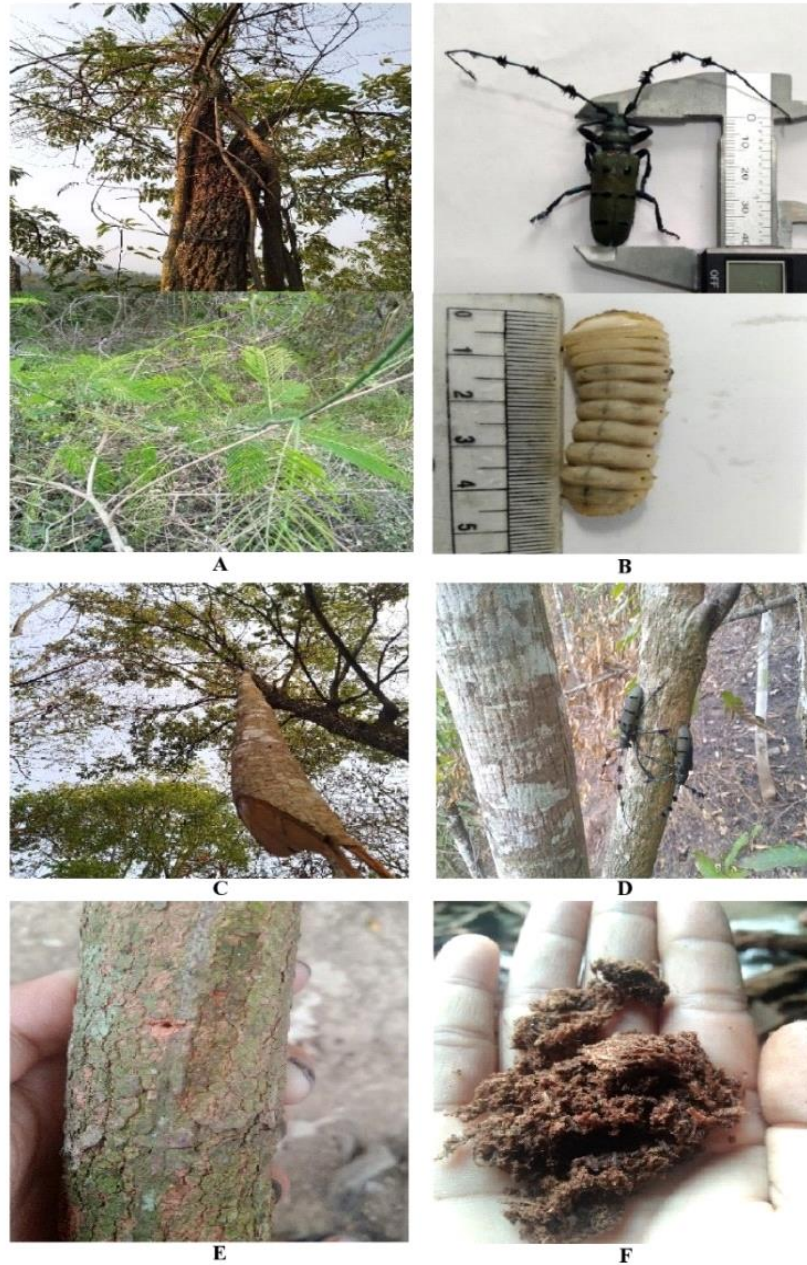


Figure 3.3(b) A- *A. pennata* tree and leaves; B-T. *wallichii* adult and larva; C- Cut wedged shape end of *A. pennata*; D- *T. wallichii* adults on host tree; E- Hole created in the host tree by adults for egg-laying; F- sawdust expelled from the host tree by feeding larva.

It has been observed that the productivity of the larvae depends on the rainfall received in the area of semi-domestication. The factors considered for optimal growth of the larvae and good harvest are: (a) good timing to start cultivation when the adults are abundant, (b) a good amount of rainfall and high temperature for the growth of the larvae as it helps the host tree to decompose at a faster rate allowing the larvae to feed better on the tree bark and (c) knowing the right time for harvest just before the larvae pupate. Thus, the seasonal rainfall pattern plays an important role as has also been observed in certain other insects like *R. palmarum* showing the highest density during the end of the rainy season (Hagley, 1965). However, in Brazil, Costa Rica, and Honduras, it has been found to be maximal during the dry season (Schuiling and Van Dinther, 1981). Bark beetles are generally sensitive to temperature, which affects their reproduction and development. (Bakke, 1968; Annila, 1969).

Along with the semi-domestication practice followed in the forest, certain rearers show certain flexibility in rearing *T. wallichii* at home, maintaining an optimal moisture and temperature. However, in this case, cutting and slicing the end of the host tree to attract the adults till the laying of the egg, is carried out in the wild. Once eggs are laid, the farmer detaches the wood and brings it home. The woods are stacked together and placed horizontally or vertically (Figure 3.4(b)-C and 3.4(b)-D) according to convenience in an open space with ample exposure to sun and rainfall. In case of low rainfall, water is sprinkled on the wood frequently to speed up the decaying process which then facilitates the larvae to feed and grow faster. Interestingly, while *T. wallichii*s reared at home, the larvae can be harvested for self-consumption as required quite unlike the one-time harvest in case of the semi-domestication of the larvae in the wild.

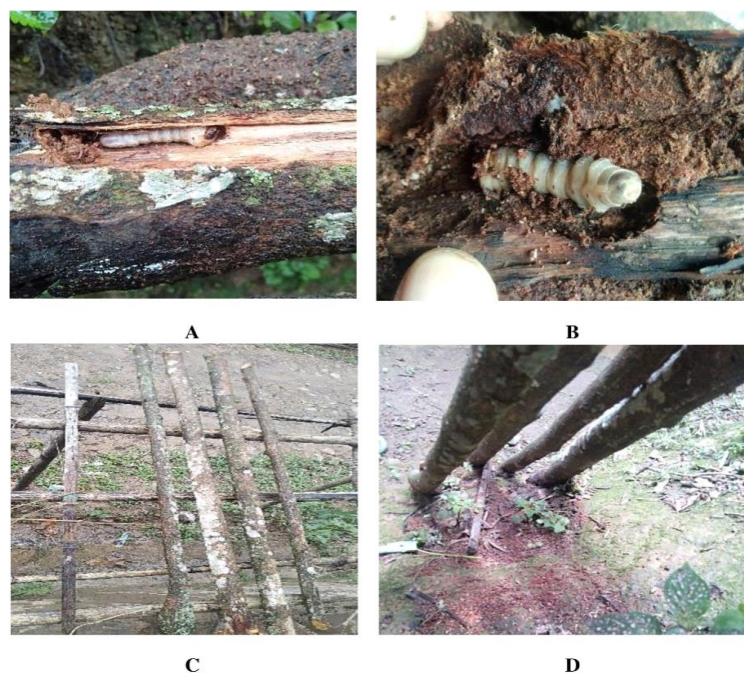


Figure 3.4(b) A-B- Larva of *T. wallichii* were found infesting the host plant; C-D- Stem of *A. pennata* kept horizontally and vertically in open space at home for domestication

3.3.3(b) Harvesting

Harvesting begins in August, but the best time is from September to October when the larvae attain their maximum weight ($2.24 \pm 0.54\text{g}$). For harvesting the larvae, the hanging portion of *Acacia pennata* is detached from the main tree and cut into 50 to 60 cm long pieces and stacked in a clean, well levelled plain area for easy collection of the larvae (Figure 3.5(b)-A). Thereafter, the cover of the stem (bark) is peeled off by hand and the main trunk is cut deeper using a machete so that the larvae which have moved inward fall onto the clean ground (Figure 3.5(b)-C). Collecting the larvae is usually done by hand without any protective hand cover, although sometimes rubber or woolen gloves are used (Figure 3.5(b)-B) to prevent being pricked by the thorny stem. It

has been estimated that host plant stems of 270 cm and 430cm heights may fetch 3kg and 5kg of larvae, respectively. This would be considered a good harvest. Harvesting involves intensive manual labour and hard work as the weather at these periods is humid with dry-sunny afternoons accompanied by heavy rainfall. Insect bites as well as injuries from *A. penneta*'s thorny covering present an additional inconvenience.

Once the larvae are collected, they are kept along with sawdust inside a cloth bag or dry container (Figure 3.5(b)-D and 3.5(b)-E) until taken home. The reason for the usage of sawdust is to prevent the larvae from biting one another resulting in death and blackening of their bodies (Figure 3.5(b)-F), instantly rendering them inedible. Usually, the mouth region of a larva is crushed using the thumb and index finger to prevent the larvae from biting one another. Larvae of *T. wallichii*, semi-domesticated at home, are collected by hand after splitting open the bark of the host trunk with bare hands or the aid of a machete. The advantage of semi-domestication at home is that the larvae can be harvested based on the need of the domesticator for the purpose of self-consumption or market. One single *T. wallichii* larva weighs on average 2.24 ± 0.54 g during September when it is harvested. The whole process involved from preparing the host tree till harvesting the larvae of *T. wallichii* takes 7 to 8 months. In the Democratic Republic of the Congo, it is customary for women to detect the most opportune moment to harvest to harvest edible insects– which occur in standing or rotting *Elaeis*, *Raphia*, *Chamaerops* and *Cocos nucifera* palm trees – by putting their ears against the trees and listening to the sound made by the chewing and burrowing beetle larvae. This method is also used in Cameroon to harvest palm weevil larvae of *R. phoenicis* at their most appropriate developmental stage for consumption (Van Huis, 2003). The same practice has been documented in the Central African Republic and many parts of tropical America (FAO, 2013).



Figure 3.5(b) A- *A. pennata* stem stacked for harvesting; B- A domesticator collecting the larva using woolen gloves; C- Collected on the clean ground; D-E- Larva kept along with sawdust in a plastic container and sackcloth; F- Dead larva turned black.

3.3.4(b) Traditional laws protecting the domesticators

The host plant *A. pennata* is a semi-parasitic species that has no value as timber and is not considered as firewood by the local people. Hence, rearing of *T. wallichii* does not pose any problem in terms of cutting down the host plant. Therefore, any individual, irrespective of whether he or she owns the forest, can adopt the semi-domestication practice. Being commonly cultivated in and around human habitation, larvae are sometimes being destroyed or stolen. However, traditional customary laws

exist and allow the harvesting of the larvae only by those who have cut the host tree for rearing. As the complete process of rearing is labor intensive and requires a considerable amount of time, the village leaders take serious note of any culprit and impose fines/penalties and sometimes ask the culprit to return the harvest to the owners. However, in countries like Thailand, most insects are collected from the wild and this can lead to overharvesting. The government of Thailand has therefore passed legal regulations to protect species (Boongird, 2010). To improve semi-domestication techniques for palm weevils and bamboo borers was encouraged due to the declining number in the wild and to ensure sustainable harvesting (Hanboonsong et al., 2013).

3.3.5(b) Socio-Economic potential

Although the socio-economic potential of *T. wallichii* is still understated, our market survey has highlighted the species as a promising alternative to conventional food items particularly with regard to nutrient supplementation of the poorer sections of society in Nagaland. The study has revealed that semi-domestication of *T. wallichii* is a regular feature for self-consumption and marketing by members of the Angami tribe; thus, making it an integral part of the socio-economic lives of tribal inhabitants of Nagaland.

In Nagaland insects are preferred as a food item based on an insect species' size, availability, associated taboos, individual tastes, market price, and traditional ethno-medicinal knowledge (Mozhui et al., 2020). *T. wallichii*, a very common wood borer larva, is semi domesticated and consumed by the Angami Nagas of Nagaland. With a UV of 0.92, *T. wallichii* is widely used as an alternative source of protein, indicating the potential demand for this insect species in the market. Among the respondents, 8% do not consume it, because of certain reasons such as unavailability, stomach upsets after consumption and feelings of aversion, but the remainder take a positive view on its use

as a food item. Both men and women have active parts to play in the semi-domestication process and in the marketing of the species. Although domesticators of *T. wallichii* are male who also sell the harvest directly at the village level, the women play an important role in taking the larvae to the urban market for sale.

It is estimated that 1 kg of fresh, live *T. wallichii* larvae fetch INR.700/kg at rural markets and INR.1000/kg at urban markets. This is considerably less than *V. mandarinia* (INR 7290/kg) (Kiewhuo, et al., 2022) and *Cossus sp.* larvae (INR 10,000/kg) (Aochen, et al., 2020). However, due to their palatability and high demand, these highly appreciated insect larvae are quickly sold out despite their exorbitant prices compared with conventional animal meat sources (Figure 3.6(b)). In developing countries with high demand for edible insects, collection and domestication can provide livelihood opportunities at the household or industrial level (Van Huis et al., 2013). An overview of the insect’s price around the world highlighted that 50 g of the *Tenebrio molitor* cost € 4.85 in Netherlands, and in Kenya, 1 kg of termites was sold at € 10 (Van Huis et al., 2013). Another study in South Cameroon showed an earning of USD 5-10 from selling termites (Tamesse et al., 2018).

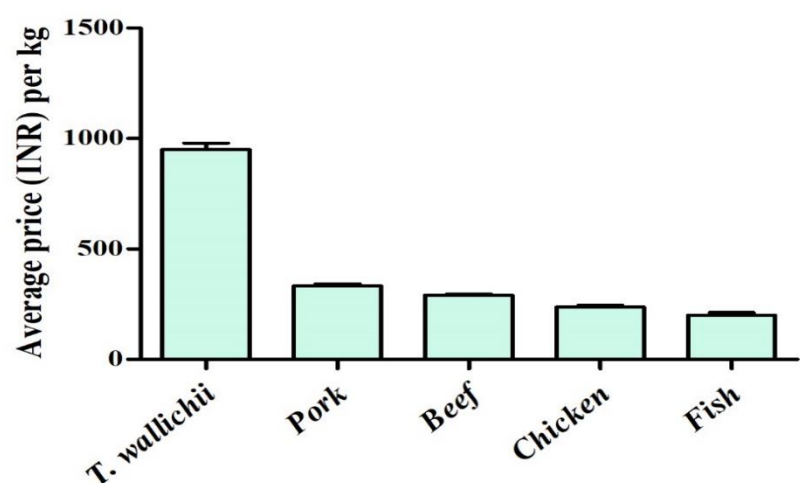


Figure 3.6(b) Market value (in rupees) of *T. wallichii* compared to other conventional meat sources in Nagaland.

3.3.6(b) Consumption process

T. wallichii larvae are consumed during the larval stage just before they pupate. The larvae are mostly preferred as a cooked item (74%), followed by being fried (18%) and in roasted form (8%) (Figure 3.7(b)). This is similar to *V. mandarinia* (Kiewhuo et al., 2022), *Cossus sp.* (Aochen et al., 2020), and other edible insects in Nagaland (Mozhui et al., 2020). The demand for these insects at the local markets is increasing due to their high nutritive value and excellent taste, which consumers relish. Preparation of *T. wallichii* larvae involves proper washing with water and completely removing the gut by simply splitting the larvae open lengthwise. Incomplete removal of the gut before cooking can cause the consumer to develop a stomach ache. The main ingredients used in the traditional method include chili, salt, and fermented bamboo shoots. The cooked larvae are eaten as a side dish with rice or as the main dish with local beverages. The dried cooked larvae can be kept up to 3 to 4 days. The larvae can also be simply fried in cooking oil with salt or gently roasted over warm charcoal ashes for 3 to 4 minutes and eaten.

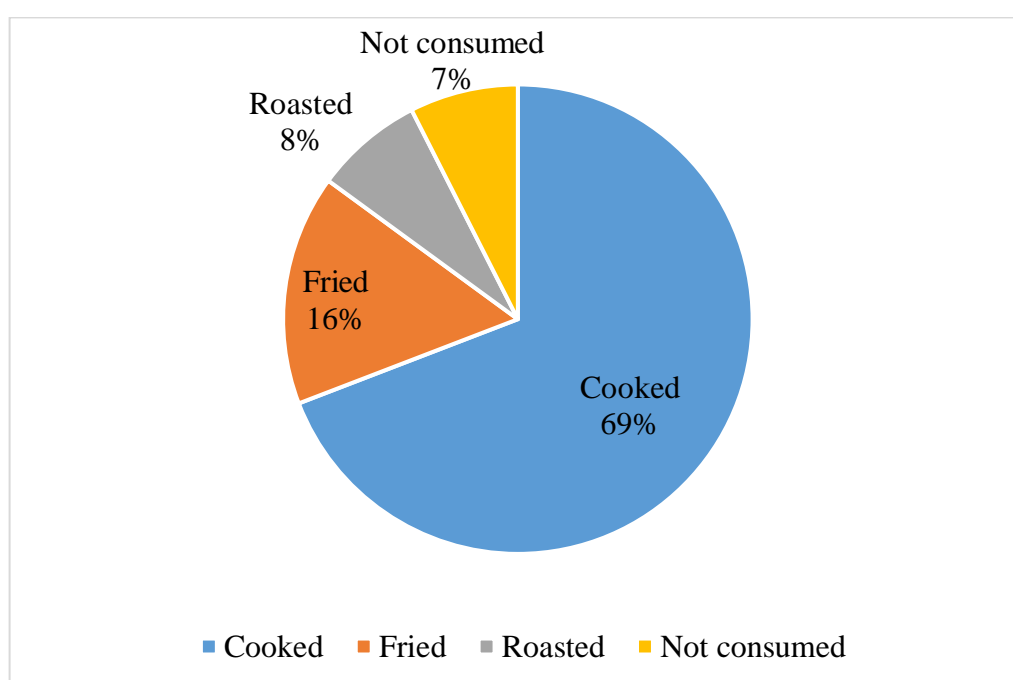


Figure 3.7(b) Different modes of consumption of *T. wallichii* larvae.

Insects are generally considered a good source of most nutrients (DeFoliart, 1992; Ghosh et al., 2017) deemed essential by the FAO, although the nutritional value of insects is not necessarily higher than that of conventional sources of protein such as chicken and beef (Payne et al., 2016). The value of insects as a human food item is primarily based on its far more efficient feed conversion ratio and much lower CO₂ footprint (Vauterin and Kahiluoto, 2021). The proximate nutritional composition of *T. wallichii* larvae is presented in Figure 8(b). Moisture content of *T. wallichii* was found to be 83.46% while ash content was 2.06%. The crude protein content of *T. wallichii* larvae was found to be 56.18±4.06%, which is within the range of 40-75% reported for other edible insect species (Bukkens, 1997; Rumpold and Schlüter 2013; Ghosh et al. 2017). Chen et al. (2010) also reported that the protein content of coleopteran larvae ranged between 23 and 66%. The larvae of coleopteran beetles such as *Oileusrimator*, *Scyphophorusacupunctatus* and *Callipogan barbatus* had been reported to contain 21%, 36% and 41% protein respectively (Ramos-Elorduy et al., 1997). Adult edible beetles, e.g., *Holotrichiaparallella*, contain less protein (24% protein) and heavier cuticle than their larvae (Yang et al., 2014). Kouřimská and Adámková (2016) and Meyer-Rochow et al. (2021) have reported that the protein content of insects can vary between 20-76% depending on their developmental stage, sex, etc. The protein content of *T. wallichii* larvae was found to be higher than that of *V. mandarinia* larvae (52.82%) (Kiewhuo et al., 2021) and *Cossus* sp. larvae (48±0.068 %) (Aochen et al., 2020) from the same region. A high protein content with essential amino acids indicates that an insect can be regarded as an alternative for other animal proteins that are often absent in the diet of the poorer sections of society, especially rural dwellers (Banjo et al., 2006). The nutritional value of edible insects ranges widely because of the diverse number of species and variability within species. Within the same group of species, the nutritional

value varies considerably depending on the developmental stage, the origin of the insect, the insect's diet, preparation method for consumption (e.g., boiling, frying, drying, pickling, etc.) and storage conditions (Meyer-Rochow et al., 2021).

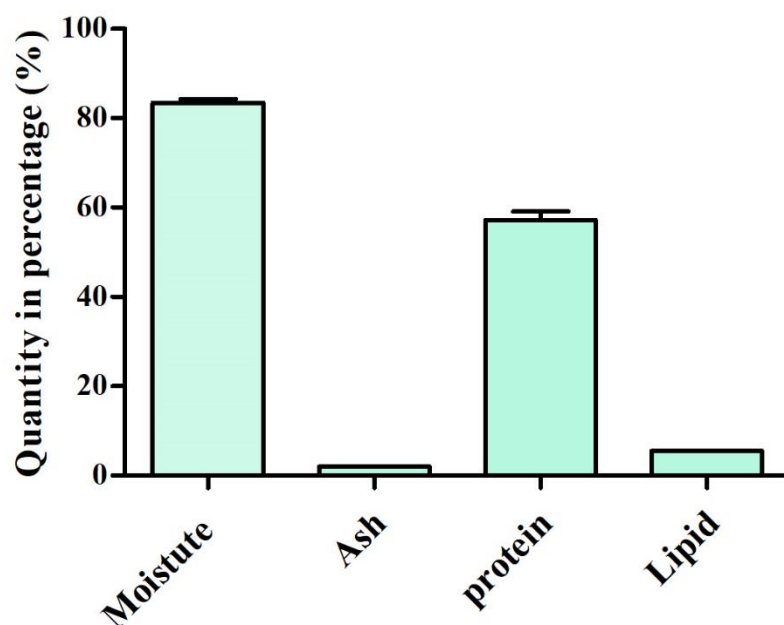


Figure 3.8(b) Proximate composition of *T. wallichii* larvae

The lipid content of *T. wallichii* larvae (5.51 ± 0.07) is less than that of many other insect larvae but comparable to that of June beetle females (5.41% to 36.87%), which depends on seasons, habitats, places, and the species (Raksakantong et al., 2010). Usually, the lipid content in insects is higher in the larval stages than in the pupa and adults (Dossey et al., 2016; Aguilar, 2021), and on average edible insects contain 13-57% of fats in dry matter (Bednářová et al., 2013). For instance, the larvae of coleopteran beetles such as *Allomyrina dichotoma* and *Protaetia brevitarsis* contain 20.24% and 15.36% fat (expressed as dry matter), reported by Ghosh et al. (2017). It has been shown that the lipid content of insect varies widely in different species, but also with regard to their stage of growth, environmental conditions and the insects' diet (Meyer-Rochow et al., 2021; Lorrette and Sanchez, 2022). Lorrette and Sanchez (2022)

observed that lipid content differed in young and older larvae of *Tenebrio molitor*, which contained respective lipid amounts of 6.6% (fresh matter), and 11.7% (fresh matter). A further increase of fat content was not observed; in fact, in lipid content decreased to 6.2% in the adult stage. Similar findings of variations of the lipid content in different insects like the giant water bug *Lethocerus indicus* (26.63%) and the water scavenger beetle *Hydrous cavistanum* (3%) were reported (Yang et al., 2006b; Sarmah et al., 2022), indicative of the roles that habitat and diet play in the total amount of lipid in an insect. The proximate analysis of protein and lipid contents in *T. wallichii* shows Similar findings of variations of the lipid content in different insects like the giant water bug *L. indicus* (26.63%) and the water scavenger beetle *Hydrous cavistanum*(3%) (Yang et al., 2006; Sarmah et al., 2022), has been indicative of the roles that habitat and diet play in the total amount of lipid in an insect. The proximate analysis of protein and lipid contents in *T. wallichii* shows promising results and suggests that the larvae of this species can supplement the nutrient requirement for consumers in Nagaland especially disadvantaged sections of the society. However, the price of the insects must come down, and for that to happen, successful domestication of *T. wallichii* has to be achieved.

Insect farming, with the exception of the silkworm and honey bee, has remained an almost unknown practice until recently. Usually, insects were collected from the wild. Land use for agriculture and urbanization has progressively caused a decline in wild insect collection. At the same time, farming edible insects have begun to get entrepreneurs interested as a valid, more sustainable alternative to wild harvesting. At present, insect rearing attracts increasing interest also in countries that are not traditionally involved in collecting insects, such as those of Europe and northern America. Insect farming, indeed, combines economic benefits with the production of

food and feed ingredients that can beneficially affect a population's diet in both developed and developing countries (Cadinu et al., 2020).

Chapter 4

Biology and Ecology of *Tarbinskiellus portentosus*
(Lichtenstein, 1796): a relished edible insect in Nagaland,
Northeast India

Contents	2.1	<i>Introduction</i>
	2.2	<i>Materials and Methods</i>
	2.3	<i>Results</i>
	2.4	<i>Discussion</i>

4.1 Introduction

Expansions of food production are the primary source of greenhouse gas emissions, with livestock products being one of the most significant contributors that trouble the ideas of modern sustainable means of livelihood (FAO, 2017). Insect productions have little environmental consequences compared to traditional livestock, and due to their physiology, insects have better feed conversion rates and growth efficiency (Oonincx et al., 2015). For instance, *Acheta domesticus* has a feed conversion ratio of 2.1, meaning 2.1 kg of feed is required to produce 1 kg of edible products. In comparison, for other conventional livestock such as cattle, pigs, and poultry, 25, 9.1, and 4.5 kg of feed is required to produce 1 kg of meat (Van Huis, 2013). Edible insects provide 5 to 10 percent of the animal protein as well as fat and calories, and various vitamins (A, B1, B2 and D) and minerals (iron, calcium) (Gullan and Cranston, 2005; McCluney and Date, 2008). Therefore, developing a new sustainable source of edible insects have been recommended (Nikkhah et al., 2021). Despite serving as a potential source of nutrients, only a few species of insects are mass-reared. According to the European Food Safety Scientific Committee, only nine different species of insects are being reared and farmed in mass to be used as food (Halloran et al., 2017).

One of the downfalls of unsuccessful mass-rearing of edible insects could be lack of essential information on biology, growth, and reproduction. There are approximately 20,000 insect farms in Thailand that produce 7500 metric tons per year, and cricket is one of the promising insect that is farmed, produced in mass, and used for domestic consumption as well as for selling in the market (van Huis et al. 2015). South Korea is another leading consumer of edible insects, and their consumption has resulted in increased demand. The edible insect market's value in south Korea increased from 143 million in 2011 to 259 million in 2015 (Shin et al., 2018). Similarly, entomophagy

practices are widespread in India (particularly Nagaland, Northeast India) (Kiewhuo et al., 2022). Among the 106 edible insects consumed and relished, cricket (*Tarbinskiellus portentosus*) is one of the most preferred insects in the region (Mozhui et al., 2020). Therefore, studies on its biology and reproduction are essential to enable the efficient usage of this promising insect.

T. portentosus belongs to the order Orthoptera, family Gryllidae, and is commonly found in Southeast Asia, including India, Thailand, and Indonesia. This species spends most of its time under burrows with a single individual per burrow (Tantrawatpan et al., 2011). It feeds on fresh plants and is considered crucial human food as this species has higher economic value in Southeast Asia, especially Thailand (Sverdrup-Jensen, 2002). Yhoun-Aree (2010) reported protein and fat content of 12.8g/100g and 5.7 g/100g respectively in *T. portentosus*. Due to its high nutritional value, *T. portentosus* has been consumed in many countries (Buzzetti and Devrisese, 2008; Yi et al., 2010). In Thailand, *T. portentosus* is available all year round at 4.8 USD per kg (Siriamornpun and Thammapat 2008). *T. portentosus* is also an important protein source for fisheries and poultry production (Razak et al., 2012; Sverdrup-Jensen, 2002). A noteworthy study on the life history of *T. portentosus* in the laboratory was conducted at $24.68 \pm 1.26^\circ\text{C}$ by Hanboonsong and Rattanapan et al. (2001). The authors highlighted information on its longevity, number of instars, incubation period, and food habits. A recent study on genetic variation in mainland Southeast Asia, such as the Lao People's Democratic Republic, Cambodia, and Myanmar, shows that three morphotypes of *T. portentosus* are available (Pradit et al., 2022). Although considered highly relished insect with potential economic value, detailed information on their biology under Nagaland's Climatic condition is lacking.

In Nagaland, *T. portentosus*, commonly known as “viituo,” is primarily available during the rainy season, with its population peaks during August- October. They are preferred as food by many sections of society due to the nutritional and cultural values associated with them. Although *T. portentosus* is preferred as a food supplement it is not fully explored in Nagaland due to lack of knowledge of its biology. Given the availability of technique to mass-produce, *T. portentosus* has enormous potential to be made available for consumption at a minimal cost of environmental pollution. In its available season (June to August), *T. portentosus* adults are sold at INR 150 per 300g in the local market. However, before mass production, life history study is a vital footstep that can facilitate the efficiency of insect utilization. Therefore, the present study assesses the biology and life cycle of *T. portentosus* under laboratory conditions to fill the gap in the existing knowledge on this novel insect.

4.2 Material and Methods

4.2.1 Study area

One-year-old abandoned Jhum cultivated lands at Lumami and Zaphumi villages, Zunheboto district (26°13'42.82''N and 94.28'24.70''E) (Figure 4.1).

4.2.2 Sample collections

Field visits were carried out to collect *T. portentosus* adults from the above-mentioned sites during 2019-22. Crickets were collected by digging their burrows using a spade/machete and kept in containers with holes to provide sufficient oxygen till they

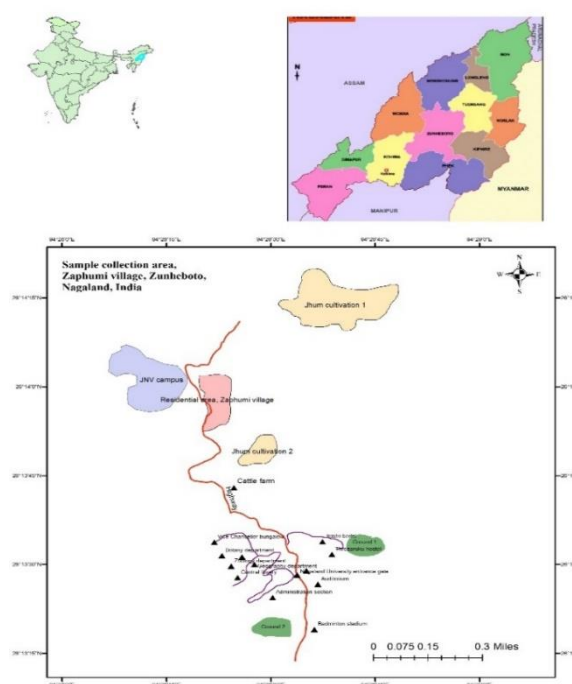


Figure 4.1. Map showing the sample collection areas at Zaphumi and Lumami, village, Zunheboto, Nagaland.

are brought to the laboratory for rearing. Morphologically, identification was done by Mr. Sawapan Pal, Assistant Zoologist, Orthoptera Section, Zoological Survey of India (ZSI), Kolkata. The key morphological identification features for *T. portentosus* are Body length, coloration, Head shape, Antenna length, Wing pad pattern, Cerci length, etc.

All data were presented as mean \pm SD. Analysis of variance (One-way ANOVA) at 95% interval ($p<0.05$) was done to find the mean significance difference in *T. portentosus* instars. Each test was followed by a multiple comparisons test (Tukey test) to find the mean difference between the variables. Statistical analysis was performed using SPSS-22 software.

4.2.3 Rearing

The natural light: dark cycle during insect rearing ranged from 10-13 hrs to 12-14 hrs. Adult crickets were kept in large plastic containers with dimensions 60cm in length, 40 cm in breadth, and 45 cm in height filled with 40cm of loose soil. The adults of *T. portentosus* were provided with natural food (leaves found in their burrows such as *Brassica oleracea var capitata* (cabbage) belonging to the family Brassicaceae, *Ageratina adenophora* (Mexican devil), belonging to the family Asteraceae) and water. The moisture content of the soil was maintained at 35-45% and the temperature of the rearing room varied between 20 to 25°C. During the year 2019 to 2021, life cycle studies were repeated thrice, and the results were based on a sample size of around 75 males and 75 females.

For growth rate studies, *T. portentosus* was weighed (wet weight) using a portable weighing machine (iScale i-400c) at intervals of 7 days, and the growth rate was calculated using the formula.

Growth rate = $\frac{\text{maximum weight} - \text{minimum weight}}{\text{number of days to gain maximum weight}}$. The final growth rate was reported as mg per day.

To estimate eggs laid per female, penultimate nymphs were segregated in a separate container at a 2:1 ratio of male to female. This experiment was performed in five containers, consequently, egg counting per female was carried out using five females. Egg collection was done daily, prior to eggs collections adults were shifted to another container with the help of insect-catching nets. Once all the adults were carefully shifted, eggs were collected manually using a spatula by searching through the soil (Figure 2A). Eggs were counted, observed using a Labomed CZM6 microscope, and kept in small plastic containers (100mm length, 40mm breadth, 30mm height) containing moist soil for further morphological observations. In five containers, a single egg was kept, and changes in their color, length (alloeet -vernier caliper), weight (iScale i-400c), and incubation period were observed till they hatched. Ten (10) containers were utilized for life cycle studies, each containing soil with an adequate moisture level. Subsequently, a single nymph (which instar) was introduced into each container, and observed daily to understand the changes in body coloration, weight, size and length.

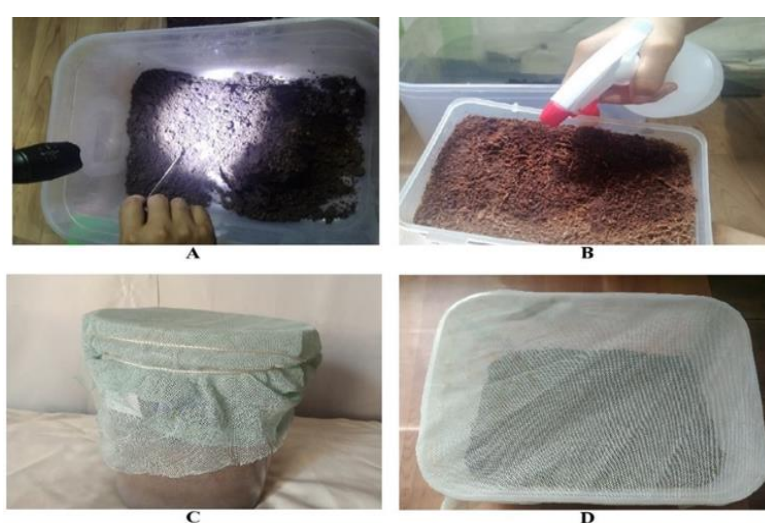


Figure 4.2. A- Collection of the egg using high-intensity light; B- Water sprayed to keep the soil moist for eggs; C- The round container; D- Rectangular box for rearing adult's crickets.

The size of the container for rearing was as per the size of the instar. Water was sprayed regularly to moisten the soil (Figure 4.1-B). The first four instars were kept in rounded containers of 110mm diameter and 120mm height filled with soil up to 50mm and covered with net (Figure 4.1-C). The last 3 instars were kept in circular plastic containers of 180mm in diameter, 200mm height, filled with soil up to 100mm, and covered with nets. Adults were kept in plastic containers 280mm in length, 200mm in breadth, and 150mm in height (Figure 4.1-D) filled with loose soil up to 120mm and provided with sufficient leaves (*Brassica oleracea capitata*, *A. adenophora*). The excess food in the rearing containers was removed and cleaned by handpicking on a regular basis. Once the nymphs matured into adults, the interactions between males and females were observed in five containers containing a 5:5 male-to-female ratio each.

4.2.4 Observations of burrows in the wild

The length of the burrows was estimated by digging from the entrance to the bottom and measuring using a scale. At the same time, the distance between burrows was measured from the entrance of one burrow to the other.

4.3 Result

4.3.1 Life stages

Eggs: Five eggs were observed where freshly laid eggs are oblong in shape, glabrous, and yellowish-white, 3 ± 0.05 mm in length, and 36 ± 0.57 mg in weight. The weight of eggs were observed on day 2 (36 ± 1.04 mg), day 7 (56 ± 0.21 mg), and day 11 (66 ± 1.23 mg), and it constantly increased till the final weight on day 31 (74.8 ± 1.67 mg). While no difference in egg weight between day 1 (on laying) and day two was observed, a significant increase in weight ($p<0.05$) was noticed, and eggs became heavier prior to the hatching. There were not many changes in the length of the eggs from the initial to the final stage. The eggs are slightly more pointed towards one end and round towards

the other (Figure 4.3-A). The chorion being translucent, the embryo is visible to some extent.

On the 7th day after oviposition, the middle part of the egg becomes bent, forming a convex plane on one side and a concave plane on the other (Figure 4.3-B). On the 13th day, the egg becomes slender and more bent towards the ends (Figure 4.3-C). On day 20, the eggs begin to break open and the embryonic eyes became visible (Figure 4.3-D). The eyes, outlines of the wings, and body appendages became distinct on day 28 (Figure 4.3-E). The forelegs, hind legs, wings, and cerci became visible as the egg starts to break open widely (Figure 4.3-F) and subsequently hatching took place. By the 28th, 29th, and 30th days, the eyes became more prominent, and body appendages were faintly visible until the cracks began to occur till hatching. The final weight and length of egg before hatching was 89.0 ± 0.13 mg and 4.89 ± 0.16 mm. The incubation period for *T. portentosus* eggs was 33.8 ± 0.96 days. The hatching efficiency was 45.20 ± 5.28 percent.

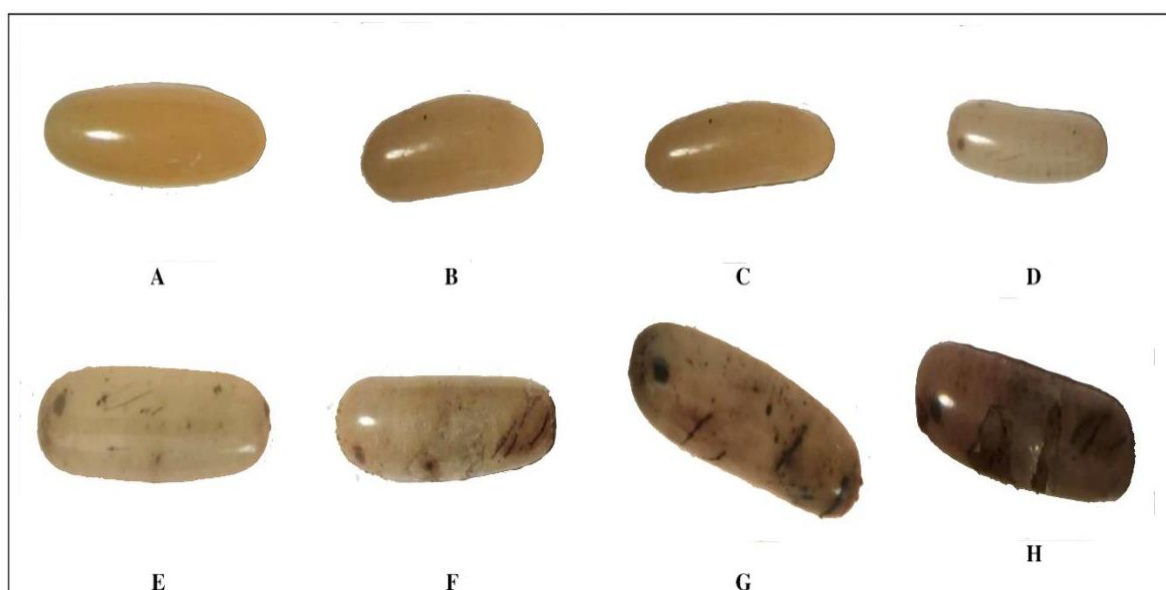


Figure 4.3. A- Day 1- Oblong compact yellowish in color; B- Day 7- Yellowish-white in color and bent at the center; C- Day 13 – more slender and thinner; D- Day 20 – Eyes become visible, egg white; E- Day 28 – Egg white and more compact, body appendages visible; F-H- Day 29 to 31- Brown dirty look on the inside with stretch marks, cracks appear.

4.3.2 Nymphs

In *T. portentosus*, seven (7) instars were observed. The newly hatched nymph of *T. portentosus* was white and possessed a soft body until transitioning into light brown. The main morphological changes between the instars were the development of the wing pad, coloration, body size, body mass, ovipositor development, and an increase in antennal length. A newly hatched instar weighed 34 ± 1.13 mg and had a body length of 4.0 ± 0.00 mm with an 8 mm antenna and 3 mm cerci (Table 4.1). The coloration of the body was grey-blackish on the abdomen, and its head region was light brown during the first three instars. The first five instars had a round head and abdomen with not-so-visible segments on the body (Figure 4.4A-E). No differences were observed during the first five instars concerning their body color and shapes except for increased body sizes, weight, antennal length, etc. The wing pad developed in the sixth instar (Figure 4.4-F)

in both male and female nymphs. In females, the ovipositor appeared in the seventh instar (Figure 4.4-G) while the body color was darker, and the nymphs became broader and less rounded than the first five instars. With further development, the head, legs, and abdomen became more turgid, and the color became dark brown. After the final molt, the adults weighed 2100 ± 379.86 mg, length 31.7 ± 1.0 mm, antennae 40 mm, and cerci 16 mm.

Table 4.1. The instars of *T. portentosus* with details of body weight, body length, antennal length, and cerci length

Instar	Fresh body weight (mg)	Body length (mm)	Antennal length (mm)	Cerci length (mm)
1 st	34 ± 19.13^a	4.0 ± 0.00^a	8.0 ± 0.21^a	3.0 ± 0.76^a
2 nd	137 ± 90.37^b	13.3 ± 1.7^b	10 ± 0.44^b	4.0 ± 0.01^b
3 rd	360 ± 124.34^c	16.6 ± 2.0^c	15 ± 0.19^c	6.0 ± 0.34^c
4 th	561 ± 68.00^d	21.6 ± 1.5^d	20 ± 0.32^d	8.0 ± 0.12^d
5 th	1044 ± 434.72^e	24.0 ± 1.0^e	31 ± 1.53^e	10.0 ± 2.00^e
6 th	1802 ± 296.02^f	31.0 ± 4.3^f	37 ± 1.41^f	12.0 ± 0.45^f
7 th	2100 ± 379.86^g	31.7 ± 1.0^g	40.0 ± 1.89^g	16.0 ± 2.90^g

Mean with different superscript indicate the statistically significant difference at 95% interval ($p < 0.05$) by multiple comparisons Tukey's test (SPSS 22).

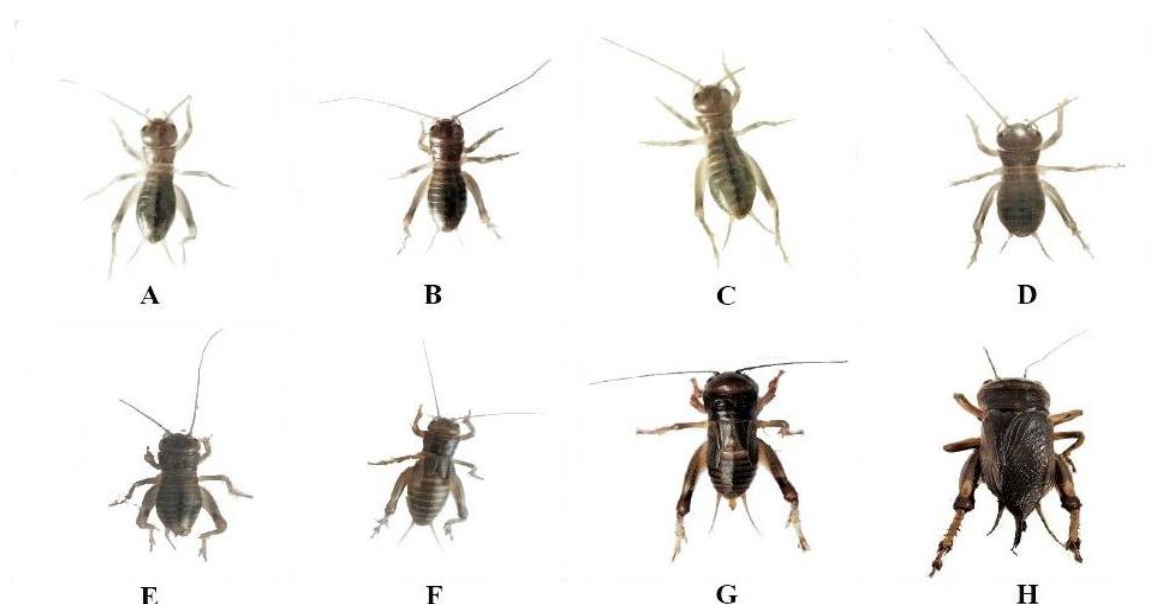


Figure 4.4. A-G- The seven instars and; H- an adult *T. portentosus*

Body weight, length, antennae, and cerci of *T. portentosus* were significantly different ($p<0.05$) from instar to instar (Table 4.1). As shown in Figure 5, the first, second, third, fourth, fifth, sixth, and seventh instars molted on day 42, 77, 119, 147, 182, 210, and 252, respectively. During the rearing period, the growth rate of *T. portentosus* ranged from 4.2 to 7 mg/day. Wide variations in growth rate were observed, where the maximum growth rate was observed between 70-80 days. With further maturity, the average growth rate of *T. portentosus* declines gradually. The development from the first instar to the final molt of *T. portentosus* required 298 ± 8.24 days. After the final molt, *T. portentosus* lived for another 43 ± 6.5 days. Therefore, the total number of days required to complete the entire life cycle of *T. portentosus* was found to be 341 ± 4.29 days.

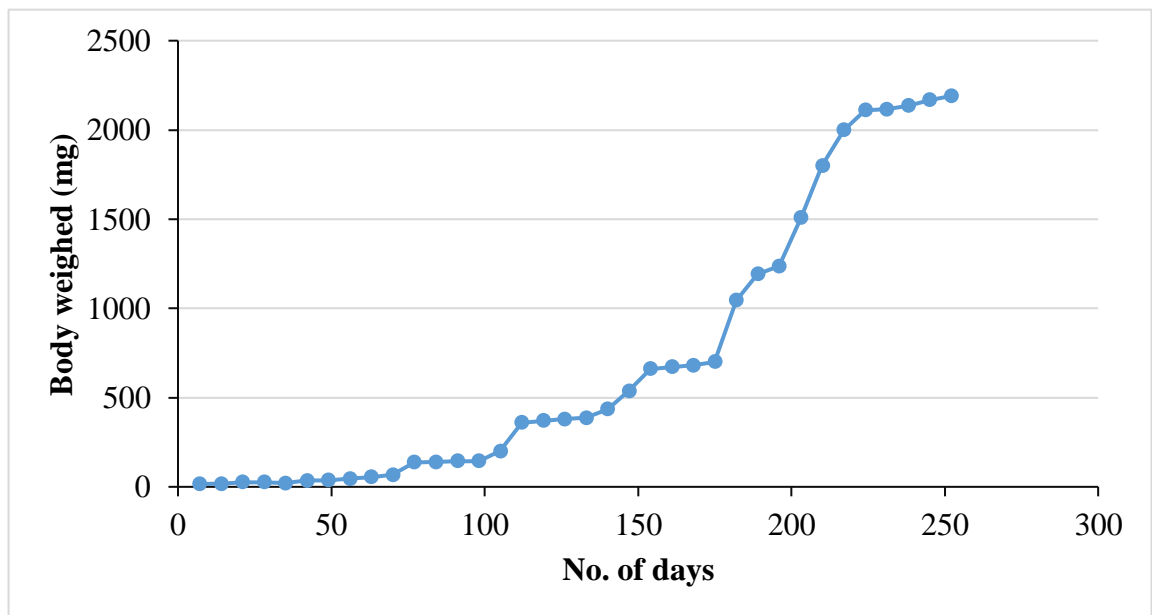


Figure 4.5. Increase body weight (mg) of *T. portentosus* during different stages of development.

4.3.3 Adults

Adult crickets had a cylindrical body, long slender antennae (34-40mm), a round head, and two long cerci (15-16mm) and a long ovipositor (11mm) for females. The hind leg has an enlarged femur; followed by three tarsal segments. The forewings ($30\pm1.4\text{mm}$) are smooth in females and rough in males (Figure 4.6A-C). The hind wings ($41.5\pm2.1\text{mm}$) are longer than the fore wings and are lighter in color. The female body measured about $36\pm1.7\text{mm}$ in length, and weight about $2980\pm200\text{mg}$, while the male measured about 37.3 ± 0.05 long, weight about of $2940\pm93.0\text{mg}$.

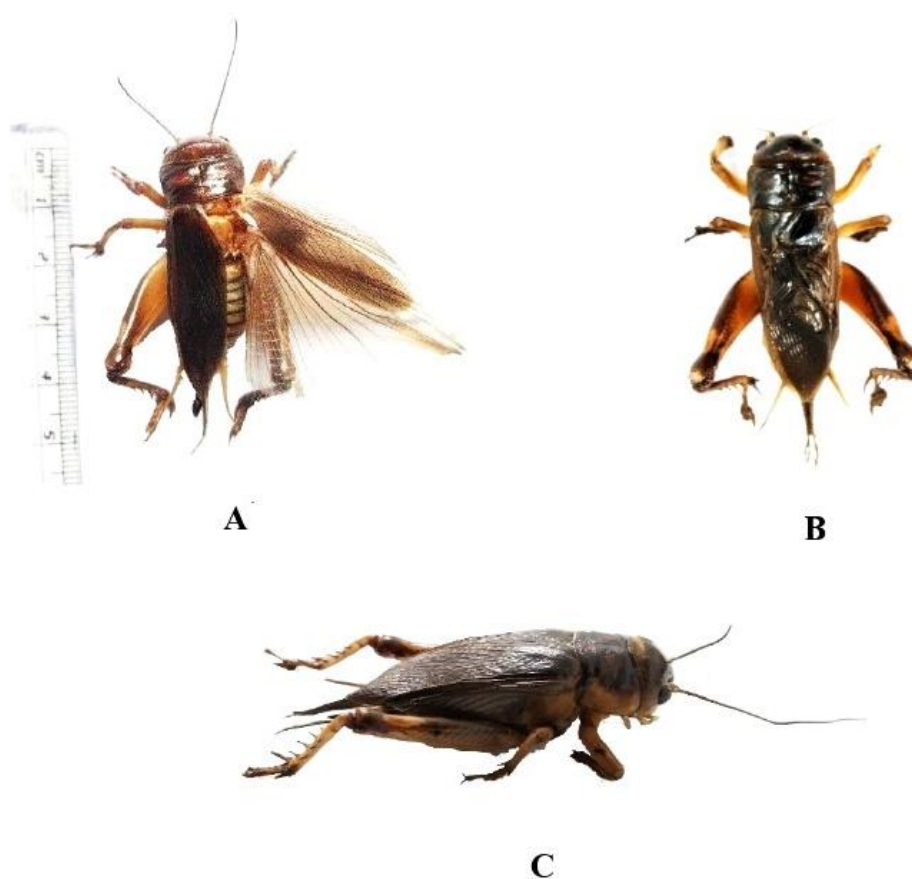


Figure 4.6. (A) Adult female and (B) Adult male and; C- lateral view of female *T. portentosus*.

4.3.4 Ecology of *T. portentosus*

In the wild, *T. portentosus* burrows were found in grassland-type vegetation with sparse trees. Burrows were located by searching for a heap of loose soil mounted around the entrance (Figure 4.7-A). A heap of moist and finely-grained soil at the entrance indicates the presence of *T. portentosus* inside the burrow. Depending on the instar and texture of the soil, the burrows can go as deep as 50mm to 800mm. In loose soil areas, adult burrows go as deep as 800mm, while in rocky soil, if less moisture and roots of trees are present, burrows can be as shallow as 50-200mm. The burrows do not have any branching at any angle, but towards the bottom, they become less vertical, and that is where crickets store their food brought from outside. The burrows are constructed in a way that the bottom is not exposed directly to sunlight. At the end of the burrow, *T. portentosus* were found embedded in soil with its back towards the entrance (Figure 4.7-B). Fresh plant leaves such as *Brassica oleracea* (cabbage) and *A. adenophora* (Mexican devil) were found inside the burrow (Figure 4.7-C). During the spring season (March-April of the year of your observation), in its nymphal stage, cricket burrows were found to be located very close to one another, just 20mm to 50mm apart. However, in the summer season (August- September, 2019-22), adult burrows were never found close to each other. Single adult per burrow was observed except during mating season, i.e., August to mid-October, when a male and female were found together in one burrow. Adults begin to appear on the ground and are attracted towards the light source at night during the month of May, peaking from July to September, with a few still present in October. During mating season, male crickets create a mating call, known as stridulation, by rubbing their rough wings together. These males were observed near burrow entrances (Figure 4.8-A), emitting stridulation calls during the evening and night hours to attract females.

T. portentosus adults and nymphs mostly stay inside the burrows and are not seen much as they do not venture out during daylight except for food collection and mating purposes. All stages of *T. portentosus* are nocturnal, and individuals construct their burrows using the mandibles to clear the soil substrate, which is pushed outside the burrows using their hind legs backward (Figure 4.8-B). During mid-August, most *T. portentosus* are in their nymphal stage, while few are in their adult stage.



Figure 4.7. A- Heap of loose soil mounted around the burrow's entrance; B- *T. portentosus* embedded at the end of the burrow; C- Food stored in burrows.

Behavioral observation in the laboratory showed that adults of the same gender showed more aggression towards each other when put together for the first time. However, within 5 to 10 minutes, they start constructing their burrows, which keeps them away from each other. Aggressive behavior such as kicking, chirping, and biting one another was observed mainly in males during mating calls and in ovipositing females. Nymphs showed no sign of aggression towards each other. At the end of the burrows, the eggs are deposited by the female into the soil through ovipositor.



Figure 4.8. A- A male *T. portentosus*; B- Adult *T. portentosus* digging its burrow.

4.4. Discussion

Due to its bigger body size than most crickets, it is one of Nagaland's most preferred edible insects (Mozhui et al., 2020). It is a giant edible cricket found in Asia, with a body length and weight of $37.30 \pm 5.0\text{mm}$ and $2980 \pm 200\text{mg}$ (ref), and is nocturnal. As per the findings of Hanboonsong and Durst (2014) in Lao PDR, the crickets were reported to be more prominent, with a body length of 50mm. The slight variations in body size could be due to differences in climatic conditions, food availability, and nutritional content of their food.

Hanboonsong and Rattanapan (2001) reported that a single female laid about 123.00 ± 46.44 eggs, and the incubation period of eggs was 56.10 ± 15.03 days, with 40.70 ± 4.74 percent of hatching, while the whole growth period including seven instars of nymph and adult was 173.70 ± 19.86 days. In the present study, the incubation period of eggs was 33.8 ± 0.96 days and showed a moderate percent of hatching efficiency ($45.20 \pm 2.8\%$). Lesser incubation period and more hatching efficiency could be attributed to difference in climatic conditions. The present study records that females individually lay 98 ± 11.4 eggs throughout its reproductive life cycle. *T. portentosus* is found in abandoned jhum cultivated areas and is available for consumption from May

till September. The crickets live in burrows, where they store food and lay eggs. With the growth and development of the nymph, its burrow goes as deep as 50mm to 800mm, and only a single generation of *T. portentosus* is produced per year. The use of mandibles and forelegs during the construction of burrows recorded in this study agrees with the study on *Anurogryllus muticus* (Lee and Loher, 1996), which uses its forelegs to push out the soil substrates when digging burrows. *T. portentosus* lives inside its burrows and comes out primarily for food collection and mating. Usually, collected food is taken back to the bottom of the tunnel for feeding. During mating season, adult males come out of burrows in search of a mate and call for partnering by chirping at the edge of their burrows. After attracting a female, mating takes place, and the mated female remains within the burrow to lay eggs at the bottom. The present study observed that mated females lay eggs at the bottom of the tunnel, and similar behavior is also found in *A. muticus* (Lee and Loher, 1996).

The burrow serves as a social environment for *T. portentosus*, allowing mating and provide protection against predators, rain, sun, and wind and acts as a convenient environment for reproduction and brood laying. Burrow-making is a common phenomenon in social insects such as earwigs or Dermaptera (Hsieh et al., 2022), ants (Tschinkel, 1987), wasps (Evans and West-Eberhard, 1970), and bees (Packer and Knerer, 1986). In addition, a sealed burrow offers protection from predators, further enhanced by increased aggression of the females during oviposition and brood care (West and Alexander, 1963; Alexander and Otte, 1967). Females of *Brachytrupes achatinus* Stoll (Brachytrupinae), the "big-brown cricket" of India, deposit their eggs in shallow burrows at the end of one of their galleries, and the young nymphs leave the parental burrow a few days after hatching (Ghosh, 1912).

After the final molt, *T. portentosus* lived for another 43 ± 6.5 days; therefore, the total number of days required to complete the entire life cycle of *T. portentosus* was 341 ± 4.29 days. Hanboonsong and Rattanapan (2001) also reported that *T. portentosus* completes its life cycle in 333.30 ± 20.06 days and has 6-7 instars at 24.68 ± 1.26 °C. In the present study, seven (7) nymphal instars were observed. The first molting took the most extended period, which could be attributed to the colder climate and lesser humidity (October, November, December, and January, 2019-22). The subsequent instars required lesser days for molting, which could be due to warmer season.

Egg morphology shows that on the seventh day after oviposition, it bent towards the middle, and by the twentieth day, eyes were visible, and the body appendages were visible on the twenty-eight days. In *Acheta domesticus*, also called house cricket, after four days of oviposition, as the eggs undergo developmental changes it become slightly curved for a few days. By the twelfth day, eyes become visible; by the 16th day, just before hatching, inner body appendages are visible (Douan et al., 2021). A study on *T. portentosus* also shows similar morphological changes in the egg, but the number of days differed. The variation between species explains most of this difference, although experimental conditions could also affect the developmental time and growth of the egg (McCluney and Date, 2008; Doherty et al., 2018).

Chapter 5

Quantitative characterization of microbial load in wild-harvested edible insects of Nagaland, India

Contents

- 5.1 *Introduction*
 - 5.2 *Materials and Methods*
 - 5.3 *Results and Discussion*
-

5.1 Introduction

Recently, edible insects have garnered much interest for their potential as an alternative food source and feed with comparable nutritional content to the commonly available conventional animal products (Labu et al., 2021). Entomophagy is a common practice in many regions of Asia, Africa, Australia, and Latin America (mainly Mexico). Still, in Western countries, insects use a new and exciting food matrix (Stoops et al., 2016). In the context of sustainable development, there is an urgent need to propose and implement new methods for production without affecting the quality of food, natural habits, and biodiversity (Megido et al., 2017). Food safety, processing, and preservation are closely related (FAO, 2013), and there are safety concerns associated with the consumption of insects, such as contaminating chemical and biological agents (Garofalo et al., 2019). The increasing introduction of insects into the human diet imposes increasing attention on their safety concerns. It needs proper evaluation of edible insect safety by monitoring the presence of harmful microorganisms (Baiano, 2020). Many studies have reported edible insects as a valuable source of proteins, lipids, carbohydrates, and specific vitamins and minerals. However, there are limited studies on their food safety and shelf life (Stoops et al., 2016). Banjo et al. (2006) reported that insects could harbor many pathogenic bacteria and fungi, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Aspergillus niger*. Earlier studies have also highlighted the presence of different genera of bacteria and fungi in fresh and processed (ready-to-eat) insects such as *Locusta migratoria*, *Tenebrio molitor*, *Acheta domesticus*, *Gryllodes sigillatus* and *Ruspolia differens* (Stoops et al., 2016; Megido et al., 2017; Vandeweyer et al., 2017; Osimani et al., 2018; Garofalo et al., 2019; Labu et al., 2021). Food safety is influenced by how it is produced, stored, processed, and eaten. The rearing environment, rearing procedures, hygiene measures, and insect feed affect

the microbiota of insect species (Wynants et al., 2018). The microbial community of *R. differens* has been reported to be influenced by the environment from which it is harvested and the post-harvest handling conditions and processes (Ssepuuya et al., 2019). Even though insects can act as biological vectors of pathogenic microorganisms, they are considered safe if properly processed and stored (Van Huis et al., 2013). Some bacterial genera such as *Leuconostoc*, *Lactococcus*, *Lactobacillus*, and *Streptococcus* identified in *R. differens* are associated with beneficial effects on human health if ingested in sufficient quantity (Ssepuuya et al., 2019). Edible insects are considered an essential component of traditional food and are highly appreciated as alternative protein sources compared to other conventional meat sources (e.g., beef, chicken, fish, and pork). They are essential nutritional food for the people of Nagaland and are prepared for consumption based on palatability. However, studies on microbiological contaminants are essential for food and consumer safety. In Nagaland, edible insects are mainly collected from the wild and made available at local markets. Members of the Naga tribes are reported to consume 106 species of insects and are particular about how an insect species is cleaned/prepared for consumption. Generally, insects are consumed as boiled, cooked, fried, or roasted (Mozhui et al., 2020). The consumption of dragon nymphs, *Cybister limbatus*, *Hydrophilus caschmirensis*, *Oecophylla smaragdina*, *Provespa barthelemyi*, and *Vespa affinis indosinensis*, as boiled, chutney, cooked or fried is reported (Mozhui et al., 2020). While insects are consumed after being well-cooked by the ethnic communities in Nagaland, the quality and safety of insect consumption is a significant concern for consumer safety globally. In view of the above, this study was conducted to analyze fresh and roasted insect samples for microbial loads to determine the efficiency of heat treatment on microbial contaminants.

5.2 Materials and Method

5.2.1 Insect samples

The study was conducted from July 2021 to December 2021. Insect samples (dragonfly nymphs, *C. limbatus*, *H. caschmirensis*, *O. smaragdina*, *P. barthelemyi*, *V. affinis indosinensis* and *O. fuscidentalis*) for the present study were obtained from different local markets at Dimapur and Kohima (Nagaland, India). Insects were starved for 24 hr before being killed by freezing. Thereafter, the samples were cleaned thoroughly with water. and roasted over low flame (approx.130°C) for 15 minutes. Fresh as well as roasted insect samples were used for further analysis.

5.2.2 Plate counts

Insect samples (0.1g) were crushed using a mortar and pestle. The crushing instrument was autoclaved before use. Samples (n=3) were suspended in 2 ml sterile half-strength nutrient broth solution containing the composition of peptone 15.0 g^l⁻¹, yeast extract 3.0g, Sodium chloride 6.0 g, D(+)-Glucose 1.0 g^l⁻¹, final pH 7.5±0.2 at 25°C temperature and homogenized for 1 min. The suspensions were serially diluted 10 fold (up to 10⁻¹⁰) in isotonic half-strength nutrient broth solution. Growth observed was only visible up to 10⁻⁴ dilutions. From the dilutions, 0.1 ml suspension was placed on pre-prepared nutrient agar Petri plates. Total numbers of aerobic mesophilic microorganisms were determined on plate count agar (BioRad, Hercules, CA, USA) after incubation at 30°C for 72 hr or until colonies appeared on the plate (Megido et al., 2017). After the incubation period, the plates were monitored for several aerobic bacterial colonies that appeared on the surface of nutrient agar. All microbial counts were analyzed in triplicates and expressed as log Cfu g⁻¹.

5.2.3 Statistical analyses

Data were analyzed using the software SPSS version 22. Kruskal-Wallis test was performed to determine significant differences ($p=0.05$) in microbial count between fresh and roasted samples, followed by a pair-wise comparison test.

5.3 Results and Discussion

Microbial load in insects was quantified by calculating the colony count in each insect extract. Differences in the number of microbial colonies were observed in fresh and roasted samples.

Table 5.1 Insect samples tested for the presence or absence of microbial load (+ shows the presence of a single colony, ++ shows the presence of double colonies).

Sl. No.	Insect sample	Edible stage	Mode of sample preparation	Presence/absence of microbes
1	Dragon fly	nymph	fresh	+
			roast	+
2	<i>C. limbatus</i>	adult	fresh	++
			roast	+
3	<i>H. caschmirensis</i>	adult	fresh	++
			roast	+
4	<i>O. smaragdina</i>	larva	fresh	+
			roast	+
5	<i>P. barthelemyi</i>	adult	fresh	+
			roast	++
		pupa	fresh	++
			roast	++
6	<i>V. affinis</i>	larva	fresh	+
	<i>indosinensis</i>		roast	+
7	<i>O. fuscidentalis</i>	larva	fresh	++
			roast	+

Microbial colonies were more in fresh samples than in roasted samples (Table 5.1), which could be due to the heat treatment applied. The moisture content was high in fresh samples and more susceptible to microbial contamination. Similar observations with a higher microbial count in fresh insects such as *Tenebrio molitor*, *Acheta domesticus*, and *Gryllodes sigillatus* were reported by Murefu et al. (2019). The insect samples were further analyzed for the aerobic microbial load. After serial dilution, most

of the samples with 10^{-1} dilution showed confluent growth in aerobic bacteria, following which the population density decreased up to 10^{-4} dilution, where only a countable amount of colony forming units was observed in all the insect samples. However no or less microbial contamination was observed in roasted samples as most of the microbes were killed due to heat during roasting. The aerobic microbial colonies were morphologically characterized by size, color, texture, margin, and surface. Each microorganism followed a particular pattern when grown in the form of culture on plates.

Out of all the colony forming units, some colonies were selected having prominently different morphological features and purified by streaking on nutrient agar Petri plates (Table 5.2). Microbial counts of fresh and roasted samples of different insect species are presented in Table 5.3. The microbial load ranged from 6.30-8.75 log Cfu g⁻¹ (Figure 5.1). The average log Cfu g⁻¹ in fresh insets samples (7.57 ± 0.87) was significantly higher ($t_{(23)}=4.47$, $p<0.05$) as compared to roasted samples (7.07 ± 0.76). From the present study, it is plausible to say that even after roasting, edible insects should be kept in a proper storage facility to avoid any risk of further contamination. A short blanching process before roasting is more effective in reducing the microbial load than dry heat treatment (Murefu et al., 2019). The reason for the high range in the total viable count of fresh and roasted samples could lie in the fact that certain bacteria that are commonly present in insect samples can be inactivated by proper heat treatment. However, spore forming bacteria cannot be entirely eliminated by boiling/roasting (Klunder et al., 2012). In insects such as *Bombyx mori*, *Omphisca fuscidentalis*, and *Gryllus bimaculatus*, moderate amounts of microbial load in frozen forms were also reported (Kurdi et al., 2021). Re-contamination or cross-contamination risks arise if roasted or fried edible insects are not handled or stored hygienically before

consumption. Edible insects will likely be contaminated with pathogenic and spoilage microorganisms during harvesting, packaging, and storage. Therefore, properly storing heat-treated edible insects is essential to reduce the risks associated with consumption. Processing methods such as boiling, followed by open-pan roasting and hot-ash roasting, can reduce microbial contamination. Such reduction in microbial load was observed in *Gonimbrasia belina* (Mujuru et al., 2014; Murefu et al., 2019). Also, simple interventions like drying and acidifying insects with vinegar to pH 4.5 prevent rapid spoilage during storage at room temperature (Klunder et al., 2012). In different households, heat treatments on fresh insects (*T. molitor*), total microbial counts reduced with levels ranging from 2 log CfU after oven cooking to 4/5 log CfU after vacuum cooking and frying until 7 log CfU reduction after boiling (Megido et al., 2018).

Table 5.2 Morphological characters of microflora extracted from different insect samples

Insect sample	Edible stage	Processing mode	Morphological features
Dragon fly	nymph	fresh	Two types of colonies 1. Medium in size, opaque with smooth edges. 2. Small, opaque in the center, and translucent at the edges.
		roast	Two types of colonies 1. Medium size, white, smooth 2. Large smear of orange texture, wavy margin
<i>C. limbatus</i>	adult	fresh	Small, translucent, convex surface and smooth margin
		roast	Single large colony, cream, smooth margin, and flat surface
<i>H. caschmirensis</i>	adult	fresh	Two types of colonies observed 1. Medium, cream with slightly rough margin, convex surface. 2. Small, cream smooth margin, convex surface.
		roast	Single small cream colony, smooth margin, convex surface
<i>O. smaragdina</i>	larva	fresh	Two types of colonies 1. Very large, cream, flat surface, smooth margin

		roast	2. Small, pale yellow, convex surface, smooth edges. Two types of colonies
<i>P. barthelemyi</i>	adult	fresh	1. Large, opaque, smooth margin 2. Small, opaque, smooth
			Two colonies observed
	pupa	fresh	1. Medium size, creamy texture colony with smooth surface and margin 2. Small colonies, smooth surface, and convex margin
			Medium size, cream texture, translucent margin
<i>V. affinis indosinensis</i>	larva	roast	Two types of colonies observed
		fresh	1. Large, creamy pink with a slightly rough margin and flat surface 2. Creamy in the center and translucent margin, convex surface.
			Single large white colony with a flat surface and rough margin
<i>O. fuscidentalis</i>	larva	roast	Heavy load of bacteria with two types of colonies.
		fresh	1. Medium, cream texture, flat surface 2. Medium, cream texture, convex surface
			Small colonies, cream color, smooth surface, soft margin.
		roast	Single large colony with rough and wavy edges and flat surface
		roast	Small colonies, creamy texture, convex surface, and opaque

However, considering cultural preferences and organoleptic aspects of edible insects, further investigation of these promising interventions is needed. The presence of contaminants even after roasting shows that roasting may not entirely kill the microbiota and needs further heating treatment. Therefore, the effectiveness of various traditional ways of preparation and decontamination of an insect as food, such as drying, frying, boiling, and smoking, needs to be assessed for microbial safety as most of the contamination occurs during sun drying and poor storage conditions (Mpuchane et al., 1996; Gashe et al., 1997; Simpanya et al., 2000; Abu-Ghannam and Crowley, 2006; Rumpold et al., 2014). After spreading the insects' cultures at different dilutions, a change in the number of colonies and counts was observed. Treating insects up to

100°C removed most bacterial contaminations from insects, however, results showed that temperature treatment was insufficient to kill the microbiota. The persistence of microbial load in insects could be due to microorganisms in the insects' gut. While insects are consumed after proper heat treatment, consumers should avoid consuming the insects' digestive tracts as different microbes are found in their gut. Despite proper heat treatment, wrong methods of storage conditions can cause food-borne illness and higher risks of microbial contamination (Klunder et al., 2012). Generally, hyalinization by sterilization, pasteurization, blanching (i.e., immersion in hot water in a temperature range of 80–100°C), or roasting is recommended for consumer safety (Abu-Ghannam and Crowley, 2006). In a study conducted by Adamek et al. (2018), the processing of insects by boiling in water, drying at 103°C for 12 hr, and hermetic packaging proved to be promising for long-term storage of *T. molitor*, *Gryllus assimilis*, *L. migratoria* and *Alphitobius diaperinus*. As observed in the present study, fresh and roasted insects require further treatment for consumer safety. Drying or dehydration by sun drying, roasting, freeze-drying, smoke drying, and oven-drying of insects is done to increase the shelf life of insect products (Hernández-Álvarez et al., 2021). Fresh insects should freeze at -20°C, while for dried and powdered insects, refrigeration is reported to be the best way to prevent oxidative and microbial degradation (Grabowski and Klein, 2017). Additionally, vacuum storage conditions for ready to-eat *Ruspolia nitidula* helped to maintain microbial, sensory, and chemical properties for 22 weeks, similar to low-temperature storage (Ssepuuya et al., 2017). A species-specific processing step with anti-microbial effect and precise characterization of insects' microbiota is required to avoid or minimize risks involved with edible insects' consumption and ensure that the product is harmless (Megido et al., 2017; Vandeweyer et al., 2017).

Table 5.3 Microbial counts (log cfu/g) from insect samples

Insect samples	Stage analyzed	Processing mode	Microbial counts (log Cfu g ⁻¹)
Dragonfly	nymph	fresh	8.57±0.03 ^{ab}
		roast	8.65±0.01 ^a
<i>C. limbatus</i>	adult	fresh	6.78±0.00 ^{cd}
		roast	6.30±0.00 ^c
<i>H. caschmirensis</i>	adult	fresh	6.97±0.06 ^{bcd}
		roast	6.30±0.00 ^{de}
<i>O. smaragdina</i>	larva	fresh	7.84±0.03 ^{abc}
		roast	7.20±0.05 ^{abcde}
<i>P. barthelemyi</i>	adult	fresh	8.40±0.00 ^{abc}
		roast	7.46±0.02 ^{ab}
	pupa	fresh	6.97±0.06 ^{bc}
		roast	6.30±0.00 ^{cde}
<i>V. affinis indosinensis</i>	larva	fresh	8.75±0.01 ^a
		roast	7.50±0.03 ^{ab}
<i>O. fuscidentalis</i>	larva	fresh	6.30±0.00 ^a
		roast	6.86±0.07 ^{bcd}

#Data are the mean of three replicates ± standard deviation.

Mean values with different letters in the column differed significantly at $p=0.05$.

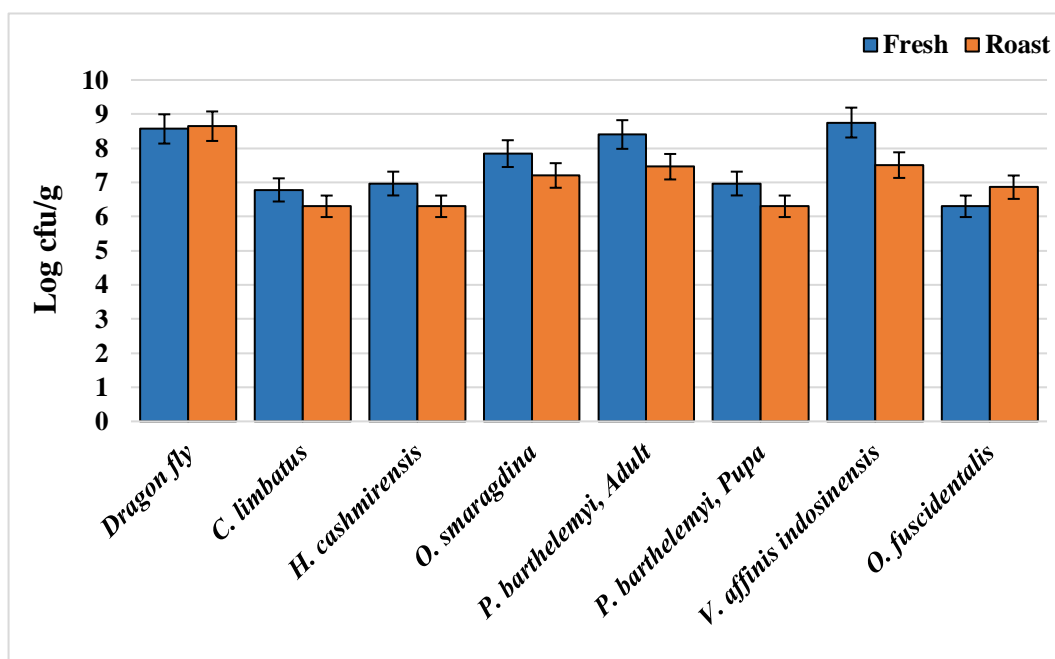


Figure 5.1 Microbial count (log cfu/g) in fresh and roasted insect samples.

Several studies have suggested that microbes present in a particular matrix or ecological niche represent only a tiny fraction of the total microbial community. Food uncultivable pathogens need to be addressed. Therefore, the limited data on the

microbial community structure of edible insects could be incomplete and require next-generation sequencing technologies to study microbial communities in diverse environments (Stoops et al., 2016). The potential presence of microbiological hazards for human health in insects is affected by combining the substrates used and the processing steps applied between farming and consumption (van der Fels-Klerx et al., 2018). Wynants et al. (2019) investigated the transmission of *Salmonella* sp. to mealworms (*T. molitor*), from contaminated wheat bran as substrate. They reported that the survival of *Salmonella* sp. in larvae and bran depended on the contamination level. *Salmonella* sp. was not detected in the larval samples at a starting contamination level of 2 log CfU g⁻¹. However, it was higher in bran initially contaminated with 7 log CfU g⁻¹. Insects can remain free from pathogens contamination if the farms are properly managed with no contact of wild insects that are pathogens carriers (Belluco et al., 2013). Therefore, possible contamination with microbiological hazards is likely influenced by the nature of the substrate, the hygienic conditions, and the farming environment (Van der Fels-Klerx et al., 2018). As the study shows that more microbial colonies are found in fresh insects than in heat-treated samples, roasting is sufficient for some insect species to inactivate/eliminate contamination. However, most edible insects need more processing to deem them safe for consumption. Even after freezing or heat treatment, contamination occurs due to improper handling of the insect samples, which leads to re-contamination. The preferable edible insects are frequently sold in a dried form packed in plastic film, with or without oxygen and/or moisture absorber (Fasolato et al., 2018). Further research needs to be undertaken to identify specific microbes/food pathogens to develop microbial quality and parameters to ensure consumer safety as well as safe production of edible insects, devising better insect species-specific processing methods, proper storage of processed insects for consumer safety of the

given product, as well as transport of insects and insect-based products to successfully facilitate the introduction of such products into the global market. Human food safety has to be considered before opening the insect's product to the global market. Therefore, the present study highlights the efficiency of roasting edible insects in reducing or eliminating microbial contamination that could help preserve the product for a longer period making it safer for consumers.

Chapter 6

Nutritional and antinutritional properties of certain edible insects in Nagaland, India

Contents

- 6.1 *Introduction*
 - 6.2 *Materials and Methods*
 - 6.3 *Results*
 - 6.4 *Discussion*
-

6.1 Introduction

The potentialities of edible insects as food and feed get its attention and have been identified to be a promising alternative protein source to alleviate the global food insecurity. Edible insects are deemed an excellent source of nutrients consisting of high-quality protein, essential amino acids, unsaturated fatty acids, vitamins, fiber, and minerals, with a comparable level of animal protein sources such as beef or chicken (Aguilar-Toala et al., 2022). Several studies on edible insect exhibited variety of health benefits (antioxidant, antimicrobial, anti-inflammatory, anti-tumor, anti-aging, and hypolipidaemic activity) which may be attributed to the presence of bioactive compounds such as fatty acids, phenolic compounds, peptides, chitin and chitosan (Zielińska et al., 2018; Di Mattia et al., 2019; Mohan et al., 2020; Nino et al., 2021). Thus, edible insects not only serve as an alternative protein source but can be explored in the nutraceutical and food industries due to the presence of a wide spectrum of bioactive compounds.

Many of the world's indigenous populations rely heavily on edible insects as a food source (Hlongwane et al., 2020) and insects are one of the best alternative protein requirements with a low environmental impact (Van Huis, 2013). In Nagaland also Insects have played important dietary components from time immemorial and the indigenous people still depend on cultural diet prepared in ethnic ways from edible insects which are either collected wild or from local markets (Chakravorty, 2014; Mozhui et al., 2017, 2020; Pongener et al., 2019; Kiewhuo et al., 2022). A large number of edible insects such as grasshoppers, cicadas, crickets, water beetles and giant water bugs etc are collected mainly from nature and are consumed more in rural area than urban.

Given the fact that the indigenous people of this geographical area are associated with nature, certain insects such as *Omphisa fuscidentalis*, *Cybister limbatus*, *Samia cynthia ricini*, *Cossus sp.* larva and *Vespa mandarinia* are steamed/boiled/fried or roasted and considered as a delicacy thus substituting a major protein diet in Nagaland. They also often rely on insects as a primary source of remedy for various physical ailments *i.e.* boiled pupa and larva of *Vespa mandarinia* are used for controlling blood sugar level and sharpen memory; oral administration of *Lethoceros indicus* nymph to treat gastrointestinal diseases and as blood purifier (Mozhui et al., 2020; Mozhui et al., 2021; Kiewhuo et al., 2022). Regardless of the high consumption rate of edible insects in the study area, information about the nutritive value of these edible insects is scarce. Further, processing of insects for human consumption influences not only their nutritive value, but also the consumer's choice/preference and acceptance (Ghosh et al., 2018) and indicate consumers about the quality and ideal quantity of insect intake. Hence the present study is designed to evaluate the nutritional profiles, amino acids, fatty acids and minerals of most popular edible insects (both fresh and processed form) with regard to locally available traditional diets along with their mode of consumption and edible form (adult, larva or pupa). The taxonomic position, consumption stage, and method of preparation are noted in Table 6.1.

6.2 Materials and methods

6.2.1 Insect samples collection and extraction

The present investigation was designed to evaluate proximate composition, antioxidant and antinutritional properties; amino acids, fatty acids and minerals of fourteen different species viz., *Tarbinskiellus portentosus*, *Mecopoda nipponensis*, *Schizodactylus monstrosus*, *Aspongopus nepalensis*, *Lethoceros indicus*, *Laccotrephes ruber*, *Oecophylla smaragdina*, *Provespa barthelemyi*, *Cybister limbatus*, *Hydrophilus*

caschmirensis, *Samia cynthia ricini*, *Cossus sp.* , and Dragon fly, Wood larva (Figureure 1A-N) which were collected from 12 districts of Nagaland (26°45' N to 27°12' N latitude and 93°39' E to 94°35' E longitudes) during 2018-2022. It is to be noted that along with the adult stages, analysis for larval sages of *Oecophylla smaragdina* and pupal and larval stages of *Provespa barthelemyi* was conducted. However, as adult stage is not consumed, biochemical analysis *Samia cynthia ricini* was restricted to pupal and larval stages only. The collected insects/insect samples were later killed by freezing at 0°C, and the sorted species were washed and cleaned of dust before being sun-dried and then oven-dried at 50 °C for 24 hrs. To keep the edible portion, the inedible parts such as the wings and legs were removed with scissors. After that, the edible portions of each insect species were pulverized separately in a mechanical grinder to obtain a fine powder. The insect powder was sieved with a fine pore strainer to obtain a homogeneous powder, which was stored in airtight plastic containers at - 80°C for further biochemical analysis/downstream experiments. All the estimation was done in different phases of edibility i.e fresh, fried and roasted form.

Oven-dried powdered samples (10g) were extracted using a homogenizer in 100 ml of 20 m M phosphate buffer, pH 7.2. The extract was filtered using double-layered cheese cloth followed by centrifugation at 12,000 rpm for 30 min at 4 °C. The supernatant was used for the estimation of total protein, total starch, total sugar, total phenolic, and anti-oxidant content.

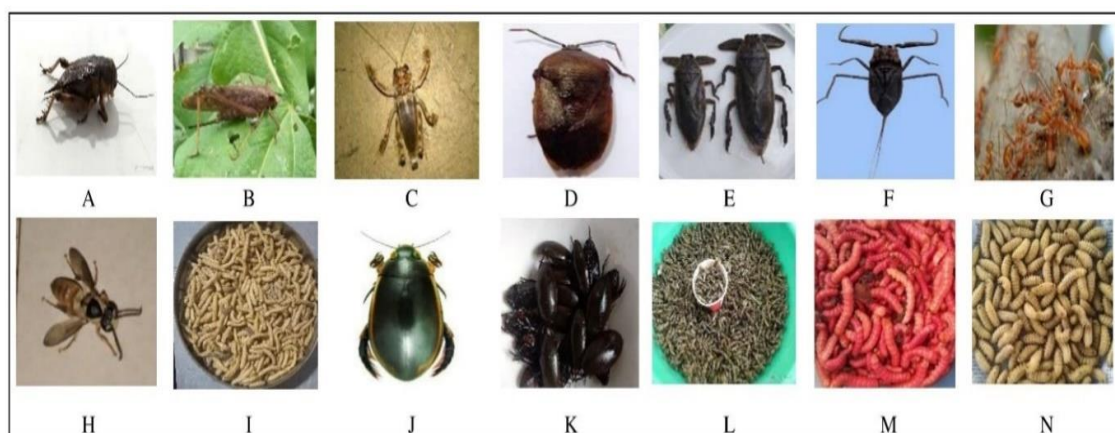


Figure 6.1. Insect species used for the study. A- *Tarbinskelius portentosus*; B- *Mecopoda nipponensis*; C- *Schizodactylus monstrous*; D- *Aspongius nepalensis*; E- *Lethoceros indicus*; F- *Laccotrephes ruber*; G- *Oecophylla samaragdina*; H- *Provespa barthelemyi*; I- Wood larva; J- *Cybister limbatus*; K- *Hydrophilus caschmirensis*; L- Dragon fly; M- *Cossus* sp.; and N- *Samia cynthia ricini*.

6.2.2 Crude protein

Protein content was estimated following Bradford (1976). Homogenate samples were diluted in deionized water, and 50 μ l of homogenate samples were transferred into the microtiter plate well and mixed with 125 μ l of Bradford's reagent (Sigma, USA). The reaction was incubated for 5 min and the absorbance was taken at 595 nm with multi-plate reader (SpectraMax plus 384). The protein content was calculated using a linear equation obtained from the BSA calibration curve.

6.2.3 Crude lipid

Oven-dried powdered samples (20 g) were extracted using Soxhlet's apparatus with chloroform and methanol in a ratio of 2:1 for 6 hr. The extract was collected and evaluated for total lipid content. The total lipid content of the insect samples was estimated using slightly modified sulpho-phospho-vanillin method developed by Bligh and Dyer (1959). Briefly, 1.0 ml of the extracts were incubated in a water bath for complete evaporation of the chloroform-methanol mixture, followed by cooling for 3

min at room temperature. Further, 2.0 ml of concentrated sulphuric acid was added to the sample tube and boiled, followed by boiling in a water bath at 100 °C for 10 min. The reaction tubes were cooled to room temperature and 5.0 ml of 1% phosphovanillin was added and allowed to stand at room temperature for 15 min. Due to the reactions of lipids present in the sample with sulphuric acid, o-phosphoric acid and vanillin, the mixture turns rose red in colour. The absorbance was measured at 540 nm by multimode reader (Thermo scientific Multiskan Go). Cholesterol was used as reference compound.

6.2.4 Total carbohydrate

The phenol-sulfuric acid method according to Dubois (1956) was used for total carbohydrate content determination. Briefly, 0.5 ml of homogenate sample was transferred to screw-capped tubes, to which 2.5 ml of concentrated H₂SO₄ was added carefully, followed by 0.5 ml of 5 % phenol. The cocktail was mixed thoroughly and incubated for 5 min at 90 °C. After cooling, the absorbance were measured using a microplate reader (SpectraMax plus) at 490 nm. Data obtained were calculated for carbohydrate content in glucose equivalent using the linear equation obtained from glucose calibration curve.

6.2.5 Total phenol

Total phenol in the sample was estimated using Folin - Ciocalteau Reagent (FCR) by Singleton and Rossi (1965) with slight modification. Briefly, 0.5 ml of sample homogenate was mixed with 0.1 ml of FCR and incubated at room temperature for 15 min. To the mixture, 2.5 ml of 15% sodium carbonate was added, shaken vigorously and further incubated for 30 min. The absorbance was measured using a microplate reader at 760 nm. Gallic acid was used as the reference compound.

6.2.6 Total antioxidant

Total antioxidant was determined using phosphor-molybdenum method described by Prieto et al. (1999) with minor modification. Briefly, 0.3 ml of sample extract was mixed with 3.0 ml of reagent consisting of 0.6 M sulphuric acid, 4.0 mM ammonium molybdate, and 28 mM sodium phosphate and incubated in a water bath at 90 °C for 90 min. Once the phospho-molybdenum green complex is formed, the reaction mixture is allowed to cool and measured at 695 nm by microplate reader (SpectraMax plus 384). L-ascorbic acid was used as a standard.

6.2.7 Screening of Amino acids

The amino acid of the insect sample was analyzed according to Dhillon et al. (2014). Briefly, 100 mg of powdered insect samples were lyophilized for 90 min. The lyophilized insect samples were hydrolyzed with 200 µl of boiling 6 N HCl and 40 µl of phenol by vapour-phase hydrolysis followed oven drying at 114±2 °C for 24 hr. The excess HCL was removed; the tubes were vacuum dried for 90 min, and reconstituted with 200 µl of 20 mM boiling HCL. A 20 µl of the reconstituted insects' samples were derivatized with 60 µl AccQ-fluor borate buffer, and 20 µl of AccQ-fluor reagent (AccQ-Fluor reagent kit, WAT052880-Waters Corporation, USA). The sample vials were heated at 55 °C in a block heater prior to amino acid analysis using High Performance Liquid Chromatography (HPLC). The AccQ-fluor amino acids derivatives were separated using Waters 2707 modules HPLC Systems attached to a PDA 2998. A 10 µL of the sample derivative was injected into a C18 Column of Waters reversed phase AccQ Tag Silica-bonded Amino Acid Column (Waters Corporation, Milford, USA) equipped with an auto injector and dual absorbance detector. The Waters AccQ Tag Eluent A Concentrate (WAT052890) was diluted to 10% in Milli-Q water and used as eluent A, and 60% acetonitrile as eluent B in a separation gradient with a flow rate of 1.0 ml/min. The ultraviolet (UV) detection for amino acid was set at 254 nm and the

temperature of the column was maintained at 37 °C. The Empower pro software® by Waters Corporation (2005-08) was used for analyzing the amino acid peaks and calculated based on amino acid calibration standard (Thermo Scientific Amino Acid Standard H, Prod # NCI0180) run at 5 concentrations (10, 20, 30, 40, and 50 µl) and expressed as µg/100 g of the insect sample.

6.2.8 Screening of Fatty acids

The fatty acids profile of insects was analyzed by a standardized protocol developed by Kumar and Dhillon, 2015. Briefly, 100 mg of lyophilized powdered samples were grounded in 10 ml solvent mixture of chloroform: Hexane: Methanol (8:5:2, V/V/V) followed by overnight extraction using extraction solvent. The filtrates were evaporated at 60 °C using nitrogen gas for 30 min followed by conversion to methyl esters. A 1.0 µl of the sample was injected with a split ratio of 20:1 in gas chromatography using Rtx®-5MS column. The lipophilic compounds were separated using GCMS-QP2010 Ultra system with auto sampler AOC-20i, Shimadzu (Japan). Helium gas was used as carrier at column flow rate of 1.2 ml/min and a pressure of 123.5 kPa. The temperatures of injector, interface and the ion source were maintained at 250, 270 and 230 °C, respectively. The temperature was programmed at 180 °C consisting of an initial 2 min isothermal heating, followed by 5 °C/min ramp up to 280 °C, hold for 5 min and increased to 300 °C with a ramp rate of 20 °C/min and holding time of 10 min. Mass spectra were performed between 2.8-30.0 min run time at 2 scans/s with m/z 50–650 scanning range. The chromatograms and mass spectra were obtained using the Lab solutions GCMS solution software version 2.71 (Shimadzu, Japan). The fatty acids identification was obtained by database search using MS libraries (NIST08, Wiley8).

6.2.9 Statistical analysis

The data reported are an average of triplicate observations. Statistical analysis was performed by one way analysis of variance (ANOVA) at a 95% confidence level and Turkey's multiple comparisons test at $p < 0.05$ using SPSS (version 22).

6.3 Results

6.3.1 Total protein content (mg/g)

The average amount of protein content of all insects in fresh, fried, and roasted forms were 7.87 ± 1.18 , 3.38 ± 0.98 , and 2.89 ± 1.26 mg/g, respectively (Figure 6.2). Protein concentration in processed forms was significantly ($F_{(2)}=293.8$, $p<0.05$) lower compared to fresh samples. Further, the differences have also been recorded in various edible stages of *O. smaragdina* (adult and larvae), *P. Barthelemy* (Adult, pupae, and larvae), and *S. cynthia ricini* (Pupae and larvae). Protein content varied from 4.9 ± 0.09 (*O. smaragdina*, Adult) to 9.21 ± 0.53 mg/g (*S. cynthia ricini*, Larva) in fresh form, from 1.13 ± 0.31 (*O. smaragdina*, Larva) to 5.32 ± 0.14 (*P. barthelemyi*, Pupa) in roasted form and from 1.02 ± 0.18 (*O. smaragdina* Adult) to 4.32 ± 0.15 (*C. limbatus*) in fried form (Figure 6.3).

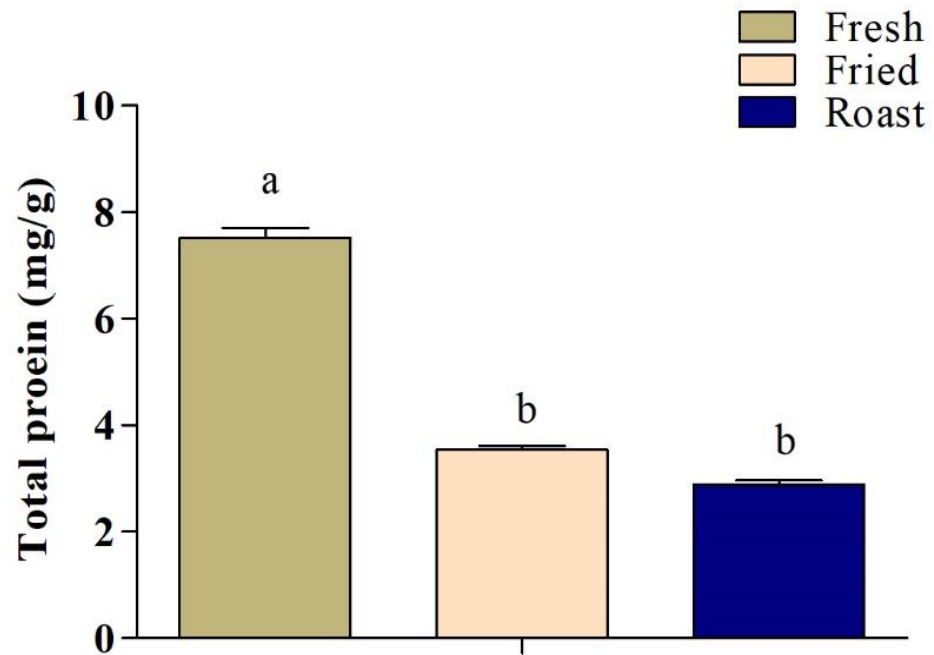


Figure 6.2. Variations of Protein content in fresh, fried, and roasted forms of insect

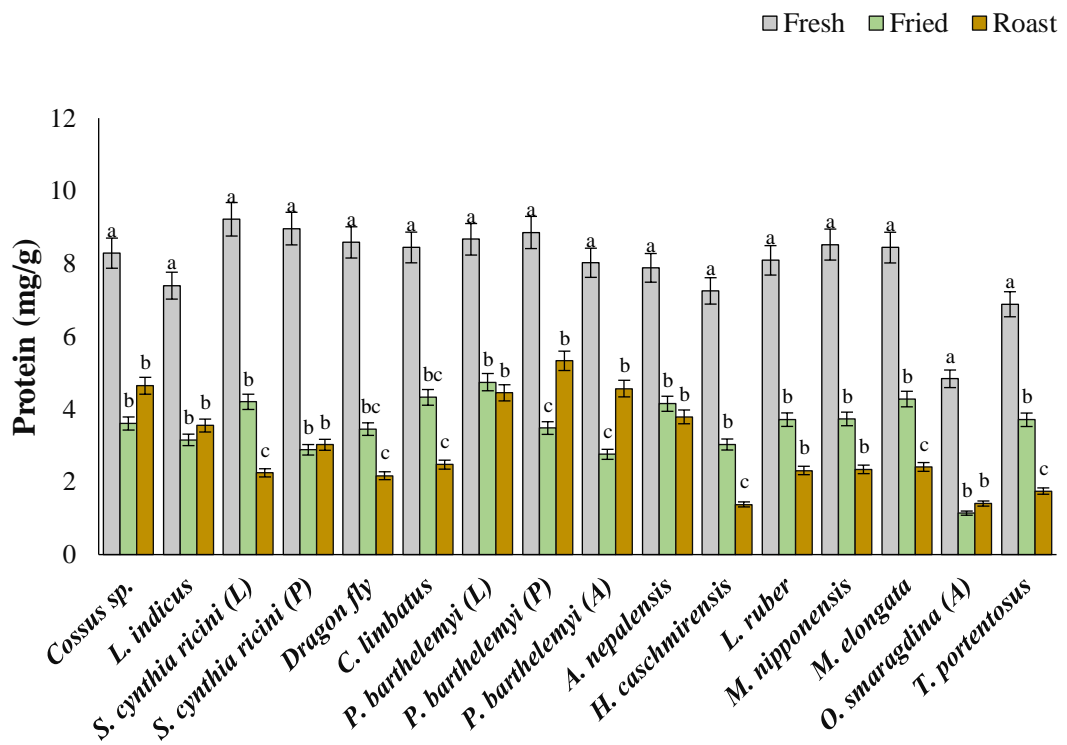


Figure 6.3 Various edible insects with their protein content in fresh friend and roasted forms.

6.3.2 Total lipid content (mg/g)

The average amount of lipid content of all insects in fresh, fried, and roasted forms were 0.32 ± 0.31 mg/g, 0.78 ± 0.33 mg/g and 1.25 ± 0.85 mg/g respectively. Multiple comparison test shows that the amount of lipids in fresh > fried > roasted samples were significantly different ($F_{(2)}=68.06$, $p<0.05$) (Figure 6.4). Further, difference has also been recorded in various edible stages of *O. smaragdina* (adult and larvae), *P. barthelemyi* (Adult, pupae and larvae) and *S. cynthia ricini* (Pupae and larvae) (Figure 6.5). Lipid content varied from 0.07 ± 0.01 (*T. portentosus*) to 0.53 ± 0.04 (*Cossus* sp.) in fresh, 1.09 ± 0.08 (*T. portentosus*) to 1.27 ± 0.08 (*Cossus* sp.) in fried, and 0.16 ± 0.01 (*P. barthelemyi* Pupa) to 5.51 ± 0.07 mg/g (*P. barthelemyi* adult) in roasted form. On frying and roasting, the concentration of lipid content increased in all insects except in roasted larval and pupal forms of *S. cynthia ricini* (Figure 6.5).

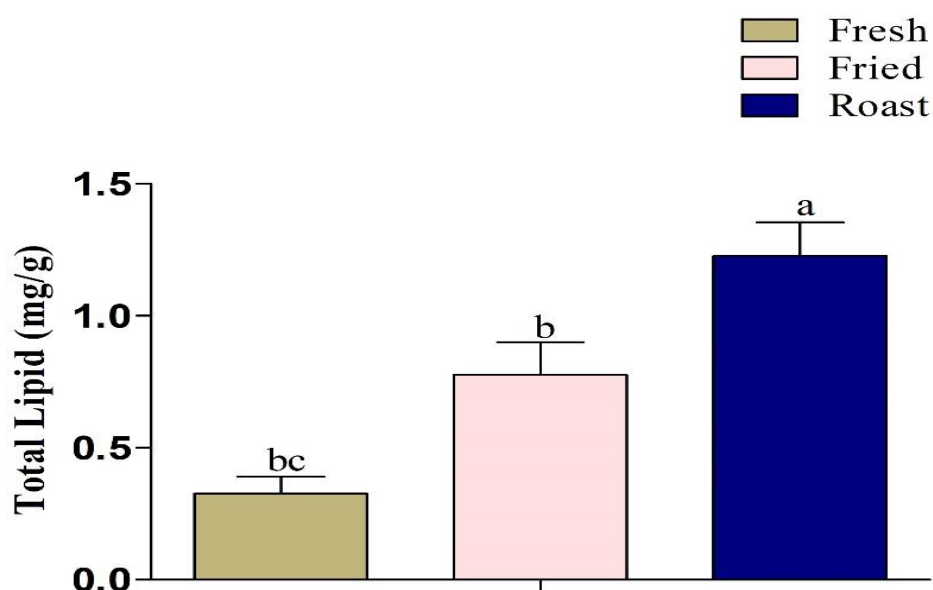


Figure 6.4 Variations of lipid content in fresh, fried, and roasted forms of insects

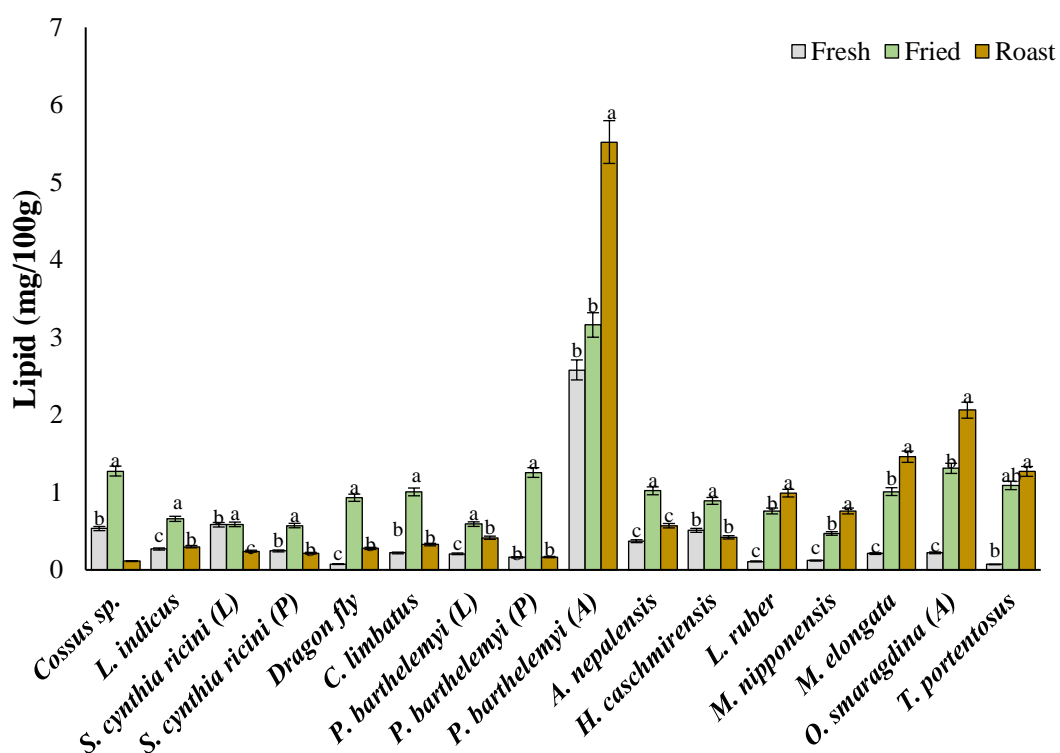


Figure 6.5 Various edible insects with their lipid content in fresh friend and roasted forms.

6.3.3 Carbohydrate content (mg/g)

The average amount of carbohydrate in fresh, fried, and roasted forms were 2.67 ± 0.15 , 3.96 ± 0.24 , and 3.86 ± 0.3 mg/g respectively (Figure 6.6). Multiple comparisons test showed that carbohydrate content varies significantly ($F_{(3)}=2.43$, $p=0.029$) in different forms of preparations. In insects such as *P. barthelemyi* and *S. cynthia ricini*, wide variations in concentrations of carbohydrate were observed in different stages of development (adult, pupa, and larva). Carbohydrate content varied from 0.26 ± 0.08 (*L. ruber*) to 6.79 ± 0.87 (dragon fly) in fresh, 0.65 ± 0.06 (*L. indicus*) to 12.17 ± 3.78 (*Cossus* sp.) in fried, and 0.56 ± 0.34 (*L. ruber*) to 13.13 ± 3.88 mg/g (*Cossus* sp) in roasted form (Figure 6.7).

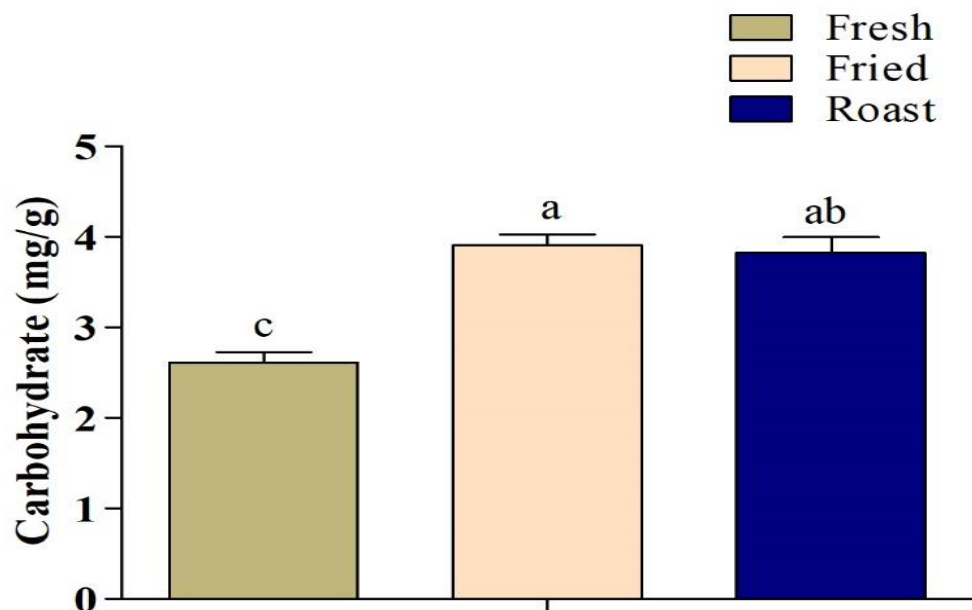


Figure 6.6 Variations of carbohydrate content in fresh, fried, and roasted forms of insects

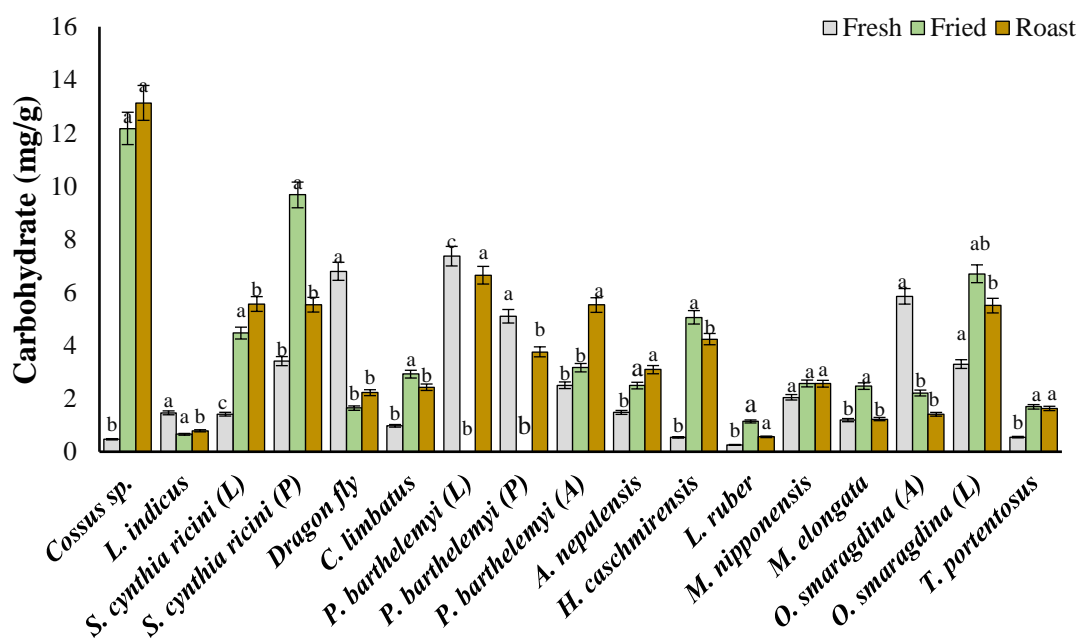


Figure 6.7 Various edible insects with their carbohydrate content in fresh, fried and roasted forms.

6.3.5 Total phenol content (mg/g)

Total phenol content varied widely depending on the species, developmental stages, and preparation methods. The average amount of total phenol in fresh, fried, and roasted forms were 3.96 ± 0.28 , 5.2 ± 0.46 , and 6.52 ± 0.41 mg/g, respectively (Figure 6.8). Multiple comparisons test shows that total phenol content was significantly different among the preparation's methods ($p < 0.05$). Total phenol ranges from 1.3 ± 0.18 (*C. limbatus*) to 5.54 ± 0.39 (*S. cynthia ricini* (P)) in fresh, 1.83 ± 0.18 (*C. limbatus*) to 10.2 ± 0.61 (*S. cynthia ricini* (L)) in fried, and 1.6 ± 0.29 (*Cossus* sp.) to 10.4 ± 1.1 (*S. cynthia ricini* (L)) in roasted form (Figure 6.9). Variations of total phenol in different species of insects and the forms of preparations are shown in Figure 6.10.

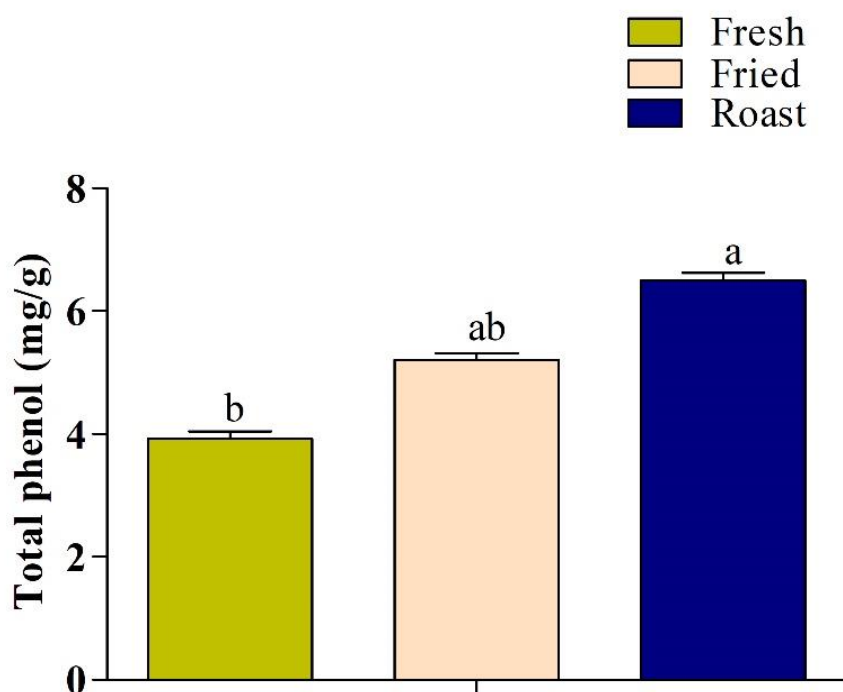


Figure 6.8 Average amount of total phenol in fresh, fried, and roasted forms of insects.

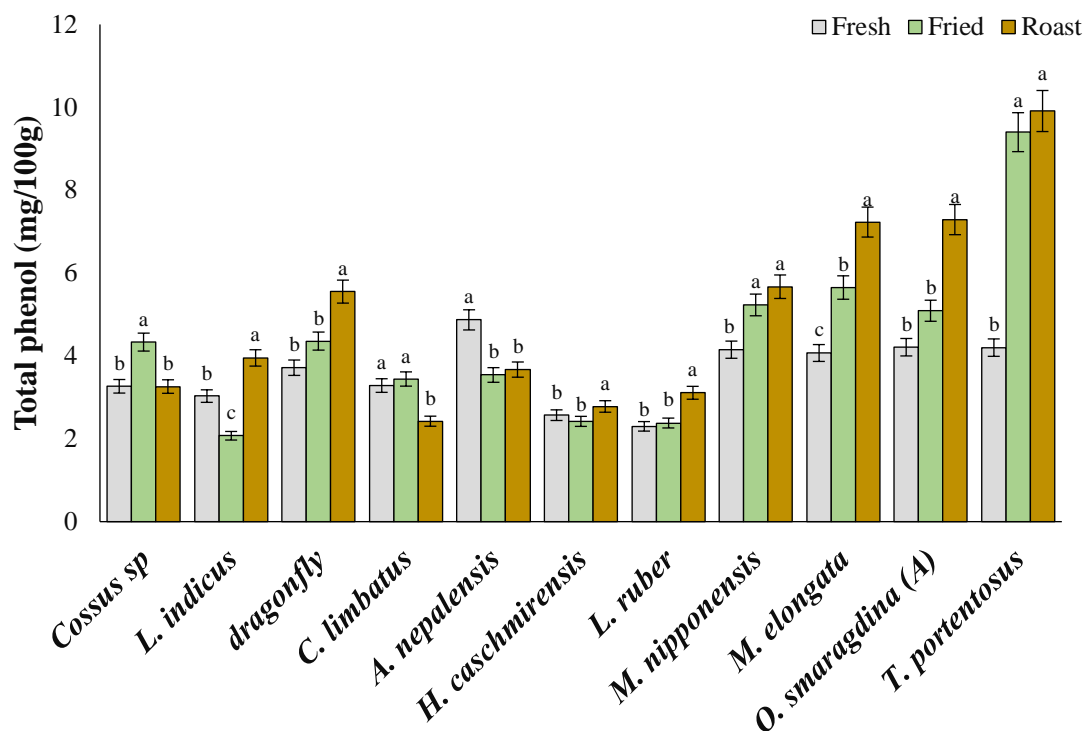


Figure 6.9 Various edible insects with their total phenol content in fresh fried and roasted forms

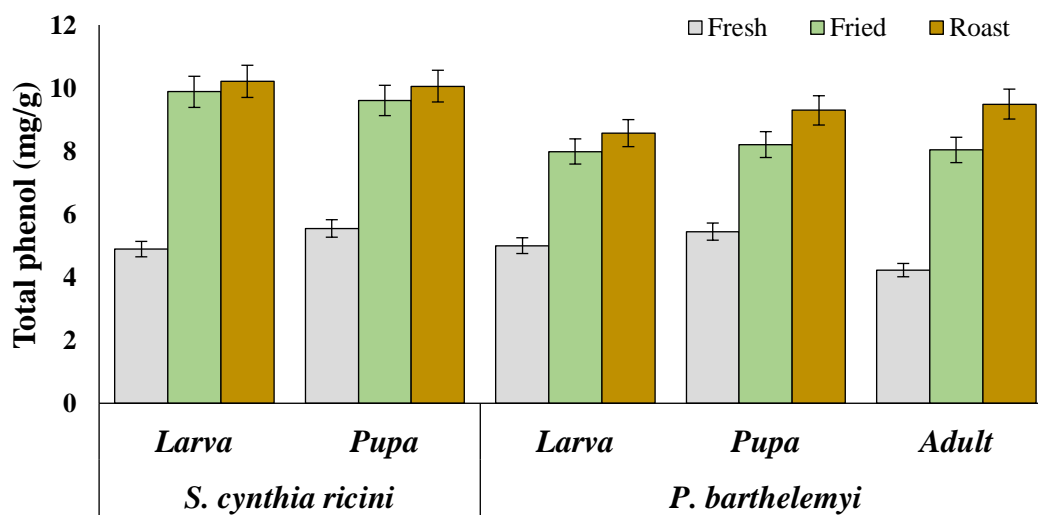


Figure 6.10 Total phenol content in various stages of development of *S. Cynthia ricini* and *P. barthelemyi*

6.3.6 Total antioxidants (mg/g L-ascorbic acid equivalent)

The average concentrations of total antioxidants in fresh, fried, and roasted forms were 4.25 ± 0.34 , 5.29 ± 0.38 , and 5.35 ± 0.34 mg/g L-ascorbic acid equivalent. Total antioxidant concentrations range from 1.02 ± 0.05 (*Cossus* sp.) to 9.18 ± 0.42 (Dragon fly) in fresh, 1.35 ± 0.13 (Dragon fly) to 13.47 ± 0.05 (*H. caschmirensis*) in fried and 1.09 ± 0.18 (*L. indicus*) to 10.4 ± 1.1 (*S. cynthia ricini* (P) in roast (Figure 6.12). In the larva and pupa forms of *S. cynthia ricini* the total antioxidant concentrations were 5.92 ± 0.45 and 5.74 ± 0.32 while in *P. barthelemyi* concentrations were 6.12 ± 1.1 , 5.37 ± 1.01 , and 6.85 ± 0.33 in larva, pupa and adult form (Figure 6.13).

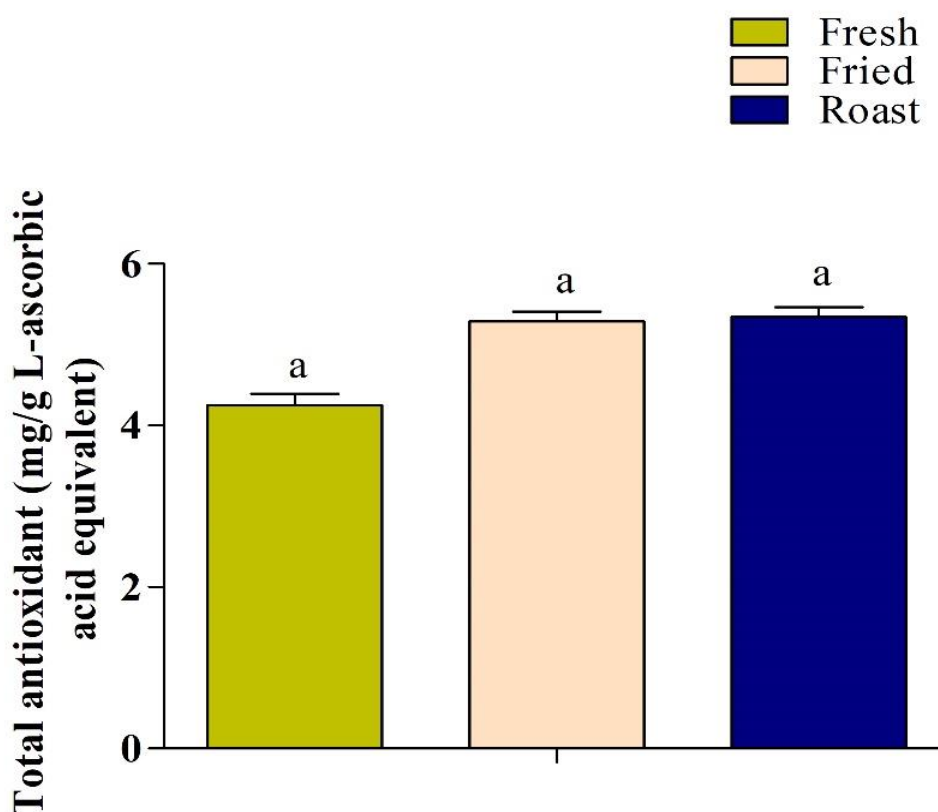


Figure 6.11 Average amount of total antioxidant in fresh, fried, and roasted forms.

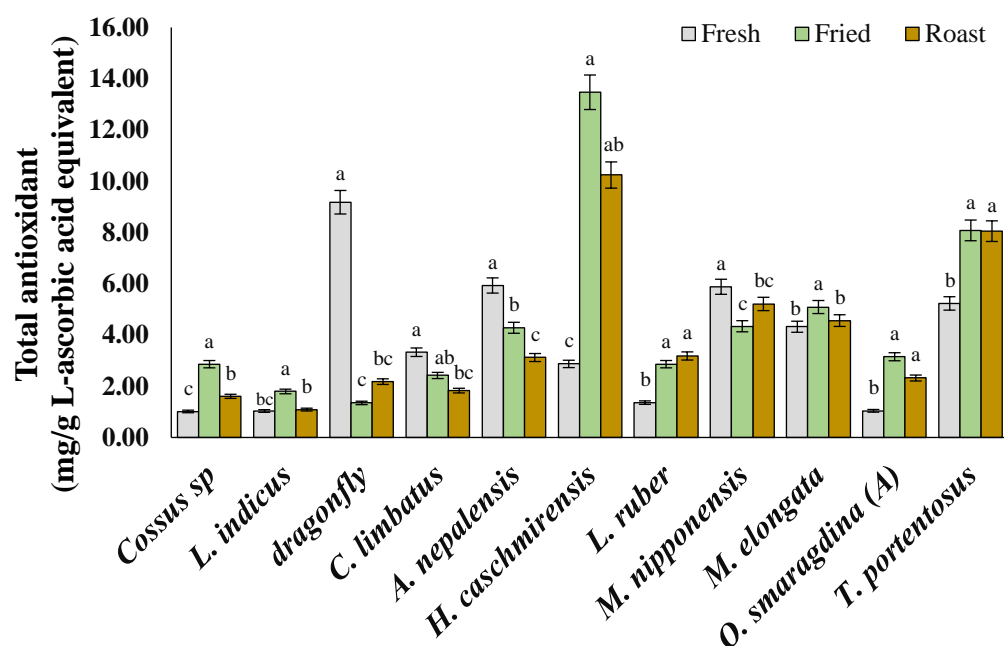


Figure 6.12. Various edible insects with total antioxidant content in fresh fried and roasted forms

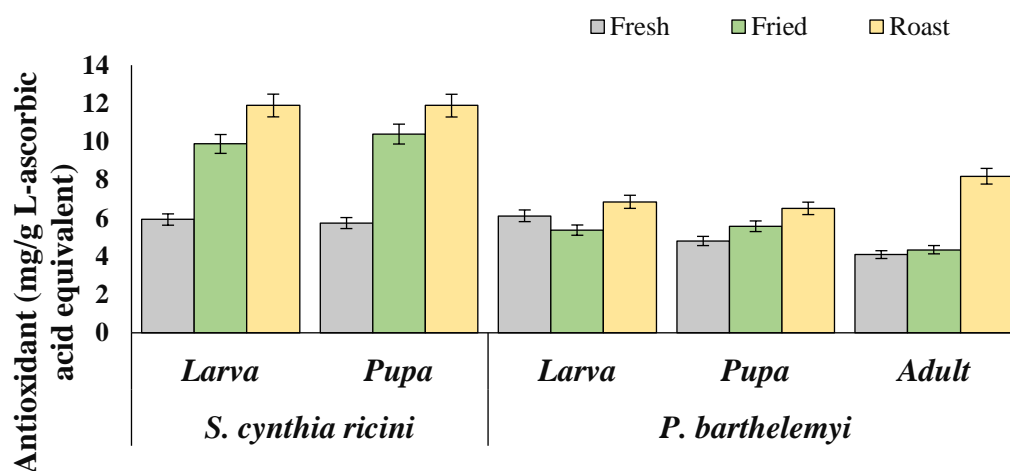


Figure 6.13. Total antioxidant content in various stages of development of *S. Cynthia ricini* and *P. barthelemy*

6.3.7 Screening of amino acids in edible insects

6.3.7.1 Essential amino acids (ug/100g) in fresh form

The present study observed a wide variation of amino acids depending on insect species, their developmental stages and preparation methods. The amount of histidine (His) ranged from 0.26 (*Cossus* sp.) to 22.56 (*P. barthelemyi*, Larva). Isoleucine ranged from 0.67 (*Cossus* sp. larva) to 78.42 (*P. barthelemyi* larva), leucine (Leu) ranged from 1.99 (*S. cynthia ricini* larva) to 81.09 (*P. barthelemyi* larva), lysine (Lys) ranged from 0.53 (*Cossus* sp.) to 48.54 (Wood larva), Methionine (Met) ranged between 0.29 (*Cossus* sp.) to 47.59 (*P. barthelemyi* larva), Phenylalanine (Phe) ranged between 0.30 (*Cossus* sp.) to 97 (*P. barthelemyi*), Threonine (Thr) ranged between 0.58 (*Cossus* sp. larva) to 27.89 (*P. barthelemyi* larva), and Valine (Val) ranged from 0.77 (*Cossus* sp. larva) to 61.18 (*P. barthelemyi* larva) (Table 1). The peaks of different amino acids in fresh forms of insects such as *O. fuscidentalis*, Wood larva, *A. nepalensis*, and *P. barthelemyi* are shown in Figure 6.14.

Table 6.1. Essential amino acid (ug/100g) profile of different edible insects in fresh form

Name	His	Ile	Leu	Lys	Met	Phe	Thr	Val
<i>A. nepalensis</i>	0.38	8.89	13.56	4.19	4.41	6.23	21.78	14.59
<i>Cossus</i> sp (larva)	0.26	0.67	2.07	0.53	0.29	0.30	0.58	0.77
<i>C. limbatus</i> (adult)	1.59	5.44	6.06	7.32	3.78	4.13	5.59	7.83
<i>H. cashmirensis</i> (adult)	1.88	11.07	16.09	5.30	4.00	7.22	7.28	12.88
<i>L. ruber</i> (adult)	0.54	12.56	38.29	6.87	7.36	10.18	0.85	18.81
<i>L. indicus</i> (adult)	1.69	3.02	4.55	2.90	4.70	2.37	5.13	4.26
<i>M. elongata</i> (adult)	0.47	4.17	4.42	1.53	1.51	1.15	1.36	5.08
<i>O. fuscidentalis</i> (larva)	1.06	2.37	3.00	2.68	1.55	1.20	13.09	4.71
<i>O. smaragdina</i> (adult)	9.10	45.78	47.51	14.03	21.48	43.32	12.81	33.23
<i>O. smaragdina</i> (larva)	5.04	8.67	26.66	9.70	5.67	10.19	1.84	14.74
<i>P. barthelemyi</i> (adult)	4.57	19.11	24.90	5.25	11.34	18.26	9.13	23.35

<i>P. barthelemyi</i> (pupa)	1.94	16.59	28.92	18.58	5.46	19.41	3.97	12.73
<i>P. barthelemyi</i> (larva)	22.57	78.42	81.09	46.79	47.59	97.00	27.89	61.18
<i>S. cynthia ricini</i> (larva)	1.03	1.21	1.99	1.96	2.31	1.11	3.53	2.42
<i>S. monstrosus</i> (adult)	21.16	65.67	76.88	31.07	39.85	75.40	12.95	60.93
Wood larva	22.49	34.33	51.82	48.54	9.38	32.17	18.81	53.35

His = Histidine, Ile= isoleucine, Leu= Leucine, Lys = Lysine, Met = Methionine, Phe = phenylalanine,

Thr = threonine, Val = Valine.

6.3.7.2 Essential amino acids (ug/100g) in roasted form

In the roasted forms of insects' samples, Histidine ranged from 0.44 (*M. elongate*) to 32.09 (*Cossus* sp.), Isoleucine ranges from 2.4 (*O. fuscidentalis*) to 62.22 (*O. smaragdina* larva), Leucine ranged from 2.37 (*O. fuscidentalis* larva) to 78.37 (*Cossus* sp.), Lysine ranges from 3.19 (*O. fuscidentalis* larva) to 25.48 (*O. smaragdina*), Methionine ranges from 2.12 (*O. fuscidentalis* larva) to 39.70 (*O. smaragdina* larva), Phenylalanine ranges from 2.01 (*O. fuscidentalis* larva) to 57.43 (*Cossus* sp. larva), Threonine ranges from 2.88 (*L. ruber*, Adult) to 35.35 (*Cossus* sp (larva), Valine ranges from 4.9 (*O. fuscidentalis* larva) to 52.4 (*Cossus* sp. larva). In insects such as *O. fuscidentalis*, Wood larva, *O. smaragdina*, and *P. barthelemyi* the variations of amino acids in the forms of peaks are shown in Figure 6.15.

Table 6.2. Essential amino acid (ug/100g) profile of different edible insects in roasted form

Name	His	Ile	Leu	Lys	Met	Phe	Thr	Val
<i>A. nepalensis</i>	4.96	29.63	38.19	11.60	16.90	34.69	29.17	27.12
<i>Cossus</i> sp (L)	25.75	53.25	78.37	21.58	28.44	57.43	35.35	52.40
<i>C. limbatus</i> (A)	14.88	18.05	42.92	10.96	22.08	27.08	15.48	23.87
<i>H. cashmirensis</i> (A)	10.86	13.22	42.43	10.14	18.17	22.20	6.84	18.30
<i>L. ruber</i> (A)	3.26	6.17	19.74	8.67	4.61	6.83	2.88	10.20
<i>L. indicus</i> (A)	10.94	14.65	18.66	12.21	9.60	14.98	15.56	20.77
<i>M. elongata</i> (A)	0.44	33.51	41.69	16.83	19.12	44.87	5.18	35.38
<i>O. fuscidentalis</i> (L)	0.94	2.40	2.37	3.19	2.12	2.01	14.04	4.90

<i>O. smaragdina</i> (A)	8.97	26.45	39.48	11.54	11.93	30.61	18.53	26.82
<i>O. smaragdina</i> (L)	15.84	62.22	59.44	25.48	39.70	53.69	20.45	39.46
<i>P. barthelemyi</i> (A)	2.60	17.61	29.25	6.36	8.41	15.39	9.42	20.99
<i>P. barthelemyi</i> (P)	3.39	37.72	37.29	20.56	32.19	29.44	4.65	31.04
<i>P. barthelemyi</i> (L)	4.11	10.84	14.61	5.71	3.49	6.04	5.67	14.52
<i>S. cynthia ricini</i> (L)	5.14	3.97	5.50	5.06	3.47	3.15	3.76	4.82
<i>S. monstrosus</i> (A)	1.25	10.15	17.98	9.61	4.56	9.87	13.84	18.43
Wood larva	32.09	10.30	8.36	7.59	3.13	4.48	6.25	17.93

A=Adult, L=Larva, P=pupa; His = Histidine, Ile= isoleucine, Leu= Leucine, Lys = Lysine, Met =

Methionine, Phe = phenylalanine, Thr = threonine, Val = Valine.

6.3.7.3 Non-essential amino acids (ug/100g) in fresh form

A total of 9 different non-essential amino acids, namely alanine, aspartic acid, arginine, cysteine, glutamic acid, glycine, proline, serine, and tyrosine, were present in the fresh insect samples (Table 6.3). The concentration of Alanine in different insects exhibited wide variations, it ranges from 3.42 (*Cossus* sp. larva) to 104.26 (Wood larva). Aspartic acid ranges between 1.04 (*O. fuscidentalis* larva) to 46.29 (Wood larva), Arginine ranges from 1.8 (*Cossus* sp. larva) to 37.93 (*P. barthelemyi*), Cystine ranges between 0 (*O. fuscidentalis* (larva) to 47.26 (*S. monstrosus*), Glutamic acid ranges between 0.24 (*P. barthelemyi* pupa) to Wood larva (16.11), Glycine ranges between 1.06 (*Cossus* sp. larva) to Wood larva (62.21), Proline ranges from 3.93 (*Cossus* sp. larva) to 92.35 (*P. barthelemyi* larva), Serine concentration ranges from 0.28 (*Cossus* sp.) to 0.43 (*A. nepalensis*) and Tyrosine ranges between 1.33 (*Cossus* sp. larva) to 39 (*S. monstrosus*) (Table 6.3). Chromatogram of non-essential amino acid in the fresh (F) form of *O. fuscidentalis*, Wood larva, *A. nepalensis*, and *P. barthelemyi* are given in Figure 6.14.

Table 6.3. Non-essential amino acid (ug/100g) profile of different edible insects in fresh form

Name	Ala	Asp	Arg	Cys	Glu	Gly	Pro	Ser	Tyr
<i>A. nepalensis</i>	29.49	18.05	13.47	2.49	0.00	18.7	25.49	4.33	9.84
<i>Cossus</i> sp (L)	3.42	3.85	1.80	0.70	0.00	1.06	3.93	0.28	1.33
<i>C. limbatus</i> (A)	16.00	3.51	17.25	0.00	0.00	2.73	15.79	1.02	1.37
<i>H. cashmirensis</i> (A)	20.00	4.11	3.94	1.80	0.00	9.20	28.59	1.25	1.95
<i>L. ruber</i> (A)	10.17	17.30	3.29	5.44	0.00	7.12	18.01	0.93	5.72
<i>L. indicus</i> (A)	7.15	2.52	3.90	4.61	0.76	9.03	12.19	1.87	3.98
<i>M. elongata</i> (A)	5.77	2.15	3.13	1.65	1.22	3.43	9.84	0.56	2.16
<i>O. fuscidentalis</i> (L)	7.39	1.04	4.77	0.00	0.47	4.41	7.51	1.89	2.60
<i>O. smaragdina</i> (A)	31.85	9.41	24.95	24.01	1.17	25.6	24.27	1.79	27.82
<i>O. smaragdina</i> (L)	29.83	12.53	13.63	4.52	0.00	26.3	23.80	2.02	10.26
<i>P. barthelemyi</i> (A)	19.83	8.03	17.41	5.06	0.00	33.7	19.98	0.85	35.39
<i>P. barthelemyi</i> (P)	22.66	9.65	10.13	2.69	0.24	22.6	20.30	1.17	8.51
<i>P. barthelemyi</i> (L)	55.57	12.07	37.93	26.36	8.29	41.2	92.35	3.63	34.40
<i>S. cynthia ricini</i> (L)	7.60	2.29	3.13	2.52	0.31	9.47	10.56	2.26	3.04
<i>S. monstrosus</i> (A)	43.82	14.48	20.68	47.26	0.27	19.2	45.56	1.22	39.61
Wood larva	104.2	46.29	15.02	4.12	16.11	62.2	91.22	33.1	13.48

A=Adult, L=Larva, P=pupa; Ala= Alanine Asp= Aspartic acid Arg= Arginine Cys= Cysteine Glu= Glutamic acid Gly= Glycine Pro= Proline Ser = Serine Tyr= Tyrosine

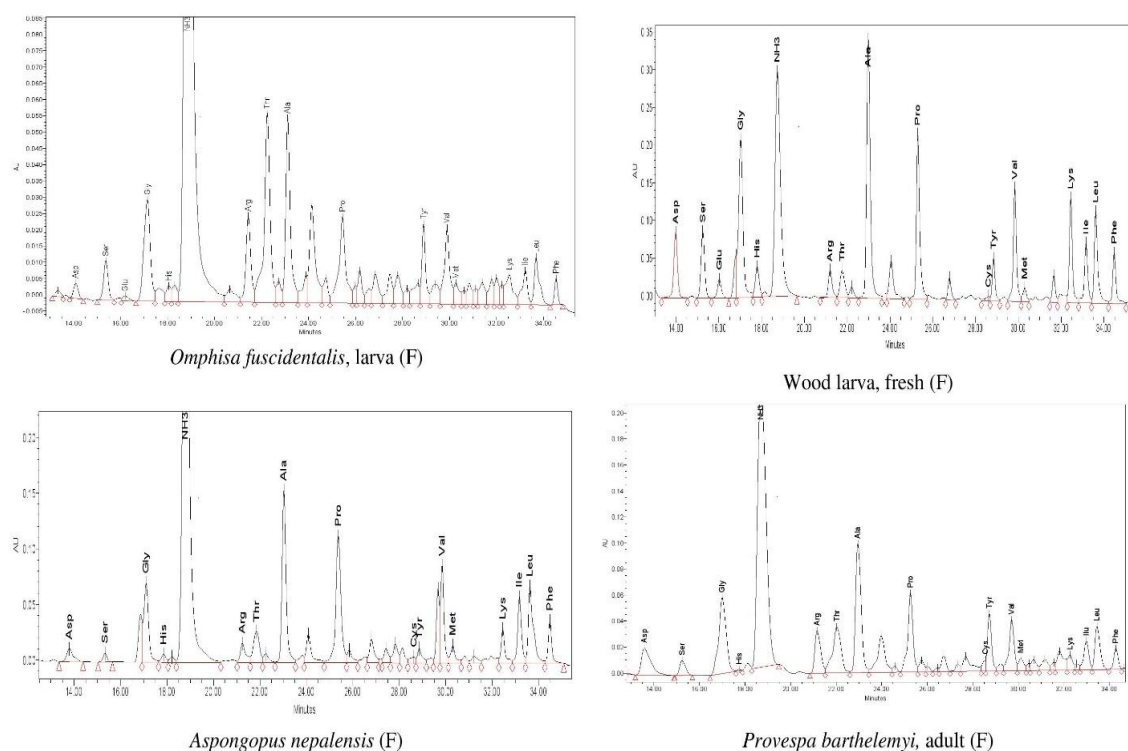


Figure 6.14. Non essential amino acid chromatogram in the fresh (F) form of *O. fuscidentalis*, Wood larva, *A. nepalensis*, and *P. barthelemyi*

6.3.7.4 Non-essential amino acids (ug/100g) in roasted form

The Alanine concentration ranges from 7.54 (*O. fuscidentalis* larva) to 88.23 (*Cossus* sp.), Aspartic acid concentration ranges from 1.12 (wood larva) to 20.23 (*A. nepalensis*), Arginine concentration ranges between 5.04 (*O. fuscidentalis* larva) to 44.37 (*Cossus* sp. larva), Cysteine concentration ranges from 1.53 (Wood larva) to 20.65 (*C. limbatus*), Comparatively, a low amount of glutamic acid was present with a concentration ranging between 0.13 (*O. fuscidentalis* larva) to 5.93 (Wood larva). In insects' such as *P. barthelemyi* adult, *S. monstrosus*, *L. ruber*, *A. nepalensis*, *M. elongata*, and *S. cynthia ricini* larva, glutamic acid was absent. Glycine showed a wide range of variations, with the highest concentration being 112.08 (*Cossus* sp. larva) and least being 4.70 (*O. fuscidentalis*), Proline varied between 5.75 (*O. fuscidentalis* larva) to 103.88 (Wood larva), Serine ranges between. Serine concentration ranged between 0.63 (*M. elongate*) to 13.35 (*L. indicus*), Tyrosine ranged between 5.82 (*L. ruber*) to

64.67 (*Cossus* sp.) (Table 6.4). Chromatogram of amino acids in roasted forms of *O. fuscidentalis*, Wood larva, *O. smaragdina*, and *P. barthelemyi* are given in Figure 6.15.

Table 6.4. Non-essential amino acids (ug/100g) profile of different edible insects in roasted form

Name	Ala	Asp	Arg	Cys	Glu	Gly	Pro	Ser	Tyr
<i>A. nepalensis</i>	30.20	20.23	15.68	8.32	0.00	21.23	25.31	1.90	32.96
<i>Cossus</i> sp (L)	88.23	7.14	44.37	11.47	5.83	112.08	76.09	4.64	64.6
<i>C. limbatus</i> (A)	22.25	12.60	16.78	19.48	0.78	37.57	21.94	2.29	18.79
<i>H. cashmirensis</i> (A)	13.58	18.87	12.63	20.65	0.18	17.49	16.01	1.07	14.04
<i>L. ruber</i> (A)	15.70	18.95	9.78	2.14	0.00	11.39	12.08	1.28	5.82
<i>L. indicus</i> (A)	37.54	17.36	18.75	3.13	0.82	27.08	31.46	13.3	14.37
<i>M. elongata</i> (A)	28.38	8.57	9.56	12.80	0.00	6.84	30.07	0.63	27.91
<i>O. fuscidentalis</i> (L)	7.54	1.12	5.04	0.00	0.13	4.70	5.75	5.79	8.48
<i>O. smaragdina</i> (A)	35.92	6.40	22.92	3.89	0.83	42.52	36.51	1.40	30.62
<i>O. smaragdina</i> (L)	45.71	9.18	23.71	20.20	4.90	24.58	59.32	2.18	34.95
<i>P. barthelemyi</i> (A)	30.31	19.94	16.14	4.50	0.00	30.64	31.04	1.66	8.66
<i>P. barthelemyi</i> (P)	15.44	6.43	8.09	16.76	0.26	9.33	16.55	0.85	19.15
<i>P. barthelemyi</i> (L)	19.45	8.03	12.37	1.90	0.71	17.82	27.73	1.13	9.45
<i>S. cynthia ricini</i> (L)	11.97	5.52	12.27	0.00	0.00	11.02	11.32	9.84	11.89
<i>S. monstrosus</i> (A)	20.14	17.21	8.87	3.43	0.00	19.31	27.26	1.29	7.21
Wood larva	16.98	1.12	17.15	1.53	5.95	9.31	103.8	6.84	11.24

A=Adult, L=Larva, P=pupa; Ala= Alanine Asp= Aspartic acid Arg= Arginine Cys= Cysteine Glu= Glutamic acid Gly= Glycine Pro= Proline Ser = Serine Tyr= Tyrosine

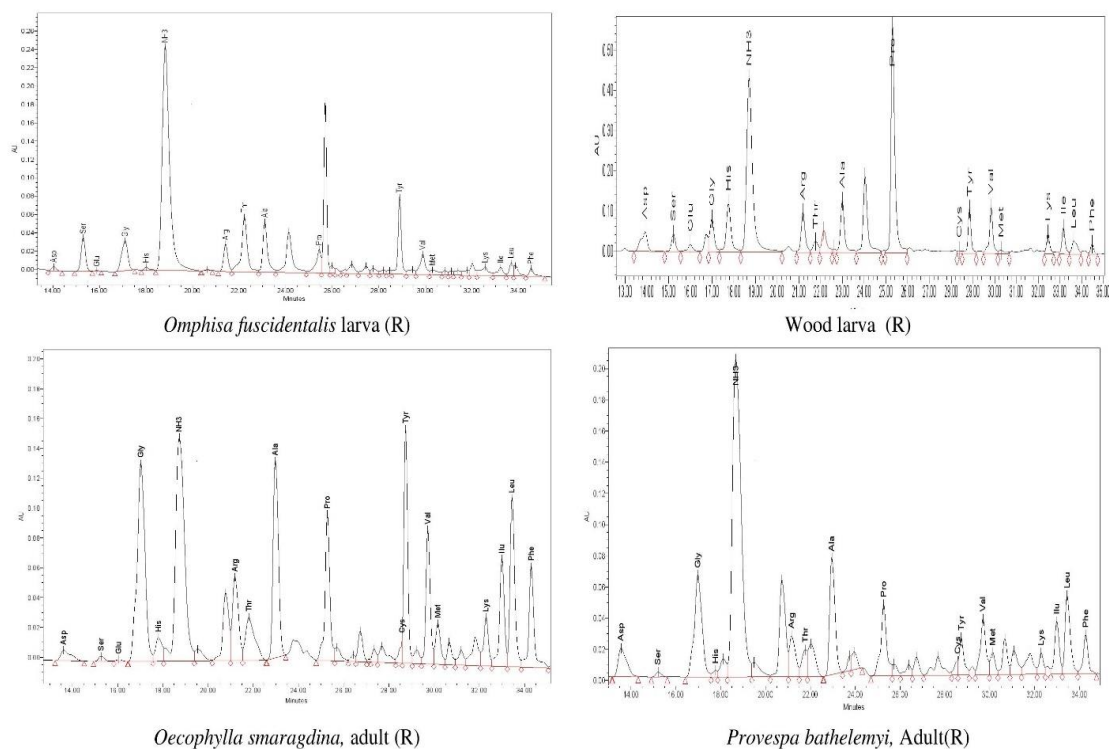


Figure 6.15. Amino acid chromatogram in the roasted (R) form of *O. fuscidentalis*, Wood larva, *O. smaragdina*, and *P. barthelemyi*

6.3.8 Screening of fatty acids (ug/100g)

6.3.8.1 Fatty acid (ug/100g) profile in fresh form

The fatty acids reported in the fresh edible insects include myristic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, total saturated fatty acid (TSFA), total monosaturated fatty acid (TMUFA), and total polysaturated fatty acid (TPUFA). Myristic acid ranges from 0.53 (*L. indicus*) to 2.86 (*H. cashmirensis*). Palmitoleic acid range from 0.67 (*P. barthelemyi*) to 13.64 (*A. nepalensis*), Linoleic acid ranges from 1.82 (*O. smaragdina* adult) to 20.12 (Dragon nymphs), Oleic acid range between 38.35 (*S. monstrosus*, adult) to 54.60 (*Cossus* sp.), Stearic acid ranged from 1.45 (*O. fuscidentalis* larva) to 12.39 (*P. barthelemyi* larva), TSFA ranged from 29.69 (*M. elongata*) to 44.24 (Wood larva). A narrow range of variations was observed for TUFMA, where the concentration range was between 38 (*S. cynthia ricini* larva) and

59.47 (*M. elongata*), TPUFA ranges between 1.820 (*S. monstrosus*) to *M. elongata* (20.12) (Table 6.5). A Chromatogram of fatty acid in *A. nepalensis*, *S. cynthia ricini*, *P. barthelemyi*, and *O. smaragdina* are shown in Figure 6.16.

Table 6.5. Fatty acid (ug/100g) profile in fresh form of edible insects

Name	M-acid	Palt acid	Pal acid	Lin acid	Ol-acid	St acid	TSFA	TMUFA	TPUFA
<i>A. nepalensis</i>	0.83	13.64	32.11	4.71	42.52	5.83	38.49	56.16	8.52
<i>Cossus</i> sp (L)	0.95	1.41	35.95	2.44	54.60	4.09	42.13	56.57	5.82
<i>C. limbatus</i> (A)	1.15	4.47	31.64	10.99	42.55	7.27	38.79	47.96	8.25
<i>H. cashmirensis</i> (A)	2.86	11.62	30.34	7.41	38.74	6.07	44.23	51.11	8.34
<i>L. ruber</i> (A)	0.75	8.18	33.19	8.55	41.08	7.87	38.77	49.26	4.71
<i>L. indicus</i> (A)	0.53	1.80	21.17	8.21	53.36	11.69	31.96	58.40	8.57
<i>M. elongata</i> (A)	0.54	1.93	20.26	8.57	52.44	11.16	29.69	59.47	20.12
<i>O. fuscidentalis</i> (L)	0.76	13.20	38.77	4.56	39.22	1.45	40.98	48.19	4.56
<i>O. smaragdina</i> (A)	2.21	3.82	33.14	1.82	51.42	7.15	40.07	55.25	5.05
<i>O. smaragdina</i> (L)	1.85	3.51	28.20	6.31	49.26	10.20	41.8	52.77	8.55
<i>P. barthelemyi</i> (A)	1.88	0.67	24.41	8.10	50.56	12.39	40.25	51.94	6.31
<i>P. barthelemyi</i> (P)	1.88	0.67	24.41	8.10	50.56	12.39	38.68	51.94	8.10
<i>P. barthelemyi</i> (L)	2.30	1.88	28.72	5.05	51.89	9.06	39.16	53.77	6.19
<i>S. cynthia ricini</i> (L)	1.35	5.61	20.65	19.37	41.31	6.96	34	38	5.6
<i>S. monstrosus</i> (A)	1.45	4.34	25.85	19.23	38.35	9.65	42.50	42.69	1.82
Wood larva	0.86	6.41	39.14	5.82	39.46	2.13	44.24	52.06	7.57

A- Adult, L-larva, P=pupa; M-acid-Myristic acid, Palt acid- Palmitoleic acid, Pal acid-Palmitic acid, Lin acid-Linoleic acid, Ol- acid-Oleic acid, St acid-Stearic acid

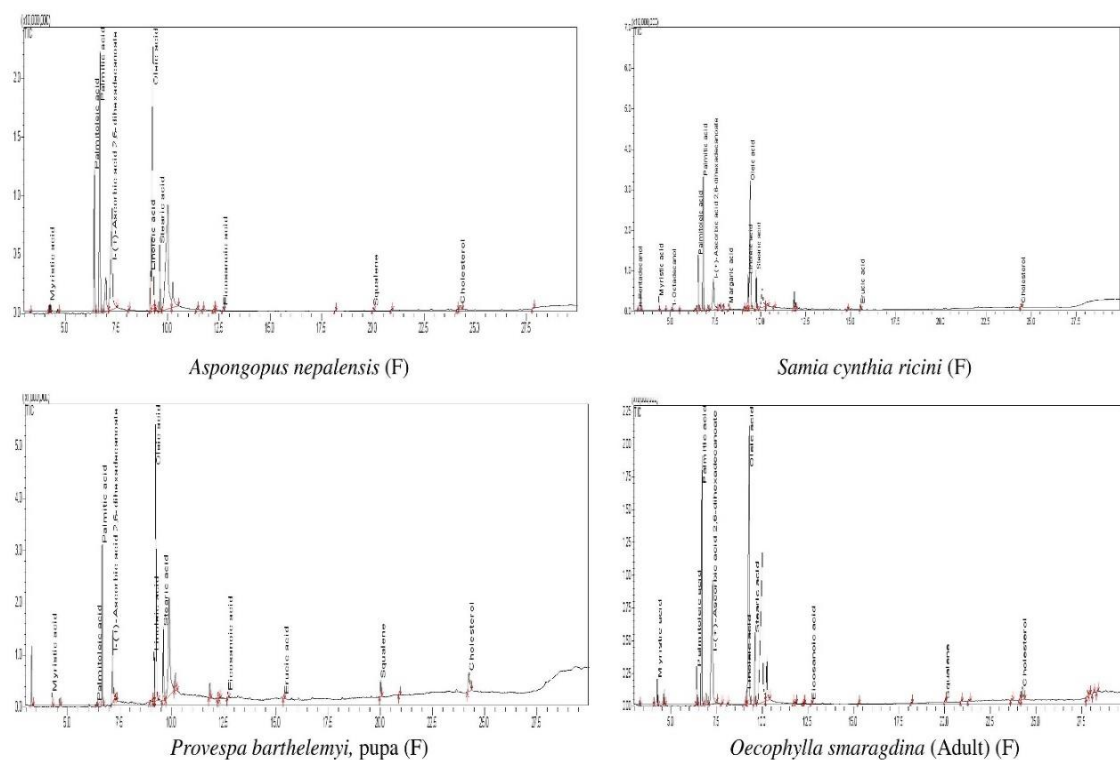


Figure 6.16. Fatty acid chromatogram in fresh (F) form of *A. nepalensis*, *S. cynthia ricini*, *P. barthelemyi*, and *O. smaragdina*

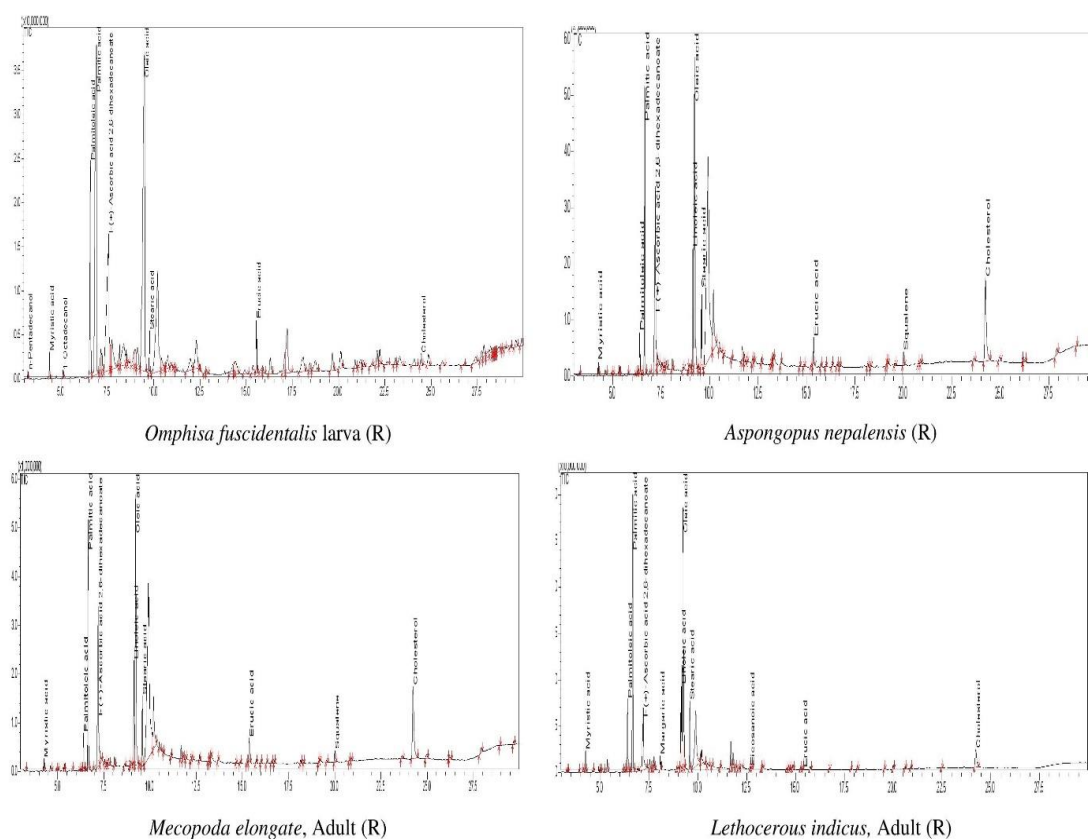
6.3.8.2 Fatty acid (ug/100g) profile in roasted form

Myristic acid ranges from 0.61 (*A. nepalensis*) to 3.31 (*O. smaragdina* larva). Palmitoleic acid range from 0.43 (*S. cynthia ricini* larva) to 13.82 (*A. nepalensis*), palmitic acid ranges from 23.42 (*P. barthelemyi* adult) to 39.72 (Wood larva), Linoleic acid ranges from 0.12 (*Cossus* sp. larva) to 17.43 (*S. monstrosus* adult), Oleic acid range between 38.56 (Wood larva) to 61.61 (*Cossus* sp. larva), Stearic acid ranged from 1.83 (*O. fuscidentalis* larva) to 12.17 (*P. barthelemyi* adult), TSFA ranged from 31.99 (*P. barthelemyi* larva) to 44.1 (*Cossus* sp. larva). TUFMA concentration range between 44.19 (*S. cynthia ricini* larva) to 62.56 (*Cossus* sp. larva), TPUFA ranges between 0.00 (*O. fuscidentalis* larva) to 19.23 (*O. smaragdina* adult). A Chromatogram of fatty acid *O. fuscidentalis*, *A. nepalensis*, *M. elongata*, and *L. indicus* are shown in Figure 6.17.

Table 6.6. Fatty acids (ug/100g) profile in roasted form of edible insects

Name	M- acid	Palt- acid	Pal- acid	Lin- acid	Ol- acid	St- acid	TSFA	TMUFA	TPUFA
<i>A. nepalensis</i>	0.61	13.82	30.43	5.38	43.43	6.21	40.54	57.37	10.38
<i>Cossus</i> sp (L)	0.73	0.95	30.79	0.12	61.61	5.80	44.10	62.56	5.46
<i>C. limbatus</i> (A)	1.56	6.46	30.09	15.48	38.97	5.87	37.05	45.86	12.82
<i>H. cashmirensis</i> (A)	1.50	6.44	28.35	12.82	39.23	7.20	37.32	50.13	0.12
<i>L. ruber</i> (A)	1.99	4.79	27.94	7.39	44.87	10.82	35.18	49.66	17.43
<i>L. indicus</i> (A)	1.92	7.76	31.29	10.38	39.22	7.33	36.92	47.18	12.69
<i>M. elongata</i> (A)	1.24	4.28	27.53	12.69	42.53	8.14	41.98	50.39	16.87
<i>O. fuscidentalis</i> (L)	0.95	13.73	37.25	0.00	43.40	1.83	40.02	54.34	0.00
<i>O. smaragdina</i> (A)	1.88	3.99	33.53	2.51	49.90	7.68	36.95	53.89	19.23
<i>O. smaragdina</i> (L)	3.31	0.91	25.32	6.19	52.19	10.53	41.43	53.11	8.71
<i>P. barthelemyi</i> (A)	2.70	0.98	23.42	8.52	50.47	12.17	41.13	51.45	7.39
<i>P. barthelemyi</i> (P)	2.21	1.95	28.38	8.71	46.18	10.84	38.19	48.20	7.04
<i>P. barthelemyi</i> (L)	2.77	1.75	25.69	7.04	53.02	9.73	31.99	54.77	18.36
<i>S. cynthia ricini</i> (L)	3.22	0.43	34.18	8.34	43.65	6.83	36.91	44.91	7.17
<i>S. monstrosus</i> (A)	1.21	4.31	24.11	17.43	41.97	9.86	43.09	46.28	2.51
Wood larva	2.08	8.79	39.72	7.57	38.56	2.44	40.98	48.19	4.56

A-Adult, L-larva, P=pupa; M-acid-Myristic acid, Palt acid- Palmitoleic acid, Pal acid-Palmitic acid, Lin acid-Linoleic acid, Ol- acid-Oleic acid, St acid-Stearic acid



6.4 Discussion

With the application of processing methods such as frying and roasting, the average concentration of protein content reduced significantly ($p<0.05$) from 7.87 ± 1.18 (Fresh) to 3.38 ± 0.98 (Fried) and 2.89 ± 1.26 (Roasted). Oonincx and Finke, (2020) reported that factors such as high pressure and temperature decrease protein quality and the amino acid availability of edible insects. Dobermann et al. (2019) demonstrated that the protein content of insects increased by 1% when the insects were dried at lower temperatures. Manzano-Agugliaro et al. (2012) highlighted that the protein content of coleopteran ranged from 23-66% while studies by Kourimska and Adamkova (2016) reported the protein content of insects between 20-76% depending on their developmental stages. High protein content indicates that insects can be valued and used as an alternative for other animal proteins that are usually absent in the diet of poor sections of society, especially for rural dwellers (Banjo et al., 2006). Reports also showed that the protein content in edible insects ranged from 35–60 g/100 g dry weight and 10–25 g/100 g fresh weight (Melo et al., 2011; Schluter et al., 2017) which was significantly higher than the protein content of cereal, soybeans, and lentils (Bukkens,1997).

Lipids are essential in diets as they increase the palatability of foods by absorbing and retaining their flavors (Aiyesanmi and Oguntokun, 1996). Lipids are also vital in the cells' structural and biological functioning and help transport nutritionally essential fat-soluble vitamins (Omotoso, 2006). It is reported that, in insects, lipid contents are higher in the larval stage than in pupa and adults (Dossey et al., 2016). The present findings also showed that in insects such as *Samia cynthia ricini*, the lipid content in the larval form was higher when compared to the pupa. Contrastingly in *P. barthelemyi*, the highest amount of lipid was observed in adults, followed by pupa, and larvae. From an initial concentration of 0.32 ± 0.31 mg/g in fresh form the average

amount of lipid content increased to $0.78 \pm 0.33 \text{ mg/g}$ and $1.25 \pm 0.85 \text{ mg/g}$ in fried and roasted forms of insects. A high amount of lipids in fried samples was observed, possibly due to the insects' body's absorption of fats from cooking oil. Oliveira et al. (1976) also reported that processing methods like frying also change the fatty acids profile of insects due to a mixture of naturally occurring fats in the insects' bodies and fats from cooking oils. Yang et al. (2006) reported that lipid content showed a wide variation in giant water bugs, beetles, and water scavengers. Similarly, in the present study, lipid content was observed to vary among larval, pupal, and adult, indicating that habitat and diet significantly contribute to the total lipid content of insects.

Carbohydrates also known as saccharides are group that include starch, sugar and cellulose. Insects may store their energy reserves as carbohydrates (sugar, glycogen) or fat (Steppuhn and Wackers, 2004), and variations in nutritional content can possibly be due to differences in dietary habitats (Banjo et al., 2006). In insects such as *P. Barthelemy* and *S. cynthiaricini* a wide variation of carbohydrates was observed in the different stages of development, such as adult, larva, and pupae. Also, after frying and roasting, carbohydrates increased to 3.96 ± 0.24 and $3.86 \pm 0.3 \text{ mg/g}$, respectively, from 2.67 ± 0.15 in roasted form. It is suggested that in general, carbohydrates are present in very small quantities in insects (Finke, 2002, 2013), for instances carbohydrates content in yellow mealworms can vary between 1-7% (Chen et al., 2010). Oonincx and Finke (2020) suggested that variations of carbohydrates in insect' samples can be attributed to food remaining in the gastrointestinal tract. Similarly, several researchers (Shantibala et al., 2014; Narzari and Sarmah, 2015; Aochen et al., 2020) have also reported the variations of carbohydrates in different edible insects.

In the present study, TPC varies widely depending on the insect species, processing methods, and developmental stages of insects. As shown in Figure 6.5 and

Figure 6.6, total phenol content (TPC) varies significantly ($p<0.05$) depending on the insect's species and their processing forms. The TPC concentration in the present study is very low, which could be due to fresh weight-based analysis. Also, TPC can be affected by various factors, such as the specific part of the insect body used to extract, the method of extraction, and the solvent or methodology used for measurement. Del Hierro et al. (2020) found that the phenolic content of lyophilized flours from *A. domesticus* and *T. molitor* ranged from 0.3 and 5g GAE/100g, this concentration varied greatly depending on the extraction method and solvent used. Similarly, Di Mattia et al. (2019) observed that TPC values obtained from defatted flours of various edible insects and invertebrates varied between 0.125 and 0.496 g GAE/100g. Kunatsa et al. (2020) also reported varied TPC values for different types of edible insects. Di Mattia et al. (2019) reported that factors such as dietary habits can also affect TPC, as vegetarian insects tend to have higher phenolic content than carnivorous ones. As shown in Figure 5, phenol content increased significantly with the application of processing methods such as frying and roasting ($p<0.05$). Phenol group consist of compounds such as phenolic acids, flavonoids, and tannins (Vuolo et al. 2019; Panzella et al., 2020). They are considered one of the most diverse and important biological compounds. They are also considered an important ingredient and component of food preparations because of their antioxidant and antimicrobial capacity (Torres-Castillo and Olazarán-Santibáñez, 2023). As an alternative source of nutrients, phenol content in edible insects is highly relevant in the present scenario and has become a subject of food research. It is reported that the presence of phenolic compounds in food products provides color and taste to prepared meals, adding further value and usefulness from the food industry's perspective (Rosales-Escobar et al., 2018; Zielinska, 2021).

Antioxidants are a group of compounds that has inhibiting or reducing effects caused by the free radicals or no free radicals' reactive species (Barbosa et al., 2010). In the present study, similar to total phenol compound, antioxidants concentration also increased significantly with different processing methods. The average amount of antioxidants increased to 5.29 ± 0.38 , and 5.35 ± 0.34 mg/g L-ascorbic acid equivalent in fried and roasted state from 4.25 ± 0.34 in fresh form. Also, in different forms of development such as larva, pupa and adult of *S. cynthia ricini* and *P. barthelemyi* total antioxidant concentrations were substantially varied. Serpen et al. (2012) reported that with the thermal treatment applications, the antioxidant concentration may increase due to the changes in their tertiary and quaternary structures. While other researchers reported that, hydrophobic and aromatic amino acids improve the force of antioxidant peptides through proton-donation capacity, electron-donation capacity, or by radical scavenging capacity (Da Rocha et al., 2018; Najafian and Babji, 2012). Liu et al. (2016) reported that peptides with a low molecular weight, such as methionine and lysine, interact with free radicals, and this increases their antioxidant effect. During the production of the silkworm powder, Anuduang et al. (2020) reported that different forms of thermal processing methods affect the antioxidant and other physicochemical properties and suggested that shorter exposure time to hot water and a lower drying temperature should be applied to prevent the loss of bioactive compounds in silkworm powder. In contrast to the present study, De Carvalho Tavares et al. (2019) reported a decrease in antioxidant concentration in an insect sample (Silkworm) when exposed to high temperature which was attributed to the formation of pro-oxidants by the Maillard reaction.

As shown in Table 6.1, eight essential amino acids, namely Histidine, isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine were present

in the edible insects indicating edible insects as rich source of nutrients. The nonessential amino acid observed in the present study include Alanine, Aspartic acid, Arginine, Cysteine, Glutamic acid, Glycine, Proline, Serine and Tyrosine. The concentration of amino acids varied widely among the species. Munro and Crim (1988) reported that 8 amino acids such as Isoleucine, lysine, Methionine, Phenylalanine, Threonine, Valine, and Histidine, are essential in human diets. In *Protaetia brevitarsis* powder essential amino acid is composed of 17.68% and 33.97% of non-essential amino acids (Chung et al., 2013). Cameron and Hofvander (1980) reported that Leucine and histidine enhance the growth of young children and infants; therefore, according to Dreyer and Wehmeyer (1982), consuming insects may, to some degree, supplement protein requirements. Studies showed that amino acids are higher in insects than in beef, pork, and chicken meat. Ekpo and Onigbinde, (2005) reported that the histidine content is 3.44g/100g, lysine 3.99g/100g, and methionine 2.05g/100g in *Rhynchophorusphoenicis*. For the metabolism of proteins, amino acids are required, and the essential amino acids must be acquired from the diet as the human body cannot synthesize them naturally (WHO, 2007).

In the present study, the fatty acids present in the edible insect were Myristic acid Palmitoleic acid, Palmitic acid, Linoleic acidOleic acid, Stearic acid, TSFA, TMUFA, TPUFA (Table 6.5, 6.6 and 6.7). Wide variations in fatty acids depending on the insect's species and developmental stages were observed. Similarly, Kulma et al. (2020) reported that developmental stages of insects have a great influence on the amino acids and fatty acids present in a particular insect. Chung et al. (2013) reported that *Protaetia brevitarsis* powder contained 61.10% unsaturated fatty acid with oleic acids, while unsaturated fatty acid was found to be 76.80 - 80.55% of the total fatty acids in mealworms. The linoleic acid contents in the Korean and Chinese mealworms

were 20.8 ± 1.1 , 20.8 ± 1.1 and 34.69 ± 1.9 , 34.69 ± 1.9 ; the linolenic acid contents were 0.47% and 1.31%; and the oleic acid contents were 51.40 ± 0.9 , 51.40 ± 0.9 and 40.20 ± 1.5 , 40.20 ± 1.5 , respectively (Yoo., et al., 2013). Saturated fatty acids (SFA) in edible insects ranged from 25.32% (*T.molitor*) to 35.3% (*S. gregaria*). Palmitic acid and stearic acid are the two main components of SFA, and 18.2% palmitic and 3.84% of stearic was found in *T.molitor*, and 7.35% and 9.27% of stearic acid in *G. sigillatus* and *S. gregaria*. (Zielinska et al., 2015). A study by Yang et al., (2006) on grasshoppers showed 18.2% of palmitic acid and 6.4% of stearic acid. The monounsaturated acids (MUFA) ranged from 34.33% in *G. sigillatus* to 43.27% in *T.molitor*. The maximum amount of oleic acid was found in *T.molitor* i.e. 40.86%. The range of polyunsaturated acids (PUFA) was from 26.28% in *S.gregaria* to 31.37% in *G. sigillatus*. (Zielinska et al., 2015). Yang et al. (2006) also reported similar values in crickets which is 33.8%. Similarly, 30% of the linoleic acid was shown in *G. sigillatus* and *T.molitor*. (Zielińska et al., 2015). Many studies pointed out that the composition of fatty acids of insects' lipids is greatly influenced by insects' feed (Bukkens, 1997; Tzompa-Sosa et al., 2014). *Imbrasiabelina* combines saturated and unsaturated acids where palmitic acid, stearic acid and linolenic acid are 31.9%, 15% and 18 % respectively. (Amadi and Kiin-Kabari, 2016). In *Cirinaforda* rich amount of linolenic acid (33.8%), stearic acid (27.4%), palmitic acid (17.2 %,) and oleic acid (12.9 %) (Ande, 2003). Due to the high presence of linolenic acid, *Cirinaforda* larva can be used as infant food since this fatty acid enhances the healthy development of children and infants (Michaelsen, et al., 2009). Fatty acids play a major role in biological, functional, and structural roles in the human body. The human body is capable of synthesizing most fatty acids except for a few essential PUFAs such as omega-6 linoleic acid (LA) and omega-3- α -linolenic acid (ALA) (Nagy and Tiuca, 2017). A study by Lehtovaara et al. (2017) showed that the amount of fatty

acids in *Ruspolia differens* can be manipulated by changing the diet. Dreassi et al. 2017 reared yellow mealworms on six different substrates with increasing total fat content (0.46–9.34%). In the present study SFA, MUFA, and PUFA contents of the substrates were 19.6–29.2, 32.0–41.4, and 37.6–47.9, respectively.

Chapter 7

Summary and Conclusion

The present study not only comprehensively documented the diversity of edible insects in Nagaland but also underscored the profound significance of the unique cultural practices associated with their harvesting, culinary preparations, and consumption across different districts. These practices, deeply rooted in local traditions, not only add a layer of intrigue but also evoke a sense of appreciation for the rich cultural heritage of Nagaland.

A staggering total of 108 edible insect species belonging to 9 orders and 32 families were meticulously documented. Orthoptera (25 species) and Hymenoptera (25 species) comprised the highest number of species, followed by Hemiptera (22 species), Coleoptera (16 species), Odonata (8 species), Lepidoptera (8 species), Mandotea (2 species), Isoptera (1 species), and Diptera (1 species). This abundance and variety of edible insects truly reflect the richness and uniqueness of Nagaland's food culture.

Crickets and grasshoppers are abundant from early summer to early winter. They are easily collected by handpicking and are commonly roasted, fried, or cooked for consumption. Certain species, like mole crickets, require specific collection methods due to their habitat preferences.

Cicadas and stink bugs are consumed after removing wings, typically roasted or fried. Their availability ranges from August to November, with nymphs collected from the soil and adults captured using glue-coated poles or near light sources. Bees and wasps are valued for their honey and larvae. Honey is considered medicinally important and is extracted using traditional methods, a testament to the meticulous knowledge and practices of the local communities. Larvae are often eaten raw or cooked, and different species are reared in wooden hives. Wood borer larvae are harvested from infested trees and prepared by roasting or frying. They are available from June to October and are

collected by handpicking or splitting open wood, a process that requires careful attention and respect for the environment.

Silkworms, caterpillars, and other moth larvae are collected from trees and consumed cooked or fried. Certain species are preferred for their taste and aroma and are available from May to November. The insects' preferences vary across districts, reflecting the unique food cultures of each region. Grasshoppers, crickets, and cicadas, for instance, have high use value in many districts, while insects like weaver ants and rhino beetles are less utilized. Districts like Phek exhibit a higher average use value for edible insects, indicating their profound cultural and dietary importance in these areas. Overall, the edible insect diversity sheds light on Nagaland's rich variety of edible insects and enhances our understanding of their importance in local diets and traditions.

The consumption of edible insects in Nagaland is influenced by various factors such as availability, size, taboos, personal taste preferences, market value, and traditional medicinal knowledge. While different Naga tribes have their preferences, the giant hornet (*Vespa mandarinia*) is a delicacy enjoyed by all ethnicities, with its larvae, pupae, and adults being prepared in various ways, including frying and cooking with local spices.

Harvesting and consuming hornets are predominantly male activities, with women usually marketing the harvested combs/nests. Traditional methods of locating and capturing hornets involve tactics passed down through generations, such as baiting and teamwork to track the insects to their nests. Semi-domestication of hornets involves constructing artificial structures for rearing, with careful attention paid to site selection for successful rearing.

Hornets hold significant socio-economic value in Nagaland, serving as an alternative protein source and contributing to the livelihoods of rural communities. The

demand for hornets is increasing due to their nutritional value and unique taste, leading to their incorporation into various culinary dishes. Additionally, hornets are utilized in traditional medicine and other cultural practices, highlighting their importance in Naga society.

There are opportunities to commercialize hornet culture through mass-rearing techniques and industrial-scale production. However, sustainable practices and government support are essential to ensure the conservation of hornets and the preservation of traditional knowledge associated with their harvesting and consumption.

Private enthusiasts and professional growers globally primarily undertake rearing beetles and other insects. Despite this, there's untapped potential for rearing these insects in their native habitats, with initiatives like providing suitable sites for their growth. Semi-domesticating of *T. wallichii* involves manipulating the environment to encourage infestation of host plants. In the case of *T. wallichii*, the process includes inducing infestation of *Acacia pennata* plants, which are native to South and Southeast Asia. This semi-domestication process requires careful attention to environmental conditions and natural cues for optimal results. Once the infestation is initiated, the life cycle of *T. wallichii* progresses through various stages, including mating, oviposition, larval development, and harvesting. Harvesting typically occurs after several months, with larvae being collected manually and prepared for consumption or sale. Traditional laws exist to protect domesticators' rights and regulate harvesting practices. The socio-economic potential of *T. wallichii* is highlighted, particularly in regions like Nagaland, where it serves as a significant source of protein and supplementary income. Market surveys indicate a growing demand for insect-based products due to their nutritional value and taste. Consumption of *T. wallichii* larvae involves various cooking methods, with the larvae being prized for their high protein content and taste. Proximate analysis

reveals promising nutritional attributes, suggesting insect farming could address dietary deficiencies, especially in rural communities.

The consumption of edible insects, such as giant hornets and *T. wallichii*, is deeply ingrained in Naga culture and plays a pivotal role in the socio-economic fabric of Nagaland. A combination of factors shapes preferences for insect consumption, and traditional harvesting and rearing practices are integral to ensuring the sustainability of insect populations. Mass-rearing insects like *T. wallichii* present a promising opportunity for sustainable food production and economic development, particularly in regions where traditional knowledge and practices support their semi-domestication. The study underscores the need for continued research, conservation efforts, and the promotion of insect-based livelihoods to harness the full potential of these bio-resources while preserving indigenous knowledge and cultural heritage. By recognizing the benefits of hornets and adopting sustainable practices, Nagaland can pave the way for a future where insect consumption contributes to economic prosperity and food security in harmony with the environment. However, challenges such as labor-intensive processes, environmental variability, and market dynamics need to be addressed to realize the full potential of insect farming.

Regulatory frameworks and community-led initiatives are crucial in ensuring sustainable harvesting practices and equitable distribution of benefits. Overall, insect farming represents a valuable contribution to addressing global food security challenges and promoting environmentally friendly agricultural practices. Continued research, innovation, and collaboration are essential for unlocking insect cultivation's socio-economic and nutritional benefits on a larger scale.

The study of *T. portentosus*, a cricket species, offers an in-depth exploration of its life cycle, and behaviour, covering its egg development, nymphal instars, adult

morphology, and habitat characteristics. Freshly laid eggs are described as oblong, with a gradual increase in weight until hatching, which occurs around day 33.

Nymphs undergo seven instars, characterized by significant morphological changes, including wing pads and ovipositors development. Adults exhibit cylindrical bodies with distinct antennae, wings, and reproductive organs. The life history of *T. portentosus* includes burrow construction, mating behavior, and nocturnal activity patterns.

Beyond morphological characteristics, the study delves into the species' ecological niche, highlighting its preference for grassland-type vegetation with sparse trees, where burrows are constructed to depths ranging from 50mm to 800mm. Behavioral observations reveal nocturnal habits, burrow construction techniques, and mating behaviors, providing valuable insights into the species' existence and interactions within its environment.

Despite the significant strides made in understanding *T. portentosus*, further research can explore population dynamics, genetic diversity, and ecological interactions, offering promising avenues for refining mass-rearing techniques. By leveraging advancements in these areas, researchers can fine-tune rearing protocols to address specific needs and challenges, thereby improving the scalability and sustainability of mass-rearing initiatives.

While insects are consumed after being well-cooked by the ethnic communities in Nagaland, the quality and safety of insect consumption are a significant concern for consumer safety globally. In view of the above, the present study also analyzes fresh and roasted insect samples for microbial loads to determine the efficiency of heat treatment on microbial contaminants.

The study revealed notable differences attributable to heat treatment. Fresh samples exhibited higher microbial colonies than roasted ones, likely due to the susceptibility of fresh samples to contamination owing to their higher moisture content. This finding aligns with previous studies reporting similar trends in other insect species. Aerobic microbial analysis showed that roasted samples had significantly lower microbial counts, indicating an effective reduction of microbial contamination through heat treatment. However, certain bacteria, particularly spore-forming ones, persisted even after roasting, suggesting further processing or storage interventions to ensure microbial safety. The study also emphasizes the importance of proper storage facilities and processing methods to mitigate the risk of contamination in edible insects. While heat treatments like roasting can reduce microbial load, additional steps such as blanching or storage in acidic conditions may enhance safety. Moreover, the investigation highlights the necessity of species-specific processing methods and comprehensive microbial quality parameters to ensure consumer safety and facilitate the global market integration of insect-based products.

Continued research is warranted to identify specific microbes and food pathogens associated with edible insects, enabling the development of tailored processing techniques and storage protocols. Furthermore, a comprehensive assessment of farming practices and environmental factors influencing microbial contamination is crucial for ensuring food safety throughout the production chain. By addressing these challenges, edible insects can be safely introduced into the global market, offering a sustainable and nutritious alternative food source while minimizing health risks for consumers.

The preparation of insects for human consumption not only affects their nutritional content but also plays a significant role in consumer preferences and

acceptance, guiding consumers on the quality and recommended quantity of insect consumption. The present study was also aimed at assessing the nutritional composition of various edible insects across different developmental stages and preparation methods, focusing on protein, lipid, carbohydrate, total phenol, total antioxidant, amino acid, and fatty acid content.

The average protein content in fresh, fried, and roasted forms decreased significantly from 7.87 ± 1.18 mg/g to 3.38 ± 0.98 mg/g to 2.89 ± 1.26 mg/g. Similarly, lipid content significantly decreased from fresh to roasted samples, with concentrations of 0.32 ± 0.31 mg/g, 0.78 ± 0.33 mg/g, and 1.25 ± 0.85 mg/g, respectively. Carbohydrate content varied among preparation methods, with concentrations of 2.67 ± 0.15 mg/g in fresh, 3.96 ± 0.24 mg/g in fried, and 3.86 ± 0.3 mg/g in roasted samples. Total phenol content increased from fresh to roasted samples, with concentrations of 3.96 ± 0.28 mg/g, 5.2 ± 0.46 mg/g, and 6.52 ± 0.41 mg/g, respectively. Total antioxidant concentrations followed a similar trend, increasing from 4.25 ± 0.34 mg/g in fresh samples to 5.29 ± 0.38 mg/g in fried samples and 5.35 ± 0.34 mg/g in roasted samples. Amino acid profiling revealed wide variations depending on insect species, developmental stages, and preparation methods. Essential amino acid concentrations ranged widely, with histidine concentrations ranging from 0.26 to 22.56 mg/g, isoleucine from 0.67 to 78.42 mg/g, and leucine from 1.99 to 81.09 mg/g in fresh samples. Fatty acid profiling showed variations in myristic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, total saturated fatty acid (TSFA), total monounsaturated fatty acid (TMUFA), and total polyunsaturated fatty acid (TPUFA) concentrations across different insect species and preparation methods.

Overall, the study underscores the nutritional diversity of edible insects and highlights the impact of preparation methods on their nutritional composition. The

findings provide valuable insights for further research and developing insect-based food products catering to diverse nutritional needs and preferences. Further investigation into the bioavailability and health benefits of insect-derived nutrients is warranted to fully exploit the potential of edible insects as a sustainable and nutritious food source.

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List of Publications

Kiewhuo, P., Mozhui, L., Kakati, L. N., & Meyer-Rochow, V. B. (2022). Traditional rearing techniques of the edible Asian giant hornet (*Vespa mandarinia* Smith) and its socio-economic perspective in Nagaland, India. *Journal of Insects as Food and Feed*, 8(3), 325-335.

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Kiewhuo, P., Jing, L., Rutsa, M. C., Kakati, L. N., Ao, B., Mozhui, L., & Meyer-Rochow, V. B. (2024). Semi-domestication of the longhorn beetle *Thysia wallichii* (Hope, 1831) and its nutritive value. *Journal of Asia-Pacific Entomology*, 102211.

Mozhui, L., Kakati, L. N., **Kiewhuo, P.**, & Changkija, S. (2020). Traditional knowledge of the utilization of edible insects in Nagaland, North-East India. *Foods*, 9(7), 852.

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Conferences Attended/Workshops Presented / Attended

Oral Presentation in a national seminar on “Recent Breakthroughs and Innovations in Science and Technology” organized by Assam Science Society, AdtU Branch and Faculty Science, AdtU on 30th March 2024.

Oral Presentation at an International Conference on Multidisciplinary Aspects of Environment and Sustainable Development organized by the Department of Zoology at Raj Rishi Govt. Autonomous College from 15th – 18th Dec. 2021.

Oral Presentation at an International E-Conference on Sustainable and Futuristic Materials (SFM-2021) Organized by International Research Centre and Department of Chemistry, Kalasalingam Academy of Research and Education, Krishnankoil, Department of Chemistry, J. M. Patel Arts, Commerce & Science College, Bhandara, and Department of Chemistry, Kamla Nehru Mahavidyalaya, Nagpur on 29-30th November 2021.

Oral Presentation at an International Conference on “Impacts & Consequences of Environmental Degradation on Animal Health and Human Wellbeing, organized by Abhayapuri College in association with the Department of Zoology, Guwahati University and Aaranyak, Assam, on 2nd to 4th September 2021.

Oral Presentation in Two day National Seminar on Biodiversity for Human Welfare: Current and Future Trends India (BHCFTI-2019)- Sponsored by National Biodiversity Authority and India Academic Researchers Associations on 7-8th Feb 2019 organized by Department of Zoology St. Joseph University, Dimapur, Nagaland.2019.

Attended a one-day State Level workshop on Intellectual Property Rights (IPR) for Accelerating Creativity and Innovation organized by Nagaland Science and Technology Council (NASTEC), Department of Science & Technology, Government of Nagaland, in collaboration with Technology Information, Forecasting, Assessment Council (TIFAC), Department of Science & Technology, Government of India, held on 24th April 2023, at Capital Convention Centre, Kohima.

Attended a voice-virtual 3rd International Conference on Environmental, Agricultural, Chemical, and Biological Science ICEACBS 2022 United Nations SDGS Jan 22 to 26, 2022.

Attended a one-day seminar on advances in Bioscience and technology organized by the Department of Biotechnology, St. Joseph University, on 6th May 2022.

Attended workshop on Remote Sensing and GIS applications in Forestry and Ecology organised by North Eastern Space Applications Centre (NESAC) from 01–05 February 2021.

Three Day Virtual International Conference on

**“Impacts & Consequences of Environmental Degradation
on Animal Health and Human Wellbeing”**



Aaranyak

Certificate

This is to certify that **Kiewhuo P** of Nagaland University, Lumami, Nagaland, India participated and presented her research paper titled **“Microbial load check in commonly available edible insects of Nagaland in north-east region”** in the three day virtual International Conference on **“Impacts & Consequences of Environmental Degradation on Animal Health and Human Wellbeing”** held on 2nd to 4th September, 2021, organized by Abhayapuri College in association with Department of Zoology, Gauhati University and Aaranyak, Assam.

We wish him/her all success in life.

Prof. Jogen Chandra Kalita
(Chief Adviser)
Head, Dept. of Zoology, GU

Dr. Sadananda Nath
(President)
Principal, Abhayapuri College

Dr. Pori Devi
(Convener)
Asst. Prof., Abhayapuri College



International E-Conference on Sustainable and Futuristic Materials (SFM-2021)

Organized by

International Research Centre and Department of Chemistry, Kalasalingam Academy of Research and Education, Krishnankoil

Gondia Education Society's

Department of Chemistry, J.M. Patel Arts, Commerce & Science College, Bhandara

And

Amar Sewa Mandal's

Department of Chemistry, Kamla Nehru Mahavidyalaya, Nagpur

CERTIFICATE

This is to certify that **Patricia kiewhuo** of **Nagaland university** has participated and delivered Oral Presentation in the **International E-Conference on Sustainable and Futuristic Materials (SFM-2021)** held during **29-30th November, 2021** organized by **International Research Center and Department of Chemistry, Kalasalingam Academy of Research and Education, Krishnankoil, Department of Chemistry, J. M. Patel Arts, Commerce & Science College, Bhandara, and Department of Chemistry, Kamla Nehru Mahavidyalaya, Nagpur.**

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Dr. V. P. Dhomne
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Dr. W. B. Gurnule
Organizing Secretary
SFM-2021

**RAJ RISHI GOVT.
AUTONOMOUS COLLEGE,
ALWAR(RAJASTHAN)**



CERTIFICATE OF PRESENTATION

THIS IS TO CERTIFY THAT

Patricia Kiewhuo

from

Nagaland university, Lumami, Nagaland, India
has presented the Research Paper "Entomophagy practices by
Angami tribe and its socio-economic potential for the
future in Nagaland, India." in the **International Conference
on Multidisciplinary aspects of Environment and
Sustainable Development** organized by **Department of
Zoology** at Raj Rishi Govt. Autonomous College from 15th - 18th Dec.
2021.

Prof. (Dr.) Hukam Singh
Patron & Principal

Dr. Kalpana Soni
HOD, Zoology

Dr. Mamta Sharma
Organising Secretary

Academic achievements

NET, UGC, Environmental Science (Assistant Professor) 2019.

Best oral Presentation (2nd Prize) in Two day National Seminar on Biodiversity for Human Welfare: Current and Future Trends India (BHCFTI-2019)- Sponsored by the National Biodiversity Authority and India Academic Researchers Associations on 7-8th Feb 2019, organized by the Department of Zoology St. Joseph University, Dimapur, Nagaland.

The story “Giant with no Good” was shortlisted in the National “Popular Science Story Writing”, jointly organized by the Department of Science and Technology (DST) and Vigyan Prasar (VP).